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The relationship between cardiovascular disease and other chronic conditions

Edited by
Cristina Vassalle, Junjie Xiao and Laura Sabatino

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The relationship between cardiovascular disease and other chronic conditions

Topic editors

Cristina Vassalle — Gabriele Monasterio Tuscany Foundation (CNR), Italy

Junjie Xiao — Shanghai University, China

Laura Sabatino — Institute of Clinical Physiology, Department of Biomedical Sciences, National Research Council (CNR), Italy

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Editorial: Relationship Between Cardiovascular Disease and Other Chronic Conditions

Cristina Vassalle[1]*, *Junjie Xiao*[2] and *Laura Sabatino*[3]

[1] Fondazione National Council of Researches-Regione Toscana Gabriele Monasterio, Pisa, Italy, [2] Shanghai Engineering Research Center of Organ Repair, School of Life Science, Shanghai University, Shanghai, China, [3] Institute of Clinical Physiology, National Council of Researches, Pisa, Italy

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Editorial on the Research Topic

Relationship Between Cardiovascular Disease and Other Chronic Conditions

BIOMARKERS AND RISK FACTORS

Cardiovascular disease (CVD) remains the most common cause of mortality and comorbidity all over the world.[1] Risk stratification plays an important role in the prevention of the onset of cardiovascular disease and the development of its complications. As CVD is multifactorial, several associated risk factors have long been identified (e.g., smoking habit, improper diet, low physical activity, hypertension, hypercholesterolemia, diabetes) (1). The key question is whether we have enough biomarkers to better stratify CV risk. Although the CV burden is generally featured by these traditional risk factors, the importance of non-traditional biomarkers may be critical to fill the gap related to the so-called "residual risk." In fact, CV load cannot be entirely explained by traditional cardiovascular risk factors, as many patients at risk for adverse prognosis do not present any of these traditional determinants (2). Such evidence suggests the involvement of possible overlooked non-traditional biomarkers that may play an important role in the pathogenesis and development of CVD (2). Thus, there is an increasing interest for additional biomarkers and risk factors providing potential new tools against CVD onset and progression. Discovery of such factors and, most important, of their mechanism of action, may help in the development of advanced care strategies aiming to the reduction of cardiovascular harmful impact. For example, between biochemical factors, beside the widely employed troponins (ischemic damage) and natriuretic peptides (cardiac stress and dysfunction), it would be important to better clarify the role of already known biomarkers in this new context, such as vitamin D well known for bone health, or other new proposed parameters (e.g., oxidative stress biomarkers, hemoglobin, galectin-3-fibrosis, cystatin-renal dysfunction, cytokines and neutrophils/lymphocytes-inflammation, d-dimer-coagulation). However, despite the best efforts, so far, no reliable applicable additive biomarkers have been identified.

A list of all contributions to this special issue is reported in **Table 1**.

Majority of the contributions in this special issue explore the significance of different biomarkers and risk factors in the various CVD manifestations. Among the 21 manuscripts dealing with this issue, we hereby report a few representative examples:

[1] https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1

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Hendrik Tevaearai Stahel, Bern University Hospital, Switzerland

***Correspondence:**
Cristina Vassalle
cristina.vassalle@ftgm.it

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Inflammation

Regarding inflammation, Hou et al. investigated the "neutrophil to lymphocyte ratio (NLR)," which is a novel inflammatory biomarker, calculated from old well known hemochrome parameters, association with CVD in children with newly diagnosed essential hypertension. Results obtained suggested that a high NLR might be a potential indicator of increased risk of the development of hypertension and LV diastolic dysfunction in children.

In another article, Hu et al. explored the relationship between neutrophil respiratory burst and coronary artery lesions (CAL), suggesting this biomarker as significant in the pathogenesis of CAL and CAL prediction of Kawasaki disease in children.

Gender/Sex Related Differences

Till now, CVD is perceived as a condition essentially regarding the male gender, although CV events account for the main cause of mortality and morbidity in postmenopausal women (3). Obviously, if it is clear that many aspects of CVD are similar in male and female patients, there are significant differences as well (4, 5). Cellular and molecular mechanisms and clinical manifestations of CVD in women are far from being fully understood. Advances in this field are essential to improve CVD pathophysiological knowledge as well as diagnostic and clinical strategies in women, in order to develop specific female-based algorithms. In this special issue, in particular, two studies addressed different aspects of gender-related characteristics. Wei et al. investigate the relationship between sex-specific associations of adverse health outcomes, left ventricular structure/function and microalbuminuria in patients with heart failure with preserved ejection fraction (HFpEF). The authors found that microalbumin/creatinine ratio (ACR) was significantly associated with LV diastolic function, hospitalization, and myocardial infarction in men, while ACR was associated with mortality in women. The interactions of sex with ACR were significant in heart failure. Moreover, the article of Surrati et al. deals with the important topic of awareness of CV risk. The study was conducted on Saudi Arabia University female employers. Authors reported limited knowledge and awareness of CVD risk, which evidenced the pressing need of educational interventions to enhance the awareness of CVD risk factors and prevention in the female population.

Arterial Stiffness

Although circulating biochemical markers are extensively studied, other physiological parameters can be effective (6). It is the case of the cardio-ankle vascular index, measured in the study of Watanabe et al. as an indicator of arterial stiffness, which resulted independently associated with impaired exercise capacity and adverse prognosis in HF patients.

Environmental Pollution

It should not be forgotten the effect of the environment on CVD, a risk factor completely neglected in clinical practice. Interestingly, Zou et al. faced the complex relationship between pollution and cardiovascular disease, reporting the latest evidence on this topic. In their systematic review and meta-analysis including exclusively cohort studies, the authors evidenced the relationship between PM2.5 and PM10 and the risk of myocardial infarction. Sensitivity analyses confirmed and even reinforced these findings. Subgroup analyses by geographical area and year of publication did not show any statistically significant difference in results. Moreover, the study of Liang et al. evidenced as short- and medium-term exposure to PM2.5 significantly increased the risk of emergency room visits in atrial fibrillation patients, suggesting the importance of air quality and providing a rationale to implement actions for reducing CVD risk in the population.

Multi-Marker Approach

Combining more biomarkers in a multi-marker approach capturing different aspects of CVD (e.g., ischemia, necrosis, thrombosis, inflammation, and fibrosis), may increase the diagnostic and prognostic capacity (7, 8). In this context, Yao et al. investigated the prediction factors of poor prognosis (mortality and/or readmission) after acute myocardial infarction (AMI) during a 6-month follow-up and suggested the multi-biomarker approach using Killip classification 2–4 and myoglobin or creatinine effective for 6-month prognosis prediction in AMI patients. Moreover, Wang et al. investigated the relationship between N-terminal pro-B-type natriuretic peptide (NT-proBNP), Glomerular Filtration Rate (GFR), and outcomes in patients hospitalized with acute heart failure (AHF). The authors found that the risk of death of patients with NT-proBNP>2,137 pg/ml and GFR<61.7 ml/(min·1.73 m^2) was significantly higher, and suggested that the combination of GFR and NT-proBNP improved the predictive value for the long-term prognosis of AHF patients.

Genetics, Chromosomal Abnormality, Metabolomics

The study of the genetic components may offer important advances in the pathophysiological aspects of CVD, in the effort of developing more efficient predictive tests for those at high risk (9). Genetic biomarkers are already present at birth and, thus, risk prediction can be evaluated before CV risk factors' onset and development, in a primordial prevention strategy. Accordingly, Cheng et al. investigated the role of neutrophil-derived S100B genetic variants in atherosclerosis progression of acute myocardial infarction (AMI), evidencing how the S100B rs9722 AA homozygous might promote the development of AMI.

Interestingly, Cheng et al. showed that ventricular septal defect (VSD) is closely related to chromosomal aneuploidies by reporting a pedigree with VSD associated with a balanced paracentric inversion of chromosome 6, inv (5) (p21.3p23). This evidence might represent a new genetic etiology for VSD.

New "omic" fields (e.g., metabolomics) are also emerging, rendering reasonable phenotype identification of patients on the basis of biomarker cluster analysis (where multiple co-occurring pathological factors can simultaneously be found in a single clustering) clinically useful in the next future time (10). In this context, the study from Yang et al. suggested that non-targeted metabolomics could evidence biochemical pathways associated with Ang II-induced hypertension in an experimental model. Available data in this field may improve knowledge of systemic metabolic response to sustained release of Ang II, providing a

TABLE 1 | List of manuscripts included in the special issue.

Topic	Publication type	Title	Authors	Key words
Biomarkers and risk factors	SR	Long-Term Exposure to Ambient Air Pollution and Myocardial Infarction: A Systematic Review and Meta-Analysis	Zou L et al.	PM10; PM2.5 (AQI); air pollution; meta-analysis; myocardial infarction; particulate matter
	SR	Effect of Uric Acid-Lowering Agents on Patients With Heart Failure: A Systematic Review and Meta-Analysis of Randomised Controlled Trials	Xu H et al.	uric acid, hyperuricemia (HUA), heart failure, left ventricular ejection fraction, six minute walk test, B type natriuretic peptide, mortality
	SR	Hypertension in Children and Adolescents: A Position Statement From a Panel of Multidisciplinary Experts Coordinated by the French Society of Hypertension	Bouhanick B et al.	French position statement; adolescents; children; high blood pressure; hypertension
	OR	Increased Neutrophil Respiratory Burst Predicts the Risk of Coronary Artery Lesion in Kawasaki Disease	Hu J et al.	Kawasaki disease, coronary artery lesion, neutrophil, flow cytometry, brain natriuretic peptide
	OR	Prognostic Value of N-Terminal Pro-B-Type Natriuretic Peptide and Glomerular Filtration Rate in Patients With Acute Heart Failure	Wang K et al.	acute heart failure (AHF), N-terminal pro-B-type natriuretic peptide (NT-proBNP), glomerular filtration rate (GFR), outcomes, prognosis
	OR	Prediction Factors of 6-Month Poor Prognosis in Acute Myocardial Infarction Patients	Yao J et al.	acute myocardial infarction, prognosis, death, readmission, biomarker
	OR	Vitamin D Deficiency and Vasovagal Syncope in Children and Adolescents	Zhang Q et al.	children and adolescents, vasovagal syncope, vitamin D, heart rate variability, autonomic nervous function
	OR	Neutrophil to Lymphocyte Ratio Is Increased and Associated With Left Ventricular Diastolic Function in Newly Diagnosed Essential Hypertension Children	Hou M et al.	hypertension, children, neutrophil-lymphocyte ratio, left ventricular hypertrophy, left ventricular diastolic function
	OR	Sex-Specific Associations of Risks and Cardiac Structure and Function With Microalbumin/Creatinine Ratio in Diastolic Heart Failure	Wei F-F et al.	heart failiure, chronic kindney disease, echocardiography, microalbuminuria, risk stratification, gender
	OR	Cystatin C-Based Renal Function in Predicting the Long-Term Outcomes of Chronic Total Occlusion After Percutaneous Coronary Intervention	Li B et al.	cystatin C, creatinine, estimated glomerular filtration rate, chronic total occlusion, all-cause mortality, cardiac death
	OR	Role of Neutrophil-Derived S100B in Acute Myocardial Infarction Patients From the Han Chinese Population	Cheng M et al.	S100B; acute myocardial infarction; genotype; plasma biomarkers; thrombosis
	OR	Cardio-Ankle Vascular Index Reflects Impaired Exercise Capacity and Predicts Adverse Prognosis in Patients With Heart Failure	Watanabe K et al.	cardio-ankle vascular index, arterial stiffness, cardiopulmonary exercise testing, heart failure, prognosis
	OR	Cardiovascular Risk Awareness and Calculated 10-Year Risk Among Female Employees at Taibah University 2019	Qasem Surrati AM et al.	cardiovascular disease, knowledge, awareness, risk factors, Madinah-KSA, calculated 10 year risk
	OR	Effect of Air Quality on the Risk of Emergency Room Visits in Patients With Atrial Fibrillation	Liang B et al.	PM2.5, atrial fibrillation, risk of emergency room visit, monsoon climate region, patients
	OR	Prevalence and Predictors of Left Ventricular Diastolic Dysfunction in Malaysian Patients With Type 2 Diabetes Mellitus Without Prior Known Cardiovascular Disease	Han Chee KH et al.	diastolic dysfunction, diabetes mellitus, left ventricular dysfunction, prevalence, Asian

(Continued)

TABLE 1 | Continued

Topic	Publication type	Title	Authors	Key words
	OR	Longitudinal Effect of Hemoglobin Concentration With Incident Ischemic Heart Disease According to Hepatic Steatosis Status Among Koreans	Jung DH et al.	hemoglobin, hepatic steatosis, cohort study, ischemic heart disease, risk factor, extrahepatic complications
	OR	Association Between C-Peptide Level and Subclinical Myocardial Injury	Chen Z et al.	C-peptide, subclinical cardiac injury, NHANES III, association, cross sectional study
	OR	UPLC-MS-Based Serum Metabolomics Reveals Potential Biomarkers of Ang II-Induced Hypertension in Mice	Yang S et al.	hypertension, LC-MS, angiotensin II, metabolomics, serum metabolites, biomarkers, mice
	OR	Correlation Analysis of Anti-Cardiolipin Antibody/D Dimer/C-Reactive Protein and Coronary Artery Lesions/Multiple-Organ Damage in Children With Kawasaki Disease	Xu Y-M et al.	anticardiolipin antibody (ACA), D dimer, C reactive protein (CRP), coronary artery lesions (CALs), multiple organ damage, Kawasaki disease (KD), children
	OR	Hyperuricemia Predicts Adverse Outcomes After Myocardial Infarction With Non-obstructive Coronary Arteries	Mohammed A-Q et al.	myocardial infarction, MINOCA, serum uric acid, hyperuricemia, outcome
	OR	Relative contribution of plasma homocysteine levels vs. traditional risk factors to first stroke: a nested case-control study in rural China	Zhou F et al.	homocysteine, systolic blood pressure, first stroke, ischemic stroke, population attributable risk
	CS	A Chromosomal Inversion of 46XX, inv (6) (p21.3p23) Connects to Congenital Heart Defects	Cheng L et al.	congenital heart disease, ventricular septal defect, chromosomal rearrangement, human chromosome 6, proband
CVD and other conditions	R	Impact of Increased Oxidative Stress on Cardiovascular Diseases in Women With Polycystic Ovary Syndrome	DuicăF et al.	polycystic ovary syndrome, cardiovascular disease, oxidative stress, C-reactive protein, homocysteine, miRNA
	R	Association Between Periodontal Disease and Atherosclerotic Cardiovascular Diseases: Revisited	Zardawi F et al.	periodontal therapy, relation, periodontal disease, cardiovascular diseases, atherosclerosis
	R	Metabolism and Chronic Inflammation: The Links Between Chronic Heart Failure and Comorbidities	Li Z et al.	heart failure, comorbidities, metabolism, chronic inflammation, reactive oxygen species, mitochondria
	R	Relationship Between Sarcopenia and Cardiovascular Diseases in the Elderly: An Overview	He N et al.	sarcopenia, cardiovascular diseases, elderly people, comorbidity, aging
	OR	Gallbladder Polyps Increase the Risk of Ischaemic Heart Disease Among Korean Adults	Lee Y-J et al.	gallbladder, polyps, coronary disease, comorbidity, cohort study
	OR	Risk Factors of Atrial Arrhythmia in Patients With Liver Cirrhosis: A Retrospective Study	Lu X et al.	age; ascites; atrial arrhythmia; liver cirrhosis; risk factor
	OR	Clinical characteristics of cryoglobulinemia with cardiac involvement in a single center	He K et al.	cryoglobulinemia, cardiac involvement, clinical characteristics, treatment outcome, retrospective study
	CS	Chronic Thromboembolic Pulmonary Hypertension in a Child With Sickle Cell Disease	Spencer R et al.	CTEPH—chronic thromboembolic pulmonary hypertension; hematology; pediatric cardiology; pulmonary hypertension; sickle cell disease
	CS	Right Atrial Thrombus in a COVID-19 Child Treated Through Cardiac Surgery	Bigdelian H et al.	COVID-19, cardiac surgery, pediatric, thrombus—echocardiography, fever

(Continued)

TABLE 1 | Continued

Topic	Publication type	Title	Authors	Key words
	CS	Massive Right Atrial Thrombosis: Are You Brave Enough to Start Anticoagulation? A Case Report	Bergonti M et al.	right atrial thrombosis, pulmonary thromboembolism, thrombus, cardio-oncology, coagulation, right atrium mass
	BRR	Gout Is Prevalent but Under-Registered Among Patients With Cardiovascular Events: A Field Study	Calabuig I et al.	gout, prevalence, cardiovascular event, cardiovascular disease, urate lowering therapy
	O	Chronic Secondary Cardiorenal Syndrome: The Sixth Innovative Subtype	Zhang Y et al.	biomarker; chronic co-impairment; chronic secondary cardio renal syndrome; fibrosis; new classification; type 6 cardio renal syndrome
Prevention, diagnosis, treatment	OR	Timing of Maximal Weight Reduction Following Bariatric Surgery: A Study in Chinese Patients	Xu T et al.	bariatric surgery, Chinese patients, weight reduction, trend, follow up
	OR	Association Between Aspirin Use and Decreased Risk of Pneumonia in Patients With Cardio-Cerebra-Vascular Ischemic Disease: A Population-Based Cohort Study	Chen Y-C et al.	aspirin, pneumonia, risk, database, cardio-cerebra-vascular ischemic diseases
	OR	The Association Between Metformin Treatment and Outcomes in Type 2 Diabetes Mellitus Patients With Heart Failure With Preserved Ejection Fraction: A Retrospective Study	Wang J et al.	heart failure with preserved ejection fraction; metformin; mortality; survival analysis (source: MeSH NLM); type 2 diabetes mellitus—exenatide
	OR	The Complementary Relationship Between Echocardiography and Multi-Slice Spiral CT Coronary Angiography in the Diagnosis of Coronary Artery Thrombosis in Children With Kawasaki Disease	Xu Y-M et al.	children, Kawasaki disease, coronary artery lesion, thrombosis, echocardiography, CTCA

SR, Systematic Review; OR, Original Research; CR, Case Report; R, Review; BRR, Brief Research Report; O, Opinion.

new panel of biomarkers that may be helpful to predict blood pressure changes in the early stages of hypertension.

CVD AND OTHER CONDITIONS

Until now, the traditional medical approach to diseases has been characterized by a point of view generally focused to diagnose, and treat pathological conditions "*per se* ." Nonetheless, there is now increasingly awareness that diseases apparently independent, instead may share many risk factors and common critical pathophysiological pathways. Twelve articles in this special issue provide evidence on how each organ/system, including CV system, is not a solitary and independent entity, but is part of a whole, interacting within a complex network with other organs (11). This is an important issue, and we need to broaden our knowledge on the differences/similarities between diseases, often considered and managed as separate entities in the common clinical practice. In fact, many pathological conditions, traditionally considered unrelated, emerge as interactive with the cardiovascular system, able to evoke similar different systemic responses, and share underlying cellular pathways and biomarkers. Accordingly, several risk factors, traditionally classified as relevant for the CVD onset and progression (e.g., diet, physical inactivity, hypertension, diabetes), result to be significant in the development of other pathological states. In particular, chronic inflammation and oxidative stress represent commonly underlying aspects in the pathogenesis and progression of different diseases, although additional overlapping mechanisms and further biochemical pathways may have other acting roles. Please, refer to table and find some example below:

Dealing with the interaction between diseases, Bigdelian et al. reported an interesting case discussing the presence of a right atrial vegetation in an 11-year-old child infected by COVID-19. They hypothesized that the etiology is the result of hypercoagulation and acute thrombosis in COVID-19 patients, which raised the issue of possible targeted treatment strategies in COVID-19 patients, to avoid hypercoagulative status and thrombus development.

Calabuig et al. brought their attention to a high gout prevalence among patients admitted for CV events, often undetected and, as such, under suboptimal treatment, despite being a well recognized CV risk factor.

Zardawi et al. drew the attention of dental practitioners and cardiologists on the reciprocal role of periodontal and CVD. This is an intriguing topic for both dentists and cardiologists in recent years, suggesting that CVD patients may benefit from periodontal check, whereas patients with periodontal disease may benefit from periodical CV evaluation. Moreover, authors also discussed available evidence on common factors that may

drive the progression of both diseases. Essentially, the main mechanisms involved are the direct invasion of bacteria and inflammation. For what concerns therapy, periodontal treatment was effective in reducing the level of inflammatory biomarkers and improving endothelial function, whereas local application of statin could also improve periodontitis through its anti-inflammatory effect.

Duica et al. reviewed the current literature and provided a new perspective of polycystic ovary syndrome, a reproductive endocrine condition, in the context of key inflammatory and oxidative stress factors and cardiovascular risk. In particular, in women with polycystic ovary syndrome, a link with the increased incidence of CVD was found, highlighting the possibility to apply an antioxidant strategy in this population.

PREVENTION, DIAGNOSIS, AND TREATMENT

Critical aspects related to prevention and treatment are faced by four more articles. In the therapeutic field, Chen Y-C et al. provided evidence on long-term low-dose aspirin association with reduced risk of pneumonia in CV patients, suggesting an important role of this drug in the prevention of this critical complication. Instead, Wang et al. evaluated the association between metformin and adverse outcome in T2DM patients with HFpEF, evidencing how metformin in this population was not independently associated with clinical outcomes in patients with T2DM and HFpEF, but resulted related with lower all-cause mortality in the subgroup of patients with poor glycemic control. Moreover, Xu et al. dealt with strengths and limitations of two imaging techniques (transthoracic echocardiography and multi-slice spiral CT coronary angiography) for identifying coronary artery thrombosis in children with Kawasaki disease (KD), evidencing their reciprocal complementarity, and the utility of their combination to improve the diagnosis rate for coronary thrombosis.

CONCLUSIONS

In conclusion, identification of new biomarkers may provide additional pathophysiological information improving biological knowledge of the disease, as well as to help in better risk stratification and identification of new targets of interventional strategies. In this context, the adoption of a multimarker panel may provide significant gain, especially if incorporating biomarkers with a low degree (or even absence) of correlation, as a reflex of different pathophysiological pathways, and, as such, capturing more levels of information.

Nonetheless, more information from non-traditional risk factors and biomarkers is needed before their introduction into the clinical practice, and warnings about possible numerous biases need to be considered (e.g., statistics).

A biomarker is considered helpful when leads to reclassification, with the potential of an incremental gain in subjects at low and intermediate risk, and especially in specific patient groups where traditional biomarkers/scores may be not optimal (e.g., women, elderly population). Thus, the assessment of the incremental value of a proposed biomarker over traditional risk models is a critical step. In this context, advances for "omic" technologies can be considered critical to reveal novel unknown molecular pathways and biomarkers, which may be of importance to characterize a particular disease state when added to traditional algorithms. Although in their developmental phase and still presenting some shortcomings, the integration these technologies in a multi-omics approach and the generation of big data may provide an exceptional opportunity to further understand processes and dynamic interactions underlying human pathophysiology, with a great potential for their relatively rapid diffusion in the routine use (12).

Generally, for the new proposed biomarkers there is a consistent insufficiency of quality controls, primary aspects of assay performance and reliability (e.g., reference material availability, quality assurance programs). Moreover, other different issues must be faced and standardized before biomarker introduction in the clinical practice, for example:

- Preanalytical factors, i.e., all factors generating variability, but controllable or minimized by standardizing the time and condition of sampling, including circadian rhythm and seasonal variation, menstrual cycle, food intake, posture and exercise, sample type (e.g., whole blood, serum, plasma or other specimens), interferences (i.e., lipemia, hemolysis), handling and storage;
- Biological heterogeneity, analytical interferences (e.g., heterophilic antibodies, immunocomplexes);
- Limits of detection (lowest concentration that can be detected) and quantitation (lowest concentration quantitatively measured with accuracy);
- Linearity;
- Reference limits (at least cut-off values);
- Ratio of cost on effectiveness;
- Easiness of use;
- Context of application as:
  - Clinical purpose (screening, diagnosis, prognosis, monitoring, treatment),
  - Condition status (risk or presence or stage of the disease),
  - Target population (e.g., males vs. females, elderly individuals).

The leitmotiv of most manuscripts reported in this issue is that no organ or system, including the CV system, is an "island," therefore the simultaneous consideration of different conditions, so far treated separately, may be helpful to develop strategies aiming to multi-disease benefits and optimize precision medicine approaches. In this scenario, a close collaboration between cardiologists and other clinical professionals and basic science researchers (e.g., biologists, laboratorists, epidemiologists, radiologists, and physicians in other fields) is desirable.

AUTHOR CONTRIBUTIONS

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Prognostic Value of N-Terminal Pro-B-Type Natriuretic Peptide and Glomerular Filtration Rate in Patients With Acute Heart Failure

Kai Wang†, Gehui Ni†, Qianyun Wu†, Yanli Zhou, Wenming Yao, Haifeng Zhang and Xinli Li**

Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

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Edited by:
Junjie Xiao,
Shanghai University, China

Reviewed by:
Ke Xiao,
Hannover Medical School, Germany
Zhanpeng Huang,
First Affiliated Hospital of Sun Yat-Sen University, China

****Correspondence:***
Haifeng Zhang
haifeng_zhang@163.com
Xinli Li
xinli3267_nj@hotmail.com

†These authors have contributed equally to this work

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Aims: To investigate the relationship between N-terminal pro-B-type natriuretic peptide (NT-proBNP), Glomerular Filtration Rate (GFR), and outcomes in patients hospitalized with acute heart failure (AHF).

Methods: The trial was registered at http://www.chictr.org/cn/. (ChiCTR – ONC - 12001944). A total of 493 patients hospitalized for AHF in cardiology department of the First Affiliated Hospital of Nanjing Medical University from March 2012 to October 2016 were enrolled into registry. The end event was the occurrence of all-cause death within an 18-month follow-up. The data collected from the participants in admission were used to calculate the GFR by chronic kidney disease epidemiology collaboration equation (CKD-EPI) and performed the according statistical analysis.

Results: There were 74 participants (13.8%) dropped out and 91 (21.7%) passed away within the 18-month follow up. Comparison of clinical indicators between survival and death group were analyzed for the long-term prognosis of patients with AHF. In the single factor analysis, both NT-proBNP and GFR were statistically significant ($P < 0.001$). Combined NT-proBNP and GFR in multi-factor COX regression analysis showed significant predictive value ($P < 0.001$). In receiver operator characteristics (ROC) analyses, the area under the curves (AUC) for NT-proBNP was 0.648 [95%CI: 0.598–0.695, $P < 0.001$] and for GFR was 0.677 [95%CI: 0.627–0.723, $P < 0.001$]. According to the Youden index, the best prediction point of NT-proBNP was 2,137 pg/ml and GFR was 61.7 ml/(min·1.73 m^2). After using the Binary Logistic Regression to combine the two indicators, the AUC was 0.711, which was significantly compared to the AUC of either single factor. The sensitivity of the combined indicators were 0.535, the specificity were 0.853. According to the cut-off point, these two indexes were separated into four groups for further analysis by Kaplan-Meier survival curve comparison (log-rank test), which showed that patients in the group with higher NT-proBNP and lower GFR had the worst prognosis.

Conclusions: In patients with NT-proBNP > 2,137 pg/ml and GFR < 61.7 ml/(min·1.73 m^2), the risk of death was significantly higher. The combination of GFR and NT-proBNP improved the predictive value for the long-term prognosis of AHF patients.

Keywords: acute heart failure (AHF), N-terminal pro-B-type natriuretic peptide (NT-proBNP), glomerular filtration rate (GFR), outcomes, prognosis

INTRODUCTION

Heart failure (HF) is one of the common diseases of the cardiovascular system. The current annual incidence of HF in the United States and Europe are more than one million (1, 2). Acute heart failure (AHF) refers to the insufficient tissue perfusion and acute systemic or pulmonary stasis syndrome caused by a sharp decrease in cardiac output. It is clinically dangerous and progresses rapidly, which is likely to cause systemic hemodynamic disorder and multi-organ failure (3). All-cause death of AHF patients were recorded in 10% at 30-day follow-up and 50.1% at 1-year follow-up (4). In recent years, new ideas have been proposed for the treatment of HF, such as exercise, microRNAs, etc. (5, 6). However, the prognosis of hospitalized patients with HF is relatively limited. With the deepening of the research on the pathogenesis of AHF, the application value of biomarkers in the early diagnosis, risk stratification, and prognosis assessment of AHF has become the focus of research. N-terminal pro-B-type natriuretic peptide (NT-proBNP) is widely used as a clinical AHF prediction factor. Due to the large number of interfering factors, the prognosis of patients with complex HF evaluated by NT-proBNP alone has limits, which results in an urgent need for a more effective method in clinical practice. In this study we evaluated renal function indicators in patients with AHF. To provide more information about AHF prognosis to medical worker, the new CKD-EPI formula was used to calculate the glomerular filtration rate (GFR) and to evaluate the long-term survival rate of AHF patients combined with NT-proBNP.

In recent years, NT-proBNP measurement has been widely recognized as an auxiliary diagnostic condition for AHF (7). Currently in the clinical practice guidelines, it is included in the level I recommendation, evidence level: A (8–10). However, some studies have shown that the interference factors of NT-proBNP level are various, including race, sex (higher in females), obesity, anemia, common kidney disease, atrial fibrillation, chemotherapy drugs, and enkephalinase inhibitor drug (10). The interaction between heart diseases and kidney diseases is a hot topic in recent years. To elucidate the interaction between heart function and kidney function, Ledoux first proposed the concept of "cardiorenal syndrome" (CRS), kidney injury induced by HF, in 1951 (11). In 2005, Bongartz et al. redefined CRS as the dysfunction of one organ in the heart and kidneys leading to acute or chronic insufficiency of the other (12). When both heart insufficiency and renal insufficiency occur at the same time, the fatality rate will increase significantly. In this paper, we mainly discuss with type I CRS (AHF with acute kidney injury), which is common in patients hospitalized for acute decompensated HF with an incidence up to 25% (13, 14). Especially in the acute stage, the interaction between cardiac and renal functions causes more serious clinical events and deteriorates the prognosis of patients. For those patients, the potential for kidney damage should be identified timely for intervention as soon as possible in the treatment of AHF.

As a recognized independent prognostic factor, NT-proBNP has a higher sensitivity, but its specificity is affected by many non-cardiac factors, which often brings certain limitations to clinical diagnosis and evaluation. At present, for most countries in the world, medical workers analyze the condition and prognosticate AHF by using the way that combines cardiac troponin I/T(cTnI/T), hemoglobin, cystatin, and others in clinical practice (15–18). In this study, renal related markers were analyzed to explore the prognostic value of NT-proBNP combined with GFR for long-term survival in AHF patients. We adopted a prospective method by enrolling 493 AHF patients, collecting their clinical data, and ending the 18-month follow-up on time. Finally, SPSS and MedCalc statistical software were used to analyze the clinical data of 419 AHF patients who completed 18-month follow-up. It was concluded that the combination of GFR and NT-proBNP can effectively predict the long-term survival of AHF patients and improve the predictive value of NT-proBNP on mortality.

METHODS

Subjects

A total of 493 patients hospitalized for AHF (including the initial onset of AHF and acute decompensated HF) in Cardiology Department of The First Affiliated Hospital of Nanjing Medical University from March 2012 to October 2016 were enrolled into registry. The diagnosis of AHF was referred to the guidelines for AHF in China. Inclusion criteria: (1) Age ≥ 18 years old, gender is not limited; (2) The onset of AHF or CHF usually includes symptoms and signs of pulmonary congestion, systemic congestion or cardiac output reduction, the NT-proBNP > 300 pg/ml, and echocardiography indicates abnormalities in cardiac structure or function; (3) Subjects should participate in the study voluntarily and sign the informed consent. Exclusion criteria: (1) Patients with malignant tumors; (2) Patients with cognitive impairment and dementia; (3) Patients with severe hepatorenal insufficiency, and primary chronic nephropathy; (4) Patients with other serious uncontrollable systemic diseases; (5) Patients who were unwilling to sign the informed consent forms or were unable to complete all follow-up. This study has been approved by the hospital ethics committee, follows the principles of clinical

practice and the Helsinki declaration, and requires each enrolled patient to sign an informed consent form.

Data Collection

Clinical data of enrolled patients were collected and basic database was established. After enrollment, the patient's hospitalization history was checked, and their age, gender, contact information, height, weight, and other basic information were recorded. Besides, patient's admission diagnosis, etiology of AHF and history of concomitant diseases such as hypertension, diabetes, myocardial infarction, atrial fibrillation, renal insufficiency, and thyroid dysfunction were recorded. At the same time, the routine examination results of the patients on admission were collected, including blood routine, blood biochemistry, NT-proBNP, myocardial markers, coagulation function, routine 12-lead electrocardiogram, echocardiography, dynamic electrocardiogram, chest film, etc., and the diagnosis and treatment in the hospital and drug regimen were recorded.

Specimen Collection and Detection

For all patients signed the informed consent, the blood was collected through the cubital vein and injected into the anticoagulant tubes and coagulant tubes with patients' limosis condition on the morning of the second day after admission. All serological tests were completed by the laboratory division of our hospital using AU 5800 automatic biochemical analyzer (Beckman Coulter, USA) and an automatic analyzer for NT-proBNP (Roche Elecsys®proBNP immunoassay, Switzerland). The unit of NT-proBNP is pg/mL and the unit of GFR is ml/(min·1.73 m^2).

Ckd-Episcr Formula Was Used for GFR Calculation (19)

(1) Women: ①serum creatinine $\leq$ 0.7 mg/dl, GFR = 144 × (serum creatinine (mg/ dl)/0.7)$^{-0.329}$ × (0.993)^{age}; ②the serum creatinine > 0.7 mg/dl, GFR = 144 × (serum creatinine (mg/dl)/0.7)$^{-1.209}$ × (0.993)^{age}.

(2) Men: ①serum creatinine $\leq$ 0.9 mg/dl, GFR = 141 × (serum creatinine (mg/dl)/0.9)$^{-0.411}$ × (0.993)^{age}; ②serum creatinine > 0.9 mg/dl, GFR = 141×(serum creatinine (mg/dl)/0.9)$^{-1.209}$ × (0.993)^{age}.

Creatinine units conversion: 1 mg/dl = 88.4 μmol/l.

Routine 12-Lead Electrocardiogram and Echocardiography

The routine 12-lead electrocardiogram was completed by qualified clinicians with FS-8322 12-channel automatic analysis electrocardiogram machine from Beijing Futian electronic medical instrument Co., LTD.

Echocardiograph was collected by VIVID E9 color doppler ultrasonography (GE, USA). The probe frequency was 3.5 mhz, and the four-chamber heart section of the apex was taken. The left ventricular ejection fraction, left ventricular diastolic diameter, left ventricular systolic diameter, and pulmonary artery systolic pressure were measured.

Follow-Up

Patients were treated normatively by professional cardiologists during hospitalization according to the 2009 and 2014 Chinese Guidelines on Diagnosis and Treatment of AHF, and provided the optimized treatment plans before hospital discharge. Follow-up was conducted by telephone or outpatient service every 3 months after discharge, which was mainly to evaluate the occurrence of end-point events and to record the cause and time of end-point events. No intervention was performed during the follow-up. The total follow-up time was 18 months and the outcome event was all-cause death.

Statistical Analysis

All data were processed by SPSS 22.0 and MedCalc 11.4.2.0 statistical software. Kolmogorov-smironov (k-s) was used to test the normality of measurement data. The data of normal distribution were expressed as mean±standard deviation, and independent sample *t*-test was used to analyze the difference between two groups. The data of non-normal distribution were represented as median (M) and range (Q1-Q3), and the differences between groups were compared by non-parametric tests. Enumeration data were expressed as frequency or rate, and x^2 test was used for differences between groups. Multiple Cox stepwise regression analysis (forward) was used to identify independent predictors of 18-months mortality in the study. Variables associated with 18-months mortality in univariate analysis ($P < 0.05$) were selected to be adjusted. The specificity and sensitivity of each indicator in the diagnosis of AHF patients' death were calculated by combining receiver operator characteristics (ROC) curve. Kaplan-meier method was used to draw the survival curve, and log-rank test was used for comparison between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Basic Population Data

Among the 493 patients enrolled in this study, 74 patients (13.8%) failed to complete the follow-up due to midway withdraw, and a total of 419 patients completed the follow-up. Ninety-one patients (21.7%) died during the 18-month follow-up period. The mean age of follow-up patients was 60.9 ± 15.7 years, and 277 patients (66.1%) were male. HF is classified according to the New York heart association (NYHA) classification standard. Patients were selected according to the NYHA heart function classification: 67 cases of cardiac function level II, 227 cases of cardiac function level III, and 125 cases of cardiac function level IV. According to the occurrence of all-cause death during the 18 months follow-up, the enrolled patients were divided into the death group and the non-death group for test comparison. Detailed results are shown in **Table 1**. Statistical analysis of 36 variables showed that compared with survival group, the age, NT-proBNP, D-dimer, serum uric acid, urea nitrogen (BUN), aspartate aminotransferase (AST), and pulmonary artery systolic blood pressure (PASP) of the death group were higher; while GFR, systolic pressure, diastolic blood pressure, serum sodium, albumin, and hemoglobin were comparatively lower ($P < 0.05$).

TABLE 1 | Baseline clinical characteristics of patients.

	All patients ($n = 419$)	Survival ($n = 328$)	Death ($n = 91$)	*P*-value
Demographic characteristics				
Age, years	60.9 ± 15.7	60.0 ± 15.7	64.1 ± 15.5	0.03
Gender(%) male	277 (66.1)	222 (67.7)	55 (60.4)	0.20
female	142 (33.9)	106 (32.3)	36 (39.5)	
SBP, mmHg	124.8 ± 20.4	127.1 ± 20.4	116.5 ± 18.1	< 0.01
DBP, mmHg	77.7 ± 13.6	79.5 ± 13.7	71.2 ± 11.3	< 0.01
Heart rate, beat/min	85.7 ± 21.1	86.3 ± 21.1	83.5 ± 21.3	0.25
BMI, kg/m^2	24.3 ± 4.4	24.4 ± 4.7	24.0 ± 3.4	0.39
Biochemistry examination				
Hemoglobin, g/dl	13.3 ± 2.1	13.5 ± 2.1	12.6 ± 2.2	< 0.01
Albumin, g/dl	3.7 ± 0.5	3.7 ± 0.4	3.6 ± 0.6	0.07
Sodium, mM	139.7 ± 3.9	140.1 ± 3.6	138.2 ± 4.5	< 0.001
Potassium, mM	3.96 ± 0.47	3.96 ± 0.45	3.99 ± 0.52	0.474
NT-proBNP, pg/ml	1227.0–5254.0 (2178.0)	1091.5–4643.5 (2035.0)	1662.3–7972.8 (2926.0)	< 0.001
BUN, mM	5.8–9.1 (7.1)	5.6–8.5 (6.8)	6.5–11.6 (9.1)	< 0.001
GFR, ml/(min·1.73 m^2)	61.2–96.5 (75.8)	63.9–97.6 (79.3)	42.5–91.1 (62.3)	< 0.001
Uric acid, μM	479.5 ± 167.1	463.5 ± 148.8	537.4 ± 212.0	0.003
ALT, U/l	16.8–45.8 (26.0)	16.9–42.9 (26.0)	15.9–51.5 (29.3)	0.632
AST, U/l	22.0–41.9 (28.1)	21.7–39.2 (27.3)	23.3–58.0 (33.8)	0.004
D-dimer, mg/l	0.3–1.5 (0.7)	0.3–1.4 (0.6)	0.5–2.5 (1.1)	< 0.001
Device inspection				
LVEF, %	30.0–55.3 (38.9)	29.9–53.1 (38.5)	30.4–58.8 (40.5)	0.227
LVEDd, mm	61.9 ± 2.5	61.9 ± 11.9	61.6 ± 14.6	0.85
LVEDs, mm	49.3 ± 14.1	49.5 ± 13.6	48.7 ± 15.9	0.64
QRS duration, ms	129.6 ± 40.2	128.4 ± 40.7	133.9 ± 38.3	0.25
QTc, ms	443.6 ± 93.3	443.7 ± 94.4	443.2 ± 89.9	0.96
PASP, mmHg	44.1 ± 16.6	42.6 ± 15.1	50.0 ± 20.2	0.008
Oral medication at admission, *n* (%)				
Loop diuretics	397 (95.2%)	309 (94.8%)	88 (96.7%)	0.449
Aldosterone antagonists	387 (92.8%)	300 (92%)	87 (95.6%)	0.243
Digoxin	174 (41.5%)	136 (41.5%)	38 (41.8%)	0.994
ACEI/ARB	340 (81.5%)	263 (80.7%)	77 (84.6%)	0.392
β-blockers	331 (79.4%)	256 (78.5)	75 (82.4%)	0.417
Aspirin	187 (44.8%)	139 (42.6%)	48 (52.7%)	0.086
Etiology, *n* (%)				
CHD	106 (25.3%)	82 (25.0%)	24 (26.4%)	0.790
VHD	109 (26.0%)	86 (26.2%)	23 (25.3%)	0.856
Cardiomyopathy	169 (40.3%)	136 (41.4%)	33 (36.3%)	0.371
Comorbidity, *n* (%)				
Hypertension	203 (48.4%)	164 (50%)	39 (42.9%)	0.228
Diabetes Mellitus	91 (21.7%)	68 (20.7%)	23 (25.3%)	0.352
Atrial fibrillation	152 (36.3%)	118 (36.0%)	34 (37.4%)	0.808
NYHA class, *n* (%)				
NYHA class II	67 (16.0%)	57 (17.4%)	10 (11.0%)	0.162
NYHA class III	227 (54.2%)	181 (55.2%)	46 (50.5%)	
NYHA class IV	125 (29.8%)	90 (27.4%)	35 (38.5%)	

ACEI, angiotension-converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotension receptor blocker; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; CHD, coronary heart disease; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; LVEDd, left ventricular end-diastolic dimension; LVEDs, left ventricular end-systolic dimension; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; SBP, systolic blood pressure; VHD, valvular heart disease.

TABLE 2 | COX stepwise regression analysis (forward step, entry only if $P \leq 0.10$ and removal only if $P > 0.10$) of mortality on the significant ($P < 0.05$) variables in baseline characteristics of patients.

	β value	Std. error	HR	95%CI	*P* value
Lg(GFR)+ Lg(NT-proBNP)	3.194	0.625	24.384	7.163~83.005	< 0.001
SBP	−0.020	0.007	0.981	0.968~0.993	0.003
Sodium	−0.056	0.028	0.946	0.895~0.999	0.047
PASP	0.021	0.007	1.022	1.007~1.036	0.003
Hemoglobin	−0.014	0.005	0.981	0.968~0.993	0.003
Lg(D-dimer)	0.498	0.256	1.645	0.996~2.716	0.052

The data of non-normal distribution were changed by Lg10.

Multivariate Cox Stepwise Regression Analysis

Single factor analysis was performed on all variables in the baseline data (**Table 1**). Results showed that NT-proBNP, GFR, sodium, systolic blood pressure (SBP), diastolic blood pressure (DBP), AST, D-dimer, hemoglobin, BUN, PASP, uric acid, and age were valuable on the prognosis of HF ($P < 0.05$). The data of non-normal distribution were changed by Lg10. Lg(GFR) was combined with Lg(NT-proBNP) in binary Logistic regression equation, and then multivariate COX stepwise regression analysis (forward step, $P \leq 0.1$ into the equation and $P > 0.1$ out of equation) was performed for the statistically significant variables in baseline data to screen out the independent predictors of all-cause death after 18 months follow-up in AHF patients ($P < 0.05$, **Table 2**). Results showed that the combination of Lg(GFR) and Lg(NT-proBNP) had significant predictive value for long-term survival prognosis in patients with AHF patients ($P < 0.001$). In addition, SBP, sodium, hemoglobin, and PASP were independent predictors of all-cause mortality at 18 months. The role of these variables in predicting HF had been reported, so it was not further described in this paper (15, 20–22).

ROC Curve Analysis

ROC curve showed that NT-proBNP and GFR had certain value in predicting the prognosis of AHF ($AUC_{GFR} = 0.677$, 95%CI: 0.627–0.723, $P < 0.001$; $AUC_{NT-proBNP} = 0.648$, 95%CI: 0.598–0.695, $P < 0.001$). The ROC curves of NT-proBNP and GFR were compared in pairs, and the results showed no statistical difference ($P > 0.05$), as shown in **Figure 1**. However, the combination of two factors could further improve the AUC ($AUC_{NT-proBNP+GFR} = 0.711$, 95% CI: 0.663–0.756, $P < 0.001$, sensitivity was 53.5%, specificity was 85.3%), which was significantly different from that of either single factor (**Figure 1**). According to the ROC curve coordinate and the cutting value of the Yueden index, the optimal prediction value of GFR was 61.7 ml/(min•1.73 m^2) (sensitivity 49.5%, specificity 81.0%), the optimal prediction value of NT-proBNP was 2,137 pg/ml (sensitivity 68.6%, specificity 53.2%).

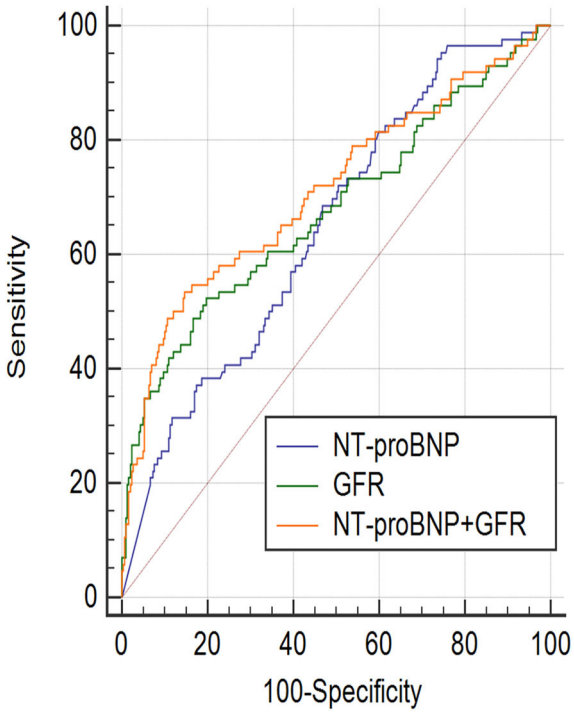

FIGURE 1 | ROC curve (Receiver operator characteristic curves) showed, NT-proBNP and GFR had certain prognosis value of acute heart failure in the 18-month. NT-proBNP combined with GFR had a higher prognostic value. $AUC_{GFR} = 0.677$, 95%CI: 0.627–0.723, $P < 0.001$; Sensitivity = 49.5%, Specificity = 81.0%, Cut off point: 61.7; $AUC_{NT-proBNP} = 0.648$, 95%CI: 0.598–0.695, $P < 0.001$; Sensitivity = 68.6%, Specificity = 53.2%, Cut off point: 2,137; $AUC_{NT-proBNP+GFR} = 0.711$, 95%CI: 0.663–0.756, $P < 0.001$, Sensitivity = 53.5%, Specificity = 85.3%; AUC_{GFR}~$AUC_{NT-proBNP+GFR}$, $P = 0.0117$; $AUC_{NT-proBNP}$~$AUC_{NT-proBNP+GFR}$, $P = 0.0384$; $AUC_{NT-proBNP}$~AUC_{GFR}, $P = 0.471$.

Chi-Square Test and Kaplan-Meier Curve Analysis

Kaplan-meier curve analysis was performed on NT-proBNP and GFR, respectively according to the optimal prediction points of NT-proBNP and GFR obtained by ROC curve as the tangent value (**Figure 2**), which showed that mortality significantly increased when NT-proBNP > 2,137 pg/ml and GFR < 61.7 ml/(min•1.73 m^2). Two groups of variables were divided into the following four groups:

A: NT-proBNP ≤ 2,137 pg/ml, GFR ≥ 61.7 ml/(min•1.73 m^2);
B: NT-proBNP ≤ 2,137 pg/ml, GFR < 61.7 ml/(min•1.73 m^2);
C: NT-proBNP > 2,137 pg/ml, GFR ≥ 61.7 ml/(min•1.73 m^2);
D: NT-proBNP > 2,137 pg/ml, GFR < 61.7 ml/(min•1.73 m^2).

Chi-square test was performed on the four groups of variables and pairwise comparisons were calculated (**Figure 3**). The mortality rates of groups A, B, C, and D were 9.9, 34.3, 20.0, and 47.1%, respectively. Group A was statistically lower compared with group B, C, and D, and the difference between group C and group D was statistically significant, while that between group B and group D was insignificant ($P = 0.188$), which showed that

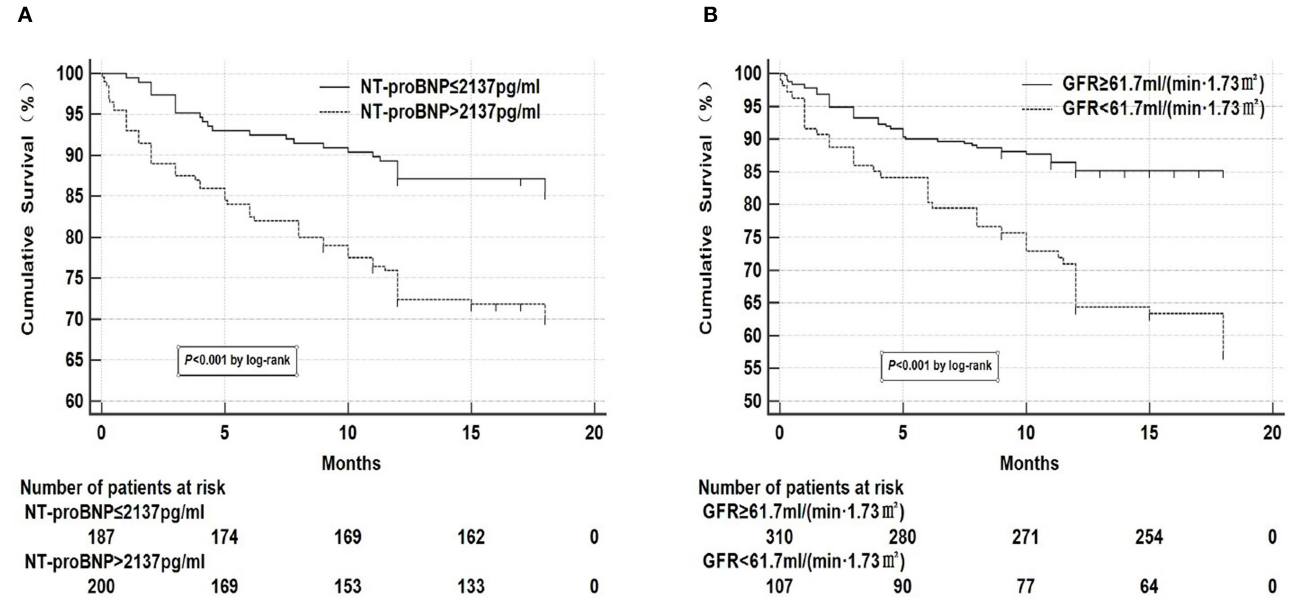

FIGURE 2 | Kaplan-meier curve analysis of NT-proBNP and GFR. **(A)** Kaplan-meier curve analysis showed that mortality significantly increased when NT-proBNP > 2,137 pg/ml. There were 32 missing data for NT-proBNP, 27 in the survival group, and 5 in the death group. **(B)** Kaplan-meier curve analysis showed that mortality significantly increased when GFR <61.7 ml/(min•1.73 m^2). There were 2 missing data for GFR in the survival group.

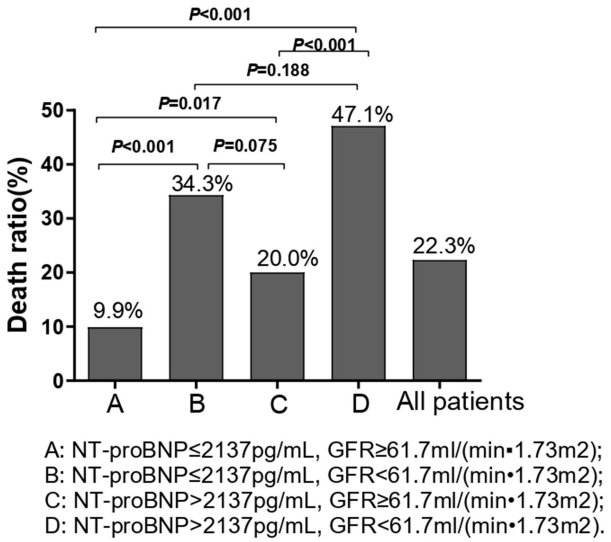

FIGURE 3 | Chi-square test was performed on the four groups of variables and pairwise comparisons were calculated. We used the optimal prediction points of NT-proBNP and GFR obtained by ROC curve as the criteria to divide groups in the figure. A: NT-proBNP ≤ 2,137 pg/ml, GFR ≥ 61.7 ml/(min•1.73 m^2); B: NT-proBNP ≤ 2,137 pg/ml, GFR < 61.7 ml/(min•1.73 m^2); C: NT-proBNP > 2,137 pg/ml, GFR ≥ 61.7 ml/(min•1.73 m^2); D: NT-proBNP > 2,137pg/ml, GFR < 61.7 ml/(min•1.73 m^2).

mortality had no significant difference between NT-proBNP > 2,137 pg/ml and NT-proBNP < 2,137 pg/ml when GFR < 61.7 ml/ (min•1.73 m^2). In order to further compare and analyze the Kaplan-Meier survival curve among the four groups which was used to predict survival of patients with AHF at 18 months (**Figure 4** and **Table 3**), it was found that the risk of death was more than 2 times higher in group B than in group A (HR = 3.71, 95% CI: 1.71–8.03), group D was more than 1 times higher compared with group C (HR = 2.69, 95% CI: 1.41–5.15). Namely, with similar NT-proBNP level, patients with lower GFR had significantly higher risk of death. The risk of death in group D was up to 4 times higher than that in group A (HR = 5.93, 95%CI: 3.17–11.10).

DISCUSSION

NT-proBNP is a focus in the study of HF, which effects on blood vessels, heart, and kidney. NT-proBNP has the effects on increasing GFR, inducing diuresis, reversing ventricular remodeling and reducing angiotasis. Based on its pathophysiology characteristic, level of brain natriuretic peptide can be used to guide the clinical medication, such as lyophilized recombinant human brain natriuretic peptide (rh-BNP) (23). Due to its ability to lower the excitability of sympathetic nervous system, this peptide can relax vascular smooth muscle, decrease blood pressure, and reduce cardiac afterload. In addition, its vasoconstriction against the renin-angiotensin-aldosterone system (RAAS) can inhibit the secretion of renin, dilate the arteries, increase the renal blood flow, and produce strong diuretic and natriuretic effects, so as to relieve the symptoms of AHF. In conclusion, NT-proBNP has become a recognized biomarker for diagnosis and prognosis of HF (24), and had a certain predictive value for the risk assessment of death in patients with atrial fibrillation (25).

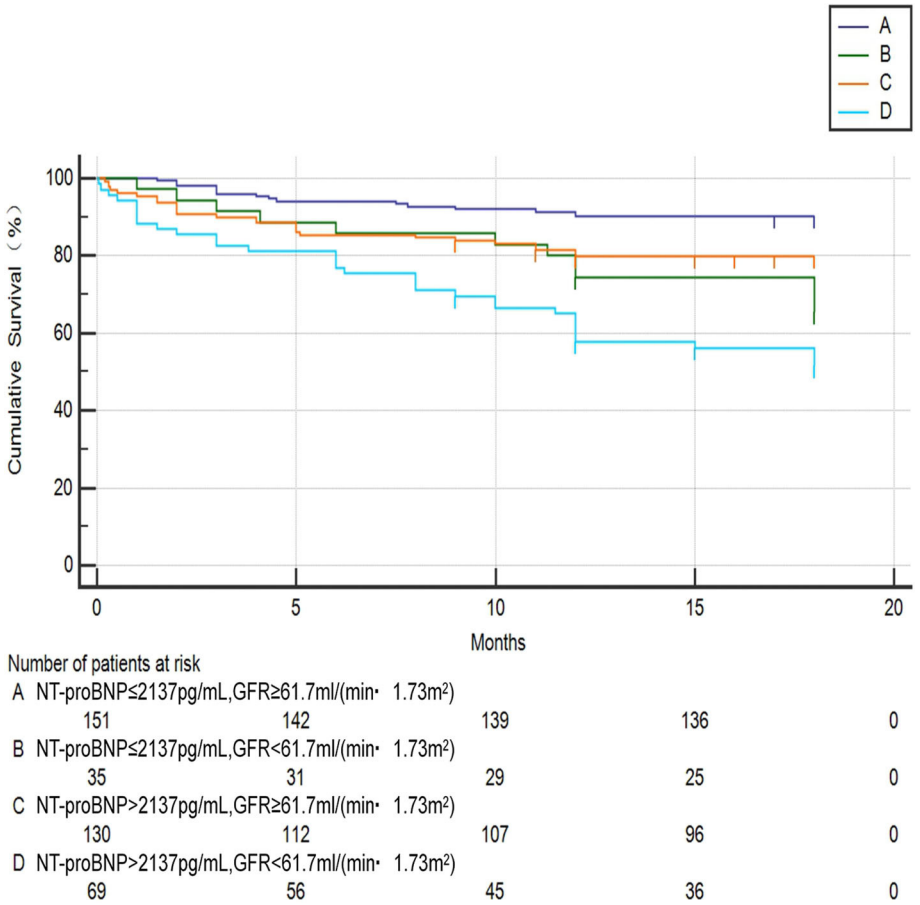

FIGURE 4 | Kaplan-meier curve analysis for predicting survival status of AHF patients between the four groups. A: NT-proBNP ≤ 2,137 pg/ml, GFR ≥ 61.7 ml/(min•1.73 m^2); B: NT-proBNP ≤ 2,137 pg/ml, GFR < 61.7 ml/(min•1.73 m^2); C: NT-proBNP > 2,137 pg/ml, GFR ≥ 61.7 ml/(min•1.73 m^2); D: NT-proBNP > 2,137 pg/ml, GFR < 61.7 ml/(min•1.73 m^2).

In addition, NT-proBNP also has a high value of negative prediction. HF can be almost excluded when NT-proBNP is normal.

Heart and kidney interact with each other through various mechanisms, including hemodynamic abnormalities, activation of neurohormones and inflammatory activities, oxidative stress, anemia, renal sympathetic nerve activity, and vitamin D metabolism, etc. In the progression of cardiorenal syndrome, impaired cardiac function may be based on the following aspects:(1) In the case of renal dysfunction, the kidney usually fails to excrete sodium properly, which results in water-sodium retention, elevated blood pressure, increased cardiac preload, and ultimately aggravates pulmonary congestion and HF. (2) HF is easy to induce hypohemoglobinemia. In severe renal dysfunction, insufficient erythropoietin will aggravate anemia, speed up heart rate compensation, strengthen myocardial contractility, activate sympathetic nerves, constrict pulmonary vessels, increase pulmonary artery pressure, and consequently aggravate cardiac remodeling.

AHF, renal insufficiency and anemia interact in a vicious circle known as cardiac anemia syndrome. The mechanism of AHF injury to renal function is considered as follows: (1) with the development of acute heart pump failure and severe water-sodium retention, the kidneys will have insufficient circulating blood volume and impaired self-regulation function. Meanwhile, hypoxia and endotoxin will cause renal vasoconstriction and further reduce renal perfusion. Then, continuous hypoperfusion can increase the susceptibility of kidney to various risk factors, which results in renal unit necrosis and apoptosis, and leads to renal cortical ischemia and infarction (19). (2) The release of inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-1, and interleukin-6 leads to chronic injury and apoptosis of kidney cells, and ultimately aggravates deterioration of renal function (26). In addition, both AHF and renal insufficiency are prone to electrolyte disturbances during progression, which can disrupt homeostasis balance in the body and further worsen the disease.

The results of this study are consistent with the previous reported predictive value of NT-proBNP for the prognosis of AHF. It showed that univariate analysis of NT-proBNP had profound statistical significance. Its high sensitivity and low specificity could be due to the fact that plasma NT-proBNP

TABLE 3 | Hazard ratio of different groups according to NT-proBNP and GFR levels (Kaplan-Meier method).

HR (95%CI)	A	B	C	D
A	–	3.71* (1.7–8.03)	2.20* (1.35–3.60)	5.93* (3.17–11.10)
B	0.27* (0.12–0.58)	–	0.59 (0.27–1.31)	1.60 (0.66–3.86)
C	0.45* (0.28–0.74)	1.68 (0.76–3.71)	–	2.69* (1.41–5.15)
D	0.17* (0.09–0.32)	0.62 (0.26–1.51)	0.37* (0.19–0.71)	–

The data in the table were HR values and 95% confidence intervals compared between each group.
**represented comparison between groups $P < 0.05$.*

is mainly metabolized in the kidney. When the kidney is damaged, the decrease of GFR further promotes the increase of serum NT-proBNP. Moreover, since NT-proBNP is affected by many factors such as race, gender, anemia, obesity, and atrial fibrillation, simple detection of NT-proBNP for diagnosis could lead to misdiagnosis of etiology (27), which also explains why NT-proBNP can be interfered by other factors in multi-factor analysis. Therefore, renal function and other related factors must be considered when using NT-proBNP as cardiac biomarker. Related studies on nephrology have found that NT-proBNP is also of certain value in the diagnosis of renal function level and can assist in predicting the prognosis of patients with middle and advanced renal insufficiency (28).

This study also suggested that GFR can be used as a powerful biomarker for predicting long-term cardiovascular events in patients with AHF after discharge. GFR has a high specificity in predicting the prognosis of AHF (81.0%). Its optimal predictive value is 61.7 ml/(min•1.73 m^2), which is close to the classification of moderate renal insufficiency. GFR, the best comprehensive index to evaluate renal function, was significantly increased its predictive value and accuracy after combination with NT-proBNP, and additional information can be added in combined analysis (sensitivity 53.5%, specificity 85.3%). A study have suggested that patients with severe renal insufficiency (GFR < 30 ml/min/1.73 m^2) account for 30% of acute systolic HF cases and GFR was independent predictor of 1-year mortality in the community (29). In addition to the certain prediction effect of prognosis of HF, large sample statistics have found that the reduction of GFR could also lead to increased risk of atrial fibrillation events, and its evaluation value was higher when combined with NT-proBNP (30). In conclusion, timely detection and diagnosis of renal insufficiency during hospitalization are of great significance to the prognosis of patients. Although prerenal renal injury is mainly caused by renal insufficiency and it is possible to recover after timely replenishment of circulating blood volume, this study showed that the mortality rate of such patients is still high. During prerenal injury, the factors of cardiogenic renal insufficiency cannot be modified quickly, and the improvement of cardiac function requires a long time, therefore the effective circulation capacity of the kidney cannot be guaranteed, which affects the recovery of renal function and the prognosis of patients.

Because of the interaction between NT-proBNP and GFR, both of them should be considered when assessing the long-term survival risk assessment of patients. In this study, the optimal prediction value of NT-proBNP and GFR was the cut-off point for grouping comparison. In the grouping comparison, there were statistically significant differences in GFR between the two groups based on the classification of NT-proBNP. In particular, the 18-month all-cause mortality was significantly increased in patients in group D (NT-proBNP > 2,137 pg/ml, GFR < 61.7 ml/(min•1.73 m^2). In the GFR-based classification, for patients with GFR > 61.7 ml/(min•1.73 m^2), there were statistically significant differences in the mortality between the two groups with different NT-proBNP. However, for patients with GFR < 61.7 ml/min. 1.73 m^2), there were no significant differences in mortality between the two groups separated by NT-proBNP (cut off point is 2,137 pg/ml), which suggested that for patients with moderate or above renal insufficiency, the concentration of NT-proBNP were susceptible to the GFR, and it could be indespensible to adopt combined judgment. The all-cause mortality rates in both groups were higher than those in the two groups with relatively preserved renal function, which further confirmed the high predictive value of GFR, therefore the combined evaluation could more accurately reflect the risk of death in patients with AHF.

Overall, this study investigated the effect of NT-proBNP and GFR on long-term prognosis and risk stratification in patients with AHF. The results showed that both GFR and NT-proBNP were significantly correlated with the risk of death in AHF patients. Moreover, the combination of these two values was of higher predictive value for the long-term prognosis of patients with AHF. This discovery could increase clinicians' attention to renal function in patients with AHF, which is helpful for adjusting the therapeutic regimen and taking effective intervention in patients with renal insufficiency.

STUDY LIMITATIONS

This study has the following shortcomings: (1) this study is a single-center study, which may have population bias. Currently, multi-center studies have been carried out synchronously. The objective is to collect data in 2,000 AHF patients and establish a database, the results of this study will be confirmed by larger sample size population data analysis in the later stage. (2) In this study, we also found that SBP, sodium, hemoglobin, and PASP were independent predictors of all-cause mortality at 18 months, which is consistent with previously reported findings. However, the prognosis of hospitalized patients with HF is still limited. More new predictors need to be discovered and the joint prediction of multiple factors should be widely used in future studies. (3) GFR is now considered as the best comprehensive indicator of renal function, but it is only

a part of renal function, and CKD-EPI formula has some limitations. However, it is difficult to use inulin or iodide phthalate clearance rate to calculate accurate GFR in clinical work. At present, CKD-EPI$_{CysC}$ formula based on cystatin is still applicable according to the kidney disease guidelines and its accuracy has been well-recognized in clinical trials. Due to the lack of cystatin data in this experiment, the applicability of this formula could not be verified. Instead, CKD-EPI$_{SCR}$ formula based on serum creatinine, which is widely used in clinical practice and has high international recognition. CKD-EPI is closer to the accurate value compared with the previous MDRD algorithm (31).

CONCLUSION

In patients with NT-proBNP > 2,137 pg/ml and GFR < 61.7 ml/(min•1.73 m^2), the risk of death is significantly higher. The combination of GFR and NT-proBNP improves the predictive value for the long-term prognosis of AHF patients. Early identification of these high-risk patients could help clinicians to modify and strengthen treatment regimens, thereby improving the clinical prognosis of these patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of the first affiliated Hospital of Nanjing Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL and HZ: study design and interpretation of results. KW, QW, GN, YZ, and WY: data collection. KW, QW, and GN: data analysis. KW, XL, and HZ: preparation of manuscript. KW, GN, QW, XL, and HZ: revision of manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Chronic Thromboembolic Pulmonary Hypertension in a Child With Sickle Cell Disease

Robert Spencer[1]*, *Gerson Valencia Villeda*[2], *Koji Takeda*[3] and *Erika B. Rosenzweig*[1]

[1] Division of Pediatric Cardiology, Morgan Stanley Children's Hospital, New York, NY, United States, [2] Division of Pediatric Cardiology, Arnold Palmer Hospital for Children, Orlando, FL, United States, [3] Division of Cardiothoracic Surgery, Columbia University Medical Center, New York, NY, United States

Chronic thromboembolic pulmonary hypertension is a potentially curable form of pre-capillary pulmonary hypertension (PH) resulting from incomplete resolution of pulmonary thromboemboli. We describe an 11-year-old boy with homozygous sickle cell disease with an indwelling catheter found to have severe PH on routine screening echocardiography. The diagnosis was confirmed by CT, ventilation-perfusion scintigraphy, and right heart catheterization. The patient was medically managed until undergoing pulmonary thromboendarterectomy with resolution of his PH. This case highlights the need for pediatric providers to be aware of this underdiagnosed form of PH, particularly for patients at high risk.

Keywords: pediatric cardiology, pulmonary hypertension, CTEPH—chronic thromboembolic pulmonary hypertension, sickle cell disease, hematology

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Junjie Xiao,
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University of Freiburg, Germany
Muhammad Ali Mumtaz,
Baylor College of Medicine,
United States

***Correspondence:**
Robert Spencer
ros9225@nyp.org

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INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH) is a distinct form of pulmonary hypertension (PH) that results from unresolved acute pulmonary embolism. The disease is caused by mechanical obstruction of the pulmonary arteries by chronic, fibrotic organized thrombi (1, 2). It is rarely diagnosed in children and has an unknown incidence in the general population (3–5).

Early diagnosis and treatment are critical, particularly because patients will develop severe PH and right heart failure. When recognized in a timely manner, the disease is often curable by pulmonary thromboendarterectomy (PTE) (6–9). We report an 11-year-old boy with sickle cell disease and an indwelling venous catheter found to have elevated right ventricular (RV) systolic pressure on routine echocardiography. Further workup led to the diagnosis of CTEPH, and he was successfully treated with PTE.

CASE

An 11-year-old boy with hemoglobin SS sickle cell disease (SCD) was referred to our hospital for further treatment of CTEPH. He had a history of multiple pain crises, acute chest syndrome, and acute ischemic strokes at ages 3 and 7 years. His SCD was further complicated by moyamoya syndrome, for which he underwent encephalodurosynangiosis at age 7 years. His hypercoagulability workup had been negative for antithrombin deficiency, protein C deficiency, protein S deficiency, factor V Leiden, plasminogen deficiency, and anticardiolipin antibodies.

Approximately 10 months before his referral, routine screening transthoracic echocardiogram (TTE) revealed a right atrial (RA) thrombus thought to be related to his Broviac central venous catheter, which had been used for exchange transfusions. He was started on enoxaparin and his Broviac catheter was replaced. TTEs over the ensuing months demonstrated persistent RA thrombus without change in size, with normal RV pressure and normal biventricular systolic function.

Seven months later, the patient was electively admitted to the referring institution in anticipation of a bone marrow transplant (BMT), at which time routine TTE showed an elevated RV systolic pressure (58 mmHg plus the RA pressure), a change from his previous echocardiograms. CT scan of the chest at that time revealed multiple bilateral lower lobe and left upper lobe pulmonary emboli. Clinically, he reported mild dyspnea on exertion and exercise intolerance for several weeks. Medical management was started with bosentan and the patient's enoxaparin dose was increased.

Follow-up TTE performed 3 months later showed severe PH, with RV systolic pressure of 90 mmHg plus the RA pressure and a corresponding blood pressure of 108/52 mmHg. This raised suspicion for CTEPH, which was supported by a ventilation-perfusion (VQ) scan showing multiple areas of wedge-shaped mismatched perfusion defects consistent with chronic bilateral thromboembolic disease and secondary PH consistent with CTEPH. The following day, right heart catheterization demonstrated pulmonary arterial pressure of 80/35 mmHg with a mean of 52 mmHg, pulmonary capillary wedge pressure of 14 mmHg, cardiac index of 5.7 L/min/m^2, and pulmonary vascular resistance index of 6.6 WUm^2. Pulmonary angiography revealed multiple areas of abrupt tapering of the pulmonary arteries, confirming the diagnosis. The patient was switched from bosentan to macitentan and riociguat, and he was referred to our center for PTE.

At admission, his blood pressure was 116/72 mmHg, heart rate was 114 beats per minute, respiratory rate was 18 breaths per minute, and oxygen saturation was 97% on room air. The result of the physical examination was unremarkable. Quantitative hemoglobin S was abnormal at 13.5%, and NT-ProBNP was elevated at 880.0 pg/mL (normal range, 10.0–242.0 pg/mL). The results of the remaining laboratory tests, including coagulation tests, were normal.

He successfully underwent bilateral PTE and removal of a calcified organized thrombus from the right atrium (**Figure 1**) without reported intraoperative complications. Post-operative transesophageal echocardiography demonstrated an estimated RV systolic pressure of 32 mmHg plus the RA pressure in the setting of a systolic blood pressure of 110 mmHg, with mildly decreased RV function. Central venous pressure was maintained under 8 mmHg with a furosemide infusion to avoid reperfusion injury. PH medications were discontinued at the time of surgery. Ten days after the procedure, the patient was completely asymptomatic with normal oxygen saturation and was discharged home on long-term warfarin. Four months later, he successfully underwent BMT, after which he had weekly TTEs to monitor for the development of PH (10). As of 6 months following surgery, the patient has remained clinically asymptomatic without echocardiographic evidence of RV hypertension based on tricuspid regurgitant jet and systolic septal position.

Abbreviations: BMT, bone marrow transplant; CTEPH, chronic thromboembolic pulmonary hypertension; RA, right atrial; RV, right ventricular; PH, pulmonary hypertension; PTE, pulmonary thromboendarterectomy; SCD, sickle cell disease; TTE, transthoracic echocardiogram; VQ, ventilation-perfusion.

DISCUSSION

To our knowledge, successful PTE in a child with CTEPH and SCD has not been reported in the MEDLINE database to date. Our patient was noted to have unexplained PH on routine screening echocardiography in the setting of a chronic hypercoagulable state and recent RA thrombus associated with a central venous catheter, and his diagnosis was confirmed by lung VQ scan and right heart catheterization. While a recently published case report described a 12-year-old with CTEPH successfully treated with PTE, that patient had severe comorbidities including paraplegia, and he was found to have factor V Leiden and antiphospholipid antibodies during his hypercoagulable workup (11).

CTEPH is a rare and life-threatening condition that can result in progressive right-sided heart failure and death. It occurs as a result of unresolved thrombi obstructing the pulmonary arteries. The following criteria are used to make the diagnosis after 3 months of anticoagulant therapy: (1) mean pulmonary artery pressure >25 mmHg with a pulmonary capillary wedge pressure ≤15 mmHg, and (2) at least one (segmental) perfusion defect detected by lung scan, CT angiography, or pulmonary angiography (1, 2, 12).

Despite increasing awareness of it, the disease remains underdiagnosed. Studies suggest an incidence of 0.56–3.2% in adult pulmonary embolism survivors, while incidence in the pediatric population is unknown (3–5). Of note, risk factors for thromboembolism are identified in the majority of children with CTEPH, and approximately one third of patients have a positive family history of thromboembolism or a known hypercoagulable state. Children with lupus anticoagulant and anticardiolipin antibodies are at the highest risk of the disease (9). Other risk factors include splenectomy, infected ventriculo-atrial shunts, thyroid replacement therapy, history of malignancy, chronic inflammatory conditions, and indwelling catheters (13).

Our patient did not have a history of an acute pulmonary embolism. Nevertheless, he had several risk factors for CTEPH. Specifically, he had a history of homozygous SCD, which is recognized as a chronic hypercoagulable state with an increased risk of thromboembolic events and PH (14–17). He also had an indwelling catheter for monthly exchange transfusions and history of catheter-related RA thrombus. Homozygous SCD additionally confers a significant risk of autosplenectomy, for which the patient did not undergo sonographic assessment at our institution (18).

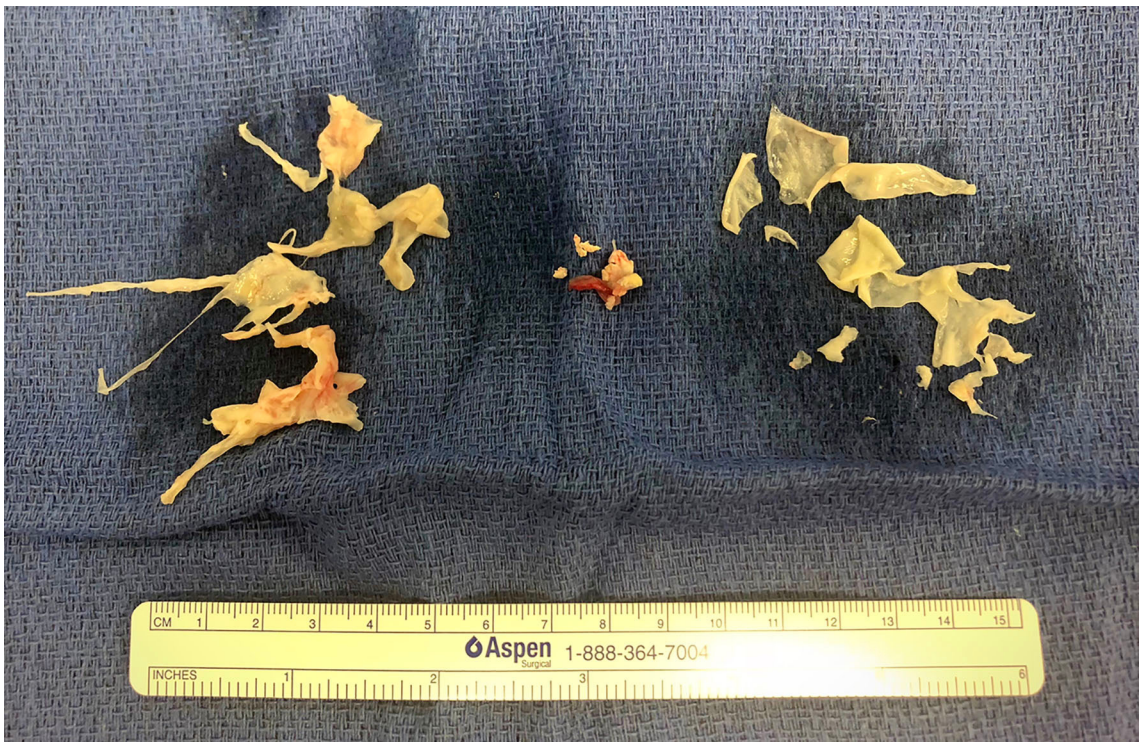

FIGURE 1 | Right atrial thrombus (center) surrounded by pulmonary thromboendarterectomy specimens.

Because there are no pathognomonic signs or symptoms for CTEPH, the diagnosis is often delayed or missed. Patients may present with exertional dyspnea, exercise intolerance, and non-specific abnormalities on physical examination. As the disease progresses, there is a high risk of developing right heart failure. While the natural history of acute pulmonary embolism is near-complete resolution of emboli within 3–6 months, the persistence of any signs or symptoms after this duration of antithrombotic therapy warrants further investigation.

Diagnostic workup begins with chest radiography, pulmonary function studies, an ECG, and an echocardiogram. If CTEPH is suspected, a lung VQ scan should assess for subsegmental or larger unmatched perfusion defects. Given its high sensitivity, a normal lung VQ scan can effectively rule out the disease, while an abnormal test result prompts further evaluation with right heart catheterization, catheter-based pulmonary angiography, CT pulmonary angiography, or MRI (1, 19, 20).

The first step in management is anticoagulant therapy. Our patient was initially on subcutaneous low molecular weight heparin, and he was later transitioned to an oral anticoagulant. Once the diagnosis was established, he was also started on targeted PH therapy, including macitentan, an endothelin receptor antagonist, and riociguat, a soluble guanylate cyclase stimulator, while awaiting definitive surgery. Riociguat was chosen because it has been shown to improve exercise capacity and pulmonary vascular resistance in patients with CTEPH, and because it is safe and well-tolerated in patients with SCD (21, 22).

PTE is the treatment of choice for operable patients, and its success has been demonstrated in children (9, 23). To be considered operable, a patient must have sufficient surgically accessible thromboembolic material without extensive distal disease (24). Patients with SCD may have additional risks of the PTE, given the need for prolonged cardiopulmonary bypass, deep hypothermia, and intervals of circulatory arrest, factors that increase the likelihood of sickling (25). Balloon pulmonary angioplasty is an emerging option for inoperable CTEPH or patients with recurrent or persistent PH after PTE; however, this approach is rarely used in children and long-term results are lacking (26). In our case, a multidisciplinary team including pulmonary hypertension, cardiology, hematology, critical care, and cardiothoracic surgery specialists reviewed the patient's clinical data and elected to proceed with surgery, which the patient underwent without complication. Given the success of this case, it is important to consider CTEPH in any children with unexplained PH, particularly when risk factors are present.

CONCLUSIONS

We describe a rare case of CTEPH in a child with SCD and an indwelling catheter who was found to have unexplained PH. CTEPH is a rare and life-threatening disease. Unlike other forms of PH, it is potentially curable with PTE. For this reason, early recognition and treatment are critical. Practitioners should consider this diagnosis in patients with unexplained

PH, particularly in patients with risk factors, including but not limited to those with a hypercoagulable state, a history of thromboembolism, or an indwelling catheter.

ETHICS STATEMENT

Parental informed consent was obtained for the publication of this case report.

AUTHOR CONTRIBUTIONS

RS was the consulting cardiology fellow for the patient. GV was the referring cardiologist who diagnosed the patient and he contributed references and revisions to the manuscript. KT performed the patient's thromboendarterectomy and he contributed revisions to the manuscript. ER was the precepting attending for RS and she contributed references and revisions to the manuscript. All authors approved the final version.

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Increased Neutrophil Respiratory Burst Predicts the Risk of Coronary Artery Lesion in Kawasaki Disease

Jing Hu[1,2†], Wei Qian[2†], Zhiwei Yu[2], Tao Xu[2], Liang Ju[2], Qi Hua[2], Yan Wang[2], Jing Jing Ling[2] and Haitao Lv[1*]*

[1] Children's Hospital of Soochow University, Suzhou, China, [2] Wuxi Children's Hospital Affiliated to Nanjing Medical University, Wuxi, China

Background: Kawasaki diseases (KD) is a febrile systemic vasculitis in infants associated with coronary aneurysm. The etiology of KD remains unclear. Human neutrophils have great capacity to cause tissue damage in inflammatory diseases via their inappropriate activation to release reactive oxygen species (ROS). Brain natriuretic peptide (BNP) is a substantial modulator of neutrophil activation to regulate ROS production. It is increasingly released from the myocardium in heart failure and myocardial inflammatory states.

Objective: The purpose of this study was to explore the potential role of neutrophil respiratory burst in the pathogenesis of coronary artery lesions (CAL) in KD.

Materials and Methods: A total of 78 children were enrolled. Of all the cases, 20 cases are healthy control (HC), 20 are with coronary artery lesion (CAL), and 38 are with non-coronary artery lesion (NCAL). The activation ratio of neutrophils was evaluated by flow cytometry. In addition, plasma levels of BNP were detected.

Results: Our results showed that the activation ratio of neutrophils in KD with CAL is significantly higher than the other two groups (HC and NCAL). Besides, the plasma levels of BNP in KD (with or without CAL) were higher than that in HC.

Conclusions: These findings suggested that neutrophil respiratory burst may play a significant role in the pathogenesis of CAL, and predicts the risk of CAL in Kawasaki disease.

Keywords: kawasaki disease, coronary artery lesion, neutrophil, flow cytometry, brain natriuretic peptide

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Jack Wong,
The Chinese University of Hong Kong, China
Zijian Li,
Peking University Third Hospital, China

***Correspondence:**
Haitao Lv
haitaosz@163.com
Jing Jing Ling
lingjingjing77@163.com

† These authors have contributed equally to this work and share first authorship

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INTRODUCTION

Kawasaki disease (KD), first reported by the Japanese physician Tomisaku Kawasaki, is an acute febrile disease characterized as systemic vasculitis. Almost 50 years have passed since its initial description. The incidence of KD is increasing worldwide, and in more economically developed countries, KD is now the most common cause of acquired heart disease in children. The cause or etiology and pathogenesis of KD is still being debated. Previous pathological studies in KD patients showed all cardiac tissues are associated with the acute inflammatory phase of the disease. Vasculitis leads to the destruction of normal arterial structure, followed by aneurysm expansion, especially affecting the proximal coronary artery, destruction of intima and media, and replacement of

myocytes by fibroblasts and connective tissue (1). The most important complication is serious coronary artery lesions (CAL). It may lead to serious formation of coronary aneurysm, which is a major cause of cardiac sequelae such as myocardial infarction (MI) and sudden death. These events are caused by coronary stenosis due to intimal proliferation or thrombotic formation (2). Timely high-dose intravenous immunoglobulin (IVIG) treatment effectively resolves the inflammation and reduces the occurrence of CAL in patients with KD (3–5). The reduction of risk for CAL by IVIG therapy in KD may be due partially to the decreased number of activated neutrophils in circulation (6).

Neutrophils are involved in the damage that occurs in coronary arteries in the early stage of KD, especially in the patient who died on the 10th day of the course of KD. Vasodilation might occur as a result of injury to vascular walls caused by neutrophil. Neutrophil infiltration reached a peak earlier than the infiltrations of monocytes/macrophages and lymphocytes. A large number of neutrophils were found to deform and penetrate the coronary artery wall in dead cases (7). Study revealed that the apoptosis of peripheral neutrophils was down-regulated persistently during the acute phase of KD (8). This suggests that the prolonged life span of activated neutrophils may contribute to the pathogenesis of KD vasculitis.

Neutrophils are important innate immune cells, which play a role of phagocytosis and killing in innate immunity and participate in infectious inflammation and other inflammatory reactions. When microbial pathogens, especially pyogenic bacteria, invade and inflammation occurs, neutrophils rapidly recruited and tended to inflammation sites mediated by various inflammation and cytokines, killing and eliminating pathogens by secreting cytokines, degranulation, and release reactive oxygen species (ROS) by the NADPH oxidase system, which is called respiratory burst. Neutrophil respiratory burst is related to the short but significant increase of neutrophil's absorption and utilization of oxygen, which is also characterized by the release of cytokines, the activation of monocytes and macrophages, and the increased release of ROS to extracellular space (9). But neutrophils inappropriate respiratory burst and elevated concentrations of ROS induce cell death and perpetuate more highly reactive radicals that lead to adverse cardiac remodeling might cause sustained tissue infiltration with neutrophils and monocytes, and persistent vasomotor dysfunction (10).

Brain natriuretic peptide (BNP) is mainly synthesized in ventricular myocytes and secreted by the left ventricle through the coronary sinus into the circulation. The production of BNP is regulated by the stretch of cardiac wall caused by myocardial volume overload, and BNP is increasingly released into systemic circulation in many clinical diseases characterized by heart failure and/or myocarditis (11). Recently a study found that under physiological conditions, BNP may play a potential regulatory role in neutrophil respiratory burst and ROS production (12). Thus, to investigate the role of neutrophils respiratory burst in KD, we detected the ROS production and the level of serum BNP in children with KD.

MATERIALS AND METHODS

Study Design and Subjects

A total of 78 children were recruited from January to December in 2019. For the healthy control group, inpatients with inguinal hernia and other selected surgery eliminating fever, allergic purpura, and other immune diseases were selected. We asked the parents to provide an additional blood sample (2 ml from their children). Patients who have a course of disease more than 10 days or have been treated with IVIG were not included. All of the KD patients were identified according to the criteria proposed by the Japanese Circulation Society Joint Working Group. Echocardiography was performed at 1, 2, and 4 weeks after the onset of fever. CALs were diagnosed on the basis of the Z scores of the left main coronary artery, proximal left anterior descending coronary artery, and proximal right coronary artery, and were denied as the Z scores (13). Z score = the internal dimension of the coronary artery expressed as the number of SD units normalized for body surface area of 2.0 or more. This study was approved by the Ethics Committee of Children's Hospital of WUXI, and the informed consent forms were obtained from the parents of all subjects.

All patients are divided into three groups: (1) healthy control group (HC); (2) patients with coronary artery lesion (CAL); (3) patients with non-coronary artery lesion (NCAL).

Flow Cytometry Measured ROS Production (14)

Venous blood samples using EDTA as an anticoagulant were collected from KD patients before intravenous immunoglobulin and oral aspirin administration on the day of admission, sent for examination within 2 h. Flow cytometry assay using dihydrorhodamine (DHR) for the measurement of the neutrophil respiratory burst in whole blood. DHR is only weakly fluorescent and can be passively loaded into neutrophils. Upon neutrophils activation, ROS produced by NOX2 is transformed by superoxide dismutase (and possibly myeloperoxidase) into hydrogen peroxide (H_2O_2) that oxidizes DHR into the strongly fluorescent molecule, rhodamine (RHO).

Transfer 50 μl whole blood sample into each of two polypropylene tubes labeled "Basal" and "PMA-stimulated." Add 50 μl PBS (phosphate buffered saline) or 10 μg/ml PMA 50 μl (phobol myristate acetate, Fluka USA) to appropriate tubes and incubate for 15 min at 37°C in shaking water bath. Twentyfive microlitre DHR were added to each tube. After mixing, 37°C water bath was taken for 5 min; add 1 ml hemolysin to each tube; after 10 min, 1,500 rpm centrifugation for 5 min. Abandon the supernatant, add 4 mL PBS, wash twice, 1,000 rpm centrifugation for 5 min. Abandon the supernatant, add 500 μl PBS for detected.

To determine neutrophil oxidative burst capacity, DHR conversion into the fluorophore rhodamine (RHO) was detected by flow cytometry fluorescence at 488 nm from detectors both below (bottom read) or above (top read) the samples. After neutrophils door setting, they provide basic graphics to show the basic level of neutrophils activation. On the flow cytometry, the number of DHR positive cells was counted after granulocyte gating. The unstimulated tube provides a

basic figure to show the basic level of granulocyte activation. Under normal conditions, the DHR stained positive cells in the tube stimulated by phorbol ester (PMA) should be significantly increased. After PMA stimulation, determine whether the neutrophil activation state response has related functional defects.

Plasma Levels of BNP

Blood samples were placed in EDTA-treated tubes, transported to the laboratory department of our hospital, and analyzed by fluorescence immunoassay (Ortho Clinical Diagnostic, Johnson & Johnson, USA). The measurable range of the BNP assay was 125–100,000 ng/L.

Statistical Analysis

Statistical analyses were performed using SPSS version 20.0 for windows (SPSS Inc., Chicago, IL, USA). The measurement data of each group were expressed as mean ± SD, and the independent sample *T*-test was used for comparing the basic level of neutrophils activation with PBS and the level of BNP. Intergroup differences neutrophils activation with PMA were analyzed with F-test, $P < 0.01$ was used for statistical significance. ROC curve analysis was performed to determine the cut-off values of neutrophils activation with PBS. Binary logistic regression analysis was used to statistically analyze risk factors.

TABLE 1 | Comparison of laboratory data and clinical characteristics between KD groups and the control group.

	HC	KD (CAL)	KD (NCAL)
N	20	20	38
Age (months)	21.50 ± 11.40	25.55 ± 22.77	20.75 ± 19.76
Male, *n*	8	26	12
Weigh (kg)	13.84 ± 6.36	12.09 ± 3.89	13.38 ± 5.73
WBC ($\times 10^9$)	6.12 ± 1.28	16.63 ± 34.91	13.70 ± 5.34
ESA (mm/hr)	16.55 ± 4.81	53.23 ± 19.54	38.38 ± 15.57

RESULTS

Children Characteristics

A total of 58 children were recruited from January to December in 2019 before intravenous immunoglobulin therapy. Twenty patients belong to the normal control group (HC), including 12 males and 8 females. Twenty patients with coronary artery lesion (CAL) include 11 males and 9 females; 38 patients with non-coronary artery lesion (NCAL) include 14 males and 4 females. The levels of WBC and ESR in KD group was higher than that in HC group, which was consistent with the characteristics of KD. In addition, there are no significant differences in gender, age, and weight between these groups (**Table 1**).

Differences of the Activation Ratio of Neutrophil in KD Among CAL, NCAL, and HC Groups

To confirm the activation of neutrophil, we detected the activation ratio of neutrophil in three groups of CAL, NCAL, and HC reflecting ROS production. As shown in **Figure 1**, the basal neutrophil activation ratio with PBS in KD with CAL group was significantly higher than that in NCAL group ($t = 4.79$, $P < 0.01$). Besides, an obvious difference was found between KD groups (NCAL or CAL) and HC group ($t = 6.424$, $P < 0.01$, $t = 4.79$, $P < 0.01$, respectively). Furthermore, there was no significant difference of activation index between the three groups after PMA stimulation ($F = 0.476$, $P = 0.478$).

Differences of BNP Level in KD Among CAL, NCAL, and HC Groups

We examined the level of plasma BNP in all groups (**Figure 2**). Results showed that the plasma level of BNP is higher in KD group (1061.16 ± 1914.21) than that in HC group (125.35 ± 38.25) with significant difference ($P < 0.01$). However, the level of BNP between CAL (1064.78 ± 2064.01) and NCAL (1055.93 ± 1651.93) did not show significant difference ($t = 0.16$, $P = 0.982$).

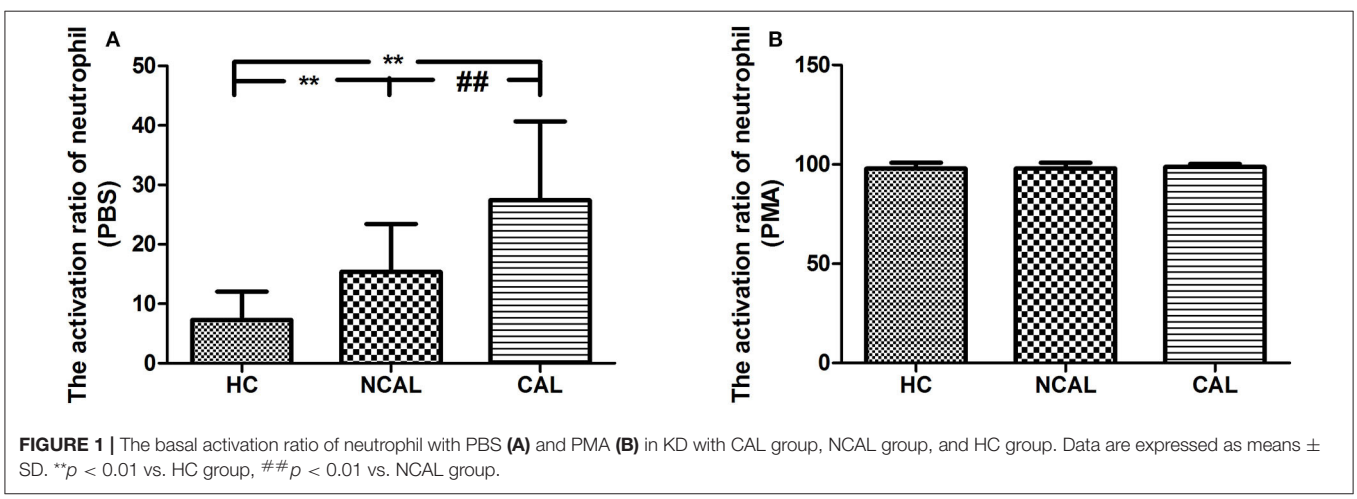

FIGURE 1 | The basal activation ratio of neutrophil with PBS **(A)** and PMA **(B)** in KD with CAL group, NCAL group, and HC group. Data are expressed as means ± SD. $^{**}p < 0.01$ vs. HC group, $^{\#\#}p < 0.01$ vs. NCAL group.

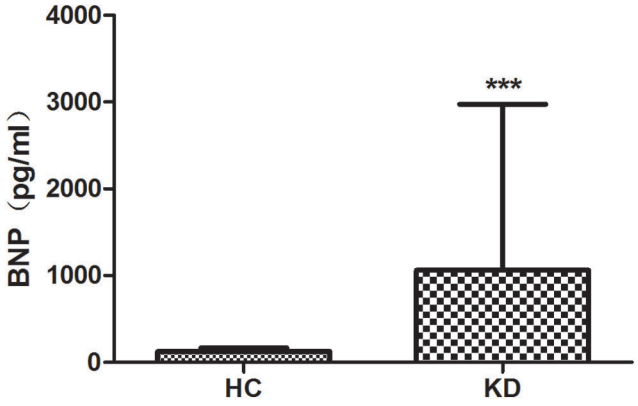

FIGURE 2 | The plasma lever of BNP in KD with CAL group, NCAL group, and HC group. Data are expressed as means ± SD. ***$p < 0.001$ vs. HC group.

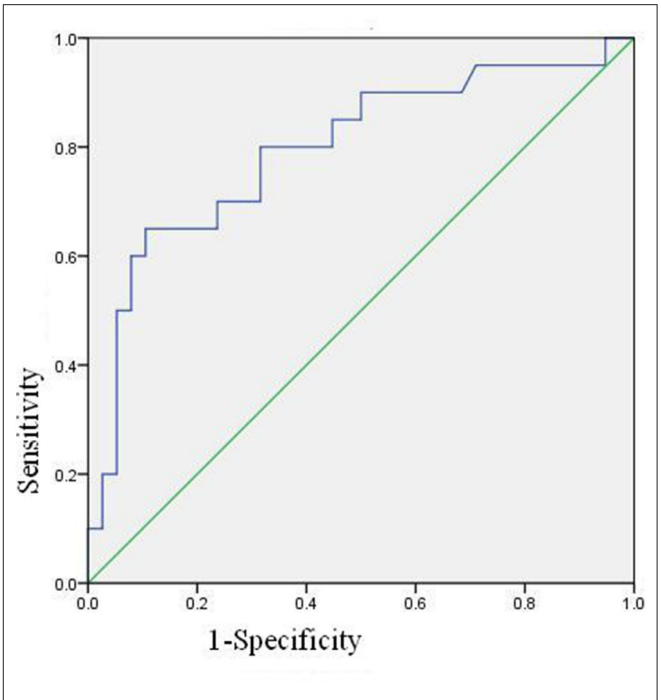

FIGURE 3 | ROC curves describing the basal activation ratio of neutrophil with PBS.

The ROC Curve of Neutrophil Activation Ratio of PBS

The ROC curve of neutrophil activation ratio with PBS for predicting coronary artery damage is shown in **Figure 3**. The area under the curve is 0.795 (95% confidence interval is 0.000–0.925, $p = 0.000$), which means the ROC curve is credible and of statistical significance. When the cut-off value is 24.4, i.e., PBS is more than 24.4, the predicted results are positive, and the sensitivity is 13/20 = 65%, the specificity is 34/38 = 89.47%.

Binary logistic regression analysis suggested that basal activation ratio of neutrophil was the risk factor of coronary artery damage, and the risk of coronary artery damage increased by 1.123 times for every unit increased ($P = 0.001$, 95% confidence interval is 1.047–1.204).

DISCUSSION

In the present study, 20 healthy controls and 38 KD patients were recruited. It was found that the activation rate of neutrophils in KD group was significantly higher than that in HC group under PBS basal control, while that in KD group with CAL was higher than that in NCAL group. Risk analysis indicated that neutrophil activation was a high risk factor for coronary lesion in KD. The higher the basal activation rate of neutrophil, the more likely the occurrence of coronary lesion.

Endothelial cells (ECS) play an important role in the physiological and pathological processes of hemostasis, inflammation, and angiogenesis. The imbalance of ROS production and antioxidant defense system is the main cause of endothelial dysfunction, which leads to the damage of endothelial cells or vascular smooth muscle cells (15). Because of the damage of blood vascular walls, vessel dilation might occur (16). These pathological changes may be the cause of vasculitis and coronary artery lesion in Kawasaki disease.

Neutrophil respiratory burst producing ROS as a means of attacking pathogens is the prerequisite as an efficient first line, and it is an important mechanism through which macrophages protect the host (17, 18). However, the imbalance of production and the detoxification ability of biological system to reactive intermediates of ROS might cause oxidative stress. Oxidative stress contributes to the mechanism of inflammation and tissue injury. The ROS acts as both a signaling molecule and a mediator of inflammation (19). The most important mechanism for neutrophils phagocytic and antimicrobial activity is the release of granular products (i.e., metalloproteinase [MMP]-8 and -9, myeloperoxidase [MPO], neutrophil gelatinase-associated lipocalin release cytokines), leading to the damage of membranes; proteins and DNA are believed to play a critical role in vascular disease (20), bring about further macrophage recruitment and the proliferation of smooth muscle cells within the vascular wall. In addition, protease secretion leads to endothelial damage of the coronary vessels, exposing thrombogenic collagen, and predisposing the vessels to thrombus formation (21–23). To clarify the relationship between neutrophil respiratory burst and KD with CAL, a multi-parameter flow cytometry technique using dihydrorhodamine 123 (DHR) was applied. Results showed that the basal neutrophil activation ratio with PBS in KD with CAL group was significantly higher than that in the NCAL group, indicating that neutrophil respiratory burst plays an important role in the occurrence of CAL in KD. The ROC curve of neutrophil activation ratio with PBS is credible and of statistical significance. Binary logistic regression analysis suggested that basal activation ratio of neutrophil was the risk factor of coronary artery damage. Besides, the neutrophils can be activated by PMA in healthy adults and children, but not in some immune deficient

diseases such as chronic granulomatous diseases. So after PMA stimulation, the neutrophil activation of three groups without functional defects have no significant difference.

BNP can stimulate macrophages to produce ROS and increase NO2 release by NADPH oxidase. It can also modulate cytokine production in several cell types, up-regulating the production of IL-10 by macrophages and inhibiting IL-12 and TNF-a release by dendritic cells (DCs), suggesting an anti-inflammatory cytokines profile induction (24). The anti-inflammatory effect of BNP may be also related to the inhibition of ROS formation by neutrophils (25). BNP inhibited the release of ROS in a cGMP/PKG dependent manner (26). BNP may present both anti- and proinflammatory actions. These findings have a number of potential therapeutic sequelae. In our study, the plasma lever of BNP is significantly higher in KD group than that in HC group.

CONCLUSION

In conclusion, our study showed that neutrophil respiratory burst evidenced by ROS production was significantly increased in the acute stage of KD patients, which was more pronounced in the CAL group and could be used as an indicator to predict CAL. BNP levels are elevated in the plasma of children with Kawasaki disease but there is no difference between KD with and without CALs.

Although we demonstrated that ROS and BNP are important to the pathogenesis of KD, the specific mechanisms still need further studies.When neutrophils were activated by microbial or inflammatory stimuli, the web-like structures known as neutrophil extracellular traps (NETs) were released. NETs are composed of chromatin DNA and neutrophil granule protein. Several of the molecules decorating the NETs (e.g., MPO, ds-DNA, histones, etc.) are autoantigens in systemic autoimmune diseases such as antineutrophil cytoplasmic antigen (ANCA)-positive vasculitis and systemic lupus erythematosus (SLE) (27). Next, we want to investigate the relationship between NET and Kawasaki disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Children's Hospital of WUXI (WXCH2015-10-003). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JH, WQ, JL, and HL: conceptualization. ZY, TX, and QH: formal analysis. HL and JL: funding acquisition. WQ, TX, LJ, QH, and YW: methodology. JH and WQ: writing-original draft. JH, JL, and HL: writing-review and editing. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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A Chromosomal Inversion of 46XX, inv (6) (p21.3p23) Connects to Congenital Heart Defects

Liangping Cheng[1,2,3†], Yanlai Tang[4†], Yuese Lin[2], Hongjun Ba[2], Yiqian Ding[1], Dubo Chen[5], Min Liu[5], Peizhen Pan[2], Youzhen Qin[2] and Zhan-Peng Huang[1,3*]*

[1] Department of Cardiology, Center for Translational Medicine, Institute of Precision Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [2] Department of Pediatric Cardiology, Heart Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [3] NHC Key Laboratory of Assisted Circulation, Sun Yat-sen University, Guangzhou, China, [4] Department of Pediatrics, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, [5] Department of Laboratory Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Congenital heart defects (CHDs) represent the most common human birth defects. Ventricular septal defect (VSD) is the most common subtype of CHDs. It has been shown that about 20–40% of VSDs are closely related to chromosomal aneuploidies or Mendelian diseases. In this study, we report a pedigree with VSD associated with a balanced paracentric inversion of chromosome 6, inv (6)(p21.3p23), a rarely reported CHD-associated chromosomal abnormality related to the fragile site at 6p23. We have found that the major clinical features of the proband include CHDs (ventricular septal defect, severe pulmonary hypertension, tricuspid regurgitation, and patent foramen ovale), severe pneumonia, and growth retardation. Our study reports a rare chromosomal abnormality connected to CHDs, which may represent a new genetic etiology for VSD.

Keywords: congenital heart disease, ventricular septal defect, chromosomal rearrangement, human chromosome 6, proband

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Pingzhu Zhou,
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***Correspondence:**
Youzhen Qin
qyouzhen@163.com
Zhan-Peng Huang
Huangzhp27@mail.sysu.edu.cn

†These authors have contributed equally to this work

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INTRODUCTION

Congenital heart defects (CHDs) represent the most common human birth defects, affecting about 1% newborns worldwide (1). Ventricular septal defect (VSD) is the most common form of CHDs. Around 30% of cases of CHDs are diagnosed after birth and 10% of all fetal cases are associated with VSD (2, 3). Data showed that ∼20–40% of VSDs occurred due to chromosomal aneuploidies or Mendelian diseases, while the rest of the cases remained idiopathic (4–8). Furthermore, 33–47% of fetuses with VSD had chromosomal abnormalities, while trisomy 18, trisomy 21, and DiGeorge syndrome were the most common cases among these mutations (5, 7, 9, 10). The defect in the ventricular septum causes the leakage of blood from the left ventricle to the right ventricle of the heart. Instead of pumping out to the body, a portion of oxygen-rich blood pumps back to the lungs, which causes the heart to work harder. Meanwhile, pulmonary arteries thicken or grow rigid and become narrowed inside where the blood flows, and then pulmonary arterial hypertension (PAH) occurs (11). When PAH happens, the patient's body cannot get the oxygen it needs. As a result, he/she grows tired more easily. Other symptoms will also turn on, like shortness of breath, chest pain or pressure, heart palpitations, dizziness, fainting, swelling in their arms and legs, racing pulse, etc.

Defects in cardiac development often lead to congenital heart disease. It has been shown that both genetics and environmental factors affect the pathogenesis of CHDs (12, 13), but the

underlying mechanism is still not fully understood. Ventricular septal defect may be inherited and sometimes is associated with other congenital disorders, such as Down syndrome. As the commonest congenital cardiac malformations (14), VSDs were widely studied. In many cases, VSD is not simply induced by a specific genetic problem, but genes probably play a role along with environmental factors. Some cases of VSD are passed from generation to generation, while non-genetic-related VSD is not inherited.

In this report, we describe a pedigree with congenital heart defects, including VSD, which carries a balanced paracentric inversion of chromosome 6, inv (6) (p21.3p23). Although a similar case was found in a pedigree with hereditary hemochromatosis (15), the chromosomal rearrangement related to the fragile site at 6p23 is rarely reported to be linked to congenital heart disease. Our study indicates that this reported chromosomal abnormality may represent a new genetic etiology for VSD.

MATERIALS AND METHODS

Genetic pedigrees are mapped with the Panogram software (https://github.com/panogram) through a detailed medical history inquiry. Panogram is an offline, stand-alone multiplatform pedigree drawing tool based on the Phenotips (https://github.com/phenotips/phenotips) platform. This platform included clinical symptoms and physical findings, family information and history (including pedigree), diagnosis (mapped to OMIM or Orphanet), genes and variants of interest, measurements (with support for the instant computation of percentiles and generation of growth charts), and demographic information (name, date of birth, etc.).

Standard phytohemagglutinin-stimulated lymphocyte chromosomes were prepared from peripheral blood lymphocytes from the patients. A karyotype analysis of the patients was performed by KingMed Diagnostics using GTG banding (Giemsa-trypsin) approach.

A clinical examination of the child was carried out. Chromosomal microarray analysis (CMA) was carried out to detect chromosomal imbalances and copy number variants in the proband. Echocardiogram and chest radiographs were carried out to detect disease progression in the proband. Her family members also had echocardiogram and detection of a series of biochemical indicators of ferritin according to the standard clinical protocol.

Ethics Statement

All individuals involved in the study have signed an informed consent.

THE PROBAND CARRIES MULTIPLE CONGENITAL HEART DEFECTS

The proband is an 8-month-old baby girl (V-4) who came to our facility for chromosomal examination at 1 month of age since she was found to have a cardiac abnormality before she was born. She was born at full term by cesarean section and was admitted to the local hospital due to continued low blood oxygen saturation after birth. In our facility, blood samples were collected for CMA testing (based on the Affymetrix CytoScan HD array). However, no abnormalities in the chromosome copy number variants and absence of heterozygosity were found (data not shown). After 4 months, the proband was hospitalized in our facility due to a decrease in the amount of milk consumed. A heart ultrasound examination showed that she had large ventricular septal defect (perimembranous), atrial septal defect (secondary foramen), and severe pulmonary hypertension (**Figure 1**). Her weight gain was only 1.5 kg in 4 months (from 3.4 to 4.9 kg, ∼40% increase). The VSD progressed from 0.484 to 1.04 cm in 4 months, as determined by echocardiogram (**Figure 1**), indicating that the defect becomes more severe (a larger percentage of VSD enlargement than her body growth). The proband was given cedar orchid digitalis, oral digoxin, and spironolactone, to adjust the heart function, and related symptomatic supportive treatment. Her milk consumption volume was gradually increased to 50–70 ml/q3h after treatment, and the patient was discharged. The proband was re-admitted to our facility at 6 months of age for a cardiac surgery. The surgery was successfully performed, which has repaired the interventional inferior ventricular septal defect in the proband.

A VSD PEDIGREE WITH CHROMOSOMAL INVERSION IN CHROMOSOME 6

A follow-up medical survey revealed that one of proband's aunts (IV-5) had undergone surgical correction for ventricular septal defect when she was an adolescent. Another biological aunt (IV-2) also had a child (V-1) with VSD 13 years ago and who died 3 months after birth (**Figure 2**). We speculated that the occurrence of VSD in this pedigree is related to genetic factors. Since no abnormality was shown in CMA testing, the proband's karyotype was further examined. As a result, a chromosomal inversion in chromosome 6 <46, XX, inv (6) (p21.3p23)> was found (**Figure 3**). Furthermore, blood samples were collected from six other individuals in this pedigree, including the proband's grandmother (III-2), parents (IV-3 and IV-4), aunt (IV-2), elder brother (V-3), and cousin (V-2), for karyotype examination. The result indicated that the proband's father (IV-3), aunt (IV-2), elder brother (V-3), and cousin (V-2) carry the same mutation as the proband's mutation. The proband's grandmother (III-2) carries a polypeptide change on chromosome 1, which was not detected in the other members. No obvious VSD was found from their provided information nor from the on-site examination of echocardiogram. It is worth noting that several members from this pedigree (II-4, III-3, III-5, and III-7) died of heart disease according to the information provided by the proband's father.

THE PEDIGREE IS NOT ASSOCIATED WITH HEMOCHROMATOSIS

Given that a similar chromosomal inversion in chromosome 6 <inv (6) (p21.1p23)> was reported, linking to hereditary

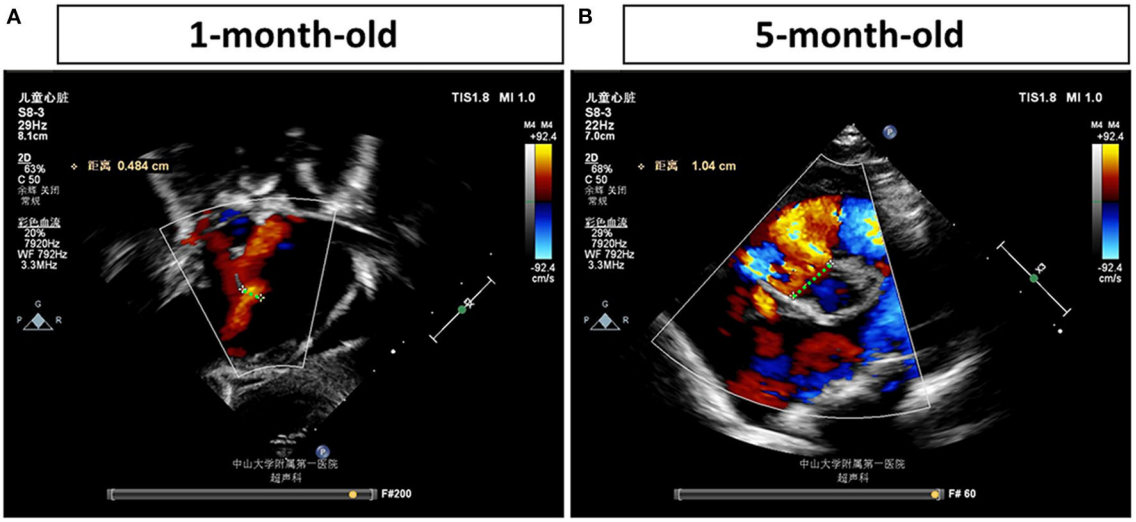
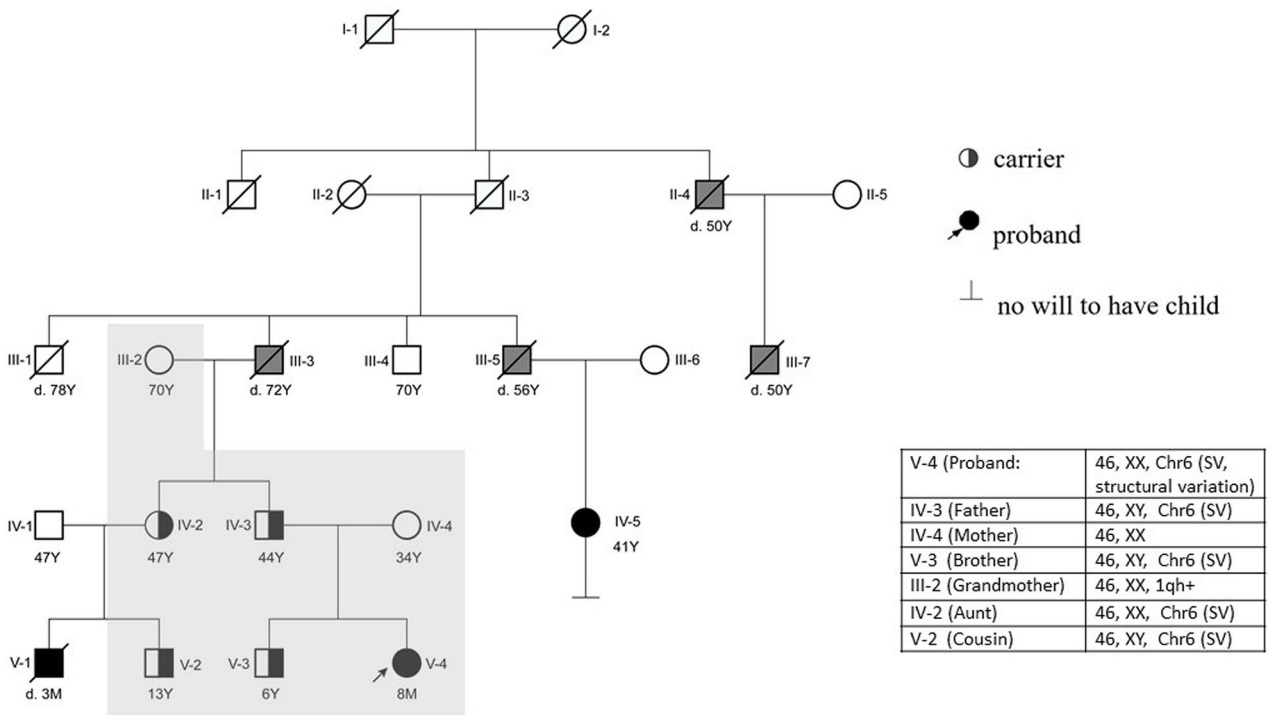


FIGURE 1 | Echocardiogram examinations of the proband at different time points. Representative image of the echocardiogram of the proband's left ventricle obtained in the parasternal long-axis view **(A)** shows a ventricular septal defect (VSD) (0.484 cm) at 1 month of age. Representative image of the echocardiogram **(B)** shows a more severe VSD, which is enlarged to 1.04 cm, in the proband's heart at 5 months of age. The largest distances of VSD are marked by green dash lines and measured.



V-4 (Proband:	46, XX, Chr6 (SV, structural variation)
IV-3 (Father)	46, XY, Chr6 (SV)
IV-4 (Mother)	46, XX
V-3 (Brother)	46, XY, Chr6 (SV)
III-2 (Grandmother)	46, XX, 1qh+
IV-2 (Aunt)	46, XX, Chr6 (SV)
V-2 (Cousin)	46, XY, Chr6 (SV)

FIGURE 2 | Pedigree of the reported family. The proband (V-4) is indicated with an arrow. The proband's mother (IV-4) has a normal phenotype. V-1, V-4, IV-5, III-3, III-5, III-7, and II-4 were reported to have cardiac abnormalities. The proband (V-4) had a surgery at 6 months. IV-5 and V-1 were found to suffer from VSD earlier than the proband. V-1 died at 90 days postnatal; IV-5 is alive after she had a surgery 30 years ago. The karyotype of the proband's father (IV-3), brother (V-3), aunt (IV-2), and cousin (V-2) are the same as the proband's karyotype. The proband's grandfather (III-3) and other relatives (II-4, III-5, and III-7) died of heart disease according to information provided by the proband's father (Y, years; M, month; d.3M, died at the age of 3 months). The individuals with a confirmed VSD are filled in black. The individuals reported to be having other cardiac diseases are filled in gray. The individuals who have done the karyotype examination in this study are indicated with a shadow.

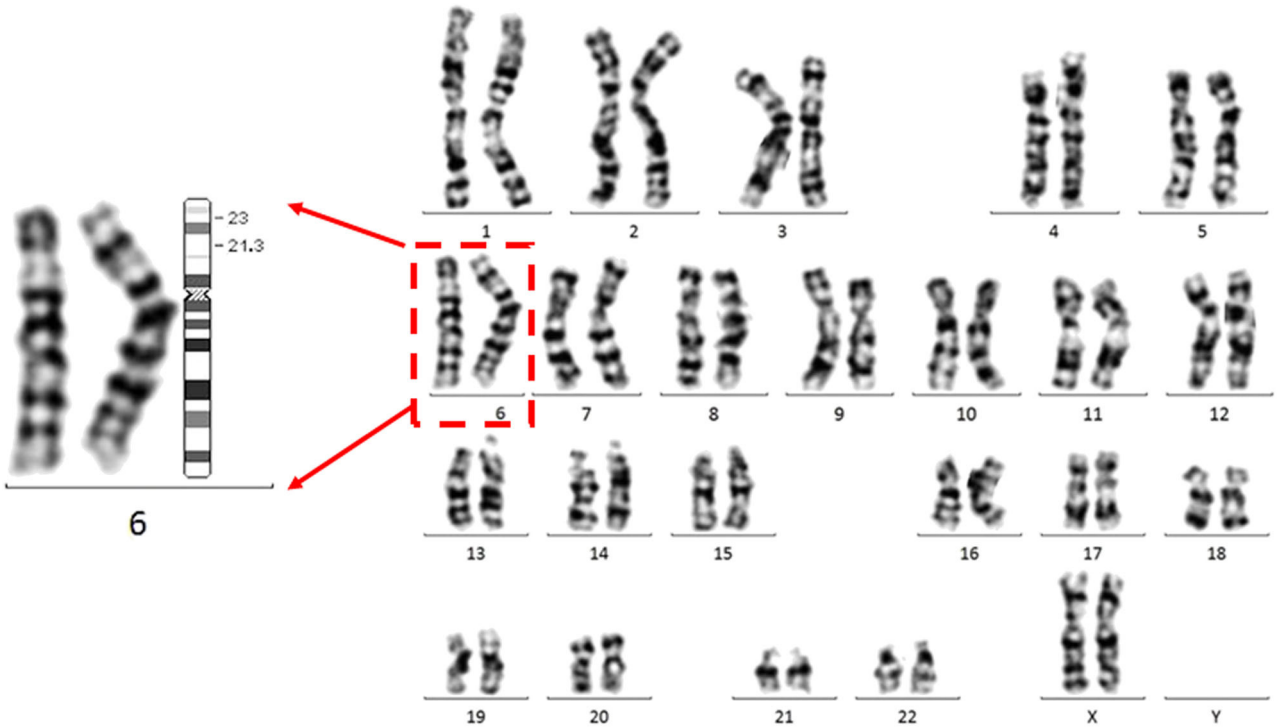


FIGURE 3 | The proband carries a chromosomal inversion in chromosome 6.Representative G banded chromosomes from the proband. Enlarged chromosome 6 is shown at the left. The karyotype represents a p21.3 in chromosome 6 invasion with p23 <46, XX, inv (6) (p21.3p23)>. An ideogram of chromosome 6 is shown. The breakpoints are indicated in the ideogram.

TABLE 1 | Blood examination of members in the pedigree detecting hemochromatosis.

	Mother (IV-4, 34 years old)	Father (IV-3, 44 years old)	Brother (V-3, 6 years old)	Grandmother (III-2, 70 years old)	Aunt (IV-2, 47 years old)	Cousin (V-2, 13 years old)
FER (ng/mL)	60.75	275.95	38.73	430.88	129.55	42.73
TIBC (g/L)	56.8	48.4	56.6	53.5	42.6	69.3
FE (umol/L)	12.2	15.1	14.3	16.7	13.6	11.8
TRF (umol/L)	2.77	2.43	2.60	2.33	2.05	3.00

FER, total iron in serum (normal range value: 21.81–274.66 for male; 4.63–204.00 for female); TIBC, total iron binding capacity (normal range value: 40.8–76.6); FE, ferritin (normal range value: 5.8–34.5); TRF, transferrin (normal range value: 2.00–3.60).

hemochromatosis with potential cardiomegaly (15), blood samples from six members in the pedigree, including four mutation carriers, were examined. Iron in the serum of the proband's father and grandmother was found to be increased, with no other abnormality found (**Table 1**). Their cardiac function was also examined, and no abnormality was shown in the echocardiogram (**Table 2**). No cardiac disease was diagnosed in a routine cardiac examination for these family members.

DISCUSSION

In this study, a mutation of chromosomal inversion in chromosomal 6 is identified to be associated with multiple congenital heart defects, including VSD and atrial septal defect. The enlargement of the septal defect in the heart (double from 0.48 to 1.04 cm) of the patient is more significant than the growth of her body, indicating that the defect does not tend to grow on its own after birth, and it is more likely to become larger, which leads to increased shunting and the progression of pulmonary hypertension that significantly affect the patient's heart function. Therefore, cardiac surgery is required in this case. The possession of the same type of congenital heart defect and, most likely, the same genetic mutation by the proband (V-4), her cousin (V-1), and her aunt (IV-5) in this pedigree indicates that chromosomal rearrangement is a major factor for the pathogenesis of VSD. However, the proband's father (IV-3), aunt (IV-2), elder brother (V-3), and cousin (V-2) carry the same mutation but with lack of an obvious congenital heart defect. There could be several possibilities: (1) these individuals, carrying the genetic mutation, have a less severe VSD that grew itself after birth and (2)

TABLE 2 | Echocardiogram examination of the members in the pedigree.

	Mother (IV-4, 34 years old)	Father (IV-3, 44 years old)	Brother (V-3, 6 years old)	Grandmother (III-2, 70 years old)	Aunt (IV-2, 47 years old)	Cousin (V-2, 13 years old)
Aortic valve (mm)	27	29	19	26	28	27
Left atrium (mm)	29	33	23	28	31	27
Right atrium (mm*mm)	47*38	44*40	32*27	42*33	46*37	44*34
LVIDd (mm)	45	48	32	41	40	47
IVSd (mm)	11	11	7	9	9	9
LVPWd (mm)	8	7	6	8	7	9
The left ventricular wall motion	Normal	Normal	Normal	Normal	Normal	Normal
TDI (cm/s)	14	10	17	9	13	16
Ejection fraction (%)	70	74	65	78	71	65

Data were obtained from a routine transthoracic echocardiogram. Cardiac function is normal for all individuals without sign of cardiomegaly.
LVIDd, left ventricular internal dimension—diastole; IVSd, interventricular septal thickness—diastole; LVPWd, left ventricular posterior wall thickness—diastole; TDI, tissue Doppler imaging.

other factors, such as environmental factors or genetic modifiers, contributing to the VSD are missing; therefore, no developmental cardiac defect is formed in these mutation carriers.

The human chromosomal fragile site at 6p23 is associated with multiple chromosomal rearrangements, which lead to congenital disorders. For example, the chromosomal translocation <t(6;9)(p23;q34)> often links to acute myeloid leukemia due to the generation of chimera genes in the translocation event (16, 17). A balanced translocation t(6;9)(p23;q22.3) is tightly associated with orofacial clefting (18). Endothelin 1 in Chr 6p23 is indicated to have a significant linkage with the orofacial cleft defects (18, 19). It is worth noting that a similar balanced paracentric inversion of chromosome 6, inv(6)(p21.1p23), is reported to be associated with hereditary hemochromatosis (HFE) (15). The locus of HFE has been further shown by linkage analysis to localize on the short arm of chromosome 6, adjacent to the major histocompatibility complex (15). While the examination excluded HFE in this reported pedigree, the indicated gene structure alteration involved in chromosomal inversion between these two pedigrees are different.

Although VSD has been shown often as caused by chromosomal abnormalities, to the best of our knowledge, the current study is the first report to link VSD to chromosomal abnormalities associated with the fragile site at 6p23, which may represent a new genetic etiology for VSD. However, the lack of identifying gene/locus, responsible for VSD, in the chromosomal abnormality is the limitation of the current study. Interestingly, several genes that reside in the proximal regions of these breakpoints, including TNXB (20) and CDKN1A (21) at around 6p21.3 and TFAP2A (22), EDN1 (23), and JARID2 (24) at around 6p23, were reported to be involved in the pathogenesis of VSD. Furthermore, duplication of the TNXB locus was considered as pathogenic to pulmonary atresia with ventricular septal defect in the human patient (25). In the future, the coverage of this genetic mutation in VSD and the major linked gene(s) in this chromosomal inversion need to be further determined in detail.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical ethics committee of the First Affiliated Hospital, Sun Yat-sen University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LC, YT, and Z-PH planned the manuscript. LC and YT collected and analyzed the clinical data. YL and HB collected information and samples from members of the pedigree. YD performed echocardiogram for members of the pedigree. DC and ML performed karyotype examination and blood examination for members of the pedigree. Z-PH drafted the final version of the manuscript. PP and YQ revised the manuscript. All authors read and approved the final manuscript.

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Prediction Factors of 6-Month Poor Prognosis in Acute Myocardial Infarction Patients

Jianhua Yao[1], *Yuan Xie*[2], *Yang Liu*[2], *Yu Tang*[2] *and Jiahong Xu*[2]*

[1] *Department of Cardiology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China,*
[2] *Department of Cardiology, Tongji Hospital, Tongji University School of Medicine, Shanghai, China*

Background: Acute myocardial infarction (AMI) is among the leading causes of death worldwide. Patients with AMI may have the risk of developing recurrent cardiovascular events leading to rehospitalization or even death. The present study aimed to investigate the prediction factors of poor prognosis (mortality and/or readmission) after AMI during a 6-month follow-up.

Methods: A total of 206 consecutive patients hospitalized for the first visit with AMI were enrolled. Data collection included demographic characteristics, medical history, clinical information, laboratory results, and oral medications within 24 h of admission. At 1, 3, and 6 months after discharge, AMI patients were followed up to assess the occurrence of composite endpoint events including in-hospital and out-of-hospital death and/or readmission due to recurrent myocardial infarction (MI) or exacerbated symptoms of heart failure following MI.

Results: After 6-month follow-up, a total of 197 AMI patients were available and divided in two groups according to good prognosis ($n = 144$) and poor prognosis ($n = 53$). Our data identified serum myoglobin ≥651 ng/mL, serum creatinine ≥96 μM, Killip classification 2–4, and female gender as independent predictors of 6-month mortality and/or readmission after AMI. Moreover, we demonstrated that Killip classification 2–4 combined with either myoglobin ($AUC_{Killip\ class\ 2-4+myoglobin} = 0.784$, sensitivity = 69.8%, specificity = 79.9%) or creatinine ($AUC_{Killip\ class\ 2-4+creatinine} = 0.805$, sensitivity = 75.5%, specificity = 77.1%) could further enhance the predictive capacity of poor 6-month prognosis among AMI patients.

Conclusions: Patients with AMI ranked in the higher Killip class need to be evaluated and monitored with attention. Multibiomarker approach using Killip classification 2–4 and myoglobin or creatinine may be an effective way for 6-month prognosis prediction in AMI patients.

Keywords: acute myocardial infarction, prognosis, death, readmission, biomarker

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First Affiliated Hospital of Sun Yat-sen University, China

***Correspondence:**
Jiahong Xu
xujiahong@tongji.edu.cn

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INTRODUCTION

Acute myocardial infarction (AMI) is among the leading causes of death worldwide (1, 2). Despite that great progress has been made in the pharmacological and interventional therapy of AMI, patients with AMI may have the risk of developing recurrent cardiovascular events leading to rehospitalization or even death (3, 4). A deep understanding of the prediction factors of AMI prognosis can provide important information for disease stratification and clinical treatment of patients.

Because of the rapid advancement of laboratory techniques, a number of biomarkers have been identified for diagnosis of AMI. Among the biomarkers of cardiac necrosis injury, cardiac troponins, especially troponin I (cTnI) and troponin T, are considered as highly specific and sensitive markers of AMI diagnosis (5). Myoglobin and creatine kinase-MB (CK-MB), although with less specificity, are also valuable diagnostic biomarkers because of their rapid elevation in the early stage of AMI (6). For prognosis of AMI, cardiac troponins, brain natriuretic peptide (BNP), and N-terminal pro–brain natriuretic peptide (NT-proBNP) were proved to have prognostic values of heart failure and/or mortality in AMI patients (7, 8). In addition, increased heart-type fatty acid–binding protein and C-reactive protein (CRP) levels were reported to be possibly predictive of heart failure or mortality during the follow-up of AMI patients (9, 10). However, there is actually no gold standard prognostic biomarker for AMI (11). Multiple factors such as demographics, clinical presentations, and comorbidities are associated with AMI prognosis (12–14). Clinical studies are still highly needed to evaluate the factors predicting prognosis of AMI, especially with continuous advances in cardiovascular care (15).

In the present study, we aimed to analyze the prediction factors of poor prognosis (mortality and/or readmission) of AMI patients during a 6-month follow-up.

PATIENTS AND METHODS

Patients

A total of 206 consecutive patients hospitalized for the first visit with AMI were enrolled from October 2015 to August 2017 at Department of Cardiology in Tongji Hospital affiliated to Tongji University (Shanghai, China). This cohort of patients was previously used to analyze gender-specific predictive markers of poor AMI prognosis, which was an independent analysis from the present study (16). The diagnosis of AMI was made by cardiologists according to Guidelines for the Diagnosis and Treatment of AMI in China. Those with malignant tumors, severe mental illness, and/or uncontrolled systemic diseases were excluded from the present study. The study protocol was approved by the independent ethics committee of Tongji Hospital affiliated to Tongji University (Shanghai, China). The written informed consent form was provided by all patients.

Data Collection Flow

Data collection included demographic characteristics, medical history, clinical information, laboratory results, and oral medications within 24 h of admission. At admission or on the next morning, venous blood was taken and immediately analyzed in the Core Laboratory of Tongji Hospital for examinations of blood biochemistry, markers of myocardium injury (e.g., CK, CK-MB, myoglobin, cTnI, NT-proBNP), CRP, hemoglobin A_{1c} (HbA_{1c}), glycated serum albumin (GSA), D-dimer, and folic acid.

Follow-Up and Primary Endpoint

At 1, 3, and 6 months after discharge, AMI patients were followed up to assess the occurrence of composite endpoint events by trained researchers. The primary endpoint events were a composite of all-cause mortality (including in-hospital and out-of-hospital death) and/or readmission due to recurrent myocardial infarction (MI) or exacerbated symptoms of heart failure following MI. During 6-month follow-up, nine patients (4.4%) were lost over time as reported before, because they provided wrong telephone number or disconnected the call (16). The primary endpoint events were eventually confirmed by patients themselves, their families, and local hospital doctors. For analysis of predictive markers, AMI patients were divided into good prognosis vs. poor prognosis groups according to the occurrence of death and/or readmission.

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) and MedCalc version 19.0.2 (MedCalc Software, Mariakerke, Belgium). Continuous variables with normal distribution were presented as mean ± standard deviation. For the cases of skewed distribution, median with interquartile range would be selected. All categorical variables and frequency of events were shown as numbers (percentage). The comparison between groups was performed with the independent-samples *t*-test, Mann-Whitney *U* test, or χ^2 test as appropriate. Based on the significant ($P < 0.05$) variables between good prognosis and poor prognosis groups, forward stepwise COX regression analyses (entry only if $P \leq 0.10$ and removal only if $P > 0.10$) were further applied to identify the independent predictors of 6-month prognosis. Receiver operating characteristic (ROC) curves and Kaplan–Meier curves were then constructed to determine the cutoff point and predictive value of these markers in the prediction of poor AMI prognosis. $P < 0.05$ was considered as statistically significant.

RESULTS

Clinical Characteristics of AMI Patients

After 6-month follow-up, a total of 197 AMI patients were available and divided into two groups according to prognosis. Those with death and/or readmission events ($n = 53$) were defined as poor prognosis, whereas the other AMI patients without death and/or readmission ($n = 144$) were defined as good prognosis (**Table 1**). In patients with poor prognosis ($n = 53$), 41 patients were rehospitalized (including three patients died during readmission), and 15 patients died (including both in-hospital and out-of-hospital death). Demographics showed that AMI patients with poor prognosis were older than those with good prognosis (71.3 ± 14.1 vs. 61.7 ± 13.3 years, $P < 0.001$). Patients with AMI with poor prognosis also had lower

TABLE 1 | Baseline clinical characteristics of patients.

	All patients ($n = 197$)	Good prognosis ($n = 144$)	Poor prognosis ($n = 53$)	*P*-value
Demographic characteristics				
Age, years	64.3 ± 14.2	61.7 ± 13.3	71.3 ± 14.1	<0.001
BMI, kg/m^2	24.8 ± 3.2	24.8 ± 3.2	24.7 ± 3.5	0.715
SBP, mmHg	126.0 ± 24.2	127.7 ± 23.9	121.5 ± 24.4	0.109
DBP, mmHg	73.9 ± 12.7	75.2 ± 12.2	70.5 ± 13.5	0.022
Heart rate, bpm	79.5 ± 16.0	78.5 ± 14.9	82.1 ± 18.6	0.162
Current or past smoker, *n* (%)	112 (56.9%)	89 (61.8%)	23 (43.4%)	0.021
Gender, *n* (%)				
Male	157 (79.7)	122 (84.7)	35 (66.0)	0.004
Female	40 (20.3)	22 (15.3)	18 (34.0)	
Classification of AMI, *n* (%)				
STEMI	167 (84.8)	120 (83.3)	47 (88.7)	0.354
NSTEMI	30 (15.2)	24 (16.7)	6 (11.3)	
Previous history, *n* (%)				
Hypertension	127 (64.5)	91 (63.2)	36 (67.9)	0.538
Diabetes mellitus	53 (26.9)	31 (21.5)	22 (41.5)	0.005
Atrial fibrillation	12 (6.1)	7 (4.9)	5 (9.4)	0.393
Stroke	35 (17.8)	25 (17.4)	10 (18.9)	0.806
Killip classification, *n* (%)				
Killip classification 2–4	66 (33.5)	29 (20.1)	37 (69.8)	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AMI, acute myocardial infarction; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non–ST-segment elevation myocardial infarction.

diastolic blood pressure (70.5 ± 13.5 mmHg vs. 75.2 ± 12.2 mmHg, $P < 0.05$), less current or past smoking experience (43.4 vs. 61.8%, $P < 0.05$), and higher prevalence of diabetes mellitus (41.5 vs. 21.5%, $P < 0.01$) when compared to those with good prognosis. A significantly larger proportion of AMI patients with poor prognosis had Killip classification 2–4 compared to those with good prognosis (69.8 vs. 20.1%, $P < 0.001$). Meanwhile, AMI prognosis was analyzed according to different genders, which showed that the proportion of AMI patients with poor prognosis (including death and/or readmission) was 45.0% in females ($n =$ 18 among 40 females), which was significantly higher than 22.3% in males ($n = 35$ among 157 males) ($P < 0.01$).

Oral medications were recorded at admission. Among the commonly used medicine, a larger proportion of AMI patients in poor prognosis group were prescribed with loop diuretics compared to those with good prognosis (41.5 vs. 12.5%, $P < 0.001$). No difference was found in other oral medications, including angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, β-blockers, antiplatelet drugs, anticoagulant drugs, and statins, between the good prognosis and poor prognosis groups (**Table 2**).

TABLE 2 | Oral medications at admission.

	All patients ($n = 197$)	Good prognosis ($n = 144$)	Poor prognosis ($n = 53$)	*P*-value
Loop diuretics, *n* (%)	40 (20.3)	18 (12.5)	22 (41.5)	<0.001
ACEI/ARB, *n* (%)	128 (65.0)	99 (68.8)	29 (55.8)	0.092
β-Blockers, *n* (%)	128 (65.0)	99 (68.8)	29 (55.8)	0.092
Antiplatelet drugs, *n* (%)	196 (99.5)	144 (100.0)	52 (98.1)	1.000
Anticoagulant drugs, *n* (%)	87(44.2)	68 (47.2)	19 (36.5)	0.184
Statins, *n* (%)	192 (97.5)	142 (98.6)	50 (94.3)	0.859

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Biochemical Examinations of AMI Patients

At admission or on the next morning, biochemical examinations were performed for AMI patients and were further compared between AMI patients with good and poor prognosis (**Table 3**). Compared to those with good prognosis, patients with poor prognosis were present with slightly lower serum levels of albumin, hemoglobin, and sodium, but higher levels of CRP, blood urea nitrogen (BUN), creatinine, and uric acid. Most of the biochemical data mentioned above had a mean value or median value within the normal range, except for BUN, creatinine, and uric acid, which were slightly above the normal range in the group of AMI patients with poor prognosis. Meanwhile, AMI patients had obviously higher levels of CK, CK-MB, myoglobin, cTnI, and NT-proBNP. Among these biochemical data indicating myocardial necrosis and heart failure, myoglobin and NT-proBNP were significantly elevated in AMI patients with poor prognosis. In particular, AMI patients with poor prognosis had a median value of NT-proBNP more than 5-fold higher than those with good prognosis [1,517.3–12,885.3 (4,448.5) U/L vs. 388.4–1,619.8 (788.4) U/L, $P < 0.001$]. Moreover, AMI patients with poor prognosis had slightly higher levels of HbA_{1c}, GSA, and D-dimer when compared to those with good prognosis.

Myoglobin, Creatinine, Killip Classification 2–4, and Gender Are Independent Predictors of Poor AMI Prognosis

We then constructed multivariate COX stepwise regression analysis to identify the independent predictors of poor AMI prognosis during 6-month follow-up in the present study. With stepwise variable selection using the covariates based on the significant ($P < 0.05$) variables in baseline characteristics and biochemical data between good vs. poor prognosis groups, myoglobin, creatinine, Killip classification 2–4, and gender were identified as potential independent predictors of poor AMI prognosis (**Table 4**).

Receiver operating characteristic curves further demonstrated that area under the curve (AUC) was $AUC_{myoglobin} = 0.632$ (95% CI = 0.561–0.700, sensitivity = 41.5%, specificity = 82.6%), $AUC_{creatinine} = 0.706$ (95% CI = 0.637–0.768, sensitivity = 58.5%, specificity = 78.5%), $AUC_{Killip\ class2-4} = 0.748$ (95% CI = 0.682–0.807, sensitivity = 69.8%, specificity = 79.9%), and $AUC_{gender} = 0.593$ (95% CI = 0.521–0.663, sensitivity = 34.0%, specificity = 84.7%), respectively (**Figure 1**). Using the cutoff point calculated from ROC analysis, myoglobin ≥651 ng/mL, creatinine ≥96 μM, Killip classification 2–4, and female gender

TABLE 3 | Biochemical examinations of patients.

	All patients ($n = 197$)	Good prognosis ($n = 144$)	Poor prognosis ($n = 53$)	*P*-value
Albumin, g/dL	3.7 ± 0.4	3.8 ± 0.4	3.6 ± 0.5	0.015
Hemoglobin, g/dL	13.2 ± 1.9	13.5 ± 1.6	12.3 ± 2.2	<0.001
Sodium, mM	137.7–140.9 (139.4)	137.9–141.1 (139.5)	136.7–140.6 (138.4)	0.027
Potassium, mM	3.9 ± 0.5	3.8 ± 0.4	4.0 ± 0.7	0.062
BUN, mM	4.5–7.0 (5.5)	4.2–6.3 (5.3)	5.2–11.6 (7.8)	<0.001
LDL-C, mM	3.3 ± 0.8	3.3 ± 0.8	3.1 ± 0.9	0.151
HDL-C, mM	1.03 ± 0.21	1.03 ± 0.21	1.00 ± 0.22	0.311
CRP, mg/dL	0.3–2.0 (0.8)	0.3–1.4 (0.7)	0.5–5.1 (1.4)	<0.001
CK, U/L	543.5–2,574.0 (1,162.0)	559.5–2,685.3 (1,179.5)	505.0–2,134.0 (1,048.0)	0.649
Myoglobin, ng/mL	141.5–644.9 (317.9)	130.4–567.4 (286.0)	201.0–935.8 (470.0)	0.004
CTnI, ng/mL	20.9–78.0 (63.9)	17.7–78.0 (54.1)	27.2–78.0 (75.0)	0.377
CK-MB, ng/mL	72.8–299.0 (194.2)	73.6–300.0 (193.3)	61.6–295.0 (197.1)	0.367
HbA_{1c}, %	5.7–6.6 (6.0)	5.6–6.4 (5.9)	5.8–7.0 (6.1)	0.008
GSA, %	12.6–16.1 (14.3)	12.5–15.5 (13.9)	13.3–17.9 (15.3)	0.002
Creatinine, μM	77.0–101.0 (88.0)	74.0–95.0 (85.5)	83.0–140.0 (101.0)	<0.001
NT-proBNP, U/L	437.4–2,653.8 (1,037.5)	388.4–1,619.8 (788.4)	1,517.3–12,885.3 (4,448.5)	<0.001
Uric acid, μM	393.8 ± 121.6	372.4 ± 101.0	451.4 ± 151.6	0.001
ALT, U/L	28.0–68.5 (39.0)	28.0–66.5 (38.0)	29.5–78.5 (43.0)	0.407
AST, U/L	79.0–296.5 (158.0)	75.0–297.3 (157.5)	80.5–286.5 (165.0)	0.964
d-Dimer, mg/L	0.3–0.9 (0.4)	0.2–0.7 (0.4)	0.4–2.4 (0.7)	<0.001
Folic acid, mM	7.5 ± 3.7	7.7 ± 3.7	7.1 ± 3.8	0.365

BUN, blood urea nitrogen; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; CK, creatine kinase; CK-MB, creatine kinase MB; cTnI, cardiac troponin I; HbA_{1c}, hemoglobin A1c; GSA, glycated serum albumin; NT-proBNP, N-terminal pro–brain natriuretic peptide; ALT, alanine aminotransferase; AST, aspartate transaminase.

were found to be significant predictors of poor prognosis in AMI patients (**Figure 2**).

Combined Analysis of Independent Predictors of Poor AMI Prognosis

We further performed combined analysis of these independent markers to explore whether this could further enhance their predictive capacity for poor AMI prognosis (**Figure 3**). We found that a combination of Killip classification 2–4 with myoglobin was sufficient to enhance AUC ($AUC_{Killip\ class\ 2-4+myoglobin}$ = 0.784, 95% CI = 0.720–0.839, sensitivity = 69.8%, specificity = 79.9%) when compared to $AUC_{Killip\ class\ 2-4}$ or $AUC_{Myoglobin}$ alone ($P < 0.05$ and $P < 0.001$, respectively). Similarly, a combination of Killip classification 2–4 with creatinine was also able to enhance AUC ($AUC_{Killip\ class\ 2-4+creatinine}$ = 0.805, 95% CI = 0.743–0.858, sensitivity = 75.5%, specificity = 77.1%) when compared to $AUC_{Killip\ class\ 2-4}$ or $AUC_{creatinine}$ alone ($P < 0.05$ and $P < 0.01$, respectively).

As expected, a combination of Killip class 2–4, myoglobin, and creatinine could also increase AUC ($AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ = 0.800, 95% CI = 0.737–0.853, sensitivity = 73.6%, specificity = 78.5%) compared to either predictive marker alone (**Figure 4**). Noteworthy, although $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ was significantly larger than $AUC_{creatinine+myoglobin}$, no significant difference was found for $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ when compared to $AUC_{Killip\ class\ 2-4+myoglobin}$ or $AUC_{Killip\ class\ 2-4+creatinine}$. Taken together, these data suggest that a combination of Killip classification 2–4 either with myoglobin ($AUC_{Killip\ class\ 2-4+myoglobin}$) or creatinine ($AUC_{Killip\ class\ 2-4+creatinine}$) was sufficient to enhance the predictive capacity for AMI poor prognosis.

TABLE 4 | Forward stepwise COX regression analysis (entry only if $P \leq 0.10$ and removal only if $P > 0.10$) for poor AMI prognosis.

	β Value	Standard error	Hazards ratio	95% CI	*P*-value
Variables in the Equation (Entry Only if $P \leq 0.10$)					
Myoglobin	0.000	0.000	1.000	1.000–1.001	0.005
Creatinine	0.011	0.003	1.011	1.006–1.016	<0.001
CRP	0.007	0.004	1.007	0.999–1.014	0.098
Killip class 2–4	1.420	0.377	4.139	1.976–8.672	<0.001
Gender	−0.934	0.354	0.393	0.196–0.787	0.008

	Score	Degrees of freedom	*P*-value
Variables not in the Equation (Removal Only if $P > 0.10$)			
Age	2.068	1	0.150
DBP	2.393	1	0.122
Current or past smoker	0.230	1	0.631
Diabetes mellitus	0.567	1	0.451
Loop diuretics	0.480	1	0.488
Albumin	0.081	1	0.776
Hemoglobin	0.001	1	0.971
Sodium	0.379	1	0.538
BUN	0.441	1	0.506
NT-proBNP	1.156	1	0.282
HbA_{1c}	0.537	1	0.464
GSA	0.694	1	0.405
Uric acid	0.082	1	0.775
d-Dimer	0.340	1	0.560

CRP, C-reactive protein.
DBP, diastolic blood pressure; BUN, blood urea nitrogen; NT-proBNP, N-terminal pro–brain natriuretic peptide; HbA_{1c}, hemoglobin A_{1c}; GSA, glycated serum albumin.

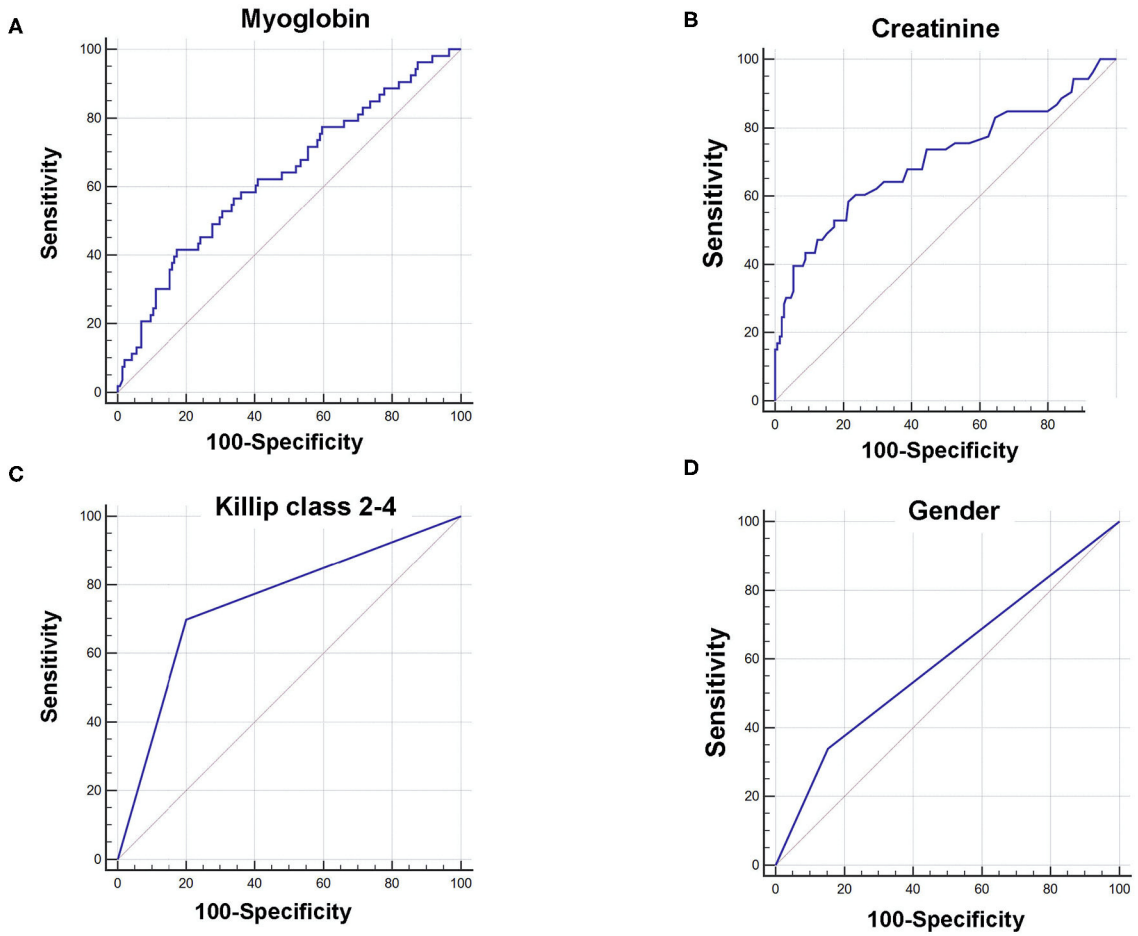

FIGURE 1 | Receiver operating characteristic curve (ROC) of myoglobin, creatinine, Killip classification 2–4, and gender for predicting 6-month prognosis in AMI patients. **(A)** $AUC_{myoglobin}$ = 0.632, 95% CI = 0.561–0.700, sensitivity = 41.5%, specificity = 82.6%, cutoff point: 651; **(B)** $AUC_{creatinine}$ = 0.706, 95% CI = 0.637–0.768, sensitivity = 58.5%, specificity = 78.5%, cutoff point: 96; **(C)** $AUC_{Killip\ class\ 2-4}$ = 0.748, 95% CI = 0.682–0.807, sensitivity = 69.8%, specificity = 79.9%. **(D)** AUC_{gender} = 0.593, 95% CI = 0.521–0.663, sensitivity = 34.0%, specificity = 84.7%.

DISCUSSION

A great number of AMI patients are at risk of recurrent cardiovascular events, which leads to readmission or even dearth. Biomarkers are useful in the prediction of AMI prognosis, which may differ from endpoint events and follow-up durations. Based on a cohort of 197 AMI patients followed up for 6 months, our study shows that serum myoglobin ≥651 ng/mL, serum creatinine ≥96 μM, Killip classification 2–4, and female gender are independent predictors of 6-month mortality and/or readmission. Our data also demonstrate that the combination of Killip classification 2–4 either with creatinine or myoglobin could further enhance the predictive capacity for AMI poor prognosis.

Demographic characteristics and oral medications at admission were first compared in AMI patients with good prognosis ($n = 144$) and poor prognosis ($n = 53$). Compared to those with good prognosis, patients with poor prognosis were about 10 years older and more likely to have previous history of diabetes mellitus. Aging and diabetes are both well-known risk factors for worse outcomes after MI that have strong associations with death or recurrent cardiovascular events (17, 18). The increased risk of adverse outcomes in patients with older age or diabetes is likely multifactorial, which may be explained by more complicating diseases, higher prevalence of multivessel disease, and less implementation of evidence-based therapies (19–21).

Gender-related difference exists in the assessment, treatment, and outcomes of coronary artery diseases (22, 23). Based on the same cohort of AMI patients followed up for 6 months, we previously analyzed and reported gender-specific predictive markers of poor AMI prognosis in male and female patients; in that study, although there was no significant difference in 6-month mortality between males and females, the readmission rate was significantly higher in females than in males (16). Here, when we analyzed death and readmission events together in AMI patients, we observed that 45.0% of females had death and/or readmission events during 6-month follow-up, which was significantly higher than 22.3% in males. COX regression analyses and Kaplan–Meier curves further demonstrated that

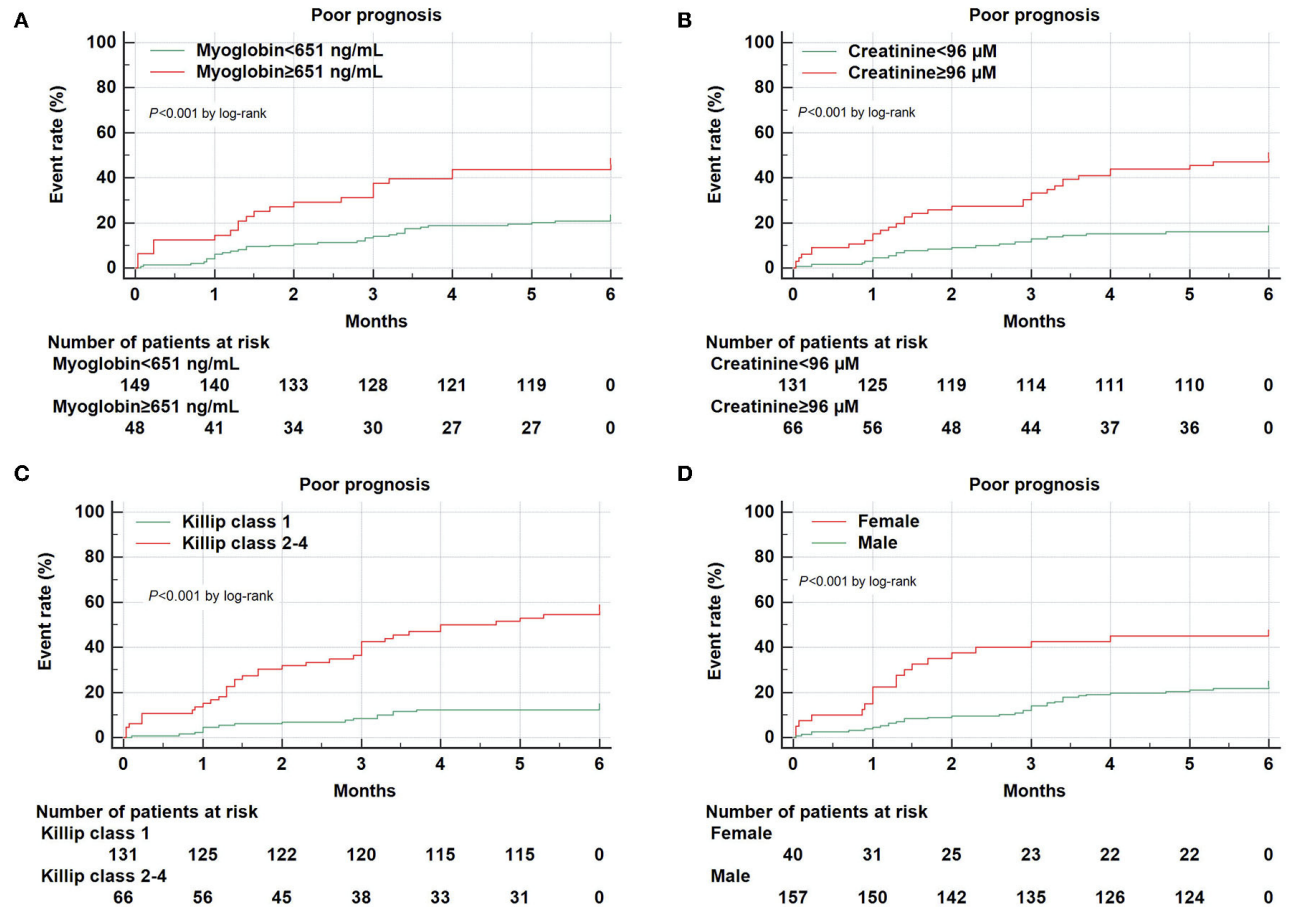

FIGURE 2 | Kaplan–Meier survival curves for 6-month prognosis of AMI patients. Kaplan–Meier survival curves were constructed according to myoglobin **(A)**, creatinine **(B)**, Killip classification 2–4 **(C)**, and gender **(D)**.

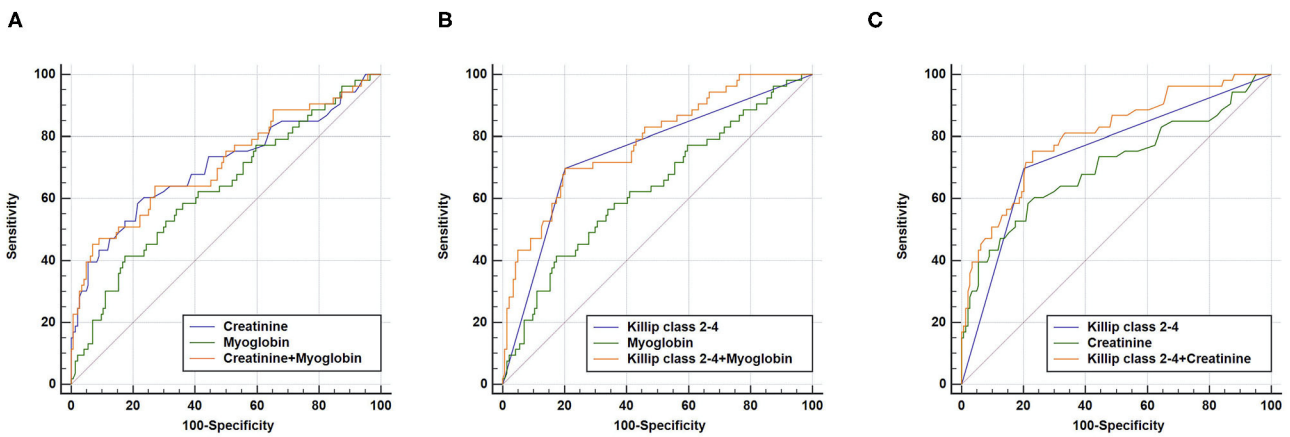

FIGURE 3 | Combination of two independent predictors in construction of Receiver operating characteristic (ROC) curve. **(A)** $AUC_{creatinine+myoglobin}$ = 0.713, 95% CI = 0.645–0.775, sensitivity = 45.3%, specificity = 93.1%, $AUC_{creatinine+myoglobin}$ vs. $AUC_{creatinine}$ (P = 0.5736), $AUC_{creatinine+myoglobin}$ vs. $AUC_{myoglobin}$ (P = 0.1094); **(B)** $AUC_{Killip\ class\ 2-4+myoglobin}$ = 0.784, 95% CI = 0.720–0.839, sensitivity = 69.8%, specificity = 79.9%, $AUC_{Killip\ class\ 2-4+myoglobin}$ vs. $AUC_{Killip\ class\ 2-4}$ (P = 0.0369), $AUC_{Killip\ class\ 2-4+myoglobin}$ vs. $AUC_{myoglobin}$ (P = 0.0003); **(C)** $AUC_{Killip\ class\ 2-4+creatinine}$ = 0.805, 95% CI = 0.743–0.858, sensitivity = 75.5%, specificity = 77.1%, $AUC_{Killip\ class\ 2-4+creatinine}$ vs. $AUC_{Killip\ class\ 2-4}$ (P = 0.0108), $AUC_{Killip\ class\ 2-4+creatinine}$ vs. $AUC_{creatinine}$ (P = 0.0038).

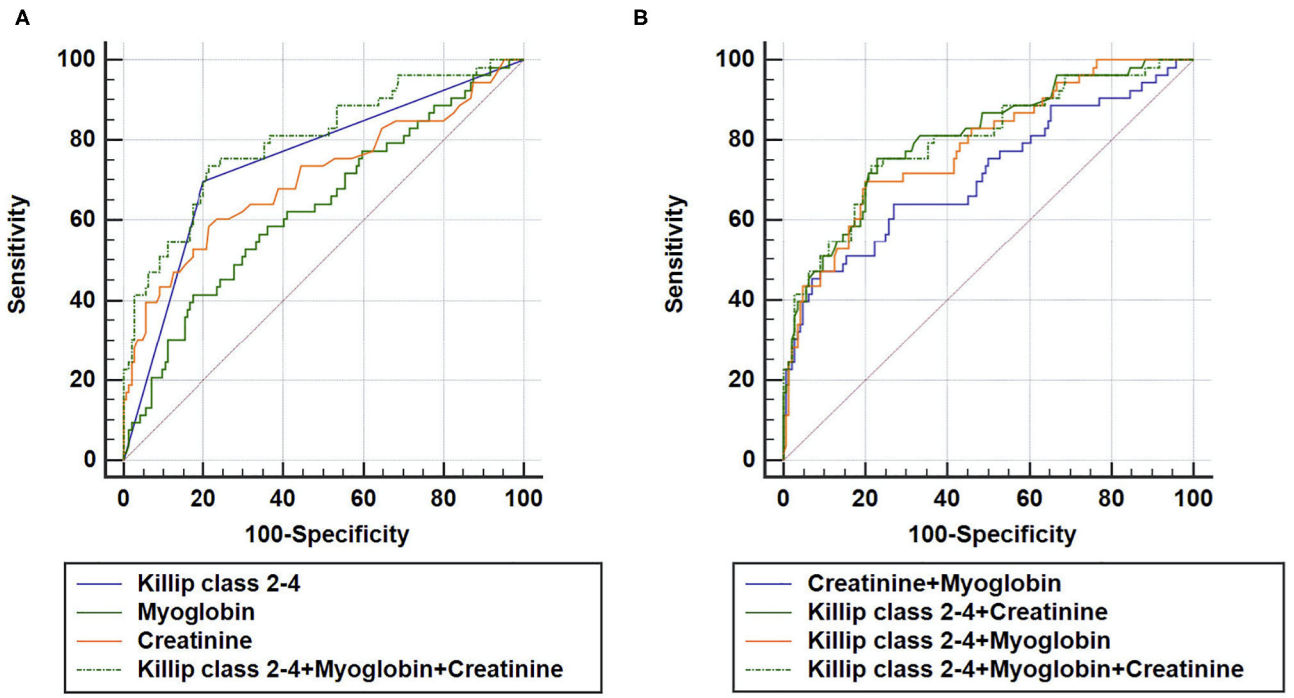

FIGURE 4 | Combination of three independent predictors in construction of Receiver operating characteristic (ROC) curve. $AUC_{Killip\ class\ 2-4+creatinine+myoglobin} = 0.800$, 95% CI = 0.737–0.853, sensitivity = 73.6%, specificity = 78.5%. **(A)** $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ vs. $AUC_{Killip\ class\ 2-4}$ ($P = 0.0224$), $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ vs. $AUC_{myoglobin}$ ($P = 0.0007$), $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ vs. $AUC_{creatinine}$ ($P = 0.0088$). **(B)** $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ vs. $AUC_{creatinine+myoglobin}$ ($P = 0.0056$), $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ vs. $AUC_{Killip\ class\ 2-4+creatinine}$ ($P = 0.3557$), $AUC_{Killip\ class\ 2-4+myoglobin+creatinine}$ vs. $AUC_{Killip\ class\ 2-4+myoglobin}$ ($P = 0.4398$).

female gender was an independent predictor of 6-month mortality and/or death after AMI. The predictive value of female gender in AMI prognosis may differ by age as well as by endpoint events analyzed in different studies. A great number of studies support that younger female patients with AMI were at higher risk of both short- and long-term mortality than male patients; however, this difference was diminished in the old population (24–26). Additionally, most studies used mortality as endpoint event (27–29), whereas our study defined poor prognosis including both death and hospital readmission. Indeed, a deeper understanding of the impact of gender on the outcomes of AMI may help guide better therapeutic strategies for male and female patients.

Biochemical examination data were then analyzed between AMI patients who had good vs. poor prognosis. Among the biochemical parameters different between good and poor prognosis groups, serum myoglobin and serum creatinine were found to be independent indicators for death and/or readmission among AMI patients. Myoglobin is a widely used biomarker for early diagnosis of MI that rises earlier than troponins. However, the diagnostic value of myoglobin is limited because of its less specificity to cardiomyocyte death. Indeed, a combined analysis of myoglobin, CK-MB, and cTnI is the most often used biochemical examination for MI diagnosis among suspected patients (30). In the present study, our data showed that serum myoglobin, CK-MB, and cTnI were all markedly elevated in AMI patients. However, only myoglobin was proved to be an independent predictor for poor AMI prognosis. It was previously reported that elevated myoglobin was a predictive biomarker better than cTnI for 5-year mortality in patients evaluated in the emergency department for possible acute coronary syndromes (ACS) (31). In comparison to the long-term prognosis of patients with undifferentiated chest pain, our study analyzed the short-term prognosis of AMI patients and observed that myoglobin ≥651 ng/mL was predictive for 6-month poor prognosis after AMI. Our findings were consistent with previous studies that reported the prognostic value of myoglobin to predict mortality in patients with ACS (32) and MI (33). Indeed, despite the absence of cardiac specificity, the prognostic value of myoglobin and its cutoff point for clinical use of predicting mortality and/or readmission after AMI deserve further investigation.

Additionally, we observed that serum creatinine level was significantly higher in AMI patients with poor prognosis [83.0–140.0 (101.0) μM] than those with good prognosis [74.0–95.0 (85.5) μM]. Furthermore, serum creatinine ≥96 μM was able to independently predict 6-month death and/or readmission among AMI patients. Serum creatinine is a commonly used biomarker for kidney function. In addition to its ability to reflect

kidney dysfunction, serum creatinine has been demonstrated to be an independent predictor for in-hospital and out-of-hospital mortality among patients with ACS or AMI (34, 35). In addition, subclinical serum creatinine elevation was reported to have prognostic value of adverse in-hospital outcomes among MI patients, which was independent of baseline renal function (36). The predictive value of high creatinine level for poor AMI prognosis is usually closely related to the kidney damage or dysfunction developed in those patients with impaired cardiac function (37). In our study, the median value of serum creatinine was slightly above the normal range in the group of AMI patients with poor prognosis compared to those with good prognosis. A cutoff point $\geq$96 μM was found to independently predict 6-month death and/or readmission among AMI patients. These data suggest that the serum creatinine elevation (even moderate elevation) also needs to be taken seriously in clinical evaluation, which may provide important information for poor prognosis among AMI patients.

Killip classification is usually evaluated for AMI patients, which is classified as Killip 1 (without heart failure), Killip 2 (with mild heart failure), Killip 3 (with pulmonary edema), and Killip 4 (with cardiogenic shock). Increasing evidence has indicated that higher Killip classification is associated with poor prognosis in patients with ACS (38, 39). Killip class $\geq$2 at presentation was previously found to be an independent predictor of in-hospital and long-term mortality of AMI patients (40, 41). Additionally, AMI patients were reported to have higher glucose level at presentation (42). In the present study, Killip classification 2–4 was identified as a strong independent predictor of 6-month death and/or readmission among AMI patients ($AUC_{Killip\ class\ 2-4}$ = 0.748, 95% CI = 0.682–0.807, sensitivity = 69.8%, specificity = 79.9%). Moreover, our data showed that Killip classification 2–4 combined with either myoglobin ($AUC_{Killipclass2-4+myoglobin}$ = 0.784, 95% CI = 0.720–0.839, sensitivity = 69.8%, specificity = 79.9%) or creatinine ($AUC_{Killip\ class\ 2-4+creatinine}$ = 0.805, 95% CI = 0.743–0.858, sensitivity = 75.5%, specificity = 77.1%) further enhanced the predictive capacity for AMI poor prognosis. Our data, together with previous reports, highly suggest that it is important to better evaluate AMI patients ranked in the higher Killip classes. Multibiomarker approach could provide more information for the risk stratification of AMI. Moreover, AMI patients with Killip classification 2–4 need to be monitored and managed differently to improve the prognosis after AMI (43).

There are some limitations that need to be noted. First, the cohort of patients is not large enough. A relatively small number of female AMI patients were enrolled in the present study. Second, despite that the medication information was not available for each patient after 6-month follow-up, whether the medication was changed or not is important information that may also influence AMI prognosis (44). Third, the prognostic value of the four identified predictors and its cutoff point for clinical use of poor AMI prognosis deserve further investigation.

In conclusion, our study identifies serum myoglobin $\geq$651 ng/mL, serum creatinine $\geq$96 μM, Killip classification 2–4, and female gender as independent predictors of 6-month mortality and/or readmission after AMI. Noteworthy, Killip classification 2–4 combined with either myoglobin or creatinine further enhances the predictive capacity of poor AMI prognosis. Multibiomarker approach using Killip classification 2–4 and myoglobin or creatinine may be an effective way for 6-month prognosis prediction in AMI patients.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Tongji Hospital affiliated to Tongji University (Shanghai, China). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JY collected clinical and biochemical data, took charge of follow-up, and analyzed the data. YX obtained informed consent from patients and participated in the follow-up of patients. YL and YT collected patient serum and ensured the quality of serum collection. JX designed and supervised the study and wrote the paper. All authors contributed to the article and approved the submitted version.

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Timing of Maximal Weight Reduction Following Bariatric Surgery: A Study in Chinese Patients

*Ting Xu[1], Chen Wang[1], Hongwei Zhang[1], Xiaodong Han[1], Weijie Liu[1], Junfeng Han[2], Haoyong Yu[2], Jin Chen[3], Pin Zhang[1] and Jianzhong Di[1]**

[1] Department of Bariatric & Metabolic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, [2] Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, [3] Department of Computer Science, Institute for Biomedical Informatics, University of Kentucky, Lexington, KY, United States

Introduction: Bariatric surgery is a well-received treatment for obesity with maximal weight loss at 12–36 months postoperatively. We investigated the effect of early bariatric surgery on weight reduction of Chinese patients in accordance with their preoperation characteristics.

Materials and Methods: Altogether, 409 patients with obesity from a prospective cohort in a single bariatric center were enrolled retrospectively and evaluated for up to 4 years. Measurements obtained included surgery type, duration of diabetic condition, besides the usual body mass index data tuple. Weight reduction was expressed as percent total weight loss (%TWL) and percent excess weight loss (%EWL).

Results: RYGB or SG were performed laparoscopically without mortality or complications. BMI generally plateaued at 12 months, having decreased at a mean of 8.78 kg/m^2. Successful weight loss of >25% TWL was achieved by 35.16, 49.03, 39.22, 27.74, 20.83% of patients at 6, 12, 24, 36, and 48 months after surgery. Overall, 52.91% of our patients had lost 100% of their excess weight at 12 months, although there was a rather wide range among individuals. Similar variability was revealed in women of child-bearing age.

Conclusion: Chinese patients undergoing bariatric surgery tend to achieve maximal weight loss and stabilization between 12 and 24 months postoperatively, instead of at >2 years. The finding of the shorter stabilization interval has importance to earlier intervention of weight loss related conditions and women's conception planning.

Keywords: bariatric surgery, Chinese patients, weight reduction, trend, follow up

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***Correspondence:**
Jianzhong Di
dijianzhong@sjtu.com

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INTRODUCTION

Obesity is a chronic health condition that is becoming a global issue. The World Health Organization stated in 2016 that more than 1.9 billion adults were overweight, and more than 650 million were obese. Besides lifestyle intervention and medication, bariatric surgery has been proved to be a safe, effective, and durable procedure for weight loss among morbidly obese patients (1). In addition, a number of comorbidities including essential hypertension, type 2 diabetes mellitus, hyperlipidemia, bronchial asthma, obstructive sleep apnea, and osteoarthritis can be ameliorated or

even resolved following bariatric surgery. In China, the number of bariatric operations conducted has increased from around 4,000 as of the previous 5 year period to more than 10,000 during the past 5 years (2).

In western countries, research evidences support the consensus that postoperative body weight decreases to a trough at 12–36 months after bariatric surgery (3–5). However, there is currently no published investigation on standardized weight loss after bariatric surgery in Asian settings, except a few studies in Japan and Singapore showed that the average percent total weight loss (%TWL) following bariatric surgery was 20–25% within the first 3 years (6, 7). On account of differences in the baseline body height and weight, and body composition, it is not completely grounded to interpret the weight loss on the Asian communities according to westerner physical standards.

The degree of weight loss and the time when it most likely occurs after bariatric surgery has been insufficiently addressed in China. In addition, we recognized in our patients that women who suffer from obesity-related infertility are more likely to have unplanned pregnancies within the first year following bariatric procedure. These altogether necessitate the establishment of the timing of maximal weight reduction during preoperative counseling. Patients who gain a realistic expectation of weight loss tend to stick to lifestyle changes and better cooperate with medical intervention, leading to uneventful recovery and satisfactory (8). To fulfill the need, we examined the up to 4 years' weight loss responses in Chinese patients following bariatric surgery according to their body mass index (BMI), age, sex, surgery type, and duration of type 2 diabetes mellitus (T2DM).

METHODS

We retrospectively reviewed the medical records of 409 patients who had undergone RYGB between February 2011 and August 2018. The Human Research Review Board of our institution approved the study and all patients provided their written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki. Our previous studies focused on the improved renal, respiratory, and reproductive function after surgery (9–13). The present study includes bariatric surgery patients who underwent at least one follow-up evaluation during the first year after their surgery. Exclusion criteria included missing preoperative BMI record, unknown surgery type, death, and/or the presence of surgical complications.

Altogether, 409 patients were enrolled in the study (252 women, representing 61.6%). Among them, 227 underwent Roux-en-Y gastric bypass (RYGB) (55.5%), and the rest underwent sleeve gastrectomy (SG). Data were collected concerning the patients' demographic data, including their age and sex, T2DM diagnosis, type of surgery, initial BMI, and postoperative weight loss for up to 4 years (**Table 1**). Postoperative weight loss was expressed using the postoperative BMI, change in BMI, %TWL and %EWL.

TABLE 1 | Baseline Characteristics.

Parameter (n = 409)	Value
BMI, kg/m^2, Mean ± SD (range)	34.37 ± 6.25 (25.5,67.5)
BMI group, *n* (%)	
25–27.5 kg/m^2	22 (5.4%)
27.5–32.5 kg/m^2	177 (43.3%)
32.5–37.5 kg/m^2	104 (25.4%)
37.5 kg/m^2 and above	106 (25.9%)
Male sex, *n* (%)	157 (38.4%)
T2DM duration, *n* (%)	
Non-diabetic	90 (22.0%)
0–5 years	168 (41.1%)
5–10 years	91 (22.2%)
10–15 years	43 (10.5%)
above 15 years	17 (4.2%)
Age, yrs, Mean ± SD	42.43 ± 13.27
Age, *n* (%)	
18–45	235 (57.5%)
45–60	118 (28.9%)
60 and above	56 (13.7%)
RYGB surgical type, *n* (%)	227 (55.5%)

BMI, body mass index; T2DM, type 2 diabetes mellitus; RYGB, Roux-en-Y gastric bypass.

Statistical Analyses

IBM SPSS Statistics software Version 20.0 (IBM Inc., Armonk, NY, USA) was used to analyze the data. Continuous data were analyzed for normality using the Shapiro–Wilks test. Differences between values were analyzed using the unpaired *t*-test (normal data), paired *t*-test (normal data), Mann–Whitney test (unpaired, non-normal data), or the Wilcoxon matched pairs test (non-normal data). A value of $p < 0.05$ indicated statistical significance.

RESULTS

Detailed characteristics of our cohort are shown in **Table 1**. There were more female than male patients (252 vs. 157). No patients had a BMI <25 kg/m^2 (overweight), and there were 12 patients whose BMIs were >50 kg/m^2 (super morbidly obese) at the time of their surgery. In all, 319 (78%) patients were diagnosed with T2DM preoperatively. More patients (182 vs. 227) underwent RYGB than SG.

Postoperative changes in the BMI for up to 4 years are shown in **Figure 1**. The patients' BMI generally plateaued at 12 months, then decreased at a rate of 8.78 kg/m^2. Following a slight rebound, an insignificant downward trend was observed around the mid-term, with the BMI decreasing to ∼25 kg/m^2. Weight loss of >20% TWL was achieved by 75.62, 64.66, 57.42, and 50.83% of patients at 12, 24, 36, and 48 months after surgery. Up to 53.74% of our patients lost more than 100% of their excess weight (compared with a BMI of 25 kg/m^2) at 12 months. Although the mean weight loss stabilized at ∼24 kg at 12 months, there was wide variability among the individual values, especially between

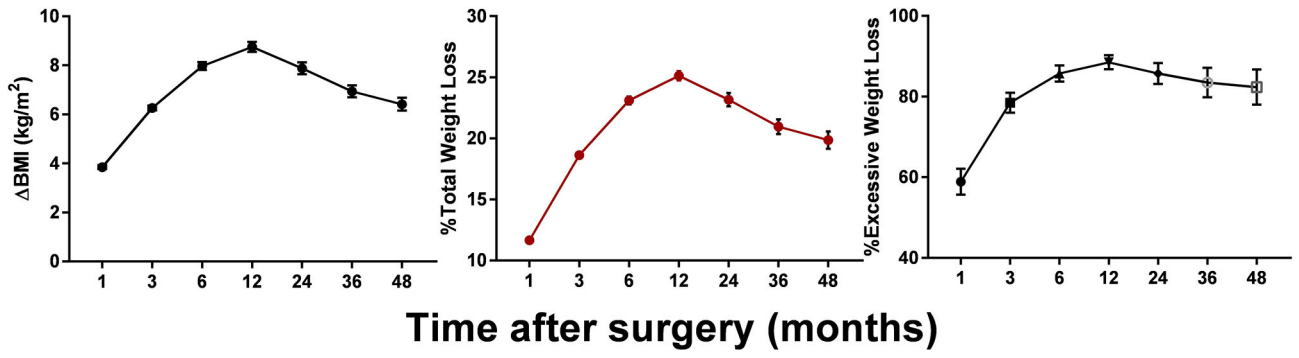

FIGURE 1 | Postoperative changes of ΔBMI, %TWL, and %EWL. Results are shown in mean ± SEM.

the two surgical types. As can be seen from **Table 2**, weight loss after SG rebounded at 3 years after surgery, whereas after RYGB the weight loss was maintained.

Patients were also divided into groups based on certain characteristics to explore other possibilities (**Figure 2**). For example, there was no significant difference between the sexes, although the female patients had generally lost more weight than the male patients at the mid-term point. In addition, post-RYGB weight loss followed a general pattern, whereas post-LSG weight loss fluctuated dramatically during the 3 years after the surgery. Divided into groups according to the preoperative BMI, patients with higher BMIs tended to lose more weight, although in a temperate slope, with the body weight dropping to its lowest 2 years postoperatively (vs. 1 year in other groups). Non-diabetic patients were more likely to have a significant weight rebound, and patients who had been diagnosed with T2DM for more than 15 years tended to face weight rebound as early as 6 months postoperatively.

The most common recipients of bariatric surgery were women of child-bearing age, accounting for 35.70% of our cohort (146 cases). Their mean BMI decreased from a baseline of 35.69 kg/m^2 to an ideal 25.23 kg/m^2 at 12 months and remaining at that level for 3 years postoperatively. Of these cases, we have also observed 17 pregnancies following bariatric surgery that result in different outcomes due to surgery-to-conception (S-C) interval. Two pregnancies were electively terminated during the first trimester for non-medical reasons. Five resulted in spontaneous abortions (defined as loss of pregnancy before 20 wk's gestation). The remaining 10 were carried to delivery.

DISCUSSION

This attempt to explore common bariatric weight loss criteria for Chinese patients showed that the body weight of bariatric surgery patients decreased sharply during the first 12–24 months, instead of at >2 years. It then stabilized unless the patient was non-diabetic, underwent SG, or had a high preoperative BMI, all of which make relapse more likely.

It is of interest to note that, the longer the patient's history of T2DM, the more likely it is that the body weight will decrease unsatisfactorily (84.7% for T2DM duration >5 years), if not rebound. This might be due to the different composition of body fat. For example, in a comparison with their BMI-matched normoglycemic counterparts, patients with T2DM have increased hepatic and pancreatic fat, which causes a severe systemic inflammatory state. A conclusive explanation for this phenomenon, however, requires further study.

Comparison of the surgical effects of the gold standard RYGB vs. the relatively new, yet predominating, SG has become a topic of debate. Our data suggest that patients who undergo SG have a significantly higher BMI than those who undergo RYGB. Both groups plateaued at 12 months, but the SG group then relapsed by a mean 3.4 kg/m^2 3 years later. This conclusion differs from that derived in western countries, where plateaus usually start at 18 months, probably because of the higher baseline body weight and different dietary preferences. Long-term evidence of the effectiveness of SG remains sparse, although short- to medium-term results suggest that SG may not be inferior to RYGB (14). Our results may be biased because of a larger drop-off in the follow-up of our SG patients (35.5% less than in the RYGB group). Also, because data were collected retrospectively in our study, some of the criteria selected were strongly related to whether SG was performed, including the duration of diabetes and baseline BMI. To justify the use of SG, randomized, controlled trials or cohort studies will be necessary to examine its benefit. In addition, a model that can better represent the effectiveness of bariatric surgery should be created, thereby neutralizing baseline differences that cannot be obviated.

Additionally, because most bariatric surgery is performed in women of reproductive age, defining the ideal interval between surgery and pregnancy is an emerging issue, which is partly based on the remission of obesity, which remains controversial in the present literature (15). Especially obese women in their late thirties who have failed many attempts to lose weight may look forward to a shorter interval for the good of both themselves and their children (16). Our research has indicated that women of reproductive age attain body weight stability at 12 months, which

TABLE 2 | Reduction in BMI after surgery.

	1-m post-op	3-m post-op	6-m post-op	1-y post-op	2-y post-op	3-y post-op	4-y post-op
n	242	322	347	361	232	155	120
Follow-up rate	59%	79%	85%	88%	57%	38%	29%
BMI, kg/m^2	29.07 ± 4.33	27.20 ± 4.40	26.01 ± 4.29	25.28 ± 3.88	25.40 ± 3.86*	25.68 ± 3.83*	25.58 ± 3.84*
RYGB	27.84 ± 3.18	25.59 ± 2.90	24.50 ± 3.03	24.08 ± 2.91	24.66 ± 3.34*	25.16 ± 3.34*	25.26 ± 3.64*
SG	33.30 ± 5.04	30.27 ± 5.09	28.26 ± 4.87	26.89 ± 4.42	27.38 ± 4.54*	28.46 ± 5.04	27.98 ± 4.59*
ΔBMI, kg/m^2	3.85 ± 1.22	6.26 ± 1.78	7.97 ± 2.89	8.75 ± 3.72	7.88 ± 3.66	6.94 ± 3.07	6.42 ± 2.85
RYGB	3.65 ± 1.16	5.77 ± 1.52	6.84 ± 2.05	7.18 ± 2.24	6.81 ± 2.60	6.34 ± 2.45	6.07 ± 2.52
SG	4.53 ± 1.21	7.21 ± 1.86	9.67 ± 3.14	10.87 ± 4.22	10.75 ± 4.47	10.18 ± 4.01	8.98 ± 3.79
%TWL	11.65 ± 3.11	18.63 ± 3.92	23.11 ± 5.88	25.12 ± 7.28	23.16 ± 8.13	20.96 ± 7.41	19.86 ± 7.76
RYGB	11.55 ± 3.22	18.32 ± 4.10	21.67 ± 5.53	22.81 ± 6.15	21.47 ± 7.14	20.01 ± 7.40	19.30 ± 7.40
SG	12.00 ± 2.71	19.22 ± 3.50	25.27 ± 5.75	28.24 ± 7.54	27.72 ± 8.90	26.10 ± 8.63	24.02 ± 9.29
%EWL	58.88 ± 25.04	78.46 ± 22.31	85.71 ± 18.60	88.48 ± 16.43	85.72 ± 20.03	83.50 ± 22.85	82.33 ± 24.02
RYGB	64.15 ± 24.43	85.59 ± 18.57	91.27 ± 15.20	92.23 ± 14.28	87.64 ± 19.63	85.06 ± 22.34	83.48 ± 23.56
SG	40.83 ± 17.79	64.85 ± 22.59	77.31 ± 20.11	83.44 ± 17.79	80.55 ± 20.35	75.08 ± 24.19	73.81 ± 26.56
Percentage of follow-up patients who reached ≥50%EWL	11.98%	38.20%	49.86%	52.91%	51.29%	52.90%	50.83%
Percentage of follow-up patients who reached ≥25%TWL	0.41%	5.28%	35.16%	49.03%	39.22%	27.74%	20.83%

$\%TWL = \frac{Initial\ BMI - Final\ BMI}{Initial\ BMI} \times 100\%$, $\%\ EWL = \frac{Initial\ BMI - Final\ BMI}{Initial\ BMI - Ideal\ BMI} \times 100\%$, *$P > 0.05$ (vs. previous visit).

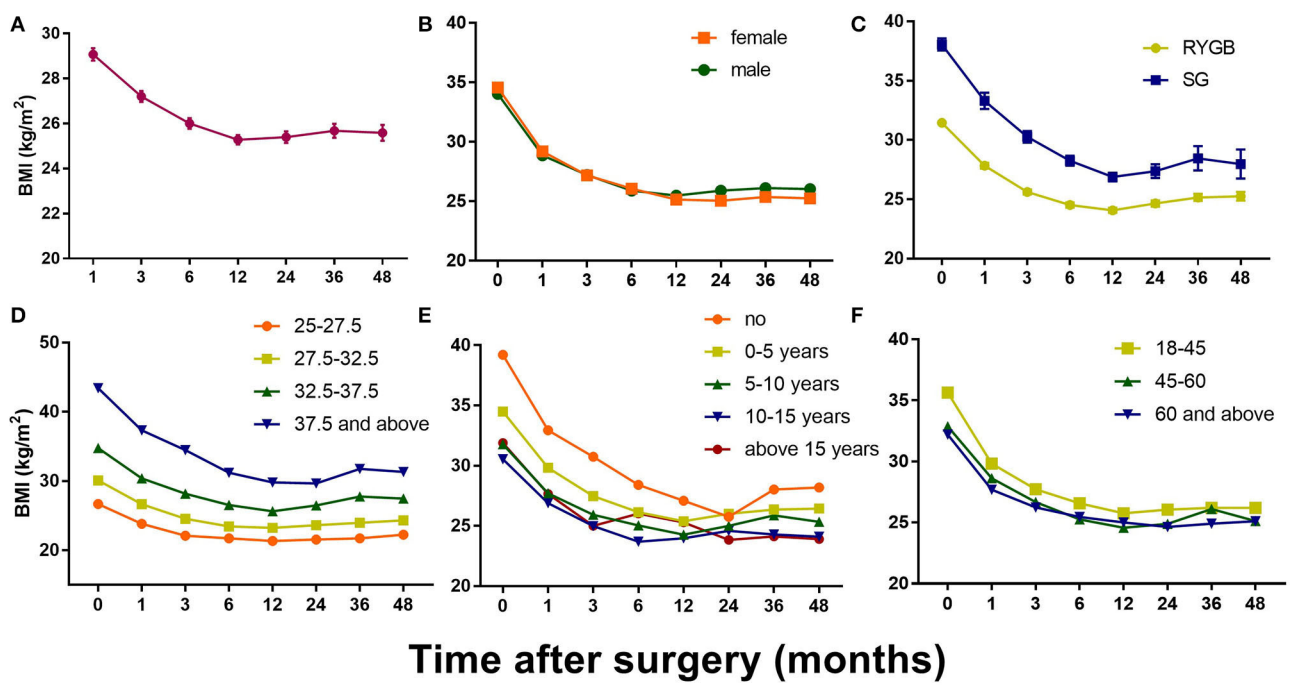

FIGURE 2 | Postoperative changes of BMI in all patients and in different groups. **(A)** Mean BMI change after bariatric surgery; **(B)** Mean BMI change in different gender groups; **(C)** Mean BMI change in different surgery type groups; **(D)** Mean BMI change in different initial BMI groups; **(E)** Mean BMI change in different T2DM duration groups; **(F)** Mean BMI change in different age groups. Results are shown in mean ± SEM.

indicates a mesomeric state suitable for pregnancy. Previous studies have shown that, in obese women with polycystic ovary syndrome, hormones such as estrogen and progestogen achieve a normalized balance as early as 3 months after bariatric surgery, which has led to unplanned pregnancies (17). Therefore, guidelines for bariatric surgery often suggest that pregnancy be avoided during the first 18–24 months postoperatively—which is generally based on consensus instead of evidence (18). However, some studies have shown that there were almost no maternal or neonatal complications in women who became pregnant even within 6 months from the surgery (18, 19). In Asian settings, some research should be dedicated to determining whether the period between surgery and pregnancy can be shortened, and by how much. With our current cohort, the number of pregnancy cases was too small, thus more ought to be monitored in extended studies, in order to draw a rigorous conclusion over suggestions for conception.

The findings of this study have the potential to improve the perioperative care of patients undergoing bariatric surgery, highlighting areas for improvement in the follow-up protocol. As can be seen, although international standards (for RYGB) were followed regarding the follow-up routine, we could not identify a more precise "lowest body-weight period," which is vital to prevent regaining the lost weight. Therefore, more frequent monthly measurements between the 6 and 12 month follow-up evaluations can be scheduled and carried further beyond the first year. With this protocol, we hope to be able to identify patients who are at high risk for treatment failure at an early stage, so help can be provided promptly. Studies have reported the importance of developing good exercise and eating habits during the early postoperative period (20). Appropriate support can also be given to patients suffering from mental health issues such as depression or anxiety (21, 22).

There are some limitations of our study. First, as a retrospective trial, there is a risk of reporting and selection bias. To lessen the impact, data were collected prospectively, and all patients who met the inclusion criteria were enrolled consecutively in the study. Second, there was a large dropout rate, especially after 2 years postoperatively. More attention should be given to long-term follow-up, including the use of specialized personnel, standardized questionnaires, and professional databases. Third, the criteria we used to divide patients into groups were not sufficiently rigid (e.g., the surgery type may be dependent on diabetes duration and the BMI). The most essential criteria will be established in future studies.

CONCLUSIONS

Patients in a Chinese population achieved maximal weight loss and stabilization most probably between 12 and 24 months after surgery, instead of at >2 years. Further patient follow-up should include the time point of 18 month after surgery to establish more precise monthly time frames. The finding of the shorter stabilization interval is meaningful to earlier intervention of weight loss related conditions and women's conception planning.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shanghai Jiao Tong University affiliated Sixth People's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JD and PZ conceived the presented idea. TX developed the theory and performed the computations. CW and HZ verified the analytical methods. XH and WL performed the surgery. JH and HY helped with follow-ups. JC encouraged TX to investigate the impact of pregnancy on bariatric patients. JD supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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frontiers in Medicine

BRIEF RESEARCH REPORT
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Gout Is Prevalent but Under-Registered Among Patients With Cardiovascular Events: A Field Study

Irene Calabuig[1*†], *Miguel Gómez-Garberí*[2] *and Mariano Andrés*[1,2†]

[1] Sección de Reumatología, Hospital General Universitario de Alicante, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, Spain, [2] Departamento de Medicina Clínica, Universidad Miguel Hernández, Alicante, Spain

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***Correspondence:**
Irene Calabuig
irenecalabuig.md@gmail.com

†ORCID:
Irene Calabuig
orcid.org/0000-0002-8755-0149
Mariano Andrès
orcid.org/0000-0002-0219-9055

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Objectives: Gout is an independent cardiovascular (CV) risk factor with significant morbidity and mortality. We aimed to estimate the prevalence of gout, characteristics and management in a hospitalized population for CV disease, a topic that remains to be defined.

Methods: An observational, descriptive, cross-sectional study was carried out in patients admitted for CV events in the Cardiology, Neurology, and Vascular Surgery units of a tertiary center. Patients were selected following a non-consecutive, systematic sampling. Data about CV disease and gout were obtained from face-to-face interviews and patients' records. Gout diagnosis was established using the 2015 ACR/EULAR clinical classification criteria. The registration rate of gout was assessed by auditing patients' records and hospital discharge reports of CV events from the units of interest in the previous 2 years. To predict the presence of gout, multivariate logistic regression models were built to study the possible explanatory variables.

Results: Two hundred and sixty six participants were recruited, predominantly males (69.9%) and Caucasians (96.6%) with a mean age of 68 years. Gout was identified in 40 individuals; thus, the prevalence was 15.0% (95% CI 10.9–19.2%). In 35% of cases, the diagnosis was absent from patients' records. Gout was found in 1.4–2.6% of hospital discharge reports of CV events, also indicating under-registration. The disease was long-standing, but with low reported rates of flares, involved joints, and tophi. At admission, only half of the gout patients were on urate-lowering therapy, being 38.5% of them on serum urate <6 mg/dl. The only independent predictor of gout was the existence of previous hyperuricemia (median serum urate in previous 5 years ≥ 7 mg/dl), with an odds ratio of 2.9 (95% CI 1.2–7.1); if hyperuricemia is not included in the model, the only independent predictor was chronic kidney disease (odds ratio 3.0; 95% CI 1.4–6.6).

Conclusion: Gout is highly prevalent among patients admitted for CV events, with significant lack of awareness and suboptimal management, despite being a well-established independent CV risk factor.

Keywords: gout, prevalence, cardiovascular event, cardiovascular disease, urate lowering therapy

INTRODUCTION

Gout is a disease of monosodium urate (MSU) crystal deposition. It is the most frequent type of inflammatory arthritis in Western countries, with prevalence rates of up to 1% of the adult population and up to 5% of males aged 65 and over. Concerning Spain, a prevalence rate of 2.4% has been recently announced (1).

Many causes contribute to hyperuricemia, the key element in the pathophysiology of gout. One factor is urate overproduction, including inherited enzymatic defects and conditions with high cell turnover (psoriasis, hematological disorders) or dietary factors, such as elevated intake of fructose, proteins, purines, and alcohol. However, urate underexcretion, especially in the kidneys but also in the gut, constitutes the principal determinant of hyperuricemia (2). Also included are drug-induced hyperuricemia (especially by loop diuretics and thiazides), chronic kidney disease, hypertension, lead poisoning, and genetic disorders. In most cases, different genetic and environmental factors coexist and interact. Over recent years, urate transporter defects are gaining importance in the pathogenesis of hyperuricemia. Several genome-wide association studies have disclosed significant single-nucleotide polymorphisms (SNPs) in genes encoding these transporters, which result in a higher risk of hyperuricemia and gout (3). Interestingly, these studies often provide clues to uncover new urate transporters (4). Transporters URAT1 and NPT1 (present at proximal renal tubule) and also GLUT9 and ABCG2 (present at both proximal renal tubule and enterocytes) are the key regulators of serum urate (SU) levels in patients with hyperuricemia and gout (5). The rs2231142 variant of the ABCG2 gene and multiple variants of SLC2A9 (gene encoding GLUT9) are likely the most influent SNPs in SU concentrations (6). Besides, the ABCG2 rs2231142 allele is strongly associated with early-onset gout (7), with a sex-specific effect (as men present higher SU levels compared to women) (6, 8), and with a poor response to allopurinol (9).

MSU crystals form and deposit in tissues when SU levels remain above its saturation point (7 mg/dl)—the threshold for hyperuricemia (10). MSU crystal deposition depends on the level and duration of hyperuricemia, among other factors, such as advanced age, elevated body mass index, and male sex (11). When formed, MSU crystals are recognized by the innate immune system as danger signals, leading to recurrent episodes of acute arthritis. Between gout flares, a sustained low-grade inflammation persists (12). If not properly treated, clinically evident chronic inflammation with massive crystal deposits may take place. Therefore, gout is a systemic inflammatory disease beyond its well-known flares and is curable by proper treatment (13).

There is a firm association between gout and cardiovascular (CV) disease. Gouty patients develop atherosclerotic complications more frequently than the general population. This association was considered related to the traditional CV risk factors that are common in gout (14, 15). However, several recent studies have revealed that this association persists after adjusting for these factors, thus indicating that gout is an independent CV risk factor (16, 17). A 29% increase in mortality from any CV disease and a 42% increase in mortality from coronary heart disease is directly attributed to gout (18). Such increased CV risk is linked to systemic inflammation associated with MSU crystals, and to endothelial dysfunction and oxidative stress that occurs in hyperuricemia (19). Recently, potential deposition of MSU crystals at artery walls has been proposed (20, 21) but remains to be firmly established and deserves further research (22).

There is cumulative evidence that hyperuricemia has a pathogenic role in CV disease (23). Besides MSU crystals, soluble urate is also capable of unleashing an inflammatory response through innate immunity, which may intervene in the development of these diseases (24). Studies with animal models have described how hyperuricemia precedes and favors CV and renal diseases. Uric acid is able to stimulate NADPH oxidase, activate the renin-angiotensin system and impair nitric oxide release, which induces oxidative stress, endothelial dysfunction, renal vasoconstriction, and ischemia (25–27). A large amount of high quality evidence from epidemiological studies supports this hypothesis (28–31). Furthermore, urate-lowering agents have demonstrated CV and renal benefits (32, 33), especially in early-onset hypertension. Conversely, there are still Mendelian randomization, experimental, and epidemiological studies supporting the notion that hyperuricemia is not an independent CV risk factor (34, 35). At this point, we might recall the importance of the intracellular-extracellular uric acid dissociation. While extracellular uric acid takes part in the development of MSU crystal deposits (gout, kidney stones, and perhaps vascular calcification), intracellular uric acid is involved in the biological effects (hypertension and metabolic disease) (36). Also, no prospective study has assessed the occurrence of CV events on asymptomatic hyperuricemia according to the presence of subclinical MSU crystal deposits. These are estimated to be present in around 20% of subjects (37) and preliminarily linked to severe coronary atherosclerosis (38).

As with other chronic inflammatory diseases (39), early and proper management of gout likely allows control of the CV risk. When SU levels fall below the saturation point, MSU crystals dissolve, and gout manifestations subside. Consequently, the use of urate-lowering therapy (ULT) should also reduce the proatherogenic state in these patients. Available data are so far contradictory and derive from population-based studies. While some studies failed to demonstrate a CV benefit of ULT in gout (40, 41), Chen et al. (42) from Taiwan noted that the use of ULT significantly improved both CV and global survival rates. Besides, the failure to reach the SU level target (<6 mg/dl) (13) has been associated with poorer survival rates, mainly due to CV diseases (43). In addition to ULT, colchicine may also have a CV benefit. This agent, used in gout to prevent and treat flares, has been recently linked to lower rates of CV disease and mortality (44, 45). Also, there are positive data for secondary prevention of CV disease in the non-gouty population (46).

As the evidence supports the hypothesis that gout is an independent CV risk factor, with derived morbidity and mortality potentially avoidable, we consider it necessary and relevant to gauge the frequency and characterization of gout in patients admitted for CV events. The confirmation of high prevalence with suboptimal management would impact the interpretation

and approach to both hyperuricemia-gout and CV disease. The CV risk control strategies initiated at the time of the event (secondary prevention)—usually focused on blood pressure, plasma lipids, and platelet activity—might benefit from proper gout management, based on the available evidence (42, 44).

The primary objective of this study was to estimate the prevalence of gout in patients admitted for CV events. The secondary objectives were (i) to assess the registration rate of gout in patients' records and discharge reports of CV events, (ii) to describe the characteristics and management of gout in this population, and (iii) to identify predictors for the presence of gout in patients with established CV disease. Our hypotheses were (a) that prevalence of gout in patients admitted for CV events would be markedly higher than in the general population, (b) that gout would be under-registered both in patients' records and discharge reports, and (c) that gout management in CV patients would remain suboptimal, with SU levels above the therapeutic target and insufficient use of ULT.

MATERIALS AND METHODS

We conducted an observational, descriptive, cross-sectional study in patients admitted for CV events in the Cardiology, Neurology, and Vascular Surgery units of our hospital, over 10 months (January to October 2018). It is an academic tertiary public hospital with a population coverage of 267,960 inhabitants (2017), eminently urban and with a slight female predominance (+4.4%). The coverage is broader, as Vascular Surgery and Neurology are reference units for other health departments, precisely in the care of atherosclerotic disease.

Adult patients hospitalized for a CV event were eligible. Events were defined by the clinical diagnosis registered in patients' records, as (i) acute coronary syndrome or coronary artery disease that requires revascularization, (ii) new or congestive heart failure, (iii) stroke or transient ischemic attack, or (iv) acute or chronic peripheral artery disease that requires revascularization. Patients were excluded if they presented a background of other inflammatory arthritis to avoid misclassification. Other exclusion criteria were declination or inability to sign the informed consent form. If excluded, minimal data (age, gender, CV event) were collected to ensure representativeness.

We followed a non-consecutive, systematic sampling. Screening for enrollment was applied to all patients admitted to the Cardiology unit the second week of odd months, the Neurology unit the fourth week of odd months, and to the Vascular Surgery unit the third week of even months.

Study variables were prospectively collected using a pre-established data collection form. Sources were primary care (SIA®) and specialized care (Orion Clinic®) electronic records and face-to-face interviews.

The primary outcome variable was the presence of gout, which was either previously registered (clinical or crystal-proven) at patients' records or defined by a face-to-face interview. For the interview, 2015 ACR/EULAR clinical classification criteria were used (47). Sensitivity of 85%, specificity of 78%, and area under the curve of 89% were published for the set clinical criteria (48).

To assess the registration rate of gout, we compared the face-to-face interviews with the patients' records. Likewise, we examined discharge reports by CV events from the units of interest in 2016 and 2017 with respect to the inclusion of gout as a secondary diagnosis (M10.X codes according to International Classification of Diseases, 10th edition); these data were provided by the Admissions and Clinical Documentation unit of our hospital.

Secondary outcomes and additional explanatory variables included SU levels at the time of the CV event (mg/dl); median SU levels in the previous 5 years (mg/dl); hyperuricemia (defined as a median SU level of $\geq$7 mg/dl in the previous 5 years or the level at admission if previous data were missing); being at the SU target (<6 mg/dl) (13); and others regarding the characteristics and treatments of gout. Other variables were demographic, clinical, and therapeutic related to CV disease (full list of variables available in the **Supplementary Appendix**). Chronic kidney disease (CKD) was defined as a median eGFR <60 ml/min/1.73 m^2 in the previous 2 years or at admission if previous data were missing, estimated according to CKD-EPI formula (49).

The study complies with the Declaration of Helsinki and was evaluated and approved by the HGUA-ISABIAL Clinical Research and Ethics Committee [2018/07 act]. Informed consent was obtained from all subjects (or their legally authorized representative). This investigation is reported according to the criteria of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (50) and gout-related terms followed G-CAN nomenclature (51).

Plan of Analysis

Sample Size

At the time of the study design, the prevalence of gout in Spain was undetermined. Later, a general population-based study communicated a prevalence of 2.4% (1). A former regional study carried out in Catalonia at the primary-care level estimated a gout prevalence of 3.3% (52), and this data point was used for sample size estimation. The present research focuses on population with CV disease, with a known increased risk of developing gout, so the prevalence was deemed to be close to 6%. On the basis of this estimation, a power of 80%, a statistical significance of 95%, and the assumption that 10% of patients would decline to participate in the study, a minimum sample size of 262 patients was calculated.

Statistical Analysis

Quantitative variables are shown as measures of central tendency (mean and median) with dispersion (standard deviations, interquartile ranges), and qualitative variables such as frequencies and percentages. Age was taken as continuous and categorized by tertiles.

For the primary outcome variable (prevalence of gout), 95% confidence intervals (95% CI) were calculated. For subgroup comparisons (clinical features according to gout diagnosis), Student's *t*-test, chi-square, and Fisher's exact test were used.

TABLE 1 | Characteristics of the study sample and comparison of clinical features regarding the diagnosis of gout.

	Total ($n = 266$)	Gout diagnosis		*p*-value
		No ($n = 226$)	Yes ($n = 40$)	
Mean age (SD)	68.0 (12.0)	68.0 (13.0)	72.0 (9.0)	0.026
Mean BMI; (SD)	27.8 (5.1)	27.7 (5.1)	28.9 (5.1)	0.152
Males	186 (69.9)	154 (68.1)	32 (80.0)	0.132
Caucasians	257 (96.6)	217 (96.0)	40 (100)	0.363
Hyperuricemia ($n = 252$)	49 (19.4)	34 (15.8)	15 (40.5)	<0.001
Hypertension	201 (75.6)	168 (74.3)	33 (82.5)	0.268
Diabetes mellitus	143 (53.8)	122 (54.0)	21 (52.5)	0.862
Dyslipidemia	176 (66.2)	150 (66.4)	26 (65.0)	0.866
Tobacco consumption	70 (26.3)	61 (27.0)	9 (22.5)	0.552
Alcohol consumption	50 (18.8)	42 (18.6)	8 (20.0)	0.833
CV events leading to admission				
– Acute coronary syndrome	50 (18.8)	42 (18.6)	8 (20.0)	0.811
– Heart failure	35 (13.2)	29 (12.8)	6 (15.0)	
– Stroke	55 (20.7)	49 (21.7)	6 (16.0)	
– Peripheral artery disease	126 (47.4)	106 (46.9)	20 (50.0)	
Background of CV disease	147 (55.3)	122 (54.0)	25 (62.5)	0.318
Chronic kidney disease	74 (27.8)	52 (23.0)	22 (55.0)	<0.001
Use of diuretics	109 (41.0)	87 (38.5)	22 (55.0)	0.050

Data shown as n (%), unless otherwise specified. BMI: body mass index; CV: cardiovascular; SD: standard deviation.

To assess which CV variables predict the presence of gout, a univariate analysis was initially performed for each explanatory variable with a chi-square test, and odds ratios were estimated by simple logistic regression. Those explanatory variables that were statistically significant for gout were included in a multivariate logistic regression model.

Statistical analyses were performed using IBM SPSS Statistics® Version 25 (Armonk, NY). For the significance level, a p-value <0.05 was established.

RESULTS

Two hundred and ninety nine patients were screened for enrollment. After we excluded 33, the final study sample was 266 participants. The reasons for exclusion were a background of other types of arthritis ($n = 16$), inability to sign the consent ($n = 9$), and declination of participation ($n = 8$).

The general characteristics of the study participants are shown in **Table 1**. They were predominantly males, Caucasians, and elderly people, of advanced age. The CV events leading to admission were peripheral artery disease (47.4%; $n = 126$), stroke or transient ischemic attack (20.7%; $n = 55$), acute coronary syndrome (18.8%; $n = 50$), and heart failure (13.1%; $n = 35$).

Gout was identified in 40 individuals (prevalence: 15.0%; 95% CI 10.9–19.2%). Prior gout diagnosis in records was found in 26 patients (65.0%), either clinical-based (50.0%; $n = 20$) or crystal-proven (15.0%; $n = 6$). Thus, in the remaining 14 patients (35.0%), the diagnosis was unregistered but confirmed by interview. Rheumatology had previously seen all crystal-proven gout patients. Regarding discharge reports from the units of interest in 2016 and 2017 ($n = 1{,}322$ and 1,263, respectively), gout was coded as a secondary diagnosis in 19 (1.4%) and 33 (2.6%), respectively, also indicating under-registration.

The distribution of gout across age groups, gender, and type of CV event is shown in **Figure 1**. The prevalence of gout in those patients with established CV disease was 17.0%, while in those with a first event it was 12.6% ($p = 0.318$).

Table 1 shows the comparisons regarding the diagnosis of gout. Patients with gout were significantly older and showed higher rates of CKD and use of diuretics. No differences in other variables were observed.

The disease was long-standing though low numbers of flares and involved joints were referred (**Table 2**). Tophi were not seen in many patients. SU levels were not adequately controlled, either at the time of the CV event or after analyzing the previous 5 years. Despite 70% of patients' having received ULT at some point, only half remained treated at admission. Only one-third of the patients were at the SU target at admission (under ULT, 38.5%). Use of prophylactic colchicine was scarce, in contrast to the ample use of NSAIDs despite the CV context.

Simple and multivariate logistic regression models are shown in **Table 3**. Hyperuricemia was the only independent predictor of gout (OR 2.9; 95%CI 1.2–7.1). After having excluded hyperuricemia from the model, the only variable significantly associated with the presence of gout was CKD (OR 3.0; 95% CI 1.4–6.6).

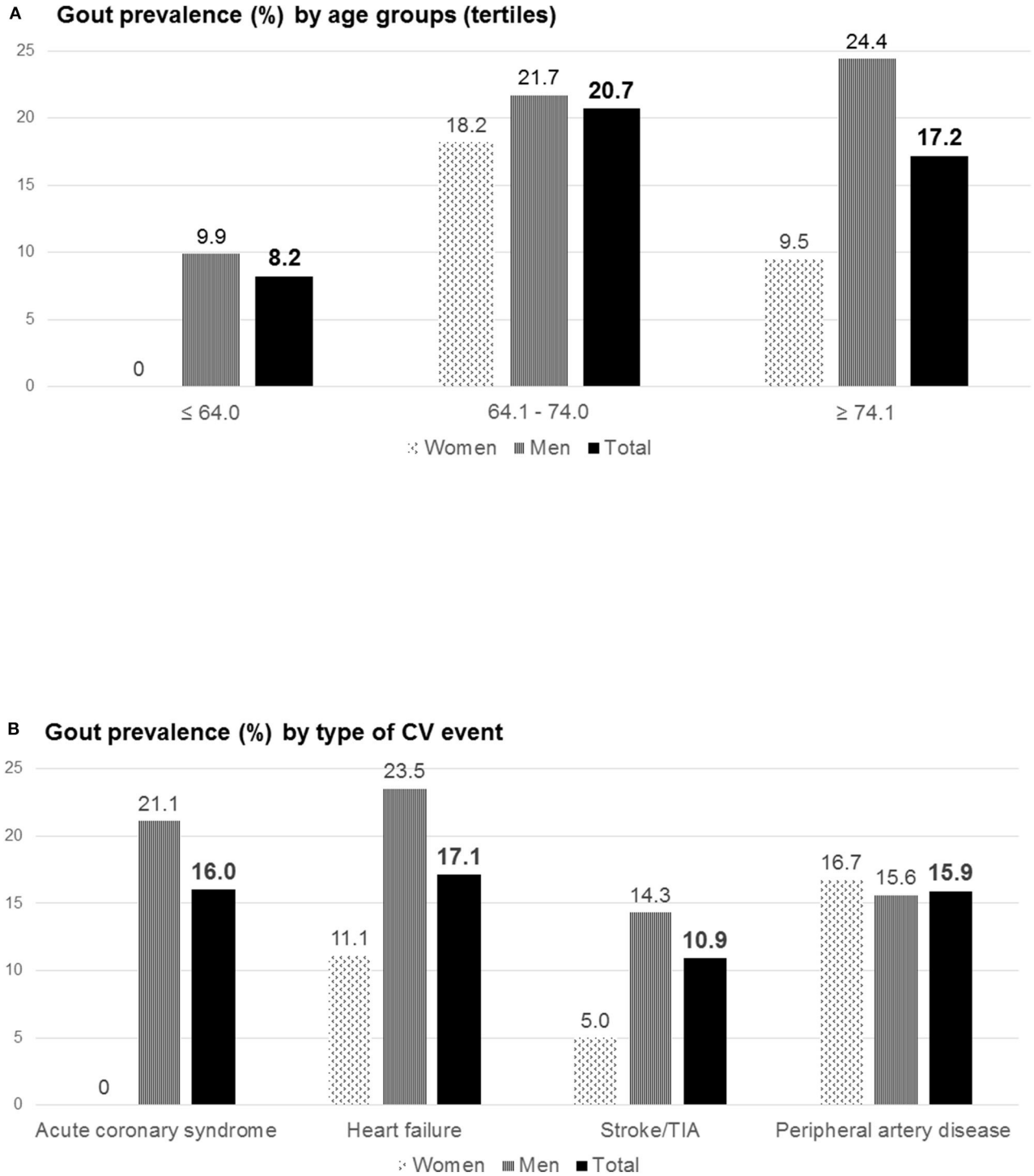

FIGURE 1 | Gout prevalence (%) by age groups (tertiles) **(A)** and by type of CV event **(B)**. This figure shows gout prevalence in the whole population, separated by gender. The age groups were constituted according to the tertiles of the range of values in the distribution of age. The first group corresponds to patients aged 64 years or less, the second group ranges from 64.1 to 74 years, and the third group includes patients aged more than 74 years. CV, cardiovascular; TIA, transient ischemic attack.

TABLE 2 | Clinical features and management of the 40 identified patients with gout.

	Gout (*n* = 40)
Years since the first flare, median (IQR)	15 (10–30) (*n* = 39)
Number of flares, median (IQR)	3 (1–9) (*n* = 39)
Number of involved joints, median (IQR)	2 (1–3) (*n* = 39)
Presence of tophi	3/39 (7.7)
Serum urate levels (mg/dl), mean (SD)	
- at admission	7.1 (2.6) (*n* = 24)
- median levels of the previous 5 years	6.8 (1.6) (*n* = 33)
Serum urate <6 mg/dl	
- at admission	8/24 (33.3)
- median levels of the previous 5 years	7/33 (21.2)
Urate-lowering agents (ever)	28/40 (70.0)
Urate-lowering agents (current)	20/40 (50.0)
Allopurinol	
- current use	16/20 (80.0)
- dose (mg/day), mean (SD)	200 (102)
Febuxostat	
- current use	4/20 (20.0)
- Febuxostat dose (mg/day), mean (SD)	80 (0)
Prophylactic colchicine (ever)	11/40 (27.5)
Prophylactic colchicine (current)	5/40 (12.5)
Use of NSAIDs for gout flares	25/40 (62.5)

Data shown as n (%) unless otherwise specified. Number in brackets accounts for complete data. IQR, interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs; SD, standard deviation.

DISCUSSION

This is the first field study attempting to estimate the prevalence of gout in patients admitted for CV events (thus, at very high CV risk). Following chart reviews and face-to-face interviews using the ACR/EULAR clinical classification criteria (47), the disease rate in this population was 15%, present in one out seven of these patients. This data point is in contrast to the low rate of disease registration in patients' records and discharge reports. Hyperuricemia was the only predictor of gout in the admitted CV population; if the SU level was not available, then CKD would predict it. Gout was associated with older age, CKD, and use of diuretics. As for its characteristics, it was a long-standing disease but with few reported accumulative flares and low rates of tophi. Despite a CV background, the use of ULT and SU therapeutic levels was inadequate. In summary, the present study has unveiled a large population with gout and high CV risk but low standards—people who are candidates for dedicated education and management strategies.

In Western Europe and North America, the prevalence of gout in adults ranges between 0.3 and 4.8% (53). In Spain, a prevalence rate of 2.4% has been recently communicated (1). In patients with CKD, numbers are larger: 16.6% in an Irish cross-sectional study and 24.3% in a German cohort (54, 55). To date, there are scarce data on the prevalence of gout in a CV setting—a recent analysis of the Swedish heart failure registry reported 4.2% (56)—and no study had focused on patients admitted for CV events. In the present study, a prevalence rate of 15.0% has been obtained, six-fold the incidence in the Spanish adult population. Moreover, in the population hospitalized for CV events, gout is present in one out of seven patients, demonstrating the close relationship between CV and inflammatory diseases.

Compared to population-based or claims databases, a field study ensures high accuracy, here given the good performance that 2015 ACR/EULAR clinical criteria have shown for epidemiological studies in the absence of crystal-proven diagnosis (48). To reinforce this assertion, we audited discharge reports of CV events admissions for the inclusion of gout as a secondary diagnosis. The records at our hospital in the previous 2 years showed a limited number of cases registered, compared to the results obtained by interview in this field study. Numbers in discharge reports were similar to the prevalence of gout in general population. It is possible that the occurrence of a gouty flare during hospitalization influenced the inclusion. Other option would be the unawareness of gout as an independent CV risk factor.

Secondary CV prevention strategies are initiated when the first clinical event occurs. They aim to make a very high CV risk subside by reaching stricter lipid levels (residual lipid risk) and greater control of the atherothrombotic process (residual thrombotic risk). However, many patients still develop new events. The role of inflammation—measured by high-sensitivity C-reactive protein—as an independent risk factor (57) and the recently proven CV benefit of anti-inflammatory therapies such as blocking interleukin-1beta (58) have confirmed the existence of residual inflammatory risk (59). Gout is a well-established independent CV risk factor, but in one-third of the identified patients with gout and CV events, the diagnosis was not recorded (either in admission or in primary and specialized care records).

Gout is not included as a variable of interest in the different guidelines for CV management, which are firmly focused on traditional risk factors. Some of them include recommendations only to check SU levels, and in the case of heart failure following use of diuretics, the management of SU is guided (60, 61). Therefore, in line with the present results, identifying gout at the time of hospitalization is undoubtedly an excellent opportunity to start ULT and accordingly improve both secondary CV prevention and gout management itself. In this sense, our study shows hyperuricemia as the only independent predictor for the presence of gout in this population. Despite recommendations, which are relatively recent, 89 out of 266 patients (33.5%) did not have their blood tested for SU during admission, and 14 (5.3%) had not had it done in the previous 5 years either. If SU levels are not available, gout should be suspected if CKD is present, according to our results.

Gout was noted as long-standing but with a low number of flares and joints involved. In gout, flares are usually spaced at early stages, and as the crystal deposit grows, they become more frequent. Although a flare is usually intense enough to remember it, the number of subsequent episodes might not be retained in the same way, entailing a potential recall bias. Tophi were detected in 7.7% of patients, a rate considered low for a population with long-standing gout. This is probably an observer

TABLE 3 | Clinical predictors for the presence of gout in the study sample.

	Univariate		Multivariate (with hyperuricemia)		Multivariate (without hyperuricemia)	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**	**OR (95%CI)**	**p-value**
Sex (male)	1.87 (0.82–4.26)	0.136				
Age*	1.03 (1.00–1.06)	0.065				
Age >65 years	2.60 (1.15–5.90)	0.022	2.04 (0.78–5.34)	0.147	1.59 (0.66–3.87)	0.305
BMI*	1.05 (0.98–1.11)	0.153				
Obesity	1.93 (0.96–3.89)	0.065	1.56 (0.70–3.47)	0.275	1.66 (0.78–3.54)	0.190
Hypertension	1.63 (0.68–3.88)	0.272				
Diabetes	0.94 (0.48–1.85)	0.862				
Dyslipidemia	0.94 (0.47–1.91)	0.866				
Median uricemia 5 PY* ($n = 219$)	1.94 (1.45–2.60)	<0.001				
Hyperuricemia ($n = 252$)	3.63 (1.71–7.70)	<0.001	2.64 (1.12–6.19)	0.026		
Tobacco	0.79 (0.35–1.74)	0.553				
Alcohol	1.10 (0.47–2.55)	0.833				
Current CV event						
- ACS	1.00 (ref)	0.814				
- CHF	1.09 (0.34–3.46)	0.889				
- Stroke or TIA	0.64 (0.21–2.00)	0.446				
- PAD	0.99 (0.41–2.42)	0.983				
Previous CVD	1.42 (0.71–2.84)	0.320				
Median GFR 2 PY* ($n = 231$)	0.97 (0.96–0.99)	<0.001				
CKD	4.09 (2.04–8.20)	<0.001	2.13 (0.92–4.95)	0.078	3.05 (1.40–6.64)	0.005
Beta-blockers	1.98 (1.00–3.94)	0.050	1.52 (0.66–3.50)	0.329	1.48 (0.67–3.30)	0.336
ACE inhibitors	0.85 (0.35–2.05)	0.723				
ARB	1.53 (0.78–3.01)	0.218				
CCB	2.50 (1.20–5.22)	0.014	1.74 (0.76–3.98)	0.190	1.68 (0.76–3.70)	0.200
Thiazide diuretics	1.10 (0.47–2.55)	0.833				
Loop diuretics	2.54 (1.26–5.11)	0.009	1.17 (0.47–2.90)	0.740	1.22 (0.52–2.88)	0.648
MRA	1.68 (0.52–5.40)	0.382				
Antiplatelets	1.16 (0.59–2.28)	0.665				
Anticoagulants	1.57 (0.76–3.26)	0.225				
Lipid-lowering drugs	1.12 (0.57–2.20)	0.745				
Antidiabetics	0.82 (0.42–1.62)	0.572				

Serum urate level at admission was not included due to the high rate of missing values (89/266, 33.5%). Ancestry was not included as no cases with gout were found in non-Caucasians. Hyperuricemia was defined as median serum urate level ≥7 mg/dl in the previous 5 years. Chronic kidney disease was defined as median GFR <60 ml/min/1.73 m^2 in the previous 2 years, estimated according to CKD-EPI formula.

**Quantitative variables.*

OR, odds-ratio; 95% CI, 95% confidence interval; BMI, body mass index; CV, cardiovascular; PY, previous years; ACS, acute coronary syndrome; CHF, congestive heart failure; TIA, transient ischemic attack; PAD, peripheral artery disease; CVD, cardiovascular disease; GFR, glomerular filtration rate; CKD, chronic kidney disease; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; CCB, calcium channel blockers; MRA, mineralocorticoid-receptor antagonists.

bias, as they were assessed by physical exam but not confirmed by imaging or sampling (62). Dedicated research to establish the prevalence of tophaceous gout in this population is needed, since tophi are a strong prognostic factor for mortality, mainly from CV origin (63).

Use of ULT and SU levels were inadequate in the sample. When admitted for CV events, patients initiate an intensive control of traditional CV risk factors. However, the poor management of gout observed is an added risk. Pagidipati et al. (64) found that after coronary revascularization, suffering from gout increased the risk of CV death by 19% and of all-cause death by 21%, despite adequate control of other risk factors. Appropriate gout management may likely help to reduce this risk (13, 15). Low-dose colchicine was also underused in the sample, despite its demonstrated anti-inflammatory properties (65) and potential clinical benefit as suggested by observational studies (44, 45).

Some strengths and limitations must be addressed. The sample size of this field study may be considered small. However, the minimal size was pre-calculated with respect to available prevalence estimates. Increasing numbers would add precision to the 95% CI estimation, though the current prediction of one gout patient in every five to ten CV inpatients is relevant and quite above the numbers in the general population (Spanish data,

2.4%). The value of a field study is to make disease diagnoses more correct, in contrast to medical records-based studies. Gout diagnosis by crystals was not feasible due to the epidemiological nature of the study, but the 2015 ACR/EULAR clinical criteria (47) have shown the best performance for epidemiological studies in the absence of crystal-proven diagnosis (48). Moreover, patients with known inflammatory arthritis were excluded to prevent misclassifications. Some gout cases could have been lost in cases of coexisting diseases (66); again, to differentiate them accurately, synovial fluid analysis would have been necessary. Determining the achievement of an SU target based on the time of admission may be problematic; in situations of acute inflammation, SU levels tend to decrease (67), whereas deterioration in renal function—occasionally occurring during a CV event—would cause an increase in SU levels. Also, study participants were not tested per protocol for SU levels, which were obtained from routine blood tests performed during hospitalization. In order to control this issue, the average of SU testing from the previous 5 years was obtained. The study centered on patients admitted for CV events, so the findings should only be generalized to the hospital setting. Some patients with established disease (TIA, stable angina, heart failure) may not require admission; as lower severity of atherosclerosis can be presumed, the prevalence of gout would then be lower. However, it is convenient to replicate the results in the outpatient setting.

CONCLUSIONS

Gout was present in 15% of hospitalized patients for CV events, a prevalence six times higher than in the Spanish adult population and hospital records. A significant rate of under-registration was detected, both in patients' records and discharge reports. Hyperuricemia was the only predictor of gout in admitted CV population; if the SU level is not available, CKD would be taken as a predictor. Gout was long-standing but with a low number of flares and involved joints, and suboptimal management was identified. Therefore, this field study was able to uncover a subpopulation with high CV risk, candidates for both secondary CV prevention strategies and dedicated gout management.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Alicante Institute of Sanitary and Biomedical Research (ISABIAL) (2018/07 act). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IC, MG-G, and MA designed the study project and analyzed and interpreted the results. IC and MG-G acquired the data. MG-G and MA wrote the first draft of the manuscript. All authors contributed, revised, and approved the final version. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00560/full#supplementary-material

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Sex-Specific Associations of Risks and Cardiac Structure and Function With Microalbumin/Creatinine Ratio in Diastolic Heart Failure

Fang-Fei Wei[1,2], *Ruicong Xue*[1,2], *Yuzhong Wu*[1,2], *Weihao Liang*[1,2], *Xin He*[1,2], *Yuanyuan Zhou*[1,2], *Marvin Owusu-Agyeman*[1,2], *Zexuan Wu*[1,2], *Wengen Zhu*[1,2], *Jiangui He*[1,2], *Jan A. Staessen*[3,4], *Yugang Dong*[1,2,5]* and *Chen Liu*[1,2,5]*

[1] Department of Cardiology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [2] NHC Key Laboratory of Assisted Circulation, Sun Yat-sen University, Guangzhou, China, [3] Studies Coordinating Centre, Research Unit Hypertension and Cardiovascular Epidemiology, Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium, [4] Non-profit Research Institute (NPA) Alliance for the Promotion of Preventive Medicine, Mechelen, Belgium, [5] National Guangdong Joint Engineering Laboratory for Diagnosis and Treatment of Vascular Disease, Guangzhou, China

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Junjie Xiao,
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Reviewed by:
Dachun Xu,
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Yi-Fei Dong,
The Second Affiliated Hospital of Nanchang University, China

***Correspondence:**
Chen Liu
liuch75@mail.sysu.edu.cn
Yugang Dong
dongxg@mail.sysu.edu.cn

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Background: Heart failure with preserved ejection fraction (HFpEF) affects women more frequently than men. However, data on sex-specific associations of adverse health outcomes and left ventricular structure and function and with microalbuminuria in patients with HFpEF are scarce.

Methods: In 1,334 participants enrolled in the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) Trial, we estimated the sex-specific multivariable-adjusted risk and LV traits with urine microalbumin/creatine ratio (ACR), using Cox or linear regression.

Results: In total, 604 (45.3%) were women. In multivariable-adjusted analyses, a doubling of ACR in both men and women was associated with higher posterior (+0.014 cm, $p = 0.012$/+0.012 cm, $p = 0.033$) wall thickness and left ventricular mass index (+2.55 mg/m^2, $p = 0.004$/+2.45 mg/m^2, $p = 0.009$), whereas was also associated with higher septal (+0.018 cm, $p = 0.002$) and left atrial volume index (+1.44 mL/m^2, $p = 0.001$) in men. ACR was a key predictor of all-cause (HR, 1.11; $p = 0.006$) and cardiovascular (HR, 1.17; $p = 0.002$) death in women, whereas in men ACR was associated with HF hospitalization (HR, 1.23; $p < 0.001$), any hospitalization (HR, 1.06; $p = 0.006$), and myocardial infarction (HR, 1.19; $p = 0.017$). The interactions of sex with ACR were significant for hospitalization for heart failure and any hospitalization ($p \leq 0.034$).

Conclusions: Outcomes and cardiac structure and function in patients with HFpEF appear to be influenced by ACR that vary according to sex. In men, ACR was significant associated with LV diastolic function, hospitalization, and myocardial infarction, whereas in women was associated with mortality.

Keywords: heart failiure, chronic kindney disease, echocardiography, microalbuminuria, risk stratification, gender

INTRODUCTION

Heart failure (HF) is a major global health problem (1) and the leading cause of morbidity and mortality worldwide (2). HF with preserved ejection fraction (HFpEF) accounts for ≈50% of cases (3). HFpEF is characterized by multiple co-morbidities, including chronic kidney disease (CKD) (4). The co-existence of HF and CKD is associated with extremely poor prognosis (5–7). The urine albumin/creatine ratio (ACR) is commonly used to evaluate the severity of CKD (8). In the general population (9), or in the patients with hypertension (10), diabetes mellitus (11), and heart failure (5), albuminuria behaves as a well-established risk factor for mortality.

Sex is a critical determinant of cardiovascular structure and function and various adverse health outcomes. Women are more likely to develop HFpEF than men (12, 13). However, our literature review did not reveal any previous study that addressed the possible sex differences in the cardiac structure and function and adverse health outcomes in relation to urine microalbumin/creatinine ratio in the setting of HFpEF. Thus, we addressed this knowledge gap to explore the sex-specific associations of cardiac structure and function and adverse health outcomes with urine ACR using data of the TOPCAT (the Treatment Of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial; NCT00094302) (14, 15).

METHODS

Study Participants

The TOPCAT study was an international, multicenter, randomized, double-blind, placebo-controlled trial. The TOPCAT trial was designed to investigate whether spironolactone improved clinical outcomes in patients with HFpEF compared with placebo. The TOPCAT study complies with the Declaration of Helsinki and the Institutional Review Board has approved this research. There were 3,445 participants at 233 sites in six countries randomly assigned to spironolactone or placebo. Inclusion criteria were age ≥50 years, ≥1 sign and at least one symptom of HF, left ventricular (LV) ejection fraction ≥45%, controlled systolic blood pressure, and serum potassium <5 mmol/L. All patients signed informed consent prior to randomization. A detailed description of the TOPCAT design and protocol has previously been reported (14). To obtain access to the TOPCAT data, we first registered at the website of the Biologic Specimen and Data Repository Information Coordinating Center of National Heart, Lung, and Blood Institute (NHLBI; https://biolincc.nhlbi.nih.gov/). Next, we submitted a request for accessing the TOPCAT data along with a protocol for the intended *post-hoc* analysis and the approval by the ethics committee of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China. After we signed a Research Materials Distribution Agreement, NHLBI transferred anonymized data. Of 3,445 patients, 1,334 patients had qualified urine ACR measurement.

Abbreviations: ACR, urine albumin/creatine ratio; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HF, heart failure; HFpEF, heart failure with preserved ejection fraction.

Laboratory Measurement

The participants were asked to provide a spot urine specimen to measure urine ACR at baseline. Laboratory measurements for urine chemistries were performed locally at the enrolling site. Albuminuria was defined by urine ACR ≥30 mg/g (16).

Echocardiographic Measurement

Echocardiographic measurements and laboratory measurement for urine ACR were performed at the baseline visit. Of 3,445 randomized HFpEF patients, 935 (27.1%) underwent echocardiography prior to the initiation of randomized treatment (17). The echocardiographic examination was performed according to the recommendations of the American Society of Echocardiography, as preciously described (17). Quantitative measurements on all study echocardiograms were performed by dedicated analysts at the core laboratory blinded to clinical information. Previous publications describe the procedures applied for acquisition and the off-line analysis of the echocardiographic measurements in detail (14). In this study, we statistically analyzed LV structure including LV dimensions, wall thickness and mass index, diastolic function including left atrial volume index, transmitral blood flow, and mitral annular tissue velocities, and systolic function including ejection fraction and longitudinal strain. Intra-observer variability for key echocardiographic measures of cardiac structure and function have been previously reported (15, 18). Intra-observer variability performed in 60 studies, was as follows: wall thickness: coefficient of variation 12%, bias 0.02 ± 0.1 cm; LV end-diastolic volume: coefficient of variation 12%, bias 1.6 ± 10.5 mL; LV end-systolic volume: coefficient of variation 18%, bias 2.6 ± 5.9 mL; LV EF: coefficient of variation 6.6%, bias 2.0 ± 4.3%; tissue Doppler imaging e': coefficient of variation 7.0%, bias 0.1 ± 0.4 cm/s; E/e' ratio: coefficient of variation 11%, bias 0.2 ± 1.2 (15, 18).

Ascertainment of Endpoints

The primary outcome was a composite of all-cause death, non-fatal myocardial infarction, non-fatal stroke, or hospitalization for HF. In the present study, we also included all-cause death, cardiovascular death, HF hospitalization, any hospitalization, myocardial infarction, and stroke. All events were adjudicated by a clinical end-point committee at Brigham and Women's Hospital, Boston, USA, according to pre-specified criteria (14). More detailed information on the evaluation of outcomes has previously been reported (14, 15).

Statistical Analysis

For database management and statistical analysis, we used SAS software, version 9.4 (SAS Institute Inc., Cary, NC), maintenance level 5. We compared means and proportions by the large-sample *z*-test or ANOVA and Fisher exact test, respectively. We normalized the distributions of urine ACR by a logarithmic transformation. The central tendency (spread) was represented by the arithmetic mean (SD) for normally distributed variables and by the geometric mean (interquartile range) of

logarithmically transformed variables. A p-value of $\leq$0.05 was considered statistically significant.

In exploratory analyses, we determined differences in participants' baseline characteristics across thirds of the urine ACR. In unadjusted and multivariable-adjusted linear regression analyses, we expressed association sizes of the echocardiographic indexes for a doubling of a urine ACR. In multivariable-adjusted analyses, in line with previous publications (5), we accounted for randomly assigned treatment (spironolactone vs. placebo), sex, age, ethnicity, body mass index, systolic blood pressure, heart rate, current smoking, use of medications (diuretics, β blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium-channel blockers, lipid-lowering drugs, aspirin, other cardiovascular medications, and hypoglycemic agents), and prevalence of diabetes mellitus and dyslipidemia. We applied Cox proportional hazards regression models to assess the associations of the urinary ACR with the primary endpoint, all-cause mortality, cardiovascular mortality, hospitalization, myocardial infarction, and stroke. We also compared the sex differences in the associations of urine ACR with various adverse health outcomes. In sensitivity analyses, concentrations of serum creatinine were added simultaneously to the model with urine ACR to assess risk associated with increased ACR, independent of renal dysfunction. Sensitivity analyses excluding those on spironolactone treatment were performed to assess the association between various adverse health outcomes and urine ACR.

RESULTS

Baseline Characteristics of Participants

Among 1,334 participant, 604 (45.3%) were women. In all participants, mean values (±SD) were 70.9 ± 9.6 years for age, 33.0 ± 7.6 kg/m^2 for body mass index, and 127.9 ± 15.1/73.2 ± 11.6 mm Hg for systolic/diastolic blood pressure. **Table 1** lists the characteristics of patients by tertiles of the urine ACR. Across increasing categories (**Table 1**), systolic blood pressure, serum creatinine, the prevalence of diabetes mellitus and dyslipidemia, the frequencies of use of β-blockers, calcium channel blockers, hypoglycemic agents increased ($p \leq 0.005$).

Women compared with men had smaller ($p < 0.001$) LV end-diastolic and end-systolic volumes, septal and posterior wall thickness, and LV mass index, but higher ($p \leq 0.005$) ejection fraction and longitudinal strain (**Supplementary Table 1**).

Echocardiographic Traits Associated With ACR

In unadjusted analyses (**Table 2**), the association sizes in relation to each doubling of urine ACR were +0.015 cm/+0.015 cm ($p < 0.001$) for septal and posterior wall thickness, +2.43 mg/m^2 ($p < 0.001$) for LV mass index, 0.005 ($p = 0.012$) for relative wall thickness, 0.32 ($p = 0.047$) for E/e' and 0.65 mL/m^2 ($p = 0.020$) for left atrial volume index (**Table 2**). With adjustments applied for potential confounders, the associations of septal and posterior wall thickness, LV mass index, and left atrial volume index with ACR remained significant ($p \leq 0.003$; **Table 2**). The septal and posterior wall thickness, LV mass index, and left atrial volume index were positively associated with urine ACR in men ($p \leq 0.012$; **Table 2**), whereas only posterior wall thickness and LV mass index remained significant ($p \leq 0.033$; **Table 2**) in women. The multivariable-adjusted analyses additionally accounted for serum creatine produced consistent results (**Table 2**).

Risks Associated With ACR

In unadjusted models (**Table 3**), urine ACR predicted primary endpoint (hazard ratio [HR] for doubling increment, 1.15; $p < 0.001$), all-cause mortality (HR, 1.08; $p = 0.003$), cardiovascular mortality (HR, 1.10; $p = 0.002$), HF hospitalization (HR, 1.18; $p < 0.001$), any hospitalization (HR, 1.06; $p < 0.001$), and incidence of myocardial infarction (HR, 1.13; $p = 0.009$). In multivariable-adjusted models (**Table 3**), urine ACR remained predictive for those adverse health outcomes with hazard ratios ranging from 1.03 to 1.16 ($p \leq 0.032$). There was no association between urine ACR and stroke in all models ($p \geq 0.062$). Sensitivity analyses of various adverse outcomes in relation to urine ACR in placebo group produced confirmatory results with the exception for stroke ($p \leq 0.036$; **Table 3**). In **Table 3**, multivariable-adjusted models additionally accounted for serum creatinine produced confirmatory results.

Table 4 shows the sex-specific risks in relation to urine ACR. In women, per doubling of urine ACR predicted primary endpoint (HR, 1.13), all-cause mortality (HR, 1.12), cardiovascular mortality (HR, 1.16), and HF hospitalization (HR, 1.12) in unadjusted models (**Table 4**; $p \leq 0.002$). The corresponding multivariable-adjusted hazard ratios remained significant with the exception for HF hospitalization ($p = 0.082$; **Table 4**). In men, urine ACR predicted primary endpoint, HF hospitalization, any hospitalization, and incidence of myocardial infarction. The adjusted hazard ratios were 1.17, 1.25, 1.08, and 1.19 in **Table 4** ($p \leq 0.017$), respectively. The multivariable-adjusted analyses additionally accounted for serum creatine produced consistent results (**Table 4**). The interactions of sex with ACR were significant for hospitalization for HF and any hospitalization ($p \leq 0.034$).

In men compared with the low tertile of ACR distribution, the incidence of any hospitalization was slightly higher in the middle tertile ($p = 0.16$) and significantly higher ($p < 0.001$) in the top tertile (**Figure 1**), while the incidence of any hospitalization was similar across the categories of urine ACR distribution in women (**Figure 1**). In women, albuminuria was associated with all-cause (1.97; CI, 1.30–3.00; $p = 0.002$) and cardiovascular (2.28; CI, 1.30–3.97; $p = 0.004$) mortality in multivariable-adjusted analyses. In men, albuminuria was important predictor of primary endpoint (HR,1.90; $p < 0.001$), HF hospitalization (HR, 2.76; $p < 0.001$), and any hospitalization (HR, 1.28; $p = 0.025$).

Sensitivity Analysis

Sensitivity analyses of adverse health outcomes related to ACR by sex in various subgroups without diabetes mellitus (**Supplementary Table 2**), dyslipidemia (**Supplementary Table 3**), and smoking (**Supplementary Table 4**) generated confirmatory results.

TABLE 1 | Baseline characteristics of participants by tertiles of the microalbumin/creatinine ratio distribution.

Characteristics	Category of microalbumin/creatinine ratio			*p*-value
Limits, mg/g	<14.0	14.0–61.9	≥61.9	
Number (%) with characteristic	445 (33.4)	441 (33.1)	448 (33.6)	0.97
Women	208 (46.7)	196 (44.4)	200 (44.6)	0.75
Race				
White	377 (84.7)	372 (84.4)	353 (78.8)*	0.032
Black	56 (12.6)	44 (10.0)	75 (16.7)†	0.011
Asian	2 (0.45)	5 (1.13)	6 (1.34)	0.37
Others	12 (2.7)	20 (4.5)	18 (4.0)	0.33
Current smoking	36 (8.1)	36 (8.2)	32 (7.1)	0.82
Office hypertension	389 (87.4)	398 (90.2)	402 (89.7)	0.35
Diabetes mellitus	159 (35.7)	162 (36.7)	219 (48.9)‡	<0.001
Dyslipidemia	272 (61.1)	276 (62.6)	332 (74.1)‡	<0.001
eGFR <60 mL/min/1.73 m^2	176 (39.6)	204 (46.3)*	224 (50.0)	0.006
Medications				
β-blockers	335 (75.4)	329 (74.6)	371 (82.8)†	0.005
Diuretic	386 (86.9)	401 (90.9)	406 (90.6)	0.097
ACE inhibitors or ARBs	373 (84.0)	367 (83.2)	353 (78.8)	0.092
Calcium channel blocker	154 (34.7)	173 (39.2)	211 (47.1)*	0.001
Hypoglycemic agent	136 (30.6)	141 (32.0)	211 (47.1)‡	<0.001
Other CVD medication	406 (91.4)	402 (91.2)	412 (92.0)	0.91
Mean (±SD) of characteristic				
Age, year	70.4 ± 9.6	71.4 ± 9.8	70.8 ± 9.4	0.30
Body mass index, kg/m^2	32.9 ± 7.7	32.6 ± 7.1	33.4 ± 8.1	0.28
Waist circumference, cm	107.8 ± 17.4	108.2 ± 17.8	109.7 ± 18.3	0.28
Systolic pressure, mmHg	125.9 ± 13.6	127.2 ± 15.1	130.7 ± 16.2‡	<0.001
Diastolic pressure, mmHg	73.4 ± 11.1	72.7 ± 11.4	73.4 ± 12.2	0.63
Heart rate, beats/min	68.3 ± 11.0	68.5 ± 11.0	69.7 ± 10.9	0.14
Serum creatinine, mg/dL	1.10 ± 0.28	1.15 ± 0.31*	1.21 ± 0.37†	<0.001
eGFR, mL/min/1.73 m^2	68.0 ± 19.8	65.0 ± 20.3*	63.4 ± 20.8	0.003
Geometric mean (IQR)				
ACR, mg/g	6.32 (5.00–9.00)	27.6 (19.0–38.0)‡	270.3 (113.5–562.0)‡	<0.001

*ACE, indicates angiotensin-converting enzyme; ARB, angiotensin receptor blockers; ACR, urine microalbumin/creatinine ratio; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range. p-values denote the significance of the difference in prevalence (chi-squared test) or means (ANOVA) across tertile of the distribution of serum creatinine. Significance of the difference with the adjacent lower third: *$p \leq 0.05$; †$p \leq 0.01$; ‡$p \leq 0.001$.*

DISCUSSIONS

The key findings can be summarized as follows: (i) in men, urine ACR was positively associated with septal and posterior wall thickness, LV mass index, and left atrial volume index, whereas in women the associations remained significant for posterior wall thickness and LV mass index; (ii) in men urine ACR predicted primary endpoint, HF hospitalization, any hospitalization, and incidence of myocardial infarction, whereas in women ACR predicted primary endpoint, all-cause mortality, and cardiovascular mortality; and (iii) the interactions of sex with ACR were significant for hospitalization for HF and any hospitalization.

Previous studies demonstrated the pre-disposition of women to HFpEF (12). The sex-specific associations between echocardiographic traits and adverse health outcomes might be partially explained by sex differences in risk factors for cardiovascular disease (19) and cardiovascular pathophysiology, including cardiac remodeling (20) and diastolic dysfunction (21). Aging, obesity, hypertension, diabetes mellitus have a different impact on cardiac and vascular structure and function, and endothelial function in women and men (20, 22, 23), indicating an important role of sex disparities in cardiovascular remodeling in patient with HF. Gori and colleagues investigated the association between sex and cardiovascular structure and function in 279 patients (mean age, 71 years; 57% women) with HFpEF from PARAMOUNT study (21). In line with our findings, they found that women had worse diastolic function (lower e' and higher E/e') and higher LV ejection fraction (21). The other mechanisms underlying women's pre-disposition to HFpEF include an activated renin-angiotensin-aldosterone system in response to low estrogen after menopause (24), calcium handling (25), and myocardial substrate metabolism (26).

TABLE 2 | Baseline cardiac structure and function in relation to the urine microalbumin/creatinine ratio.

Characteristics	Unadjusted		Adjusted		Fully adjusted	
	Estimates (95% CI)	*p*-value	Estimates (95% CI)	*p*-value	Estimates (95% CI)	*p*-value
All						
Septal wall thickness, cm	0.015 (0.006–0.023)	<0.001	0.015 (0.006–0.023)	<0.001	0.015 (0.007–0.023)	<0.001
Posterior wall thickness, cm	0.015 (0.006–0.022)	<0.001	0.014 (0.006–0.021)	<0.001	0.014 (0.006–0.021)	<0.001
LV mass index, mg/m^2	2.43 (1.23–3.62)	<0.001	2.51 (1.30–3.72)	<0.001	2.58 (1.37–3.79)	<0.001
Relative wall thickness	0.005 (0.001–0.009)	0.012	0.004 (-0.0004–0.008)	0.078	0.004 (−0.0004–0.008)	0.073
E/A ratio	−0.026 (−0.063–0.011)	0.16	−0.021 (−0.060–0.018)	0.29	−0.018 (−0.057–0.021)	0.36
TDI e', cm/s	−0.10 (−0.23–0.044)	0.18	−0.061 (−0.21–0.087)	0.42	−0.062 (−0.21–0.086)	0.41
E/e' (septal)	0.32 (0.005–0.64)	0.047	0.20 (−0.13–0.54)	0.24	0.20 (−0.14–0.51)	0.25
Left atrial volume index, mL/m^2	0.65 (0.10–1.19)	0.020	0.86 (0.29–1.42)	0.003	0.90 (0.33–1.46)	0.002
Ejection fraction, %	0.085 (−0.22–0.39)	0.58	0.051 (−0.25–0.36)	0.85	0.062 (−0.24–0.37)	0.69
TDI longitudinal strain, %	−0.13 (−0.32–0.060)	0.18	−0.12 (−0.33–0.072)	0.22	−0.12 (−0.31–0.073)	0.22
Women						
Septal wall thickness, cm	0.012 (0.001–0.024)	0.037	0.010 (−0.002–0.022)	0.11	0.010 (−0.002–0.022)	0.10
Posterior wall thickness, cm	0.013 (0.002–0.024)	0.018	0.012 (0.001–0.024)	0.033	0.012 (0.001–0.024)	0.033
LV mass index, mg/m^2	2.56 (0.88–4.24)	0.003	2.43 (0.59–4.27)	0.010	2.45 (0.61–4.29)	0.009
Relative wall thickness	0.004 (−0.001–0.010)	0.13	0.003 (−0.003–0.009)	0.31	0.003 (−0.003–0.009)	0.31
Left atrial volume index, mL/m^2	0.27 (−0.44–0.97)	0.46	0.36 (−0.41–1.12)	0.36	0.36 (−0.40–1.13)	0.35
Men						
Septal wall thickness, cm	0.018 (0.006–0.029)	0.002	0.018 (0.007–0.029)	0.002	0.018 (0.007–0.030)	0.002
Posterior wall thickness, cm	0.016 (0.005–0.027)	0.004	0.014 (0.003–0.025)	0.012	0.014 (0.003–0.025)	0.012
LV mass index, mg/m^2	2.36 (0.74–3.98)	0.004	2.46 (0.78–4.14)	0.004	2.55 (0.85–4.26)	0.004
Relative wall thickness	0.006 (0.0003–0.012)	0.040	0.004 (−0.002–0.010)	0.16	0.004 (−0.002–0.011)	0.16
Left atrial volume index, mL/m^2	0.98 (0.17–1.78)	0.018	1.34 (0.50–2.19)	0.002	1.44 (0.59–2.30)	0.001

CI, indicates confidence interval; LV, left ventricular; TDI, tissue doppler imaging. Association sizes (95% CI) express the difference in indexes of cardiac structure and function associated with a doubling of urine microalbumin/creatinine ratio. Adjusted models accounted for randomly assigned treatment (spironolactone vs. placebo), sex, age, ethnicity, body mass index, systolic blood pressure, heart rate, current smoking, use of medications (diuretics, β blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium-channel blockers, lipid-lowering drugs, aspirin, other cardiovascular medications, and hypoglycemic agents), and prevalence of diabetes mellitus and dyslipidemia. Fully adjusted models additionally adjusted for serum creatinine.

Albuminuria is present in 30∼50% of patients with HFpEF and confers poor prognosis (5–7). The albuminuria has been considered as a target to reduce cardiovascular outcomes in patients with HFpEF. Jackson and coworkers assessed the prevalence and prognostic value of a spot urinary ACR in 2,310 patients (mean age, 66.2 years; 33.4% women) with HF (5). In 967 patients with HFpEF, 281 (29%) had microalbuminuria and 119 (12%) had macroalbuminuria. HRs per unit ACR (100 mg/mmol) for the primary composite outcome was 1.12 (1.04–1.21) in those with HFpEF (5). In categorical analyses, HRs were 2.03 (1.45–2.85) for macroalbuminuria vs. normoalbuminuria, and 1.31 (0.99–1.74) for microalbuminuria vs. normoalbuminuria (5). The investigators of the Chronic HF Analysis and Registry in the Tohoku District 2 study demonstrated measurement of albuminuria in addition to estimated glomerular filtration rate (eGFR) is useful for risk stratification in 2,465 patients with HFpEF (6). They divided the patients into four groups: group 1 (eGFR $\geq$ 60, normal ACR), group 2 (eGFR $\geq$ 60, abnormal ACR), group 3 (eGFR $<$ 60, normal ACR), and group 4 (eGFR $<$ 60, abnormal ACR) (6). Over 2.5 years (mean) of follow-up, compared with group 1, HRs for all-cause death were 2.44 (1.47–4.05) for group 2, 1.43 (0.92–2.23) for group 3, and 2.71 (1.72–4.27) for group 4 (6).

Study Strength and Limitations

Our current study must be interpreted within the context of its strength and potential limitations. Strengths of our study include its relatively large-sample size, long-term follow-up of this randomized clinical trial, and adjustments applied for a plenty of confounders in line with previous studies. There are some possible limitations of the study. First, we used spot measurements to estimate albuminuria. However, it is impracticable to collect 24 h urine samples in the context of a large clinical trial. Furthermore, previous studies reported good concordance between ACR estimates from spot and 24-h urine collections (27). Second, several baseline characteristics were self-reported and might have introduced recall bias in our analyses. Third, urine specimens were not available for all participants. However, participants analyzed compared with those not analyzed had similar heat rate, but were on average 3.8 years older, had a slightly higher body mass index (33.0 vs. 31.5 kg/m^2), higher prevalence of diabetes mellitus (40.5

TABLE 3 | Adverse outcomes in relation to the urine microalbumin/creatinine ratio.

Characteristics	Unadjusted models		Adjusted models		Fully adjusted models	
	HRs (95% CI)	*p*-value	HRs (95% CI)	*p*-value	HRs (95% CI)	*p*-value
All (*n* = 1,334)						
Primary end point (*n* = 345)	1.15 (1.11–1.20)	<0.001	1.13 (1.09–1.18)	<0.001	1.12 (1.07–1.17)	<0.001
Death (*n* = 252)	1.08 (1.03–1.13)	0.003	1.08 (1.03–1.14)	0.002	1.07 (1.01–1.12)	0.012
Cardiovascular death (*n* = 152)	1.10 (1.03–1.17)	0.002	1.10 (1.03–1.17)	0.005	1.08 (1.01–1.15)	0.023
HF hospitalization (*n* = 259)	1.18 (1.13–1.24)	<0.001	1.16 (1.10–1.22)	<0.001	1.14 (1.09–1.20)	<0.001
Any hospitalization (*n* = 709)	1.06 (1.03–1.09)	<0.001	1.03 (1.003–1.07)	0.032	1.03 (0.99–1.06)	0.10
Myocardial infarction (*n* = 60)	1.13 (1.03–1.25)	0.009	1.13 (1.02–1.26)	0.016	1.13 (1.02–1.25)	0.020
Stroke (*n* = 52)	1.10 (0.99–1.22)	0.062	1.09 (0.98–1.22)	0.13	1.08 (0.97–1.21)	0.17
Placebo (*n* = 669)						
Primary end point (*n* = 185)	1.16 (1.10–1.22)	<0.001	1.15 (1.08–1.21)	<0.001	1.13 (1.07–1.20)	<0.001
Death (*n* = 137)	1.08 (1.01–1.15)	0.021	1.09 (1.02–1.17)	0.011	1.08 (1.01–1.16)	0.029
Cardiovascular death (*n* = 88)	1.11 (1.03–1.20)	0.007	1.13 (1.04–1.24)	0.004	1.12 (1.03–1.22)	0.010
HF hospitalization (*n* = 141)	1.18 (1.12–1.26)	<0.001	1.16 (1.09–1.24)	<0.001	1.14 (1.07–1.22)	<0.001
Any hospitalization (*n* = 359)	1.08 (1.04–1.12)	<0.001	1.05 (1.01–1.10)	0.017	1.04 (0.999–1.09)	0.052
Myocardial infarction (*n* = 29)	1.14 (0.998–1.30)	0.054	1.17 (1.004–1.36)	0.044	1.16 (0.996–1.35)	0.056
Stroke (*n* = 28)	1.15 (1.01–1.32)	0.036	1.10 (1.02–1.39)	0.028	1.17 (1.003–1.38)	0.047

CI, indicates confidence interval; HF, heart failure; HR, hazard ratio (95% CI) express the risk of adverse outcomes associated with a doubling of urine microalbumin/creatinine ratio. Adjusted models accounted for randomly assigned treatment (spironolactone vs. placebo), sex, age, ethnicity, body mass index, systolic blood pressure, heart rate, current smoking, use of medications (diuretics, β-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium-channel blockers, lipid-lowering drugs, aspirin, other cardiovascular medications, and hypoglycemic agents), and prevalence of diabetes mellitus and dyslipidemia. Fully adjusted models additionally adjusted for serum creatinine.

TABLE 4 | Adverse outcomes in relation to the urine microalbumin/creatinine ratio by sex.

Characteristics	Unadjusted models		Adjusted models		Fully adjusted models	
	HRs (95% CI)	*p*-value	HRs (95% CI)	*p*-value	HRs (95% CI)	*p*-value
Women (*n* = 604)						
Primary end point (*n* = 150)	1.13 (1.07–1.19)	<0.001	1.09 (1.02–1.16)	0.010	1.08 (1.01–1.15)	0.020
Death (*n* = 100)	1.12 (1.05–1.20)	0.002	1.12 (1.03–1.21)	0.006	1.11 (1.03–1.20)	0.006
Cardiovascular death (*n* = 59)	1.16 (1.06–1.27)	0.001	1.17 (1.06–1.30)	0.002	1.17 (1.06–1.30)	0.002
HF hospitalization (*n* = 118)	1.12 (1.05–1.19)	0.001	1.07 (0.99–1.14)	0.082	1.06 (0.98–1.13)	0.14
Any hospitalization (*n* = 331)	1.03 (0.99–1.07)	0.16	0.99 (0.95–1.04)	0.81	0.99 (0.95–1.04)	0.68
Myocardial infarction (*n* = 29)	1.09 (0.95–1.25)	0.21	1.07 (0.92–1.25)	0.38	1.07 (0.92–1.24)	0.40
Stroke (*n* = 23)	1.05 (0.90–1.23)	0.54	1.05 (0.89–1.25)	0.55	1.05 (0.88–1.24)	0.61
Men (*n* = 730)						
Primary end point (*n* = 195)	1.17 (1.11–1.24)	<0.001	1.17 (1.11–1.24)	<0.001	1.15 (1.08–1.22)	<0.001
Death (*n* = 152)	1.04 (0.98–1.11)	0.24	1.04 (0.98–1.12)	0.14	1.03 (0.96–1.10)	0.42
Cardiovascular death (*n* = 93)	1.05 (0.97–1.14)	0.22	1.05 (0.97–1.15)	0.24	1.02 (0.94–1.12)	0.60
HF hospitalization (*n* = 141)	1.26 (1.18–1.34)	<0.001	1.25 (1.17–1.34)	<0.001	1.23 (1.15–1.32)	<0.001
Any hospitalization (*n* = 378)	1.09 (1.05–1.13)	<0.001	1.08 (1.03–1.12)	0.001	1.06 (1.02–1.11)	0.006
Myocardial infarction (*n* = 31)	1.18 (1.04–1.36)	0.014	1.19 (1.03–1.36)	0.017	1.19 (1.03–1.38)	0.017
Stroke (*n* = 29)	1.15 (1.00–1.33)	0.051	1.15 (0.98–1.34)	0.080	1.14 (0.98–1.33)	0.11

CI, indicates confidence interval; HF, heart failure; HR, hazard ratio (95% CI) express the risk of adverse outcomes associated with a doubling of urine microalbumin/creatinine ratio. Adjusted models accounted for randomly assigned treatment (spironolactone vs. placebo), age, ethnicity, body mass index, systolic blood pressure, heart rate, current smoking, use of medications (diuretics, β-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium-channel blockers, lipid-lowering drugs, aspirin, other cardiovascular medications, and hypoglycemic agents), and prevalence of diabetes mellitus and dyslipidemia. Fully adjusted models additionally adjusted for serum creatinine.

vs. 27.4%) and dyslipidemia (66.0 vs. 56.6%), and had a lower blood pressure (127.9 vs. 130.0 mm Hg). Finally, not all patients randomized into TOPCAT underwent echocardiography at baseline. Compared with TOPCAT participants not included in the echocardiographic study, those included were on average 1.80 years older ($p < 0.001$) and had a slightly higher body

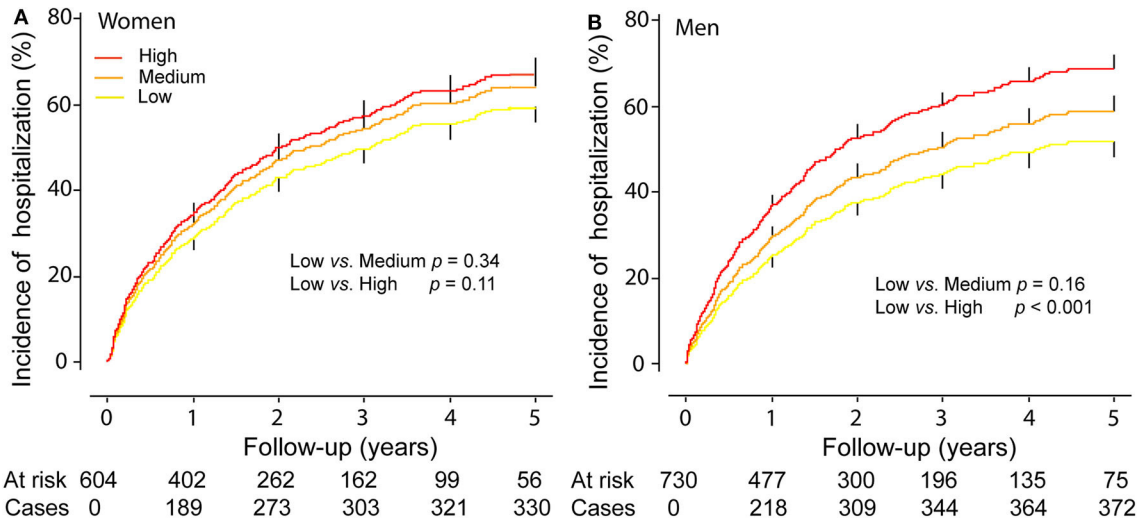

FIGURE 1 | Cumulative Incidence of any hospitalization in women **(A)** and men **(B)** by tertiles of the distribution of urine microalbumin/creatinine ratio. *p*-values refer to the differences between the low and the middle and the top tertiles of urine microalbumin/creatinine ratio distribution. Vertical lines denote the standard error.

mass index (+0.71 kg/m^2, $p = 0.009$), which, although relatively minor, may limit the generalizability of these findings. However, participants with and without baseline echocardiogram included proportionally a similar number of women, hypertensive patients, and smokers ($p \geq 0.093$).

CONCLUSION

In TOPCAT, women and men presented with different echocardiographic traits and long-term clinical outcomes in HFpEF. The interaction between ACR and sex was significant for hospitalization, and particularly, HF hospitalization. Our findings suggest that sex can be applied to better characterize patients with HFpEF regarding the association of echocardiographic traits and adverse outcomes with urine ACR. Sex-specific health promotion efforts may be warranted to improve the prevention of adverse health outcomes in both women and men. Furthermore, improving the sex-specific application of evidence-based treatments in patients with HFpEF may help reduce the observed sex disparities in various adverse health outcomes (28, 29).

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the requests to access the dataset should be sent to the NHLBI. Requests to access the datasets should be directed to https://biolincc.nhlbi.nih.gov/.

ETHICS STATEMENT

The current analysis was approved by the ethics committee of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China. TOPCAT complied with the Declaration of Helsinki and received ethical clearance. All patients signed informed consent prior to randomization.

AUTHOR CONTRIBUTIONS

JS, YD, and CL: conceptualization. F-FW, WL, and XH: formal analysis. RX, YW, YZ, MO-A, and WZ: methodology. JH, JS, YD, and CL: supervision and validation. F-FW, XH, and ZW: writing and revision. All authors interpreted the results, commented on successive versions of the manuscript, and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.579400/full#supplementary-material

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Cystatin C-Based Renal Function in Predicting the Long-Term Outcomes of Chronic Total Occlusion After Percutaneous Coronary Intervention

Bolin Li[1†], Jie Rong[2†], Bobo Wang[3], Ke Gao[1], Xing Wen[4], Hongbing Li[1], Lele Cheng[1], Yi-Ming Hua[1], Shanshan Li[1], Zhijie Jian[1], Yujing Zhang[5], Hui Huang[5], Youlong Pan[6], Yue Wu[1] and Xiao-Zhen Zhuo[1*]*

[1] Department of Cardiology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [2] Department of Encephalopathy, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, China, [3] Health Science Center, School of Pharmacy, Xi'an Jiaotong University, Xi'an, China, [4] The Center of Gastrointestinal and Minimally Invasive Surgery, The Third People's Hospital of Chengdu, Chengdu, China, [5] Cardiovascular Department, The General Hospital of Ningxia Medical University, Yinchuan, China, [6] Cardiovascular and Cerebrovascular Diseases Hospital of Qinghai Province, Xining, China

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***Correspondence:**
Yue Wu
yue.wu@xjtu.edu.cn
Xiao-Zhen Zhuo
imxiaozhen@xjtu.edu.cn

[†] These authors have contributed equally to this work

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Renal function estimated by various biomarkers predicting for adverse cardiovascular events has not been well-identified in received percutaneous coronary intervention (PCI) for chronic total occlusion (CTO), the advanced stages of atherosclerosis. We aim to determine whether the serum cystatin C-based-estimated glomerular filtration rate (eGFR) can have an improved predictive value in patients with CTO lesions undergoing PCI as compared with multiple creatinine-based estimates of kidney function. Six hundred and seventy-one patients received CTO PCI were retrospectively included in the study. The eGFR was calculated by modification of diet in renal disease equation for Chinese (cMDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations at baseline, respectively. Then, the cohort was categorized into three groups according to standard KDIGO kidney stages based on eGFR. The primary endpoint was all-cause mortality, and the secondary endpoint was cardiac death. Strikingly, cystatin C-based eGFR showed a better performance with the greater area being under the receiver operating characteristic (ROC) curve (0.73 for all-cause mortality and 0.73 for cardiac death, separately) and a better assessment for survival free from adverse event across renal levels among four eGFR equations. Compared with eGFR calculated by other formulas, serum cystatin C-based eGFR showed the highest prognostic value for both all-cause mortality (adjusted HR 3.6, 95% CI 1.6–8.1, $P = 0.002$) and cardiac death (adjusted HR 2.9, 95% CI 1.0–8.1, $P = 0.028$). Moreover, cystatin C-based eGFR significantly improved the risk reclassification of event with a high value of net reclassification improvement and integrated discrimination improvement. This study may prove that cystatin C-based eGFR is a better predictor of both all-cause mortality and cardiac death than other equations in populations with CTO undergoing PCI.

Keywords: cystatin C, creatinine, estimated glomerular filtration rate, chronic total occlusion, all-cause mortality, cardiac death

INTRODUCTION

Renal insufficiency has been found to increase the incidence of both cardiovascular diseases and adverse outcome (1). Estimated glomerular filtration rate (eGFR) has been widely used in clinical practice. Current recommendations and guidelines (2) have pointed out that the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation can be applied to estimate GFR with better accuracy than the modification of Diet in Renal Disease (MDRD) equation, as the actual GFR in patients with preserved renal function may be underestimated by using MDRD (3, 4).

Recently, a number of endogenous biomarkers, especially cystatin C, have been established and used to estimate GFR. Cystatin C is a 13-kDa protein and a member of the family of competitive lysosomal cysteine protease inhibitors. It can be freely filtered by the glomerulus. Compared to creatinine-based eGFR, cystatin C depends less on health status, muscle mass, or other demographic characteristics and appears to be more consistent across cohorts. It has been proved to be a more reliable and sensitive marker for estimating GFR in patients with normal creatinine-based eGFR (5) or with mildly renal insufficiency (6). According to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (2), cystatin C-based eGFR may be used to verify the diagnosis of a reduced creatinine-based eGFR and, therefore, recategorize patients into normal group in the absence of albuminuria. Several studies have shown a very close relationship between cystatin C and cardiovascular events, especially cardiac death and all-cause mortality (6), among different cohorts, such as patients with chronic kidney disease(CKD) (7) and the elderly people (8). These studies have revealed that cystatin C-based eGFR can be an earlier and stronger predictor for predicting adverse cardiovascular outcomes as compared with creatinine-based eGFR.

Renal dysfunction can be estimated by various equations and has been identified as an important predictor of mortality for patients with coronary heart disease after percutaneous coronary intervention (PCI) and surgical revascularization. Chronic coronary total occlusion (CTO) is advanced atherosclerotic lesions and represents a challenging subset of coronary artery disease. However, the value of eGFR in predicting adverse cardiovascular events has not been well-established within the patients with CTO after PCI. This deficiency forms the basis for this study, aiming to determine whether or not the decreased eGFR calculated by cystatin C can have a higher risk predictive value for assessing risk and risk grading in terms of all-cause mortality and cardiac death in CTO cohort available in our Cardiology Department.

PATIENTS AND METHODS

Population and Outcomes

A total of 762 patients with CTO lesions confirmed by coronary angiography in the Cardiology Department of the First Affiliated Hospital of Xi'an JiaoTong University (Xi'an, Shaanxi, China) undergoing PCI between June 2013 and October 2017 were included in the study. The follow-up for all-cause mortality and cardiac death was carried out via telephone contacts between 2018 and 2019. The study was approved by both Research and Ethics Committees of the First Affiliated Hospital of Xi'an JiaoTong University. Recanalization of CTO lesion was performed according to current guidelines (9, 10) by the highly experienced CTO operators with contemporary techniques. A coronary CTO lesion was defined as the presence of Thrombolysis in Myocardial Infarction [TIMI] flow grade 0 within an occluded coronary artery segment of an estimated duration of at least 3 months (11, 12). Procedural success was defined by complete restoration of antegraded blood flow (TIMI flow grade 3) in the occluded segment with <30% residual diameter stenosis. The primary endpoint was all-cause mortality, and the secondary endpoint was cardiac death.

Measurements of Related Parameters

We collected several demographic, clinical, and analytical parameters. Age, gender, height, and weight were recorded, and body mass index (BMI) (kg/m^2) was calculated on the basis of weight and height. Blood pressure (BP) was measured three times or more by a nurse while patients were seated after 5 min of rest. Patients either with persistent BP>140/90 mmHg or those currently taking antihypertensive drugs were considered hypertensive. Presence of type 2 diabetes mellitus (T2DM) was determined by means of a medical history of diabetes, fasting glucose levels >126 mg/dL (7.0 mmol/L), and/or glycated hemoglobin (HbA1c)>6.5%. Several parameters, including low-density lipoprotein-cholesterol (LDL-c), HDL cholesterol (HDL-c), and C-reactive protein, were measured. Serum levels of cystatin C were determined by an automated particle-enhanced immunoturbidimetric method on a Siemens Dade Behring BN II Nephelometer. Renal function was assessed by means of serum levels of creatinine and cystatin C at baseline and calculated by MDRD for the Chinese equation (13) (eGFR-cMDRD) and chronic kidney epidemiology collaboration (CKD-EPI) equations (4), respectively (**Supplementary Table 1**). CKD-EPI equations are composed of eGFR from serum creatinine (eGFRcre), eGFR from serum cystatin C (eGFRcys), and eGFR from the equation incorporating both creatinine and cystatin C (eGFRcre-cys).

Categorization of the Cohorts

The cohort was categorized according to its prognostic category, defined by an existing classification system—the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (14)—that is, the level of GFR (known as stages). Therefore, patients were stratified according to eGFR (group 1: eGFR $\geq$ 90 ml/min/1.73 m^2, group 2: 60–89 ml/min/1.73 m^2, group 3: <60 ml/min/1.73 m^2) as shown in **Supplementary Table 2**.

Statistical Analysis

Continuous variables are presented as the mean $\pm$ standard deviation if normally distributed or median (lower quartile, upper quartile) otherwise, and Shapiro–Wilk was used for normality test. Categorical variables are presented as numbers and percentages. Differences in the parameters among groups were analyzed using analysis of variance (ANOVA) for normally

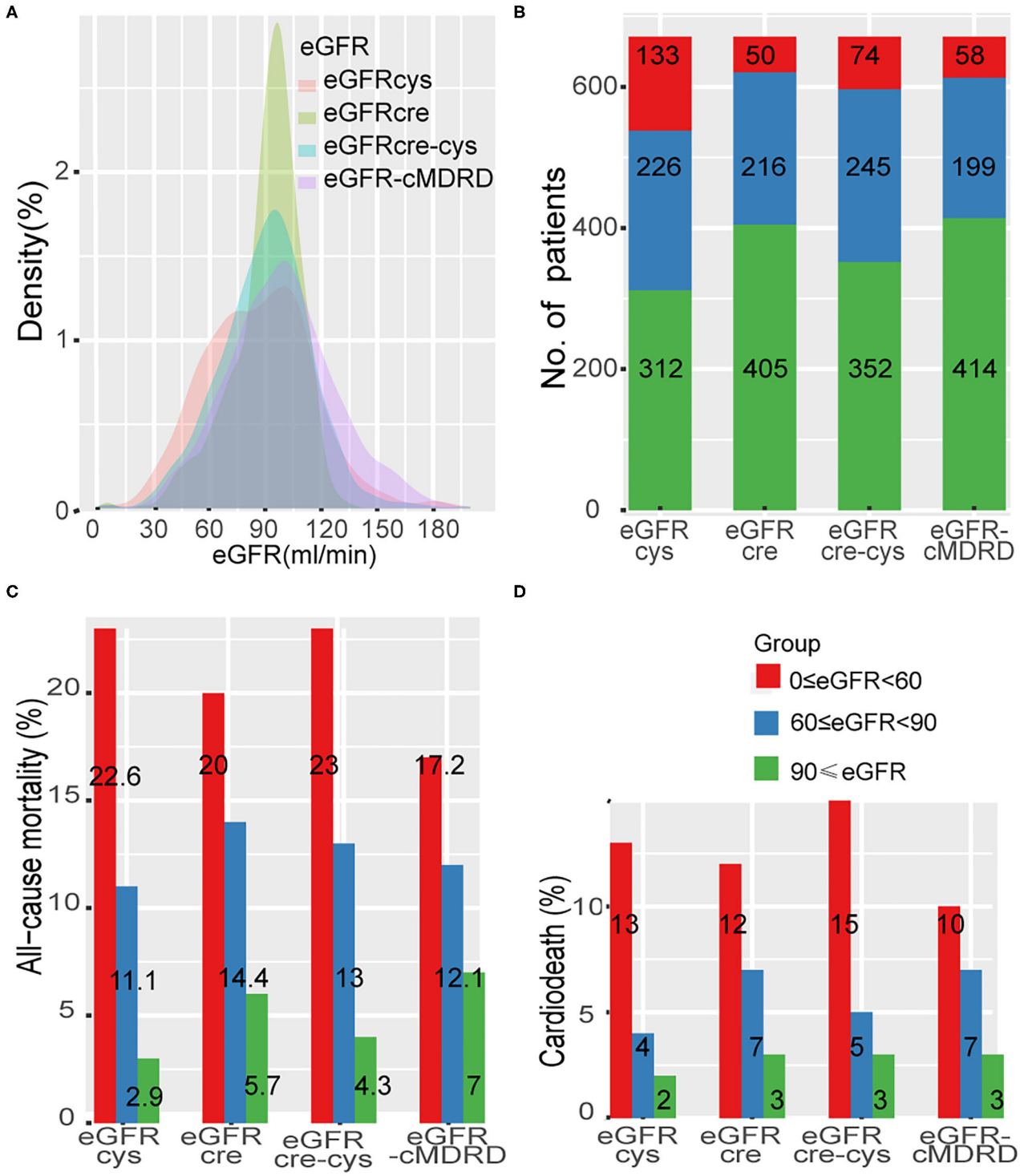

FIGURE 1 | Distribution of eGFR and the rate of adverse event across different renal levels. **(A)**, Distribution of eGFR according to each of the four equations in a density plot. **(B)**, Thresholds of eGFR according to each equation and number of patients in each CKD stage. **(C,D)**, Incidence of all-cause mortality and cardiac death at different renal levels by different formulas. eGFR-cMDRD, calculated by MDRD for Chinese equation. eGFRcre, CKD-EPI equations composed of eGFR from serum creatinine; eGFRcys, eGFR from serum cystatin C; eGFRcre-cys, eGFR from equation incorporating both creatinine and cystatin C.

TABLE 1 | Baseline characteristics, by GFR, estimated using the eGFRcys equation.

	eGFRcys > 90 (ml/min/1.73 m2)	eGFRcys 90–60 (ml/min/1.73 m2)	eGFRcys < 60 (ml/min/1.73 m2)	*p*
Patients	312 (46.5%)	226 (33.7%)	133 (19.8%)	
Age (years)	62 (54–68)	68 (61–73)	72 (66–78)	<0.001
Sex (men)	271 (86.9%)	195 (86.3%)	95 (71.4%)	<0.001
BMI	24.2 (22.8–26.2)	24.2 (22.6–25.7)	24.2 (23.4–25.4)	0.12
Drunk	77 (24.7%)	50 (22.1%)	22 (16.5%)	0.17
Smoking	166 (53.2%)	116 (51.3%)	50 (37.6%)	0.008
Previous MI	96 (30.8%)	76 (33.6%)	45 (33.8%)	0.77
Procedural success	220 (70.5%)	157 (69.5%)	94 (70.7%)	0.96
Multivessel disease				0.14
Single vessel	30 (9.60%)	15 (6.60%)	5 (3.80%)	
Double vessel	60 (19.2%)	35 (15.5%)	22 (16.5%)	
Triple vessel	222 (71.2%)	176 (77.9%)	106 (79.7%)	
J-CTO score				0.097
0	21 (6.7%)	7 (3.1%)	4 (3.0%)	
1	42 (13.5%)	22 (9.7%)	17 (12.8%)	
2	84 (26.9%)	54 (23.9%)	28 (21.1%)	
≥3	165 (52.9%)	143 (63.3%)	84 (63.2%)	
Stroke	25 (8.00%)	30 (13.3%)	13 (9.80%)	0.14
DM	144 (36.5%)	72 (31.9%)	52 (39.1%)	0.33
HT	179 (57.4%)	112 (49.6%)	82 (61.7%)	0.06
HR	70 (65–74)	70 (66–75)	70 (67–76)	0.14
SysBP (mmHg)	125 (118–135)	124 (116–134)	127 (117–140)	0.19
DiaBP (mmHg)	73 (67.1–78.8)	72 (66–77.6)	72 (66–78.3)	0.59
CRP (mg/L)	1.40 (0.800–1.40)	1.40 (1.28–1.80)	1.40 (1.40–3.20)	<0.001
WBC (10^9/L)	6.39 (5.42–7.81)	6.22 (5.33–7.01)	6.41 (5.32–7.56)	0.15
Neutrophils (10^9/L)	4.00 (3.33–5.07)	4.03 (3.21–4.95)	4.23 (3.40–5.17)	0.25
Cholesterol (mmol/L)	3.71 (3.08–4.33)	3.61 (3.05–4.17)	3.64 (3.07–4.58)	0.38
Triglyceride (mmol/L)	1.47 (1.02–2.10)	1.40 (0.980–1.96)	1.38 (1.04–1.95)	0.53
HDL (mmol/L)	0.910 (0.790–1.08)	0.920 (0.800–1.04)	0.950 (0.800–1.10)	0.57
LDLc (mmol/L)	2.12 (1.66–2.66)	2.01 (1.56–2.58)	2.09 (1.56–2.79)	0.40
LVEF (%)	60.0 (48.3–80.0)	58.5 (46.0–47.0)	53.0 (42.0–63.0)	<0.001

Values are given as median and interquartile range or numbers and percentages.
BMI, body mass index; LVEF, left ventricular ejection fraction; DM, diabetes mellitus; HT, hypertension; CRP, C-reactive protein; HR, heart rate; SysBP, systolic blood pressure; DiaBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; eGFRcys, eGFR from serum cystatin C.

distributed variables, the Kruskal–Wallis test was used for non-normally distributed continuous variables, and the chi-square test was used for categorical variables. We assessed performance through the receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC). Clinical event rates were compared with the Kaplan–Meier method using the log rank test for comparisons between groups. Cox proportional hazards regression models were used in univariate analyses and multivariate analyses to determine the prognostic value of different kidney stage levels estimated by each eGFR equation. Multivariate analyses adjusted for significant baseline variables and the factors closely related to the outcome of patients with cardiovascular disease, such as age, sex, smoking, BMI, diabetes mellitus (DM), hypertension (HT), and low-density lipoprotein cholesterol (LDL-c), left ventricular ejection fraction (LVEF), C-reactive protein (CRP), and procedural success. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were performed to analyze the degree to which eGFR improved the predictive ability of the eGFRcys. Both NRI and IDI values were analyzed with their 95% CI. SPSS version 25.0.0 (IBM, USA) and R version 3.6.1 (https://www.r-project.org) were used for conducting statistical analyses, and a two-sided $P < 0.05$ was considered statistically significant. There is no adjustment for multiplicity.

RESULTS

A total of 97 patients with acute coronary syndrome at admission were excluded. A total of 762 patients with CTO lesions confirmed by coronary angiography were treated with PCI, and the rate of CTO lesion revascularized was 70.2%. After a median follow-up of 33 months, 91 patients were lost to follow-up, leaving 671 in the study population at the end of follow-up (**Supplementary Figure 1**). In our cohort, 64 patients (9.5%) died

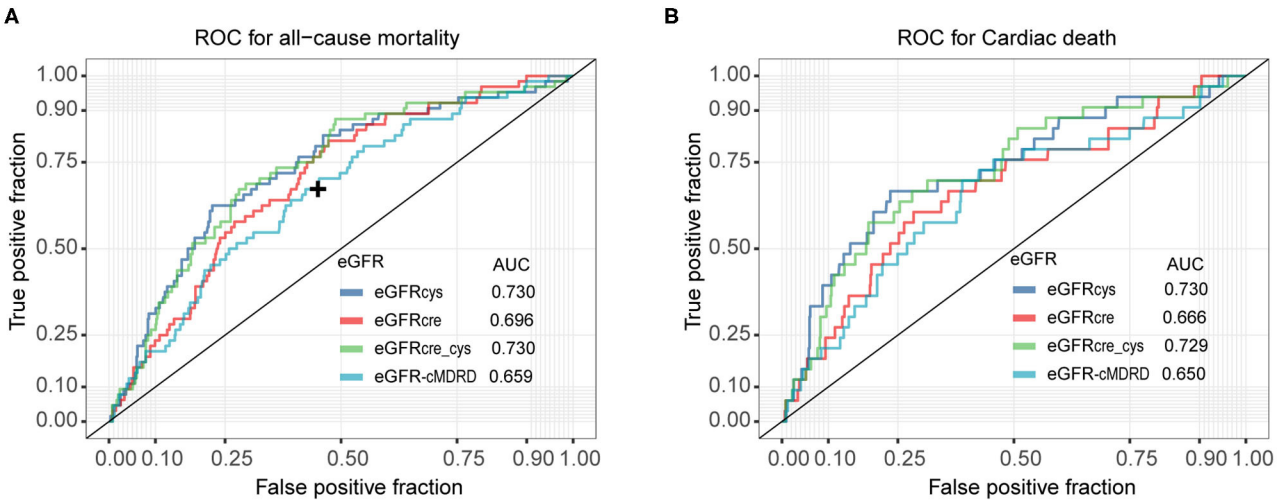

FIGURE 2 | ROC for predicting all-cause mortality and cardiac death. **(A)**, accuracies of eGFRcys, eGFRcre, eGFRcre-cys, and eGFR-cMRDR for predicting all-cause mortality presented as areas under the receiver operating characteristic curves, individually. **(B)**, accuracies of eGFRcys, eGFRcre, eGFRcre-cys, and eGFR-cMRDR for predicting cardiac death presented as areas under the receiver operating characteristic curves, separately. ROC, receiver operating characteristic curve; AUC, area under the receiver operating characteristic curve. DeLong's test to check the difference of AUC for eGFRs calculated by other formulas, compared to eGFRcys. + represents significant differences ($P < 0.05$).

and 33 patients (4.9%) died from cardiac death. Distribution of eGFR according to each of the four equations is shown in **Figure 1A**. Differences in grouping situation of four equations are shown in **Figure 1B**.

The baseline characteristics of the cohort are shown in **Table 1**. With decrease of renal function, patients were older ($p < 0.001$) with fewer men ($p < 0.001$) and had a higher prevalence of smoking ($p < 0.01$), a lower (although within normal range) ejection fraction ($p < 0.001$), and increased levels of CRP as well. Procedural success rate, prevalence of DM and HT, serum levels of LDLc, and other baseline parameters did not differ among the three groups.

Levels of renal function estimated by each eGFR equation (eGFR-cMDRD, eGFRcys, eGFRcre, and eGFRcre-cys) were classified according to the risk event of all-cause mortality and cardiac death (**Figures 1C,D**, **Supplementary Table 3**). All the groups showed a trend that all-cause mortality was increased with the decreased renal function (eGFRcys: group 1 to group 3, 2.9–22.6%; eGFRcre: group 1 to group 3 5.7–20.0%; eGFRcre-cys: group 1 to group 3, 4.3–23.0%; and eGFR-MDRD: group 1 to group 3, 7.0–17.2%). The same was true when cardiac death rates were tested (eGFRcys: group 1 to group 3, 1.90–12.80%; eGFRcre: group 1 to group 3, 2.70–12.00%; eGFRcre-cys: group 1 to group 3, 2.60–14.90%; and eGFR-MDRD: group 1 to group 3, 3.4–10.3%).

The accuracies of eGFR-cMDRD, eGFRcys, eGFRcre, and eGFRcre-cys for predicting all-cause mortality and cardiac death are shown as the AUC in **Figure 2**, **Supplementary Table 4**. For predicting all-cause mortality, the eGFRcys (AUC = 0.73) and eGFRcre-cys (AUC = 0.73) exhibited higher predictive accuracy (**Figure 2A**). For predicting cardiac death, eGFRcys (AUC = 0.73) showed the best prediction performance (**Figure 2B**).

Kaplan–Meier curves were used to illustrate the survival free from adverse events. Overall, patients at a lower eGFR levels had a significantly worse outcome of survival free from all-cause mortality during the follow-up period (**Figures 3A–D**). eGFRcys showed better performance on grading and risk assessment. Alike, the lower renal function is, the worse survival free from cardiac death is (**Figures 3E–H**). Also, eGFRcys showed the superior performance on grading and risk assessment as well as eGFRcre-cys to other equations.

We performed Cox regression hazard models on eGFR calculated by each equation as the categorical variable grouped as previously mentioned. The risk of each adverse outcome was assessed by comparing Group 1 (reference) with Groups 2 and 3 in every equation. Cystatin C-based eGFR (adjusted HR, 3.6; 95% CI, 1.6–8.1; $P = 0.002$) was a stronger predictor than eGFRcre (adjusted HR 1.7, 95% CI 0.7–4.1, $P = 0.203$), eGFRcre-cys (adjusted HR, 2.3; 95% CI, 1.0–5.3; $P = 0.049$), and eGFR-cMDRD (adjusted HR, 1.3; 95% CI, 0.6–2.9; $P = 0.516$) for the risk of all-cause mortality for moderate-to-severe reduction in GFR (eGFR < 60 ml/min/1.73 m^2). Likewise, cystatin C-based eGFR (adjusted HR, 2.9; 95% CI, 1.0–8.1; $P = 0.028$) was able to predict the risk of cardiovascular mortality better than eGFRcre (adjusted HR, 2.3; 95% CI, 0.7–7.6; $P = 0.155$), eGFRcre-cys (adjusted HR, 2.4; 95% CI, 0.8–7.2; $P = 0.12$), and eGFR-cMDRD (adjusted HR, 1.5; 95% CI, 0.5–4.2; $P = 0.498$) for moderate-to-severe reduction in renal function.

The above data showed that in terms of outcome of all-cause mortality, eGFRcre was able to estimate an increased risk that was 1.7 times higher than that of the population without CKD (patients whose eGFR was above 90 ml/min/1.73 m^2). On the other hand, eGFRcys showed an increased

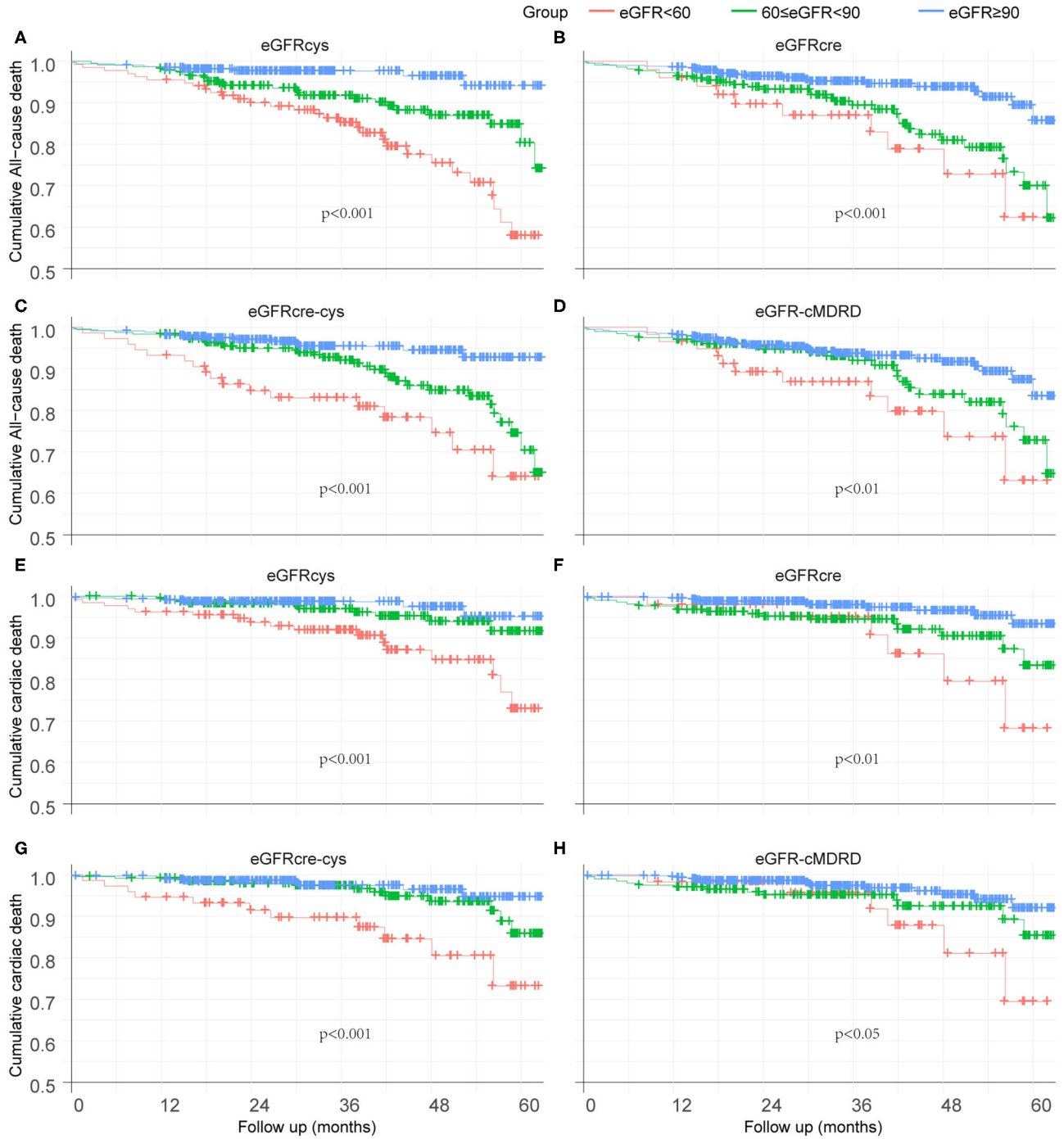

FIGURE 3 | Kaplan–Meier curves to illustrate the survival free from adverse event. **(A–D)** Survival analysis of eGFRcys, eGFRcre, eGFRcre-cys, and eGFR-cMRDR for all-cause mortality, individually. **(E–H)** Survival analysis of eGFRcys, eGFRcre, eGFRcre-cys, and eGFR-cMRDR for cardiac death, separately. No. at risk = number of patients at risk for all-cause mortality or cardiac death.

risk rate of 3.6-folds, respectively, as compared with that of the population without CKD. This means that cystatin C is able to improve the predictive value by 111.7% (for the eGFRcys) in patients with moderate-to-severe reduction in GFR in terms of all-cause mortality. Likewise, cystatin C-based eGFR may improve the estimation of risk by 26.1% (for the cystatin C-based equation) in terms of cardiovascular mortality, as compared with those estimated with other equations. A forest plot summarizing these data is shown in **Figure 4**, **Supplementary Tables 5**, **6**.

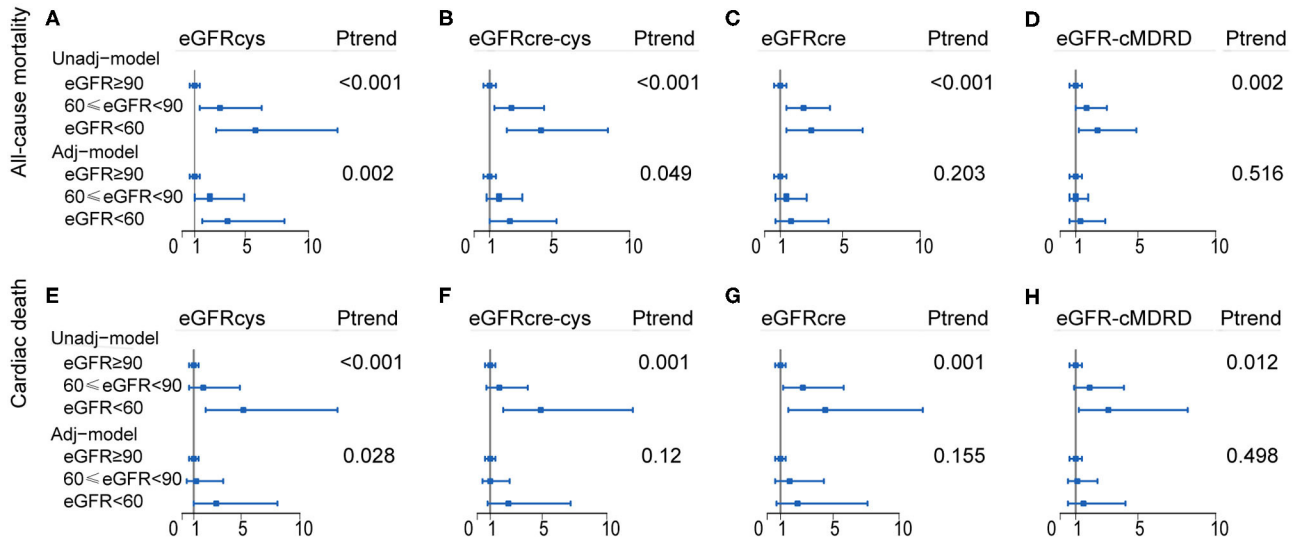

FIGURE 4 | Forest plot showing the risk assessment of event across renal levels. HR and 95% CI of the association between both all-cause mortality **(A–D)** and cardiac death **(E–H)** and the eGFR calculated by of four equations. Group 1 (eGFR ≥ 90 ml/min/1.73 m2) as reference group, Group 2 (60 ≤ eGFR<90 ml/min/1.73 m2), and Group 3 (eGFR < 60 ml/min/1.73 m2) were compared with it, separately. Adj-model was adjusted for baseline variables significantly and factors closely related to the outcome of patients with cardiovascular disease, such as age, sex, smoking, body mass index (BMI), diabetes mellitus (DM), hypertension (HT), and low-density lipoprotein cholesterol (LDL-c), left ventricular ejection fraction (LVEF), C-reactive protein (CRP), and procedural success. Unadj-model, Unadjusted model; Adj-model, Adjusted model; Ptrend, P for trend.

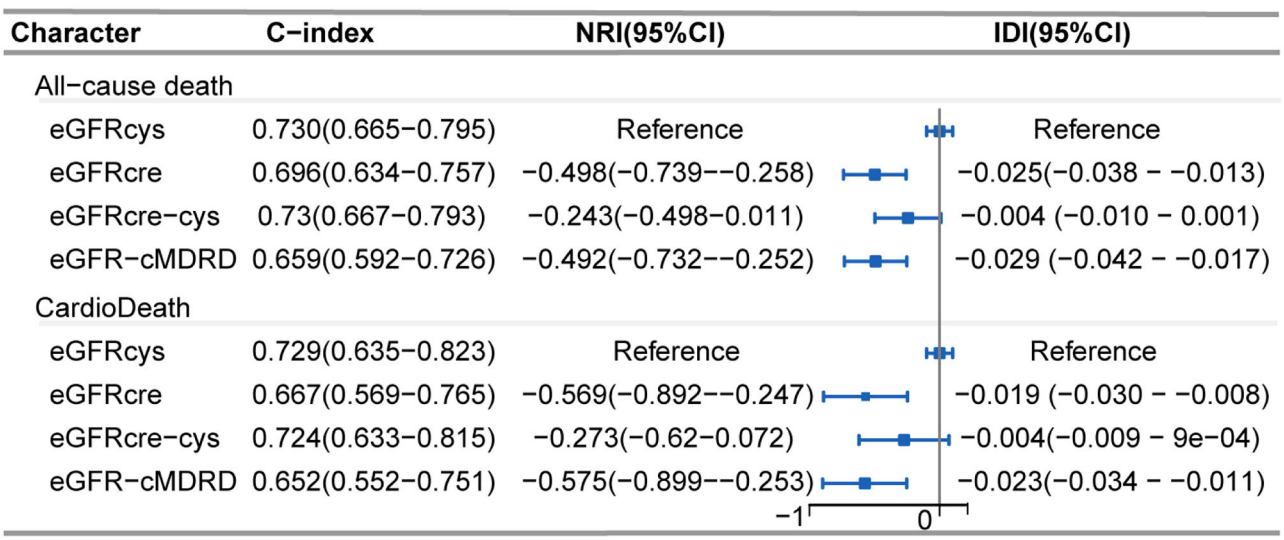

FIGURE 5 | Forest plot showing net reclassification improvement of renal function calculated by various eGFR equations. Net reclassification improvement in all-cause mortality and cardiac death was assessed by comparing eGFRcre, eGFRcre-cys, and eGFR-cMRDR with eGFRcys (reference), individually. C-index: Harrell's concordance index. NRI, Net reclassification improvement; IDI, integrated discrimination improvement; CI, confidence interval.

Regarding discrimination, NRI and IDI in all-cause mortality and cardiac death were assessed by comparing eGFRcre, eGFRcre-cys, and eGFR-cMDRD to eGFRcys (reference). Overall, the reclassification of other equations performed worse both on all-cause mortality and cardiac death (**Figure 5**). Cystatin C-based eGFR also showed the best performance with the maximum C-index (**Figure 5**) both in all-cause mortality and cardiac death.

DISCUSSION

This study showed an increase in all-cause mortality and cardiac death after CTO PCI as baseline renal function declined. Patients with renal insufficiency represent a high-risk patient subset. The values of the four measures showed similar accuracy using the area under the receiver operating characteristic curves. The classification into standard kidney stages showed significant

differences in the survival curves and Cox regression models for both endpoints.

Given the rapidly aging population in the majority of regions of the world, along with the growing prevalence of chronic disease, chronic kidney disease has become a significant contributor to the increased morbidity and mortality (1). Consistent with the findings made in previous studies for patients suffering from both cardiovascular disease and renal impairment (15, 16), we found that patients age and the prevalence of adverse cardiovascular event were increased across stages of renal dysfunction in patients received CTO-PCI. Patients with renal impairment more frequently presented with a significantly lower (although within normal range) ejection fraction ($p < 0.001$) and increased levels of CRP. We also found some HR *p*-values which are significant in the univariate analyses become non-significant after adjusting for confounders, and this statistical phenomenon mostly occurred in creatinine-based eGFR. The true causal effect between eGFR values and outcome is affected by confounders (17), defined as variables that are associated with both eGFR values and outcome, but influenced by neither. Differences among the eGFR values with respect to risk relationships probably reflect confounding by non-GFR determinants of the filtration markers. It is well-known that the non-GFR determinants of serum creatinine, including age, sex, BMI, DM, and others can confound the associations between the creatinine-based eGFR and outcomes (18). Non-GFR determinants of cystatin C also exist, though they are quantifiably smaller than those of creatinine (19).

The main novelty of our research centers on the fact that it was specifically performed in patients with CTO. To the best of our knowledge, this is the first study to address the prognostic usefulness of eGFRcys, by comparing it with eGFRcrea, eGFRcre-cys, and eGFR-cMDRD in patients that received CTO PCI. The analysis of our series of patients suggested that eGFRcys might be a better predictor for cardiovascular events in patients with renal impairment (< 60 ml/min/1.73 m^2) when compared with the individuals with normal renal function (>90 ml/min/1.73 m^2). The main finding in our research is that the eGFRcys performed better than other three eGFR equations in assessing the adverse cardiovascular risk in both cardiac death and all-cause mortality in our CTO population.

Consistent with our findings, Shlipak et al. (7, 20) found that serum levels of cystatin C were strongly predictive of all-cause mortality and cardiovascular disease risk, whereas serum levels of creatinine showed relatively poorer performance in predicting adverse outcomes. Peralta et al. (21) demonstrated that the eGFRcys played a more important role than eGFRcre in identifying the high risk of adverse outcome, both in patients with a moderate to severe renal insufficiency (eGFR < 60 ml/min/1.73 m^2) and in patients with a mild renal insufficiency (60–89 ml/min/1.73 m^2). Recently, Shlipak et al. (6) demonstrated the increased risk of death for eGFRcys for patients with a mild reduction in renal function (60–89 ml/min/1.73 m^2). Pedro et al. (22) provided the evidence suggesting that the use of eGFRcys improved the role of eGFR in risk stratification of all-cause mortality for patients with non-ST-segment elevated acute coronary syndrome, as compared with eGFR-MDRD. Helmersson-Karlqvist et al. (23) and Lim et al. (24) found that cystatin C-based eGFR was more closely associated with mortality as compared with both eGFRcre and eGFRcre-cys. Peralta et al. (21) also found the benefits of the eGFRcys in reclassifying mortality risk among adults diagnosed with CKD using the eGFRcre who had the highest risk for complications. Similar clinical applications of cystatin C in a subset of patients would be its use as a confirmatory test, where cystatin C would predict risk for all-cause mortality and cardiovascular events among hypertensive patients (25). Thus, above results are consistent with our findings that eGFRcys can improve the risk-grading by identify patients at different levels of risk for adverse outcome whose risk has been estimated with eGFRcre.

STRENGTHS

One strength of our research was the use of eGFRcys to estimate the risk of cardiac death and all-cause mortality with standard statistical methods, which could be better than the use of the other equations. It should be noted that of more significance to our study was the assessment of a distinct subset of population, i.e., CTO patients. Our results may provide physicians with a support for the need of reconsidering which eGFR equation is the best one for assessment of long-term adverse events in CTO patients.

LIMITATIONS

The research is a single-center retrospective observational design and is restricted in generalization of the findings. Future multicenter prospective study with a larger number of patients and a longer follow-up is required.

CONCLUSION

This study has demonstrated that all-cause mortality and cardiac death are increased across stages of renal dysfunction, and the decreased eGFR calculated with cystatin C has a higher risk predictive value to assessing risk and risk-grading in terms of all-cause mortality and cardiac death in our CTO population than eGFR calculated by other three equations.

DATA AVAILABILITY STATEMENT

The data generated during this study are included in this article. Raw data are available upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research and Ethics Committees of the First Affiliated Hospital of Xi'an

JiaoTong University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BL: conceptualization, project administration, writing - original draft, and writing - review and editing. JR: methodology, project administration, data analysis, and writing - review and editing. BW, KG, Y-MH, SL, and ZJ: investigation and data curation. XW: data curation and writing - review. HL: resources and data curation. LC: writing - review and editing. YZ and HH: writing - review and editing and data curation. YP: data curation. YW: resources, funding acquisition, and writing - review and editing. X-ZZ: supervision, project administration, and writing - review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2020.586181/full#supplementary-material

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frontiers in Cardiovascular Medicine

CASE REPORT
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Right Atrial Thrombus in a COVID-19 Child Treated Through Cardiac Surgery

Hamid Bigdelian[1], Mohsen Sedighi[2], Mohammad Reza Sabri[1], Bahar Dehghan[1], Zahra Pourmoghaddas[3], Rana Saleh[3], Alireza Sherafat[4], Gholamreza Massoumi[1], Mohammad Kazem Rezaei[1] and Masoud Shahbazi[1]*

[1] Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran, [2] Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran, [3] Pediatric Department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [4] School of Medicine, University of Central Lancashire, Preston, United Kingdom

We herein report a case of large intracardiac thrombus in a child with SARS-CoV-2 infection (COVID-19). The diagnosis of COVID-19 was confirmed through HRCT and RT-PCR. Transthoracic echocardiography revealed a large thrombus in the right atrium treated successfully *via* cardiac surgery. The underlying mechanisms of this thrombus in the COVID-19 infection may be attributed to the hypercoagulation and inflammatory condition incurred by the COVID-19 virus.

Keywords: COVID-19, cardiac surgery, pediatric, thrombus—echocardiography, fever

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***Correspondence:**
Mohsen Sedighi
sedighi.mo@iums.ac.ir
orcid.org/0000-0003-0608-7840

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INTRODUCTION

In December 2019, an outbreak of new viral pneumonia caused by the novel coronavirus (SARS-CoV-2) occurred in Wuhan, China (1). This virus is responsible for COVID-19 (Corona Virus Disease 2019) and can lead to different symptoms ranging from a mild viral disease to acute respiratory distress syndrome (ARDS), multi-organ failure, and death (2, 3). Although cardiovascular complications of COVID-19 has been well-described in the current literature, intracardiac thrombus complication in children has been rarely reported. Intracardiac thrombus caused by SARS-CoV-2 infection is a serious and life-threatening complication in COVID-19 patients (4) and the size of thrombus at the time of diagnosis correlates strongly with increased risk of thromboembolism and sudden death (5). Here, we describe a case report of large thrombus formation in the right atrium (RA) of a child with COVID-19 infection required emergency cardiac surgery, as the only preferred treatment to remove such a large thrombus.

CASE REPORT

On 7 April 2020, an 11-years-old boy was admitted to the pediatric hospital in Esfahan with high fever, dyspnea, and skin rashes. Past medical history of patient showed that he was a known case of seizure disorder and neurodevelopmental delay, and had been under treatment with phenobarbital. Blood gas analysis on the admission time showed a pH of 7.27, pCO_2 of 49.5 mmHg, pO_2 of 44.8 mmHg, and oxygen saturation (O_2 Sat) of 73%. Also, his laboratory test results were as follows: white blood cell count (WBC) of 11.4 × 10*9/L (reference range: 4.5–11), C-reactive protein (CRP) 38 mg/dL (reference range: up to 6), erythrocyte sedimentation rate (ESR) 38 mm/h (reference range: up to 20), and lactate dehydrogenase (LDH) 427 U/L (reference range: 140–280). Chest x-ray and high-resolution computed tomography (HRCT) showed typical ground-glass opacitiesin both lungs, suggesting viral pneumonia (**Figure 1**). Throat swab samples analysis by reverse transcription-polymerase chain reaction (RT-PCR) confirmed COVID-19 infection and treatment of disease was started according to the Iranian pediatric protocol including hydroxychloroquine (5 mg/kg/dose), lopinavir /ritonavir (230 mg/m^2/dose), ceftriaxone (75 mg/kg/dose), and vancomycin (10 mg/kg/dose) (6). On the 2nd day of hospitalization, he presented shortness of breathing and decreased level of consciousness (LOC) that led to intubation and he put on a ventilator. After extubation, Bi-level positive airway pressure (BiPAP) was administrated for respiratory support. Despite receiving drug treatment for COVID-19, his laboratory test results were significant for continuous leukocytosis and neutrophilia (**Table 1**). Because of persistent fever and tachycardia, pediatric cardiology consultation was requested and transthoracic echocardiography revealed a large mobile homogenous mass (2.5 × 1.5 cm) on the tricuspid valve leaflet extended to the RA and right ventricle (RV) with attachment to the tip of central venous catheter (CVC) that was in favor of thrombus or vegetation (**Figure 2**). Besides, trivial tricuspid regurgitation (TR) and trivial pulmonary insufficiency (PI) were found. Consequently, he was transferred to the Chamran Heart Center on 16 April 2020 for surgical intervention. A sternotomy with cardiopulmonary bypass was performed and intraoperative observations revealed a large thrombus in RA which was removed completely together with CVC while cardiac valves were preserved (**Figure 3**). After the surgery, he was admitted to the ICU, and the pediatric ward thereafter. Culture result of the thrombus showed no fungal and bacterial infection and also SARS-CoV-2 RNA was not detected in the mass. Histological analysis of the mass showed inflammatory infiltrate (mostly neutrophils) formation along with organizing thrombus and partial necrosis of tricuspid valve leaflet. He was discharged after 2 weeks in good health condition.

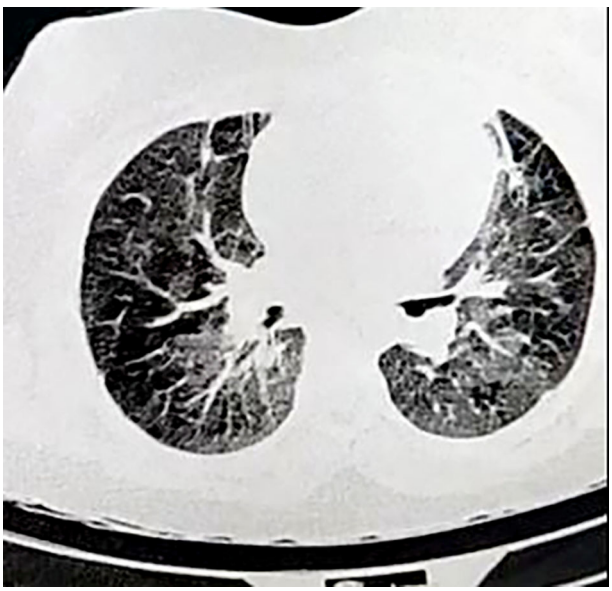

FIGURE 1 | High-resolution computed tomography (HRCT) of the lungs in COVID-19 child showing bilateral multiple patchy areas of ground glass opacities.

TABLE 1 | Laboratory test results of patient during the course of disease.

Variables	Reference range	Admission	Day 5	Day 10	Before surgery	After surgery
RBC count (×10*12/L)	4.5–6.5	3.84	4.04	4.13	3.88	4.63
Hematocrit (%)	41–51	36.9	38.3	38.4	31.6	41.3
Hemoglobin (g/dL)	13–17	11.5	12.5	13.3	10.9	14.5
WBC (×10*9/L)	4.5–11	11.4	12.2	13.5	21.3	11.2
Lymphocyte count (%)	20–40	27	15	16	15.1	17.5
Neutrophil count (%)	50–70	66.4	78	72.1	75.7	72.9
Platelet count (×10*9/L)	150–450	252	279	247	400	238
PT (second)	11–13	13	12.8	12.2	17.1	16
PTT (second)	26–45	38	37	33	30	28
INR	0.8–1.2	1.1	1.1	1.1	1.4	1.2
Blood culture (×3)	+ / -	-	-	-	-	-

RBC, red blood cell; WBC, white blood cell; PT, prothrombin time; PTT, partial thromboplastin time; INR, international normalized ratio.

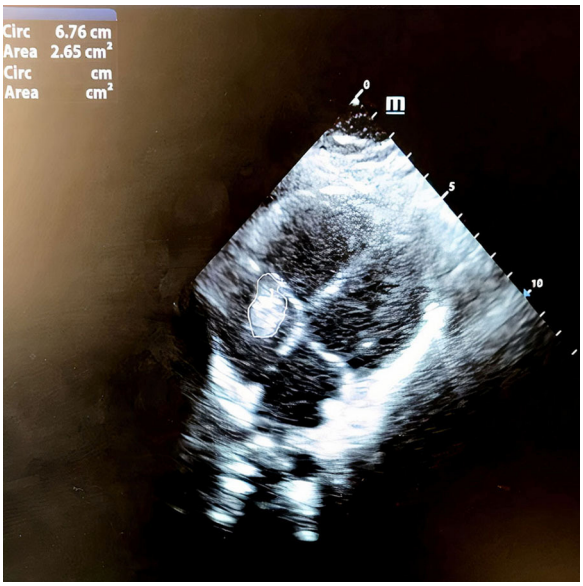

FIGURE 2 | Transthoracic echocardiogram showing a large 2.5 × 1.5 cm mass on tricuspid valve leaflet protruded to the right atrium and right ventricle.

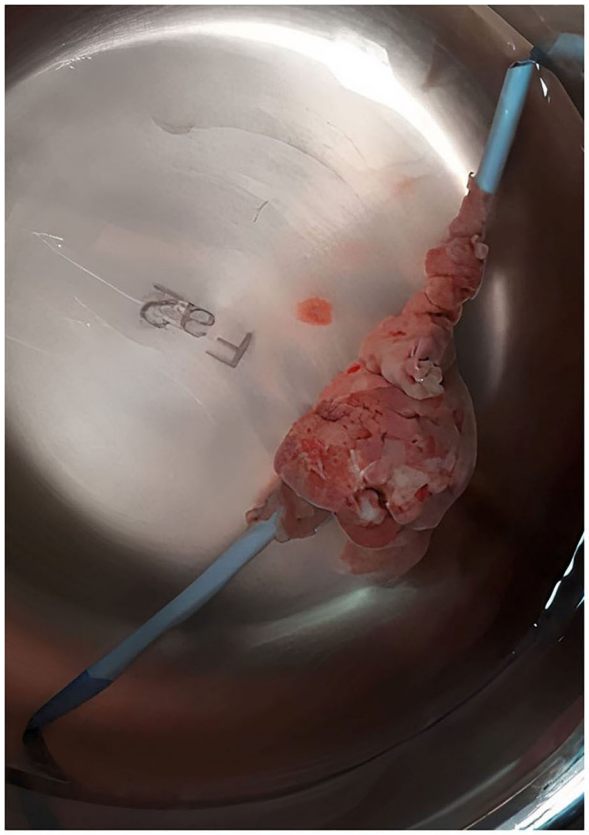

FIGURE 3 | Formation of a large thrombus around the CVC that was removed along with catheter via open heart surgery.

DISCUSSION

Accumulating evidence has indicated cardiovascular involvement of COVID-19 infection which can lead to the poor clinical prognosis of this disease. Discriminating between a cardiac or pulmonary cause of symptoms can be difficult because each may present mostly with dyspnea. Recent reports have shown that COVID-19 is associated with the increased incidence of cardiovascular complications including myocarditis, acute cardiac injury, and arrhythmias (7). Moreover, it has been demonstrated that inflammation and thrombosis mutually reinforce each other and COVID-19 infection can lead to the coagulopathy likely due to infection-induced inflammatory changes (8). Remarkable inflammation is observed in patients with COVID-19 infection presenting by elevated levels of IL-6, increased CRP and erythrocyte sedimentation rate (ESR). This inflammatory condition and subsequent activation of coagulation are the probable causes for the hypercoagulable state in COVID-19 infection (9). The intracardiac thrombus formation has rarely been described in the COVID-19 patients and our finding supports current reports that indicate higher thrombotic risk in COVID-19 patients (10, 11). The presence of a large thrombus in RA was an unexpected finding in our case because he had no history of heart disease or coagulopathy. Similar to the other reports, we believe that the bigger the size of the clot, the greater the chance that medical treatment will fail (12). Removal of such a large thrombus needs surgical intervention and surgical thrombectomy should be opted to treat large right atrial thrombus (more than 2 cm) in the absence of any contraindication for surgery. In parallel with us, a literature review by Negulescu et al. has reported a lower mortality rate in the surgical intervention compared to the anticoagulation treatment. Meanwhile, anticoagulation treatment correlates with the theoretical risk of lysed clots lodging in pulmonary arteries and consequent pulmonary thromboembolism (13). In conclusion, there is an increasing concern about hypercoagulation and acute thrombosis in patients with COVID-19 infection. Therefore, conservative treatment with anticoagulation along with vigilant observation is recommended in all COVID-19 patients to prevent subsequent hypercoagulation and thrombus formation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study involving human participant was reviewed and approved by Isfahan University of Medical Sciences (IR.MUI.MED.REC.1399.198). Written informed consent to

participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors contributed to the analysis, interpretation of data, wrote the manuscript, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

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REVIEW
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Association Between Periodontal Disease and Atherosclerotic Cardiovascular Diseases: Revisited

Faraedon Zardawi[1], *Sarhang Gul*[1]*, *Ali Abdulkareem*[2], *Aram Sha*[1] *and Julian Yates*[3]

[1] *Periodontics Department, College of Dentistry, University of Sulaimani, Sulaymaniyah, Iraq,* [2] *Department of Periodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq,* [3] *Division of Dentistry, School of Medical Sciences, University of Manchester, Manchester, United Kingdom*

Atherosclerotic cardiovascular disease (ACVD) is an inflammatory disease of the coronary arteries associated with atheroma formation, which can cause disability and often death. Periodontitis is ranked as the sixth most prevalent disease affecting humans affecting 740 million people worldwide. In the last few decades, researchers have focused on the effect of periodontal disease (PD) on cardiovascular disease. The aim of this review was to investigate the association between these two diseases. PD is a potential risk factor that may initiate the development, maturation, and instability of atheroma in the arteries. Two mechanisms were proposed to explain such association, either periodontal pathogens directly invade bloodstream or indirectly by increasing systemic level of inflammatory mediators. Interestingly, it has been suggested that improvement in the condition of one disease positively impact the condition of the other one. Highlighting the association between these two diseases, the importance of early diagnosis and treatment of PD and its impact on cardiovascular status may be of great value in reducing the complications associated with ACVDs. Further *in vitro* and *in vivo* studies with longer follow up are necessary to confirm the causal relationship between PD and ACVDs.

Keywords: periodontal therapy, relation, periodontal disease, cardiovascular diseases, atherosclerosis

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***Correspondence:**
Sarhang Gul
sarhang.hama@univsul.edu.iq

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INTRODUCTION

Periodontal disease (PD) is an inflammatory disease primarily initiated in response to a specific group of bacteria and characterized by a complex host-biofilm interaction (1). According to the World Health Organization, the severe form of periodontitis causes tooth loss in about 5–15% of the population worldwide, and it is considered the sixth most common disease affecting humans (2). Aberrant immune–inflammatory responses determine a patient's susceptibility to developing periodontitis, which may be modified by a range of risk factors (3). The transition from gingivitis to periodontitis initiates when the population and activity of a specific group of periodontal pathogens, predominantly Gram-negative anaerobic bacteria such as *Porphyromonas gingivalis* (*P. ginigvalis*), *Aggregatibacter actinomycetemcomitans* (*A. a*), *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*) and spirochetes, increase in the subgingival biofilm (4). These quantitative and qualitative alterations in the bacterial composition of the biofilm are responsible for disturbing the normal symbiotic relationship between the host and its resident microbiota,

leading to an alteration in the hosts immune response. This response can be a "double-edged sword," in that it is an integral defense mechanism but is also simultaneously responsible for periodontal tissue breakdown proportional with the severity of the disease (5).

The inflammatory response in periodontal tissues is characterized by the local production of various pro-inflammatory mediators and enzymes such as C-reactive protein (CRP), interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and matrix metalloproteinases (MMP) (6). Consequently, the rate of periodontal tissue destruction is accelerated with an increase in such mediators. Deep periodontal pockets represent a micro-environment for increased levels of inflammatory cytokines either directly or indirectly. Given the cumulative increase in inflammatory cytokines, and its potential influence on systemic disease processes, this in turn acts as a possible risk factor for several systemic diseases including atherosclerotic cardiovascular disease (ACVD) (7).

ACVDs are a group of disorders affecting the heart and blood vessels, including coronary heart disease, cerebrovascular and other peripheral artery diseases, congestive heart failure, carotid heart disease, and aneurysms. Some ACVDs include two major conditions: ischemic heart disease and cerebrovascular disease which are considered as the first and third cause of death, respectively (7). In Europe, ACVD is responsible for approximately 3.9 million deaths (45% of deaths) annually (8). According to a global survey, there were an estimated 422.7 million subjects with ACVD and 17.9 million deaths due to ACVD in 2015 (9). The pathogenic process causing ACVD is very complex. It is recognized that elevated level of low density lipoprotein cholesterol is the principle element in the pathogenesis of ACVD that change cellular permeability and has impact on arterial walls. Inflammatory cells and cytokines induce plaque formation in the walls of blood vessels, and are also responsible for propagation and rupture of the established plaque along with the resultant thrombotic complications (10).

Mechanisms that have been proposed to explain the link between PD and ACVD include the inflammatory pathways common to both diseases, such as increased levels of white blood cells (WBC), CRP, fibrinogen, intercellular adhesion molecule-1 (ICAM-1), and proinflammatory cytokines (11). Additionally, both diseases share similar risk factors such as smoking, poor oral hygiene, diabetes mellitus (DM), obesity, stress and reduced physical activities. Despite these common features, it is difficult to conclude that periodontitis is a primary causal factor of ACVD, as a result of the complexity in the confounders that correlate PD to ACVD (12).

Although stronger and more suggestive evidence has emerged to highlight a causal relationship between the two pathologies, but it was still insufficient for PD to be classified as a causal risk factor (13). Therefore, further studies are required to provide a more robust, consistent link in order to confirm PD as an independent and potentially adjustable risk factor for ACVD (12). Therefore, the current review attempts to review and update the current evidence and provide further insight into the relationship between PD and ACVD.

PATHOGENESIS OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASES INCLUDING THE ROLE OF PERIODONTAL DISEASE

Atherosclerotic disease is a focal thickening of vascular intima residing between the endothelial lining and smooth muscle cell (SMC) layers of blood vessels in response to an immune response (14). Endothelial dysfunction is the earliest change in atherosclerotic formation. The primary etiological factor of atherosclerosis is un known (15). However, other risk factors significantly contribute to the development and progression of this pathology, such as aberrant profile of plasma cholesterol, smoking, hypertension, DM, and increased levels of inflammatory mediators including CRP and cytokines (15).

Atherosclerosis starts with accumulation of low density lipoprotein (LDL) within the intima layer where it is oxidized. This in turn activates increased expression in nearby endothelial cells of cell surface proteins such as ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and selectins (15). Adhesion of circulating inflammatory cells (monocytes, lymphocytes) to these adhesion molecules is increased by their diapedesis into the inflamed intima site (15). The initial development of the atherosclerotic lesion occurs through differentiation of monocytes to macrophages that scavenge on LDL, thus forming foam cells and subsequently fatty streaks (15, 16). Later, a T-leukocyte induced-cell-mediated immune response with increased level of inflammatory cytokines such as INF-γ, TNF-α, and IL-1β further accelerate atherogenesis (17). T-cell-associated mediators stimulate migration and mitosis of SMC to form a fibrous pseudo-capsule around the lesion (17). Macrophages loaded with lipids undergo apoptosis leading to formation of a necrotic core underneath the fibrous capsule, which renders it susceptible to rupture, thus leading to formation of fatal thrombosis (14).

Cumulative evidence from literature over the last decades have supported the role of PD as an independent risk factor for ACVD (18). The presence of certain periodontal pathogens, Gram-negative anaerobes in particular, in subgingival biofilm has been associated with increased risk of MI; the odds ranging between 2.52 and 2.99 with the presence of *T. forsythia* and *P. gingivalis*, respectively, in comparison to controls (19). The hallmark of periodontitis is elevation in the levels of Gram-negative bacteria that are characterized by their ability to trigger an intense immune response via their mechanism of pathogenicity, such as lipopolysaccharide (LPS) (20). Moreover, some of these bacterial species possess the potential to invade deeper tissues, reaching the circulation and inducing a systemic immune response away from their original habitat (21). Results from several *in vivo* and *in vitro* studies have suggested that periodontal bacteria associated with chronic inflammation may compromise the epithelial-barrier function by epithelial-mesenchymal transition (22–24). Epithelial-mesenchymal transition comprises cellular events starting with loss of polarity, cytoskeletal, and adhesion proteins, ending with loss of epithelial-phenotype and acquisition of mesenchymal-like characteristics (25). This results in loss of epithelial sheet coherence and formation of

microulceration; thus, facilitating the penetration of motile periodontal pathogens/virulence factors to the underlying connective tissue and exposed blood vessels. On the other hand, periodontal bacteria can invade host cells as part of their defensive strategy to evade host immune responses (26). This intracellular localization provides not only protection from the body's defensive mechanisms but also a shelter from action of antimicrobials (26). Periodontopathogens such as *P. gingivalis* residing within the cells either stay dormant or multiply by modulating cellular machinery (27). Once multiplied, *P. gingivalis* leaves the epithelial cells via the endocytic recycling pathway to infect other cells or gain access to the circulatory system (28). The trafficking of *P. gingivalis* into endothelial cells is positively influenced by bacterial load, and certain virulent proteins such as gingipains, fimbriae, and hemagglutinin A (29). Further, invasion of gingival epithelial and endothelial cells by *P. gingivalis* could be synergized by *Fusobacterium nucleatum* (30) and *T. forsythia* (31).

Two mechanisms have been proposed to explain how PD influences ACVD. First, a direct mechanism by which periodontal pathogens directly invade endothelial cells. This notion is supported by polymerase chain reaction assays for atherosclerotic plaques (32). Analysis of cardiovascular specimens containing thrombus tissues demonstrated that *Streptococcus mutans* was the most prevalent bacteria (78%), followed by *A. a* (33). Atherosclerotic lesions formed in coronary arteries also exhibited the presence of other bacteria such as *P. gingivalis*, *Prevotella intermedia*, and *T. forsythia* (34). It is not clear how the presence of bacteria intracellularly influences atherosclerosis but some pathogens, e.g., *P. gingivalis*, could trigger foam cell formation or their persistence within the cells, and thereby provoke a state of secondary inflammation that leads to endothelial dysfunction (35).

Increased systemic levels of inflammatory cytokines due to PD is the second suggested mechanism (indirect pathway). PD stimulates a systemic inflammatory response which results in chronically elevated levels of different cytokines, also related to atherosclerotic vascular disease, such as IL-1β, IL-6, IL-8, TNF-α, and monocyte chemoattractant protein-1. Some can enhance rapid hepatic synthesis and secretion of intravascular plasma proteins such as CRP protein and fibrinogen (36, 37). Additionally, bacterial products such as LPS could enter the circulation and induce a potent immune response. These aforementioned factors could initiate atherosclerosis by their action on endothelial cells, modulating lipid metabolism, and increasing oxidative stress (38). This was supported by results from a previous study that indicated endothelial dysfunction in patients with periodontitis (39).

Despite robust evidence drawn from many studies linking PD to the initiation and progression of ACVD (which is discussed in section Effects of Periodontal Disease on the Incidence of Atherosclerotic Cardiovascular Diseases), these results require further support to pinpoint the exact pathological mechanism(s) between PD and ACVD. The presence of atherosclerotic diseases and their hypothesized relation with PD are illustrated in **Figure 1**.

CONFOUNDERS BETWEEN PERIODONTAL AND ATHEROSCLEROTIC CARDIOVASCULAR DISEASES

As previously discussed, the available literature has provided ample evidence in relation to the existence of a relationship between PD and ACVD. However, this link is not easily comprehended and it could be further complicated by the presence of other systemic diseases, genetic factors, and lifestyle-related habits. These factors could simultaneously influence the progression of PD and ACVD.

Chronic stress is a response associated with stimulation of a sympathetic nervous system which induces the adrenal glands to increase secretion of adrenalin and cortisol in order to cope with the stress (40). Furthermore, stress activates the hypothalamus-pituitary-adrenal axis, which together with a trigger from the sympathetic nervous system causes upregulation of catecholamines, glucocorticoids and inflammatory cytokines (41). Limited studies are available on the influence of stress on the progression of PD in humans; however, it is well-documented that the systemic level of inflammatory cytokines is significantly increased in response to prolonged stress (41). These cytokines are common to destructive events of PD. Experimental *in vitro*, and *in vivo* animal studies have provided potential mechanisms by which stress can contribute to periodontal tissue breakdown (42). Concomitantly, several types of stress are involved in the development of cardiovascular disease, including oxidative stress, mental stress, hemodynamic stress and social stress. The relationship of stress and ACVD has been thoroughly investigated and the results suggest that individuals suffering from ACVD and under psychological stress are more prone to transient myocardial ischemia, risk of recurrent ACVD and increased mortality (41).

Smoking is a well-recognized risk factor for PD and ACVD. Among toxic products generated during smoking, nicotine is one of the most harmful (43). This substance is responsible for vasoconstriction that compromises delivery of nutrients to the periodontium. In addition, nicotine significantly suppresses cellular/humoral immune responses and causes neutrophil dysfunction (43). Similarly, toxic products produced during burning of tobacco induce the atherogenic mechanism by increasing oxidation of LDL, causing chronic inflammation in the intima layer and subsequent endothelial dysfunction (44, 45). Smoking increase platelet aggregation, blood viscosity and shifts the pro- and antithrombotic balance toward increased coagulation. The contribution of smoking to the pathophysiology of PD and ACVD has been demonstrated by a significant reduction in the strength of association between these two diseases after adjustment of smoking (46). A systematic review showed that 11 out of 15 cross-sectional studies had suggested a modest relationship between ACVD and PD after adjusting for several risk factors including smoking (47).

Diabetes mellitus (DM) is a metabolic disease that adversely affects the body through different mechanisms including periodontal and cardiovascular health. Persistent increase in glucose level is expressed as microvascular changes leading to

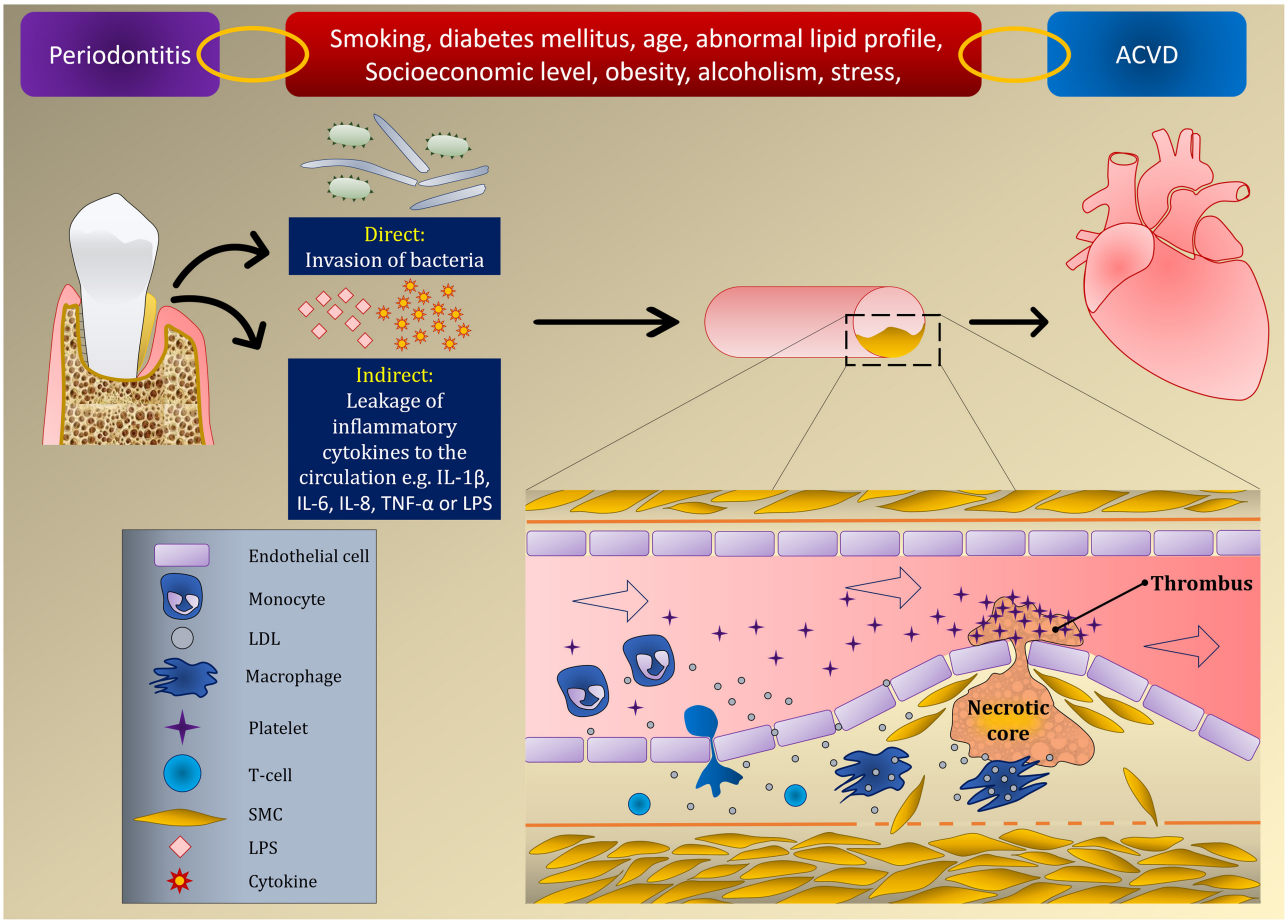

FIGURE 1 | Relationship between PD and ACVD induced by endothelial dysfunction.

endothelial cells dysfunction (48), mainly via increased level of TNF-α, interleukins and proteinases that enhances apoptosis of endothelial cells (49). DM-associated complications also affect the bone healing capacity, mineral density, and turn-over rate (50). These DM-associated systemic events significantly increase progression rate of PD. The odds of periodontitis are increased by almost three- to four-fold in diabetic patients compared to healthy controls (51). This is supported by several studies that demonstrated an increase in the prevalence and severity of PD regardless of age, gender, and ethnic group (52). Undoubtedly, DM is a profound risk factor for initiation and progression of periodontitis and ACVD that should be considered when studying the association between these two diseases.

Both periodontitis and ACVD are multifactorial diseases whose development and progression require interaction between several factors, among which is genetic predisposition. A study conducted on twins, utilizing quantitative genetic analyses, showed evidence of an association between ACVD and PD (53). Interestingly, three of the loci among four genes significantly associated with PD, namely ANRIL/CDKN2B-AS1, PLG, and CAMTA1/VAMP3, showed association with ACVD (54). Furthermore, results from a candidate-gene association study also concluded that periodontitis and ACVD are genetically related through at least one susceptibility locus (55). Despite the fact that these studies highlighted a novel shared pathologic pathway between the two conditions, larger scale genetic studies are highly recommended.

Any potential contribution of periodontitis to the pathology of ACVD should be carefully interpreted as many confounding factors could affect both conditions and result in overestimation of this relationship. Thus, adjustment of these risk factors need to be taken into consideration during statistical analysis.

BIOMARKERS SHARED BY PERIODONTAL AND ATHEROSCLEROTIC CARDIOVASCULAR DISEASES

Endothelial dysfunction is the earliest stage of atherosclerosis and a possible link between PD and ACVD (56). Several studies have linked periodontitis to endothelial dysfunction and this relationship is sustained by several shared biomarkers of periodontitis, ACVD and endothelial dysfunction (47). Despite the potential for these biomarkers to identify the

strength of this correlation, they are still not considered as "gold standard" diagnostic markers (47). Upon initiation of periodontitis, expression of inflammatory cytokines markedly increases together with alteration in the lipid profile which could contribute to the development and aggravation of thrombogenesis and thromboembolic events (57). It has been reported that PD is significantly associated with upregulation of biomarkers responsible for endothelial dysfunction and dyslipidemia such as CRP, tissue plasminogen activator (t-PA), and LDL-cholesterol (C), TNF-α (58). Additionally, periodontitis is associated with higher levels of other inflammatory serum biomarkers including von Willebrand factor (vWF), fibrinogen, and endothelial progenitors' cells (58). Interestingly, levels of these serum biomarkers are reduced following periodontal therapy (59, 60).

A systematic review investigated the serum level of a group of mutual biomarkers in order to define the strength of evidence relating PD, CVD, and endothelial dysfunction. The analysis of results indicated that the levels of different inflammatory markers, IL-6 and CRP in particular, were elevated. These outcomes of this systematic review suggested that endothelial dysfunction may be the link between PD and ACVD (61). Furthermore, it was found that ACVD is associated with more severe periodontitis and this was marked by higher serum level of high sensitivity (hs)-CRP (62). Elevated level of hs-CRP due to periodontitis exerts stress additional to the previously existing inflammatory activity of atherosclerotic lesion; consequently, increasing the risk of ACVD (63). Recently, periodontitis was found to be associated with high levels of IL-6, PTX3, and sTWEAK in patients with cerebral small vessel disease, increasing by almost 3 times the likelihood of having this type of ACVD (64). This was supported by results from an *in vivo* study that indicated changes in vascular inflammatory biomarkers, IL-6, PTX3, and sTWEAK, in systemic circulation after injection of LPS from *P. gingivalis* in rats (65).

Indeed, the current literature has provided valuable information about shared biomarkers between PD and ACVD which may offer predictive and diagnostic potential to significantly reduce the risk of developing undesirable cardiac events at earlier stages (**Table 1**). However, further studies are required in this regard as the exact signaling downstream of ACVD and PD biomarkers has not yet been fully elucidated.

EFFECTS OF PERIODONTAL DISEASE ON THE INCIDENCE OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASES

The joint workshop between the European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) in 2012 presented evidence linking PD and ACVD (75). The evidence included the role of periodontopathogenic bacteria in ACVD and clinical (epidemiological and intervention) studies that support the association between these two diseases (76) which will be highlighted in this section.

Microbiological Studies

Clinically, it is very difficult to find the causative agents of atherosclerosis. Firstly, the endothelial injury usually develops and progresses without symptoms, potentially masking the initiating agent. Secondly, multiple factors can lead to a common inflammatory response such as an atherosclerotic lesion, and these factors could be co-existent, which further complicates identifying the causative factor (as discussed in section Confounders Between Periodontal and Atherosclerotic Cardiovascular Diseases). Additionally, studies relating to interventions performed in this respect have reported mixed results, such as no change, temporary worsening of signs after periodontal treatment or improvement in signs (75, 77). Nevertheless, any reported evidence on the potential role of periodontal pathogens in promoting atherosclerosis has to fulfill the following seven proofs (76).

Proof 1: Periodontal bacteria can reach systemic vascular tissues Undoubtedly, many studies have shown that oral bacteria in general and periodontopathogenic in particular can enter the systemic circulation and cause bacteremia (12, 14, 76, 78–80). A previous systematic review has shown that following periodontal procedures the incidence of bacteremia can be as high as 49.4% (81). The prevalences of periodontopathogenic bacteria in systemic vascular tissue following periodontal procedures and in atheromatous lesions without periodontal procedure in subjects with chronic periodontitis are summarized in **Table 2**. It can be concluded that periodontal pathogens could potentially invade the systemic vascular tissue following periodontal procedures as well as contribute to atheromatous lesions. As Koch's postulate cannot be applied in humans, the direct cause-effect of these periodontopathogenic bacteria in development of atherosclerosis still needs to be confirmed.

Proof 2. Periodontal bacteria can be found in the affected tissues There is sufficient evidence from several studies that different oral bacterial species can be identified in atheromatous lesions using DNA, RNA, antigen and passive sequencing (91–93). Analyses of samples have shown that periodontitis subjects are at high risk for development of atherosclerosis (94).

Proof 3. Evidence of live periodontal bacteria at the affected site Detection of live periodontopathogenic bacteria is essential to fulfill this proof. Live *P. gingivalis* and *A. a* have been isolated from atheromatous lesions by at least two studies (95, 96).

Proof 4. In vitro evidence of invasion of affected cell types A number of *in vitro* studies showed that periodontopathogenic bacteria can invade different types of host cells. Studies have demonstrated invasion of endothelial cells by *P. gingivalis* (97, 98) and the mechanism as well as the importance of the particular strain type have been evaluated in a further study (99).

Proof 5. Demonstration that periodontal bacteria can promote atherosclerosis in animal models of disease The review provided by EFP and AAP in 2012 demonstrated evidence that periodontopathogenic bacteria can induce and promote atherosclerosis (75). *P. gingivalis* has been shown to enhance atherosclerosis in murine (100), rabbit (101), and pig (102) models. Furthermore, when mice with hyperlipidemic conditions were infected orally with *P. gingivalis*, *T. forsythia, T.*

TABLE 1 | Studies correlating ACVD and PD biomarkers.

References	Aims	Study groups, *N*	Sample	Biomarkers, assays[†]	Clinical parameters[¶]	Results and conclusions
Joshipura et al. (58)	Health Professional Follow-up Study to evaluate associations among periodontal disease, tooth loss, and specific biomarkers in blood	Male health professionals ($N = 18,225$)	Blood samples	• CRP measured by an ultra-sensitive immunotechnique • Fibrinogen level by the Clauss method • Factor VII, t-PA, sTNF-R, and vWF antigen concentrations assayed by ELISA.	Self-reported periodontal disease and numbers of natural teeth	• CRP, t-PA, and LDL-C significantly ↑ in subjects with self-reported periodontal disease. • Results suggested that periodontal disease is significantly associated with biomarkers of endothelial dysfunction and dyslipidemia
Gita et al. (66)	• Assessment of total cholesterol, LDL, HDL, and triglycerides in periodontal health, and disease • Assessing associations between elevated lipid profiles and periodontal disease	• Control, patients with healthy periodontium ($N = 30$) • Case, patients with PPD ≥5 mm ($N = 30$)	Venous blood	Lipid profile assessed by homogenous enzymatic calorimetry	Oral hygiene index simplified, PPD, CAL, FI, Mobility index, and OPG for assessing bone level	No association between total cholesterol, LDL, HDL, and triglycerides with periodontal disease
Domingues et al. (67)	Assessing the association of markers of cardiovascular risk with severity of periodontitis	• Control ($N = 45$) • Case ($N = 45$)	Venous blood	• LDL- measured by immunoenzymatic assay • CRP, hs-CRP, HDL-c, assayed by immunoturbidimetry automated methodology • Ratio of hs-CRP/HDL-c	• Control group, < 4 sites with PPD ≥ 4.0 mm and CAL ≥ 3.0 mm • Case group, 30% of sites with PPD ≥ 4.0 mm and CAL ≥ 3.0 mm	Severity of periodontitis was inversely and significantly associated with plasma concentrations of HDL-c
Kalburgi et al. (68)	To investigate the serum CRP level, leukocyte count in periodontitis patients and their association with severity of periodontitis	• Healthy control ($N = 10$) • Periodontitis patients ($N = 20$)	Venous blood	• CRP assayed by turbidimetric immunoassay • Determination of TLC and DLC	• Control group; PPD < 3 mm, CAL < 3 mm • Moderate periodontitis; PPD = 4-6 mm, CAL = 3-4 mm • Severe periodontitis; PPD > 6mm, CAL ≥ 5 mm	• CRP in moderate and severe was significantly higher than control • TLC count and neutrophil in particular were significantly higher in severe than moderate periodontitis • ↑ inflammatory burden during periodontitis may ↑ the risk for cardiovascular events
Ramírez et al. (69)	Evaluation of endothelial function, inflammatory biomarkers and subgingival microbial profile associations in patients with and without periodontal disease	• Control, patients with gingivitis and incipient periodontitis ($N = 20$) • Case, patients with moderate to severe periodontitis ($N = 20$)	• Venous blood • Subgingival plaque samples	• Endothelial function measured by FMD • Bacterial characterization by DNA extraction and PCR • MMP-9, MPO, PAI-1, E-Selectin, ICAM-1, adiponectin, and VCAM-1 measured by multiplexed immunocytometric assay	• Control group, clinically healthy periodontium • Case group, patients with at least 10 sites with PPD ≥5 mm + alveolar bone loss in periapical radiographs	↑ population of red complex group, E-selectin, MPO, and ICAM-1 significantly increased in moderate and severe periodontitis cases, suggesting their susceptibility to develop cardiovascular events

(Continued)

TABLE 1 | Continued

References	Aims	Study groups, *N*	Sample	Biomarkers, assays†	Clinical parameters¶	Results and conclusions
Gupta et al. (70)	Clinical trial aimed to correlate the levels of sCD40 L and MCP-1 in serum and GCF of patients with periodontitis before and after SRP	• Healthy control ($N = 15$) • Severe periodontitis patients ($N = 30$)	• Venous blood • GCF	The sCD40 L and MCP 1 levels were quantified using ELISA	• PI, GI, PPD, CAL • Control: GI < 1, PPD < 3 mm, CAL = 0 • Case: patients with two or more inter-proximal sites with CAL ≥ 6 mm, not on the same tooth, and one or more inter-proximal sites with PD ≥ 5 mm	• In periodontitis cases, the sCD40 L levels correlated strongly with MCP-1 levels in both GCF and serum before and 6 w after SRP. • The results highlighted the potential benefits of good oral hygiene level on cardiovascular health
Cotič et al. (71)	To investigate the association between oral health and serum biomarkers among the hemodialysis (HD) patients	Adult patients undergoing maintenance dialysis ($N = 111$)	Blood samples	• CRP level was determined by a chemiluminescent immunometric high sensitivity assay • TnT was measured by an electrochemiluminescence assay • NOx concentration was measured by a colorimetric non enzymatic assay Serum levels of IgA and IgG to *Aa* and *Pg* were determined by ELISA	DMF, API, SBI, and CPITN	Levels of CRP and TnT were higher in edentulous patients, indicating a need for improving dental care to retain the teeth as long as possible
Pedroso et al. (72)	Investigate the concentration of modified (m)LDL level in diabetic type 2 patients with periodontal disease and the effect of periodontal therapy on mLDL and diabetes status over 12 months	• Group 1: diabetic patients with periodontitis ($N = 24$) • Group 2: diabetic patients with gingivitis ($N = 24$)	Blood samples	Glycemia, A1c, total cholesterol, HDL-c, LDL-c, triglycerides, hs-CRP, and oxLDL determined by bicinchoninic acid (BCA) protein assay kit and Z-Scan technique for LDL-c	• PPD, CAL, BOP, PI, and GR • CAL >3 mm in two non-adjacent teeth and the CAL ≥5 mm in 30% or more of the teeth present At least 20 teeth present	• Levels of hs-CRP in Group 1 showed a significant reduction after 12 months • Periodontal treatment improved the LDL-c quality in both groups • Periodontal therapy may help with the control and prevention of hyperglycemia and precursors of cardiovascular diseases
Ameen et al. (73)	• Investigate the levels of the cardiac biomarkers in smoker vs. non-smokers in periodontitis and healthy subjects Assessing the level of cardiac biomarkers with severity of periodontitis	• Control ($N = 20$) • Smoker/periodontitis ($N = 28$) • Non-smoker/periodontitis ($N = 32$)	Venous blood	• AST, ALT, CK, and LDH measured by UV/Vis Spectrophotometer • Tr-I assayed by ELISA	• PI, BI, PPD, and CAL • Diagnosis criteria for periodontitis: Stage II and Stage III (Grade B or C) patients (CAL ≥ 3 mm)	• Tr-I, ALT, AST, LDH, and CK significantly higher in smokers than healthy group • Tr-I, CK, and LDH significantly ↑ in smoker/periodontitis vs. non-smoker/periodontitis • Cardiac biomarkers ↑ during periodontitis and their expression is further aggravated in conjugation with smoking

(Continued)

TABLE 1 | Continued

References	Aims	Study groups, *N*	Sample	Biomarkers, assays[†]	Clinical parameters[¶]	Results and conclusions
Boyapati et al. (74)	To compare and correlate the occurrences of periodontitis with serum levels of cardiac-biomarkers in patients with coronary heart disorders	• Patients with coronary artery diseases ($N = 63$) • Control: patients without periodontitis ($N = 31$) • Test: patients with periodontitis ($N = 32$)	Blood samples	LDL, HDL, VLDL, TC, hs-CRP, Troponin T, Troponin I, Myoglobin assessed by chemiluminiscence immunoassay	Periodontitis was defined as: • BOP • CAL≥ 1 site • At least 20 natural teeth • At least two sites with PPD > 3 mm	• Patients in test group exhibited significant and positive correlation between TC, VLDL, hs-CRP, Troponin T and periodontal parameters • Prevention of progression of periodontitis potentially reduces the risk of cardiovascular problems

[†]CRP, C-reactive protein; hs-CRP, high sensitive C-reactive protein; HDL-c, high-density lipoproteins; LDL-, electronegative low-density lipoproteins; FMD, Flow-Mediated Dilation; PCR, Polymerase Chain Reaction; MMP-9, matrix metalloproteinase; MPO, myeloperoxidase; PAI-1, Plasminogen activator inhibitor type-1; ICAM-1, Intercellular Adhesion Molecule-1; VCAM-1, Vascular Cell Adhesion Molecule-1; AST, aspartate transaminase; ALT, alanine transaminase; Tr-I, troponin-I; CK, creatinine kinase; LDH, lactate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; sCD40 L, soluble CD40 ligand; MCP-1, monocyte chemoattractant protein-1; TLC, total leukocyte count; DLC, differential leukocyte count; t-PA, tissue plasminogen activator; sTNF-R, soluble tumor necrosis factor receptors; vWF, von Willebrand factor; c, cholesterol; ox, oxidized; TnT, cardiac troponin T; NOx, nitrite/nitrate; Aa, A. actinomycetemcomitans; Pg, P. gingivalis; VLDL, very LDL; TC, total cholesterol.

[¶]PPD, probing pocket depth; CAL, clinical attachment loss; FI, furcation involvement; OPG, orthopantomogram; PI, plaque index; BI, bleeding index; GCF, gingival crevicular fluid; GI, gingival index; SCR, scaling and root planing; GR, gingival recession; DMF, decayed-missing-filled index; API, approximal plaque index; SBI, sulcus bleeding index; CPITN, Community Periodontal Index of Treatment Need. ↑ mean increase.

denticola and *F. nucleatum*, viable bacteria of these species were detected in oral epithelium, aorta and atherosclerotic plaque (21, 103).

Proof 6. In vitro and in vivo evidence that non-invasive mutants cause significantly reduced pathology (animal model) The importance of the strain of bacterial species in respect of invasion of vascular tissue and cells has been examined. The non-invasive fimbrillinA deficient mutant of *P. gingivalis* was not found to promote atherosclerosis and resulted in less pro-inflammatory mediators than the invasive wild type strain of *P. gingivalis* (100).

Proof 7. Fulfill modified Koch's postulate to demonstrate that a human atheroma isolate causes disease in animal models To achieve this proof, the periodontopathogen has to be isolated from human atheroma and lead to atheroma formation in an animal model after inoculation. A strain of *P. gingivalis* has been isolated from human atherosclerotic plaque (95). Furthermore, evidence is available that inoculation of periodontopathogenic bacteria has the ability to induce atherosclerosis in animal models (21, 103, 104). However, the strains used were not obtained from human atherosclerotic plaque; therefore, this proof has not been entirely fulfilled yet.

Overall, numerous studies are available to support proofs 1 to 6, but not yet for proof 7. Nevertheless, the evidence from the first six proofs supports that periodontopathogenic bacteria are associated with atherosclerosis.

Observational Studies

The association between PD and atherosclerosis has been intensively examined in cohort and case control studies. In general, studies on subjects with periodontitis as defined by probing pocket depth (PPD), clinical attachment loss (CAL) and alveolar bone loss have a higher prevalence of subclinical ACVD (18). Those subjects were also suggested to exhibit higher prevalence of ACVD and risk of stroke or MI (105), increased prevalence or incidence of peripheral artery disease (106, 107), and higher prevalence of arterial fibrillation (108). Taking all the evidence from observational studies (**Table 3**) into account, it can be concluded that the odds ratio of atherosclerotic disease is greater in patients with PD in comparison to non-PD individuals. Furthermore, The results of some interventional studies have suggested that some preventive oral hygiene measures such as regular toothbrushing and oral health interventions including self-performed oral hygiene habits (124), dental prophylaxis (103), increased self-reported visits to the dental office (123) and periodontal treatment (125, 126) can reduced the incidence of ACVD events.

Intervention Studies

The effect of periodontal therapy on primary prevention of ACVDs such as ischemic heart disease and cardiovascular death has not been examined to date due to methodological, financial and, most importantly, ethical considerations (76). Therefore, surrogate markers of cardiovascular events have been examined rigorously and periodontal therapy has shown significant influence on these markers as summarized in

TABLE 2 | Selected studies on bacteremia of periodontal pathogens and periodontal pathogens identified in atheromatous lesions in subjects with chronic periodontitis.

References	Design	Technique	Intervention	Bacterial species (prevalence)
Forner et al. (82)[a]	C-S	Lysis filtration	Chewing gum and tooth brushing	*Pg* (10), *Pi* (40%), *Fn* (40%)
Lafaurie et al. (83)[a]	C-S	Culture	SRP	*Pg* (28.5%), *Tf* (7.1%), *Fn* (11.9%)
Perez-Chaparro et al. (84)[a]	C-S	Culture	SRP	*Pg* (43.7%)
Castillo et al. (85)[a]	C-S	Nested PCR	SRP	*Pg* (31%), *Aa* (21.4)
Waghmare et al. (86)[a]	C-S	Culture	SRP	*Pg* (37.5%), *Pi* (15%), *Tf* (12.5%)
Sharmann et al. (87)[a]	RCT	Lysis centrifugation, culture	SRP and povidone iodine	*Pi* (5.2%), *Fn* (5.2%)
Marin et al. (88)[a]	C-S	Lysis centrifugation, culture, qPCR	Tooth brushing	*Fn* (33%)
Balejo et al. (79)[a]	RCT	Culture, qPCR	SRP and chlorhexidine	*Pg* (Change in levels. By culture from 113.8 to 782.4, by qPCR from 0.5 to 512.5)
Elkaim et al. (89)[b]	C-S	Hybridization	None	*Aa* (54.4%), *Pg* (72.7%),
Nakano et al. (33)[b]	C-S	Specific PCR	None	*Aa* (35%), *Pg* (20%), *Td* (20%)
Gaetti- Jardim et al. (90)[b]	C-S	Real time PCR	None	*Aa* (46.2%), *Pg* (53.8%), *Tf* (25.6%), *Pi* (59%), *Fn* (0)
Figuero et al. (91)[b]	C-S	Nested PCR	None	*Aa* (66.7%), *Pg* (78.6%), *Tf* (61.9%), *Fn* (50%)

[a]*Bacteremia after periodontal procedure.* [b]*Periodontal pathogen in atheromatous lesion. C-S, cross sectional; SRP, scaling and root planing; RCT, randomized clinical trial; Pg, Porphyromonas gingivalis; Tf, Tannerella forsythia; Td, Treponema denticola; Pi, Prevotella intermedia; Aa, Aggregatibacter actinomycetemcomitans; Fn, Fusobacterium nucleatum; FISH, florescence in situ hybridization; qPCR, quantitative polymerase chain reaction.*

Table 4. However, evidence regarding the long-term effect of periodontal therapy on these surrogate markers is scarce. Further, the effect of periodontal therapy on clinical outcomes of cardiovascular events has not been examined yet (76).

The majority of researches relating periodontal treatment to ACVD have focused on assessing and quantifying systemic inflammatory biomarkers and endothelial function, as these atherosclerotic risk factors allow the estimation of treatment outcomes over a shorter period. Previous studies have shown that intensive periodontal treatment temporarily increases the blood levels of inflammatory markers, and worsens endothelial function, possibly through the release of bacteria or inflammatory cytokines into the blood stream. However, after several weeks, local and systemic inflammatory markers as well as PD parameters are lower, with considerably better endothelial function than before treatment (142–145). Moreover, carotid intimal-medial thickness is decreased 6 months after periodontal treatment (146). Several randomized controlled clinical trials, case control studies and systematic reviews with meta-analyses have been published in the last decade (**Table 4**) and they support the notion that periodontal treatment has an effect on cardiovascular events by reducing many cardiovascular risk factors.

Future intervention researches are needed to further clarify the relationship between PD and ACVD, particularly in terms of the biological impacts of PD on the atherogenic cascade by influencing the vascular endothelium. At the same time, there is still a need for more long-term interventional studies, preferably using more homogeneous methodologies for evaluating ACVD events, to determine whether the stated advantages of periodontal treatment translate into a reduction of ACVD occurrence.

EFFECTS OF STATINS AS MEDICATION FOR ACVD ON PD

Among the different medications used for treatment and prevention of ACVD, statins have demonstrated therapeutic potential in treating PD, which was further confirmed when used via a local delivery system (147, 148). Statins are inhibitors of 3-hydroxy-methylglutaryl coenzyme A reductase (HMG-CoA reductase). These medications have different ring structures and they are known to reduce the level of LDL and cholesterol in the blood for the prevention of ACVD (149, 150). Apart from their main action of lowering lipid levels, statins have several pleiotropic effects including anti-inflammatory, antioxidative, antibacterial and immunoregulatory functions (151, 152).

The anti-inflammatory effect of statins is due to their capability to inhibit pro-inflammatory cytokines and upregulate anti-inflammatory and/or proresolution molecules. This effect is primarily attributed to the activation of extracellular signal-regulated protein kinases (ERK), mitogen activated protein kinase (MAPK), protein kinase (PI3-Akt) signaling pathway, and suppression of NF-κB activation pathways. Furthermore, statins are able to modulate host response to bacterial challenges; thereby preventing inflammation-mediated bone resorption and stimulating new bone formation (153). Local delivery of statins using experimental animal models contributed in preventing alveolar bone resorption as a result of their anti-inflammatory, anti-microbial and bone remodeling properties besides their metalloproteinases inhibitory effect (154).

A 5-year population based competitive follow-up study investigated the effect of systemically administrated statins on the tooth loss rate as compared to participants not on statin medications. The study reported a reduction in the incidence of tooth loss in patients on statin therapy compared to controls (155). In addition, significant improvement in clinical signs of

TABLE 3 | Summary of observational studies.

References	Design	N	PD parameters	ACVD parameters	Conclusion
Ajwani et al. (109)	Cohort	364	CPITN	Cardiovascular mortality	Periodontitis (CPITN code ≥3) with cardiovascular mortality (HR = 2.28)
Grau et al. (110)	C-C	471	AVL, CAL	Acute ischemic lesion on brain imaging	Severe periodontitis and stroke (OR = 4.34)
Sim et al. (111)	C-C	479	PPD, CAL	Stroke as a hemorrhagic or ischemic lesion using brain images taken by CT or MRI	Periodontitis and stroke (OR = 4)
Dietrich et al. (112)	Cohort	1203	AVL, PPD	MI, angina pectoris, and fatal CHD were considered as CHD events	Periodontitis and CHD (HR =2.12)
Jimenez et al. (113)	Cohort	1137	AVL, PPD	Cerebrovascular disease was defined as a cerebrovascular event consistent with stroke or transient ischemic attack	Periodontitis and cerebrovascular disease (HR= 3.52)
Xu and Lu (114)	Cohort	10849	PPD, CAL	Cardiovascular mortality	Periodontitis with cardiovascular mortality (HR = 1.64)
Hayashida et al. (115)	C-C	1053	PPD	Carotid intima media thickness, arterial stiffness (cardio-ankle vascular index)	Each 1-mm increase in mean PPD corresponded to a 0.02 mm increase in maximal carotid intima media thickness and 0.1 increase in mean cardio-ankle vascular index
Heaton et al. (116)	Cohort	1461	AVL	CHD events were defined as MI, angina pectoris and fatal CHD	Progression of >0.5mm AVL associated IR of 5.4 and 2.5 for new and already diagnosed periodontitis subjects.
Vedin et al. (117)	Cohort	15824	Number of present teeth, BOP	Prior MI, prior coronary revascularization	Tooth loss and BoP associated with risk factors of cardiovascular disease
Ahn et al. (106)	C-S	1343	AVL, periodontitis (no, moderate, severe)	Subclinical atherosclerosis: carotid intima media thickness ≥0.754 mm, peripheral arterial disease: ankle-brachial index ≤1.0	Severe periodontitis and subclinical atherosclerosis (OR = 1.55) Severe periodontitis and peripheral arterial disease (OR = 2.03)
Gorski et al. (118)	C-C	289	PPD, BoP, CAL, tooth loss	MI according to European Society of Cardiology guideline	Periodontitis and MI (OR = 2.4).
Hansen et al. (119)	Cohort	17691	Hospital diagnosis of periodontitis	Hospital diagnosis of cardiovascular disease	Periodontitis and cardiovascular disease death (IR= 2.02)
Rydén et al. (46)	C-C	1610	AVL, PPD, BoP	First MI according to international criteria (120)	Increased risk for first myocardial infarction with periodontitis (OR = 1.49)
Bengtsson et al. (121)	C-S	499	AVL, PPD, BoP	Carotid calcification in panoramic dental radiographs	Periodontitis and carotid calcifications (OR = 1.5)
Beukers et al. (13)	Cohort	9730	CPITN	Acute cardiovascular disease	Periodontitis and acute cardiovascular disease (OR = 2.52)
Nordendahl et al. (122)	C-C	1577	AVL, PPD, BoP	First MI according to international criteria (120)	Increased risk for first MI with periodontitis in females aged <65 years (OR = 3.72)
Sen et al. (123)	C-S	17098	2017 classification (seven classes A-G) according to periodontal severity (A = periodontally healthy, G = severe periodontitis)	Subjects from atherosclerosis risk communities	Increased stroke risk with worse periodontal severity (IR = 5.03)

C-C, case control; C-S, cross sectional; OR, odds ratio; HR, hazard ratio; IR, incident rate; PPD, probing pocket depth; CAL, clinical attachment loss; BoP, bleeding on probing; AVL, alveolar bone loss; CPITN, community periodontal index of treatment need; CHD, coronary heart disease; CT, computerized tomography; MRI, magnetic resonance imaging; MI, myocardial infarction.

periodontal inflammation was recorded compared to those not taking statins (148).

The effect of statins on periodontitis in otherwise healthy patients was assessed in a review by Petit et al. (153). The primary outcomes included CAL, PPD, and gingival index as well as level of inflammatory biomarkers in serum and gingival crevicular fluid. For clinical parameters, contradictory results were reported on using statins as local adjunct to non-surgical therapy. However, most of these studies consistently reported significant reduction in proinflammatory mediators such as IL-8 and IL-6 associated with upregulation of anti-inflammatory cytokines such as IL-10 (156–158). To the contrary, local application of statins with different periodontal surgeries resulted in significant improvement in PPD, CAL, and bony defects (159–162). The systemic impact of statins on the outcomes of non-surgical periodontal treatment have not been fully elucidated. There are discrepancies in the results of the available studies that are mainly due to variations in the follow-up period and/or

TABLE 4 | Selected studies that have demonstrated effects of periodontal therapy on cardiovascular risk markers.

References	N	Study design	Cardiovascular risk markers	Intervention	Duration	Effect
López et al. (120)	315	RCT	TC, HDL, and LDL and glucose, CRP, and FGN	NSPT+ amoxicillin and metronidazole	6 months	Only CRP and Fibrinogen ↓
Bokhari et al. (127)	246	RCT	CRP, FGN and white blood cells.	NSPT	2-months	All ↓
Bresolin et al. (60)	33	Prospective clinical study	CRP, TC, VLDL, HDL, TGs, FGN, IL-6, and TNF-α	NSPT	180 days	All ↓ except TNF-α
Vidal et al. (59)	26	Cohort pilot study	CRP, FGN, IL6, SBP, DBP, LVM, and PWV	NSPT	3 months 6 months	All ↓ after 6 months
Banthia et al. (128)	40	Clinical study	TLC, DLC and platelet count, BT and CT	NSPT	2 weeks	All ↓
Caúla et al. (36)	66	RCT	CRP, ESR, TC, HDL, LDL and TGs	NSPT	2 months 6 months	All ↓ except HDL ↑
Kiany and Hedayati, 2015 (129)	25	RCT	IgM aCLA, IgG aCLA	NSPT	6 weeks	All ↓
Gupta et al. (130)	150	cross-sectional	CRP	SPT	3 months	All ↓
Graziani et al. (131)	38	RCT	CRP, IL6 and TNF-α	NSPT	1 day 1 week 3 months	All ↑ after 24 hrs. but ↓ after 1week and 3 months
Houckenet al. (132)	109	Case–control and pilot intervention	Pulse-wave velocity (PWV), SBP, DBP, TC, HDL, and LDL	NSPT	3 months 6 months	PWV not changed and the others ↓
Torumtay et al. (133)	50	case–control	CRP, IL6, IL-10, TAC, TOS, FPG, HbA1c, TRG, TC, HDL, LDL, SBP and DBP	NSPT	3 months 6 months	All ↓ except HbA1c, SBP, DBP unchanged after 6 months.
Siddeshappa et al. (134)	30	clinical trial	TLC, platelet count	NSPT	1 week and 2 weeks	All ↓
Arvanitidis et al. (135)	25	clinical trial	Binding of PAC-1, P-selectin and CD63, TLC and platelet count	NSPT	3 months	All ↓
Zhou et al. (136)	107	RCT	SBP, DBP, EM, CRP, IL-6	intensive periodontal treatment	1 months 3 months 6 months	SBP ↓ but DBP, EM, and CRP ↓ after 3 and 6 months but IL-6 ↓ only after 6 months
de Souza et al. (137)	44	RCT	CRP	NSPT	60 days	All ↓
Jockel-Schneider et al. (138)	55	RCT	PWV, PPao, RRsys, Aix, and MAP	NSPT + amoxicillin (500 mg) and metronidazole (400 mg),	12 months	PWV ↓, PPao ↑ RRsys and MAP not changed
Saffi et al. (139)	69	RCT	FMD, sVCAM-1, sICAM-1, and P selectin	NSPT	3 months	All ↓ except FMD
Morozumi et al. (140)	31	RCT	CRP, IFN-γ, IL-5, IL-6, IL-12, TNF-α	NSPT	1 day 6 weeks	All ↑ after 1 day After 6 weeks: CRP, IFN-γ and IL-6 ↓ IL-5, IL-12, TNF-α ↑
Moeintaghavi et al. (141)	30	RCT	TC, LDL, HDL, TGs, CRP), and FBS.	SPT and NSPT	3 months	All ↓ except HDL

RCT, randomized clinical trial NSPT, non-surgical periodontal therapy; SPT, surgical periodontal therapy; TC, total cholesterol; HDL, high-density lipoprotein; TGs, triglycerides; LDL, low-density lipoprotein; FGN, fibrinogen; CRP, C-reactive protein; IL, interleukin; TNF, tumor necrosis factor; ESR, erythrocyte sedimentation rate; LVM, left ventricular mass; TAC, total antioxidant capacity; TOS, Total oxidant status; TLC, Total leucocyte count; DLC, differential leucocyte count; BT, bleeding time; CT, clotting time; EM, Endothelial Microparticles; FMD, flow-mediated dilation; PWV, pulse wave velocity; Aix, augmentation index; PPao, central pulse pressure; RRsys, peripheral systolic pressure; MAP, Mean arterial pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; FBS, Fasting Blood sugar. ↓ mean decrease, ↑ mean increase.

their design (153, 163). Cellular and molecular effects of statins on periodontal tissues and their clinical impact are illustrated in **Figure 2**.

In general, local application of statins was found to achieve better treatment outcome than systemic application when used as an adjunct to periodontal therapies. Despite the promising results of statins, their effects on different aspects of soft and hard tissue healing need further exploration, especially on wound healing and regeneration.

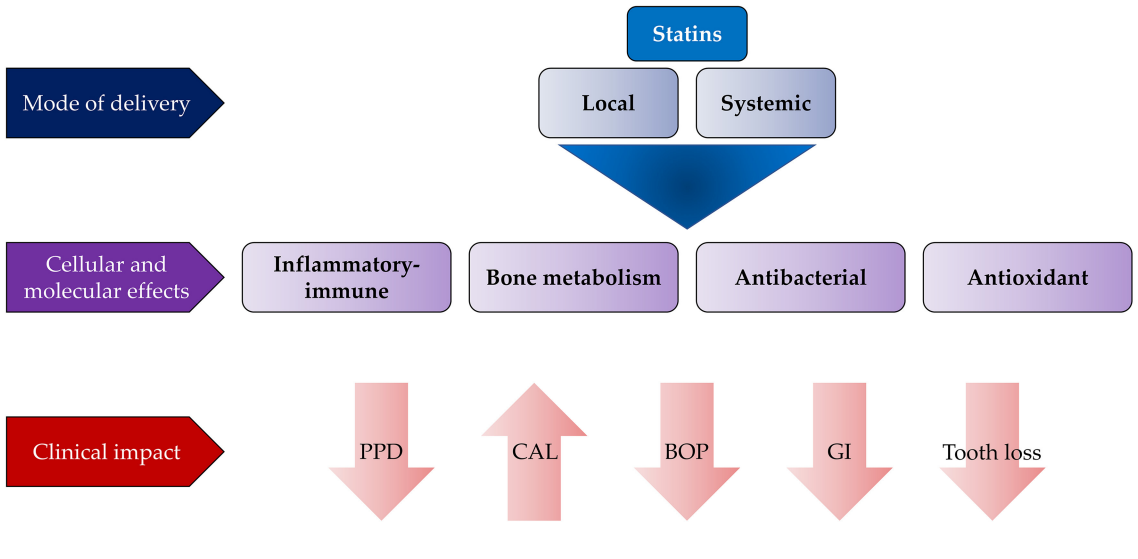

FIGURE 2 | Effect of local and systemic use of statins on the outcome of conventional periodontal treatment.

CLINICAL SIGNIFICANCE OF THE LINK BETWEEN PD AND ACVD FOR DENTAL PRACTITIONERS AND CARDIOLOGISTS

As detailed previously, a substantial body of evidence supports the relationship between PD and ACVD. Although many studies have reported that periodontal therapy significantly increases surrogate markers of ACVD within a short time, followed by improvement in systemic inflammation and endothelial function (76, 164), invasive dental procedures including periodontal treatment have not been associated with increased risk of MI (165). Furthermore, hemoglobin A1c (Hb A1c) has been found to decrease after periodontal therapy, which is of clinical relevance (143).

Dental practitioners have to be aware of the association between these two diseases. Patients with severe periodontitis should be advised to see a physician to check for signs of ACVD. Those patients should be informed that PD is associated with increased risk of cardiovascular complications and therefore their periodontal condition requires treatment. Furthermore, subjects with ACVD have to adhere to proper oral hygiene measures and regular check-ups with a dental practitioner (18, 76).

Although there is lack in evidence of a direct cause-effect relationship between PD and ACVD, evidence from published studies have confirmed the reduction in the systemic burden of inflammation following periodontal therapy. Thus, cardiologists should notify patients with atherosclerosis about the importance of good oral and dental health. Patients should be advised of the need to have regular home and professional dental care. Furthermore, the physician can recommend referral to a dentist or periodontist for oral and periodontal examination, assessment and treatment when necessary (76). Cooperation between the dentist and the cardiologist is of paramount importance for patients on anticoagulant/antiplatelet medication prior to any oral or periodontal surgeries to avoid any complications such as excessive bleeding and ischemic events.

CONCLUSIONS AND FUTURE RESEARCH

Evidence from the studies detailed in this review supports the notion that there is a link between PD and ACVD. These two diseases share several systemic inflammatory mechanisms including increases in levels of inflammatory mediators, lipids, and hemostatic and thrombotic factors. Furthermore, they share several risk factors such as smoking and genetics. However, the extent of the impact of PD on the initiation and progression of ACVD is not clear yet and needs to be further examined. Microbiological studies have shown that periodontal pathogens can cause bacteremia and invasion of distant tissues. Evidence from epidemiological studies shows that the odds ratio of atherosclerotic disease is greater in patients with PD in comparison to non-PD individuals. Interventional studies could not examine the effect of periodontal therapy on primary prevention of ACVD such as ischemic heart disease and cardiovascular death due to methodological, financial and, most importantly, ethical considerations. Therefore, surrogate markers of cardiovascular events have been examined rigorously and periodontal therapy has shown significant influence on these markers in the short term. On the other hand, amongst medications used for treatment and prevention of ACVD, statins have shown positive impact on periodontal treatment outcome. Several mechanisms have been proposed regarding the effect of statins on PD outcome, but again this needs to be further

investigated. Therefore, it is too early to mark PD as a causal factor with direct relation to the etiology and incidence of ACVD. Further studies should be conducted *in vivo* and *in vitro* to determine the cause-effect relationship between PD and ACVD, besides longitudinal studies and with longer follow up are advised to provide solid confirmation to support this relationship and clarify the link between PD and ACVD.

AUTHOR CONTRIBUTIONS

FZ: conceptualization and first draft of sections Introduction, Biomarkers Shared by Periodontal and Atherosclerotic Cardiovascular Diseases, and Clinical Significance of the Link Between PD and ACVD for Dental Practitioners and Cardiologists. SG: conceptualization and first draft of sections Effects of Periodontal Disease on the Incidence of Atherosclerotic Cardiovascular Diseases and Conclusions and Future Research. AA: drawing the figures and first draft of sections Pathogenesis of Atherosclerotic Cardiovascular Diseases Including the Role of Periodontal Disease, Confounders Between Periodontal and Atherosclerotic Cardiovascular Diseases, and Biomarkers Shared by Periodontal and Atherosclerotic Cardiovascular Diseases. AS: first draft of sections Effects of Periodontal Disease on the Incidence of Atherosclerotic Cardiovascular Diseases and Effects of Statins as Medication for ACVD on PD. JY, SG, and AA: critically reviewed the manuscript and final version editing. All authors have read and agreed to the published version of the manuscript.

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REVIEW
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Impact of Increased Oxidative Stress on Cardiovascular Diseases in Women With Polycystic Ovary Syndrome

Florentina Duică[1†], Cezara Alina Dănilă[1†], Andreea Elena Boboc[1], Panagiotis Antoniadis[2], Carmen Elena Condrat[1,3], Sebastian Onciul[4], Nicolae Suciu[1,5,6], Sanda Maria Creţoiu[7], Valentin Nicolae Varlas[8,9] and Dragoş Creţoiu[1,7]*

[1] Fetal Medicine Excellence Research Center, Alessandrescu-Rusescu National Institute for Mother and Child Health, Bucharest, Romania, [2] Division of Molecular Diagnostics and Biotechnology, Antisel RO SRL, Bucharest, Romania, [3] Doctoral School of Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [4] Department of Cardiology, Clinical Emergency Hospital, Bucharest, Romania, [5] Division of Obstetrics, Gynecology and Neonatology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [6] Department of Obstetrics and Gynecology, Polizu Clinical Hospital, Alessandrescu-Rusescu National Institute for Mother and Child Health, Bucharest, Romania, [7] Department of Cell and Molecular Biology and Histology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, [8] Department of Obstetrics and Gynecology, Filantropia Clinical Hospital, Bucharest, Romania, [9] Faculty of Dental Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Polycystic ovary syndrome (PCOS) is a complex disorder that affects around 5% to 10% of women of childbearing age worldwide, making it the most common source of anovulatory infertility. PCOS is defined by increased levels of androgens, abnormal ovulation, irregular menstrual cycles, and polycystic ovarian morphology in one or both ovaries. Women suffering from this condition have also been shown to frequently associate certain cardiovascular comorbidities, including obesity, hypertension, atherosclerosis, and vascular disease. These factors gradually lead to endothelial dysfunction and coronary artery calcification, thus posing an increased risk for adverse cardiac events. Traditional markers such as C-reactive protein (CRP) and homocysteine, along with more novel ones, specifically microRNAs (miRNAs), can accurately signal the risk of cardiovascular disease (CVD) in PCOS women. Furthermore, studies have also reported that increased oxidative stress (OS) coupled with poor antioxidant status significantly add to the increased cardiovascular risk among these patients. OS additionally contributes to the modified ovarian steroidogenesis, consequently leading to hyperandrogenism and infertility. The present review is therefore aimed not only at bringing together the most significant information regarding the role of oxidative stress in promoting CVD among PCOS patients, but also at highlighting the need for determining the efficiency of antioxidant therapy in these patients.

Keywords: polycystic ovary syndrome, cardiovascular disease, oxidative stress, C-reactive protein, homocysteine, miRNA

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****Correspondence:***
Carmen Elena Condrat
drcarmencondrat@gmail.com

†These authors have contributed equally to this work

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INTRODUCTION

Assessment of the clinical interaction between cardiovascular diseases and other interrelated pathophysiological conditions, such as polycystic ovary syndrome (PCOS), in terms of molecular and cellular changes, common biochemical and immunological pathways leading to the development of these diseases, have been intensively studied in the latest decades. To this extent, it has been shown that a variety of cardiovascular diseases (CVD) have heterogenous pathophysiologic mechanisms, where oxidative stress (OS) has been considered as one of the potential etiologies.

Under normal conditions, when the body is not subjected to a high level of oxidative stress, there is a fine balance at the physiological intracellular level of reactive oxygen species (ROS), which is maintained at low levels by various antioxidant systems. A basal concentration of ROS is essential for performing pivotal cellular functions such as gene expression or complex processes involved in signal transduction pathways (1, 2). Dysregulation of the fine balance between ROS and antioxidants at cellular level leads to the occurrence of oxidative stress that has been demonstrated to be involved in a series of pathological conditions, including cardiovascular diseases and inflammatory processes, known to be associated with a high ROS levels. Excessive ROS concentrations act on cell macromolecules by promoting cell necrosis and apoptosis, thus affecting the normal course of multiple cellular functions (1, 3–6).

With regard to the female reproductive tract, although ROS indeed play certain physiological roles, including the modulation of several functions such as ovarian steroidogenesis, corpus luteal function and luteal regression, fertilization, and the development of the early embryo, numerous studies have demonstrated the pathological effects of these molecules, involved in a multitude of diseases (7). Further on, in relation to the mechanisms by which oxidative stress affects the cardiac function at cellular level, it has been shown that the occurrence of hypertension may be due to the process of vasoconstriction that takes place as a result of a decreased availability of nitric oxide due to increased ROS levels, concentrations which further impact the cardiac function by negatively influencing calcium signals, thus leading to arrhythmia. Additionally, it has been speculated that the increase in ROS levels could also influence cardiac remodeling and atherosclerotic plaque formation (1, 8). Although several studies have evaluated the correlation between cardiovascular diseases and PCOS, the association of this syndrome with subclinical and/or clinical forms of cardiovascular disease, independent of the risk factors common to the two diseases, the exact interrelationship between these conditions has not been clearly elucidated.

PCOS is a disease that presents heterogeneous clinical variants, in which the pathogenesis involves the existence of several cardiometabolic abnormalities such as metabolic syndrome, glucose intolerance, dyslipidemia, hypertension, diabetes, all of which are also risk factors for CVD diseases (9, 10). Furthermore, PCOS is characterized by polycystic ovarian morphology that leads to ovarian dysfunction such oligo- or anovulation, where the central neuroendocrine systems perform an important role, due to excessive luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH) levels and relative follicle-stimulating hormone (FSH) deficiency, that contribute to the ovarian hyperandrogenemia and altered folliculogenesis, characteristic features of PCOS (11–13).

PCOS is a heterogenous syndrome that manifests through changes in the metabolic balance in which mitochondrial dysfunctions have been shown to facilitate the progression and occurrence of various complications of this disease (13). Although the etiology and pathophysiology of PCOS are not yet fully elucidated, it is currently considered that the main pathophysiological mechanism leading to this syndrome is the excess of androgen hormones, which results in metabolic, reproductive, and not least cosmetic changes, consisting of an increased body mass index due to a predisposition to obesity, as well as changes in the appearance of the skin due to acne outbreaks (12–15). Moreover, recent studies have highlighted the link between the pathogenesis of PCOS and chronic inflammatory status, with published data showing that numerous inflammatory markers are elevated in women suffering from PCOS (13, 16, 17). An additional possible cause of PCOS has been shown to be oxidative stress that could cause genetic changes such as point mutations, DNA strand breaks, aberrant DNA cross-linking, DNA-protein cross-linking, and DNA methylation, ultimately leading to the silencing of certain tumor suppressor genes (18–22).

PCOS—Definition

PCOS is a heterogeneous ailment described in women of childbearing age, characterized by ovulatory dysfunction, androgen excess, and polycystic ovarian morphologic features (23, 24). Also known as the Stein-Leventhal syndrome, it is a common endocrinopathy among women of reproductive age. PCOS affects 6% to 15% of women at the reproductive age, depending on diagnostic criteria (25, 26). The Rotterdam criteria (2013) are the most commonly used criteria to diagnose PCOS, and include the following: ovulation disorder, hyperandrogenism diagnosed by biochemical testing and/or clinical aspects, and ovarian volume over 10 ml or 12 or more ovarian cysts. The diagnosis can be established when two of the three conditions are fulfilled (27). Based on these criteria, four PCOS phenotypes can be detected, namely ovulation disorders, polycystic ovary, and hyperandrogenism, making up the classic phenotype, normal ovarian ultrasonography with hyperandrogenism and ovulation disorder, polycystic ovary ultrasonography image and hyperandrogenism, with no ovulation abnormalities, and no evidence of hyperandrogenism, but with polycystic ovary ultrasonography image and ovulation disorders (28, 29). Several endocrinopathies can mimic PCOS, such as Cushing's syndrome, non-classic adrenal hyperplasia, drug-induced androgen excess, and androgen-producing tumors (30). Ovulatory dysfunction can further be found in conditions like hyperprolactinemia or thyroid dysfunction (31). Therefore, in order to proper diagnose PCOS, these disorders need to be excluded.

PCOS is characterized by the overproduction of ovarian androgen hormones, especially testosterone, as a result of an

excessive production of LH in the pituitary gland or due to hyperinsulinemia, if the ovaries are sensitive to insulin. Common symptoms noticed in women with PCOS are infertility, signs of androgen excess such as hirsutism, virilization, acne, alopecia, and menstrual irregularities, including amenorrhea and dysfunctional bleeding (32). Women with PCOS also have an increased prevalence of certain comorbidities, such as dyslipidemia, excess weight, metabolic syndrome, type 2 diabetes, and hypertension. Along with other features such as chronic low-grade inflammatory state and endothelial dysfunction, PCOS poses an elevated risk of developing cardiovascular disorders (33).

PCOS—Pathophysiology

There are many hypotheses regarding the pathophysiology of PCOS, including among them ovarian hyperandrogenism, follicles resistant to rupture due to shell thickness, hypersecretion of luteinizing hormone, increased anti-Mullerian hormone (AMH), which is a blocker paracrine factor for follicular development, and hyperinsulinemia (34). These abnormalities can appear due to hormonal, metabolic, or even toxic factors occurring during the embryonic stage and/or in the early development of the female gonad, or because of certain epigenetic changes (35). The genetic basis of PCOS is suspected on the grounds of the aggregation of this syndrome in families, since it has been shown that within first-degree relatives, about 20 to 40% of women also have the disorder (23, 34, 36, 37).

Gonadotropins

Gonadotropin-releasing hormone (GnRH) neuropeptides released from neurons into the portal vein and median eminence stimulate the adenohypophysis gland to secrete gonadotropins, which mediate ovarian steroidogenesis and folliculogenesis. The follicle-stimulating hormone (FSH) binds to FSH receptor on the granulosa cells and stimulates follicular maturation and ovulation (38). On the other hand, the luteinizing hormone (LH) stimulates steroidogenesis, follicular growth, and corpus luteum formation (39, 40). Anovulation is determined by inappropriate gonadotropin secretion. Specifically, modified pulsatility of GnRH consisting of elevations in the amplitude and frequency of secretion, generates an increased production of LH compared to that of FSH. It is unknown whether hypothalamic dysfunction is a determining cause of PCOS or is caused by an abnormal steroid feedback. In both cases, the level of LH is reported to be high, while the LH/FSH ratio is increased to over 2/1 (36).

The impact of peripheral hormones on the brain function in the pathogenesis of PCOS has been explained through four suggested hypotheses. The first hypothesis is based on the negative feedback of steroid hormones which appears after setting up changes of the critical neuronal circuits determined by hyperandrogenism (40). The second hypothesis revolves around the hyperinsulinemia that stimulates the activity of GnRH neurons and the response of the pituitary gland to GnRH (41). The third hypothesis refers to the low concentration of progesterone in serum that is followed, in PCOS, by anovulation, which eventually eliminates the influence of the progesterone negative feedback on the release of GnRH (42). The fourth hypothesis states the function of the pulse generator of GnRH that reduces the activity of GnRH inhibitors (40, 43). Overall, the hypothalamic-pituitary-gonadal axis remains one of the principal regulators of female reproduction, its dysfunction leading to ovulation disorders.

Hyperandrogenism

A fundamental characteristic of PCOS is the increased production of androgens in ovaries, due to excessive activity in the theca cells stimulated by intraovarian or extraovarian factors (44). LH and insulin stimulate the production of androgens, determining elevated levels of dehydroepiandrosterone (DHEAS) and testosterone (36) (**Figure 1**). High levels of free testosterone were noticed in about 70% to 80% of patients with PCOS, while 25% to 65% expressed elevated DHEAS levels. This leads to increased estrone levels by peripheral conversion mechanism, which converts androgens to estrogens using aromatase. Furthermore, low levels of sex hormone-binding globulin (SHBG) were reported in women diagnosed with PCOS (36). Liver synthesis of SHBG is reduced by insulin as well as progestins, androgens, corticoids, and growth hormones (45). Reduced SHBG production leads to lower levels of bound circulating androgens, thus resulting in more available androgens capable of binding to organ receptors. Consequently, clinical hyperandrogenism is determined by high levels of free testosterone, although total testosterone might be within the normal range (46).

Exposure to androgens throughout fetal development has been speculated as another reason for hyperandrogenism determining the phenotypes of PCOS in adulthood (47). In this regard, there are four hypotheses for the exposure to additional androgens during the embryo stage. Firstly, the evolution of the hypothalamic-pituitary axis simultaneously with certain hypothalamic-pituitary axis disorders in embryonic development are thought to increase the production of androgen hormones (48). Secondly, in mothers with PCOS, the placenta is limitedly capable of aromatization and increasing of SHBG concentration, thus causing the fetus to receive maternal androgens through the placenta (49). The third hypothesis suggests a fatal genetic disorder with undifferentiated ovaries that can be the source of increased androgen production (35). The fourth hypothesis refers to malformations that increase the androgen production, such as hyperplasia of the adrenal glands (50). Either way, in order to diagnose PCOS in women, one must look at the biochemical androgen profile, which includes free and total testosterone, SHBG, DHEAS, 17-hydroxy-progesterone and the free androgen index (FAI), estimated as the total testosterone level divided by SHBG and multiplied by 100 (51).

The steroidogenic cells of the adrenal cortex and the ovary stand at the origin of the hyperandrogenemia that characterizes PCOS, using similar enzymes for steroidogenesis (52). The Cytochrome P450 Family 19 Subfamily A Member 1 (CYP19A1) gene encodes the aromatase, enzyme which turns androgens into estrogens. In the ovarian follicles, reducing the activity of aromatase leads to hyperandrogenism, and a positive correlation between the incidence of PCOS and mutations in this gene

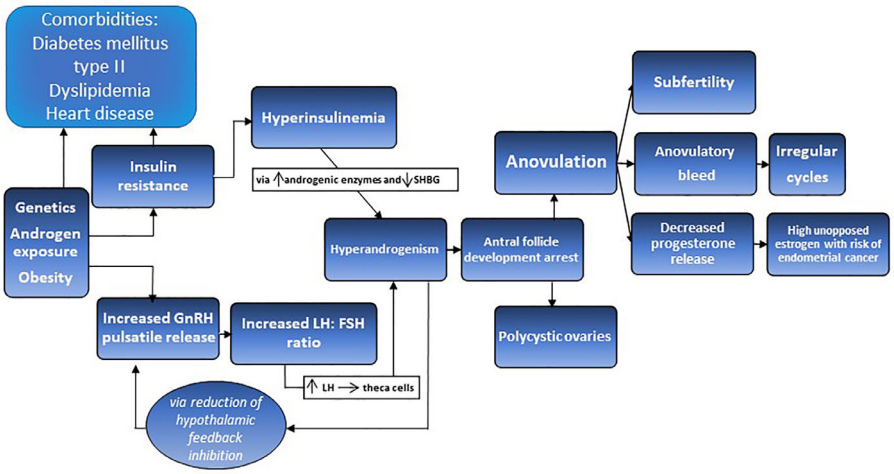

FIGURE 1 | The proposed pathophysiology of PCOS is a synergistic relationship between perturbed gonadotrophin releasing hormones (GnRH) pulsatility and insulin resistance, accompanied by hyperinsulinemia and hyperandrogenism leading to antral follicle development arrest, anovulation, irregulate cycles, subfertility, and polycystic ovaries.

has been observed (53). Furthermore, an androgen excess has been indicated to determine hypertension by stimulating the expression of adipose tissue aromatase (54, 55).

Hyperinsulinemia

Insulin is the hormone primarily responsible for lipogenesis and glucose homeostasis. Insulin has effects on fat, protein metabolism, carbohydrates, while also acting as a mitogenic hormone (56). The ovary and adrenal cortex are steroidogenic tissues in which insulin promotes steroidogenesis by potentiating the cognate trophic hormones (57). Insulin resistance associated with compensatory hyperinsulinemia determines excessive adrenal and/or ovarian androgen secretion and decreases the synthesis of SHBG in the liver, thus resulting in an increase of circulating testosterone concentration. Intrinsic insulin resistance is characteristic of women with PCOS independent of the magnitude of androgen levels and extent of obesity, with lean PCOS patients also experiencing it (28). Insulin resistance leads to reduced glucose-uptake response in spite of high insulin levels. This is the result of decreased insulin sensitivity due to abnormal signal transduction at receptor and post-binding level (36).

Alternate theories emphasize the fact that LH levels negatively correlate with insulin levels in women, an aspect demonstrated experimentally in both normal and PCOS women under euglycemic/hyperinsulinemic clamps (58, 59). Loss of negative feedback in the hypothalamus elevates LH, which may drive increased androgen production, but it is androgen that results in insulin resistance (60, 61). Elevated androgen levels positively correlate with LH levels, suggesting a failed compensatory mechanism prompting elevated LH output. Thus, loss of negative feedback in the hypothalamus can lead to both PCOS and increased heart disease, which may also be aggravated by increased obesity (62). The paradox of insulin signaling witnessed in PCOS is that the adipose tissue, liver, and skeletal muscles exhibit insulin resistance, whereas the pituitary and steroid-producing tissues retain insulin sensitivity. This aspect has been illustrated by observing the different actions of insulin in granulosa lutein cells from patients with PCOS and anovulation (28). In women with PCOS, the prevalence of metabolic syndrome is approximately threefold higher and is defined as the association of hyperglycemia, obesity, dyslipidemia, and hypertension (63). However, the definition of metabolic syndrome is incomplete in adolescents, being characterized by a combination of low high-density lipoprotein (HDL) cholesterol levels, high triglyceride concentrations, increased waist circumference, elevated fasting blood glucose, and hypertension for age (28, 64, 65).

Ovaries

Ovulation results from coordinated signaling by the hypothalamus-pituitary axis, ovarian granulosa cells, ovarian theca cells, and the developing follicle (66). In women with PCOS, this process malfunctions because of the abnormal development and failure in selecting a dominant follicle, thus inducing anovulation (67). The ovulatory dysfunction is characterized by increased activation of the follicles, followed by arrested growth before the maturation of these follicles can occur. Furthermore, PCOS follicles also have lower rates of atresia, which may explain why premature depletion of the follicular pools seldom occurs in the ovaries of these women (68). Due to anovulation, progesterone is lacking, thus leading to chronic estrogen exposure. This has an impact on the endometrium by constant mitogenic stimulation with endometrial thickening which leads to unpredictable bleeding or endometrial cancer (69).

In normal folliculogenesis, growth factors such as growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP15), also referred to as oocyte-secreted growth factors (OSFs), aid in the development from primordial to primary stage follicles, while subsequent stages, up to the selection of the dominant follicle are regulated by FSH to (70).

Throughout folliculogenesis, insulin and androgens have a synergistic aspect with LH, which exerts its effect from the middle to the late follicular stage (71). The equivalence between AMH and FSH may play a primary role in the aromatase activity, both during and after dominant follicle selection. Moreover, increased estradiol emission by the dominant follicle suppresses FSH levels, leading to subordinate follicle dissolution resulting in mono-ovulation (72). Under excessive androgen exposure, accelerated early follicular growth in PCOS tends to take place, leading to small-follicle occurrence. Decreased OSFs levels further lead to intensified early folliculogenesis (73). Further on, small follicle excess promotes high AMH levels, which in turn mediate follicle responsiveness to FSH (74). To this extent, low FSH responsiveness and premature granulosa cell luteinization denature the dominant follicle selection, producing follicular arrest (75). High insulin levels can further induce premature luteinization along with LH receptor expression (76).

Follicular defects associated with PCOS are defined by early and accelerated follicular growth as well as distortion in the subsequent stages in relation to dominant follicle selection, leading to follicular arrest (77). In this regard, Webber et al. have reported a greater density of small preantral, especially primary follicles in analyzed ovarian biopsies belonging to women diagnosed with PCOS in comparison with control groups (78). Atresia deceleration, later demonstrated by the same team of researchers, may answer for the increased recruitment and explain why premature follicle depletion does not occur in polycystic ovary (79). Arrested follicle development in women with PCOS can be explained by the relatively low levels of circulating FSH, which hinder the normal maturation process (80). Additionally, LH hypersecretion is detrimental to ovulation and follicular growth, since it determines decreasing FSH sensitivity, thus contributing to the premature luteinization of granulosa cells (32).

Anovulation can also be determined by altered GnRH pulsatility and improper gonadotropin secretion, both leading to menstrual irregularity (81). Moreover, anovulation can also be facilitated by insulin resistance, as many anovulatory patients diagnosed with PCOS express ovulatory cycles after treatment with insulin sensitizers such as metformin (82, 83). Increased intraovarian androgens from large antral follicles may also cause anovulation in patients with PCOS, fact which is supported by the improvement of menstrual regularity in patients who underwent laparoscopic ovarian drilling or ovarian wedge resection (36).

Inflammation

Low-grade systemic inflammation associated with PCOS is indicated by the high levels of inflammatory markers such as interleukin-18 (IL-18), C-reactive protein (CRP), white blood count, and monocyte chemoattractant protein-1 (MCP-1), along with increased oxidative stress and endothelial dysfunction (84). These inflammatory markers stimulate the proliferation of theca cells, while also promoting steroidogenesis, and contributing to follicular atresia and hyperandrogenemia (13).

Hyperglycemia further plays a role in PCOS-related inflammation, due to mononuclear cells utilizing glucose as a redox substrate, thus leading to high levels of ROS and inducing oxidative stress (85). ROS production by immune cells as a result of oxidative stress plays a primordial role in both the development and progression of endothelial dysfunction, which significantly contributes to the occurrence of arterial hypertension along with other cardiovascular diseases. Furthermore, insulin resistance and chronic inflammation play important roles in the etiopathogenesis of diabetes mellitus type II and metabolic syndrome, common comorbidities among PCOS women (33, 86).

CARDIOVASCULAR DISEASE IN PCOS

While significant improvement in the incidence and general outcome of cardiovascular diseases has been observed in the past decades, they go on being the leading cause of death among women worldwide (87, 88). Furthermore, preventive care including counseling and prophylactic treatment is less likely to be offered to women than men with similar atherosclerotic cardiovascular disease risk (87, 89), while medical management of these patients tends to be less vigorous, thus more rarely achieving optimal results (90, 91). While most cardiovascular risk factors in women overlap with those in men, several circumstances remain characteristic of women (92, 93), as it can be seen outlined in **Figure 2**.

Cardio-metabolic disturbances have been found in women with PCOS regardless of age, posing significant risks for the occurrence of CVD. These disturbances are represented primarily by atherogenic dyslipidemia, hypertension, obesity, along with insulin resistance, impaired glucose tolerance and type II diabetes (94, 95). The association between PCOS and CVD has been related to this partial overlapping of risk factors. While PCOS is influenced by race, BMI and age, with symptoms becoming less thunderous with increasing age and most of them disappearing after the onset of menopause, cardio-metabolic disorders can, however, continue to pose a threat to the patients' health (94, 96–99). If earlier studies regarding the higher risk of CVD in women with PCOS could not establish its absoluteness (100), more recent data confirm that the metabolic dysfunction typical of women with PCOS leads to a definite increase in CVD events (101–103).

Hypertension

The pathophysiology of hypertension in PCOS is multifactorial, depending on factors such as obesity, hyperandrogenism, elevated sympathetic nervous system activity, and insulin resistance (104). Several studies indicated that patients with PCOS are more likely to develop hypertension as opposed to the normal population. However, this fact is somewhat unclear, since PCOS is associated with obesity as well, which also represents a significant risk factor for hypertension. Therefore, the interpretation of these studies is rather complicated, since obesity is a variable not usually considered in many types of research (105–111). Still, a meta-analysis performed by Amiri et al. showed that hypertension is more common in women with PCOS than in the control population. Moreover, they have

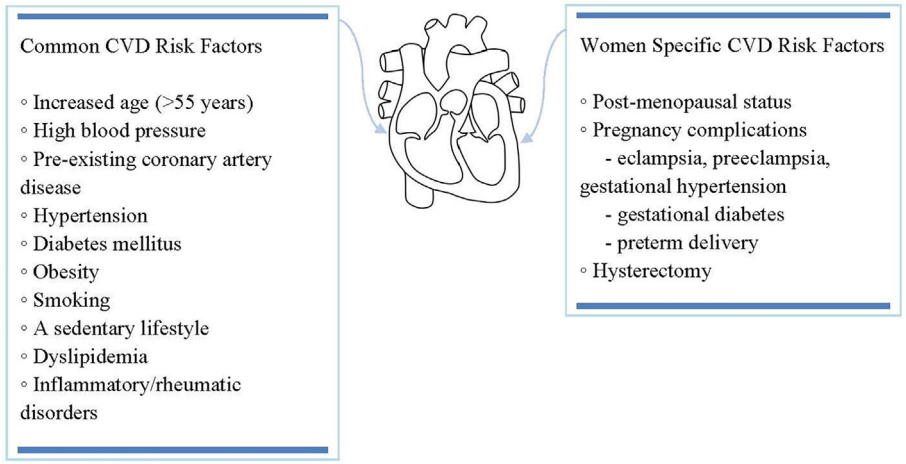

FIGURE 2 | While most cardiovascular risk factors in women overlap with those in men, several circumstances remain characteristic of women.

separately evaluated women during post-menopause and reproductive-age women with PCOS because, since it is well known that the prevalence of hypertension is higher with aging and with menopause onset. The result was that, even after adjusting diabetes mellitus and BMI variables, PCOS women during reproductive age were more likely to develop hypertension (112).

As mentioned previously, a significant risk factor for hypertension is represented by obesity. In this regard, it has been shown that the prevalence of obesity and overweight status among PCOS patients is 80% higher compared with non-PCOS women, with PCOS women associating BMIs over 30 kg/m^2 and higher waist-hip ratios (113), more commonly in Caucasian than Asian women (114). Obesity in females suffering from PCOS may be correlated with insulin resistance, which generates hyperinsulinemia that triggers ovarian steroidogenesis. This way, sex hormone-binding globulin production is downregulated and, as a consequence, the availability of free androgens is elevated, causing visceral accumulation of fat, thus facilitating central obesity (113–115). In PCOS patients, it was observed that a combination of factors like insulin resistance, obesity, and hyperandrogenism leads to an elevated sympathetic nervous system activity, each factor being a possible mediator of hypertension (116–118).

Deficiencies in the hypothalamic-pituitary axis produce an excessive secretion of LH and a low excretion of FSH, hormonal imbalance that leads to secretory changes in the inner sheath of ovarian follicles. In turn, an excess of androgenic hormones is released, which is responsible for both clinical and paraclinical signs of hyperandrogenism (119, 120). Numerous women with PCOS, especially those with hyperandrogenic phenotype, have various cardio-metabolic disturbances that increase the risk of developing hypertension (121, 122). One study demonstrated that, with age, almost half of women with PCOS improve due to the decrease of serum androgens as a consequence of adrenal and ovarian aging (123). Testosterone levels drop with age in both PCOS and healthy women, the decrease being observed years before the onset of menopause (124). This fact may lead to a progressive reduction of CVD risk factors (125). However, the processes that determine the lowering of hypertension risk remain slightly vague. An analysis of daytime ambulatory blood pressure revealed that young and obese women suffering from PCOS had elevated blood pressure in comparison with non-PCOS females (107). Other variables that must be taken into consideration are background aspects of the individual such as ethnicity and race. To this extent, Lo et al. revealed that, even after adjusting for diabetes mellitus, age, and BMI, the prevalence of hypertension and/or high blood pressure was increased in black women with PCOS when compared to the Caucasian population, and among the latter, Hispanic and Asian women were the least affected (126).

Atherosclerosis and Vascular Disease

Dyslipidemia is a cardio-metabolic disturbance distinguished by high levels of LDL cholesterol and triglycerides and low levels of HDL cholesterol, found in both obese and lean women with PCOS (127, 128). This imbalance, together with obesity and insulin resistance, predisposes these females to a subclinical vascular disease characterized by intimal-medial thickening in the carotid arteries, coronary artery calcifications, and endothelial dysfunction (129–131). These modifications could put PCOS patients at risk for developing cardiac events, both fatal and nonfatal, as well as strokes (132).

Carotid intima-media wall thickness (cIMT) is a determination of the tunica media and tunica intima of the arteries, evaluated usually by ultrasound performed on large vessels close to the skin, as is the carotid artery. This measurement is utilized for the detection of atherosclerosis and for tracking its regression or progression, and it is correlated with the prevalence of myocardial infarction or stroke (133–139). cIMT is known to be associated with visceral adiposity, dyslipidemia, hyperinsulinemia, and raised systolic blood pressure, risk factors also encountered in

PCOS (140–146). Meyer et al. performed a meta-analysis that showed that cIMT is elevated in females with PCOS compared with the control group, suggesting an elevated risk for accelerated atherosclerosis in PCOS patients (147). Talbott et al. further demonstrated that increased cIMT was noticed in females ≥ 45 years, explaining that CVDs have long incubation periods, with metabolic disturbances occurring in young age converting into carotid damage by older age, and it seems that cIMT is more affected by the combination of age and PCOS than by aging alone (131).

The severity of coronary atherosclerosis is indicated by the coronary artery calcium (CAC) score, an independent risk marker for sudden cardiac death and myocardial infarction in both symptomatic and asymptomatic patients (148), with several studies focusing on elevated CAC scores in PCOS patients. For instance, Christian et al. performed a study that included premenopausal women at 30 to 45 years old suffering from PCOS and found a higher prevalence of elevated CAC scores in PCOS women than in the control group (149). Another study performed by Talbott et al. reported that elevated CAC had a higher prevalence among PCOS females between 40 and 61 years old (46%) than the control group (31%), even after controlling for BMI and age (150). Shroff et al. further conducted a research study designed to discover early-onset increased CAC score as an indicator of subclinical atherosclerosis in young and obese PCOS females (151). Compared with weight and age-matched controls, early coronary atherosclerosis was detected in young females suffering from PCOS. Due to the young age, the subjects in the study did not associate other CVD risk factors, therefore PCOS was speculated to contribute to the risk of elevated CAC scores (151).

It is broadly accepted that chronic inflammation is correlated with endothelial dysfunction. Abnormal morphology, disposition, and function of the adipose tissue in PCOS females are correlated with the generation of chemokines, cytokines, and low-grade inflammation, which lead to the activation of hypoxia-induced pathways, with the consequential reduction of adiponectin production (152). This pro-inflammatory condition is correlated with the progression of insulin resistance, thus promoting type II diabetes development along with increasing cardiovascular risk (153). Furthermore, it is presumed that androgens are mediators in the transformation of preadipocytes into mature adipocytes, while also having an impact on oxidative stress, lipid, and glucose metabolism (99, 154). Overall, females with PCOS, due to their underlying pathophysiology, could be at risk for cardiac and cerebrovascular disease. Contrasting results that were obtained during several studies prompted more research, especially in the form of longitudinal studies, focusing on cardiovascular assessment and follow-up of these women for a better understanding and management of PCOS complications.

OXIDATIVE STRESS IN PCOS AND CVD

At a biological level, oxidative stress refers to the physiological disturbances between free radical species such as ROS or reactive nitrogen species (RNS), and the body's ability to eliminate them. Oxidative stress can also be defined as the discrepancy between signaling systems and redox control systems (155–157). Living organisms have developed several mechanisms to respond to oxidative stress by producing antioxidants. A change in the balance between oxidizing and antioxidant substances in favor of excess oxidants leads to oxidative stress. These systems include enzymes (superoxide dismutase, catalase, and glutathione peroxidase), antioxidant macromolecules (albumin, ceruloplasmin, and ferritin), antioxidant micro-molecules (ascorbic acid, α-tocopherol, β-carotene, ubiquinone, flavonoids and glutathione, methionine, uric acid) bilirubin (1, 158–161).

ROS are highly reactive molecules with a very short lifespan, and are classified into two categories, namely non-radical species, including hydrogen peroxide (H2O2), hypochlorous acid (HOCl-), ozone (O3-), lipid peroxides (LOOH), along with hydroperoxides (ROOH), and radical species, consisting of superoxide anion (O2•−), singlet oxygen (1O2), hydroperoxyl radical (HOO•), hydroxyl radical (•OH), with •OH being considered the most important ROS (158). RNS on the other hand comprise a range of various chemical compounds derived from nitric oxide (NO) in the reaction of biologically generated free radicals that tend to form more stable species, a process that generates multiple biological effects (162). Free radical species are extremely unstable molecules that tend to gain stability by acquiring electrons from neighboring molecules such as nucleic acids, carbohydrates, proteins, and lipids, which leads to a cascade of chain reactions, that cause cell damage (155, 163–167). Free radicals fulfilling important roles in physiological and pathological conditions, come from both endogenous and exogenous sources. They are the result of cellular processes such as oxygen reduction through the electron transport chain in the mitochondria, but could also be generated in the endoplasmic reticulum, phagocytic cells, peroxisomes, as well as other cell compartments, as a result of central processes such as protein phosphorylation and activation of certain factors specific for transcription, apoptosis, and immunity (168).

When the body's ability to eliminate excessive ROS and/or RNS molecules is exceeded, and they remain in the intercellular space for longer periods of time, oxidation of sensitive biomolecules takes place, such as lipid peroxidation (LPO), essential fatty acid oxidation, or oxidation of guanine DNA-base, causing damage to proper cellular function (169). Among the cellular components involved in regulating OS levels, mitochondria play an important role, dysfunctions at this level having been demonstrated to assist in the pathogenesis of several diseases, including PCOS, metabolic syndrome and diabetes mellitus, cardiovascular disease, and cancer (13, 170, 171). Thus, mitochondrial dysfunction in combination with systemic inflammation is thought to play an essential role in the occurrence of complications associated with metabolic disorders in patients with PCOS, and in the predisposition to cardiovascular disease. In this regard, as a result of mitochondrial dysfunctions, systemic increase of OS occurs in patients of reproductive age who develop symptoms of PCOS, and who have been found to have elevated serum levels of inflammatory markers such as C-reactive protein, interleukins, and proinflammatory cytokines, increased cell counts

of leukocyte series such as lymphocytes and monocytes, change in tumor necrosis factor (TNF-α), as well as increases in some metabolites resulting from the processes of carbonylation and oxidation of proteins and lipids (172–174).

Previous studies have revealed that women with PCOS, due to their altered lipid profile, may present certain dysregulated markers, such as increased body mass index, triglycerides, total cholesterol and LDL levels, along with decreased total HDL and HDL2 levels (175–177). In this regard, in PCOS women, several specific metabolites such as nitric oxide (NO) and malondialdehyde (MDA), resulting after lipid metabolization through the reduction of mono- and polyunsaturated fatty acids (MUFAs and PUFAs) and considered oxidative stress markers, have been found at higher levels when compared to control lots (178). On the other hand, Sulaiman et al. have demonstrated the decreased levels of antioxidant molecules glutathione (GSH) and total antioxidant capacity (TAC), capable of cancelling out the destructive impact of free radicals (179). Furthermore, it has been postulated that, especially among women with PCOS, dietary factors may accomplish an important role in promoting the metabolic imbalance (177). For instance, Kazemi et al. have evaluated the relationship between four dietary patterns and the overall ovarian function, and found that the latter was affected by diets that influence obesity, metabolic status and hyperandrogenism regulation (176). The Dietary Approaches to Stop Hypertension (DASH) eating plan has also been previously analyzed by Asemi and colleagues, who highlighted the effect of the DASH diet not only on lipid profiles, but also on oxidative stress markers in PCOS women. They found that women with PCOS undergoing the DASH diet could register significant reduction in insulin, triglyceride and very low-density lipoprotein cholesterol (VLDL-C) values, along with an increased capacity of prooxidant status by elevated levels of total antioxidant capacity (TAC) and GSH (175).

The etiology and circumstances that define the severity of PCOS and the occurrence of risk factors in the development of cardiovascular disease involve the endothelial dysfunction caused by an imbalance between the production and bioavailability of vasoactive molecules that either contract or relax the vessel. Molecules such as endothelium-dependent relaxing factors, endothelium-dependent hyperpolarization factors, endothelium-dependent constricting factors, vasodilator prostaglandins, nitric oxide (NO), fulfill an important role in maintaining a balance for tissue oxygen needs, while also being involved in central processes such as the remodeling of vascular structures by adjusting the vascular tone and diameter to adapt to the metabolic demand in every particular situation (1, 8, 180, 181).

CVD MARKERS IN PCOS

In light of the absence of conventional CVD risk factors in PCOS women, various studies have focused on the relevance of subclinical CVD markers among these patients. In this regard, CRP and homocysteine have consistently been shown to be increased in the plasma of patients with PCOS. At the same time, emerging microRNA (miRNA) analysis methods have enabled the identification of various dysregulated miRNAs, as a response to metabolic changes characteristic of this condition.

C-Reactive Protein (CRP)

CRP is a very common circulating marker, that is usually used as an inflammatory index for individuals. Recent studies have demonstrated the inducing function of CRP in inflammation, as the protein promotes the activation of the complement pathway, induces apoptosis, phagocytosis, and the production of proinflammatory cytokines, such as IL-6 and TNF-α (182). The fact that CRP has been observed to be increased in women with PCOS implicates chronic inflammation as a mechanism that contributes to the increased risk of CVD in women with PCOS (183). A large study performed in 2011 compared CRP levels in the serum of 2.359 women with PCOS with those from 1.289 healthy women, pointing out the significant difference between the two groups, as the group with PCOS had a mean value 95% higher than the control group (184). These findings were irrespective of the high body mass index (BMI), as they had not changed much after eliminating the bias from BMI.

Different approaches have been indicated to be beneficial for the reduction of CRP in women with PCOS, such as medication with statins or an increase in daily activity. In a study carried out in 2008, which included 40 medication naïve women with PCOS, an effective reduction of mean high sensitivity CRP (hs-CRP) in serum was demonstrated after 12 weeks of atorvastatin administration. This reduction was around 1.5 mg/liter and was accompanied by a reduction of mean levels of total cholesterol, LDL cholesterol, triglycerides, testosterone, and insulin resistance (185). Moreover, in another study, an increase of 1000 steps per day was associated with a decrease of 13% in serum CRP levels for a group of 65 women with PCOS, following 6 months of increased daily activity. For this research, data was adjusted for different parameters, such as age and baseline step count, while the observed reduction in CRP levels had a p-value of 0.005 (186).

Homocysteine

Homocysteine is a well-known marker of oxidative stress, as it has the ability to promote the production of ROS, and, when in high concentration, it can induce the injury of endothelial cells (187). In a big meta-analysis performed in 2013, a group of 4.933 women with PCOS has been compared with a control group of 3.671 healthy women for the detection of circulating markers that indicate OS and PCOS (188). The findings of this study pointed out a 23% higher mean concentration of homocysteine in the group of women with PCOS, implying the increased levels of OS in this group. Homocysteine can induce OS and increase the risk of CVD in PCOS patients by restricting the expression and the activity of glutathione peroxidase and superoxide dismutase (SOD), while promoting the expression of inducible nitric oxide synthase (iNOS). Moreover, it induces the expression of NADPH oxidase and diminishes thioredoxin, thus favoring the build-up of ROS (189).

The implication of homocysteine for the development of CVD has been noted since the 1990s, due to the promotion of atherosclerosis and hypercoagulability (190). Apart from PCOS patients, homocysteine has been associated with CVD, such as coronary artery disease (CAD), in individuals with chronic renal dysfunction (191). The fact that atherosclerosis is a pathological process with very strong associations with the onset of CVD, correlates hyperhomocysteinemia with conditions such as stroke, heart failure, and myocardial infarction (192). Moreover, there has been described a strong correlation between homocysteine and CRP expression in vascular smooth muscle cells (VSMCs). In this regard, it has been shown that increased levels of homocysteine can induce the expression of CRP at the transcriptional and the translational level, through harnessing signal pathways of N-methyl-D-aspartate receptor (NMDAr) in VSMCs (193). Therefore, a connection between hyperhomocysteinemia and inflammation comes up, which further corroborates the role of homocysteine in atherosclerosis.

The correlation of homocysteine with CAD has also been pointed out in a study where 70 patients were monitored and compared for their homocysteine serum levels and the presence of CAD through coronary angiography. The patients with CAD had considerably higher levels of homocysteine at a fasting state compared to individuals without CAD, showing increased statistical significance ($p < 0.001$) (190). In addition, the severity of CAD has been found to be associated with the levels of homocysteine, having a p-value below 0.001. Homocysteine seems to induce the proliferation of VSMCs while also augmenting the activity of HMG Co-A reductase, which promotes the synthetic production of cholesterol (190). These findings highlight once again the significant role of homocysteine in atherosclerosis.

Over and above, homocysteine has been implicated in the progress of increased arterial stiffness, as it has been correlated with increased aortic stiffness and pulse pressure. Although the mechanism that connects hyperhomocysteinemia with aortic stiffness remains to be further clarified, it seems to be triggered by the elevated oxidation and inflammation levels of vascular endothelial cells, which lack in nitric oxide production and availability (194).

Increased risk of vein thrombosis has been also connected with hyperhomocysteinemia. It has been indicated that elevated levels of homocysteine can enhance platelet adhesion on endothelial cells, while promoting the production of prothrombotic factors, such as tissue plasminogen activator and β-thromboglobulin (194, 195).

MicroRNAs

MicroRNAs are small non-coding molecules involved in the regulation of numerous genes due to their ability to recognize target sequences situated within the 3 prime untranslated region (3′-UTR) of messenger RNA (mRNA). miRNAs have a regulating effect in the post-transcriptional expression of eukaryotic genes and their role in PCOS patients is prominent. In a recent study performed in 2015, where 25 women with PCOS were compared with 24 healthy women of the same age and weight, an increased presence of miRNA-93 and miRNA-223 has been observed in the group of women with PCOS. The p values for these observations were <0.01 and 0.029 respectively, indicating miRNA-93 as a better circulating biomarker for the detection of PCOS (196). The upregulation of miRNA-93 induces insulin resistance, through targeting the CDKN1A and GLUT4 genes, therefore contributing to the increased risk of CVD in PCOS patients (197).

On the other hand, miRNA-223 which targets glucose transporter type 4 (GLUT4), has also been found significantly upregulated in patients with type II diabetes mellitus (T2DM) and left ventricular heart dysfunction (LVD) in biopsies from the left ventricle. When the effect of miRNA-223 was studied *in vivo* in rat cardiomyocytes, a GLUT4 mediated glucose uptake increase has been found as a response to miRNA-223 upregulation. The regulatory function of miRNA-223 on the post-transcriptional expression of GLUT4 and subsequently on glucose uptake was validated using a synthetic inhibitor of the miRNA *in vivo*, which diminished the levels of GLUT4 and glucose uptake (198).

Apart from miRNA-93 and miRNA-223, several other miRNAs have been found to be differentially expressed in women with PCOS in the follicular fluid. The most significant ones, which have been observed to demonstrate a more than 2-fold change, are miRNA-199b, miRNA-650, miRNA-663b, miRNA-361, miRNA-127, miRNA-382, miRNA-425, miRNA-212, miRNA-891b, miRNA-513c, miRNA-507, miRNA-32, miRNA-200c (199).

In a recent meta-analysis performed in 2020, two new miRNAs have been proposed as potential diagnostic biomarkers for PCOS, miR-29a-5p, and miR-320, respectively, indicating miR-29a-5p as a superior potential biomarker (200). Both molecules seem to be downregulated in patients with PCOS. There is a connection between miR-320 and the regulation of genes associated with PCOS morbidity, whereas miR-29a-5p is involved in several metabolic diseases and comorbidities. Moreover, the significant role of miR-29a-5p regarding cell growth, differentiation, and proliferation has also been highlighted. When the DIANA-microT-CDS tool was used for the determination of differentially expressed target genes, which are involved in pathways targeted by miRNAs and associated with PCOS, several results came up. In particular, miR-320 was found to possibly interact with the expression of ESR1, IL-1A, 10, 12B, 37, 8, RAB5B, PDK3, and HMGA2, all of which are involved in estradiol synthesis, steroidogenesis, insulin signaling, fertilization, cell adhesion, and embryo development. On the other hand, miR-29a-5p was found to potentially regulate AR, AKT2, TGFβ, MAP, KFBN3, STARD3, ITGB1, TGFB2, and INRS, which are involved in follicle growth, cell growth, insulin, and collagen synthesis (200).

Nowadays, the correlation between miRNAs and different pathological conditions has been profoundly studied, connecting the dysregulated expression of miRNAs with complex diseases, including CVD. Usually, the targets of a miRNA expand on several different mRNAs, thus affecting the expression of a collection of genes. It has been estimated that around 30% of genes are regulated by miRNAs (201), depicting their significance in human physiology. A promising field for future

research is the monitoring of serum miRNAs, so as to be used as diagnostic, prognostic, or treatment response markers.

CONCLUSION

Polycystic ovary syndrome is one of the most common endocrine disorders in women of childbearing age and the most common source of anovulatory infertility. This syndrome presents heterogeneous clinical variants, where the pathogenesis involves the existence of several cardiometabolic abnormalities that manifest through changes in the metabolic balance in which mitochondrial dysfunctions play a key role in the progression and occurrence of complications. Besides mitochondrial dysfunction, systemic inflammation characteristic of PCOS women also fulfills an important role in the occurrence of complications associated with metabolic disorders in these patients, as well as in the predisposition to cardiovascular disease.

Among the metabolic disorders associated with PCOS that occur from adolescence, insulin resistance and impaired glucose tolerance are included, as well as other manifestations that are more prominently expressed with age, such as hyperglycemia, obesity - especially visceral, hepatic steatosis, dyslipidemia, hypertension, type II diabetes, and an increased risk of cardiovascular diseases such as hypertension and myocardial infarction. Moreover, in addition to other features such as chronic low-grade inflammatory state and endothelial dysfunction, PCOS poses an increased risk of developing cardiovascular disorders. One of the diverse mechanisms that could enhance the overall cardiovascular risk especially by causing arterial hypertension is represented by endothelial dysfunction, which is tightly correlated with ROS levels that are highly dependent upon the oxidative stress in the body. In this respect, high ROS levels are further involved in genetic changes such as point mutations, DNA strand breaks, aberrant DNA cross-linking, and DNA-protein cross-linking, DNA methylation, with the effect of silencing the genes tumor suppressors, phenomena that were observed in women with PCOS syndrome. Moreover, owing to OS's ability to induce DNA injury and methylation, the activation of oncogenes along with antioncogene silencing are not out of the question among these patients, which are, in fact, also susceptible to developing endometrial cancer.

Overall, current literature suggests an evident increase in OS among PCOS women, contributing to the numerous metabolic and cardiovascular dysfunctions characteristic of this disease. The development of both preventive and therapeutic strategies aimed at the cardiovascular risk of these patients ought to therefore involve further studies regarding the reduction of oxidative stress.

AUTHOR CONTRIBUTIONS

Conceptualization, FD, AB, CD, PA. Methodology, SC, VV. Investigation, SO, DC. Writing—original draft preparation, FD, CD, AB, PA, CC. Writing—review and editing CC, SO. Supervision, DC. Funding acquisition, NS, SC. All authors contributed to the article and approved the submitted version.

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Vitamin D Deficiency and Vasovagal Syncope in Children and Adolescents

*Qingyou Zhang, Yan Sun, Chunyu Zhang, Jianguang Qi and Junbao Du**

Department of Pediatrics, Peking University First Hospital, Beijing, China

Aims: To investigate the association of vitamin D deficiency with cardiovascular autonomic nervous system function in children and adolescents with vasovagal syncope (VVS).

Methods: This study recruited 76 pediatric patients with VVS and 15 healthy children. The 25-hydroxyvitamin D levels in serum among the participants were evaluated. Heart rate variability analysis including SDNN, rMSSD, and SDANN was tested in patients with VVS. The correlation between indices of time-domain analysis and serum vitamin D status of the children with VVS was investigated.

Results: In this work, 25-hydroxyvitamin D levels in serum among VVS cases remarkably decreased compared with those among healthy controls (48.76 ± 19.25 vs. 67.62 ± 15.46 nmol/L, $p < 0.01$). The vitamin D deficient patients with VVS exhibited a lower rMSDD value compared to the non-deficient group with VVS (45.56 ± 16.87 vs. 61.90 ± 20.38 ms, $p < 0.001$, respectively). Pearson correlation analysis indicated that serum 25-hydroxyvitamin D levels had positive correlation with rMSDD values ($r = 0.466$, $p < 0.001$).

Conclusions: As suggested by our data, VVS children and adolescents with vitamin D deficiency may have cardiac autonomic dysfunction and cardiac vagal tone decreases with the reduction in vitamin D level.

Keywords: children and adolescents, vasovagal syncope, vitamin D, heart rate variability, autonomic nervous function

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****Correspondence:***
Junbao Du
junbaodu1@126.com

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INTRODUCTION

Syncope is a common occurrence in the pediatric population. Before the end of adolescence, about 15% of children and adolescents experience at least one episode of syncope (1, 2). The most common etiology of syncope in pediatric patients is vasovagal syncope (VVS) (3). Recurrence of VVS affects physical, psychological, and psychosocial activities of life, leading to impaired quality of life. However, the exact pathogenesis of VVS is currently unknown (4).

It has been reported that orthostatic intolerance including VVS in children is associated with many malnutrition diseases, including low iron storage (5), vitamin B12 deficiency (6), vitamin B1 deficiency (7), and others. Vitamin D is one of fat-soluble nutritive molecules that is crucial for calcium and phosphorus homeostasis. However, recent research has identified vitamin D as a prohormone with a wide range of actions in human diseases, particularly in the cardiovascular system (8, 9). It is related to the modulation of blood pressure, heart functions, coronary

atherosclerosis, and calcification (8, 9). Some studies show that vitamin D can also regulate the cardiovascular autonomic tone (10, 11). Hypovitaminosis D has been associated with the disturbance of the cardiovascular autonomic system contributing to the development of an array of diseases including hypertension, orthostatic hypotension, and postural orthostatic tachycardia syndrome (10–13).

VVS is one of the most common diseases in the cardiovascular autonomic system in youngsters. In adult VVS patients, Usalp et al. (14) recently reported that serum vitamin D levels were low in patients with syncope, especially in patients diagnosed with VVS by HUTT test. However, the relationship between hypovitaminosis D and the cardiac autonomic nervous function state in children with VVS have not been studied. This research was undertaken for investigating correlation of vitamin D contents in serum with cardiac autonomic function in the pathogenesis and development of VVS in youngsters.

METHODS

This study included 76 patients (7–18 years old) with VVS. These patients were referred to Pediatric Cardiology, Peking University First Hospital (China) for an evaluation of unexplained syncope between May 2018 and November 2019. Routine evaluation in all of the patients was performed including a thorough investigation of their medical history, complete physical examination, 12-lead standard electrocardiogram (ECG), head-up tilt test and basic laboratory examinations. Fifteen healthy control subjects were included in this study and they were referred to our department for a cardiovascular assessment for an innocent murmur in the same months of the year as the VVS-group. The criteria for VVS diagnosis were determined based on a previously described protocol (3). Patients with neurologic, psychiatric, cardiovascular disorders including hypertension, and obesity were excluded from the study. All enrolled patients (with VVS), and their parents signed informed consent for the tilt test and the blood tests; the control subjects and their parents signed for the blood tests. The Ethics Committee of Peking University First Hospital approved this study.

Study Design

For investigating the association of vitamin D levels in serum with VVS, we compared VVS cases and control subjects for their serum contents of vitamin D. According to their serum levels, patients with VVS were then classified in deficient vitamin D or non-deficient group. To investigate how vitamin D deficiency affected the cardiac autonomic function between these two groups, we further compared time-domain parameters of heart rate variability (HRV) and examined the association of serum vitamin D contents with the indices of time-domain analysis in VVS patients.

Abbreviations: VVS, vasovagal syncope; 25(OH)D, 25-hydroxyvitamin D; HRV, heart rate variability; BMI, body mass index; BP, blood pressure; rMSSD, square root of the mean of sum of the square of differences between adjacent normal to normal interval; SDNN, standard deviation of normal to normal; SDANN, standard deviation of the average normal to normal intervals; ECG, electrocardiogram; SD, standard deviations.

Serum Vitamin D Assessment

Vitamin D from diet and skin exposure to sunlight can be assessed based on serum 25-hydroxyvitamin D (25(OH)D) level. So, vitamin D contents in serum (chemiluminescence immunoassay) were determined by detecting the 25(OH)D contents in serum. Each specimen was collected following 12 h of overnight fasting. According to recent clinical guidelines (15), vitamin D deficiency is defined as the 25(OH)D content in serum of <50 nmol/L.

Heart Rate Variability

Twenty-four-hour electrocardiogram (Holter) was examined for all enrolled patients. In order to reduce the influence of various tests, there was no overlap between 24-h Holter ECG and other cardiovascular tests. Holter ECG recordings of each patient were of good quality, as none of the patients experienced frequent premature contractions (less or equal to one contraction per hour). HRV was analyzed with an automatic Holter analysis system (DMS version 12.5, USA). Premature beats and artifacts of ECG were adjusted by interpolation with the previous and next successive heartbeat. HRV time-domain analysis was performed following calibration. The evaluation standards, physiological explanation, and biological signal processing algorithms were done following the guidelines from the North American Society of Pacing and Electrophysiology, as well as the Task Force of the European Society of Cardiology (16). Besides, HRV indices, including standard deviations (SDs) for all normal to normal heart rate intervals over 24 h (SDNN), percentage of differences between adjacent RR intervals that are >50 ms (pNN50), root-mean-square difference in the interval between two normal heart rates (rMSSD) and SDs for 5 min average interval between two normal heart rates (SDANN), were calculated. SDNN depends on a change of the overall autonomic nervous system activity of the heart. rMSSD is an HRV parameter that shows the parasympathetic activity of the heart while SDANN shows the sympathetic activity of the heart (16).

Statistical Methods

SPSS18.0 (SPSS Inc., Chicago, IL, USA) was adopted for all statistical analyses. All measurement variables were presented in the manner of mean $\pm$ SD. Continuous variables with normal distribution were analyzed by independent t-test, whereas chi-squared test was adopted for categorical variables, and Pearson correlation coefficient was determined to examine the association of 25(OH)D content in serum with the rMSDD value. A difference of $p < 0.05$ indicated statistical significance.

RESULTS

Demographic Characteristics and Serum Vitamin D Status of Participants With VVS

A total of 76 youngsters aged 7–18 years participated in this study. Among those patients, 21 (27.6%) were male and 55 (72.4%) were female. The mean BMI in this cohort was 18.5 $\pm$ 2.3 kg/m^2. Compared with healthy control group, age, sex, BMI, baseline BP, and baseline heart rate did not differ in children and adolescents with VVS. The average 25(OH)D content in serum

among the test patients was 48.76 ± 19.25 nmol/L. Compared with healthy control group, VVS children and adolescents had significantly low 25(OH)D content in serum. Using serum level of 25(OH)D < 50 nmol/L as standard for hypovitaminosis D, there were 60.5% of hypovitaminosis D in test patients, significantly higher than the healthy control group (**Table 1**).

Associations of Vitamin D Value in Serum With HRV Indices in Youngsters With VVS

Table 2 illustrates patient demographic features according to the 25(OH)D status. Differences in 25(OH)D level between vitamin D deficient and non-deficient groups showed no significance with regard to sex, age, BMI, baseline blood pressure, or baseline heart rate. However, in the serum vitamin D deficient test patients exhibited a lower rMSDD value than the non-deficient test group (45.56 ± 16.87 vs. 61.90 ± 20.38 ms, $p < 0.001$, respectively). The other time-domain parameters, including SDNN, pNN50, and SDANN, did not differ between the two VVS groups.

Serum 25-hydroxyvitamin D levels had a positive correlation with rMSDD values ($r = 0.466$, $p < 0.05$) using Pearson correlation analysis (**Figure 1**).

DISCUSSION

Our results suggest that hypovitaminosis D was common (at a rate of 60%) in children and adolescents with VVS at a rate of 60%. This group exhibited a significantly reduced rMSSD value, and the 25(OH)D levels in serum were positively correlated with rMSSD value. rMSSD reflects the parasympathetic activity of the heart, indicating a correlation between hypovitaminosis and the disturbance of cardiac autonomic nervous function, which may participate in VSS occurrence and progression among child and adolescent subjects. We speculate that vitamin D deficiency results in a decreased cardiac vagal tone which further leads to the augmentation in basal sympathetic activity. Increased baseline sympathetic activity, combined with modulation of the cardiovascular autonomic nervous system when assuming

TABLE 1 | Demographic and clinical characteristics of patients and control subjects.

Characteristics	VVS group	Control group	*P*-value
Cases, n	76	15	
Age, years	12 ± 2	11 ± 2	0.296
BMI, kg/m^2	18.5 ± 2.3	17.4 ± 1.4	0.07
Females/males, n	55/21	9/6	0.364
Baseline systolic BP, mmHg	106.5 ± 8.0	107.3 ± 8.4	0.747
Baseline diastolic BP, mmHg	63.8 ± 6.3	65.6 ± 10.4	0.386
Baseline hear rate, beats/min	78.3 ± 10.0	77.9 ± 8.6	0.884
25(OH)D level, nmol/L	48.76 ± 19.25	67.62 ± 15.46	0.001
Children with vitamin D deficiency, n (%)	46 (60.5)	2 (13.3)	0.001

BMI, body mass index; BP, blood pressure.

TABLE 2 | Characteristics and HRV indices of time-domain analysis in children and adolescents with VVS based on 25-hydroxyvitamin D status.

Characteristics	Deficient group	Non-deficient group	*P*-value
Cases, n	46	30	
Age, years	12 ± 2	12 ± 2	0.237
BMI, kg/m^2	18.9 ± 2.4	18.0 ± 2.3	0.084
Females/males, n	33/13	22/8	0.879
Baseline systolic BP, mmHg	107.6 ± 8.2	104.9 ± 7.7	0.156
Baseline diastolic BP, mmHg	64.4 ± 6.2	62.9 ± 6.5	0.298
Baseline hear rate, beats/min	76.5 ± 10.0	79.9 ± 8.2	0.127
25(OH)D level, nmol/L	37.58 ± 11.45	65.90 ± 15.90	<0.001
SDNN, ms	150.04 ± 37.05	154.00 ±35.18	0.644
pNN50, %	20.28 ± 8.18	20.56 ± 10.07	0.893
rMSDD, ms	45.56 ± 16.87	61.90 ± 20.38	<0.001
SDANN, ms	138.91 ± 28.25	139.83 ± 34.98	0.900

BMI, body mass index; BP, blood pressure; HRV, heart rate variability; rMSSD, Square root of the mean of sum of the square of differences between adjacent normal to normal interval; SDNN, standard deviation of normal to normal; SDANN, standard deviation of the average normal to normal intervals; pNN50, percentage of differences between adjacent RR intervals that are >50 ms.

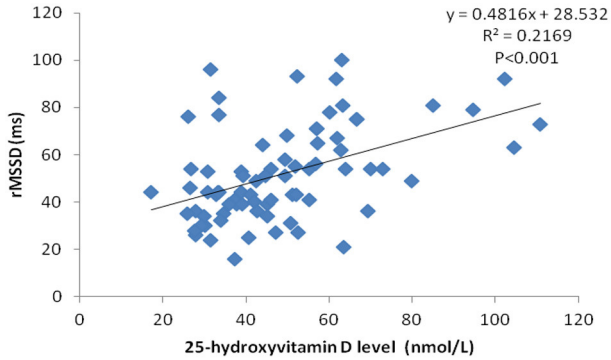

FIGURE 1 | Correlation scatter plot between serum 25-hydroxyvitamin D level and rMSSD in VVS patients.

an upright position, makes the patients more predisposed to triggering the Bezold-Jarisch reflex and leading to a syncopal attack. However, we did not find that orther HRV indexes of sympathetic activity such as SDANN increased in vitamin D deficient children with VVS. Further studies with larger size are needed to confirm these findings.

Vitamin D deficiency is becoming a widespread nutritional disorder of epidemic proportions primarily caused by insufficient sunlight exposure, a high prevalence of obesity and poor eating habits (17). Previous reports showed that low levels of serum vitamin D were related to an increased susceptibility to many cardiovascular diseases (CVDs), such as orthostatic hypotension, hypertension, heart failure, and coronary artery disease (8, 9, 11, 12). Many studies found that vitamin D supplementation could also modulate the sympathetic nervous system of the heart in vitamin D deficient, but otherwise healthy young adults (10, 18).

The autonomic nervous dysfunction has been shown to participate in the pathogenesis of VVS in children and adolescents. HRV is measured by the exact fluctuations in the beat-to-beat interval and used for qualitative and quantitative evaluation of cardiac autonomic function. At present, HRV is a commonly recognized approach for the evaluation of cardiac autonomic function. Akçaboy et al. (19) studied 24-h HRV in children with VVS and reported that they exhibited a significantly increased SDNN compared to healthy control subjects. Zygmunt and Stanczyk (20) found that rMSSD and pNN50 (the proportion of the difference between adjacent normal to normal heart rate intervals >50 ms) values in syncopal children were lower than in healthy children. In addition, the children with syncope exhibited decreased high-frequency (HF) whereas increased low-frequency (LF) using the frequency-domain analysis. These results indicate a decreased vagal and increased sympathetic modulation in VVS patients.

The exact pathogenesis of VVS, however, remains unknown. VVS, postural tachycardia syndrome and orthostatic hypotension have similar etiologies. Studies in adults showed that hypovitaminosis D was independently correlated with orthostatic hypotension (12). Antiel et al. (13) found that the incidence of hypovitaminosis D in adolescent patients with POTS was higher than the normal adolescent population (30 vs. 14%, respectively) (21). Our study reported a possible association between hypovitaminosis D and VVS in children and adolescents. We found that hypovitaminosis D was more prevalent in VVS patients than in the healthy control group and may be involved in the pathological mechanism for VVS by affecting cardiac autonomic function. These findings are significant to the understanding of the VVS pathogenesis. Tønnesen et al. (18) found that young people (18–25 years old) with low serum vitamin D improved their autonomic dysfunction following 180-day vitamin D supplementation. Thus, supplementation of vitamin D is suggested to benefit the recovery from VVS in children and adolescents with hypovitaminosis D.

Our study has several limitations. We did not compare HRV of VVS patients with the normal controls. As mentioned above, there had been several studies including Akçaboy et al. (19) and Zygmunt and Stanczyk (20) which showed that HRV in children with VVS exhibited significant abnormalities compared with normal healthy children. Further research should include multi-center-based studies to identify vitamin D deficiency involvement in the regulation of cardiac autonomic functions in a wider range of patients with VVS. Other methods of autonomic nervous function assessment including frequency-domain analysis, quantitative Valsalva maneuver and heart rate changes with deep breathing in VVS patients are worthy of further investigation. Moreover, we failed to consider that the vitamin D levels were affected by exposure to the sun in different seasons, which might affect the accuracy of the results.

CONCLUSION

Our data indicate that vitamin D deficiency may be correlated with cardiac autonomic dysfunction of pediatric vasovagal syncope. Further prospective large-scale studies are necessary to confirm these findings and better understand the role of hypovitaminosis D in the pathogenesis of autonomic dysfunction in patients with VVS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of Peking University First Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

QZ and JD designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. YS collected data, carried

out the initial analyses, and reviewed and revised the manuscript. CZ contributed to the manuscript design and data analysis. JQ reviewed the manuscript, contributed to the literature overview, and data analysis. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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OPINION
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Chronic Secondary Cardiorenal Syndrome: The Sixth Innovative Subtype

*Yipeng Zhang, Yue Jiang, Wentao Yang, Linghong Shen and Ben He**

Department of Cardiology, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China

Keywords: new classification, chronic secondary cardiorenal syndrome, type 6 cardiorenal syndrome, chronic co-impairment, fibrosis, biomarker

INTRODUCTION

Heart failure, as the leading cause of cardiovascular death, has seen an increased prevalence nowadays, along with renal insufficiency. It's estimated that 25–63% of heart failure patients have the comorbidity of renal insufficiency, an independent risk factor of various cardiovascular events and predictors of poor prognosis (1). A therapeutic principle of heart failure emphasizing decongestive treatment is limited by the demand for sufficient perfusion in terms of renal insufficiency therapy, making the treatment of both concomitant diseases more complicated and contradictory, with mild balance; hence, the great interest in cardiorenal interactions has broadened among researchers and clinicians, and the concept of cardiorenal syndrome (CRS) was first proposed in 2004.

CRS, defined as a pathophysiological process of adverse interaction between the heart and kidneys, encompasses a spectrum of diseases involving acute or chronic dysfunction in one organ that induces decompensated dysfunction in the other, and eventually they evolve into an interrelated and vicious cycle of declining function in both organs. CRS was first categorized into five subtypes in 2008 based on sequential organ involvement and the course of progression over time (i.e., acute or chronic), including acute cardiorenal syndrome, chronic cardiorenal syndrome, and acute renocardiac syndrome, chronic renocardiac syndrome, secondary cardiorenal syndrome, of which a brief definition, etiology, and pathophysiology are given in **Table 1** (2). With the consideration that the previous secondary CRS subtype has a shortcoming in that it is not as symmetrical as the first four subtypes, as well as the huge difference of pathophysiological changes, the treatment principles of the two organs, and of gradually increasing in-depth knowledge of fibrosis pathogenesis over the past few years, it's become quite necessary to propose a new CRS classification. And that's why we now first propose the sixth innovative CRS subtype on the basis of the concept of "chronic co-impairment" of the heart and kidneys, further classifying traditional secondary CRS as acute secondary CRS and chronic secondary CRS, thus making the novel six kinds of CRS categories paired and matched correspondingly. The most common precipitant of acute secondary CRS is biotoxin damage and a cytokine storm generated by acute sepsis, and the primary treatment principles are cause-related treatment as aggressive anti-infective therapy, as well as symptomatic treatment of cardiac and renal dysfunction. However, the novel type 6 CRS (chronic secondary CRS) is actually more prevalent in clinical practice, and its pathophysiological and clinical characteristics as well as diagnosis and treatment principles vary dramatically from other subtypes of CRS, which will be described in detail in the following article.

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Edited by:
Junjie Xiao,
Shanghai University, China

Reviewed by:
Yan Zhang,
Peking University, China
Leyi Gu,
Shanghai JiaoTong University, China

****Correspondence:***
Ben He
heben241@126.com

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TABLE 1 | New classification of CRS based on disease acuity and sequential organ involvement.

Phenotype	Nomenclature	Definition	Common etiology	Pathophysiology	Diagnosis	Treatment
Type 1 CRS	Acute cardiorenal syndrome	ACS or AHF resulting in AKI	ACS, AHF	Hemodynamic damage, RAAS activation, sympathetic neurohormonal activation	Descriptive diagnosis based on history and sequential organ involvement	Respective treatment of organs involved
Type 2 CRS	Chronic cardiorenal syndrome	Chronic HF resulting in CKD	Chronic HF	Chronic hypoperfusion, subclinical inflammation, accelerated atherosclerosis		
Type 3 CRS	Acute renocardiac syndrome	AKI resulting in AHF	Volume overload, inflammatory surge resulting from AKI	Hypertension, RAAS activation, sympathetic activation; electrolyte, and acid-base imbalance		
Type 4 CRS	Chronic renocardiac syndrome	CKD resulting in chronic HF	spectrum of CKD	Anemia, malnutrition, uremic toxins, electrolyte and coagulation imbalance, volume overload		
Type 5 CRS	Acute secondary cardiorenal syndrome	Acute systemic disease resulting in AHF and AKI/AKF	Sepsis, acute intoxication	Toxemia, systemic inflammation, cytokine storm, exogenous toxins mediated damage		
Type 6 CRS	Chronic secondary cardiorenal syndrome	Chronic systemic process resulting in HF and CKD	Diabetes mellitus, hypertension, obesity, amyloidosis, cirrhosis, systemic lupus erythematosus	Common fibrosis pathway mediated by systemic inflammation, oxidative stress, RAAS activation, sympathetic neurohormonal activation, vascular endothelial dysfunction, etc.	Comprehensive diagnosis based on definitive history, objective biomarkers (Gal-3, NGAL, ST-2) and visible imaging	Cardiorenal co-treatment represent as MRA, ARNI, SGLT-2i

ACS, Acute Coronary Syndromes; AHF, Acute Heart Failure; AKI, Acute Kidney Injury; CKD, Chronic Kidney Disease; AKF, Acute Kidney Failure; MRA, Mineralocorticoid Receptor Antagonist; ARNI, Angiotensin Receptor Neprilysin Inhibitor; SGLT-2i, Sodium-Glucose co-Transporter-2 inhibitor.

TYPE 6 CRS

Definition of Type 6 CRS

In clinical practice, many patients hospitalized for the first time have been examined for cardiac and renal insufficiency simultaneously, in whom it's hard to identify exactly which organ is the primary precipitant of the vicious cycle of CRS. There is the other situation that patients with chronic systematic diseases diagnosed long ago such as cirrhosis, amyloidosis, systemic lupus erythematosus, obesity, diabetes, hypertension, and hyperlipidemia may gradually evolve into declining function of both organs in the follow-up. The above two kinds of circumstances can be classified into type 6 CRS. In summary, type 6 CRS is defined as analog clinical circumstances of a clearly acknowledged onset of chronic systemic conditions in the very beginning, including diabetes, hypertension, amyloidosis, and systemic lupus, followed by gradually progressive decompensation of cardiac and renal function, culminating in cardiac and renal failure.

Pathophysiology of Type 6 CRS

Recent studies have shown that type 6 CRS shares a common pathophysiological mechanism, i.e., that chronic systemic diseases cause systemic inflammation, renin-angiotensin-aldosterone system (RAAS) activation, sympathetic neurohormonal activation, oxidative stress, vascular endothelial dysfunction, and other pathological changes, and these finally lead to cardiac and renal fibrosis and consequent insufficiency (3, 4). There exist both overlap and difference between type 6 CRS and the five traditional cardiorenal syndromes in pathophysiological mechanisms, as illustrated in **Table 1,** by the detailed multiple pathophysiology of each CRS, but the common fibrosis pathway is unique to type 6 CRS with direct significance in fundamental research and clinical applications. Take hypertension, for example: it's often accompanied by RAAS activation, sympathetic nerve activation, and vascular endothelial dysfunction, on account of which increased aldosterone has been found to mediate a series of signaling cascades of fibrosis in animal models. NGAL, ST-2, and Galectin-3, as mediators of aldosterone-induced fibrosis, can promote proliferation and differentiation of fibroblasts and the secretion of extracellular matrix proteins, leading to fibrosis in both organs eventually (5). Similar pathophysiological changes can also be seen in diabetes, such as chronic systemic vascular inflammation, endothelial dysfunction, and oxidative stress, which induce increased transcription of multiple inflammatory factors. TGF-β, a well-known inflammatory factor with the broadest spectrum of effects, could affect Smad transcription factors to mediate downstream signaling pathways of fibrosis (6). Systemic lupus erythematosus (SLE) causes apoptosis and necrosis of cardiac and renal cells through direct immune injury, thus leading to an injury-related fibrosis repair process. Fibrosis is seen primarily as a protective compensation mechanism

of external damage of systemic conditions, but it eventually causes chamber dilatation, heart failure, loss of nephrons, and decreased glomerular filtration rate, namely, the type 6 cardiorenal syndrome here.

Diagnosis of Type 6 CRS

The past diagnosis of CRS was merely descriptive, on the basis of previous history, without accurate laboratory and imaging data assisting. The new CRS classification proposed here, especially the type 6 CRS, is diagnosed with the common pathogenesis pathway of fibrosis and can be differentiated in diagnosis from type 2 or type 4 CRS according to onset characteristics and complementary examinations. In addition to a patient's previous diagnostic history of chronic diseases such as hypertension, obesity, and SLE, or if the first symptoms are chronic co-impairment of cardiac and renal function without a clear precipitant, laboratory examinations can provide an important reference for diagnosis with elevated biomarkers relevant to fibrosis and other pathophysiological targets (7). Several studies have found that Gal-3, NGAL, ST-2, cardiotrophin-1, as rising indicators in a mouse fibrosis model, can be potential diagnostic biomarkers for type 6 CRS in the future (8–11). However, more evidence is needed for those markers of effective indication of fibrosis in human body. Imaging examination such as LGE-MRI has important diagnostic value for myocardial fibrosis. Kidney biopsy, as an invasive test, used to be conducted in the evaluation of the pathological type of intractable nephritis, could provide direct evidence of renal fibrosis, in which renal tubulointerstitial fibrosis, mesenchymal-epithelial transformation of interstitial cells with elevated fibroblast markers such as collagen, fibronectin, and reduced interstitial markers such as E-cadherin in immunohistochemical staining can be observed.

Treatment of Type 6 CRS

Fibrosis is not only an indicator of diagnosis of type 6 CRS, but also the creative breakthrough in the treatment of the new subtype. Previous CRS treatments were treated, respectively, not as unified as the concept of CRS (12). Clinicians tend to treat the organ perceived to be the primary precipitant with etiological treatment and provide corresponding symptomatic support for the other organ involved in CRS, and there are often contradictory conditions, such as acute CRS, so that decongestive therapy needed for heart failure may conflict enough with renal perfusion and reflexively activate the RASS system, thus aggravating renal insufficiency, in which the inappropriate balance of the two will lead to deterioration of the CRS. The new unified therapeutic target of type 6 CRS based on the common fibrosis pathway, is mediated by RASS activation, inflammation, oxidative stress, and vascular endothelial dysfunction (13). Therefore, the treatment of type 6 CRS based on the above common target has substantial theoretical support. Current evidence shows that fibrosis is partly mediated by MR pathway (14), and mineralocorticoid receptor antagonists (MRA), as diuretics in treatment of heart failure, is an important potential anti-fibrosis drug. The RASS system inhibitors ACEI and ARB, which have been widely recognized for their positive effects on ventricular remodeling, have reduced the risk of cardiovascular events and prolonged survival, also have an anti-fibrosis effect to some degree; therefore they are expected to take a prescription for treatment of type 6 CRS. Physicians have long been quite circumspect about the usage of ACEI and ARB in HF patients with severe renal insufficiency for fear of exacerbation of renal dysfunction and hyperkalemia. Recent studies have shown that above inordinate concern can be dispelled by regular monitoring of renal function and serum potassium. A propensity score analysis of 1,665 patients with HF (EF $<$ 45%) and eGFR $<$60 ml/min suggested treatment with an ACE inhibitor or ARB, which was associated with significant reductions in all-cause mortality. More clinical trials are still underway to provide evidence to confirm the clinical benefits of ACEI or ARB in patients with advanced CKD. In the future, target excavation and development of new drugs related to inflammation, endothelial dysfunction, oxidative stress, and other pathophysiological changes in CRS are of great value and prospect (15). ARNI and SGLT-2i have shown tremendous benefits to both the heart and kidneys in patients with chronic diseases, which especially demonstrates the pioneering concept of cardiorenal co-treatment (16, 17). As for the initial precipitant of the vicious circle of type 6 CRS, chronic systemic diseases should be well-controlled with respect to indispensability, such as hypertension, diabetes mellitus, obesity, etc.

CONCLUSION

The sixth innovative CRS subtype, named chronic secondary CRS, is a new concept derived from the five classical CRS, and its most important value lies in its subversive notion of the diagnosis and treatment of CRS. The past CRS classification proposed in 2008 is a descriptive diagnosis based on a medical history with no specifically derived therapeutic interventions; therefore, it exerts little influence over treatment in current clinical practice. While our new type 6 CRS is based—except for disease acuity and sequential organ involvement—on detectable and visualized markers of cardiac injury including BNP and myocardial enzyme spectrum, renal function markers such as creatinine, Cys-C, and key pathophysiological markers of fibrosis of cardiorenal chronic co-impairment, together with imaging and pathological examination to make an integrated diagnosis, for which our novel pragmatic CRS categories facilitate a differentiation diagnosis from various subtypes and streamline inclusion criteria for future clinical trials. Moreover, our brand-new concept can also guide the treatment of type 6 CRS, based on the common pathogenesis of fibrosis. Unlike previous CRS for which heart and kidney dysfunction are treated separately, anti-fibrosis therapy truly realizes a comprehensive and unitary treatment based on the concept of CRS. However, there are still many fields of the new subtype worth further exploration. In addition to fibrosis, the problems of inflammation, endothelial dysfunction, oxidative stress, and other unified pathophysiological mechanisms of

cardiorenalco-impairment of type 6 CRS deserve further investigation, as well as the establishment of diagnostic markers based on common pathogenesis, research for key targets, and development of corresponding potential new drugs (18). Furthermore, nowadays many large-scale RCT of drugs often exclude advanced CKD from the population included due to the concern of renal insufficiency affecting pharmacokinetics, which makes the clinical research data for excavating potential drugs specific to CRS less available. It is firmly believed that, as clinicians attach more importance to the novel concept of type 6 CRS, more cross-specialty cooperation and clinical trials for precise diagnosis and treatment of type 6 CRS will spring up constantly.

AUTHOR CONTRIBUTIONS

BH proposed this new classification concept and revised the manuscript. YZ wrote the manuscript. YJ, WY, and LS participated in discussions and provided useful suggestions to the conceptualization of the work. All authors contributed to the article and approved the submitted version.

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The Association Between Metformin Treatment and Outcomes in Type 2 Diabetes Mellitus Patients With Heart Failure With Preserved Ejection Fraction: A Retrospective Study

Jianfang Wang[1,2†], *Yi Lu*[1†], *Xinjia Min*[1], *Tan Yuan*[1], *Jia Wei*[3] *and Zhejun Cai*[1,4*]

[1] Department of Cardiology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2] Ningbo Medical Center Lihuili Hospital, Ningbo, China, [3] Department of Urology, Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China, [4] Jiaxing Key Laboratory of Cardiac Rehabilitation, Jiaxing, China

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Edited by:
Junjie Xiao,
Shanghai University, China

Reviewed by:
Yue Wu,
The First Affiliated Hospital of Xi'an Jiaotong University, China
Nicholas Cauwenberghs,
KU Leuven, Belgium

***Correspondence:**
Zhejun Cai
caizhejun@zju.edu.cn

[†]These authors have contributed equally to this work

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Background: Metformin is the first-line antidiabetic medication for type 2 diabetes mellitus (T2DM). However, the association between metformin and outcomes in T2DM patients with heart failure with preserved ejection fraction (HFpEF) is still unknown. We aimed to explore the association between metformin and adverse outcome in T2DM patients with HFpEF.

Methods: A total of 372 T2DM patients with HFpEF hospitalized from January 1, 2013, to December 31, 2017, were included in this retrospective cohort study. There were 113 and 259 subjects in metformin and non-metformin group, respectively. Subjects were followed up for all-cause mortality, cardiovascular death, all-cause hospitalization, and heart failure hospitalization.

Results: The median follow-up period was 47 months. Eleven patients (2.49% per patient-year) in the metformin group and 56 patients (5.52% per patient-year) in the non-metformin group deceased during follow-up ($P = 0.031$). However, a multivariable Cox regression failed to show that metformin was an independent factor of all-cause mortality [HR (95% CI) = 0.682 (0.346–1.345); $P = 0.269$]. A subgroup analysis revealed a significant association between metformin and all-cause mortality in patients with a higher hemoglobin A1c (HbA1c) level (HbA1c ≥7%) [HR (95% CI) = 0.339 (0.117–0.997); $P = 0.045$]. The 4-year estimated number needed to treat (NNT) with metformin compared with non-metformin for all-cause mortality was 12 in all populations and 8 in the HbA1c ≥7% subgroup.

Conclusions: Metformin was not independently associated with clinical outcomes in patients with T2DM and HFpEF, but was associated with lower all-cause mortality in the subgroup of patients with poor glycemic control. Prospective, randomized controlled trials are needed to further verify these findings.

Keywords: metformin, heart failure with preserved ejection fraction, type 2 diabetes mellitus—exenatide, survival analysis (source: MeSH NLM), mortality

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) might be a heterogeneous syndrome of multiple discrete phenotypes and is prone to have multiple comorbidities, such as diabetes, hypertension, pulmonary disease, chronic kidney disease, and obesity (1), resulting in systemic and cardiac microvascular dysfunction (2, 3). Conventional therapies including angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARB), beta-blockers, mineralocorticoid receptor antagonists (MRA) can improve the long-term outcomes of heart failure with reduced ejection fraction (HFrEF) (4). However, these conventional medical therapies failed to reduce the risk of all-cause and cardiovascular death in HFpEF patients (5).

Type 2 diabetes mellitus (T2DM) is a common comorbidity in HFpEF and has a conspicuous negative impact on prognosis (6). As first-line antidiabetic therapy, metformin has cardiovascular protective effect through multiple mechanisms, including decreasing glucose, lowering weight, anti-inflammatory properties, and improving insulin resistance and endothelial function (7). Several observational studies indicated that metformin was associated with reduced mortality risk compared with other traditional antidiabetic drugs in T2DM patients with HF (patients with preserved or reduced left ventricular ejection fraction were included) (8). However, the impact of metformin on the outcome of HFpEF in T2DM patients has not been elucidated. Therefore, we performed a retrospective cohort study to investigate the association between metformin and this specific group of patients suffering from HFpEF with T2DM.

MATERIALS AND METHODS

Study Population

This is a retrospective cohort study conducted among in-hospital HFpEF (4) with T2DM patients admitted in the Department of Cardiology, the Second Affiliated Hospital, Zhejiang University School of Medicine from January 1, 2013, to December 31, 2017. The main inclusion criteria were (1) $\geq$40years of age; (2) had a left ventricular ejection fraction (LVEF) $\geq$50% and New York Heart Association (NYHA) class II to IV symptoms; (3) elevated B-type natriuretic peptide (BNP) $\geq$35 pg/mL or N-terminal pro-B-type natriuretic peptide (NT-proBNP) $\geq$125 pg/mL; (4) echocardiographic evidence of relevant structural heart disease (left atrial enlargement or left ventricular hypertrophy) or diastolic dysfunction (meet at least three of the following criteria simultaneously: left atrial enlargement, tricuspid regurgitation peak velocity >2.8 m/s, septal e' <7 cm/s or lateral e' <10 cm/s, E/e' >14), or imaging findings of pulmonary congestion; (5) diagnosed as T2DM in medical records and remained antidiabetic drugs therapy regularly for at least 3 months.

Patients were excluded if they: (1) had cardiovascular disorders that may change their clinical course independently of heart failure (such as myocardial infarction, coronary artery bypass graft surgery, or other major cardiovascular surgery, stroke or transient ischemic attack in the past 90 days); (2) currently implanted left ventricular assist device, or cardiac resynchronization therapy; (3) had a history of acute decompensated heart failure within 1 week of screening; (4) had a specific heart failure etiologies including hypertrophic obstructive cardiomyopathy, amyloidosis, acute myocarditis, pericardial disease, primary valvular heart disease requiring surgery or intervention, or severe conduction disorders requiring pacemaker implantation; (5) were previously diagnosed of reduced left ventricular EF <40%; (6) significant impaired renal function (estimated glomerular filtration rate of <45 mL/min/1.73 m^2 measured by the CKD-EPI equation or requiring dialysis at the time of screening) or hepatic function; (7) short life expectation; (8) loss of follow-up; (9) metformin users who failed to taking metformin continuously.

Data Collection

The demographic characteristics, underlying diseases, laboratory reports, antidiabetic therapy, and nursing records of study patients were collected. Demographic characteristics included age, gender, body mass index (BMI), cigarette smoking, blood pressure, heart function classification, left ventricular ejection fraction. Main underlying diseases included hypertension (HTN), atrial fibrillation (AF), coronary heart disease (CHD), or cerebral infarction (CI). Main laboratory reports included BNP, NT-proBNP, hemoglobin A1c (HbA1c), hemoglobin, serum creatinine, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG). The CKD-EPI equation was adopted for estimating the glomerular filtration rate (eGFR) (9).

Outcomes

Clinical endpoints were obtained through a median follow-up of 47 months *via* the EMR database and telephone connection. The primary endpoint was all-cause mortality. Secondary endpoints were cardiovascular death, all-cause hospitalization, and hospitalization for heart failure.

Statistical Analysis

Baseline data were expressed as means $\pm$ standard deviation or median (25th and 75th percentiles). Student's *t*-test or Mann–Whitney-test was used to compare continuous variables between the two groups. Data were presented as the number (percentage), and the χ^2-test was used to compare qualitative variables. The association between metformin and clinical outcomes was analyzed by Kaplan-Meier analysis. A multivariable Cox regression was performed using the stepwise regression, with a threshold of 0.1, to assess the independence of this association. Adjusted confounders included age, gender, body mass index, cigarette smoking, systemic blood pressure, diastolic blood pressure, left atrial diameter, left ventricular ejection fraction, NYHA class, duration of diabetes and heart failure, comorbidities including hypertension, atrial fibrillation, coronary heart disease, and cerebral infarction, laboratory findings including glycated hemoglobin, estimated glomerular filtration rate, hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride, and usage of sulfonylureas, glinides, glucosidase inhibitors, and insulin.

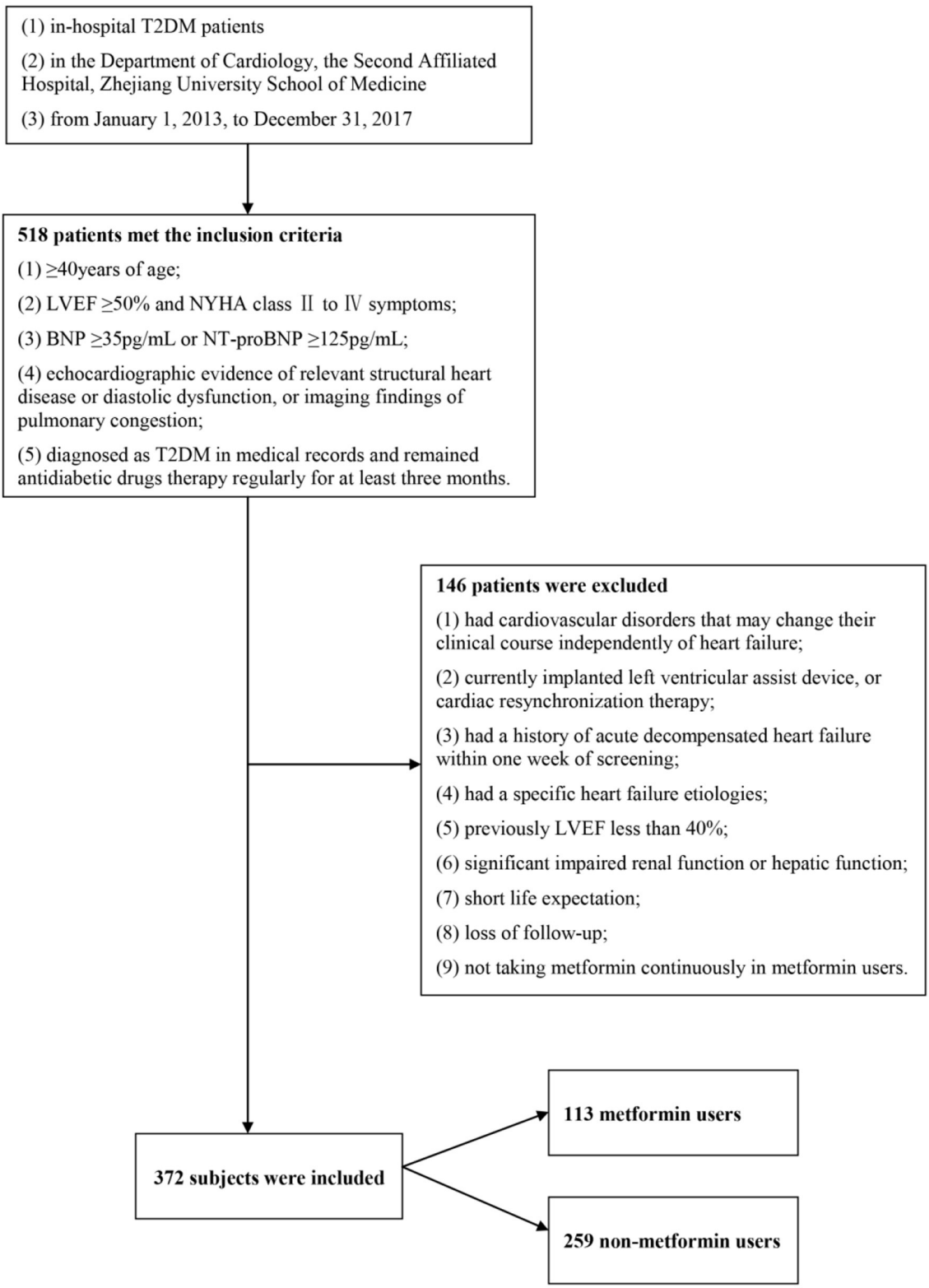

FIGURE 1 | Study population. Among the 518 subjects, we excluded 146 subjects who did not meet our inclusion criteria. The remaining 372 subjects were divided into the metformin group ($n = 113$) and the non-metformin group ($n = 259$).

Further, we explored the association between metformin and the primary outcome in selected subgroups. Multivariable Cox regressions with the same way and adjustments as the above were used in the subgroup analysis. The best glycaemic targets are still unclear, but most agree on HbA1c thresholds <7.0% for the majority of adults with DM. We categorized the subjects as HbA1c <7% and HbA1c ≥7% and did a subgroup analysis among patients with higher HbA1c levels. Major imbalances were found in age, sex, eGFR, and GI treatment between metformin and non-metformin users in the HbA1c ≥7% subgroup. We performed propensity score matching (PSM) of age and sex for patients in two groups. Matching was performed using the nearest neighbor matching, with a default caliper of 0.1.

The number needed to treat (NNT) values for metformin therapy incremental to non-metformin treatment were estimated for years 1 to 4 for the primary endpoint. NNT values were estimated as the inverse of the difference in estimated absolute risk between the metformin and non-metformin groups at each time point. The absolute risk for the non-metformin group was calculated directly from Kaplan-Meier estimates.

A two-sided P <0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (IBM, Armonk, New York).

TABLE 1 | Demographic and clinical characteristics of the patients.

Characteristics	Metformin (n = 113)	Non-metformin (n = 259)	P-value
Demographic characteristics			
Age (yr)	68.00 (62.00, 73.00)	73.00 (66.00, 80.00)	0.000
Male	44 (38.9%)	151 (58.3%)	0.001
Smoking	29 (25.7%)	79 (30.5%)	0.344
BMI (kg/m²)	25.26(23.37, 27.51)	25.00(23.01, 27.04)	0.151
SBP (mmHg)	130.00 (119.00, 139.00)	130.00 (120.00, 140.00)	0.858
DBP (mmHg)	75.00 (67.00, 83.00)	71.00 (63.00, 78.00)	0.001
NYHA class III-IV	26 (23.0%)	73 (28.2%)	0.299
LVEF (%)	64.97 ± 7.24	63.09 ± 7.14	0.021
Underlying diseases			
DM duration (yr)	10.00 (5.00, 10.00)	8.00 (4.00, 10.00)	0.280
HF duration (yr)	1.00 (0.10, 5.00)	1.00 (0.17, 5.00)	0.808
HTN	94 (83.2%)	214 (82.6%)	0.895
CHD	64 (56.6%)	173 (66.8%)	0.061
AF	49 (43.4%)	98 (37.8%)	0.316
CI	14 (12.4%)	37 (14.3%)	0.625
Laboratory reports			
HbA1c (%)	7.20 (6.50, 8.35)	7.30 (6.60, 8.46)	0.782
eGFR (ml/min/1.73 m²)	91.53 (77.11, 100.24)	81.55 (66.55, 93.32)	0.000
BNP (pg/mL)	84.55 (47.85, 171.63)	104.90 (57.25, 240.10)	0.074
NT-proBNP (pg/mL)	543.00 (305.00, 1470.00)	999.00 (324.00, 2194.00)	0.303
Hb (g/L)	125.00 (117.50, 133.00)	128.00 (117.00, 137.00)	0.169
LDL (mmol/L)	1.89 (1.39, 2.61)	2.02 (1.56, 2.57)	0.212
HDL (mmol/L)	1.06 (0.89, 1.23)	1.08 (0.92, 1.23)	0.417
TG (mmol/L)	1.43 (1.07, 1.96)	1.29 (0.91, 1.78)	0.081
LA (cm)	3.97 (3.70, 4.36)	4.11 (3.80, 4.53)	0.103
Antidiabetic therapy			
Sulfonylureas	43 (38.1%)	134 (36.0%)	0.590
Glinides	18 (15.9%)	47 (18.1%)	0.604
GI	158 (61.0%)	48 (42.5%)	0.001
TZD	4 (3.5%)	10 (3.9%)	0.881
DPP4 inhibitor	3 (2.7%)	9 (3.5%)	0.681
Insulin	25 (22.1%)	80 (30.9%)	0.084

Values are means ± standard deviation or median (interquartile range) or the number of participants (%). AF, atrial fibrillation; BMI, body mass index; BNP, B-type natriuretic peptide; CHD, coronary heart disease; CI, cerebral infarction; DBP, diastolic blood pressure; DM, diabetes mellitus; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GI, glucosidase inhibitors; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HF, heart failure; HTN, hypertension; LA, left atrial/atrium; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; SBP, systemic blood pressure; TG, triglyceride; TZD, thiazolidinediones.

RESULTS

Baseline Characteristics

Among the 518 subjects, we excluded 146 subjects who did not meet our inclusion criteria. The remaining 372 subjects were divided into the metformin group (n = 113) and the non-metformin group (n = 259) (**Figure 1**). The median age was 71 years old (interquartile range, 65–79), and 52.4% were male gender. 26.6% of the subjects were categorized as NYHA class III-IV, and few patients have taken thiazolidinediones (TZD) or dipeptidyl peptidase-4 (DPP4) inhibitor. Major imbalances were spotted in age (P = 0.000), gender (P = 0.001), diastolic blood pressure (P = 0.001), LVEF (P = 0.021), eGFR (P = 0.000) and glucosidase inhibitor usage (P = 0.001) between metformin and non-metformin users (**Table 1**). The date of the last patient follow-up was August 2, 2020. The median duration of participation in the study was 47 months (interquartile range, 38–67).

Association Between Metformin and Clinical Outcomes

In current study, 11 patients (2.49% per patient-year) in metformin group and 56 patients (5.52% per patient-year) in non-metformin group deceased during follow-up. The 1-, 2-, 3-, and 4-year survival rates of the metformin group were 100, 97.3, 92.7, and 88.7%, and the non-metformin group were 97.7, 94.2, 87.1, and 80.5%, respectively, with statistically significant differences (P = 0.031) (**Figure 2A**). There was no statistically significant association between metformin and cardiovascular death (P = 0.252), all-cause hospitalizations (P = 0.900), and hospitalization for heart failure (P = 0.671) (**Figures 2B–D** and **Table 2**). However, a multivariable Cox regression failed to show that metformin was an independent factor of all-cause mortality [HR (95% CI) = 0.682 (0.346–1.345); P = 0.269] (**Table 2**).

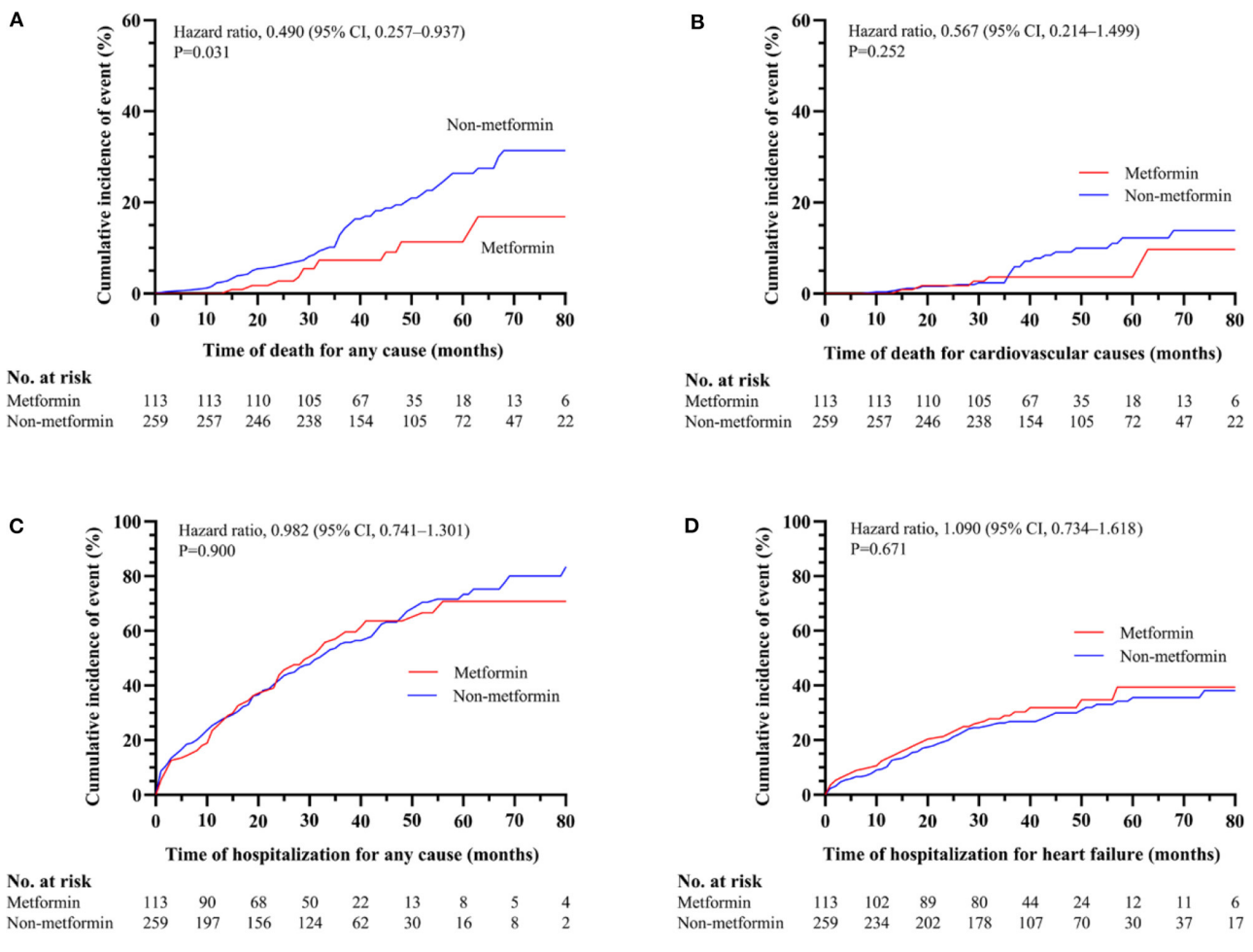

FIGURE 2 | The association between metformin and non-metformin and clinical outcomes in the overall cohort. Metformin was positively related to all-cause mortality (P = 0.031) **(A)**. However, metformin has no effect on cardiovascular death (P = 0.252) **(B)**, all-cause hospitalizations (P = 0.900) **(C)** or hospitalization for heart failure (P = 0.671) **(D)**.

TABLE 2 | The association between metformin and endpoints.

	Metformin (*n* = 113)	Non-metformin (*n* = 259)	Hazard ratio (95% CI)	*P*-value
All-cause mortality*	11 (2.49)	56 (5.52)	0.682 (0.346, 1.345)	0.269
Cardiovascular death†	5 (1.13)	22 (2.17)	0.567 (0.214, 1.499)	0.252
All-cause hospitalizations†	69 (15.59)	165 (16.27)	0.982 (0.741, 1.301)	0.900
Hospitalization for heart failure†	36 (8.13%)	78 (7.69)	1.090 (0.734, 1.618)	0.671

Data were presented as no. of patients with events (% per patient-year).

**For all-cause mortality, hazard ratios and 95% CIs were estimated using Cox regression models. Adjusted covariables included age, gender, body mass index, cigarette smoking, systemic blood pressure, diastolic blood pressure, New York Heart Association class, left ventricular ejection fraction, duration of diabetes and heart failure, whether living with hypertension, atrial fibrillation, coronary heart disease, or cerebral infarction, glycated hemoglobin, estimated glomerular filtration rate, hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, left atrial, sulfonylureas, glinides, glucosidase inhibitors, insulin.*

† Hazard ratios and 95% CIs for secondary outcomes were not adjusted for multiplicity.

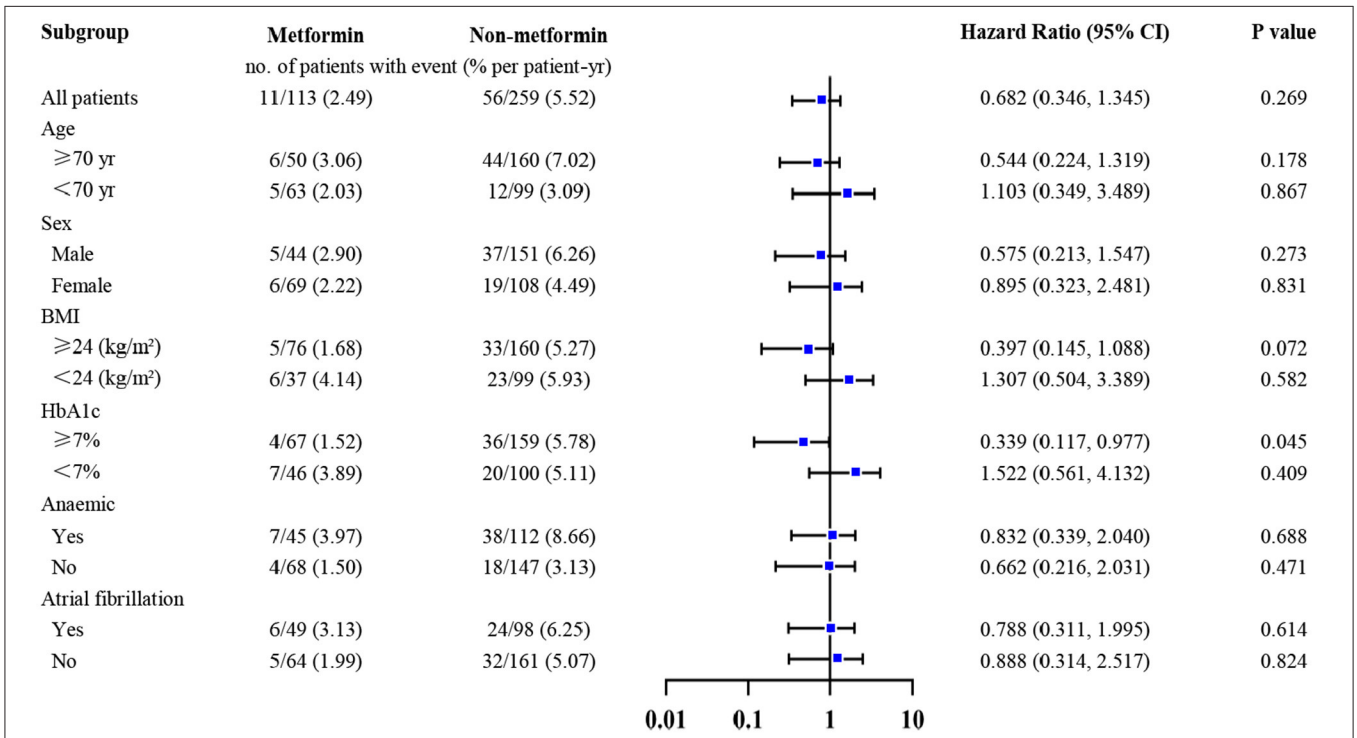

FIGURE 3 | Association between metformin and all-cause mortality in selected subgroups adjusted for multiplicity. Metformin was only associated with lower all-cause mortality in patients whose HbA1c ≥7% [HR (95% CI) = 0.339 (0.117–0.977); $P = 0.045$]. The widths of the confidence intervals were adjusted for multiplicity, using the stepwise regression, with a threshold of 0.1. Adjusted covariables included age, gender, body mass index, cigarette smoking, systemic blood pressure, diastolic blood pressure, New York Heart Association class, left ventricular ejection fraction, duration of diabetes and heart failure, whether living with hypertension, atrial fibrillation, coronary heart disease, or cerebral infarction, glycated hemoglobin, estimated glomerular filtration rate, hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, left atrial, sulfonylureas, glinides, glucosidase inhibitors, insulin.

Association Between Metformin and Primary Outcome in Selected Subgroups for Multiplicity

We explored the association between metformin and the primary outcome in selected subgroups (stratified according to age, gender, anemia, atrial fibrillation, level of HbA1c, and BMI), using a Cox proportional-hazards model to adjust the hazard ratio and widths of the confidence intervals, and the results were listed in **Figure 3**. Notably, metformin usage was associated with lower all-cause mortality only in patients with HbA1c ≥7% [HR (95% CI) = 0.339 (0.117–0.977); $P = 0.045$].

Association Between Metformin Usage and Clinical Outcomes in Subjects With HbA1c ≥7%

The demographic and clinical characteristics of subjects with HbA1c ≥7% are described in **Table 3**. Among the 226 subjects in the HbA1C>7% subgroup, 156 subjects were included for

TABLE 3 | Demographic and clinical characteristics of the patients in the HbA1c ≥7% subgroup.

Variables	Metformin usage (before PSM)		*P*-value	Metformin usage (after PSM)		*P*-value
	Metformin (*n* = 67)	Non-metformin (*n* = 159)		Metformin (*n* = 60)	Non-metformin (*n* = 96)	
Demographic characteristics						
Age (yr)	69.00 (63.00, 73.00)	74.00 (66.00, 80.00)	0.000	69.00 (64.00, 74.00)	70.00 (65.00, 75.75)	0.295
Male	28 (41.8%)	89 (56.0%)	0.051	27 (45.0%)	41 (42.7%)	0.779
Smoking	20 (29.9%)	48 (30.2%)	0.960	19 (31.7%)	24 (25.0%)	0.369
BMI(kg/m²)	25.35 (23.73, 27.63)	24.80 (22.68, 27.12)	0.043	25.31 (23.75, 27.95)	24.85 (22.67, 27.38)	0.096
SBP(mmHg)	126.00 (117.00, 139.00)	131.00 (119.00, 140.00)	0.206	126.00 (119.00, 138.75)	130.50 (119.25, 140.00)	0.291
DBP(mmHg)	74.00 (66.00, 81.00)	71.00 (63.00, 79.00)	0.263	73.50 (63.75, 80.75)	72.00 (64.00, 79.00)	0.658
NYHA class III-IV	17 (25.4%)	42 (26.4%)	0.871	16 (26.7%)	23 (24.0%)	0.709
LVEF(%)	64.42 ± 7.24	63.19 ± 7.33	0.249	64.45 ± 7.42	63.42 ± 6.64	0.410
Underlying diseases						
DM duration (yr)	10.00 (5.00, 14.00)	10.00 (5.00, 13.00)	0.262	10.00 (5.00, 16.25)	8.00 (4.00, 14.00)	0.131
HF duration (yr)	1.00 (0.10, 6.00)	1.00 (0.10, 4.00)	0.680	2.00 (0.17, 6.00)	1.00 (0.10, 5.00)	0.533
HTN	56 (83.6%)	127 (79.9%)	0.517	50 (83.3%)	74 (77.1%)	0.347
CHD	39 (58.2%)	105 (66.0%)	0.264	36 (60.0%)	61 (63.5%)	0.657
AF	27 (40.3%)	64 (40.3%)	0.995	22 (36.7%)	38 (39.6%)	0.716
CI	8 (11.9%)	24 (15.1%)	0.535	8 (13.3%)	10 (10.4%)	0.579
Laboratory reports						
HbA1C (%)	8.00 (7.50, 8.90)	8.10 (7.40, 9.10)	0.547	8.00 (7.50, 9.00)	8.15 (7.50, 9.19)	0.406
eGFR (ml/min/1.73 m²)	89.10 (76.27, 98.64)	82.49 (66.75, 94.16)	0.034	88.97 (74.96, 99.00)	86.73 (68.86, 95.47)	0.382
BNP (pg/mL)	84.55 (44.20, 174.75)	117.75 (57.08, 268.63)	0.711	84.90 (43.80, 175.20)	115.60 (56.40, 312.25)	0.094
NT-proBNP (pg/mL)	645.00 (404.00, 1869.00)	1009.50 (327.75, 2199.25)	0.273	703.00 (430.00, 1969.50)	890.00 (266.75, 2197.75)	0.767
Hb (g/L)	125.00 (119.00, 133.00)	128.00 (118.00, 137.00)	0.372	126.00 (117.00, 139.00)	128.50 (118.00, 137.00)	0.345
LDL (mmol/L)	1.84 (1.33, 2.52)	2.10 (1.59, 2.73)	0.765	1.87 (1.34, 2.51)	2.14 (1.64, 2.84)	0.030
HDL (mmol/L)	1.09(0.90, 1.24)	1.08(0.92, 1.22)	0.934	1.08(0.89, 1.23)	1.10(0.94, 1.22)	0.727
TG (mmol/L)	1.32 (1.03, 1.83)	1.31 (0.91, 1.70)	0.341	1.39 (1.06, 1.92)	1.39 (0.92, 1.85)	0.597
LA (cm)	3.95 (3.72, 4.32)	4.04 (3.75, 4.51)	0.377	3.96 (3.73, 4.34)	4.05 (3.71, 4.53)	0.699
Antidiabetic therapy						
Sulfonylureas	25 (37.3%)	52 (32.7%)	0.504	22 (36.7%)	28 (29.2%)	0.329
Glinides	10 (14.9%)	29 (18.2%)	0.547	10 (16.7%)	15 (15.6%)	0.863
GI	33 (49.3%)	108 (67.9%)	0.008	30 (50.0%)	60 (62.5%)	0.124
TZD	1 (1.5%)	7 (4.4%)	0.280	0 (0%)	5 (5.2%)	0.072
DPP4 inhibitor	2 (3.0%)	8 (5.0%)	0.494	2 (3.3%)	5 (5.2%)	0.582
Insulin	21 (31.3%)	58 (36.5%)	0.460	19 (31.7%)	39 (40.6%)	0.260

Values are means ± standard deviation or median (interquartile range) or number of participants (%). AF, atrial fibrillation; BMI, body mass index; BNP, B-type natriuretic peptide; CHD, coronary heart disease; CI, cerebral infarction; DBP, diastolic blood pressure; DM, diabetes mellitus; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GI, glucosidase inhibitors; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HF, heart failure; HTN, hypertension; LA, left atrial/atrium; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; SBP, systemic blood pressure; TG, triglyceride; TZD, thiazolidinediones.

further analysis after PSM. The significant differences between the metformin and non-metformin groups (i.e., differences in age, sex, eGFR, and GI treatment) were adjusted after match. Multivariable Cox regression analysis demonstrated that metformin usage was significantly associated with lower all-cause mortality before match [HR (95% CI) = 0.339 (0.117–0.977); $P = 0.045$] (**Table 4**). The associations remained unchanged after PSM [HR (95% CI) = 0.292 (0.093–0.913); $P = 0.034$] (**Table 5**). Same as the results in the full cohort, there was no independent association between metformin use and secondary outcomes in the HbA1c ≥7% subgroup (**Figure 4** and **Tables 4**, **5**).

Absolute Risk Reduction of All-Cause Mortality With Metformin in Comparison With Non-metformin

Cumulative incidence of event, incident rates, relative risk reduction, and number needed to treat (NNT) values for the overall cohort and the HbA1c ≥7% subgroup by year are displayed in **Table 6**. The 4-year estimated NNT with metformin compared with non-metformin for all-cause mortality was 12 in all populations and 8 in the HbA1c ≥7% subgroup.

TABLE 4 | The association between metformin and endpoints in HbA1c ≥7% subgroup before PSM.

	Metformin (*n* = 67)	Non-metformin (*n* = 159)	Hazard ratio (95% CI)	*P*-value
All-cause mortality*	4 (1.52)	36 (5.78)	0.339 (0.117, 0.977)	0.045
Cardiovascular death†	2 (0.76)	17 (2.73)	0.283 (0.065, 1.226)	0.092
All-cause hospitalizations†	44 (16.77)	103 (16.54)	1.080 (0.757, 1.541)	0.671
Hospitalization for heart failure†	25 (9.53)	49 (7.87)	1.289 (0.794, 2.090)	0.304

PSM, propensity score matching.
Data were presented as no. of patients with events (% per patient-year).
**For all-cause mortality, hazard ratios and 95% CIs were estimated using Cox regression models, stratified according to level of HbA1c. Adjusted covariables included age, gender, body mass index, cigarette smoking, systemic blood pressure, diastolic blood pressure, New York Heart Association class, left ventricular ejection fraction, duration of diabetes and heart failure, whether living with hypertension, atrial fibrillation, coronary heart disease, or cerebral infarction, glycated hemoglobin, estimated glomerular filtration rate, hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, left atrial, sulfonylureas, glinides, glucosidase inhibitors, insulin.*
†Hazard ratios and 95% CIs for secondary outcomes were not adjusted for multiplicity.

TABLE 5 | The association between metformin and endpoints in HbA1c ≥7% subgroup after PSM.

	Metformin (*n* = 60)	Non-metformin (*n* = 96)	Hazard ratio (95% CI)	*P*-value
All-cause mortality*	4 (1.70)	19 (5.05)	0.292 (0.093, 0.913)	0.034
Cardiovascular death†	2 (0.85)	8 (2.13)	0.408 (0.774, 1.706)	0.257
All-cause hospitalizations†	42 (17.87)	64 (17.02)	1.149 (0.774, 1.706)	0.491
Hospitalization for heart failure†	23 (9.79)	31 (8.24)	1.267 (0.735, 2.184)	0.394

PSM, propensity score matching.
Data were presented as no. of patients with events (% per patient-year).
**For all-cause mortality, hazard ratios and 95% CIs were estimated using Cox regression models, stratified according to level of HbA1c. Adjusted covariables included age, gender, body mass index, cigarette smoking, systemic blood pressure, diastolic blood pressure, New York Heart Association class, left ventricular ejection fraction, duration of diabetes and heart failure, whether living with hypertension, atrial fibrillation, coronary heart disease, or cerebral infarction, glycated hemoglobin, estimated glomerular filtration rate, hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, left atrial, sulfonylureas, glinides, glucosidase inhibitors, insulin.*
†Hazard ratios and 95% CIs for secondary outcomes were not adjusted for multiplicity.

DISCUSSION

In the present study, we investigated the association between metformin and adverse outcome on HFpEF with T2DM populations. The major finding was that metformin was linked to a lower incidence of death from any causes in HFpEF and T2DM patients with poor glucose control.

Tremendous advancements have been made in the treatment of HFrEF employing neurohumoral activation. Nevertheless, no therapy has been shown to reduce morbidity or mortality in HFpEF patients (4). Furthermore, the pathophysiology underlying HFpEF is heterogeneous and is associated with multiple phenotypes, including cardiovascular and non-cardiovascular comorbidities (2). The cause of death and hospitalization among HFpEF patients is more likely to be non-cardiovascular than patients with HFrEF (10). Thus, management of comorbidity is an essential task for HFpEF patients.

Compared with the general population, diabetes doubles and quintuples HF's risk in males and females, respectively (11). About 45% of HFpEF patients suffer from diabetes (1). Diabetic patients tended to combine with structural and functional echocardiographic abnormalities (6). Clinical trial data suggest that among individuals with HFpEF, those with diabetes were associated with worse health-related quality of life and increased risk of hospitalization, cardiovascular mortality, and all-cause mortality (6, 12).

The most critical finding in the present study is that metformin reduced all-cause mortality in HFpEF and T2DM patients with poor glucose control. Metformin treatment improved glycaemic and reduced cardiovascular mortality, without the risk of hypoglycemia or bodyweight gains associated with the use of other antidiabetic drugs (13–15). Therefore, it is currently the preferred oral antidiabetics in T2DM and heart failure patients (16). A recent study showed that long-term prescription of metformin could improve left ventricular diastolic function and delay the progression of HFpEF in T2DM and hypertension population (17). Slater et al. reported that metformin improves diastolic function in a mouse model with HFpEF-like symptoms by lowering titin-based passive stiffness (18). The mechanisms by which metformin exerts favorable effects of metformin on HF progression are still not fully understood. Adenosine monophosphate-activated protein kinase (AMPK) is correlated with cardiac fatty acids uptake, autophagy, mitochondrial biogenesis, and energy regulation. Changes in the adenosine AMPK pathway play a major role in developing myocardial impairment (19, 20). In addition to glycaemic control, metformin appears

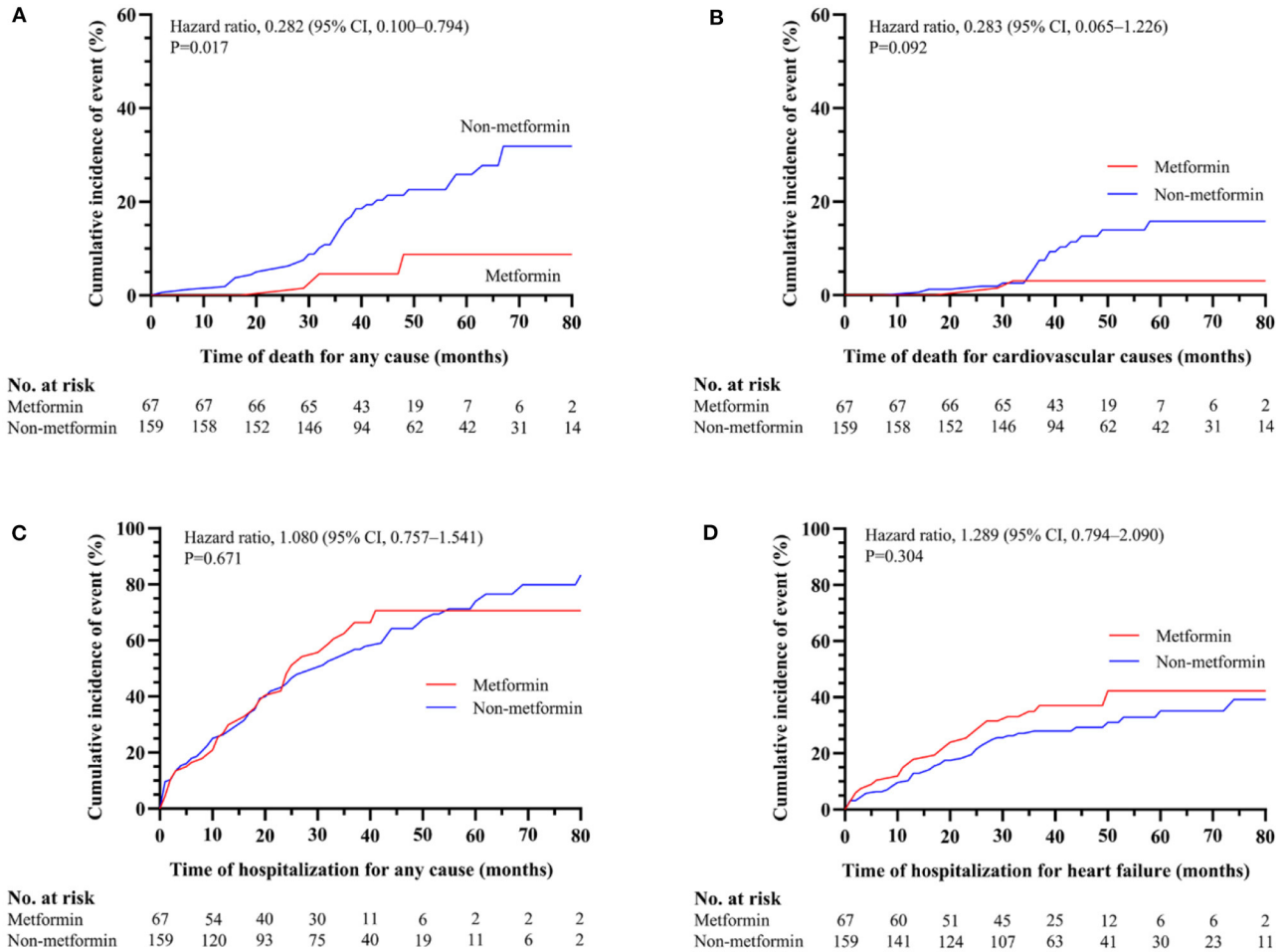

FIGURE 4 | The association between metformin and non-metformin and clinical outcomes in HbA1c ≥7% subgroup. Metformin was positively related to the all-cause mortality ($P = 0.017$) **(A)** but has no effect on cardiovascular death ($P = 0.092$) **(B)**, all-cause hospitalizations ($P = 0.671$) **(C)**, or hospitalization for heart failure ($P = 0.304$) **(D)**.

pleiotropic effects. In a diabetic heart, metformin regulates lipid and glucose metabolism *via* AMPK activation and further improves cardiac energy metabolism (20). What's more, metformin can improve mitochondria function, increasing nitric oxide bioavailability, inhibit the interstitial accumulation of collagen and cardiomyocyte apoptosis through AMPK-dependent or AMPK-independent pathways, and thereby reduce cardiac remodeling and hypertrophy, and preserve cardiac function (7, 20).

It is worth noting that metformin was linked to a lower incidence of all-cause mortality only in patients with poor glycemic control. Prolonged exposure to pronounced hyperglycemia may have a more sustainable myocardial damage than the lower glucose status, therefore increasing the relative adverse influence of hyperglycemia in HF patients. This might explain why metformin showed a more significant cardioprotective effect in those with poor glycemic control. Finally, a higher HbA1c level means a higher glucose status, a sign of insufficient insulin effect or the underlying insulin resistance. Nevertheless, the specific underlying mechanism needs to be clarified.

Historically, metformin should not be used in patients with heart failure due to lactic acidosis risk (21). Nowadays, clinical observations and experimental studies have provided increasing evidence of the safety and benefits of metformin in patients with diabetes and heart failure. A systematic review of observational studies indicates that metformin can be safely used in patients with diabetes mellitus and HF, even in heart failure with reduced left ventricular ejection fraction or chronic kidney failure. Meanwhile, none of the trials demonstrate that metformin was associated with an increased risk of lactic acidosis than other hypoglycemic agents (8).

Several limitations of our study should be acknowledged. First, our study was retrospective rather than randomized prospectively planned, and causality cannot be inferred from these retrospective findings. Second, clinical data were obtained in a single-center instead of multi-centers. Third, the amount of the subject was relatively small.

TABLE 6 | Event rates, incidence rates, and number needed to treat for all-cause mortality for comparison of metformin with non-metformin.

	All-cause mortality in whole population ($n = 372$)	All-cause mortality in the HbA1c ≥ 7% subgroup ($n = 226$)
Events/total number		
Metformin	11/113	4/67
Non-metformin	56/259	36/159
P-value*	0.269	0.045
Incidence rate, %		
Metformin	9.7	6
Non-metformin	21.6	22.6
Difference	11.9	16.6
Relative risk reduction, %	55	73
Number needed to treat		
1-year	44	46
2-year	32	20
3-year	20	10
4-year	12	8

**Events of all-cause mortality were estimated using Cox regression models. Adjusted covariables included age, gender, body mass index, cigarette smoking, systemic blood pressure, diastolic blood pressure, New York Heart Association class, left ventricular ejection fraction, duration of diabetes and heart failure, whether living with hypertension, atrial fibrillation, coronary heart disease, or cerebral infarction, glycated hemoglobin, estimated glomerular filtration rate, hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, left atrial, sulfonylureas, glinides, glucosidase inhibitors, insulin.*

Finally, since our study was conducted before the general introduction of novel antidiabetics, including sodium-glucose co-transporter-2 inhibitors and glucagon-like peptide-1 receptor agonists, they were not included in our analysis.

In conclusion, there was no independent association between metformin use and outcome in the cohort of T2DM with HFpEF. However, metformin was associated with lower all-cause mortality in the subgroup of patients with poor glycemic control. Prospective, large sample studies are necessary to determine the optimal treatment for HFpEF patients with T2DM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Board of the Second Affiliated Hospital, Zhejiang University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZC and YL: designed the study. JWa, TY, and XM: contributed data. JWa, YL, and JWe: drafted the manuscript. All authors: were involved in critically revising the manuscript.

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frontiers in Cardiovascular Medicine

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Role of Neutrophil-Derived S100B in Acute Myocardial Infarction Patients From the Han Chinese Population

Minghui Cheng†, Xu Su†, Dan Liu, Xiaoxiang Tian, Chenghui Yan, Xiaolin Zhang and Yaling Han**

Cardiovascular Research Institute and Department of Cardiology, The General Hospital of Northern Theater Command, Shenyang, China

Objective: This study aimed to clarify the novel role of homeostatic calmodulin S100B and determined whether S100B genetic variants affected atherosclerosis progression in acute myocardial infarction (AMI) patients.

Methods: Plasma levels of S100B were measured systemically in AMI patients, stable angina pectoris patients, and control subjects. S100B was obtained from the human coronary artery thrombi using a thrombectomy catheter and quantified via immunohistochemical analysis, qRT-PCR and Western blot analyse. We also screened for S100B variations (rs9722, rs9984765, rs2839356, rs1051169, and rs2186358) via direct sequencing, and investigated the relationship between these variants and AMI patients in the Chinese Han population.

Results: Plasma S100B levels increased significantly in AMI patients compared to the levels in stable angina pectoris patients and control subjects (119.45 ± 62.46, 161.96 ± 73.30, and 312.91 ± 127.59 pg/ml, respectively). Immunohistochemical staining results showed that S100B expression was increased in the neutrophils of coronary artery thrombi obtained from AMI patients, as compared to that in normal blood clot, and S100B expression was significantly increased in fresh thrombi tissues, as compared to that in organized thrombi tissues. Western blot and qRT-PCR analysis showed that S100B expression increased in coronary artery thrombi, as compared to that in normal blood clots. After pre-treating the neutrophils with siRAGE, the neutrophils migration induced by S100B were abolished through the NFκB-IL1β/IL6 signaling pathway. Compared to their corresponding wild-type genotypes, the S100B rs9722 variant was associated with increased susceptibility to AMI (OR = 1.35, 95%CI: 1.12–1.65, $P = 0.02$). Individuals with the S100B 9722 A allele had higher plasma S100B levels than those with the G allele in control subjects and AMI patients (141.70 ± 76.69 vs. 107.31 ± 56.05 and 347.13 ± 148.94 vs. 273.05 ± 133.62, respectively).

Conclusions: Levels of neutrophil-derived S100B, a novel homeostatic calmodulin, were elevated in the early stages of myocardial infarction. The S100B rs9722 allele was independently associated with AMI patients in the Han Chinese population.

Keywords: S100B, acute myocardial infarction, genotype, thrombosis, plasma biomarkers

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Junjie Xiao,
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Xiaoxiang Yan,
Shanghai Jiao Tong University, China
Xiang Xie,
Xinjiang Medical University, China

****Correspondence:***
Xiaolin Zhang
xiaolindianyu75@163.com
Yaling Han
hanyaling@163.net

†These authors have contributed equally to this work

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HIGHLIGHTS

- Levels of neutrophil-derived S100B, a novel homeostatic calmodulin, are elevated in the early stages of myocardial infarction.
- S100B induces neutrophils migration through the NF-κB-IL6/IL1β signal pathway.
- S100B rs9722 allele is independently associated with AMI patients in the Han Chinese population.

INTRODUCTION

Acute myocardial infarction (AMI), the leading cause of death in the industrialized world, is associated with high morbidity and mortality. It is challenged for doctors to rapidly and precisely diagnose AMI and take effective steps for evidence-based medical management and treatment (1, 2). Acute coronary thrombus formation, considered to be secondary to the rupture of vulnerable atherosclerotic plaques, is the main pathological basis for the occurrence of AMI. Atherosclerotic plaque disruption and intramural thrombus formation cause ischemia and hypoxia in cardiomyocytes, which leads to cardiomyocytes necrosis (3–5).

Recent studies have shown that the inflammatory response plays a vital role in the origin and development of atherosclerosis, which causes the rupture of vulnerable atherosclerotic plaques (5–7). During atherosclerotic plaque formation, the knock-on effects caused by inflammatory factors aggravated the degree of atherosclerosis, leading to systemic inflammation and an atherogenesis feedback loop (8, 9). Increasing evidence supports the involvement of circulatory inflammatory cytokines in early vascular inflammation and atherogenesis (10, 11). S100B is composed of at least 25 low-molecular-weight $Ca2^{+}$-binding protein belonging to the S100 family member mainly which is mainly localized in the central nervous system, peripheral nerves, adipocytes and a subset of lymphoid cells under normal physiological conditions. These proteins are involved in various intracellular inflammatory signaling pathways. Interestingly, the S100B bound to the receptor for advanced glycation end products (RAGE) has been shown to play multiple roles in regulating cell functions including proliferation and differentiation, under physiological and pathological conditions. Moreover, S100B also mediates the process of neuroprotection by minimizing and reducing inflammatory factors. Cai et al. (12) demonstrated that S100B participated in RAGE-activated inflammatory pathways and accelerated the onset of coronary artery atherosclerosis. Increased S100B levels were associated with the acute coronary syndrome, and S100B expression was related to myocardial injury in rat models of myocardial infarction. A growing body of evidence suggested that vulnerable plaques in coronary arteries exhibited higher levels of S100B expression which revealed that S100B could play a potential role in atherothrombosis (12).

Therefore, in this study we measured the plasma S100B level and investigated its clinical significance in relation to the occurrence of AMI. Furthermore, we determined whether the genetic variations of S100B were associated with AMI patients in Chinese Han population.

MATERIALS AND METHODS

Study Design and Participants

A total of 367 unrelated Han Chinese subjects including 125 AMI patients, 122 stable angina pectoris patients, and 120 control volunteers were recruited from the General Hospital of Northern Theater Command between August 2015 and October 2017. Information regarding conventional cardiovascular risk factors, such as smoking, hypertension, or diabetes was obtained via a standardized interview. BMI was calculated as the body weight (kg)/height $(m)^2$. Type 2 diabetes mellitus was diagnosed according to WHO criteria, and hypertension was diagnosed according to the Seventh Report of the Joint National Committee on the prevention, detection, evaluation, and treatment of high blood pressure, which defined hypertension as arterial blood pressure $\geq$140 mm Hg (systolic) or 90 mm Hg (diastolic). All recruited subjects with specific clinical presentations and angiographic findings were diagnosed as AMI, stable angina pectoris subjects, and control subjects were recruited from the emergency room. Control subjects were individuals with normal coronary angiogram results without a history of coronary artery disease, electrocardiographic signs of CAD, regional wall motion abnormalities, or relevant valvular abnormalities according to analysis echocardiograms. The design of the present study complied with the Declaration of Helsinki. Approval was obtained from the General Hospital of Northern Theater Command ethics committees, and all subjects provided their informed consent.

Neutrophils Activation

Neutrophils were isolated via centrifugation density gradient centrifugation. After centrifugation, blood was separated into several distinct bands. Mononuclear cells were placed in the upper phase, whereas the neutrophils became sedimented into the lower phase. Furthermore, we cultured the neutrophils collected from the participants in six well plates (10^6 cells/mL). Neutrophils were stimulated with 100 ng/mL lipopolysaccharide (LPS) for 4 h in a CO_2 incubator. Neutrophils not subjected to LPS treatment were used as controls for each participant. At the end of 4 h, the supernatant was separated via centrifugation and stored at −80°C. S100B levels were measured in the cell culture supernatant using a commercial ELISA kit as per the manufacturers' instructions. The LPS-induced secretion of S100B was calculated as the ratio of S100B levels in LPS-stimulated plasma to the corresponding levels in unstimulated plasma obtained from the same patient.

Human Thrombus Tissue Obtained From the AMI Patients

Human coronary artery thrombi were obtained using a thrombectomy catheter (Export catheter, Medtronic, Minneapolis, MN, USA) during percutaneous coronary intervention prior to balloon angioplasty and stent deployment in AMI patients. The coronary thrombus tissues were obtained by using an aspiration catheter. The expression of S100B and MPO (EPR20257, ABCOM, USA) in the coronary artery thrombus was examined using immunohistochemistry and

immunofluorescence staining. S100B and MPO antibodies were incubated overnight at 4°C with thrombus samples; the bound S100B and MPO antibodies were stained using avidin–biotin–peroxidase method (Vector Laboratories, Burlingame, CA, USA) and photographed under a microscope (ZEISS, Image A2, Germany).

RNA Extraction and qRT-PCR

Fresh thrombus tissues and neutrophils were suspended in 500 μL of TRIzol LS reagent (10296010; Invitrogen, USA) and stored at −80°C prior to RNA isolation. RNA was extracted from the cells in TRIzol, as per the manufacturer's recommended protocol. RNA was quantified using an Eppendorf Biophotometer (Eppendorf, USA). One microgram of RNA was used for cDNA conversion using the Prime ScriptTMRT reagent kit and the gDNA Eraser kit (RR047A, Takara, Japan). Relative levels of S100B expression in the thrombectomies were determined using qRT-PCR (13). The S100B forward primer for qRT-PCR was 5′-GCGAATGTGACTTCCAGGAA-3′ and the reverse primer was 5′-GCTTCCTAATTAGCTACAAC-3′ while the GAPDH forward primer for qRT-PCR was 5′-AGGATGGTGTGGCTCCCTTG-3′ and the reverse primer was 5′-GCAGGGCTGAGACAGCTTCC-3′. Thrombus tissue cDNA was amplified, electrophoresed on 1% agarose gel as described previously, and visualized via ethidium bromide staining. Relative gene expression levels were calculated by comparing the threshold cycle (Ct) for the target gene to that of GAPDH. The mRNA level of S100B in the thrombus tissues of AMI patients and controls was calculated using the following equation: relative gene expression $= 2^{-(\Delta Ctsample - \Delta Ctcontrol)}$.

Protein Extraction and Western Blotting

Neutrophils and thrombus tissues were dissolved in the protein lysis buffer containing a fresh protease inhibitor (14). The cell debris was extracted via centrifugation at 12,000*g* for 10 min at 4°C, and supernatants were extracted for western blotting. The supernatants were electrophoretically separated via SDS-PAGE, and transferred to activated PVDF membranes. PVDF membranes were incubated with the anti-S100B antibody (ab4066, Abcam, USA) and anti-GAPDH antibody (D16H11, Cell Signaling, USA). Immune complexes were detected using the SuperSignal @ West Pico Chemiluminescent Substrate (Thermo Scientific, USA).

Genetic Variation and Genotyping

A total of 453 AMI patients and 456 control subjects were recruited at the General Hospital of the Northern Theater Command from July 2015 to December 2017.The inclusion criteria for AMI patients were based on a recent AMI definition depending on elevated plasma hscTnT levels (≥0.05 ng/mL) (14), electrocardiogram-related changes such as pathological Q waves, ST segment elevation or depression, and ischemic-type chest pain within the last 24 h. The control participants were selected from subjects who were admitted to the General Hospital of Northern Theater Command who exhibited ≤20 % major coronary artery stenosis according to the results of coronary arteriography. Subjects with active inflammatory disease, autoimmune disease, severe heart failure, hemodynamic instability, suspected myocarditis or pericarditis, diseases of the hematopoietic system, extensive of kidney or liver disease, malignant disease, renal or hepatic diseases, taking immune-suppressing drugs, and who had renal or hepatic diseases were excluded.

Genomic DNA was extracted using the DNA extraction kit (TIANGEN, China) according to the manufacturer's instructions (15). Five polymorphisms (rs9722, rs9984765, rs2839356, rs1051169, and rs2186358) of S100B were monitored. A standard polymerase chain reaction (PCR) was used to amplify the S100B rs9722, rs9984765, rs2839356, rs1051169, and rs2186358 variants. Each PCR reaction was performed using 0.1 μg gDNA, 10 pmol each of forward and reverse primer, 0.4 mmol/L dNTPs, 1 × reaction buffer including $MgCl_2$ and 4U rTaq DNA polymerase. S100B primer sequences and PCR cycling conditions are included in **Table A1**.

Determination of Plasma S100B Levels

Blood samples obtained from AMI, stable angina pectoris patients, and control subjects were dispensed into EDTA-anticoagulation tubes. Plasma samples were isolated and stored at −80°C in plastic cryovials. S100B concentrations were evaluated via ELISA. Each sample used to perform into three experimental replicates and incubated for 2 h. Plates were washed three times, and the conjugate was added and incubated for 2 h at room temperature. Plates were washed again three times, and then incubated for 30 min before the addition of 50 μL of stop solution. Next, we next examined whether the S100B rs9722 GA variant influenced the expression of S100B by measuring the plasma S100B levels between AMI patients and control subjects.

Transfection of siRNA Into Human Neutrophils

Transient siRNA transfection was conducted in the light of the advice provide by the manufactory. We transfected the neutrophils with siRNA in a transfection medium using the liposomal transfection reagent (Lipofectamine RNAimax, Invitrogen) and the neutrophils grown in 6 well plates were transfected. In the 150 μl transfection medium added 6 μl siRNA solution and 150 μl transfection medium added 6 μl transfection reagent, thus gently mixed together. At room temperature for 30 m, we added the siRNA-lipid complexes into the neutrophils with the 1.0 ml transfection medium, and incubated the cells with this mixture for 6 h at 37°C. Furthermore normal medium replaced the transfection medium and neutrophils were cultured for 48 h.

Transwell Migration Assay

Neutrophils with or without the S100B protein, and neutrophils transfected with the siRNA RGAE medium were added to the transwell migration assay using the 8 μm pore polycarbonate membrane inserts (Corning). Moreover, adding 600 μl culture with 10% fetal calf serum in the lower chamber, and adding 105

neutrophils with 0.1% fetal calf serum in the upper chamber were incubated for 24 h. Then the neutrophils migrating to the lower surface of the membrane were fixed with methanol and stained with crystal violet. The migration neutrophils numbers were calculated by 4 fields per membrane under a microscope.

Statistical Analysis

Statistical analysis was performed using SPSS version 21.0 and data were represented as mean ± SEM values. We used the Student's *t* test or Mann-Whitney *U* test to compare groups; the plasma S100B levels were analyzed using ANOVA. The distribution of S100B genotype and allele frequencies between AMI and control groups were compared using X^2 tests. The AMI risk was evaluated by *P*-values, 95% confidence intervals (95% CIs), and odds ratios (ORs). Adjustment were made for bonferroni multiple test comparisons of the five SNPs, and a significance cutoff value of $p < 0.01$ ($0.05/5 = 0.01$) was applied. Assuming a dominant genetic model, the study had 80% statistical power at the 5% significance level for detecting the effect of the polymorphism and was associated with an OR > 1.8, which indicated a low risk of obtaining a false-negative result.

RESULTS

Increased S100B Plasma Levels in AMI Patients

We enrolled 367 participants including 120 control subjects, 122 stable angina pectoris and 125 AMI patients. Baseline characteristics and the results of plasma biochemistry tests were presented in **Table 1**. In comparison to the control subjects, there were more smokers ($P = 0.007$), diabetics ($P < 0.01$), and hypertension patients in AMI patients. The AMI group also had more comorbid conditions than the stable angina pectoris or

TABLE 1 | Baseline demographic and clinical parameters among the healthy control, stable angina pectoris and AMI subjects.

Variable	Healthy Controls (*n* = 120)	Stable angina pectoris patients (*n* = 122)	AMI patients (*n* = 125)	*P*-value
Age, years	58.68 ± 11.44	60.09 ± 11.97	60.88 ± 13.27	0.238
Female, no. (%)	42 (35.0)	47 (38.5)	47 (37.6)	0.841
Smoking	30 (25.0)	53 (43.4)	50 (40.0)	0.007
Hypertention	44 (36.7)	61 (50.0)	88 (70.4)	0.000
Diabetes	13 (10.8)	51 (41.8)	71 (56.8)	0.000
Previous stroke	0 (0.00)	7 (5.70)	8 (6.4)	0.022
Previous AMI	0 (0.00)	5 (4.10)	8 (6.40)	0.023
Family history	3 (2.50)	3 (2.50)	8 (7.20)	0.178
WBC, $\times 10^9$/L	5.97 ± 1.55	7.02 ± 1.90	13.30 ± 3.53	0.000
TG, mmol/dl	1.45 ± 0.54	1.63 ± 0.92	1.61 ± 0.81	0.216
LDL-C, mmol/dl	2.28 ± 0.66	2.30 ± 0.79	2.80 ± 0.71	0.007
hscTnT, ng/mL	0.008 ± 0.004	0.011 ± 0.012	4.53 ± 3.52	0.000
CK-MB, U/L	11.98 ± 6.51	15.69 ± 5.72	245.76 ± 235.21	0.000

TG, triglyceride; LDL-C, low-density lipoprotein; WBC, white blood cell; hs-CRP, high-sensitivity C-reactive protein; hscTnT, high-sensitivity troponin T; CK-MB, creatine kinase MB isoenzyme.

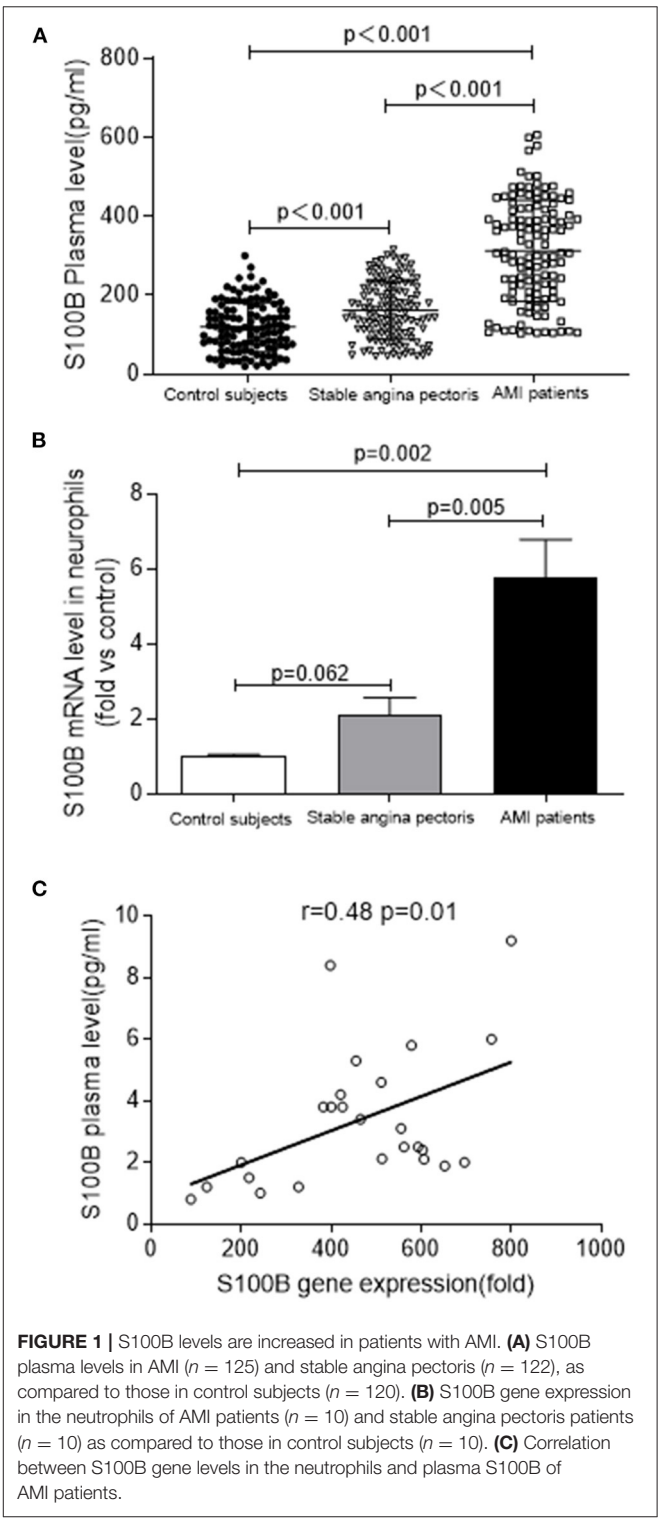

FIGURE 1 | S100B levels are increased in patients with AMI. **(A)** S100B plasma levels in AMI ($n = 125$) and stable angina pectoris ($n = 122$), as compared to those in control subjects ($n = 120$). **(B)** S100B gene expression in the neutrophils of AMI patients ($n = 10$) and stable angina pectoris patients ($n = 10$) as compared to those in control subjects ($n = 10$). **(C)** Correlation between S100B gene levels in the neutrophils and plasma S100B of AMI patients.

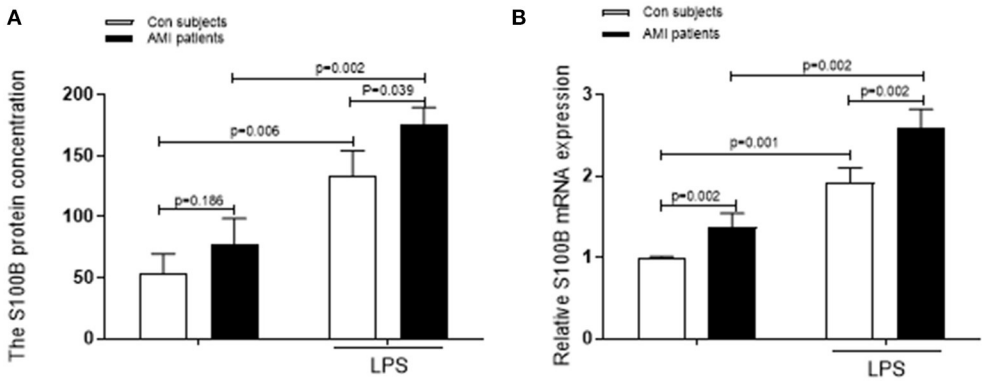

FIGURE 2 | Levels of S100B secreted by stimulated neutrophils are increased in AMI patients. **(A)** A comparision of the fold change in the levels secreted by LPS-stimulated neutrophils with that in unstimulated neutrophils from the same AMI patients ($n = 10$) and control subjects ($n = 10$). **(B)** The S100B mRNA levels and the corresponding secreted S100B levels in the unstimulated controls and LPS-stimulated subjects are shown.

control groups, and the patients were more likely to have a history of stroke ($P = 0.022$). As expected, the clinical and biochemical values of AMI patients were significantly higher than those of subjects belonging to the control and stable angina pectoris groups. Clinical parameters including higher WBC levels, which indicate poorer cardiac functioning and general conditions, and severe inflammation were more prevalent in AMI group than in the control or stable angina pectoris groups. AMI patients ($n = 125$) had significantly higher systemic S100B levels, as compared to those in stable angina pectoris ($n = 122$) and controls subjects ($n = 120$) [median (IQR):302.16 (606.50–103.18) vs. 159.50 (315.69–45.38) vs. 119.45 (300.23–20.41)] (**Figure 1A**).

To confirm the fact S100B was highly upregulated in AMI patients, we analyzed S100B expression in neutrophils using real time PCR in a cohort of patients with AMI. We observed the marked upregulation of S100B in AMI patients ($n = 10$), as compared to that control subjects ($n = 10$) and stable angina pectoris patients ($n = 10$) ($P = 0.062$; $P = 0.005$; $P = 0.002$) (**Figure 1B**). However, the S100B level was also upregulated in stable angina pectoris patients as compared to the level in control subjects, albeit at a lower level than in AMI patients. Finally, we observed that S100B mRNA expression and plasma levels were positively correlated in AMI patients, which suggested that neutrophils might act as an important source of S100B in AMI patients ($r = 0.48$, $P = 0.01$) (**Figure 1C**). In AMI patients, neutrophils released S100B into the plasma, resulting in a marked increase in plasma S100B levels during the early stages of AMI.

We also evaluated the differences in the levels of pro-inflammatory S100B secreted after activation in AMI patients and control subjects. We measured S100B levels in the supernatants of neutrophils stimulated with LPS for 4 h. In the control and AMI subjects, secreted levels of S100B increased in LPS stimulated-neutrophils, as compared to those in unstimulated neutrophils ($p = 0.006$; $p = 0.002$). In neutrophils isolated from AMI patients, S100B protein and mRNA expression levels increased similarly after LPS stimulation, with significantly different levels of S100B being expressed from the control-stimulated neutrophil supernatant ($p = 0.001$; $p = 0.002$). S100B levels were increased in LPS-stimulated cells, as compared to those in both controls and AMI subjects (**Figure 2**).

S100B Expression Increased in the Thrombi Tissues Obtained From AMI Patients

Acute coronary thrombus formation, considered to be secondary to the rupture of vulnerable atherosclerotic plaques, leads to the occurrence of AMI. To examine the S100B expression pattern in thrombus tissues, we collected coronary artery thrombi from AMI patients who underwent angiography, and demonstrated the thrombotic occlusion of the anterior descending branch of the coronary artery. Thereafter, we removed thrombus tissues via an aspiration thrombectomy followed by balloon angioplasty and stent deployment; as a result, a widely patent anterior descending branch of the coronary artery exhibited no significant luminal narrowing. The thrombectomy catheter retrieved multiple coronary artery thrombi that were then analyzed for MPO positive neutrophils and S100B expression using immunohistochemical microscopy. MPO and S100B were abundantly expressed and co-localized in the thrombus tissue. S100B expression was significantly increased in positive neutrophils from the coronary artery thrombus of AMI patients as compared to that in normal blood clots. Numerous neutrophils were visible, in fresh thrombus tissues, but were largely absent in organized thrombus tissues. The H&E staining and Masson staining of fresh thrombus tissues showed that the S100B expression of neutrophils was higher than that in fresh thrombi (**Figures 3**, **4**).

S100B expression levels in human thrombus tissues were further analyzed in five blood clots and five thrombectomy specimens using qRT-PCR ($P = 0.024$) and western blotting ($P = 0.003$). S100B expression levels increased in the thrombi, as compared to those in the normal blood clot tissues, which

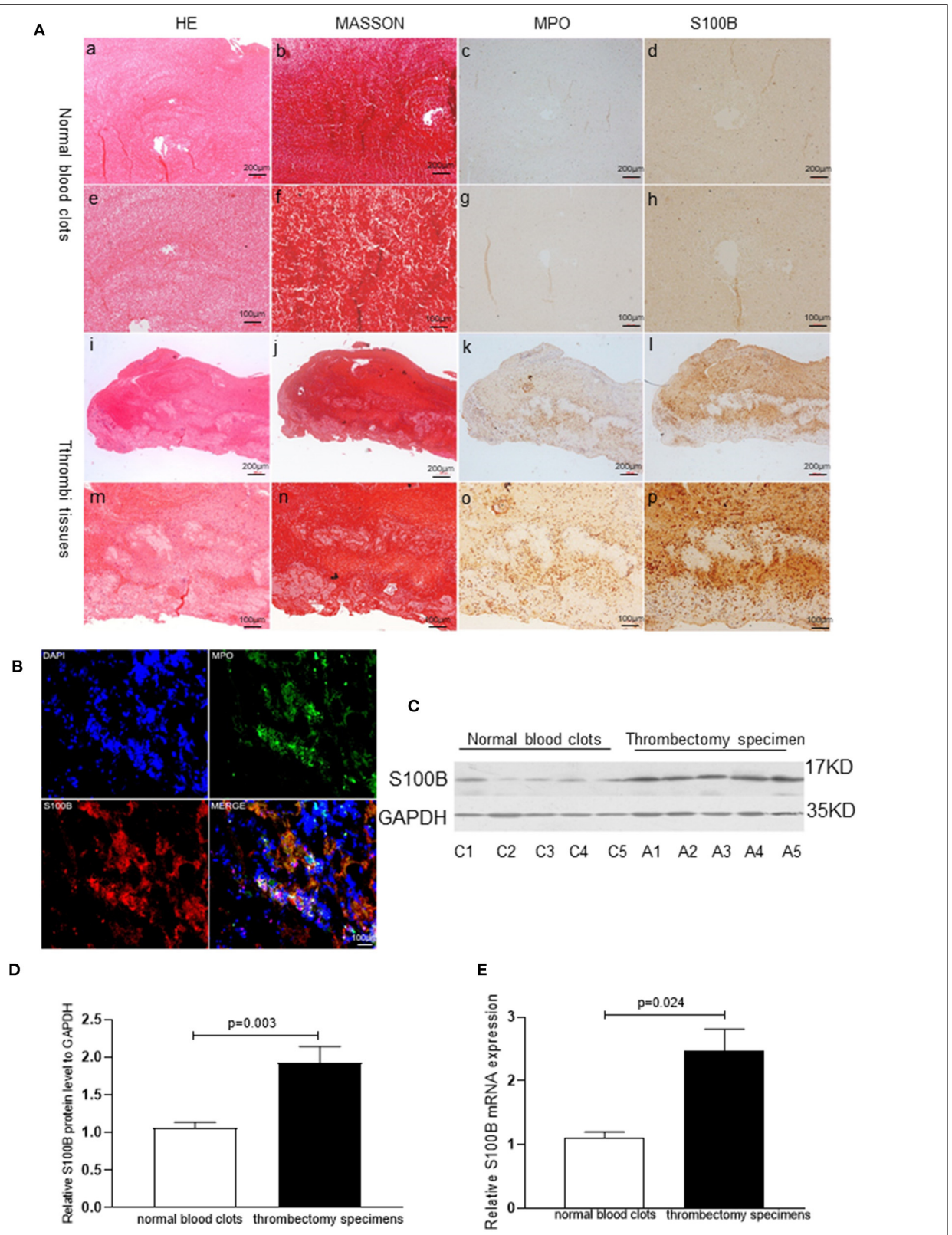

FIGURE 3 | S100B expression in human thrombi tissues of AMI patients. **(A)** Representative images showing the immunohistochemical staining of MPO and S100B in thrombi tissues coming obtained from AMI patients. Normal blood clots: a, b (×10) and e, f (×20): Thrombi tissues: i, j (×10) and m, n (×20). S100B expression was

(Continued)

FIGURE 3 | observed in thrombi tissues: d, h, i, p; MPO^+ neutrophils were identified in thrombi tissues (c, g, k, o) and S100B expression was increased in areas with high levels of neutrophils infiltration. **(B)** Representative immunofluorescence staining results of MPO and S100B from human thrombi tissues. S100B proteins were co-localized in the neutrophils of thrombi tissues. **(C,D)** Western blot analysis of S100B expression in normal blood clots and thrombectomy specimens obtained from AMI patients. S100B protein was detected in all specimens, but was present at significantly higher levels in the thrombectomy specimens ($n = 5$) as compared to levels in normal blood clots ($n = 5$). C1–C5 showed representative normal blood clots from control subject 1 control subject 5. A1–A5 show the representative images of thrombectomy specimens from AMI patient 1 to patient 5. **(E)** qRT-PCR comparison of S100B mRNA expression levels of normal blood clots and thrombectomy specimens obtained from AMI patients. Image magnification ×10 and ×20.Immunoreactivity was detected using DAB staining (brown color).

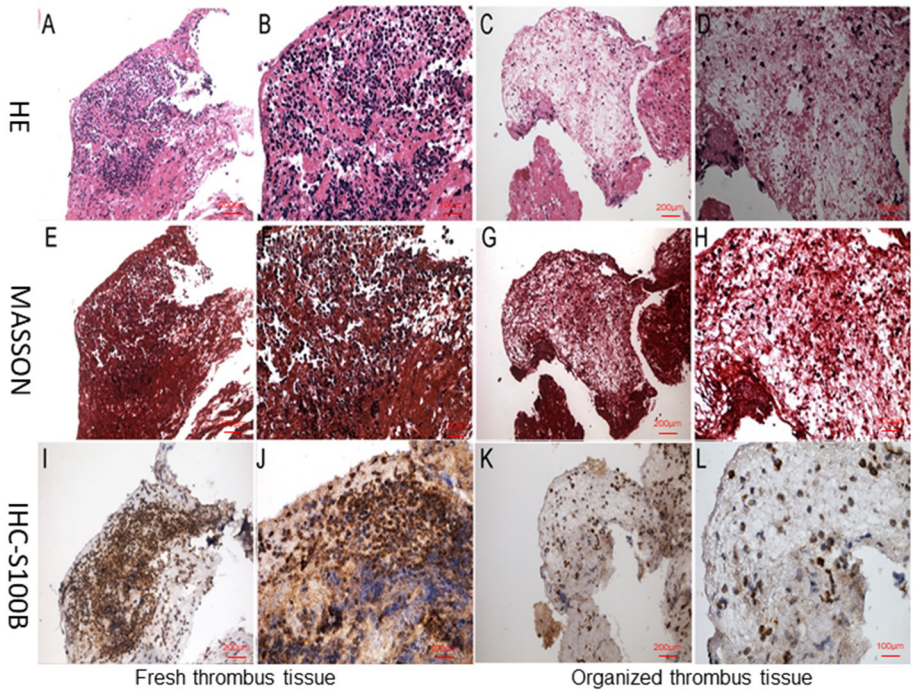

FIGURE 4 | Neutrophil staining during different stages of thrombus organization. **(A,B,E,F,I,J)** Fresh thrombus tissue with numerous visible neutrophils. **(C,D,G,H,K,J)** Organized thrombus tissues with largely absent. **(A–D)** H&E staining of thrombus tissues, **(E–H)** Masson staining of thrombus tissues, **(I–L)** Immunohistological staining of thrombus tissues. All samples were histologically graded as either fresh thrombi containing numerous neutrophils, or organized thrombi with a few neutrophils. Image magnification ×10 and ×20.Immunoreactivity was detected using DAB staining (brown color).

indicates that S100B might play an important role in AMI progression (**Figure 3**).

Association Between S100B Variants and Occurrence of AMI Patients

To avoid false positive experimental results, the DNA source, preparation, and genotyping analysis were controlled using the paradigm of blindness and randomization. The demographic data for 453 AMI patients and 456 control subjects is presented in **Table 2**. We screened for S100B variations (rs9722, rs9984765, rs2839356, rs1051169, and rs2186358) via direct sequencing and investigated the relationship between these variants and AMI in the Han Chinese population. The distribution frequency of genotypes conformed to the Hardy-Weinberg equilibrium in the control subjects. AMI patients were characterized by higher frequencies of traditional atherosclerotic risk factors, compared to the control subjects. Cardiovascular risk factors including hypertension, diabetes mellitus, and cigarette smoking were more prevalent in AMI patients than in control subjects. Furthermore, lower levels of HDL-C and increased levels of TG were found in the AMI patients as compared to those observed in the control subjects.

The differences in the allelic and genotypic frequencies of the S100B variants rs9722, rs2239574, rs881827, rs9984765, and rs1051169 were compared between AMI patients and control subjects for S100B in the Han Chinese population. Carriers of the rs9722 AA allele were significantly overrepresented among AMI patients as compared to those of control subjects (15.0% vs. 7.9%, $P = 0.002$). S100B rs9722 is independently associated with AMI patients in the Han Chinese population ($P = 0.002$, AA vs. GG; OR = 1.35,95%CI: 1.12–1.65, $P = 0.002$) (**Table 3**). After adjusting for classical cardiovascular risk factors such as age, gender, body mass index, smoking, hypercholesterolemia, hypertension and diabetes, the association between AMI patients and S100B rs9722 remained significant. There were no significant differences in the allele frequencies or genotype distributions of the other four SNPs (rs2239574, rs881827, rs9984765, and rs1051169) between AMI patients and control subjects. When the

TABLE 2 | Clinical characteristics of AMI patients and control subjects.

Variable	Control subjects ($n = 456$)	AMI patients ($n = 453$)	*P*-value
Female, no. (%)	146 (32.0)	155 (34.0)	match
Age (years)	58.97 ± 12.12	60.83 ± 10.33	0.001
BMI	25.48 ± 3.03	25.76 ± 2.94	0.459
Smoking	247 (24.2)	274 (60.5)	<0.001
Hypertension	265 (28.1)	266 (58.7)	<0.001
Diabetes	137 (30.0)	176 (38.9)	0.003
TG, mmol/dl	1.29 ± 0.53	1.78 ± 0.88	<0.001
TC, mmol/dl	3.97 ± 0.63	4.21 ± 0.54	0.012
LDL-C, mmol/dl	2.26 ± 0.72	3.12 ± 0.61	0.022
HDL-C, mmol/dl	1.01 ± 0.33	1.04 ± 0.21	<0.001

BMI, body mass index; F, female; M, male; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein chlolsterol; TC, total cholesterol; TG, triglyceride.

subjects were categorized into sub-groups according to gender, the AA genotype and A allele frequencies of rs9722 were found to be significantly higher in male patients than in male controls ($P = 0.006$; OR = 1.39;95% CI = 1.09–1.78, **Table 4**). The AA genotype and A allele frequency of rs9722 between female patients and female controls was not higher (**Table 4**).

We performed a case-control study of plasma S100B levels to explore the association between S100B expression and AMI.S100B levels were significantly higher in AMI patients as compared to those in the control group. Further analysis revealed that the disease-susceptible genotype S100B rs9722 AA was associated with higher S100B levels in the plasma as compared to the GG and GA genotypes in both controls and AMI patients (141.70 ± 76.69 vs.107.31 ± 56.05 and 23 347.13 ± 148.94 vs 273.05 ± 133.62 respectively) (**Table 5**).

S100B Influences the Effects of Neutrophils Through the RAGE–NFκB–IL1β/IL6 Signaling Pathway

S100B stimulated inflammation and S100B receptor(siRAGE)may confer some cardiovascular benefits by modulating the inflammatory process in atherosclerosis. Therefore, we examined the ability of siRAGE to modulate effect of S100B in neutrophils. To investigate whether S100B induced the inflammatory response in neutrophils which were stimulated with S100B for 2 h. siRAGE induced a significant decrease in RAGE–NFκB–IL1β/IL6 production in S100B-activated neutrophils (**Figures 5A–C**). To investigate the possible effects of S100B on neutrophils migration, we tested whether the addition of S100B fusion protein or knocking down of S100B in neutrophils would alter their migratory ability. To examine whether S100B induction increased neutrophil migration, we performed neutrophil migration assays under static conditions. S100B treatment significantly altered the migration (**Figures 5D,E**) of neutrophils. Furthermore, we transfected cultured neutrophils with either the S100B fusion protein/sicontrol and siRAGE, respectively.

TABLE 3 | Genotypic and allelic frequencies of S100B polymorphisms between the 456 control subjects and 453 AMI subjects.

	Control subjects ($n = 456$)	AMI patients ($n = 453$)	*P*	OR(95% CI)
Rs9722				
G/G no. (%)	221 (48.5)	191 (42.2)	0.002	
G/A no. (%)	199 (43.6)	194 (42.8)		
A/A no. (%)	36 (7.9)	68 (15.0)		
G allele	641 (70.3)	576 (63.6)	0.002	1.35 (1.12–1.65)
A allele	271 (29.7)	330 (36.4)		
Rs9984765				
T/T no. (%)	218 (47.8)	205 (45.3)	0.19	
C/T no. (%)	197 (43.2)	203 (44.8)		
C/C no. (%)	41 (9.0)	45 (9.9)		
C allele	279 (30.6)	279 (32.3)	0.42	1.08 (0.88–1.32)
T allele	613 (69.4)	633 (67.7)		
Rs2839356				
G/G no. (%)	226 (40.6)	221 (46.6)	0.14	
C/G no. (%)	199 (43.6)	195 (43.0)		
C/C no. (%)	31 (16.8)	47 (10.4)		
G allele	651 (71.4)	617 (68.1)	0.12	1.16 (0.95–1.42)
C allele	261 (28.6)	289 (31.9)		
Rs1051169				
A/A no. (%)	185 (40.6)	183 (40.4)	0.99	
A/C no. (%)	198 (43.4)	196 (43.3)		
C/C no. (%)	73 (16.0)	74 (16.3)		
A allele	562 (62.0)	568 (62.3)	0.91	1.01 (0.83–1.22)
C allele	344 (38.0)	344 (37.7)		
rs2186358				
A/A no. (%)	363 (79.6)	381 (84.1)	0.20	
A/C no. (%)	84 (18.4)	69 (14.6)		
C/C no. (%)	9 (0.02)	6 (0.01)		
A allele	810 (88.8)	828 (91.4)	0.06	1.33 (0.98–1.82)
C allele	102 (11.2)	78 (8.6)		

*OR, Odd Ratio; CI, Confidence Interval. * P<0.01.*

In separate experiments, cultured neutrophils were transfected with either S100B siRNA or control siRNA. Efficient S100B protein fusion or knockdown has been shown in **Figure 5**. Transfected cells were subjected to migration assays by using the transwell chamber. The experiments showed that the S100B fusion protein significantly increased the migratory ability of neutrophils whereas RAGE (S100B receptor) knockdown inhibited neutrophils migration (**Figure 5**).

DISCUSSION

Coronary heart disease, and its main complication AMI, represented major causes of morbidity and long-term disability worldwide. The exact etiology and pathophysiological mechanisms of AMI have remained unclear till date. The formation of thrombi during the progression of vulnerable plaques is an important factor contributing to AMI development. The cascade amplification effect is thought to promote the

TABLE 4 | Genotypic and allelic frequencies of rs8193037 in AMI patients and control subjects according to gender.

SNP	Genotype	Females		*P*-value	OR (95%CI)	Males		*P*-value	OR (95%CI)
		Controls subjects	AMI patients			Controls subjects	AMI patients		
		146	155			310	298		
Rs9722	GG	72 (49.3)	65 (41.9)	0.05		151 (48.7)	124 (41.6)	0.009 *	
	GA	64 (43.8)	66 (42.6)			135 (43.5)	128 (43.0)		
	AA	10 (6.8)	24 (15.5)			24 (7.7)	46 (15.4)		
	G	208 (71.2)	196 (63.2)	0.04	1.44 (1.02–2.03)	437 (70.5)	376 (63.1)	0.006*	1.39 (1.09–1.78)
	A	84 (28.8)	114 (36.8)			183 (29.5)	220 (36.9)		

*P-values are obtained for the comparison between the AMI and control subjects by $\chi 2$ -test; *P < 0.01.*

TABLE 5 | Plasma S100B levels in subgroup subjects according to S100B rs9722 G/A genotypes.

Group	Plasma S100B levels (pg/mL)	
	GG + GA	AA
Control subjects ($n = 75$)	107.31 ± 56.05	141.70 ± 76.69
AMI patients ($n = 75$)	273.05 ± 133.62*	347.13 ± 148.94*$

*Results are means ± S.D.*P < 0.05 compared with GG + GA; $P < 0.01 compared with AA control group.*

progression of atherosclerosis and cardiovascular complications, leading to the occurrence of AMI (2–4).

S100B is small molecular calmodulin that adhered to the RAGE receptor and induces the inflammatory reaction of the coronary artery unstable plaque, thus, it aggravated the rupture of unstable coronary artery plaque (13, 16, 17). In the present study, we found that plasma S100B levels were significantly higher in AMI patients than in the control subjects and stable angina pectoris patients. Moreover, we found that the S100B levels in the neutrophils obtained from AMI patients were higher with LPS stimulation compared to neutrophils from control subjects. We also found that organized thrombi from the coronary artery had lower levels of S100B than fresh thrombi from the same AMI patients. Furthermore, we found that S100B induced the neutrophil migration through the NF-KB-IL6/IL1β signal pathway; a blockade of this pathway interrupted neutrophil migration. Taken together, our results suggested that S100B might play a crucial role in the occurrence of AMI, especially in the early stages of myocardial infarction.

The current study verified the association between S100B rs9722 and the risk of AMI in the Han Chinese population. After adjusting the various risk factors, the S100B rs9722 variant was found to be associated with AMI patients. We demonstrated that AMI patients with the homozygous rs9722 AA allele exhibited increased levels of plasma S100B in AMI patients. Moreover, we found that rs9722 was located in 3′-UTR region of S100B, and the variants of S100B could alternate the potential microRNA targeting sites in S100B. Using the TargetScan version 4.0 (http://www.targetscan.org/), it was predicted that the S100B rs9722 A allele disrupted the binding sites of miRNAs (hsa-miR-3912-3p, hsa-miR-340-3p, hsa-miR-6797-3p), which could influence the expression of S100B (18–20). A minimum "seed" length of 7-mer was specified as the minimum cut-off score. It was possible that the S100B rs9722 variant at the 3′-UTR affected gene expression at the post-transcriptional level by interfering with protein binding, polyadenylation, or microRNA (miRNA) binding, which would further influence S100B expression.

The S100B expressed by the neutrophils in the coronary artery of AMI patients was examined, the results suggested that S100B were released from the shoulder region of ruptured coronary artery plaques of the AMI patients. Thus, S100B might be released into the blood immediately from the inflammatory cells in the shoulder region of ruptured coronary artery plaques, leading to a rapid increase in plasma S100B levels. Moreover, we found that organized thrombi had lower levels of S100B than fresh thrombi tissue obtained from the AMI patients which suggested that S100B may be released from the thrombi tissues into the blood during the early stages of AMI; thus, plasma S100B levels could be significantly higher at the onset of myocardial infarction.

There are several limitations associated with the present study (14, 15). As observed in numerous previous studies, the validity results are highly dependent on the study sample size. To verify these results, the study cohort needs to be expanded to include a larger number of participants, as well as a more racially heterogenous cohort. Second, the functional mechanisms underlying the association between the S100B rs9722 polymorphisms and AMI risk require further investigation, which can be achieved using high-throughput "omics" technology. By expanding the cohort across different geographical regions, the relationship between the S100B rs9722 variant and AMI risk could be further clarified, and the underlying mechanisms may be revealed. Most importantly, the elevation of S100B levels might not be AMI specific, and might represent a non-specific response to an acute body injury; it could be a secondary inflammatory response observed after the occurrence of AMI. Not only is the S100B level increased in patients with cardiovascular disease but it is also increased in those with non-cardiovascular disease. Further experiments are needed to investigate the findings of present study.

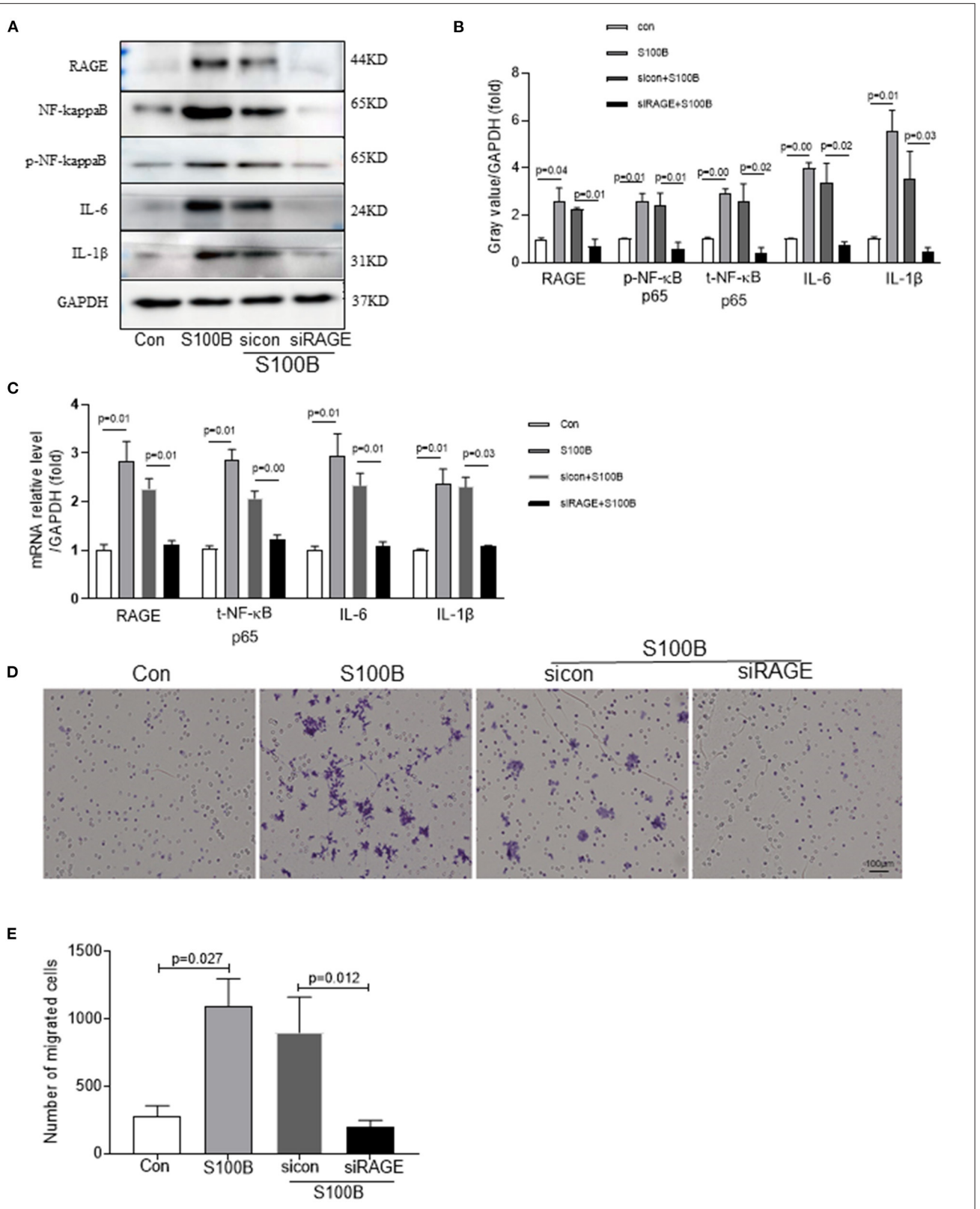

FIGURE 5 | Effect of S100B and S100B receptor knockdown influencing on neutrophils migration through the RAGE–NFκB–IL1β/IL6 signaling pathway. **(A,B)** Western blot analysis of RAGE–NFκB–IL1β/IL6 expression in neutrophils with S100B protein, sicontrol and siRAGE (S100B receptor). **(C)** Quantitative real-time PCR

(Continued)

FIGURE 5 | analysis of mRNA level of RAGE–NFκB–IL1β/IL6 in neutrophils with S100B, sicontrol and siRAGE. **(E)** Untransfected or transfected neutrophils with a control siRNA vector or siRAGE to enable the overexpression of the S100B fusion protein, control siRNA, or siRAGE were subjected to the transwell chamber migration assay. Cells that had migrated to the lower surface of the transwell chamber were stained with crystal violet and counted under a microscope. Representative microscopic images are shown. The column chart shows mean numbers of migrated cells in three experiments.

In summary, the level of novel homeostatic calmodulin in S100B is elevated in the early stages of myocardial infarction, and the S100B variant rs9722 is independently associated with AMI patients in the Han Chinese population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the General Hospital of Northern Theater Command ethics committees. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YH was responsible for the study concept and design and obtained study funding. YH, MC, and XZ wrote the manuscript. XS, DL, XT, and CY collected clinical specimens. MC and XZ did the statistical analysis. All authors critically revised the paper and approved the final version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX

Table A1 | Primer Sequence used in genotyping *S100B* variations.

SNPs	Primer sequence	Size of PCR product (bp)	Annealing temperature
Rs9722	forward:5′-AAGAGCAGGAGGTTGTGGAC-3′ reverse:5′- AAGAGGTTTTCAATTTTTCA -3′	291	58°C
Rs9984765	forward:5′- GATCCAAGGA CAGTGGAGAC-3′ reverse:5′- GGCTTTAGCTA GAAGTTTAG-3′	421	55°C
Rs2139356	forward:5′- GGGACCTTGA TGCACCTACT -3′ reverse:5′- CTGAGCTGGA GAAGGCCATG-3′	371	56°C
Rs1051169	forward:5′- TTGAGGTCTG TATTGATAAC-3′ reverse:5′- GAGTAACGGAACCCTCAGTG -3′	451	52°C
Rs2186358	forward:5′- CAGATGGATT TTAAGAGGAG -3′ reverse:5′- ATCCCTGAAT TCCCTTGCTG-3′	531	62°C

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Long-Term Exposure to Ambient Air Pollution and Myocardial Infarction: A Systematic Review and Meta-Analysis

Li Zou[1,2†], Qiao Zong[3†], Wenning Fu[4,5], Zeyu Zhang[1], Hongbin Xu[3], Shijiao Yan[2,6], Jin Mao[4], Yan Zhang[7], Shiyi Cao[3*] and Chuanzhu Lv[5,8,9,10*]*

[1] Department of Neurology, Renmin Hospital of Wuhan University, Wuhan, China, [2] Department of Neurology, Taihe Hospital, Hubei University of Medicine, Shiyan, China, [3] School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [4] School of Nursing, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [5] Key Laboratory of Emergency and Trauma, Ministry of Education, College of Emergency and Trauma, Hainan Medical University, Haikou, China, [6] School of International Education, Hainan Medical University, Haikou, China, [7] School of Medicine and Health Management, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [8] Department of Emergency, Hainan Clinical Research Center for Acute and Critical Diseases, The Second Affiliated Hospital of Hainan Medical University, Haikou, China, [9] Emergency and Trauma College, Hainan Medical University, Haikou, China, [10] Research Unit of Island Emergency Medicine, Medical University, Chinese Academy of Medical Sciences, Haikou, China

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Edited by:
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***Correspondence:**
Chuanzhu Lv
lvchuanzhu677@126.com
Yan Zhang
zhangyan1604@163.com
Shiyi Cao
caoshiyi@hust.edu.cn

†These authors share first authorship

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Background and Objective: An increasing number of epidemiological original studies suggested that long-term exposure to particulate matter ($PM_{2.}5$ and PM_{10}) could be associated with the risk of myocardial infarction (MI), but the results were inconsistent. We aimed to synthesized available cohort studies to identify the association between ambient air pollution ($PM_{2.5}$ and PM_{10}) and MI risk by a meta-analysis.

Methods: PubMed and Embase were searched through September 2019 to identify studies that met predetermined inclusion criteria. Reference lists from retrieved articles were also reviewed. A random-effects model was used to calculate the pooled relative risk (RR) and 95% confidence intervals (CI).

Results: Twenty-seven cohort studies involving 6,764,987 participants and 94,540 patients with MI were included in this systematic review. The pooled results showed that higher levels of ambient air pollution ($PM_{2.5}$ and PM_{10}) exposure were significantly associated with the risk of MI. The pooled relative risk (RR) for each 10-$\mu g/m^3$ increment in $PM_{2.5}$ and PM_{10} were 1.18 (95% CI: 1.11–1.26), and 1.03 (95% CI: 1.00–1.05), respectively. Exclusion of any single study did not materially alter the combined risk estimate.

Conclusions: Integrated evidence from cohort studies supports the hypothesis that long-term exposure to $PM_{2.5}$ and PM_{10} is a risk factor for MI.

Keywords: air pollution, particulate matter, myocardial infarction, meta-analysis, PM2.5 (AQI), PM10

INTRODUCTION

The incidence and prevalence of cardiovascular diseases have increased in recent decades, and cardiovascular disease has become one of the main causes of death among adults (1–3). Myocardial infarction (MI) is an acute and severe cardiovascular disease that generally can endanger the life of patients and has become a serious public health problem (4). The causes of MI are complex and are related to lifestyle, diet structure, genetic factors, and environmental factors, including air pollution (5–7). Studies have shown that reducing modifiable risk factors may contribute to the prevention and control of MI (8–10), which is of considerable public health importance.

Air pollution, especially particulate matter (PM), has been increasingly investigated as an environmental risk factor for MI morbidity and mortality recently. However, studies have had modest sample sizes and reported inconclusive results (11–13). These inconsistent and controversial results indicate the need to quantitatively synthesize and interpret the available evidence to provide more explicit information for policy decisions and clinical use. Meta-analysis is a statistical tool that can be used to integrate results of multiple independent studies considered to be combinable for a more precise estimation (14, 15). Although previous meta-analyses have examined associations between air pollution exposure and MI, their studies have mostly included cross-sectional literatures (13), and much new research has been recently published. Therefore, more meta-analyses with cohort studies are urgently needed.

Taking into consideration the inconsistent conclusions and the limitations of existing epidemiological studies and the flaws of previous meta-analyses, we therefore performed a systematic review and meta-analysis of cohort epidemiological studies to examine the potential associations between air pollution exposure and the risk of MI. Given the heavy economic and health burden of curing MI, the results of our study may provide additional practical and valuable clues for the prevention of MI.

METHODS AND MATERIALS

Ethical approval is not required for this systematic review.

Literature Search Strategy

We conducted this meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (16) and the checklist of items in the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) (17). A systematic literature search of PubMed and Embase was conducted through September 2019 by using the following search terms with no restrictions: "air pollution" or "particulate matter" or "air pollutants" or "PM_{10}"or "$PM_{2.5}$" or "air quality" in combination with "myocardial infarction" or "heart attack" or "acute coronary syndrome" or "cardiovascular disease" or "heart disease." The language was restricted to English. Additionally, reference lists of the retrieved original articles and relevant review articles were also scrutinized to identify further pertinent studies.

Study Selection

Studies meeting the following criteria were included in the meta-analysis: (1) the study design was cohort; (2) the exposure of interest was ambient air pollution; the endpoint of interest was the incidence of MI; and (3) the relative risk (RR) and the corresponding 95% confidence interval (CI) of MI relating to ambient air pollution were reported or could be calculated from the data provided. Animal studies, clinical trials, reviews, letters, and commentaries were excluded. Only studies with detailed information on both ambient air pollution and the incidence of MI was included.

Data Extraction and Quality Assessment

Two investigators (WF and SC) extracted the following information from the studies: first author, publication year, country, study period, age, number of cases, size of cohort, and time windows of exposure. Discrepancies were resolved by discussion with a third investigator (SC).

The Newcastle-Ottawa Scale, used to evaluate the qualities of cohort studies (18), is a nine-point scale allocating points based on the selection of participants, comparability of groups, and exposure/outcome. This scale awards a maximum of nine points to each study: four for selection of participants and measurement of exposure, two for comparability of cohorts or cases and controls on the basis of the design or analysis, and three for assessment of outcomes and adequacy of follow-up. Studies scoring 0–3 points, 4–6 points, and 7–9 points were categorized as low, moderate, and high quality of studies, respectively. When studies had several adjustment models, we extracted those that reflected the maximum extent of adjustment for potentially confounding variables. Each study was rated independently by two authors (WF and JM). Discrepancies were resolved by discussion with a third investigator (SC).

Statistical Analyses

We used RR to measure the association between ambient air pollution and the risk of MI and the random effects model was used to calculate an overall pooled RR for the main analysis.

The Q statistic with a significance level at $P < 0.10$ and I^2 statistic were used to test heterogeneity. The I^2 statistic measures the percentage of total variation across studies due to heterogeneity rather than chance. It was calculated according to the formula by Higgins (19). We used I^2 to quantify the heterogeneity, with 25%, 50%, and 75% indicating low, moderate and high degrees of heterogeneity, respectively.

In our meta-analysis the following formula was used to calculate the standardized risk estimates for each study:

$$\mathrm{RR}_{(\mathrm{standardized})} = \mathrm{RR}^{\frac{\mathrm{Increment}(10)/\mathrm{Increment}(\mathrm{original})}{(\mathrm{original})}}$$

Subgroup analyses were conducted to determine the possible influence of some factors such as state and publication years. We conducted a sensitivity analysis to explore potential sources of heterogeneity and to investigate the influence of various exclusion criteria on the pooled risk estimate. Begg's rank

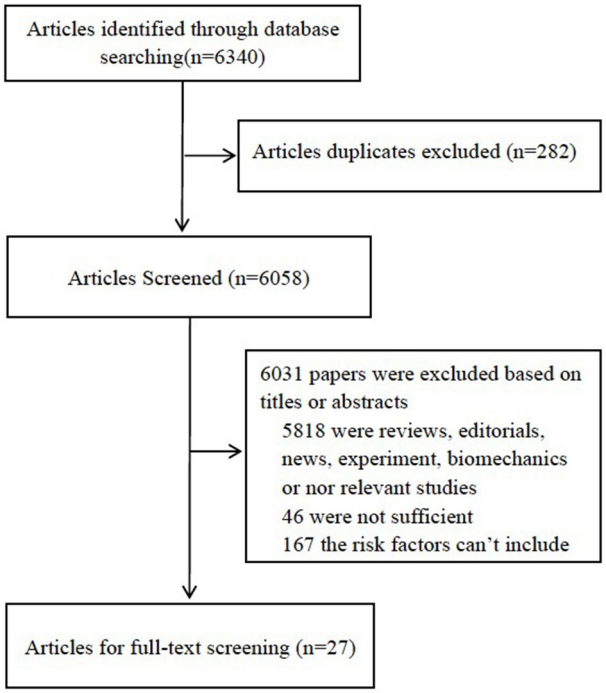

FIGURE 1 | Flow diagram of identification of relevant observational studies of ambient air pollution in relation to the risk of MI.

correlation and Egger's linear regression tests were used to assess the potential publication bias (20, 21). Using Duval and Tweedie's non-parametric trim-and-fill method to adjust potential publication bias (22). All analyses were performed using STATA statistical software (version 12.0; College Station, TX, USA), and all tests were two-sided with a significance level of 0.05.

RESULTS

Literature Search

Figure 1 shows the process of study identification and inclusion. Initially 3,097 and 3,243 citations were retrieved from PubMed database and Embase, respectively. After the exclusion of 282 duplicates, 6,058 potentially relevant studies from electronic databases were identified. Of these, we excluded 6,031 papers because they were experimental, biomechanics, reviews, or irrelevant studies. After full-text review of the remaining 213 articles, 46 articles were excluded because of insufficient data to calculate the risk estimates, and 167 were excluded because they were not a risk factor. Finally, 27 studies (23–49) were included.

Characteristics of the Studies Included

The main characteristics of the 27 studies on MI and long-term PM exposure in our meta-analysis were summarized in **Table 1**. These studies were published between 2004 and 2019. Among them, eight studies were from Europe, two studies were from Asia, and twelve studies were from America. The size of the cohorts ranged from 1,120 to 4,404,046 for a total of 6,764,987 subjects. The exposure measure was $PM_{2.5}$ in seventeen studies and PM_{10} in eleven studies; six publications investigated the association of MI with exposure to both $PM_{2.5}$ and PM_{10}.The end point was MI incidence (sixteen studies), MI mortality (twelve studies), and only one study MI hospital, and two studies included MI incidence and mortality. Twenty studies were published after 2010, and seven studies before 2010. The quality assessment scores ranged from 6 to 9, with an average score of seven points, representing satisfactory quality of the studies.

Results of Meta-Analysis

We employed meta-analysis to assess the association of $PM_{2.5}$ and PM_{10} with MI.

Association Between $PM_{2.5}$ and the Risk of MI

The results from random-effects meta-analysis of ambient air pollution ($PM_{2.5}$ and PM_{10}) and the risk of MI were shown in **Figure 2**. Twenty studies reported the effect of $PM_{2.5}$ exposure on the risk of MI and the pooled estimates suggested a positive relationship. Results from standardized data showed that a 10 mg/m^3 increase in $PM_{2.5}$ exposure was positively associated with the risk of MI (RR = 1.18, 95% CI: 1.11 to 1.26), and there was a moderate heterogeneity ($p = 0.002$; $I^2 = 54.2\%$). Subgroup analyses by state, and publication year (before 2010 vs. after 2010) showed no statistically significant difference in results (**Table 2**). Every single pooled result of subgroup showed the positive and statistically significant relationship between exposure to $PM_{2.5}$ and the risk of MI.

Association Between PM_{10} and the Risk of MI

Fourteen studies investigated the association of PM_{10} exposure with the risk of MI (**Figure 3**). The pooled estimates of these studies indicated that a 10 mg/m^3 increase in PM_{10} was associated with a higher risk of MI (RR = 1.03, 95% CI: 1.00 to 1.05), and the heterogeneity is ($p = 0.052$, $I^2 = 41.5\%$). We conducted subgroup analyses by state, and publication year (before 2010 vs. after 2010). In general, these subgroup analyses showed no statistically significant difference in results.

Sensitivity

Sensitivity analyses was conducted to find potential sources of heterogeneity in the association between air pollution ($PM_{2.5}$ and PM_{10}) and MI risk, to examine the influence of various exclusions on the combined RR and assess the robustness of all results. The pooled RR did not materially change, for $PM_{2.5}$ and PM_{10}, the both overall combined RR did not materially change, with a range from 1.17 (95% CI: 1.10–1.24) to 1.19 (95% CI: 1.11–1.26), and 1.02 (95% CI: 1.00–1.04) to 1.03 (95% CI: 1.01–1.05), respectively.

Publication Bias

Visual inspection of the funnel plot showed significant asymmetry ($PM_{2.5}$: **Figures 4**, **5**; PM_{10}: **Figures 6**, **7**). For $PM_{2.5}$ and PM_{10}, the Egger test indicated publication bias, but the Begg test did not ($PM_{2.5}$: Egger, $Z = 5.385$, $p = 0.000$, Begg, $t = 1.44$: $p = 0.168$; PM10: Egger, $Z = 2.283$ $p = 0.022$, Begg, $t = 1.22$ $p = 0.830$). We used the trim-and-fill method to evaluate the impact of any potential publication bias, and the results showed

TABLE 1 | Main characteristics of the studies included involving ambient air pollution and the risk of myocardial infarction.

Author	Year	Country (state)	Exposure	Study period	Age	Sample size	Gender, female (%)	Time windows of exposure	Endpoints	No. of cases
Jaana Hartiala	2016	American (North America)	$PM_{2.5}$	1998–2010	≥ 64	6,575	32	2001–2007	Incidence	288
Laura A	2016	American (North America)	$PM_{2.5}$	2001–2011	20–93	9,334	39	2002–2009	Incidence	704
Malik A	2019	American (North America)	$PM_{2.5}$	2003–2008	60.6 ± 12.2	5,650	25	2005–2008	Mortality	978
Hyeanji Kim	2017	Korea (Asia)	$PM_{2.5}$	NA	≥ 18	136,094	50.9	2007–2013	Incidence	1,881
Krishnan B	2016	England (Asia)	PM_{10}	2003–2006	NA	79,288	NA	2003–2006	Incidence	NA
Teresa To	2015	Canada (North America)	$PM_{2.5}$	NA	40–59	29,549	100	1980–2006	Incidence	1,233
Giulia C	2014	Sweden (Europe)	PM2.5 PM10	1980– 2006	NA	100,166	NA	1980– 2006	Incidence	5,157
Silvia Koton	2013	Israel (Asia)	$PM_{2.5}$	1995–2011	≤ 65	1,120	18.8	2005–2011	Incidence	432
Laura A	2017	American (North America)	$PM_{2.5}$	2001–2010	60.8 ± 12.1	5,679	39	2003–2009	Incidence	704
Vicki M	2013	Israel (Asia)	$PM_{2.5}$	1992–1993	≤ 65	1,120	16.3	2002–2013	Mortality	848
Michael J	2011	American (North America)	$PM_{2.5}$	1996–2005	≥ 20	124,614	NA	1999–2000	Incidence	722
Daniela N	2011	Italy (Europe)	PM_{10}	2002–2005	72.8 ± 13.0	11,450	49	2002–1005	Incidence	950
Kristin A	2007	American (North America)	$PM_{2.5}$	1994–2000	50–79	65,893	100	1998–2000	Incidence	584
Richard W	2012	London (Europe)	PM_{10}	2003–2007	40–89	836,557		2003–2006 12 months.	Incidence	13,965
George S	2018	Netherlands (Europe)	$PM_{2.5}$ PM_{10}	1993–2010	20–65	23,100	77	2005–2010	Incidence	797
Antonella Zanobetti	2007	American (North America)	PM_{10}	1985–1999	≥ 65	196,131	49.6	1 year	Incidence	22,552
Robin C	2009	South Carolina (North America)	PM_{10}	1992–2002	62.4 ± 7.6	66,250	100	48 months	Incidence Mortality	854
Robin C	2008	South Carolina (North America)	PM_{10}	1992–2002	30–55	121,700	100	4years	Incidence mortality	2,6,96
Harris He' ritier	2018	Switzerland (Europe)	$PM_{2.5}$	2000–2008	> 30	4,404,046	52	2003–2008	Mortality	19,261
Hong Chen	2016	Canada (North America)	$PM_{2.5}$	1999–2011	> 35	8,873	35	2001–2011	Mortality	4,016
C. Arden Pope	2004	Canada	$PM_{2.5}$	1982–1988	≥ 30	319,000 to 500,000,	NA	1984 1986 1988	Mortality	47.00%
Rob Beelen	2014	Netherlands (Europe)	$PM_{2.5}$ PM_{10}	1992–1996	46.0 (10.2)	22,136			Mortality	117
R Beelen	2009	Netherlands (Europe)	$PM_{2.5}$	1987–2000	55–69	120,852	30.9	1996–2000	Mortality	4,243
Cathryn Tonnea	2015	London (Europe)	$PM_{2.5}$ PM_{10}	2003–2007	≥25	18,138	32		Mortality	390

(Continued)

TABLE 1 | Continued

Author	Year	Country (state)	Exposure	Study period	Age	Sample size	Gender, female (%)	Time windows of exposure	Endpoints	No. of cases
Robin C	2011	South Carolina (North America)	$PM_{2.5}$ PM_{10}	1986–2003	40–75	17,545	0	24 36 46 months	Mortality	646
Stephanie von Klot	2005	Finland (Europe)	PM_{10}	1992–2001	≥ 35	22,006	NA	1995–1999	Hospital	2,321
Anke Huss	2010	Switzerland (Europe)	PM_{10}	1990–2005	> 30	12,122	NA	2000–2005	Mortality	8,192

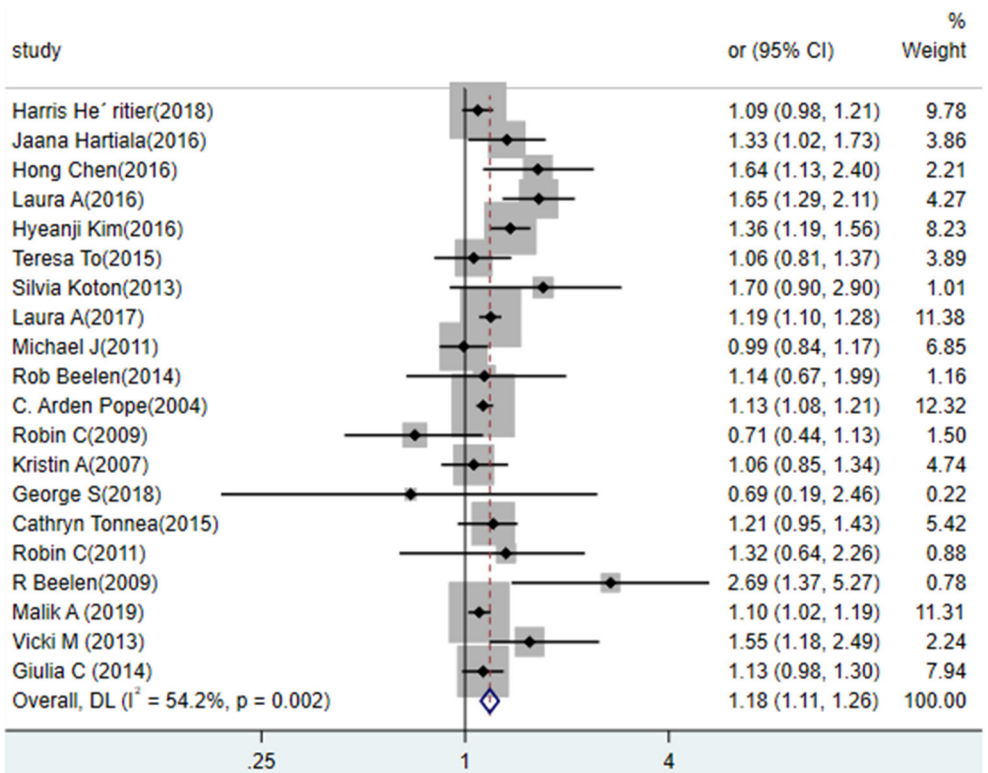

FIGURE 2 | Association between exposure to $PM_{2.5}$ and the risk of MI in a meta-analysis of cohort studies. Weights are from random- efforts model.

that one and five potentially missing studies would be needed to obtain funnel plot symmetry for the association of MI and air pollution ($PM_{2.5}$, PM_{10}), respectively. After using the trim-and-fill method, both the corrected *RR* for PM $_{2.5}$ was 1.18 (95% *CI*: 1.10 to 1.26; random-effects model, $p = 0.000$), for PM_{10} was 1.03 (95% CI: 1.00 to 1.06; random-effects model, $p = 0.027$), which suggested the both pooled RRs were not substantially changed by the correction for potential publication bias.

DISCUSSION

Myocardial infarction results in a high medical burden for families and society and remains a worldwide public health challenge (50). Ascertaining the risk factors of MI could provide significant information for the prevention of MI. Our meta-analysis has quantitatively examined the association between long-term exposure to ambient air pollution ($PM_{2.5}$ and PM_{10}) and MI. The pooled analysis, which included 27 cohort studies with more than 6.5 million people, showed an inverse association between exposures to air pollutants ($PM_{2.5}$ and PM_{10}) and the risk of MI, which was consistent with previous reviews and meta-analyses (13). However, previous meta-analyses included only studies published before 2014, while more recent studies were not included that might report lower estimates or negative results. Additionally, our meta-analysis included all cohorts from studies with reliable data, so that the effect of

TABLE 2 | Results of subgroup analyses about ambient air pollution and the risk of myocardial infarction.

Subgroup	PM2.5					PM10				
	Number of studies	RR	95% CI	*P* for heterogeneity	*I*-square (%)	Number of studies	RR	95% CI	*P* for heterogeneity	I-square (%)
State										
North America	11	1.15	1.08–1.22	0.014	55.0	2	1.06	0.97–1.15	0.001	85.2
Europe	6	1.12	1.03–1.22	0.017	61.3	5	1.02	0.99–1.05	0.026	67.6.
Asia	3	1.39	1.23–1.58	0.644	48.8	1	—	—	—	—
Publication years										
Before 2010	4	1.12	0.87–1.44	0.016	71.1	3	1.06	0.99–1.13	0.187	37.5
After 2010	13	1.16	1.09–1.24	0.000	66.6	11	1.02	1.00–1.05	0.041	47.2
Populations										
General	9	1.19	1.06–1.33	0.021	55.5	6	1.03	0.99–1.07	0.281	20.2
Specific	10	1.19	1.10–1/30	0.009	57.5	8	1.04	0.99–1.08	0.031	54.7

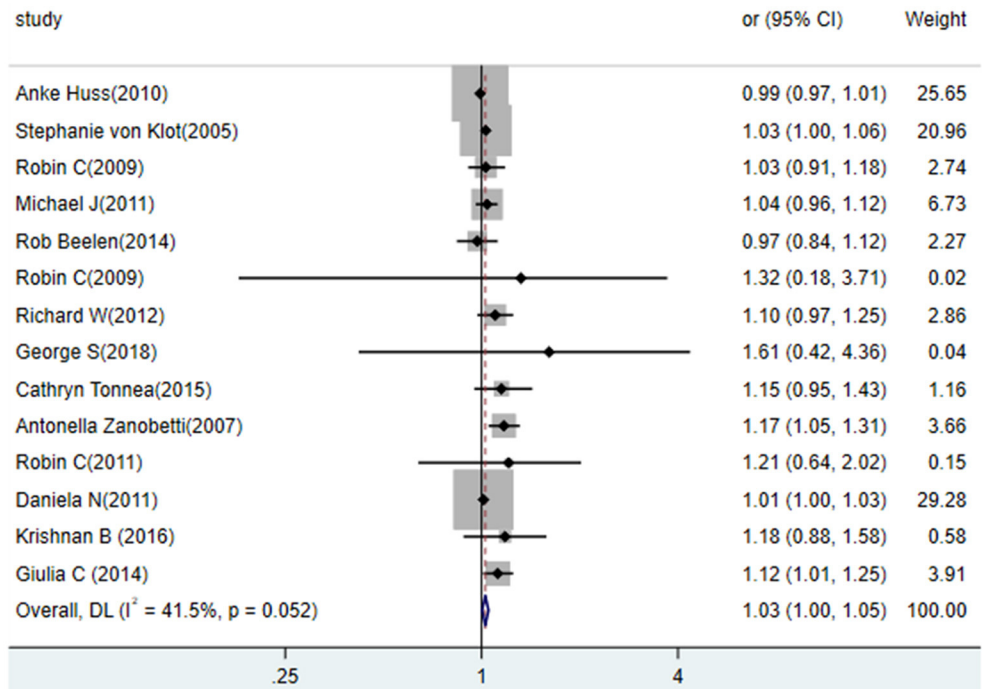

FIGURE 3 | Association between exposure to PM_{10} and the risk of MI in a meta-analysis of cohort studies. Weights are from random- efforts model.

exposure can be fully and directly analyzed and conclusions are relatively stable.

The mechanisms by which air pollution exposure could contribute to the development of MI might include inflammation, induction of autophagy, and down-regulation of membrane repair protein MG53. Researchers found that inflammation plays an important role in the formation of coronary atherosclerosis and aggravation of plaque instability, and air pollutants can also promote MI by promoting inflammation (51). The second potential mechanism is the induction of autophagy. Several observational studies have shown that autophagy is a normal process for cells to achieve their own metabolism and organelle renewal. Autophagy can maintain the body's metabolism to reduce damage and protect the organism. However, excessive autophagy can lead to apoptosis of cardiomyocytes and aggravate the damage of ischemic-related sites (52). Studies have found that autophagic levels for exposure to air pollutants are significantly higher than in the control group, while the corresponding protein expression levels, MI size decreases, and myocardial cell damage decrease

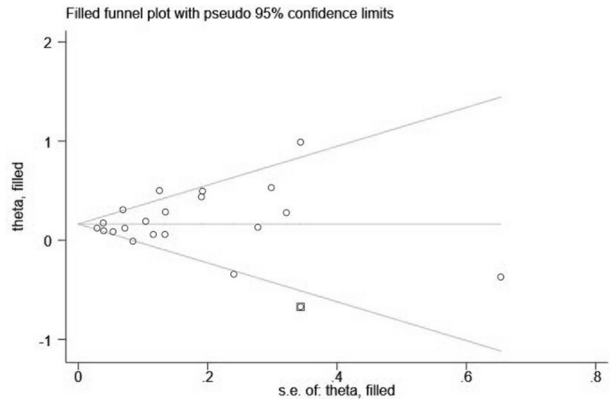

FIGURE 4 | Funnel plot with 95% confidence limits of $PM_{2.5}$ and the risk of MI.

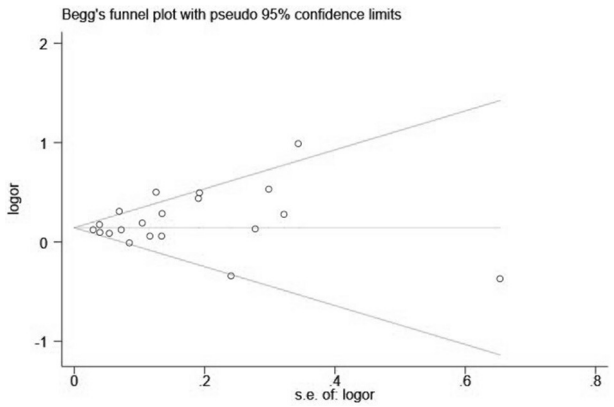

FIGURE 5 | Filled funnel plot of *RR* from studies that investigated the association between $PM_{2.5}$ and the risk of MI.

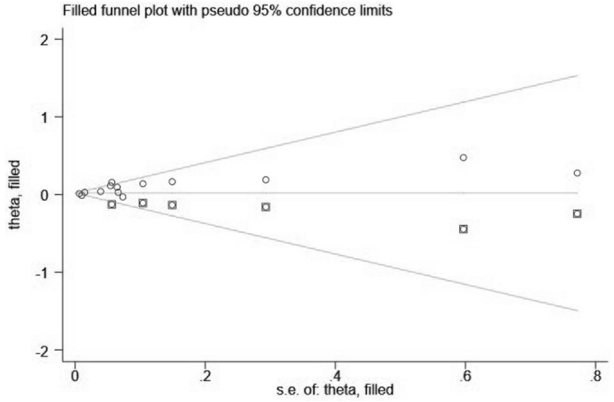

FIGURE 6 | Funnel plot with 95% confidence limits of PM_{10} and the risk of MI.

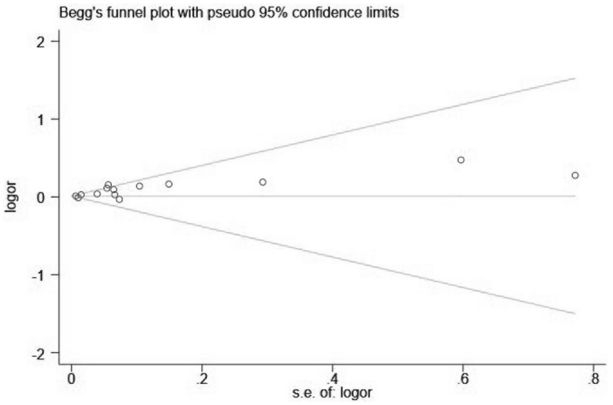

FIGURE 7 | Filled funnel plot of *RR* from studies that investigated the association between PM_{10} and the risk of MI.

in Farnesoid X receptor (FXR) knock out SD rats. Therefore, it is speculated that exposure to air pollutants promotes the development of MI through FXR-induced autophagy (53). The third possible mechanism is down-regulation of membrane repair protein MG53. Exposure to air pollutants can affect membrane repair through down-regulation of the expression of MG53 protein and aggravation of the severity of ischemia and hypoxia in MI (54).

We also found that long-term exposure to $PM_{2.5}$ has a more pronounced effect than PM_{10} on MI risk in each 10 $\mu g/m^3$ increase, which is in line with previous related research (13). Compared with PM_{10}, $PM_{2.5}$ can remain suspended for a longer time in the air and be inhaled into the respiratory tract and directly into the pulmonary alveoli. In addition, $PM_{2.5}$ has a larger superficial area and hence absorbs more chemical constituents than PM_{10}. Therefore, $PM_{2.5}$ is probably more harmful on human health than PM_{10} (55, 56).

Considering people's different diets and lifestyles and the prevalence of MI in different regions, we also conducted subgroup analysis by region, and statistically significant differences across subgroups were found except for PM_{10}. In Asia, a 10 mg/m^3 increase in $PM_{2.5}$ exposure was positively associated with the risk of MI (RR = 1.38), which was inconsistent with previous studies. The possible reasons for the differences include inconsistency of study designs and potentially selective reporting of the results for pollutants. However, it is suggested that the Asia region should pay attention to the relationship between air pollution and MI, and more work on air pollution and epidemiology remains to be done. We conducted a subgroup analysis by publication year but found the pooled result of studies before 2010 was not significantly different from that after 2010.

There are several strengths in this meta-analysis. First, all the studies in our analysis were cohort studies, which is considered as stronger measure for demonstrating causation and identification of risk factors than other observational study designs (57). Second, in this comprehensive literature review, we pooled data from 22 cohort studies from several geographical regions in one meta-analysis, thus increasing the statistical power and

allowing an investigation of regional patterns. Third, sensitivity analysis and consistent results from various subgroup analyses indicated that our findings were reliable and robust, although heterogeneity existed among the included studies. Furthermore, all the studies were published in the past decade, indicating that data on both the exposure and the outcome are recent and relevant.

Some limitations in the present meta-analysis should be of concern. First of all, the heterogeneity of the studies included was significant and existed through the whole analysis. But we explored the potential heterogeneity resources by subgroup analysis and sensitivity analysis. Second, there are relatively few studies on the mortality and hospitalization of MI, and there is a lack of data required for meta-analysis, so subgroup analysis is not conducted. Third, other air pollutants may have interaction with $PM_{2.5}$ and PM_{10}. A compounding effect of all these air pollutants should be examined and quantified on increasing the risk of MI.

Conclusion

In conclusion, our meta-analysis identified long-term exposure to $PM_{2.5}$ and PM_{10} as a significant risk factors of MI. In light of the heavy economic burden of MI, the results of our study provide additional valuable clues for the prevention of MI. For future studies, more high-quality longitudinal and interventional studies are needed to explore the underlying mechanisms of the relationships between ambient air pollution and MI, especially in Asia and other middle- and low-income countries. Besides, it is recommended that relevant departments must adopt a comprehensive particulate matter control policy to promote health and reduce the burden of MI.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

LZ, QZ, YZ, SC, and CL conceived and designed the study. LZ, QZ, WF, ZZ, HX, SY, JM, YZ, SC, and CL participated in the acquisition of data. LZ and QZ analyzed the data. YZ, CL, and SC gave advice on methodology. LZ drafted the manuscript. LZ, QZ, WF, ZZ, HX, SY, JM, YZ, SC, and CL revised the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.616355/full#supplementary-material

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Association Between Aspirin Use and Decreased Risk of Pneumonia in Patients With Cardio-Cerebra-Vascular Ischemic Disease: A Population-Based Cohort Study

Ying-Cheng Chen[1,2†], *Yin-Yang Chen*[1,3†], *Han Wei Yeh*[4], *Tung-Ying Yeh*[5,6], *Jing-Yang Huang*[1,7], *Pei-Lun Liao*[7], *Liang-Tsai Yeh*[1,8], *Shun-Fa Yang*[1,7], *Ming-Chih Chou*[1,3*] *and Chao-Bin Yeh*[1,9,10*]

[1] *Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan,* [2] *Department of Surgery, Changhua Christian Hospital, Changhua, Taiwan,* [3] *Department of Surgery, Chung Shan Medical University Hospital, Taichung, Taiwan,* [4] *School of Medicine, Chang Gung University, Taoyuan City, Taiwan,* [5] *Graduate School of Dentistry, School of Dentistry, Chung Shan Medical University, Taichung, Taiwan,* [6] *Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan,* [7] *Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan,* [8] *Department of Anesthesiology, Changhua Christian Hospital, Changhua, Taiwan,* [9] *Department of Emergency Medicine, School of Medicine, Chung Shan Medical University, Taichung, Taiwan,* [10] *Department of Emergency Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan*

This study evaluated the association between long-term low-dose aspirin use and decreased risk of pneumonia in patients with cardio-cerebra-vascular ischemic diseases (CCVDs). This retrospective cohort study used records from Taiwan's National Health Insurance Research Database of claims made between 1997 and 2013. After propensity score matching (PSM), patients who took a low dose of aspirin for more than 90 days within 1 year of diagnosis with CCVDs were identified as the exposure group (n = 15,784). A matched total of 15,784 individuals without aspirin use were selected for the non-aspirin group. The main outcome was the development of pneumonia after the index date. Multivariable Cox regression analysis and Kaplan–Meier survival analysis were performed to estimate the adjusted hazard ratio (aHR) and cumulative probability of pneumonia. The result after PSM indicated a lower hazard ratio for pneumonia in aspirin users (aHR = 0.890, 95% confidence interval = 0.837–0.945). Therefore, patients with CCVDs who took aspirin had a lower risk of developing pneumonia than those who did not. In conclusion, this population-based cohort study demonstrated that long-term low-dose aspirin use is associated with a slightly decreased risk of pneumonia in patients with CCVDs.

Keywords: aspirin, pneumonia, risk, database, cardio-cerebra-vascular ischemic diseases

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Charles Feldman, University of the Witwatersrand, South Africa
Chin Chen Chu, Chi Mei Medical Center, Taiwan

***Correspondence:**
Chao-Bin Yeh
sky5ff@gmail.com
Ming-Chih Chou
cshy236@csh.org.tw

†These authors have contributed equally to this work

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INTRODUCTION

Cardio-cerebra-vascular ischemic diseases (CCVDs), a class of disorders involving the heart and blood vessels, are the major leading causes of death worldwide, contributing to decreased quality of life and increased economic burden on patients. CCVDs include coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism (1). In Taiwan, coronary heart disease and cerebrovascular disease are the 2nd and 4th leading causes of death, respectively, with mortality rates of 48.8 and 26.1% (2). The average medical costs for the 1st year of myocardial infarction, stoke, and angina were reported to be NT$293,995, NT$141,086, and NT$60,305, respectively (3).

Moreover, CCVDs puts patients at higher risk of comorbidities such as hypertension, diabetes mellitus, pneumonia, heart failure, and arrhythmia. Among these, pneumonia is the 3rd leading cause of death in Taiwan (2). A population-based retrospective cohort study reported that patients with CCVDs had a higher risk of pneumonia than did those without [adjusted hazard ratio (aHR): 2.27, 95% confidence interval (CI): 2.01–2.56, $P < 0.001$) (4). The coexistence of CCVDs and pneumonia is associated with a high rate of pneumonia-associated 30-day mortality [hazard ratio (HR), 5.490, CI: 2.905–10.374, $P < 0.001$] (5). Prevention and early treatment of pneumonia in patients with CCVDs is crucial.

Other than lifestyle modifications, antiplatelet therapies such as aspirin play a crucial role for primary and secondary prevention of CCVDs. The cardioprotective effect of aspirin is mainly attributed to the irreversible acetylation of cyclooxygenase (COX)-1 and COX-2 in platelets, resulting in blockade of the production of thromboxane A2 and prostaglandin I2 (PGI2) and inhibiting platelet aggregation and vasoconstriction (6–9). Benefits are achieved with low-dose aspirin (75–100 mg orally daily) according to current trials (10) and guidelines (11).

The anti-infection effects of aspirin have also been studied. The mechanisms by which aspirin affects the immune system and manipulates processes involved in sepsis include the inhibition of COX (12) and nuclear factor kappa B (NF-κB) (13) and the induction of the production of nitric oxide (14) and lipoxin (15). Falcone et al. demonstrated an association between long-term use of aspirin and lower mortality rates (2.07, CI: 1.08–3.98, $P = 0.029$) in patients with pneumonia (16). Other studies have reported protective effects against viral infections of the respiratory tract (17), infectious endocarditis (18), and pyogenic liver abscess (19).

By contrast, Yayan's retrospective cohort study indicated that rather than exhibit significant anti-infection effects in patients with chronic obstructive pulmonary disease (COPD), aspirin use corresponded to increased infection rates (20). Eisen et al. designed the "AspiriN To Inhibit SEPSIS (ANTISEPSIS) randomized controlled trial protocol" for further research into this topic, but the final data have yet to be reported (21). Moreover, McNeil et al. demonstrated increased all-cause mortality in patient with daily low dose aspirin without indication (22). In response to the lack of consensus on the anti-infection effect of aspirin and the high mortality rate of pneumonia in patients with CCVDs, this study evaluated the association between long-term aspirin use and risk of pneumonia in patients with CCVDs in Taiwan. We hypothesized that aspirin can reduce the risk of pneumonia in patients with CCVDs.

Abbreviations: aHRs, adjusted hazard ratios; CCVDs, cardio-cerebra-vascular ischemic disease; NHIRD, National Health Insurance Research Database; COPD, Chronic Obstruction Pulmonary Disease; NSAIDs, Non-steroidal anti-inflammatory drugs; PPIs, Proton-pump inhibitors; CCBs, Calcium channel blockers; ACEIs, Angiotensin-converting-enzyme inhibitors.

MATERIALS AND METHODS

Data Source

We used the Longitudinal Health Insurance Database (LHID) 2000, which is a subset of the National Health Insurance Research Database (NHIRD), to evaluate the effect of aspirin on the risk of pneumonia. The NHIRD provides real-world evidence for exploring the risk factors or effects of an intervention for specific diseases (23). The LHID 2000 consists of the data of one million beneficiaries who were insured by the National Health Insurance program in 2000. The research timeframe in this study was the 17 years between January 1997 and December 2013. This retrospective population-based cohort study was approved by the National Health Insurance Administration and the Institutional Review Board of Chung Shan Medical University (registration number: CSMUH CS16183).

Study Population

We included patients with a diagnosis of a CCVDs, including coronary artery disease and ischemic stroke (using International Classification of Diseases, Ninth Revision, Clinical Modification [ICD-9-CM] codes 410.x to 414.× and 433.× to 436.×), between January 1, 1997, and December 31, 2013. To reduce the false positive rate of identifying diseases with ICD-9-CM codes (24), patients were excluded if they had outpatient visit records with CCVDs only. The index date was 365 days after the date of first diagnosis with CCVDs. We excluded patients who (a) had index dates before January 2001 (for left-censored or left-truncated data) or after December 2013 (limited due to the research timeframe), (b) were missing demographic data, (c) died before the index date, or (d) developed pneumonia before the index date. In this study, the censored data including withdraw from insurance coverage, death, and end of study (December 31, 2013). **Figure 1** illustrates the study framework.

Initially, 22,858 patients with CCVDs and low-dose aspirin use for more than 90 days after their first diagnosis were classified into the aspirin user group. We evaluated the dose of aspirin within 1 year after diagnosis of CCVDs, the median (Q1, Q3) duration of long-term (100 mg per day) aspirin usage was 275-day (152, 365) and the average medication possession ratio (MPR) was 0.71 in exposure group. By contrast, the median of duration was 1-day (0, 15) with average MPR of 0.03 in non-exposure group within 1 year. In addition, we identified the overall dosage during follow up, the median of duration

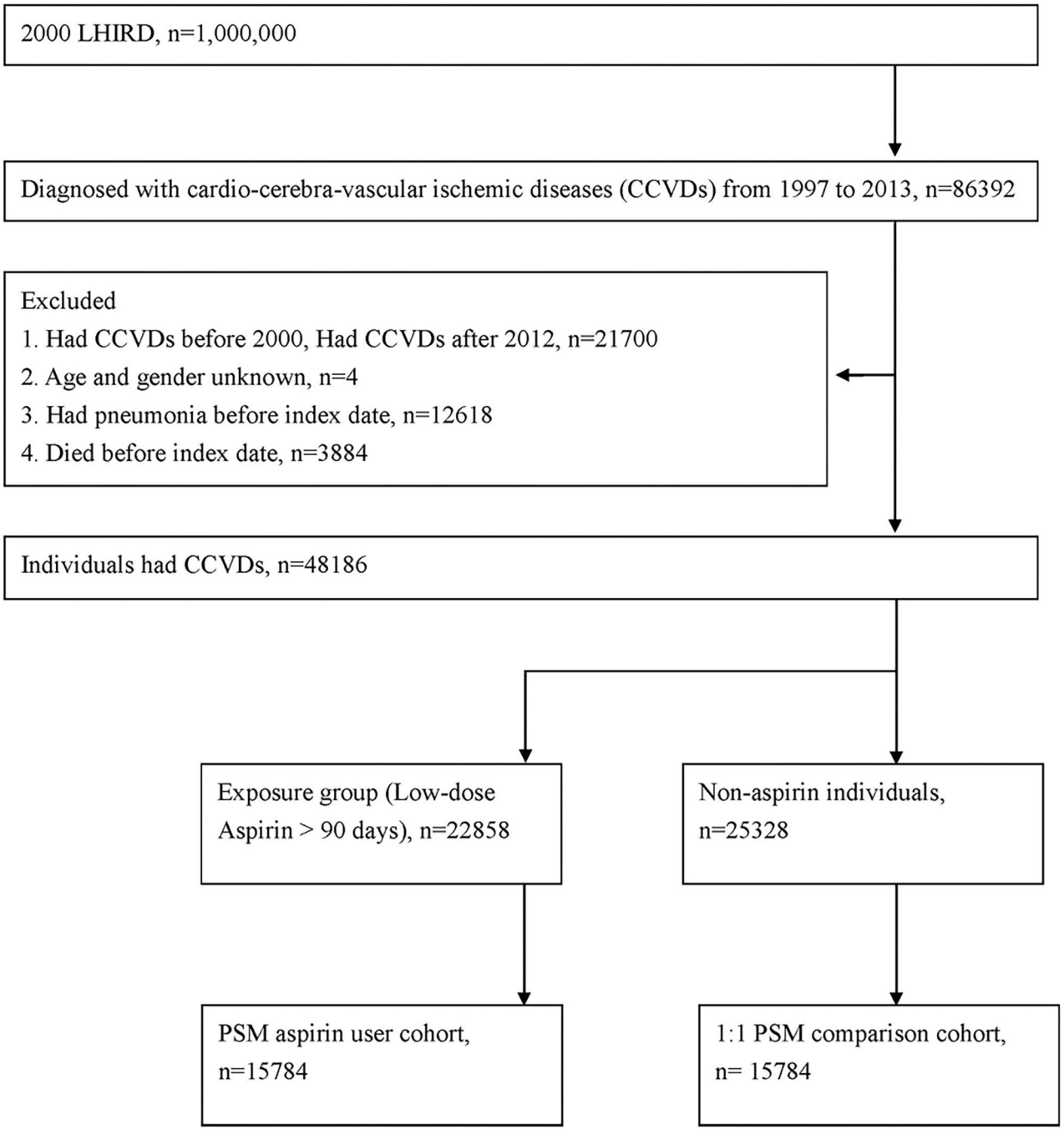

FIGURE 1 | Flowchart of patient selection.

was 807-day with average MPR of 0.58 in exposure group, and the median of duration was 26-day with average MPR of 0.08 in non-exposure group. The remaining individuals comprised the non-aspirin cohort. To reduce potential confounding bias in our results, 1:1 propensity score matching (PSM) was performed using greedy nearest neighbor, non-replacement matching with a caliper width of 0.01. PSM analysis is widely used in retrospective studies to eliminate imbalance in measured confounding factors among subjects in two study groups. The propensity score of exposure was estimated using logistic regression and covariates, including age, sex, and comorbidities (e.g., hypertension, diabetes mellitus, hyperlipidemia, chronic obstruction pulmonary disease (COPD), dementia, cancer, or major bleeding) and co-medication [including corticosteroids,

non-steroidal anti-inflammatory drugs (NSAIDs), proton-pump inhibitors (PPIs), calcium channel blockers (CCBs), Angiotensin-converting-enzyme inhibitors (ACEIs) and statin]. Standardized differences (SDs) were estimated to evaluate the success of balancing baseline covariates between the two study groups; a SD absolute value of < 0.1 indicated that the item was balanced between the two groups (25).

Characteristics, Comorbidities, and Study Outcome

Baseline demographic characteristics, such as age and sex, were recorded. Comorbidities, including hypertension, diabetes mellitus, hyperlipidemia, COPD, dementia, cancer, major bleeding, corticosteroids, NSAIDs, PPIs, CCBs, ACEIs and statin within 1 year before the index date, were documented as potential confounding factors. The study event was defined as the diagnosis of pneumonia (ICD-9-CM 480-486.) during an emergency visit or upon hospital admission. All study individuals were followed up from the index date to the study event, date of death, or end of the study (December 31, 2013).

Statistical Analysis

Categorical data are presented as numbers and percentages and were compared using a chi-square test. The incidence rate ratio with corresponding CIs and crude HR were calculated using Poisson regression. After the proportional hazard assumption was tested, Cox proportional hazard model analysis was performed to estimate the HR for pneumonia and 95% CI. Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA). The significance level was set at 0.05. The cumulative probability of pneumonia was assessed using Kaplan-Meier analysis, in which statistical significance was based on a log-rank test.

RESULTS

Characteristics of Study Subjects

We identified 48,186 patients who had received a CCVDs diagnosis during the period from 2000 to 2012. Of these, 22,858 (47.43%) were using a low dose of aspirin for secondary prevention. The aspirin group had a higher proportion of male patients (61.27%), elderly patients, and patients with comorbidities (such as hypertension, diabetes mellitus, and

TABLE 1 | Baseline characteristics among study groups.

Variable	Before PSM			After PSM		
	Non-aspirin *N* = 25,328	Aspirin *n* = 22,858	Standardized difference	Non-aspirin *N* = 15,784	Aspirin *n* = 15,784	Standardized difference
Sex			0.17834			0.03814
Female	12,039 (47.53%)	8,854 (38.73%)		6,943 (43.99%)	6,645 (42.1%)	
Male	13,289 (52.47%)	14,004 (61.27%)		8,841 (56.01%)	9,139 (57.9%)	
Age			0.40063			0.09334
≤50	6,136 (24.23%)	2,306 (10.09%)		1,806 (11.44%)	1,754 (11.11%)	
51–60	5,325 (21.02%)	4,768 (20.86%)		3,123 (19.79%)	3,340 (21.16%)	
61–70	5,458 (21.55%)	6,387 (27.94%)		4,001 (25.35%)	4,335 (27.46%)	
71–80	5,583 (22.04%)	6,755 (29.55%)		4,572 (28.97%)	4,581 (29.02%)	
≥81	2,826 (11.16%)	2,642 (11.56%)		2,282 (14.46%)	1,774 (11.24%)	
Comorbidities						
Hypertension	16,044 (63.34%)	19,573 (85.63%)	0.52873	13,312 (84.34%)	12,857 (81.46%)	−0.07661
Diabetes mellitus	7,096 (28.02%)	9,503 (41.57%)	0.28756	5,867 (37.17%)	5,831 (36.94%)	−0.00472
Hyperlipidemia	7,944 (31.36%)	12,335 (53.96%)	0.46934	6,740 (42.7%)	6,836 (43.31%)	0.01229
COPD	5,983 (23.62%)	5,515 (24.13%)	0.01185	4,113 (26.06%)	3,993 (25.3%)	−0.01740
Dementia	1,596 (6.30%)	1,690 (7.39%)	0.04325	1,216 (7.7%)	1,208 (7.65%)	−0.00190
Cancer	2,071 (8.18%)	1,673 (7.32%)	−0.03208	1,292 (8.19%)	1,240 (7.86%)	−0.01213
Major bleeding	5,970 (23.57%)	4,427 (19.37%)	−0.10250	3,557 (22.54%)	3,538 (22.42%)	−0.00288
Medication						
corticosteroids	12,963 (51.18%)	11,273 (49.32%)	−0.03727	8,147 (51.62%)	7,981 (50.56%)	−0.02104
NSAIDs	23,370 (92.27%)	20,814 (91.06%)	−0.04384	14,516 (91.97%)	14,476 (91.71%)	−0.00926
PPIs	5,329 (21.04%)	3,714 (16.25%)	−0.12327	3,128 (19.82%)	2,979 (18.87%)	−0.02390
CCBs	15,089 (59.57%)	17,737 (77.6%)	0.39579	12,073 (76.49%)	11,705 (74.16%)	−0.05410
ACEIs	8,277 (32.68%)	12,088 (52.88%)	0.41714	7,378 (46.74%)	7,368 (46.68%)	−0.00127
Statin	5,205 (20.55%)	10,906 (47.71%)	0.59790	5,015 (31.77%)	5,389 (34.14%)	0.05042

COPD, Chronic Obstruction Pulmonary Disease; NSAIDs, Non-steroidal anti-inflammatory drugs; PPIs, Proton-pump inhibitors; CCBs, Calcium channel blockers; ACEIs, Angiotensin-converting-enzyme inhibitors.

hyperlipidemia); however, a lower proportion of major bleeding was found in the aspirin group. After PSM, a matched aspirin cohort of 15,784 and non-aspirin cohort of the same number were established for analysis. A comparison of the characteristics of the aspirin users and the non-aspirin users is presented in **Table 1**. Hypertension was the comorbidity of highest prevalence in both groups, followed by hyperlipidemia and diabetes mellitus. Age, gender, comorbidities, and co-medication of corticosteroids, NSAIDs, PPIs, CCBs, ACEIs and statin were not significantly different between the propensity score matched aspirin and non-aspirin groups.

Risk of Pneumonia for Different Comorbidities and Other Conditions

After PSM, the incidence densities of pneumonia (per 1,000 person months) were 3.60 (CI = 3.48–3.73) and 3.95 (CI = 3.82–4.07) in the aspirin and non-aspirin cohorts, respectively. A Kaplan–Meier survival analysis revealed a significantly lower cumulative incidence of pneumonia in the aspirin group (**Figure 2**). Compared with patients in the non-aspirin group, those with CVD in the aspirin group exhibited a decreased risk of pneumonia (aHR: 0.890, CI: 0.837–0.945). Other significant risk factors of pneumonia in patients with CCVDs included dementia, DM and COPD (**Table 2**). The stratified analysis showed the significant protect effect of aspirin in subgroup of male, hypertension, non-DM, COPD, non-dementia, non-cancer, and patients without major bleeding (**Table 3**).

DISCUSSION

This is the first nationwide longitudinal population-based cohort study to evaluate the effect of aspirin on pneumonia in patients with CCVDs, and the findings indicate that long-term low-dose aspirin use is associated with a slightly decreased risk of pneumonia. Aspirin is well-known for secondary prevention in patients with CCVDs and reduces the mortality and disease recurrence of CCVDs (10, 11). We discovered its further ability to reduce the incidence of pneumonia in patients with CCVDs. Nevertheless, for patients with hemorrhagic stroke, how aspirin use affects the risk of major bleeding is still under debate. Hence, we only included patients with ischemic stroke in this study population.

Our findings are consistent with those of other studies on the association between aspirin and infection. A literature review

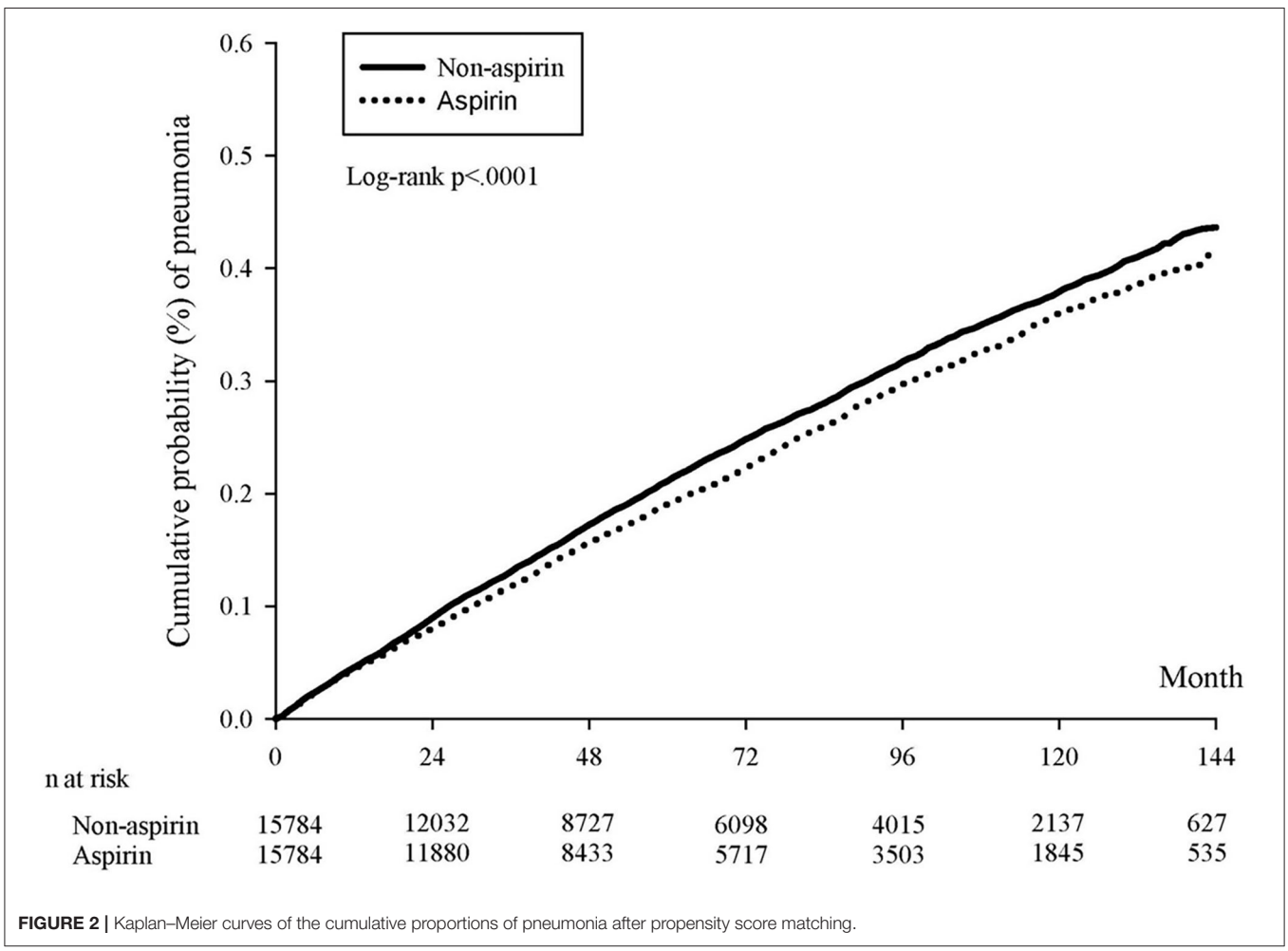

FIGURE 2 | Kaplan–Meier curves of the cumulative proportions of pneumonia after propensity score matching.

TABLE 2 | Multiple Cox proportional hazard regression results for pneumonia.

	Before PSM		After PSM	
Variable	**aHR (95% C.I.)**	***P*-value**	**aHR (95% C.I.)**	***P*-value**
Aspirin				
No	Reference		Reference	
Yes	0.952 (0.913–0.992)	0.0199	0.890 (0.837–0.945)	0.0002
Sex				
Female	Reference			
Male	1.351 (1.297–1.408)	<0.0001		
Age				
≤50	Reference			
51–60	1.437 (1.303–1.584)	<0.0001		
61–70	2.328 (2.129–2.546)	<0.0001		
71–80	4.184 (3.837–4.563)	<0.0001		
≥81	7.717 (7.037–8.463)	<0.0001		
Comorbidities (ref: non)				
Hypertension	1.034 (0.973–1.098)	0.2851		
Diabetes mellitus	1.436 (1.377–1.496)	<0.0001		
Hyperlipidemia	0.847 (0.806–0.891)	<0.0001		
COPD	1.363 (1.307–1.422)	<0.0001		
Dementia	1.929 (1.816–2.048)	<0.0001		
Cancer	1.225 (1.147–1.309)	<0.0001		
Major bleeding	1.210 (1.155–1.268)	<0.0001		
Medication				
Corticosteroids	1.134 (1.089–1.181)	<0.0001		
NSAIDs	0.943 (0.874–1.017)	0.1297		
PPIs	1.127 (1.070–1.186)	<0.0001		
CCBs	1.133 (1.073–1.196)	<0.0001		
ACEIs	1.150 (1.102–1.199)	<0.0001		
Statin	0.962 (0.912–1.016)	0.1641		

**aHR, adjusted hazard ratio; COPD, Chronic Obstruction Pulmonary Disease; NSAIDs, Non-steroidal anti-inflammatory drugs; PPIs, Proton-pump inhibitors; CCBs, Calcium channel blockers; ACEIs, Angiotensin-converting-enzyme inhibitors.*

revealed the ability of aspirin to prevent infections. Eisen et al. performed a retrospective cohort study in an intensive care unit setting that showed that the administration of aspirin was associated with 14.8% lower mortality in critically ill patients with sepsis (26). Another nationwide population-based cohort study published in 2015 reported that the administration of aspirin was associated with survival benefit in patients with sepsis (27). Furthermore, a prospective cohort study published in the same year demonstrated that elderly patients with pneumonia who received a low daily dose of aspirin (100 mg/day) had a lower total mortality rate than did those who did not receive any medication (16). In our population, 47.43% of patients were treated with long-term low-dose aspirin and were predominantly male, of old age, and with comorbidities of hypertension, diabetes mellitus, or hyperlipidemia and use of PPI before PSM.

The mechanisms by which aspirin affects the immune system has three main pathways, involving tumor necrosis factor (TNF), lipid mediators, and platelets (21). First, receptors of immune cells can recognize pathogen-associated molecular patterns and modulate intracellular signaling, resulting in the activation of NFκB and the transcription of TNF, a proinflammatory cytokine. Aspirin regulates immune response by inhibiting the activation of NFκB (28). Second, lipid mediators play a part in anti-inflammation and the restoration of homeostasis (29). Low-dose aspirin use increases the number of lipid mediators of lipoxins and resolvins, which inhibit the production of proinflammatory cytokines (30). Finally, aspirin inhibits the activation and aggregation of platelets, and its role in the interaction between platelets and pathogens in immune responses and infections has recently been researched widely (31, 32). The surface receptors of platelets are involved in direct platelet–bacteria interactions, and plasma proteins promote indirect interactions, resulting in the modulation of neutrophils, Kupffer cells, and the complement system (33).

In regard to the definition of pneumonia in our study was described as follows. According to our study design, the index date was 365 days after the date of first diagnosis of CCVDs and patients with previous pneumonia diagnosis were excluded and we only included the newly diagnosis of pneumonia with ICD 9 code from emergencies or hospitalization to confirm the accuracy

TABLE 3 | Risk of pneumonia among aspirin and non-aspirin groups and stratified analysis.

Variables	Incidence rate		Adjusted HR†	95% CI	
	Non-aspirin	Aspirin		Lower	Upper
Study groups	3.95 (3.82–4.07)	3.60 (3.48–3.73)	0.890**	0.837	0.945
Sex					
Female	3.39 (3.22–3.57)	3.46 (3.28–3.64)	1.016	0.911	1.132
Male	4.41 (4.23–4.59)	3.71 (3.55–3.88)	0.802***	0.735	0.876
Age					
<70	2.18 (2.06–2.30)	1.99 (1.88–2.11)	0.824**	0.732	0.928
≥70	6.65 (6.39–6.91)	6.30 (6.04–6.57)	0.981	0.897	1.072
Comorbidities					
Hypertension					
Without	3.04 (2.79–3.32)	2.72 (2.49–2.97)	0.932	0.760	1.143
With	4.12 (3.99–4.27)	3.81 (3.68–3.95)	0.892**	0.834	0.954
Diabetes mellitus					
Without	3.58 (3.43–3.73)	3.22 (3.08–3.37)	0.896*	0.822	0.978
With	4.63 (4.41–4.86)	4.31 (4.09–4.54)	0.910	0.815	1.015
Hyperlipidemia					
Without	4.58 (4.40–4.76)	4.13 (3.96–4.31)	0.924	0.851	1.003
With	3.11 (2.94–3.28)	2.89 (2.73–3.06)	0.915	0.813	1.028
COPD					
Without	3.25 (3.12–3.38)	3.00 (2.88–3.13)	0.889**	0.818	0.965
With	6.03 (5.73–6.35)	5.45 (5.15–5.76)	0.837**	0.743	0.944
Dementia					
Without	3.65 (3.53–3.77)	3.25 (3.13–3.37)	0.861***	0.805	0.921
With	9.18 (8.40–10.0)	10.1 (9.32–11.1)	1.127	0.863	1.472
Cancer					
Without	3.80 (3.68–3.93)	3.46 (3.34–3.58)	0.897**	0.840	0.958
With	6.04 (5.47–6.68)	5.73 (5.15–6.37)	1.066	0.779	1.457
Major bleeding					
Without	3.63 (3.50–3.77)	3.29 (3.16–3.42)	0.874**	0.811	0.941
With	5.18 (4.87–5.51)	4.86 (4.55–5.19)	0.969	0.841	1.117
Medication					
Corticosteroids					
Without	3.51 (3.35–3.69)	3.20 (3.04–3.36)	0.831**	0.748	0.923
With	4.36 (4.18–4.54)	4.00 (3.83–4.19)	0.963	0.879	1.055
NSAIDs					
Without	3.79 (3.38–4.26)	3.16 (2.79–3.59)	0.897	0.635	1.268
With	3.96 (3.83–4.09)	3.64 (3.52–3.77)	0.897**	0.841	0.957
PPIs					
Without	3.78 (3.65–3.92)	3.39 (3.26–3.52)	0.861***	0.802	0.924
With	4.81 (4.48–5.17)	4.82 (4.47–5.19)	1.114	0.938	1.324
CCBs					
Without	3.13 (2.91–3.37)	2.91 (2.70–3.14)	0.891	0.751	1.059
With	4.19 (4.05–4.34)	3.83 (3.69–3.98)	0.905**	0.843	0.972
ACEIs					
Without	3.40 (3.24–3.56)	3.38 (3.22–3.55)	0.973	0.879	1.076
With	4.54 (4.35–4.74)	3.83 (3.66–4.02)	0.842**	0.767	0.923
Statin					
Without	4.20 (4.05–4.35)	3.99 (3.84–4.15)	0.924*	0.858	0.994
With	3.32 (3.11–3.54)	2.72 (2.53–2.92)	0.844*	0.734	0.969

*$p < 0.05$, **$p < 0.01$,***$p < 0.0001$; † *Adjusted for all variables.*

COPD, Chronic Obstruction Pulmonary Disease; NSAIDs, Non-steroidal anti-inflammatory drugs; PPIs, Proton-pump inhibitors; CCBs, Calcium channel blockers; ACEIs, Angiotensin-converting-enzyme inhibitors.

of diagnosis. Hence, if the diagnosis of pneumonia was made in ER or upon admission, it should represent for community-acquired pneumonia (CAP). On the contrast, if the diagnosis of pneumonia was made during the hospitalization, it could be hospital-acquired pneumonia (HAP). We could not distinguish CAP from HAP only using ICD 9 code and this is one limitation of NHIRD. However, CAP should be the majority of the diagnosis based on the clinical condition. Hence, our study included all cause of pneumonia instead of CAP only. On the other hand, for the accuracy of using ICD 9 code to claim the data of pneumonia, there is one research on this question: International classification of diseases codes showed modest sensitivity for detecting community-acquired pneumonia. Previous study concluded that ICD-9-CM codes showed modest sensitivity for detecting CAP in hospital administrative databases, leaving at least one quarter of pneumonia cases undetected (34). Therefore, sensitivity decreased with longer duration of hospital stay.

Our study demonstrated a 2-fold risk of pneumonia in patients with COPD and patients with dementia, which is in agreement with the findings of other studies. COPD was associated with a higher risk of pneumonia and an increased mortality rate (35, 36). A recent COPD cohort study showed an incidence rate of pneumonia of 22.4 (CI: 21.7–23.2) per 1,000 person years (37). Furthermore, a single center study in Taiwan demonstrated that COPD patients with CCVDs had an increased risk of pneumonia (38). However, dementia was proven to be a risk factor of pneumonia because of prolonged latency of the swallowing reflex (39). A systemic review and meta-analysis published in 2019 reported that the rate of pneumonia-associated mortality was doubled in patients with dementia compared with those without (40). Patients with COPD and dementia require adequate chest care and cautious clinical management for pneumonia prevention and improved prognoses. The non-protection effect of aspirin in COPD patients with pneumonia was consistent with a previous study mentioned, showing that aspirin was associated with higher COPD acute exacerbation and infection events (20). On the other hand, the non-protection effect of aspirin on diabetes mellitus, dementia and cancer population found in our study needs more research to confirm the relevance. The strengths and novelties of our study are as follows. First, a large population cohort with application of PSM provided a balance of selected covariates. Second, pneumonia is associated with higher mortality in CCVDs patients. Our results demonstrate the clinical benefits of aspirin for pneumonia prevention. Finally, aspirin prescriptions are made for patients with CCVDs after careful consideration of two crucial aspects: the reduced risk of death of CCVDs and the increased risk of major bleeding. The results of our study provide supporting evidence of the reduced risk of pneumonia resulting from aspirin use in patients with CCVDs.

This study had some limitations. First, the NHIRD did not contain information on the severity of CCVDs, performance status, or patients' clinical characteristics such as obesity or smoking status. Second, covariates in this study lacked information on the initial treatment of CCVDs, such as medication usage or the necessity of therapeutic or surgical intervention. Third, data on influenza and pneumococcal vaccinations was not included in this study. In Taiwan, the health policy provided annual free influenza and pneumococcal vaccination since 2001 and 2007, respectively, but with age limitation. Annual influenza vaccination was only free for people aged more than 65 years, and pneumococcal vaccination people only for aged more than 75 years, both elder and susceptible group of general population. Finally, a lack of randomization due to the observational nature of this study was an intrinsic limitation. Carefully designed and planned studies using randomized control trials are warranted for precise analysis of the benefits of aspirin.

SUMMARY

In summary, this population-based cohort study with PSM demonstrated that long-term low-dose aspirin use is associated with a decreased risk of pneumonia in patients with CCVDs, whereas Diabetes mellitus, COPD and dementia are associated with greater risk of pneumonia. Further randomized clinical trials are required to support these findings before strategies for increasing prevalence of aspirin use among patients with CCVDs can be developed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

This retrospective population-based cohort study was approved by the National Health Insurance Administration and the Institutional Review Board of Chung Shan Medical University (registration number: CSMUH CS16183). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Y-CC, Y-YC, M-CC, and C-BY conceived and designed the experiments. HY, T-YY, J-YH, and P-LL analyzed the data. L-TY, S-FY, and C-BY contributed reagents, materials, and analysis tools. Y-CC, Y-YC, M-CC, and C-BY wrote the paper. All authors contributed to the article and approved the submitted version.

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Cardio-Ankle Vascular Index Reflects Impaired Exercise Capacity and Predicts Adverse Prognosis in Patients With Heart Failure

Koichiro Watanabe[1], Akiomi Yoshihisa[1,2], Yu Sato[1], Yu Hotsuki[1], Fumiya Anzai[1], Yasuhiro Ichijo[1], Yusuke Kimishima[1], Tetsuro Yokokawa[1], Tomofumi Misaka[1,2], Takamasa Sato[1], Takashi Kaneshiro[1], Masayoshi Oikawa[1], Atsushi Kobayashi[1] and Yasuchika Takeishi[1]*

[1] Department of Cardiovascular Medicine, Fukushima Medical University, Fukushima, Japan, [2] Department of Advanced Cardiac Therapeutics, Fukushima Medical University, Fukushima, Japan

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***Correspondence:**
Akiomi Yoshihisa
yoshihis@fmu.ac.jp

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Aims: We aimed to assess the associations of CAVI with exercise capacity in heart failure (HF) patients. In addition, we further examined their prognosis.

Methods: We collected the clinical data of 223 patients who had been hospitalized for decompensated HF and had undergone both CAVI and cardiopulmonary exercise testing.

Results: For the prediction of an impaired peak oxygen uptake (VO_2) of < 14 mL/kg/min, receiver-operating characteristic curve demonstrated that the cutoff value of CAVI was 8.9. In the multivariate logistic regression analysis for predicting impaired peak VO_2, high CAVI was found to be an independent factor (odds ratio 2.343, $P = 0.045$). We divided these patients based on CAVI: the low-CAVI group (CAVI < 8.9, $n = 145$) and the high-CAVI group (CAVI ≥ 8.9, $n = 78$). Patient characteristics and post-discharge cardiac events were compared between the two groups. The high-CAVI group was older (69.0 vs. 58.0 years old, $P < 0.001$) and had lower body mass index (23.0 vs. 24.1 kg/m^2, $P = 0.013$). During the post-discharge follow-up period of median 1,623 days, 58 cardiac events occurred. The Kaplan–Meier analysis demonstrated that the cardiac event rate was higher in the high-CAVI group than in the low-CAVI group (log–rank $P = 0.004$). The multivariate Cox proportional hazard analysis revealed that high CAVI was an independent predictor of cardiac events (hazard ratio 1.845, $P = 0.035$).

Conclusion: High CAVI is independently associated with impaired exercise capacity and a high cardiac event rate in HF patients.

Keywords: cardio-ankle vascular index, arterial stiffness, cardiopulmonary exercise testing, heart failure, prognosis

INTRODUCTION

Impaired exercise capacity is an independent predictor of poor prognosis in patients with heart failure (HF) (1–3). Cardiopulmonary exercise testing (CPX) is the widely accepted gold-standard approach to assess exercise capacity (4). However, compared with other exercise tests (6-min walk test, electrocardiography stress testing), CPX is more time-consuming, more expensive, and needs specialized equipment and personnel (4). Vascular dysfunction (e.g., arterial stiffness, endothelial dysfunction) in HF may contribute to altered ventricular-arterial coupling (4), and might be associated with impaired exercise capacity (4, 5). The cardio-ankle vascular index (CAVI) is a measure of arterial stiffness, and is useful to evaluate atherosclerosis, and moreover to predict the prognosis in patients who have multiple risk factors of cardiovascular diseases (6–9). High CAVI is an independent predictor of cardiovascular events including cardiovascular death, nonfatal myocardial infarction, or nonfatal ischemic stroke in patients with acute coronary syndrome (7).

However, the clinical implication of CAVI in patients with HF is yet unclear, especially in terms of assessing exercise capacity and prognosis. Therefore, we aimed to assess the associations of CAVI with exercise capacity in HF patients. In addition, we further examined their prognosis.

METHODS

Subjects and Protocol

The patient flow chart is presented in **Figure 1**. This was a prospective observational study of patients who (1) had been both hospitalized at Fukushima Medical University Hospital for decompensated HF and discharged alive between January 2010 and September 2019; and (2) out of the 2,715 HF patients, a total of 497 patients had undergone both CAVI measurement and CPX testing before discharge in a stable condition. Patients with decompensated HF were identified by the current guidelines (1, 2). Patients with obvious history of peripheral artery disease, those with atrial fibrillation and/or those who were receiving maintenance dialysis throughout the study period were excluded ($n = 274$). We excluded patients with concurrent peripheral artery disease and atrial fibrillation because it is difficult to accurately measure CAVI in such patients (patients receiving dialysis $n = 26$ and/or patients with atrial fibrillation $n = 208$ and/or patients with peripheral artery disease $n = 107$) (7). Peripheral artery disease was defined as in previous studies (10, 11). Other co-morbidities were also defined in accordance with our previous studies (10, 11). We defined reduced ejection fraction (EF) as left ventricular EF (LVEF) < 40%, mid-range EF as 40% ≤ LVEF < 50% and preserved EF ≥ 50% (1–3). Finally, a total of 223 patients were enrolled. For the prediction of impaired

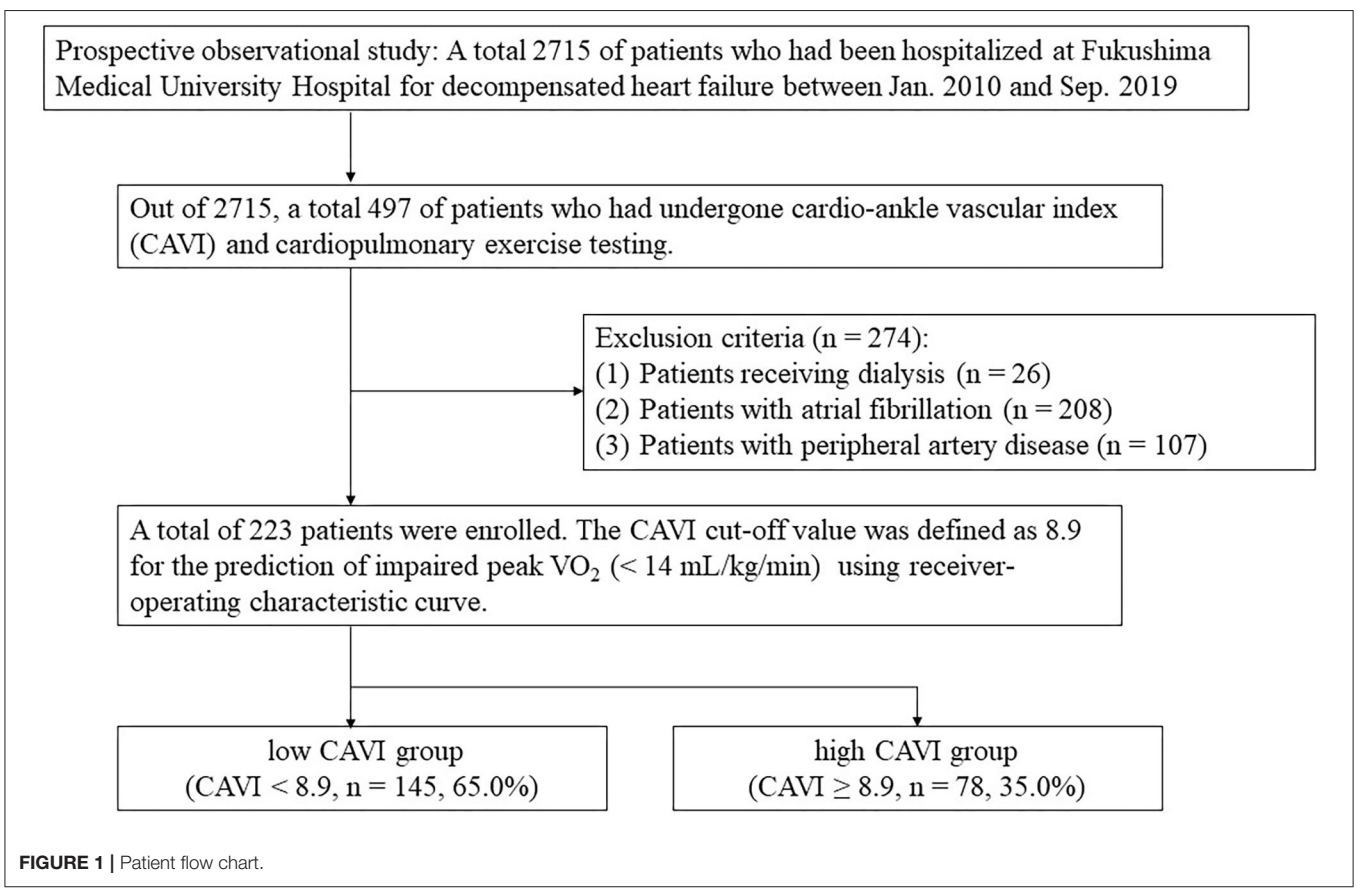

FIGURE 1 | Patient flow chart.

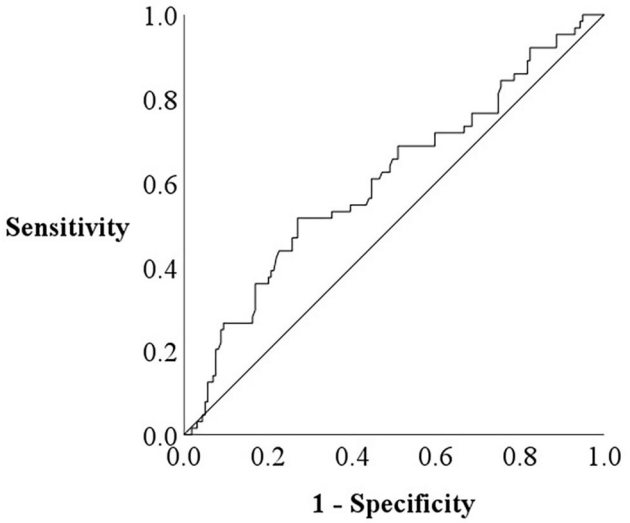

FIGURE 2 | Receiver-operating characteristic curve for the prediction of impaired peak VO_2 by CAVI.

peak VO_2, defined as < 14 mL/kg/min (12), receiver-operating characteristic (ROC) curve demonstrated that the cut-off value of CAVI was 8.9 (**Figure 2**, area under curve 0.67, 95% confidence interval, 0.52–0.69, $P < 0.05$). Next, these patients were divided into two groups based on this cut-off value: the low-CAVI group (CAVI < 8.9, $n = 145$, 65.0%) and the high-CAVI group (CAVI $\geq$ 8.9, $n = 78$, 35.0%). Patient characteristics and post-discharge prognosis were compared between the two groups. The patient characteristics included demographic data at discharge, as well as laboratory data and echocardiographic data, which were obtained within one week prior to discharge when the patient was in a stable condition. We compared post-discharge cardiac events, ischemic events and all-cause mortality.

These patients were followed up until March 2020 for cardiac events as composites of cardiac death or unplanned re-hospitalization for HF treatment, ischemic coronary events and all-cause mortality. For patients that experienced two or more events, only the first event was included in the analysis. Since these patients visited patient's referring hospital monthly or bi-monthly, we were able to follow up on all patients. Status and dates of death were obtained from the patient's medical records. This study conformed to the Declaration of Helsinki (13) and the statement of STROBE (Strengthening the Reporting of Observational studies in Epidemiology) (14). The ethical committee of Fukushima Medical University approved the study protocol. Written informed consent was obtained from all patients.

The Measurement of CAVI

We measured CAVI automatically by using VaSera VS-1000 (Fukuda Denshi Co., Ltd., Tokyo, Japan) with the patient in the decubitus position before discharge in a stable condition. We attached cuffs bilaterally to the upper arms and ankles of the patient. We placed electrocardiogram electrodes and a microphone on both wrists and on the sternum, respectively. We analyzed the average CAVI values of both sides (6–9).

Cardiopulmonary Exercise Testing

Patients underwent incremental symptom-limited exercise testing before discharge in a stable condition, using an upright cycle ergometer with a ramp protocol (Strength Ergo 8, Fukuda Denshi Co., Ltd., Tokyo, Japan). Breath-by-breath VO_2 was measured during exercise using an Aeromonitor AE-300S (Minato Medical Science Co., Ltd., Osaka, Japan). Breath-by-breath oxygen consumption (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE) were measured during exercise using an AE-300S respiratory monitor (Minato Medical Science, Co., Ltd.) (15). Peak VO_2 was measured as an average of the last 30 s of exercise, and ventilatory response to exercise (slope of the relationship between ventilation and carbon dioxide production, VE/VCO_2 slope) was calculated as the regression slope relating VE to CO_2 from the start of exercise until the respiratory compensation point (the time at which ventilation is stimulated by CO_2 output and end-tidal CO_2 tension begins to decrease) (15). We calculated the ventilatory anaerobic threshold using the V-slope method.

Statistical Analysis

Normality was confirmed using the Shapiro-Wilk test in each group. Normally distributed variables are presented as mean ± standard deviation, non-normally distributed variables are presented as median (interquartile range), and categorical variables are expressed as counts and percentages. ROC curves for predicting impaired peak VO_2 were plotted using EZR version 1.40 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (16). Non-normally distributed variables were compared using the Mann-Whitney U test, and the Fisher's exact test was used for comparisons of categorical variables. If 20% or more cells had expected count less than five, the one-sided Fisher's exact test was adopted. Logistic regression analysis was performed to assess associations between impaired exercise capacity and CAVI, as well as other variables (e.g., age, sex, blood pressure, heart rate, hypertension, diabetes mellitus, dyslipidemia, coronary artery disease, cerebral vascular disease, chronic kidney disease, anemia, BNP, and LVEF), which are generally thought to be associated with exercise capacity. The occurrence of post-discharge cardiac events, ischemic events and all-cause mortality was compared using the Kaplan-Meier analysis with a log-rank test. We assessed CAVI as a predictor for cardiac events, ischemic coronary events and all-cause mortality using the univariate or multivariate Cox proportional hazard analysis. The threshold for statistical significance was $P < 0.05$. All analyses, except for ROC, were conducted using IBM SPSS Statistics version 26 (IBM, Armonk, NY, USA).

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

TABLE 1 | Baseline patient characteristics.

	Low CAVI (CAVI < 8.9, *n* = 145)	High CAVI (CAVI ≥ 8.9, *n* = 78)	*P* value
CAVI	7.31 (6.50–8.00)	9.62 (9.36–10.14)	<0.001
Demographic data			
Age (years old)	58.0 (46.0–65.0)	69.0 (61.0–74.0)	<0.001
Male sex (*n*, %)	112 (77.2)	66 (84.6)	0.191
Body mass index (kg/m^2)	24.1 (22.2–28.1)	23.0 (21.4–26.3)	0.013
Systolic blood pressure (mmHg)	122.5 (108.0–143.0)	130.0 (115.0–151.5)	0.094
Diastolic blood pressure (mmHg)	71.5 (61.0–86.0)	77.0 (62.0–91.0)	0.436
Heart rate (/min)	78.0 (65.0–96.0)	73.0 (62.0–89.5)	0.140
NYHA functional class III or IV(*n*, %)	1 (0.7)	1 (1.3)	0.578
Ischemic etiology (*n*, %)	41 (32.5)	29 (44.6)	0.070
Reduced/mid-range/preserved EF (*n*, %)	48 (38.4)/24 (19.2)/53 (42.4)	19 (29.7)/14 (21.9)/31 (48.8)	0.496
Co-morbidities			
Hypertension (*n*, %)	99 (68.3)	64 (82.1)	0.019
Diabetes mellitus (*n*, %)	60 (41.4)	41 (52.6)	0.072
Dyslipidemia (*n*, %)	116 (80.0)	68 (87.2)	0.122
Coronary artery disease (*n*, %)	51 (35.2)	35 (44.9)	0.101
Cerebral vascular disease (*n*, %)	17 (11.7)	9 (11.5)	0.577
Chronic kidney disease (*n*, %)	48 (34.3)	37 (54.4)	0.005
Anemia (*n*, %)	31 (22.5)	23 (33.3)	0.067
Medications			
β blockers (*n*, %)	126 (86.9)	68 (87.2)	0.565
ACEIs/ARBs (*n*, %)	117 (80.7)	67 (85.9)	0.216
Loop diuretics (*n*, %)	84 (57.9)	49 (62.8)	0.286
Inotropic agents (*n*, %)	16 (11.0)	12 (15.4)	0.233
Calcium blockers (*n*, %)	40 (27.6)	28 (35.9)	0.129
Antiplatelet agents (*n*, %)	71 (49.0)	31 (60.3)	0.070
Anticoagulants (*n*, %)	68 (46.9)	35 (44.9)	0.441
Laboratory data			
BNP (pg/mL)	151.6 (43.8–509.4)	207.4 (72.5–431.3)	0.326
Echocardiographic data			
LVEF (%)	45.0 (31.9–60.4)	46.0 (36.9–63.0)	0.250
Cardiopulmonary exercise testing			
Peak VO_2 (mL/kg/min)	17.3 (14.4–21.1)	14.6 (12.8–17.7)	<0.001
VE-VCO_2 slope	30.8 (26.7–34.0)	32.7 (29.1–39.8)	<0.001

CAVI, cardio-ankle vascular index; NYHA, New York Heart Association; EF, ejection fraction; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction; VO_2, oxygen uptake; VE-VCO_2, ventilatory equivalent vs. carbon dioxide output.

RESULTS

Comparisons of patient characteristics between the low- and high-CAVI groups are shown in **Table 1**. A total of 78 (35.0%) patients belonged to the high-CAVI group. The high-CAVI group was older and showed lower body mass index. Prevalence of hypertension and chronic kidney disease was significantly higher in the high-CAVI group than in the low-CAVI group. In contrast, sex, blood pressure, heart rate, NYHA functional class, other co-morbidities, and medications did not differ between the two groups. There were no significant differences regarding BNP levels and LVEF between the two groups.

ROC analysis demonstrated that a CAVI cut-off value of 8.9 predicted impaired exercise capacity (**Figure 2**; area under the curve 0.67, 95% confidence interval, 0.52–0.69, $P < 0.05$). In the multivariate logistic regression analysis for predicting impaired peak VO_2 (**Table 2**), high CAVI was found to be an independent factor (odds ratio 2.343, 95% confidence interval 1.021–5.380, $P = 0.045$).

During the post-discharge follow-up period (median 1,623 days), 58 cardiac events including 53 worsening HF and 5 cardiac deaths, 11 ischemic coronary events and 39 all-cause deaths occurred. The Kaplan-Meier analysis showed that cardiac event rates and all-cause mortality were higher in the high-CAVI group than in the low-CAVI group (**Figure 3**; cardiac event rates, log-rank $P = 0.004$; **Figure 4**; all-cause mortality, log-rank $P = 0.015$), however ischemic coronary events did not differ between the high-CAVI and the low-CAVI

TABLE 2 | Logistic regression analysis for predicting impaired peak VO_2.

	Univariate		Multivariate	
	OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**
High CAVI (≥ 8.9)	2.697 (1.481–4.911)	0.001	2.343 (1.021–5.380)	0.045
Age	1.037 (1.012–1.063)	0.003	1.009 (0.977–1.043)	0.584
Male sex	0.224 (0.113–0.446)	<0.001	0.120 (0.049–0.292)	<0.001
Body mass index	1.004 (0.942–1.071)	0.899		
Systolic BP	1.001 (0.991–1.011)	0.864		
Diastolic BP	1.005 (0.992–1.018)	0.469		
Heart rate	1.009 (0.995–1.023)	0.210		
Hypertension	2.477 (1.166–5.261)	0.018	2.245 (0.850–5.926)	0.103
Diabetes mellitus	1.194 (0.668–2.137)	0.550		
Dyslipidemia	1.030 (0.478–2.217)	0.940		
Coronary artery disease	1.623 (0.901–2.925)	0.107		
Cerebral vascular disease	0.719 (0.275–1.882)	0.502		
Chronic kidney disease	3.043 (1.627–5.692)	<0.001	2.137 (0.973–4.694)	0.059
Anemia	2.697 (1.400–5.198)	0.003	1.795 (0.797–4.046)	0.158
log BNP	1.993 (1.162–3.419)	0.012	1.913 (0.989–3.700)	0.054
LVEF	0.999 (0.979–1.020)	0.953		

OR, odds ratio; CI, confidence interval; CAVI, cardio-ankle vascular index; BP, blood pressure; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction.

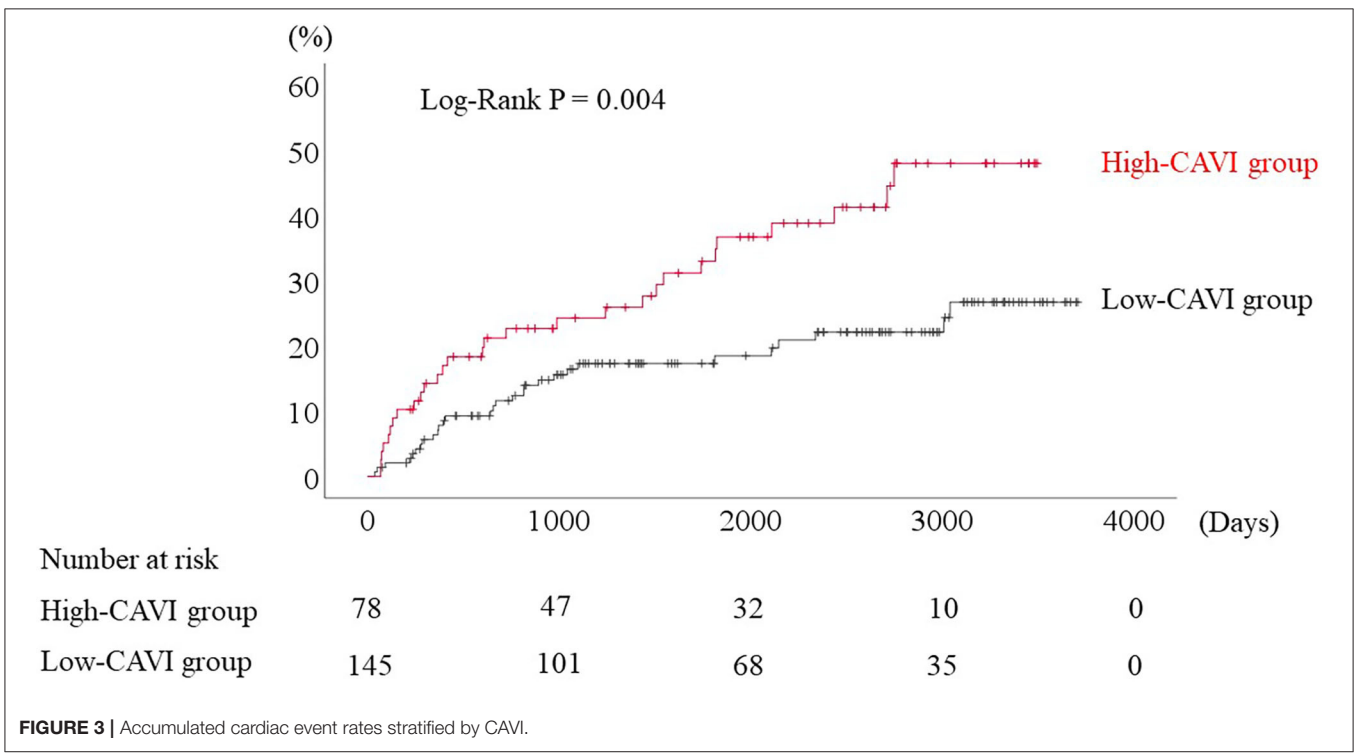

FIGURE 3 | Accumulated cardiac event rates stratified by CAVI.

group (**Figure 5**; log-rank $P = 0.822$). In the multivariate Cox proportional hazard analysis (**Table 3**), we considered possible confounding factors, which differed between the groups (i.e., age, sex, body mass index, hypertension, and chronic kidney disease), and high CAVI was found to be an independent predictor of cardiac events (hazard ratio 1.845, 95% confidence interval 1.044–3.261, $P = 0.035$). In contrast, high CAVI did not fully predict ischemic coronary events and all-cause mortality in the multivariate Cox proportional hazard analysis (**Tables 4**, **5**). Furthermore, in the subgroup analysis for predicting cardiac events (**Table 6**), there was no significant interactions between prognostic impact of CAVI and both sex ($P = 0.704$), age ($P = 0.291$), and LVEF ($P = 0.279$).

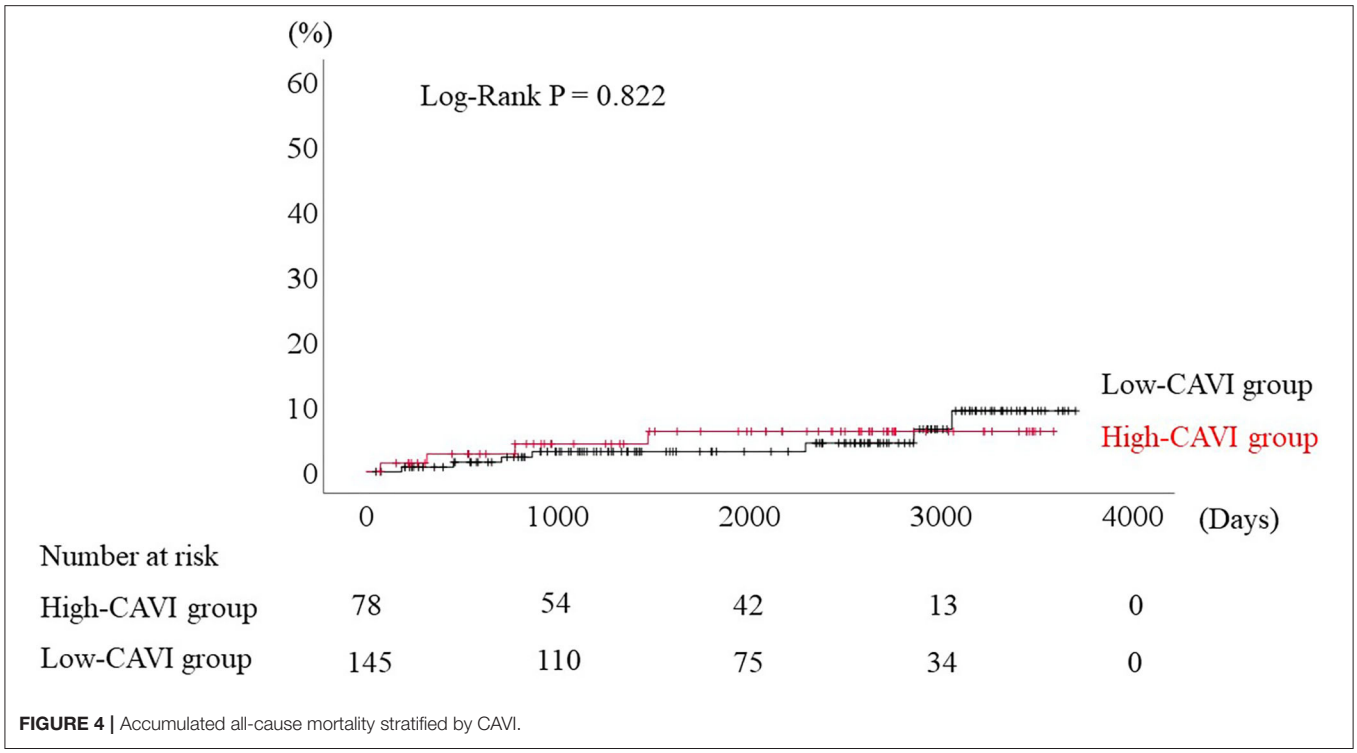

FIGURE 4 | Accumulated all-cause mortality stratified by CAVI.

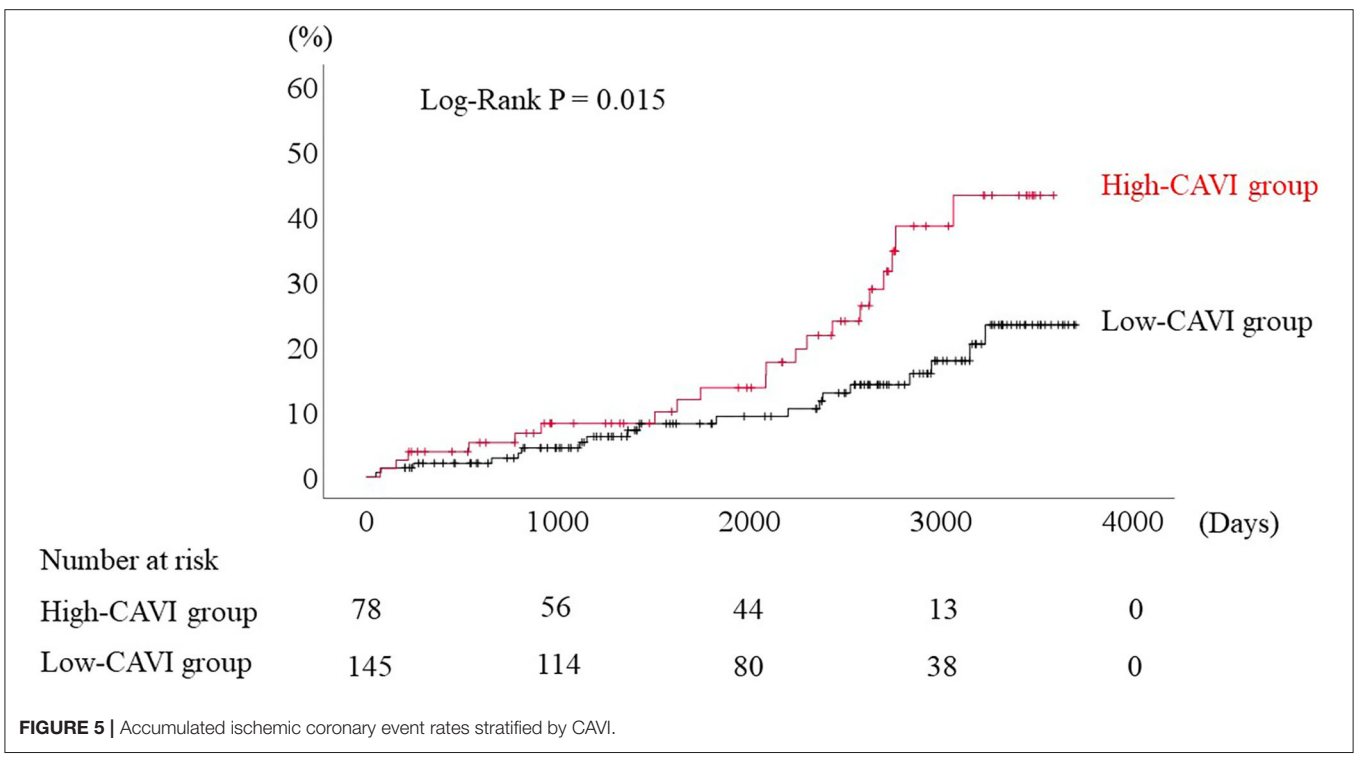

FIGURE 5 | Accumulated ischemic coronary event rates stratified by CAVI.

DISCUSSION

The present study, to the best of our knowledge, was the first to report that (A) high CAVI (≥ 8.9) was independently associated with impaired peak VO_2 (< 14 mL/kg/min), and (B) high CAVI independently predicted the cardiac events in patients with HF.

There was weak association between CAVI and exercise capacity in the present study. Concordant with our data, it

TABLE 3 | Cox proportional hazard model for cardiac events.

	Univariate		Multivariate	
	HR (95% CI)	***P* value**	**HR (95% CI)**	***P* value**
High CAVI (≥ 8.9)	2.090 (1.248–3.500)	0.005	2.090 (1.248–3.500)	0.005
Age (older vs. younger)	1.520 (0.894–2.585)	0.122		
Male sex	0.771 (0.423–1.408)	0.397		
Body mass index	0.994 (0.933–1.059)	0.858		
Hypertension	1.195 (0.644–2.217)	0.572		
Chronic kidney disease	1.600 (0.933–2.744)	0.088		

HR, hazard ratio; CI, confidence interval; CAVI, cardio-ankle vascular index.

TABLE 4 | Cox proportional hazard model for ischemic coronary events.

	Univariate		Multivariate	
	HR (95% CI)	***P* value**	**HR (95% CI)**	***P* value**
High CAVI (≥ 8.9)	1.152 (0.336–3.945)	0.822		
Age	1.061 (1.000–1.126)	0.049		
Male sex	0.600 (0.159–2.266)	0.451		
Body mass index	1.065 (0.948–1.196)	0.290		
Hypertension	31.818 (0.095–10611.11)	0.243		
Chronic kidney disease	1.134 (0.318–4.046)	0.846		

HR, hazard ratio; CI, confidence interval; CAVI, cardio-ankle vascular index.

TABLE 5 | Cox proportional hazard model for all-cause mortality.

	Univariate		Multivariate	
	HR (95% CI)	***P* value**	**HR (95% CI)**	***P* value**
High CAVI (≥ 8.9)	2.145 (1.142–4.027)	0.018	1.802 (0.912–3.561)	0.090
Age	1.027 (1.000–1.055)	0.051	1.018 (0.989–1.048)	0.227
Male sex	1.140 (0.503–2.583)	0.754		
Body mass index	0.921 (0.840–1.010)	0.081		
Hypertension	1.377 (0.607–3.125)	0.445		
Chronic kidney disease	1.612 (0.843–3.081)	0.149		

HR, hazard ratio; CI, confidence interval; CAVI, cardio-ankle vascular index.

TABLE 6 | Cox proportional hazard model for cardiac events: the impact of high CAVI (Sub-group analysis).

Factor	**Subgroup**	***n***	**HR (95% CI)**	***P* value**	**Interaction *P* value**
Total			2.090 (1.248–3.500)	0.005	
Sex	Male	178	2.057 (1.138–3.719)	0.017	0.704
	Female	45	2.491 (0.854–7.268)	0.095	
Age	Older (≥ median 61 years)	116	1.802 (0.905–3.586)	0.094	0.291
	Younger (< median 60 years)	107	2.908 (1.133–7.466)	0.026	
LVEF	Reduced and mid-range EF	105	1.934 (1.036–3.608)	0.038	0.279
	Preserved EF	84	3.572 (1.196–10.672)	0.023	

HR, hazard ratio; CI, confidence interval; CAVI, cardio-ankle vascular index; LVEF, left ventricular ejection fraction; EF, ejection fraction.

has recently been reported that CAVI was associated with 6-min walk test, and indicated that arterial stiffness may relate to partly exercise capacity (17). Regarding arterial stiffness and impaired exercise capacity in HF patients, abnormal ventricular-arterial coupling may be caused by vascular dysfunction in HF (4). Because of arterial stiffness and an impaired peripheral vasodilatory response to exercise, the timing and amplitude of the reflected pulse wave are changed, and as a result the pulsatile load arriving at the heart during late systole increases (4, 18). After that, the myocardial workload during exercise increases and contributes to functional exercise intolerance (4). Arterial stiffening and abnormal vasorelaxation during exercise elevate filling pressure and impair cardiac output reserve in HF patients, and contribute to exercise intolerance (4, 5, 19). Vascular dysfunction also decreases the O_2 delivery to the skeletal muscle at the start of exercise, and the skeletal muscle uses anaerobic energy (4, 20). The decrease of the finite energy sources needed to maintain exercise at latter exercise stages contributes to exercise intolerance (4, 20). In addition, CAVI was reportedly to be an independent risk factor for frailty (21), which is associated with adverse outcome in HF patients (22, 23). Arterial hemodynamic dysfunction may have a predictive effect on reduction in muscle mass, and the reduction results in a decrease in body mass, grip strength, and walking speed (21). Muscle blood flow decreases were partly related to the degree of atherosclerosis (24). Therefore, atherosclerosis and arterial stiffness were risk factors for frailty (21, 25).

Especially, in patients with HFpEF, arterial stiffness is increased and is correlated with decreased exercise capacity (5, 26–29). Arterial stiffening and impaired arterial vasodilator reserve with exercise are important in the pathophysiology of HFpEF that is independent of hypertension and mean blood pressure alone (5). A reduction in pulsatile arterial afterload improves functional capacity measured by the 6-min walk test (4, 30). The impairment oxygen delivery and extraction in tissue is considered an important determinant of exercise tolerance (4, 31, 32).

In the present study, CAVI was an independent predictor of impaired peak VO_2, after adjustment for important factors including age, blood pressure and LVEF. Thus, CAVI may be a useful marker for impaired exercise capacity, especially in HF patients who have difficulty undergoing CPX testing and other exercise tests.

There are stronger relationships between arterial stiffness and HF, because decreases in arterial wall compliance increase cardiac afterload and exacerbate HF (8). Meguro et al. (33) reported that the high brachial-ankle pulse wave velocity (BaPWV) group had a lower event-free survival rate than the low BaPWV group, so elevated arterial stiffness is a risk factor for rehospitalization or cardiac death of HF patients. On the other hand, PWV has a weak point; it is known to depend on blood pressure at the time of measurement, whereas CAVI is independent of blood pressure (6). Consistent with our results that the cut-off value of CAVI was 8.9, a recent review of vascular function has suggested that CAVI $\geq$ 9.0 is a marker of vascular failure (9). Additionally, it has been reported that CAVI $\geq$ 9.0 predicted higher cardiovascular events in diabetic patients (34). On the other hand, the associations between changes of CAVI and prognosis have not yet been examined (35). A prospective, large-scale, and longitudinal study with repeated measurement of CAVI in high cardiovascular risk patients, the Coupling registry, has been under way (35). The study may provide useful information on the significance of both baseline CAVI and changes in CAVI over time as indicators of cardiovascular prognosis (35).

Our study has several strengths. For example, to the best of our knowledge, the present study is the first to show associations between increased CAVI and impaired exercise as well as adverse prognosis in HF patients, taking into consideration a multifaceted background and exercise capacity within a given population. Second, we were able to follow up on all patients.

The present study has several limitations. First, since CAVI measurement is inappropriate for patients with concurrent peripheral artery disease and atrial fibrillation, which are sometimes complicated with HF, CAVI is not necessarily indicated for all HF patients. Second, the results of the current study may not represent the general population, as this was a prospective cohort study of a single center with a relatively small number of patients. We considered several confounding factors and performed multivariate Cox proportional hazard analysis, but we cannot exclude all residual confounding factors, and we might not completely adjust for the effects of the differences in the backgrounds between the groups. Third, in the present study we considered the variables during hospitalization for decompensated HF, but we did not analyze the changes in medical parameters (e.g., CAVI) throughout the clinical course and post-discharge treatment. Fourth, although we encouraged CAVI and CPX in hospitalized patients, attending physicians could not perform these measurements in all patients for various reasons (e.g., patient refusal, medical reasons, timing of hospital discharge). Thus, potential selection bias in these measurements possibly existed. Fifth, the present study was a cross-sectional and prospective observational study, therefore we could not fully explain the causal relationships and mechanisms of increased CAVI on impaired exercise capacity and worse prognosis. Therefore, the present results should be considered preliminary, and further studies analyzing larger population are required.

CONCLUSION

High CAVI is independently associated with impaired exercise capacity, and leads to a high cardiac event rate in HF patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The ethical committee of Fukushima Medical

University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KW and YS: conceptualization, methodology, formal analysis, investigation, writing–original draft, and visualization. AY: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing–original draft, visualization, supervision, project administration, and funding acquisition. YH, FA, YI, YK, TY, TM, TK, MO, and AK: conceptualization, methodology, formal analysis, investigation, and, writing–review and editing. YT: conceptualization, methodology, formal analysis, investigation, writing–original draft, supervision, and project administration. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.631807/full#supplementary-material

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UPLC-MS-Based Serum Metabolomics Reveals Potential Biomarkers of Ang II-Induced Hypertension in Mice

Shaying Yang†, Zhiwei Wang†, Mengting Guo, Mengfan Du, Xin Wen, Li Geng, Fan Yu, Liangliang Liu, Yanting Li, Lei Feng and Tingting Zhou**

Laboratory of Cardiovascular Research, School of Medicine, Jiangnan University, Wuxi, China

Hypertension is caused by polygenic inheritance and the interaction of various environmental factors. Abnormal function of the renin-angiotensin-aldosterone system (RAAS) is closely associated with changes in blood pressure. As an essential factor in the RAAS, angiotensin II (Ang II) contributes to vasoconstriction and inflammatory responses. However, the effects of overproduction of Ang II on the whole body-metabolism have been unclear. In this study, we established a hypertensive mouse model by micro-osmotic pump perfusion of Ang II, and the maximum systolic blood pressure reached 140 mmHg after 2 weeks. By ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry, the metabolites in the serum of hypertensive model and control mice were analyzed. Partial least squares discriminant analysis (PLS-DA) in both positive and negative ionization modes showed clear separation of the two groups. Perfusion of Ang II induced perturbations of multiple metabolic pathways in mice, such as steroid hormone biosynthesis and galactose metabolism. Tandem mass spectrometry revealed 40 metabolite markers with potential diagnostic value for hypertension. Our data indicate that non-targeted metabolomics can reveal biochemical pathways associated with Ang II-induced hypertension. Although researches about the clinical use of these metabolites as potential biomarkers in hypertension is still needed, the current study improves the understanding of systemic metabolic response to sustained release of Ang II in hypertensive mice, providing a new panel of biomarkers that may be used to predict blood pressure fluctuations in the early stages of hypertension.

Keywords: hypertension, LC-MS, angiotensin II, metabolomics, serum metabolites, biomarkers, mice

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Junjie Xiao,
Shanghai University, China

Reviewed by:
Dalei Wu,
Shandong University, China
Liang Zhang,
Shanghai Jiao Tong University, China

****Correspondence:***
Lei Feng
feng2008lei@163.com
Tingting Zhou
tingtingchou@126.com

† These authors have contributed equally to this work and share first authorship

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INTRODUCTION

Hypertension is a common cardiovascular disease and the leading risk factor for both cardiovascular and cerebrovascular events. It can cause functional or organic lesions of the heart, brain, blood vessels, kidneys, and other organs, contributing to a significant cause of disability and death (1). The incidence and development of hypertension are affected by both genetic and environmental factors (2). In recent studies, 60% of the main factors leading to hypertension have been associated with metabolic abnormalities, while 80% of hypertensive patients have various forms of metabolic disorder (3).

Ang II increases systemic blood pressure and glomerular capillary pressure. It is directly involved in renal arteriosclerosis and causes kidney damage (4). It also increases the pressure in the glomeruli and contracts mesangial cells, leading to an increase in the selective permeability to urine proteins. Clinical and experimental studies have shown that it regulates the processes of inflammation and fibrosis contributing to kidney pathogenesis through activating growth factors associated with fibrosis (5). The clinical manifestations of hypertension and kidney damage are persistent hypertension accompanied by persistent trace or mild-to-moderate proteinuria, and impaired renal function (i.e., increased creatinine and urea nitrogen) (6). Ang II-induced hypertension leads to the hypertrophy of smooth muscle cells and to increases in the expression of their specific markers, eventually leading to thickening of the arterial media and increasing vascular resistance (7).

A study revealed that Ang II induction increases thromboxane production in mice (8), and another indicated that prolonged Ang II-induced hypertension and massive blood-brain barrier leakage, microglia activation, myelin loss, and memory dysfunction are associated with stroke compared with control mice (9).

High-throughput full-spectrum analysis of metabolites provides an opportunity to assess disease severity, restored metabolic pathways, and homeostasis (10, 11). Identifying the disturbed biochemical pathways helps to understand changes in body components during the development of hypertension. Therefore, we aimed to find the endogenous molecular metabolites regulating the blood vessels of mice during the induction by Ang II by analyzing the metabolic spectrum that can be used as a marker for early blood pressure fluctuations.

Metabolomics based on liquid chromatography-mass spectrometry (LC-MS) is an effective method for the metabolic profiling of biological systems (12). LC-MS analysis has higher sensitivity and a more comprehensive polarity range than NMR spectroscopy (13, 14). In the current study, the UPLC-Q-TOF/MS (ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry) platform was used to analyze serum samples from control and Ang II-induced hypertensive mice to explore the differential metabolites of hypertension induced by slow-release Ang II. We identified >40 different metabolites involved in >20 metabolic pathways in the Ang II mice.

METHODS AND MATERIALS

Animals and Sample Collection

All animal experiments were performed in accordance with the laboratory animal guidelines and with the approval of the Animal Experimentation Ethics Committee, Jiangnan University (License No: JN. No 20190930c1000120[232]). The male C57BL/6J mice used in the experiments were provided by the Institute of Model Animal Research of Nanjing University, and were reared in a specific pathogen-free environment. The average body weight was ∼23 g. The mice were divided into two groups (Ang II and control groups) of 10 mice each. Mice were infused subcutaneously with Ang-II (Sigma, 600 ng/kg/min), or vehicle (0.9% saline) for 19 days using osmotic pumps (Alzet) (15). Venous blood was collected from the retro-orbital venous plexus using a blood collection capillary.

Measurement of Blood Pressure

We used a non-invasive blood pressure meter (IITC Life Science) to measure the blood pressure of the mice.

Sample Preparation

Two hundred microliters of each sample was placed in a new Eppendorf tube, to which was added 800 μL of methanol/water (v/v = 8:1) pre-cooled at −20°C for >0.5 h; then iron beads were added and the samples were lysed at 60 Hz for 5 min. After each sample is mixed in equal volume, it is used as a quality control (QC) sample.

Metabolomic Analysis Based on LC-MS/MS

An ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm, Waters Corp., UK) was used for chromatographic separation. The column temperature was 50°C and the flow rate was 0.4 ml/min, where mobile phase A was water and 0.1% formic acid, and mobile phase B was methanol and 0.1% formic acid. The sample was eluted with the following gradient: 0–2 min, 100% A; 2–11 min, 0–100% B; 11–13 min, 100% B; 13–15 min 0–100% A. For the small molecules eluted from the column, the high-resolution tandem mass spectrometry using Xevo G2-XS QTOF (Waters, UK) was used to collect positive and negative ions. In electrospray ionization positive ion mode (ESI+), the capillary voltage was 3 kV and the cone voltage 40 V; in ESI negative ion mode (ESI–), the capillary and cone voltages were 1 kV and 40 V. The mass spectrometry data were acquired in Centroid MSE mode. The TOF mass range was from 50 to 1,200 Da and the scan time was 0.2 s. For the MS/MS detection, all precursors were fragmented using 20–40 eV, and the scan time was 0.2 s. During the acquisition, the LE signal was acquired every 3 s to calibrate the mass accuracy. Meanwhile, the quality control (QC) samples were collected every 10 samples to evaluate the stability of the instrument during the sample collection process.

Data Processing, Mass Spectrometric Identification, and Statistical Analysis

Peak extraction was mainly achieved using the software Progenesis QI (version 2.2), including peak alignment, peak extraction, normalization, deconvolution, and compound identification. See the previous report for details (16). The results show the mean ± SEM. Comparisons among groups were made using ANOVA or unpaired Student's *t*-test, with $P < 0.05$ as the threshold for a significant difference.

Abbreviations: RAAS, renin-angiotensin-aldosterone system; Ang II, angiotensin II; UPLC-Q-TOF/MS, ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; QC, quality control; ESI+, electrospray ionization positive ion mode; ESI–, electrospray ionization negative ion mode; RSD, relative standard deviation; PCA, principal component analysis; PLS-DA, partial least squares-discriminant analysis; ABC, ATP binding cassette membrane transporter; PPAR, peroxisome proliferators-activated receptor.

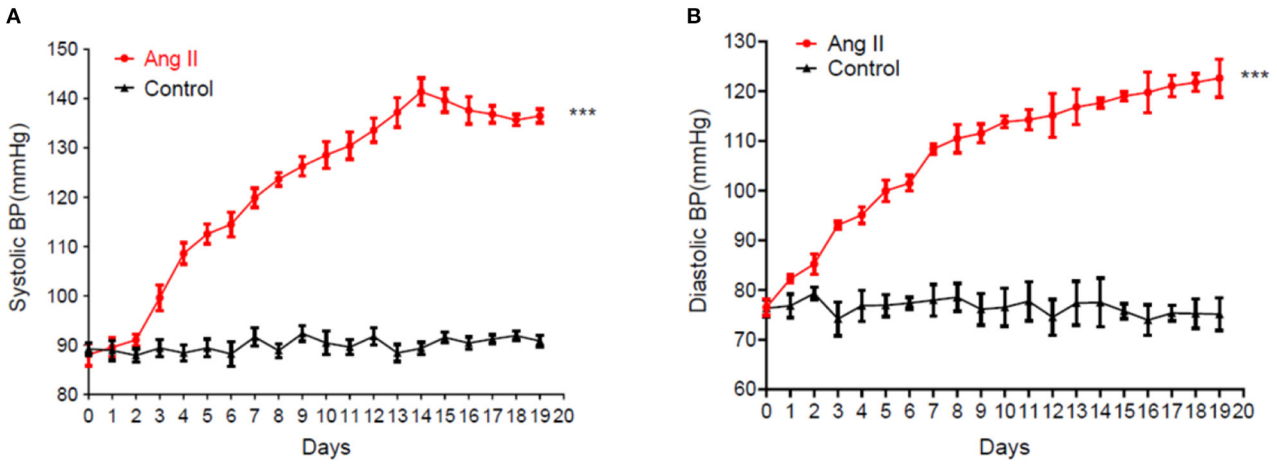

FIGURE 1 | Sustained rise of systolic blood pressure **(A)** and diastolic blood pressure **(B)** in mice induced by the continuous release of Ang II (data are the mean ± SEM, $n = 10$, $^{***}P < 0.001$ vs. Control, two-way ANOVA).

Metabolite Annotation and Pathway Analysis

Metabolites were identified by matching the exact molecular mass data (m/z) of the samples against METLIN (http://metlin.scripps.edu/) and the Human Metabolome Database (http://www.hmdb.ca/) with 10-ppm accuracy (17, 18). We quantitatively mapped the different metabolites to the reference paths in the online Kyoto Encyclopedia of Genes and Genomes database (https://www.kegg.jp/kegg/pathway.html). Statistically significant enrichment pathways were evaluated by the hypergeometric test adjusted by the false discovery rate ($P < 0.05$).

RESULTS

Mouse Model of Ang II-Induced Hypertension

Blood pressure recorded in the Ang II model significantly and continuously rose from day 5 (**Figure 1**). On day 17, the systolic blood pressure in the Ang II group was 140 mmHg while the controls remained at ∼89 mmHg. Therefore, this model of hypertension induced by slow-release Ang II was successful.

Metabolites Differ Between Control and Ang II-Induced Hypertensive Mice

None of the QC sample chromatograms showed significant retention time drift. We measured 5,904 ions in ESI+ mode and 6,937 in ESI– mode (**Figures 2A,B**). After elimination and filling in, 4,557 and 5,773 ions were finally obtained. The QC samples were tightly clustered, and were significantly separate from the test samples, indicating that the LC-MS/MS analysis platform had high stability and reproducibility (16) (**Figures 2C,D**). We further used three-dimensional principal component analysis (PCA) scatter plots to evaluate changes in the metabolite profile of mice during the development of Ang II-induced hypertension. The ordinary "unsupervised" analysis was unable to distinguish between the Ang II and control groups (**Figures 2E,F**). However, the use of partial least-squares discriminant analysis built an excellent regression model. The three-dimensional scatter diagram showed that the Ang II and control groups were significantly separated (**Figures 3A,B**), showing different metabolic phenotypes. This indicated that the sustained release of Ang II leads to metabolic disorders in mice. We selected the top 20 serum metabolites that met the variable importance in the projection threshold (VIP > 1) and Student's *t*-test ($P < 0.05$) criteria in the ESI+ and ESI– modes. Volcano maps based on *P*-values and one-dimensional test multiple changes (**Figures 3C,D**) and heat maps based on differences in metabolite abundance also showed clear separation of the Ang II and control groups (**Figures 3E,F**).

In ESI+ mode, contents of 4-Hydroxy-6-methylpretetramide, 6-Phospho-D-gluconate and Aminopentol considerably increased, and Ephedroxane and Bellendine obviously decreased in Ang II-induced hypertensive mice (**Table 1**). Likewise, variance in the production of N(omega)-Nitro-L-arginine, Deoxypeganine and Hinokitiol glucoside was also observed between Ang II and control groups in ESI- mode (**Table 2**).

Metabolic Pathway Disorders

The serum metabolites of the mice made hypertensive by Ang II were significantly different from the control group. Compared with control mice, 581 differential metabolites were obtained under ESI+ mode and 530 under ESI– mode (**Supplementary Materials**). Search of a mass-based metabolomics database showed that metabolite ions detected under the ESI+ and ESI– modes included disaccharides, glycerophospholipids, amino-acids, sphingolipids, fatty acyl groups, acylcarnitines, and other organic compounds. Here, the

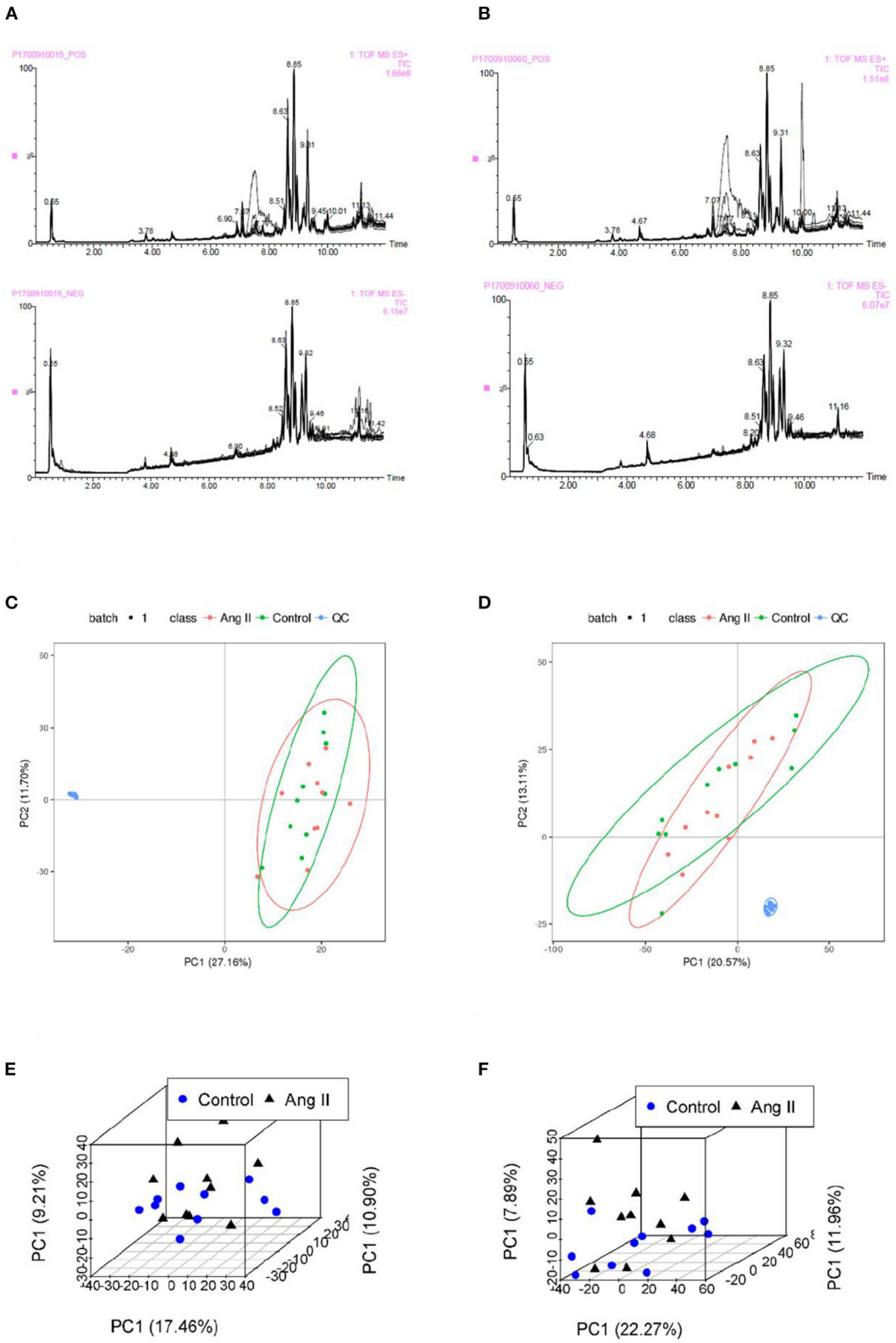

FIGURE 2 | (A,B) Total ion chromatograms of QC samples in Control **(A)** and Ang II groups **(B)** in ESI+ and ESI– mode. **(C,D)** Plots of PCA scores for serum samples from test mice and QC samples showing metabolites obtained in ESI+ mode **(C)** and ESI– mode **(D)**. **(E,F)** Scatter plots of PCA scores of metabolites from the LC-MS/MS fingerprints in ESI+ mode **(E)** and ESI– mode **(F)**.

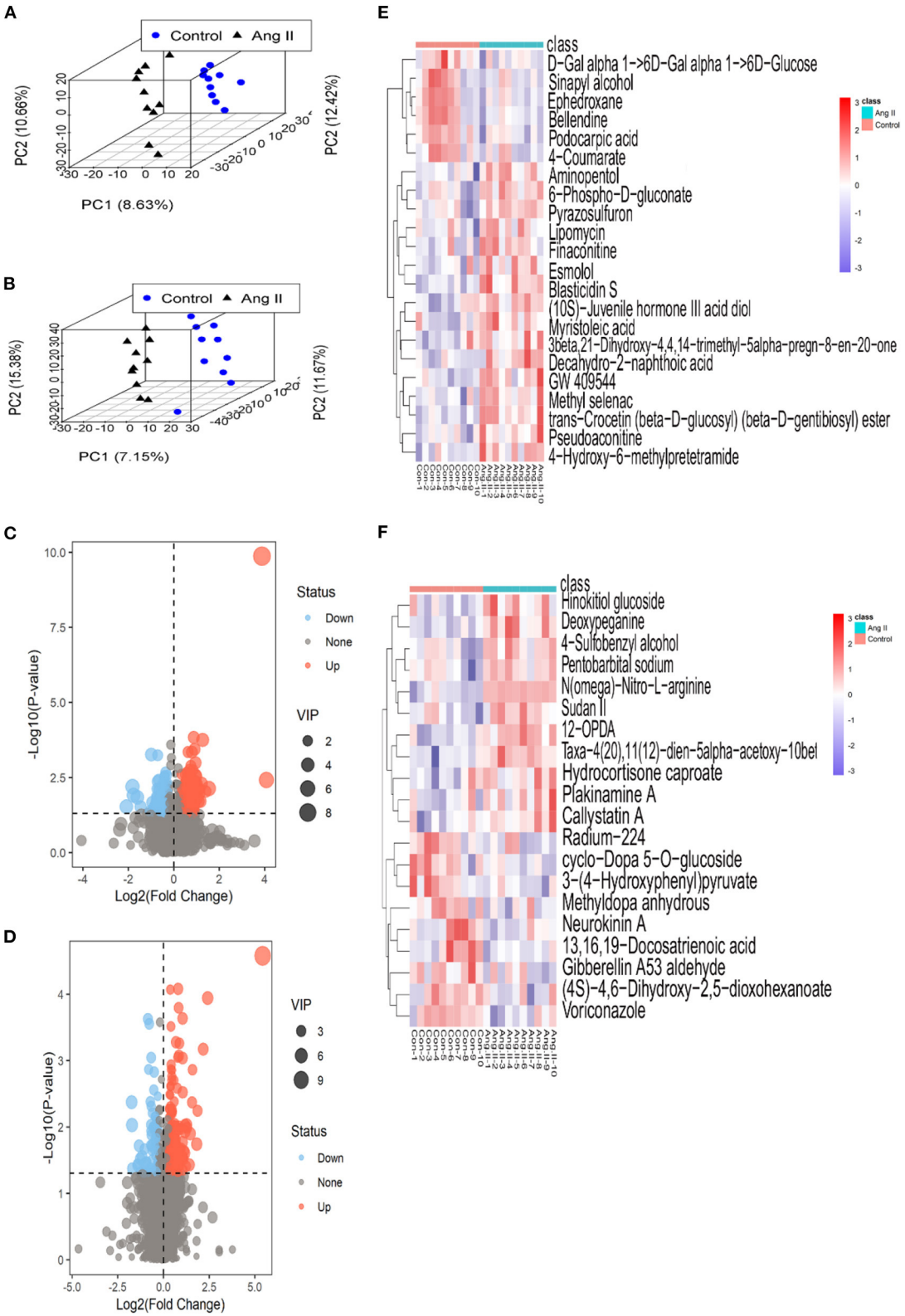

FIGURE 3 | (A,B) 3-D plots of scores from partial least-squares discriminant analysis based on the metabolic profiling data from the plasma of Ang II-induced hypertensive mice and healthy (control) mice in ESI+ **(A)** and ESI– mode **(B)** (black triangles, Ang II-induced hypertensive mice; blue circles, control mice). **(C,D)** Volcano plots based on *P*-values and fold-changes of single-dimensional tests in ESI+ mode **(C)** and ESI– mode **(D)**. **(E,F)** Heatmaps of the differential metabolites for Ang II vs. control in ESI+ mode **(E)** and ESI– mode **(F)**.

TABLE 1 | Top 20 differential serum metabolites between Ang II-induced hypertension mice and control mice in ESI+ mode.

Compound	*P*-value	Regulation	Fold change	Retention time/min
(10S)-Juvenile hormone III acid diol	0.009	Up	2.001	5.587
3beta,21-Dihydroxy-4,4,14-trimethyl-5alpha-pregn-8-en-20-one	0.020	Up	2.249	7.477
4-Coumarate	0.043	Down	0.527	4.582
4-Hydroxy-6-methylpretetramide	0.004	Up	16.918	6.664
6-Phospho-D-gluconate	0.007	Up	2.917	4.511
Aminopentol	0.009	Up	2.648	6.807
Bellendine	0.012	Down	0.293	4.262
Blasticidin S	0.0001	Up	1.839	3.827
D-Gal alpha 1->6D-Gal alpha 1->6D-Glucose	0.030	Down	0.362	0.633
Ephedroxane	0.030	Down	0.237	4.262
Esmolol	0.016	Up	2.027	3.691
Finaconitine	0.008	Up	2.084	4.055
GW 409544	0.003	Up	1.957	4.739
alpha-Lipomycin	0.039	Up	1.828	4.041
Methyl selenac	0.006	Up	1.960	4.668
Myristoleic acid	0.005	Up	2.324	8.555
Podocarpic acid	0.015	Down	0.331	3.563
Pseudoaconitine	0.011	Up	2.184	4.725
Pyrazosulfuron	0.008	Up	2.202	4.860
trans-Crocetin (beta-D-glucosyl) (beta-D-gentibiosyl) ester	0.0002	Up	2.410	4.668

TABLE 2 | Top 20 differential serum metabolites between Ang II-induced hypertension mice and control mice in ESI- mode.

Compound	*P*-value	Regulation	Fold change	Retention time/min
(4S)-4,6-Dihydroxy-2,5-dioxohexanoate	0.050	Down	0.427	0.633
12-OPDA	0.0002	Up	2.077	8.090
13,16,19-Docosatrienoic acid	0.036	Down	0.378	9.841
4-Hydroxyphenylpyruvate	0.022	Down	0.488	3.414
4-Sulfobenzyl alcohol	0.009	Up	2.436	4.497
Callystatin A	0.021	Up	1.963	8.047
cyclo-Dopa 5-O-glucoside	0.036	Down	0.484	0.633
Deoxypeganine	0.0001	Up	5.391	6.108
Gibberellin A53 aldehyde	0.019	Down	0.397	7.156
Hinokitiol glucoside	0.006	Up	3.668	5.865
Hydrocortisone caproate	0.037	Up	2.686	7.477
Methyldopa anhydrous	0.034	Down	0.524	7.202
N(omega)-Nitro-L-arginine	0.00003	Up	42.991	0.633
Neurokinin A	0.048	Down	0.480	8.603
Pentobarbital sodium	0.025	Up	2.242	4.682
Plakinamine A	0.018	Up	3.526	7.583
Radium-224	0.030	Down	0.414	4.041
Sudan II	0.009	Up	2.287	8.339
Taxa-4(20),11(12)-dien-5alpha-acetoxy-10beta-ol	0.0008	Up	1.794	8.746
Voriconazole	0.009	Down	0.306	4.262

top 20 metabolites and their regulatory changes under the two ion modes were identified as potential biomarkers in developing hypertension (**Tables 1**, **2**). In addition, these metabolites mapped to >20 metabolic pathways, mainly sugar and linoleic acid metabolism, carbohydrate digestion and absorption, ATP binding cassette (ABC) membrane transporter transport protein, peroxisome proliferator-activated receptor (PPAR), and hypoxia-inducible factor-1 signaling pathways (**Figure 4**).

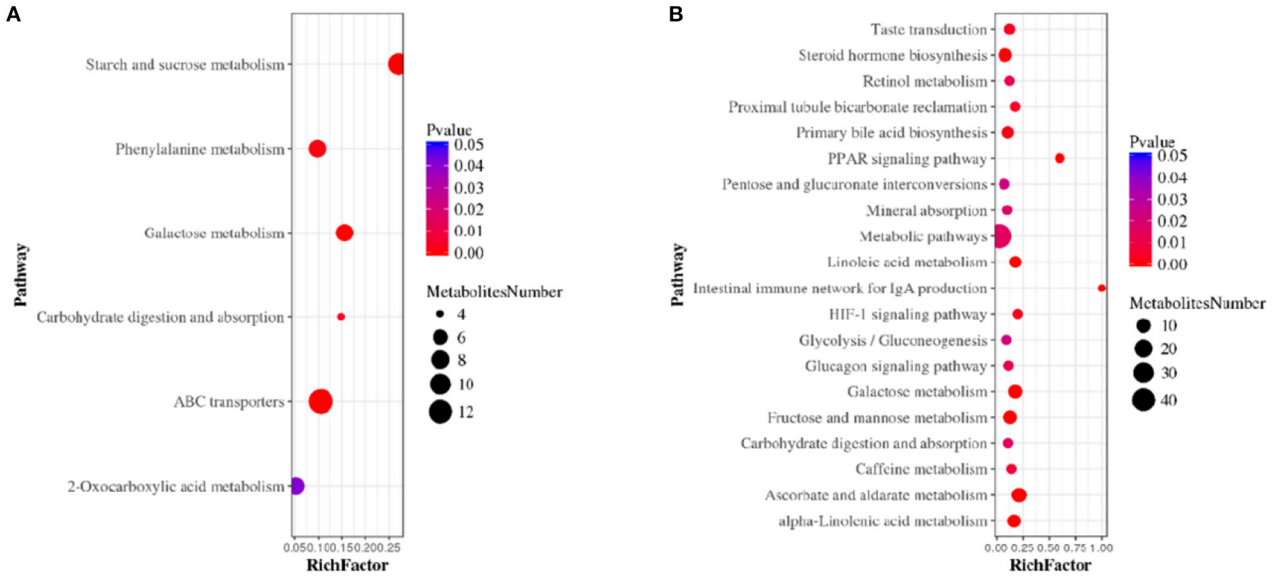

FIGURE 4 | Metabolic pathway enrichment analysis in ESI+ mode **(A)** and ESI– mode **(B)**. The size of each circle represents the number of metabolites enriched in the pathway.

DISCUSSION

The renin-angiotensin-aldosterone system (RAAS) is one of the systems closely related to vasomotor and sodium-water metabolism *in vivo*, which plays crucial roles in cardiovascular physiology and pathophysiology (19, 20). In the RAAS, the angiotensin converting enzyme (ACE) generates Ang II, which has high bioactivity and powerful vasoconstrictor effect. It is well-known that a variety of cardiovascular risk factors are closely associated with Ang II (21–23). The ability of Ang II to elevate blood pressure is 10∼40 times powerful than adrenaline (24). Ang II raises blood pressure through multiple factors, mainly through stimulating zona glomerulosa of the adrenal gland, promoting aldosterone secretion and sodium-water retention (20, 25–27). Besides, it enhances noradrenaline release from sympathetic nerve endings (28). Meanwhile, elevation of Ang II content promotes oxidative stress and endothelial dysfunction, and plays a crucial role in atherosclerosis (29, 30). Vascular damage, regulated with Ang II, is also dependent on the gut microbiome (31). It is noticed that a significant positive correlation between Ang II level and left ventricular hypertrophy in the hypertensive patients diagnosed without any treatment (32, 33). Plasma Ang II level is significantly correlated to the end diastolic interventricular septal diameter (IVSDd) in essential hypertension (34). Taken together, due to the multifactorial roles played by Ang II in the course of hypertension, it has been used in many studies to construction of hypertensive models in mice (35, 36).

In this study, we analyzed the serum metabolites of Ang II-induced hypertensive mice based on an LC-MS/MS platform. Our results showed that the slow release of Ang II induces metabolomics changes in mice, promoting the development of hypertension. According to previous research reports on Ang II-induced hypertension (37), the body weight of mice will decrease significantly during the sustained release of Ang II, which may be related to metabolic disorders in the body.

Ang II is a critical factor in hypertension, diabetes, and aging, and it induces many metabolic pathway disorders. Hypertension and diabetes are considered to be the main components of metabolic syndrome, sharing a common pathogenesis according to a large number of basic and clinical studies (38–40). About 60–70% of diabetic patients have hypertension (41), and hypertensive patients have abnormal glucose metabolism. Consistent with this, our results showed that Ang II-induced hypertension in mice was accompanied by evident glucose metabolism disorder. A significant increase in 6-phosphate-d-gluconate and decreases in maltose, lactose, and other disaccharides was found in the Ang II group. The analysis of metabolite pathway enrichment showed a disturbed glucagon signal pathway, consistent with the phenomenon of hyperinsulinemia in hypertensive patients.

In recent years, PPARs have been closely associated with energy metabolism, cell differentiation, proliferation, apoptosis, and the inflammatory response (42–44). Ang II increases the permeability of cerebral vascular endothelium *via* type 1 receptors, disrupts the membrane distribution of zonula occludens-1 and vascular endothelial-cadherin on cerebral vascular endothelium, decreases the total levels of junctional adhesion molecule-A and major facilitator superfamily domain-containing protein 2a, and increases caveolin 1 accompanied by the de-phosphorylation of PPARα. PPARα agonists improve the endothelial permeability caused by Ang II (45). Interestingly, in ESI– mode, we found that the levels of three metabolites [9-cis-retinoic acid, 9(S)-hydroxyoctadecadienoic acid (HODE), and 13(S)-HODE] are closely associated with the PPAR signaling

pathway. After 2 weeks of Ang II induction in mice, the content of 9-cis-retinoic acid, an active metabolite of vitamin A, increases significantly. The content of 9-cis-retinoic acid in the serum of Ang II-induced hypertensive mice was 1.42 times compared to control mice ($P = 0.03$; VIP = 1.66). 9-cis-Retinoic acid is an active retinoid that regulates expression of retinoid responsive genes (46). 9-cis-Retinoic mediates gene transcription acting through the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs) in cells (47). In addition to PPARs, RXRs are also an essential heterodimeric partner for other subclass I nuclear receptors, such as the farnesoid X receptor (FXR), thyroid hormone receptors, the vitamin D receptor and the liver X receptor (LXR) (48–51). However, the transcriptional complex formed by RXRs and PPARs plays a critical role in energy balance, such as glucose homeostasis, fatty acid handling and triglyceride metabolism (52). The PPAR-RXR transcriptional complex also participates in inflammatory and vascular responses in endothelial and vascular smooth muscle cells directly (52–54). It has been suggested that RXR regulates the growth and differentiation of normal and malignant cells, and inhibits the prostaglandin expression of endoperoxide-2 (55). However, retinoic acid is a toxin that can bring about fracture, skin injury and swelling, serum calcium elevation, limited dose hyperlipidemia (cholesterol and triglyceride elevation), and hypothyroidism (56). Ang II contributes to an increase in the content of two HODEs that have been used as biomarkers for assessing oxidative status (57).

The ABC is an outflow promoter of phospholipid and cholesterol, playing an essential role in the development of atherosclerosis and arterial hypertension. ABCA1 mediates the first step of reverse cholesterol transport by transporting excess cholesterol in peripheral tissues to the liver for excretion (58). Recent evidence has shown that the expression of ABCA1 is significantly decreased in patients with hypertension, and the outflow of cholesterol to apo-A1 leads to increased carotid intima-media thickness, and promotes arterial hypertension (59). Interestingly, in ESI+/ESI– modes, we identified 19 differential metabolites that were enriched in the metabolic pathway of the ABC transporter. These substances may affect lipid metabolism and increase blood vessel wall pressure by interfering with the flow of cholesterol from monocytes, macrophages, and the liver.

In this study, we found changes in the serum metabolome treated with Ang II in mice, providing new clues for the further study of the pathophysiological mechanisms in hypertension. Our non-targeted metabolomics research identified specific differences related to carbohydrate, lipid, and carbohydrate metabolism in hypertension. These results improve the understanding of systemic metabolic response to sustained release of Ang II in hypertensive mice, providing a new panel of biomarkers that may be used to predict blood pressure fluctuations in the early stages of hypertension, although researches about the clinical use of these metabolites as potential biomarkers in hypertension is still needed.

DATA AVAILABILITY STATEMENT

All relevant data are within the paper and its **Supplementary Materials**. The metabolomic data for this study can be found in the MetaboLights database (60) under Accession No. MTBLS2643 (http://www.ebi.ac.uk/metabolights/MTBLS2643). The full dataset is also available from Tingting Zhou (tingtingchou@126.com).

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Experimentation Ethics Committee, Jiangnan University (License No: JN. No 20190930c1000120[232]).

AUTHOR CONTRIBUTIONS

SY and ZW: conception, design, data analysis, and interpretation. MG, MD, XW, and FY: administrative support. LG, LL, and YL: provision of study materials or patients. LF and TZ: collection and assembly of data. All authors manuscript writing, final approval of manuscript, read, and approved this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.683859/full#supplementary-material

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REVIEW
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Metabolism and Chronic Inflammation: The Links Between Chronic Heart Failure and Comorbidities

Zhiwei Li, Hongmei Zhao and Jing Wang**

Department of Pathophysiology, State Key Laboratory of Medical Molecular Biology Institute of Basic Medicine, Chinese Academy of Medical Sciences, School of Basic Medicine, Peking Union Medical College, Beijing, China

Heart failure (HF) patients often suffer from multiple comorbidities, such as diabetes, atrial fibrillation, depression, chronic obstructive pulmonary disease, and chronic kidney disease. The coexistance of comorbidities usually leads to multi morbidity and poor prognosis. Treatments for HF patients with multi morbidity are still an unmet clinical need, and finding an effective therapy strategy is of great value. HF can lead to comorbidity, and in return, comorbidity may promote the progression of HF, creating a vicious cycle. This reciprocal correlation indicates there may be some common causes and biological mechanisms. Metabolism remodeling and chronic inflammation play a vital role in the pathophysiological processes of HF and comorbidities, indicating metabolism and inflammation may be the links between HF and comorbidities. In this review, we comprehensively discuss the major underlying mechanisms and therapeutic implications for comorbidities of HF. We first summarize the potential role of metabolism and inflammation in HF. Then, we give an overview of the linkage between common comorbidities and HF, from the perspective of epidemiological evidence to the underlying metabolism and inflammation mechanisms. Moreover, with the help of bioinformatics, we summarize the shared risk factors, signal pathways, and therapeutic targets between HF and comorbidities. Metabolic syndrome, aging, deleterious lifestyles (sedentary behavior, poor dietary patterns, smoking, etc.), and other risk factors common to HF and comorbidities are all associated with common mechanisms. Impaired mitochondrial biogenesis, autophagy, insulin resistance, and oxidative stress, are among the major mechanisms of both HF and comorbidities. Gene enrichment analysis showed the PI3K/AKT pathway may probably play a central role in multi morbidity. Additionally, drug targets common to HF and several common comorbidities were found by network analysis. Such analysis has already been instrumental in drug repurposing to treat HF and comorbidity. And the result suggests sodium-glucose transporter-2 (SGLT-2) inhibitors, IL-1β inhibitors, and metformin may be promising drugs for repurposing to treat multi morbidity. We propose that targeting the metabolic and inflammatory pathways that are common to HF and comorbidities may provide a promising therapeutic strategy.

Keywords: heart failure, comorbidities, metabolism, chronic inflammation, reactive oxygen species, mitochondria

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Edited by:
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Jianqing She,
The First Affiliated Hospital of Xi'an Jiaotong University, China

****Correspondence:***
Jing Wang
wangjing@ibms.pumc.edu.cn
Hongmei Zhao
pumczhaohm@sina.com

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INTRODUCTION

Heart failure (HF) is a global public health problem that affects more than 26 million people worldwide and causes a heavy health burden (1, 2). The prevalence of HF was 1.3% in Chinese adults (an estimated 13.7 million), in which 23% of patients had HF with preserved ejection fraction (EF), (HFpEF), 23% had HF with middle-range EF (HFmrEF), and about 54% had reduced ejection fraction (HFrEF) (3). Due to the aggravation of aging, the incidence of HF is rising, and HF is associated with increased mortality, morbidity, and hospitalization (4).

HF often coexists with multiple comorbidities. The reported prevalence of comorbidities varied with HF severity (5). As shown in **Figure 1**, we summarized the prevalence of major comorbidities according to the different organs and systems involved, such as hypertension (65%), atrial fibrillation (45%), chronic obstructive pulmonary disease (COPD)/asthma(40%), iron deficiency (30%), diabetes (40%), chronic kidney diseases (CKD) (25%), obesity (45%) (6, 7), ischaemic heart disease (50%), hyperlipidaemia (55%) (8), depression (40%) (9–11), sleep apnea (40%) (12), sarcopenia (40%) (13) and liver dysfunction (10%) (14). The high prevalence of multi morbidity is associated with poor prognosis and heavy heath burdens, and therapy for multi morbidity in HF is still a challenge (15). However, the treatment of comorbidities may have cardiovascular side effects. Therefore, understanding the underlying mechanisms and finding potential strategies for both HF and comorbidities is worthwhile.

Metabolism and inflammation play an essential role in the pathophysiology of HF and its associated comorbidities, which may be the link between them. In this review, we summarized the role of metabolism and inflammation in HF and its most common comorbidities, and review their possible links, including shared risk factors, signal pathways, and therapeutic targets.

METABOLIC REMODELING FROM NORMAL TO FAILING HEART IS BOTH CAUSE AND EFFECT OF HEART FAILURE

The heart requires a high rate of ATP production and turnover to fuel its continuous mechanical work, and it has become common knowledge that the failing heart is an "engine out of fuel" (16). We give an overview of the pathological cardiac metabolic remodeling from physiological condition to heart failure in **Figure 2**, including glucose, fatty acid (FA), amino acid, and ions metabolisms. These metabolic changes all affect cardiac energy metabolism either by directly participating in or indirectly regulating mitochondrial metabolism.

Normal Cardiac Energy Metabolism Has Compensatory Capacity

Under normal physiological conditions, the heart cycles about 6 kg of ATP every day (16). Regulation of cardiac energy metabolism is through substrate alteration. The substrate mainly consists of fatty acids (FAs), glucose, pyruvate, lactate, and ketone bodies. Glucose and fatty acid metabolism are major contributors to cardiac energy metabolism.

At rest, about 15–25% of the heart's maximum energy loading capacity is used (17). The cardiac energy metabolic pathway can be altered in only a few seconds through substrate alterations when shifting from rest to acute stress such as exercise or ischemia, or after glycogen stores have been depleted when fasting. In the normal heart, about 60–90% (depending on energy demand) of the cardiac energy budget is produced by FA β-oxidation, and the rest is produced by the pyruvate and tricarboxylic acid (TCA) cycle (18). Under non-ischemic conditions, more than 95% of the ATP in the normal heart comes from oxidative phosphorylation of FAs, glucose, and lactate in mitochondria, while in a fasting state, as the energy demand increases, there is a substrate shift from FAs to glucose (FAs produce about 70% of the ATP and glucose produces 20%) (17).

There is an auto-balance mechanism of glucose and fatty acid oxidation (FAO) pathways in the energy substrate that called the "Randle cycle," in which the activation of FAO would inhibit glucose uptake, whereas the increased utilization of glucose inhibits FAO, and inhibiting FAO increase glucose oxidation compensatorily (19). This regulation is mainly through an increase in plasma insulin level and the activation of the AMP-activated protein kinase (AMPK) pathway. Insulin would increase glucose uptake and activate the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, and finally increases myocardial contractility. Glucose uptake is an insulin-dependent process because glucose transporters (GLUT1/GLUT4) are sensitive to insulin. The activation of AMPK promotes both FA and glucose oxidation which increases cardiac energy. Moreover, AMPK inhibits ATP-consuming processes like protein synthesis (20).

Altered Energy Metabolic Substrate Utilization Is the Major Metabolic Remodeling in Heart Failure

The metabolic remodeling in the failing heart is similar to the alterations from the non-ischemic to the ischemic condition, as mentioned above, and may well be a protective compensatory mechanism to use more of its capacity. However, long term sustained high energy loading would cause some toxic substances to accumulate, which in turn may contribute to the progress of HF and comorbidities. In most cases, FAO decreases and glycolysis increases rapidly in HF, except for advanced and diabetic HF where FAO increases (18, 21, 22), this is because mitochondrial dysfunction in HF causes decreased expression and activity of enzymes associated with mitochondrial FAO (23). Several key enzymes of FAO are regulated by transcript factor peroxisome proliferator-activated receptors (PPARs). The decrease of FAO could be mainly explained by the activation of PPARγ and reduced activity of PPARα (18). Insulin plays an important role in substrate shift progression. Therefore, in cases of insulin resistance, such as diabetic HF or advanced HF, FAO is increased by activating PPARα signaling (22). Myocardial uptake of FA usually increases in HF. The imbalance of increased FA uptake and impaired utilization of FAs in HF results in FA accumulation. Accumulated FAs cause lipotoxicity and worsen HF by promoting mitochondrial dysfunction and apoptosis, and contributes to the development of insulin resistance (18).

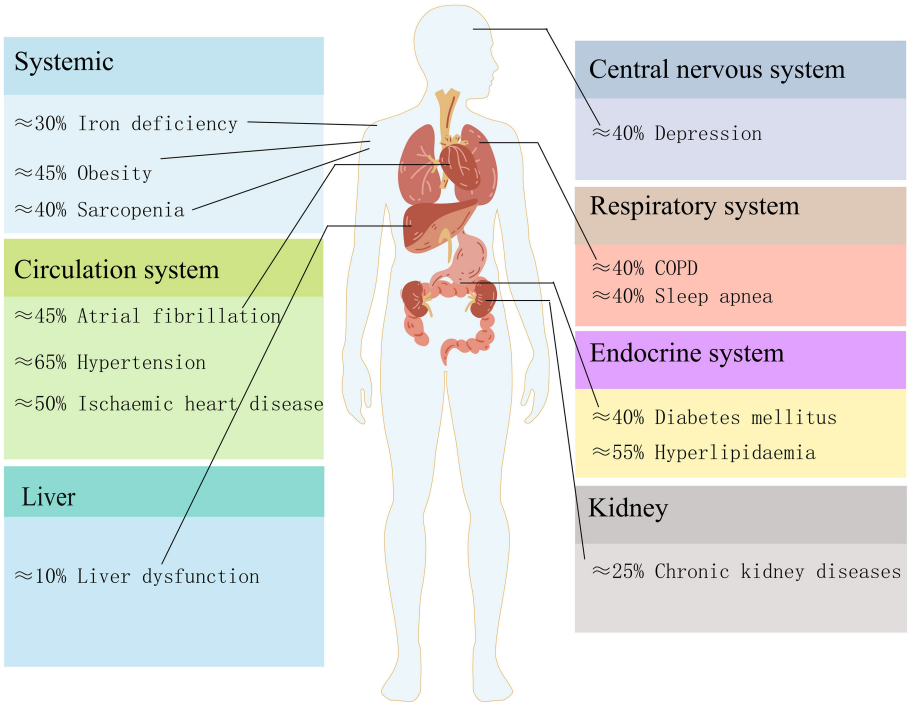

FIGURE 1 | The estimated prevalence of heart failure comorbidities in different organs and systems.

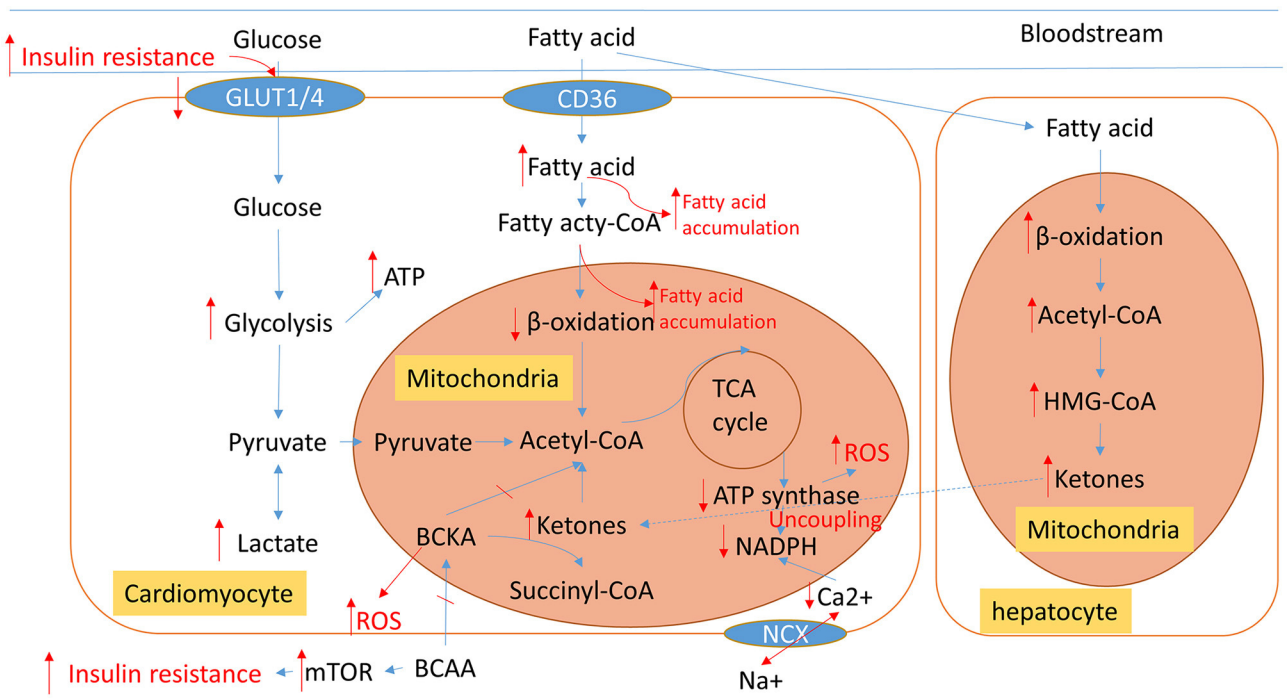

FIGURE 2 | An overview of normal physiological metabolic processes and the pathological metabolic remodeling characteristic of HF. Blue arrows show normal cardiac metabolic processes. The altered metabolic processes of HF are displayed in red; Arrows indicate changed metabolic intermediates and products. TCA, tricarboxylic acid; BCCA, branched-chain amino acids; BCKA, branched-chain alpha-keto acids; ROS, reactive oxygen species; NCX, Na^+/Ca^{2+} exchanger.

Targeting the FAO pathway is an emerging treatment for HF (24), but the significance of the shift from FAO to glucose metabolism remains controversial and there have two opposite therapeutic strategies: inhibit or facilitate FA utilization. The two therapeutic strategies are not contradictory because they both reduce the cardiac accumulation of FAs, one is by reducing the uptake of FAs and the other is by increasing the catabolism of FAs. Drugs targeted inhibition of FAO may be classified into 3 categories: (1) β-oxidation inhibition, such as malonyl-CoA decarboxylase inhibitors, (2) mitochondrial FA uptake inhibition, such as the carnitine palmitoyl transferase 1 inhibitor (CPTI), (3) plasma membrane FA uptake reduction by inhibiting related proteins, such as in the case of CD36 (the major FA transporter) or fatty acid-binding protein (FABP). CD36 inhibitor is still under preclinical investigations. However, considering that glucose provides less capacity for energy production than FAs (one FA molecule produces 120–130 ATP, while one glucose molecule produces 30–32 ATP) (22), there is an opposite opinion, which asserts that the heart reverting back to using FA may have therapeutic value for HF, such as by targeting GLUT4 to inhibit glycolysis or activate the AMPK pathway by phosphorylation to increase FAO. Studies have confirmed that reverting to the use of FA has a cardio protective effect (22, 25). Restoration of FAO could improve heart function, possibly via reduced cardiac lipotoxicity (26).

Mitochondria are a physiological source of reactive oxygen species (ROS). They are generated in the electron transport chain (ETC) during respiration, and eliminated by NADPH dependent enzyme systems, forming a "redox-optimized ROS balance" (19). The deficit in energy would cause the uncoupling of oxidative phosphorylation, and cause an increase in reactive oxygen species (ROS) and oxidative stress (27). ROS, in return, inactivates several enzymes of the TCA cycle (19).

In addition, liver energy metabolism also participates in the process of HF. Ketone bodies synthesized in liver mitochondria, especially β-hydroxybutyrate, the so-called super fuel, are more efficient than FAs or glucose. The failing heart adaptively consumes more ketone bodies (28) and this is believed to be beneficial (23, 29).

Amino Metabolism Dysfunction Indirectly Affects Cardiac Energy Metabolism

More glutamine is consumed in HF because it is the most abundant secreted amino acid (28), but branched-chain amino acids (BCAAs) played a more important role in HF. In healthy individuals, BCAAs are essential nutrition for mitochondrial biogenesis, and dietary supplementation of BCAAs has cardio protective effects (30–32). However, BCAA catabolic metabolism is impaired in HF, leading to the accumulation of BCAAs and branched-chain alpha-keto acids (BCKAs) (33). The accumulated BCAAs and their catabolic intermediates have a cardiotoxic effect. BCAA accumulation could result in insulin resistance by activating the mTOR pathway (34, 35), and accumulated BCKAs would increase reactive oxygen species (ROS) (36). Furthermore, BCAA is reported to be a potential therapeutic target for HF (37). BCAAs are not a major source of cardiac energy (below 5%) (28). but may have important indirect regulatory roles in energy metabolism as they affect mitochondrial biogenesis and BCAA toxicity affects energy metabolism.

Ion Metabolism Induces Heart Failure by Regulating Energy Metabolism

Sodium (Na^+) and calcium (Ca^{2+}) ions are closely linked to HF. Elevated intracellular Na^+ can lead to cardiac energy metabolic shift from FAO to glycolysis (38). The renin-angiotensin-aldosterone (RAAS) system has evolved to retain Na^+ homeostasis and RAAS-blockers have been widely used in HF therapy. In HF, tubular cells are often hypertrophic and Na^+ reabsorption increases. Sodium-glucose transporter-2 (SGLT-2) is a recently discovered diuretic agent that could improve the outcome of HF (39). Increases in the Na^+/H^+ exchanger may explain the phenomenon of the elevated Na^+ level in HF (40). Ca^{2+} is required for cardiac diastolic function (41). In fact, Ca^{2+} signaling plays an essential role in regulating mitochondrial ATP production (42). Ca^{2+} is a second messenger in various cells and is regulated by ion channels, ion exchangers, pumps (ATPases), and Ca^{2+}-binding proteins (43). The dysfunction of a sarcoplasmic reticulum Ca^{2+}-release channel, ryanodine receptor, can cause calcium leakage and mitochondrial damage, which contribute to the progression of HF (44). Na^+ is associated with Ca^{2+} uptake and Ca^{2+} related myofilament contraction through Na^+/Ca^{2+} exchange (45).

The Clinical Significance of Metabolic Remodeling: A Double-Edged Sword

Metabolic remodeling is a major pathophysiologic character of HF, but whether it is the cause or result of the HF, and whether it is maladaptive or adaptive is still controversial (46). Why have drugs both targeting inhibition and promotion of metabolic remodeling been used for the treatment of heart failure, and are both able to alleviate HF symptoms? FA or glucose, which is the superior energy substrate? We think that metabolic remodeling has a double effect: On one hand, metabolic remodeling is thought to be an adaptive compensatory mechanism. First, the shift toward glucose metabolism improves myocardial contractile efficiency by increasing the stoichiometric ratio of ATP production to oxygen consumption and reducing oxygen waste (47). Although glucose has a lower energy capacity, the shift is not due to a lack of substrate availability because the coronary circulation is able to provide an excess of substrates (47), and glycolysis produces ATP much faster than other ways, as epitomized by the Warburg effect (48). Second, similar metabolic remodeling can also be seen in the physiological remodeling of the heart. Many pathways, such as the activation of the AMPK and PI3K pathways, which have protective roles, are active in both physiological and pathological cardiac remodeling (20). On the other hand, metabolic remodeling is harmful when toxic substances such as accumulated excess intracellular FAs and ROS are increased, which may worsen HF and cause comorbidities. Recent evidence suggests that the accumulation of toxic intermediates, rather than alterations of substrate

utilization or ATP deficit *per-second*, is responsible for cardiac dysfunction (18).

CHRONIC INFLAMMATION

The Role of Inflammation in Heart Failure

HF is usually accompanied by highly elevated circulating pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8, TNF-α, NF-κb, etc. However, the role of inflammation in HF has long been controversial. Because most traditional anti-inflammatory drugs failed in clinical HF therapies, inflammation was considered to not be a cause, but a complication of HF. The importance of inflammation in HF was not widely accepted until the success of canakinumab, an IL-1β inhibitor, which significantly improved the prognosis of HF. The effect of it and other anti-cytokine drugs indicates the role of inflammation in HF (49). Moreover, Soluble suppression of tumorigenesis-2(sST2) and galectin-3 are inflammatory biomarkers associated with fibrosis in HF, which have reportedly even better prognoses than NT-pro-BNP, an HF biomarker not directly associated with inflammation (50, 51). Having established the causal role of inflammation in HF, in the following, we give an overview of inflammation in cardiac remodeling and various comorbidities.

The Immune Response Causes Systemic Inflammation

Both the innate and adaptive immune systems have a pro-inflammation role in HF. The immune response triggered inflammation mechanism is called immune inflammation. Innate immune cells, such as neutrophils, natural killer cells, and mast cells (52), have been revealed to participate in the progress of HF through immune inflammation. For instance, monocytes derived from HF patients have higher secreted cytokines (IL-1β, IL-6) and chemokines (CCL3, CCL4), and can stimulate T cell activation (53). Monocyte-derived macrophages have a pro-inflammation role in cardiovascular diseases (49). In addition, several pattern recognition receptors (PRRs), such as NOD-like receptors (NLRs) and Toll-like receptors (TLRs), are mainly expressed on tissue-resident immune cells, can turn on multiple signals to trigger innate immune inflammation. Finally, the activation of the innate immune system can cause the activation of the adaptive immune system by activation and infiltration of B cells and T cells (54).

Inflammatory Cascade Promotes Cardiac Structural Remodeling

The major cardiac structural remodeling of HF including cardiac hypertrophy, fibrosis, and extracellular matrix (ECM) remodeling. Systemic inflammation can drive cardiac hypertrophy and fibrosis, and the inflammation is mainly triggered by PRRs (such as NLRP3 and TLR4) mediated innate immune response. The key inflammatory factors in this process are IL-1β, IL-6, and NF-κB. They can stimulate the release of many other inflammatory cytokines and transcription factors, which may promote cardiac hypertrophy and fibrosis (55, 56). The Nod1 receptor signaling pathway can contribute to cardiac hypertrophy (57). The mechanisms of the inflammatory cascade are yet not fully clear. However, it is known that IL-1β is activated by NLRP3 receptive inflammasomes. IL-1β and IL-6 stimulate immune cells (T cells, macrophages, and monocytes) to increase the release of IL-17, TNFα, and IFN-γ (58). These cytokines are signals which may activate immune cell trans differentiation into pro-inflammatory and pro-fibrotic subsets. For instance, Th1/Th2 polarization in T cells toward Th2 has a pro-fibrotic effect. While the CCR2+ monocytes, which express CC-chemokine ligand 2(CCL2) have pro-hypertrophy and pro-fibrotic effects (54). IL-33 is a member of the IL-1 family and ST2 is the receptor of IL-33. IL-33 has an anti-hypertrophic effect, whereas sST2 can competitively inhibit the IL-33/ST2 pathway and promote cardiac hypertrophy and fibrosis (59). Additionally, micro vascular inflammation can stimulate monocyte-derived macrophages to secrete transforming growth factorβ (TGF-β) which induces pro-fibrosis effects by stimulates the differentiation of fibroblasts into myofibroblasts. Myofibroblasts deposit collagen, and its increase may cause fibrosis (60). Moreover, inflammation may cause pyroptosis and apoptosis, which may also promote cardiac fibrosis (61).

The immune-inflammation mechanism may mediate cardiac ECM remodeling by increases ventricular stiffness (62, 63). Ventricular stiffness is a common pathological feature of HFpEF which promotes diastolic dysfunction (64). In systematic inflammation, IL-1β and other cytokines cause increased extracellular deposition of collagen and reduced elasticity of titin, resulting in ventricular stiffness (60).

RECIPROCAL PROMOTION OF COMORBIDITIES AND HF ARE ASSOCIATED WITH METABOLISM AND INFLAMMATION

Comorbidities and HF interact as both cause and effect, in which metabolism and inflammation are the possible common mechanisms underlying this cyclical relationship. In the following, some common comorbidities, including atrial fibrillation, diabetes, COPD, and obesity, are discussed from the standpoint of epidemiological evidence showing the reciprocal causation associated with underlying common metabolic and inflammatory mechanisms.

Atrial Fibrillation

Atrial fibrillation (AF) frequently coexists with HFpEF and they share similar risk factors (65, 66). In a recent study, more than one-third of the AF patients had HF, and more than half of the HF patients had AF (67). Even subclinical AF was associated with about a 4-fold increase in HF risk (68). On the other hand, HF promotes AF via cardiac fibrosis, inflammation, and oxidative stress (69, 70). Cardiac resynchronization therapy with a defibrillator can reverse HF remodeling (71).

Metabolism and inflammation are the most consequential underlying mechanisms common to the two diseases. Cardiac energy alterations in HF cause subsequent oxidative stress and inflammatory cascades, and contribute to AF. Mitochondrial Ca^{2+} handling dysfunction is a shared mechanism in AF and HF,

in which intracellular calcium leakage happens through oxidative stress-induced hyperphosphorylation of ryanodine receptor (43, 72). The PI3K/AKT may be a shared signaling pathway that regulates cardiac Ca^{2+} and Na^{+} ion channels (73).

Diabetes Mellitus

The prevalence of type 2 diabetes mellitus (T2DM) in HF was about 20–50%, and T2DM may increase mortality due to HF (74). T2DM and HF coexist in about 30–40% of patients with T2DM (74–76). T1DM (77) is also associated with an increased risk of developing HF.

HF caused by coronary artery disease and hypertension secondary to T2DM is more common in HFrEF (74). Diabetic cardiomyopathy, which refers to HF occurring in the absence of related cardiovascular diseases, is generally believed to be mediated by abnormal mitochondrial calcium handling (78). HFpEF is also associated with insulin resistance-induced ventricular remodeling and mitochondrial dysfunction (79, 80). Chronic inflammation caused by excess insulin has also been found to be responsible for diabetic HFpEF (81). Moreover, the byproduct of glycolysis has recently been reported to link diabetes and HF by post-translational modifications (82, 83).

The molecular mechanisms underlying diabetic HF are associated with changes in myocardial substrate metabolism, inflammation, endoplasmic reticulum stress, aberrant insulin signaling, and autophagy (84). For one thing, hyperglycemia and insulin resistance cause excessive ROS production. Furthermore, oxidative stress causes chronic inflammation and mitochondrial metabolic disorders. Several molecular pathways are involved in these processes. ROS activates poly (ADP-ribose) polymerase (PARP) and inhibits the AMPK pathway and decreases mitochondrial biogenesis. These changes would cause disturbed circadian clock synchronization of glucose and FA metabolism. The insulin receptor may activate the PI3K/AKT pathway, which is a major mechanism responsible for insulin resistance induced cardiac dysfunction. Moreover, the activation of Na^{+}/H^{+}-exchange (NHE1/3) can promote HF (85). Finally, the NLRP3 inflammasome is activated in T2DM and triggers NLRP3/ IL-1β, IL-6, and IL-18 inflammatory pathways to contribute to cardiac fibrosis (86).

Chronic Obstructive Pulmonary Disease

About 20% of unknown HF patients have COPD or asthma (87). Asthma increases HF risk by 80% (88). COPD is associated with increased risk (89) and worse prognosis of HF (90–92). The prevalence of systolic or diastolic HF in COPD patients ranges from 20% to 70% (93). Inhaled corticosteroid/long-acting β2-agonists (LABAs) in treating COPD were beneficial to cardiac function (94).

COPD may induce HF through chronic systemic inflammation and pulmonary vascular remodeling (95). In turn, HF aggravates excess ventilation in COPD, and causes dyspnea, and exercise intolerance (96).

Obesity

The prevalence of obesity in HF was about 40% (97, 98). Obesity increases the risk of HF (99). However, there is a U-shaped relationship between BMI and survival of HF - the so-called "obesity paradox." That is, high BMI is associated with better survival in patients with HF. However, the mortality risk from HF increased for patients with extremely high BMIs of 45 or greater (98). Furthermore, high waist-to-hip ratios have been associated with increased mortality, suggesting the harmfulness of obesity in HF (100). Abdominal obesity is associated with significantly higher mortality in HFpEF, which may be a better predictor than BMI (101). Abdominal obesity is strongly associated with the circulating level of aldosterone, the main role of which is to regulate salt-water retention. Mineralocorticoid receptor antagonists have recently been discovered as targets for obesity-associated HF (102).

Metabolism and inflammation are involved in the progress of HF in patients with obesity (103, 104). Increased leptin, which is reported as the product of the obesity gene, contributes to cardiac remodeling through Leptin-Aldosterone-Neprilysin Axis (105, 106). Insulin resistance secondary to obesity can cause altered cardiac energy metabolism and HF (107, 108). Obesity can cause immune-inflammation by activating macrophages, and activate IL-1β and NF-κB pathway (104).

Obesity can suppress BNP levels in HF (109) and causing lower plasma NT-pro-BNP levels (5). Therefore, BNP may not reflect the HF severity accurately in obese patients (110, 111). BNP enacts cardiac protection via multiple actions, such as suppressing RAAS activation and regulating sodium metabolism. An insufficient BNP level may promote HF progression (112).

Cancers

Cancers and HF are often coexisting in patients with cancers, they share several common pathophysiological mechanisms and causes, such as angiogenesis, clonal haematopoiesis, and sarcopenia (113–115). Aging may cause somatic mutations of genes (typically DNMT3A and TET2) in hematopoietic stem cells, which promote peripheral blood leukocytes release proinflammatory factors such as IL-1β and IL-6. This phenomenon is called clonal hematopoiesis of indeterminate potential (CHIP), which is a risk factor of cardiovascular diseases and cancer (116). Sarcopenia is a common complication in advanced stage cancer, which may promote HF through muscle wasting and thinning of the ventricular wall, (115).

Cardio toxicity is a major risk factor for HF. It reportedly accounts for 45% of all drug withdrawals (117). Mitochondrial dysfunction is the major pathophysiologic mechanism of drug-induced cardio toxicity (117, 118). In most times a drug with cardio toxicity would not be used in clinical. However, anti-tumor drugs with cardio toxicity are common when weighing the pros and cons because of the therapeutic effect (119). For instance, aromatase inhibitors have become the preferred treatment for estrogen receptor-positive breast cancer, which targets the cytochrome P450 enzyme, but it is associated with a significantly increased risk of HF (120). Anthracyclines such as doxorubicin (121) and epirubicin (122) are commonly used for breast cancers, lymphoma (123), and a variety of other cancers, but their usage is limited by cardio toxicity. Trastuzumab, another breast cancer drug, is also associated with increased HF risk (124). The proposed biological mechanisms underlying

anthracycline cardio toxicity are mitochondrial dysfunction, mitochondrial iron overload, oxidative stress, inflammation, and impaired autophagy (125).

LINK BETWEEN HEART FAILURE AND COMORBIDITIES

Common risk factors such as aging, hyperglycemia, and lifestyle are the cause of HF and comorbidities. The underlying mechanisms of these factors are associated with common metabolic or inflammatory pathways. In this review, the major pathways were identified through gene enrichment analysis. Further, the common therapy drug targets have also be summarized by analyzing the disease-gene network. This review will be helpful for selecting the therapeutic strategy.

Major Shared Risk Factors of HF and Comorbidities Are Associated With Metabolism and Inflammation

Epidemiologic evidence has found many risk factors for cardiovascular diseases, including chronic conditions or diseases (aging, hyperlipidemia, hypertension, hypoxaemia, and metabolic syndrome), and lifestyles (dietary and sleeping patterns, smoking, and drinking (126–128). Unhealthy lifestyles may contribute to HF by dysregulated innate immunity and chronic inflammation (129). These factors are also risk factors for many comorbidities (130) and share similar mechanisms, which are associated with metabolism and inflammation.

Aging is one of the major risk factors for developing multi morbidity and HFpEF, and both multi morbidity and HFpEF are unmet needs in the therapy of HF (131–133). The main underlying mechanisms of cardiovascular aging are associated with mitochondrial metabolism (134, 135), chronic inflammation (136), autophagy (137), and oxidative stress (138).

Physical inactivity (sedentary behavior), is a risk factor of multi morbidity (139), it causes chronic subclinical myocardial injury detectable with high-sensitivity cardiac troponin and increases HF risk (140). Meta-analysis showed exercise is beneficial for people with multi morbidity (141). It can regulate mitochondrial remodeling (142), and also causes physiologic remodeling which increases cardiorespiratory fitness (143). It is improved cardiorespiratory fitness that is the physiopathological link between obesity, exercise, and HF (93, 94), primarily by increases the cardiac compensatory capacity (17). Furthermore, exercise has direct anti-inflammatory effects by inhibition of TNF-α and IL-1β, and may attenuate insulin resistance (144).

Metabolic syndrome, mainly charactered by hyperlipidemia and hypertension, shared similar mechanisms to that of diabetes and obesity, such as insulin resistance and macrophage induced inflammation, which have already been discussed (104). Taken together, metabolism and chronic inflammation are the major mechanisms underlying the major shared risk factors between HF and comorbidities.

Common Molecular Pathways Analysis

Although many metabolism and inflammation mechanisms have been reviewed previously, which pathways are most important remains unclear. To conduct an unbiased analysis of the key shared biological pathways in HF and comorbidities, we performed enrichment analysis on target genes of HF and some comorbidities of high prevalence in the database. The Target Validation platform (https://www.targetvalidation.org/) contains disease target genes from Genome-Wide Association Studies (GWAS), drug targets from the EMBL-EBI ChEMBL database, EMBL-EBI RNA expression data, and text mining of literature. First, we retrieved all the targets of HF and several comorbidities (diabetes mellitus, obesity, COPD, chronic kidney disease, and OSA) in the Target Validation platform (accessed on March 22, 2021) and intersected the disease targets as shown in the Venn diagram (**Figure 3A**). Five comorbidities (diabetes mellitus, obesity, COPD, CKD, and obstructive sleep apnea) were selected for analysis because these represent the most highly prevalent comorbidities (The major enriched pathways did not change but the Venn diagram and the latter network plot would be more complex and less understandable when adding other common comorbidities such as atrial fibrillation and depression into the analysis). There were 299 common targets associated with all the four diseases, and 1,051 common targets were shared by HF and at least four of the comorbidities. Gene Ontology and KEGG enrichment analysis was performed on 1,051 semi-common targets with the R (version 3.6.0) package cluster Profiler (version 3.14.3). The activation of metabolic and inflammatory pathways may require the expression level change or activation of a group of enzymes, cytokines, or proteins regulated by common transcription factors. To identify key transcription factors, transcription factors enrichment analysis was performed using Meta scape website tools (http://metascape.org) (145) with TRRUST (Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining) database (146) and the figure was plotted with ggplot2 (version 3.3.3).

Some known factors which play a crucial role in HF, such as the NADPH oxidase (147), and sulfur compound binding (148), and growth factor activity (149) were enriched in the Geno ontology enrich analysis (**Figure 3B**).

The enriched pathways are mainly associated with metabolism and inflammation. Some significantly enriched pathways not shown in the figure are also analyzed. According to their role in HF, most of the significantly enriched pathways can be classified into one or more of the following categories: (1) Energy metabolic associated pathways. The PI3K/Akt pathway regulates both metabolic and structural remodeling. The PI3K/AKT pathway is associated with AF (150), COPD (151), HF (152), and multi morbidity (131). The PI3K/AKT pathway regulates cardiac metabolism both in pathological remodeling in HF (143), and it also regulates heart growth (149). (2) Structure remodeling associated pathways. The MAPK pathway is the key pathway activated in response to ischemia and has a critical role in cardiac hypertrophy. Moreover, the MAPK pathway may be involved in the interplay of mitochondrial energy metabolism and systemic inflammation (57). The Hypoxia-Inducible Factor

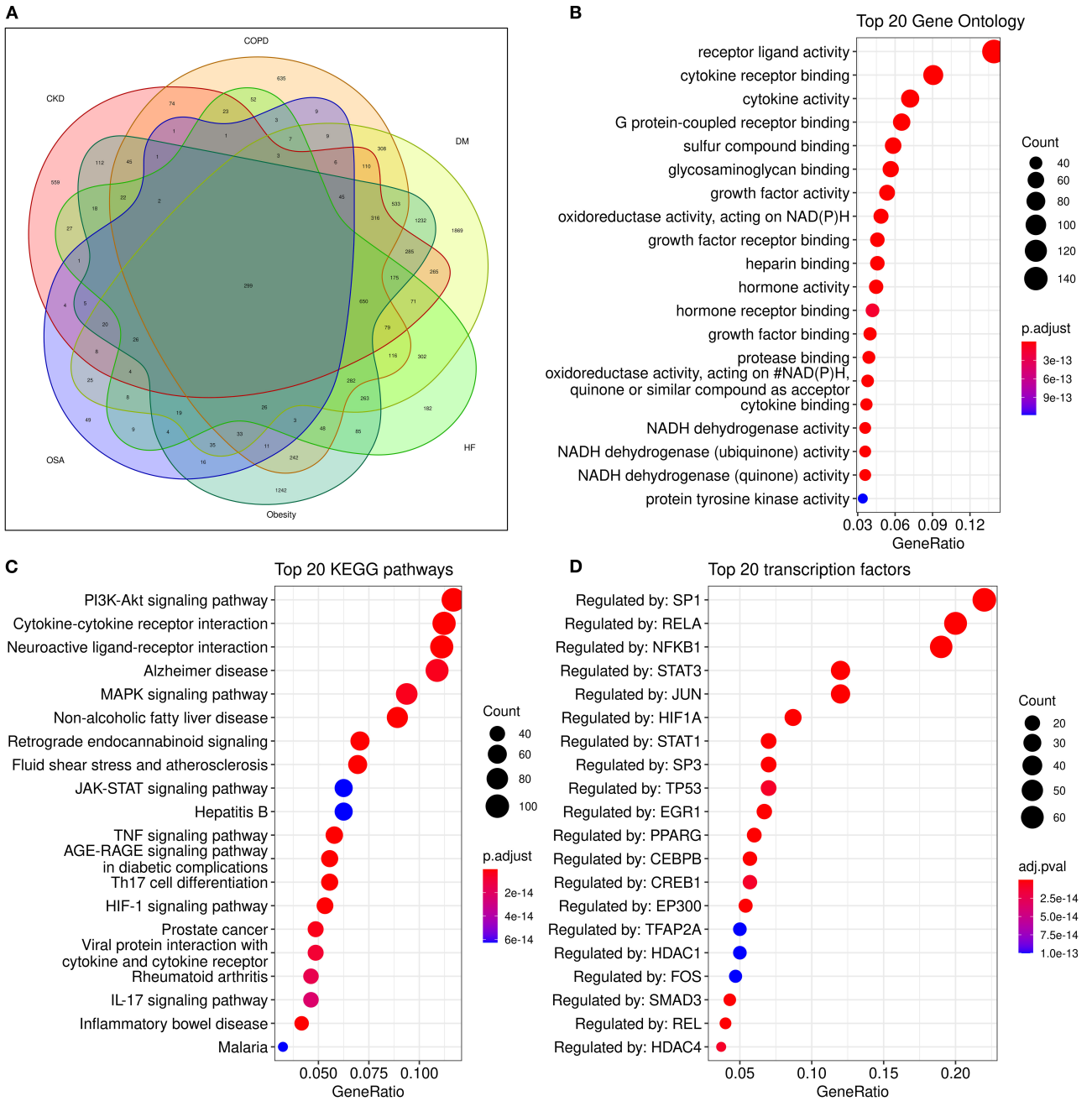

FIGURE 3 | Analysis of common genes and pathways in comorbidities and HF. **(A)**: Venn diagram of common genes between comorbidities and HF; **(B)**: Dot plot of top 20 enriched Gene Ontology biological processes enriched for common genes between HF and comorbidities; **(C)**: Dot plot of top 20 enriched KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways for common genes between HF and comorbidities; **(D)**:Dot plot of top 20 enriched transcription factors from TRRUST(Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining) database.

1 (HIF-1) pathway can regulate glucose metabolism and is adaptively activated in response to hypoxia conditions and can promote cardiac hypertrophy. HIF-1 can activate the glycation end products (AGE) advanced glycation end products (RAGE) signaling. The AGE-RAGE signaling pathway is associated with some comorbidities of HF such as OSA and diabetes (153, 154). Insulin-like growth factor (IGF) signaling is activated in HF and promotes cardiac hypertrophy (155, 156); (3) Cardiac systolic and diastolic functions associated pathways. Such as the CaMKII pathway (157), The G protein-coupled receptor (GPCR) signaling pathway is a known drug target of HF, these drugs include β-adrenergic receptor and angiotensin II receptor

antagonists (158). (4) Inflammatory pathways, majorly include the Cytokine-cytokine receptor interaction, TNF signaling, IL-17 signaling, and Toll-like receptor pathways (**Figure 3C**). Additionally, the clonal hematopoiesis pathway is a risk factor of HF enriched in the analysis and may be related to immune inflammation (53, 159). (5) Other structural remodeling in addition to hypertrophy, such as fibrosis and amyloidosis. The activation of inflammatory pathways can activate the TGF-β pathway and promote fibrosis. Alzheimer's disease is also enriched. Alzheimer's disease is major characterized by amyloidosis, and senile amyloidosis may be an overlooked causal mechanism of HFpEF (60, 66). The PI3K/AKT/GSK3β pathway is proposed as the link between diabetes and Alzheimer's disease (160).

The PI3K/AKT pathway is most significant in the pathway enrich analysis and is a key pathway in cardiac remodeling. Cardiac remodeling is a key biological process that contributes to the progression of HF. Some drugs, such as calcium antagonists and renin inhibitors, may alleviate hypertension and improve contraction function of HF, they did not improve remodeling, and therefore did not improve the prognosis of HF (161). Some downstream signaling pathways, such as PI3K/AKT/eNOS have a cardio protective role, and the activation of this pathway may be the mechanism of some cardiovascular drugs such as statins (162). PI3K/AKT pathway activation is a shared mechanism in physiological and pathological cardiac hypertrophy, and physiological hypertrophy may enhance cardiac systolic and diastolic function (143). However, in pathological conditions, such as HF, long-term sustained activation of PI3K/AKT pathway in HF promotes excessive cardiac growth, mitochondrial dysfunction, ROS production, and impaired $Ca2^{+}$ handling (163). Activation of PI3K/AKT pathway is a common mechanism in many chronic diseases, such as cardiovascular disease, metabolic diseases, COPD, and cancers (131). It has been reported improved HF syndrome with no substantial side effects when using PI3K/AKT inhibitors as a treatment of PIK3CA-related overgrowth syndrome (164). Therefore, PI3K/AKT inhibitors may be a promising treatment for HF and comorbidities. However, because the activation of PI3K/AKT pathway is essential for many cellular processes such as cell growth, proliferation, and migration, targeting PI3K/AKT pathway may have side effects, finding a more specific target of HF and comorbidities related to PI3K/AKT pathway may be a better treatment choice.

Common Mechanistic Pathways in Heart Failure and Comorbidities

Together with a review of the literature into account, the main shared mechanisms of HF-induced comorbidities can be summarized (**Figure 4**) and elucidated. The mechanism of how comorbidities promote HF may be clarified similarly by the shared mechanisms.

The metabolic mechanisms of HF promote comorbidities are associated with mitochondria injury, oxidative stress, insulin resistance, and hypoxia. HF and risk factors induce altered cardiac energy metabolism. Cardiac energy metabolic

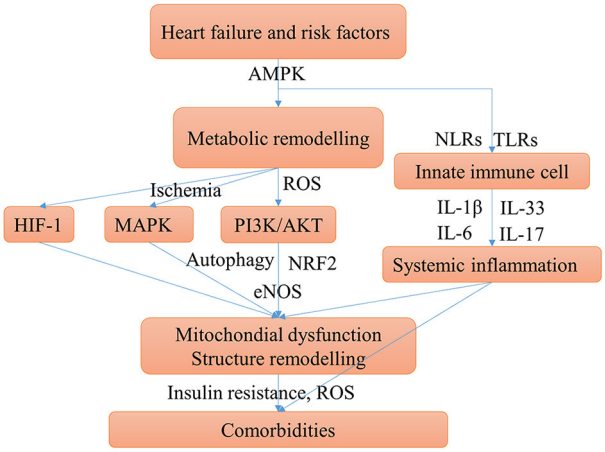

FIGURE 4 | Main possible shared mechanisms underlying HF and comorbidities. HF, comorbidities, and risk factors may have some shared chronic conditions such as insulin resistance, hypoxia, and chronic inflammation, these conditions aggravate HF and cause comorbidities. The mechanism of comorbidities contribute to HF can be clarified similarly. Cardiac energy metabolic remodeling may take place in these conditions mediated by activating the AMPK signaling. Metabolic remodeling can activate PI3K/AKT pathway, which promotes myocardial over-growth and cardiac hypertrophy and can cause mitochondrial injury. The mTOR signaling may be activated by AMPK and PI3K/AKT pathways can cause disturbed autophagy which aggravates the mitochondrial injury. Ischemia and hypoxia conditions can activate MAPK and HIF-1 pathways, which contribute to cardiac structure remodeling. Innate immune cells, mainly monocytes, macrophages, and neutrophils, can trigger the immune response and systemic inflammation by secreting IL-1β and IL-6. Moreover, the pro-inflammatory cytokines stimulate T cells to polarize to Th17 cells and release IL-17. Systemic inflammation can cause diastolic dysfunction and cardiac hypertrophy.

remodeling causes oxidative stress through NAD (P) H oxidase-derived ROS (165). Oxidative stress can trigger mitochondrial injury and inflammation. As such, antioxidants have been a therapeutic strategy for cardiovascular diseases (166). Oxidative stress, mitochondrial dysfunction, and chronic inflammation were the major mechanisms of multi morbidity in the elderly (131). There is a consensus that mitochondrial impairment is key to cardiac dysfunction in HF (167). Mitochondria injury can cause cardiac remodeling, such as hypertrophy and fibrosis (168). In addition, mitochondrial biogenesis dysfunction play important roles in multi morbidity such as diabetes (169), obesity (170), lung diseases (171, 172), depression (173), sarcopenia (142), iron deficiency (148, 174), fatty liver disease (175), obstructive sleep apnea (176), and diabetic kidney disease (177). Mitochondria injury is commonly induced by oxidative stress or inflammation mediated by the PI3K/AKT/eNOS, PI3K/AKT/mTOR, AMPK/mTOR pathway (178), or the MAPK signaling pathway (179). Mitochondria autophagy, also called mitophagy, is a cellular process in which impaired mitochondria are destroyed to protect eukaryotic cells from mitochondrial injury. Autophagy has a protective role for HF and comorbidities, and may be injured by the activation of mTOR pathway (180). Insulin resistance plays an important role in the

pathological processes of HF, and is also strongly associated with diabetes (181), as well as obesity in which is associated with the phosphorylation of PPARγ (182). Insulin resistance was associated with the worse outcomes in patients with HF and diabetes (183). Hypoxia is a common chronic condition in many comorbidities such as COPD and anemia, and the related HIF-1 pathway may have an important role in the progression of obesity and hypertension (104).

Chronic systemic inflammation associated with HF is mainly triggered by innate immune cells (monocyte, macrophage, and neutrophils). The major pro-inflammatory cytokines including IL-1β, IL-6, IL-8, IL-17, IL-18, and TNA-α (49, 184, 185). Apart from their role in HF, IL-1β and IL-6 are key pro-inflammatory factors in many diseases, like COPD (186), diabetes (187), kidney disease (188), sarcopenia, obesity, and HF (189), and the cytokine storm in COVID-19 (190). A recent study on HFpEF supported that systemic inflammation may be the association between comorbidity and HF (191). The IL-1β and IL-18 signaling pathways may be novel drug targets for HFpEF, which are important in the mitochondria-inflammation circuit (192).

The alteration of pathways is often regulated by transcription factors as switches. Many common transcription factors have been found including SP-1, RELA, NF-κB, STAT3, HIF-1α, PPARγ, c-FOS, and c-JUN (**Figure 3D**) and together with a review of the literature, the transcription factors network in HF and comorbidities are briefly summarized as follows: (1) Regulation of inflammation. NF-κB is the key transcription factor in inflammation. Both RELA and NFKB1 are genes of NF-κB subunit. NF-κB regulates inflammation initiated Ca^{2+}/Calmodulin-dependent cardiac remodeling (193). STAT3 is a predicted target regulated by NF-κB in **Figure 3D**. The activation of NF-κB and STAT3 is required for the expression of multiple inflammatory cytokines including IL-1β (194), TNA-α (195) and IL-6. The c-FOS and c-JUN are family of AP-1, which regulate the MAPK pathway, and can be inhibited by SIRT3 (196). EGR1 and c-FOS are also associated with the release of IL-1β (197). SP-1 can regulate immune responses, but it is a non-specific transcription factor involved in many other cellular processes and indicates transcriptional activation; (2) Regulation of metabolism. The activation of PPARγ is essential for the FAO process (18). The sirtuin family members SIRT1, SIRT2, and SIRT3 are important transcription factors in cardiac energy metabolism and have similar roles. SIRT3 regulates ATP production (198). SIRT2 and PPARα regulate glycose metabolism by the AMPK pathway (199), SIRT1 and NRF2 regulate energy metabolism and mitochondrial biogenesis (200).

Common Therapeutic Drug Targets

Common pathways indicate common targets, which are the basis for drug repurposing. Network analysis is often used in the repurposing of drugs (201). The known drug targets of HF, diabetes mellitus, COPD, CKD, sleep apnea, and obesity were retrieved from the Target Validation Platform (targetvalidation.org). We constructed a disease-target network in Cytoscape 3.8.0 (202). Some representative drugs were randomly chosen and listed in **Figure 5** to provide an example. The drugs range from old drugs like metformin to relatively new ones in HF treatment, like SGLT2 inhibitors. However, network analysis has some limitations and should be interpreted combined with literature review. For one thing, it is based on the database, and some drugs in the database had been investigated in HF clinical trials but have no effect. Some drugs such as calcium channel blockers could not treat HF. For another, a drug associated with multiple targets might be non-specific and does not necessarily have better effects. For instance, doxorubicin inhibits both Top2a and Top2b, inhibiting Top2a have an anti-cancer effect while inhibiting Top2b have a cardiac side effect (125). Anti-inflammatory therapy with Canakinumab (203) in clinical trials which target IL-1β can reduce the mortality of HF patients. IL-1β is an important inflammatory cytokine associated with many comorbidities. Canakinumab can improve the prognosis of cardiovascular outcomes in patients with CKD (204). However, Canakinumab could not reduce the incidence of new-onset diabetes (205), which suggests the role of inflammation in diabetes might be less important. Anakinra, a recombinant IL-1 receptor antagonist, is another drug targets IL-1β, it is under phase III clinical trial in HF and has a therapeutic effect (206). In summary, IL-1β inhibitors/antagonists are promising drugs for HF and comorbidities.

Diabetes drugs are a good example of drug repurposing applied in HF. Some therapy of diabetes may increase the risk of HF such as insulin (183), whereas some drugs such as metformin, sulphonylureas, and gliptins either alone or in combination, could significantly reduce the risk of HF (207). The SGLT2 inhibitors are originally designed for diabetes, which targets the SLC5A2 gene, and have shown benefit for HF, regardless of whether comorbid with diabetes or not (208, 209). In a clinical trial, there were unexpected excellent risk reductions in hospitalization for HF and all-cause mortality with the use of the SGLT2 inhibitor, empagliflozin (210). The benefit of empagliflozin could not be explained by the effects of classical inhibitors, such as natriuresis or neurohormonal mechanisms. It has been speculated that the shift in cardiac energy substrate may play a major role in the cardiorenal benefits of empagliflozin; that is, a shift from using glucose and fat to ketone bodies (211). Linagliptin, a DPP-4 inhibitor designed to treat diabetes, can also be used to treat HF (212, 213). Metformin affects many targets that are associated with oxidative phosphorylation in mitochondria (214), such as MT-ND5 and NDUFB7, and has been reported to have therapeutic effects on HF and comorbidities. Metformin is an indirect AMPK pathway activator, and also increases glucose transport and catabolism by increasing the residence time of GLUT4. AMPK agonists are promising HF therapy drugs, which consist of direct activators, such as A-769662 (a preclinical drug), or indirect activators, such as 5′-aminoimidazole-4-carboxyamide-ribonucleoside (22, 215).

Although many anti-tumor drugs have cardio toxicity, network analysis of shared pathways and targets enables us to find drugs beneficial for both diseases. For example, PI3K/Akt/mTOR pathway is a shared pathway in cancers and HF, drugs targeting the mTOR pathway, such as rapamycin, are novel potential

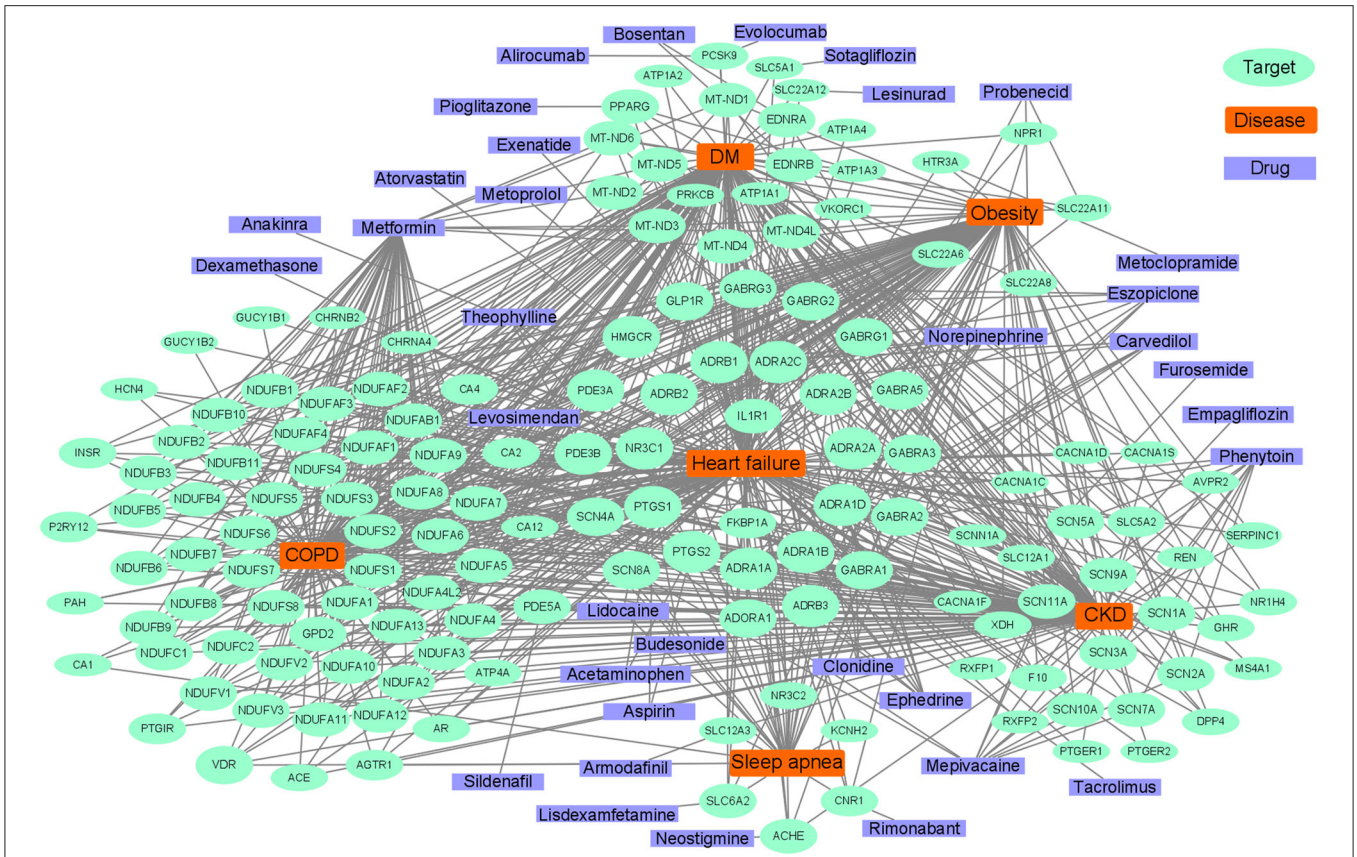

FIGURE 5 | Abridged common drug target network of heart failure and comorbidities.

drugs for HF which can reduce cardiac remodeling and HF (119).

There are some genes of the phosphodiesterase family, such as PDE5A, PDE3A, and PDE3B. PDE5 inhibitors (such as Sildenafil) regulates the nitric oxide synthases and hydrogen sulfide (H2S) generation, and may attenuate ROS induced mitochondrial dysfunction through the AMPK pathway (216). However, side effects largely limit its clinical application, probably because PDE5 is involved in a variety of biological processes not specific to HF.

Beyond known drug targets, some targets may have similar functions as they belong to the same protein family. Similar to SLC5A2, SLC25A51 is a member of the solute carrier family, and has been recently found to be a mitochondrial NAD+ transporter (217) and may perhaps serve as a new drug target.

Links Between Heart Failure Phenotypes and Comorbidity

Multi morbidity and HFpEF are both unmet needs in HF therapy. Comorbidities exist in both HFpEF and HFrEF, but the prevalence of most comorbidities is higher in the HFpEF than reduced ejection fraction (HFrEF) (6, 218), indicating a strong association between HFpEF and comorbidities (6). The prevalence of preserved ejection fraction HF (HFpEF) is rising, and mortality remains high because of the absence of effective therapies (60, 219), which gives rise to the urgent need for drug discovery targeting HFpEF. Although HFpEF has a better ejection fraction than HFrEF, the mortalities are similar, and the higher frequency of morbidities in HFpEF than HFrEF may explain the phenomena (220). The risk factors and incidence of comorbidities are different, therefore the pathways, therapeutic targets, and drugs between the subclasses of HF were different. COPD and OSA are associated with increased HFpEF disease risk and adversely impact cardiovascular disease outcomes, in which chronic inflammation and oxidative stress are responsible for the association. Therefore, drugs like statin and/or antioxidants may be beneficial (221, 222). Compared with HFrEF, there are more hypertension and fewer coronary diseases in HFpEF (218, 223). Atrial fibrillation is associated with significantly increased mortality (224), and AF is more frequently in HFpEF than HFrEF (218). Because multi morbidity is more frequent in HFpEF, targeting the common pathways between comorbidities may be a potential novel therapy for HFpEF.

Expression levels of biomarkers is also different between systolic and diastolic HF. The BNP level is lower in HFpEF

(225) and NT-proBNP/BNP-guided therapy was reportedly only beneficial in HFrEF because comorbidities may influence BNP level and provide misleading information (226).

CONCLUSION AND FUTURE PERSPECTIVES

In this review, we concluded the pathology and molecular mechanisms of comorbidities of HF. Metabolism remodeling and chronic inflammation are responsible for the major underlying pathophysiologic links between HF and comorbidities. Mitochondrial metabolism is expected to play a central role, but no drugs specifically conceived to modulate mitochondrial functions are currently available (227). The therapy for comorbidities of HF is increasingly becoming challenging. The common metabolic and inflammatory mechanisms may provide promising possible therapeutic targets for both HF and comorbidities, which may be useful for both old drug repurposing and the discovery of new drugs.

AUTHOR CONTRIBUTIONS

ZL performed an extensive literature review, drafted the manuscript, and prepared figures. JW and HZ proposed the subject of the review, critically revised, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Effect of Air Quality on the Risk of Emergency Room Visits in Patients With Atrial Fibrillation

Bin Liang[1,2†], *Xiaonan He*[3†], *Xin Du*[1], *Xiaoxia Liu*[1] *and Changsheng Ma*[1*]

[1] *Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China,* [2] *Department of Cardiology, The Second Hospital of Shanxi Medical University, Taiyuan, China,* [3] *Emergency Critical Care Center, Beijing Anzhen Hospital, Capital Medical University, Beijing, China*

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Chongqing Medical University, China

***Correspondence:**
Changsheng Ma
chshma@vip.sina.com

[†]*These authors have contributed equally to this work*

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Background: We investigated the effect of particulate matter with aerodynamic diameter <2.5 μm (PM2.5) and meteorological conditions on the risk of emergency room visits in patients with atrial fibrillation (AF) in Beijing, which is considered as a monsoon climate region.

Methods: In this case-crossover design study, medical records from patients with AF who visited the Critical Care Center in the Emergency Department of Anzhen Hospital from January 2011 through December 2014 and air quality and meteorological data of Beijing during the same period were collected and analyzed using Cox regression and time-series autocorrelation analyses.

Results: A total of 8,241 patients were included. When the average PM2.5 concentration was >430 $\mu g/m^3$, the risk of emergency room visits for patients with uncomplicated AF, AF combined with cardiac insufficiency, and AF combined with rheumatic heart disease increased by 12, 12, and 40%, respectively. When the average PM2.5 concentration was >420 $\mu g/m^3$, patients with AF combined with diabetes mellitus had a 75% increased risk of emergency room visits, which was the largest increase in risk among all types of patients with AF. When the average PM2.5 concentration was >390 $\mu g/m^3$, patients with AF combined with acute coronary syndrome had an approximately 30% increased risk of emergency room visits, which was the highest and fastest increase in risk among all types of patients with AF. The risk of emergency room visits for patients with AF was positively correlated with air quality as the time lag proceeded, with an autocorrelation coefficient of 0.223 between the risk of emergency room visits and air quality in patients with AF on day 6 of the time lag.

Conclusion: Exposure to certain concentrations of PM2.5 in a monsoon climate region significantly increased the risk of emergency room visits in patients with AF.

Keywords: PM2.5, atrial fibrillation, risk of emergency room visit, monsoon climate region, patients

INTRODUCTION

With the advancement in industrialization, air pollution is on the rise. The increase in the concentration of various pollutants, such as PM2.5, has been closely associated with various diseases, including lung, cardiovascular, kidney, and eye diseases, according to an increasing number of studies (1–4). A joint opinion from the World Heart Federation, American College of Cardiology, American Heart Association, and the European Society of Cardiology has been published against air pollution recently (5).

Epidemiological studies have shown that air pollution is associated with the development of cardiovascular disease (CVD) (6, 7). A study on the link between CVD and air pollution particulate matter found that a 10 $\mu g/m^3$ increase in PM concentration was associated with a 4% and 10% increase in the incidence of total CVD and ischemic heart disease, respectively (8). In addition, the number of CVD hospitalizations increased with increases in air pollution index (9). These studies suggested that exposure to a certain concentration of PM2.5 may significantly increase morbidity, hospitalization rates, and mortality rates of patients with CVD and that reducing PM2.5 exposure may improve cardiovascular event outcomes (10).

AF is the most common arrhythmia observed in clinical practice, with a complex etiology and an incidence that increases sharply with age (11, 12). Recent studies have shown that PM2.5 exposure may contribute to arrhythmogenesis (13). Additional studies have found that PM2.5 exposure is associated with increased risk of incident AF (14). Possible reasons include (i) the adverse effects of PM2.5 on cardiac autonomic regulation (15–17); (ii) possible inflammation, oxidative stress, and altered atrial pressure caused by PM2.5 (18–20); and (iii) other unidentified causes.

However, only a few studies have focused on the synergistic effects of air pollution and meteorological parameters on AF morbidity. Therefore, in this study, we analyzed the effect of meteorological conditions and PM2.5 exposure on the risk of emergency room visits in patients with AF in a monsoon climate region to further identify risk factors for AF and to provide a theoretical basis on the importance of air quality improvement.

MATERIALS AND METHODS

Data Related to PM2.5

Beijing is located in northern China, which is a monsoon climate region with four distinct seasons including cold and windy dry winters and hot and humid summers. In this study, four air quality monitoring stations within an average distance of 7.5 km from Beijing Anzhen Hospital were selected, and data on the 24-h average PM2.5 concentration from January 1, 2011 to December 31, 2014 were retrieved from the website of the Ministry of Ecology and Environment of the People's Republic of China and used as a representative air quality measurement for each day (http://www.mee.gov.cn/).

Data Related to Meteorological Conditions

The Beijing Regional Climate Center is an accurate and reliable electronic weather database. We collected data related to meteorological conditions from January 1, 2011 to December 31, 2014 from the measurement points of the 16 districts of Beijing through the website (http://bj.cma.gov.cn/). The main data included average temperature (°C), minimum temperature (°C), maximum temperature (°C), diurnal temperature range (DTR, °C), relative humidity (%), average wind speed, and air pressure (hPa). DTR refers to the difference between the maximum and minimum temperatures in 1 day.

Data Related to Clinical Information

Medical records from all patients with AF who visited the Critical Care Center in the Emergency Department of Anzhen Hospital from January 1, 2011 to December 31, 2014, were collected, including sex, age, comorbidities (diabetes, acute coronary syndrome, rheumatic heart disease, and cardiac insufficiency), and the time of emergency room visits. Private information, such as name and ID number, was not collected. The inclusion criteria for the medical study were residence in Beijing and its vicinity and a diagnosis of AF based on the International Statistical Classification of Diseases and Related Health Problems, 9th edition, confirmed by electrocardiography. Patients with atrial fibrillation caused by cor pulmonale, hyperthyroidism and traffic accidents were excluded.

Quality Control

Medical records were obtained from a tertiary referral center and uploaded after review to ensure accuracy. The PM2.5 concentration and meteorological conditions data were obtained from the release of the Ministry of Environmental Protection and the China Meteorological Administration, which are authoritative standards in this regard.

Statistical Methods

SPSS version 25.0 was used for statistical analysis. Normally distributed measures are expressed as mean ± standard deviation (SD), and discrete variables are described with median and interquartile range. In order to clarify the close degree and direction of the correlation between air quality and meteorological conditions, Pearson correlation was used to analyze the correlation between them. The daily frequency of atrial fibrillation was simulated by stepwise Cox regression analysis model to study the relationship between daily atrial fibrillation attacks and air quality PM2.5 concentration in patients with atrial fibrillation without other diseases, cardiac insufficiency, rheumatic heart disease, diabetes and acute coronary syndrome. The lagged correlation between air quality and AF episodes was investigated by autocorrelation analysis of the time series. $P < 0.05$ was considered as a statistically significant difference.

TABLE 1 | The information of air quality and meteorological conditions in Beijing, 2011–2014.

	Minimum	P25	Median	Mean (SD)	P75	Maximum
Average temperature (°C)	−10	1.30	13.60	12.53 (0.12)	23.30	32
Maximum temperature (°C)	−6	6.50	19.90	17.68 (0.13)	27.90	32
Minimum temperature (°C)	−14	−2.70	7.90	7.85 (0.12)	18.25	28
DTR (°C)	2	7.10	9.30	9.69	12.10	21
Relative humidity (%rh)	0	38	54	53.45 (0.22)	69.00	97
Average wind speed (m/s)	1	1.50	1.90	2.09 (0.01)	2.50	7
Average daily surface temperature (°C)	−12	0.20	14.20	13.36 (0.14)	25.10	37
Air pressure (kPa)	9,900	10,049	10,133	10,131.87 (1.09)	10,218	10,392
Air quality ($\mu g/m^3$)	3	42.42	84.04	105.96 (0.94)	146.38	552.50

Minimum refers to the minimum value; P25 refers to the 25th percentile; median refers to the median; P75 refers to the 75th percentile, and maximum refers to the maximum value. DTR:diurnal temperature range.

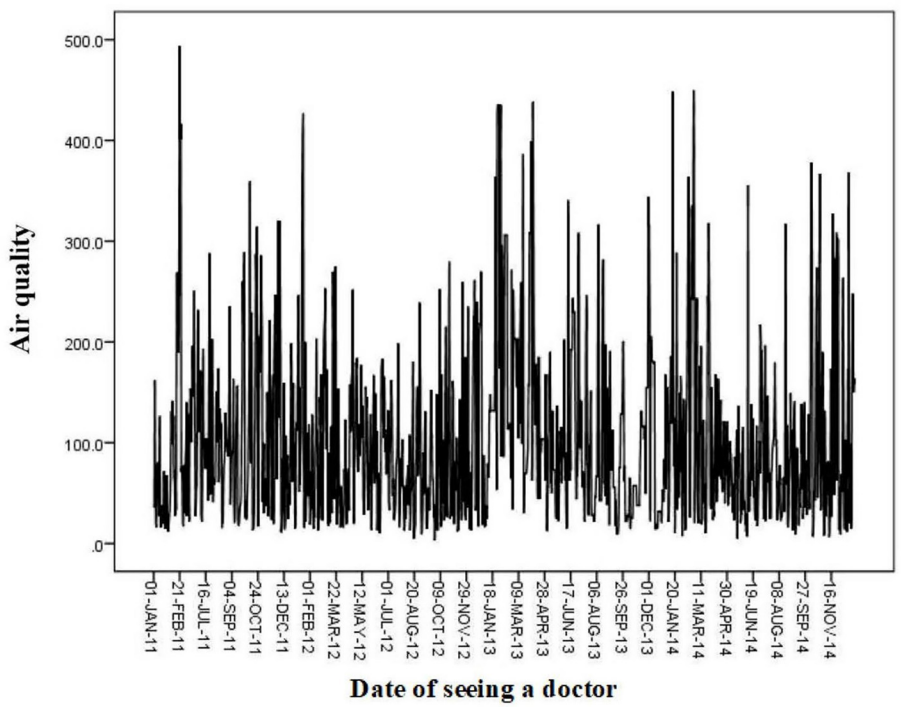

FIGURE 1 | Daily air quality, 2011–2014.

RESULTS

Characteristics of the Climate in Beijing

Beijing has four distinct seasons and a typical northern temperate and semi-humid continental monsoon climate. During 2011–2014, the annual average temperature in Beijing was 12.41–12.65°C, with the highest annual average temperature between 17.55°C and 17.81°C, the lowest annual average temperature between 7.73°C and 7.79°C, and the average PM2.5 concentration between 105.02 and 106.90 $\mu g/m^3$ (**Table 1**). Air quality was poor(PM2.5 concentration was >35 $\mu g/m^3$) in February and October 2011; February 2012; January, March, June, August, and December 2013; and January, March, June, August, September, and November 2014 (**Figure 1**). Pearson's correlation analysis, which was used to examine the correlation between air quality and meteorological conditions, showed that air quality was negatively correlated with average air temperature, maximum air temperature, minimum air temperature, diurnal temperature difference, average wind speed, and average daily surface temperature, and positively correlated with relative humidity (**Table 2**).

TABLE 2 | Pearson's correlation analysis of air quality and meteorological conditions.

	Average temperature	Maximum temperature	Minimum temperature	DTR	Relative humidity	Average wind speed	Average daily surface temperature	Air pressure	Air quality
Average temperature	1	0.988**	0.982**	0.123	0.317**	−0.009	0.988**	−0.870**	−0.143**
Maximum temperature	0.988**	1	0.949**	0.255	0.262**	−0.018	0.980**	−0.866**	−0.151**
Minimum temperature	0.982**	0.949**	1	−0.051	0.405**	−0.008	0.964**	−0.853**	−0.112**
DTR	0.123	0.255	−0.051	1	−0.429	−0.023	0.153	−0.127	−0.115**
Relative humidity	0.317**	0.262**	0.405**	−0.429	1	−0.452**	0.283**	−0.347**	0.366**
Average wind speed	−0.009	−0.018	−0.008	−0.023	−0.452**	1	0.024*	−0.012	−0.260**
Average daily surface temperature	0.988**	0.980**	0.964**	0.153	0.283**	0.024*	1	−0.860**	−0.144**
Air pressure	−0.870**	−0.866**	−0.853**	−0.127	−0.347**	−0.012	−0.860**	1	0.015
Air quality	−0.143**	−0.151**	−0.112**	−0.115**	0.366**	−0.260**	−0.144**	0.015	1

*$P < 0.05$ (two-tailed) with significant correlation; **$P < 0.01$ (two-tailed) with significant correlation.

Characteristics of Patients With AF

From 2011 to 2014, 8,241 patients with AF in Beijing and its vicinity were treated in the Critical Care Center at the Emergency Department of Anzhen Hospital. The mean age of all patients with AF observed in the Emergency Department was 60 years, and the mean age of patients with uncomplicated AF and AF combined with diabetes, acute coronary syndrome, rheumatic heart disease, or cardiac insufficiency was 60.08, 60.85, 57.37, 56.43, and 64.44 years, respectively (**Table 3**). More patients with AF visited the Emergency Department in October and December 2011; February and June 2012; January, March, June, and December 2013; and January, March, April, June, September, and November 2014 (**Figure 2**), which was generally consistent with the months with poor air quality, as shown in **Figure 1**, suggesting that poor air quality may have led to an increase in the number of emergency visits for patients with AF.

Impact of PM2.5 on the Risk of Emergency Room Visits for Patients With AF

Cox regression analysis showed that air pollution increased the risk of emergency room visits for patients with AF. When the average PM2.5 concentration was >430 $\mu g/m^3$, the risk of emergency room visits for patients with uncomplicated AF, AF combined with cardiac insufficiency, and AF combined with rheumatic heart disease increased by 12, 12, and 40%, respectively (**Figures 3A–C**). When the average PM2.5 concentration was >420 $\mu g/m^3$, patients with AF combined with diabetes mellitus had a 75% increased risk of emergency room visits, which was the largest increase in risk among all types of patients with AF (**Figure 3D**). When the average PM2.5 concentration was >390 $\mu g/m^3$, patients with AF combined with acute coronary syndrome had an ∼30% increased risk of emergency room visits, which was the fastest increase in risk among all types of patients with AF (**Figure 3E**).

Autocorrelation Analysis of Air Quality and Time Series Data of Emergency Room Visits for AF

The effect of short-term PM2.5 exposure on the risk of emergency room visits for patients with AF was investigated by autocorrelation analysis of the time series data. The results showed that air quality was positively associated with the risk of emergency room visits for patients with AF with a time lag, and the autocorrelation coefficient for air quality and the risk of emergency room visits for AF was 0.223 on day 6 of the time lag (**Table 4**).

DISCUSSION

A study published in the *Lancet* found that air pollution contributed to CVD morbidity and premature human death (21). The American Heart Association indicated that there was a clear causal relationship between air pollutants and CVD morbidity. The World Health Organization reported that air pollution was strongly associated with CVD mortality. There is also clear

TABLE 3 | Statistical description of the general information of patients with atrial fibrillation visiting the Emergency Department in 2011–2014.

	Minimum (years)	P25 (years)	Median (years)	Mean (SD) (years)	P75 (years)	Maximum (years)
Age	18	51	60	60 (14.37)	71	98
Atrial fibrillation	18	51	60	60.08 (0.16)	71	98
Atrial fibrillation combined with diabetes mellitus	21	53	61	60.85 (0.53)	73	96
Atrial fibrillation combined with acute coronary syndrome	26	54	58	57.37 (0.69)	63	88
Atrial fibrillation combined with rheumatic heart disease	18	47	49	56.43 (0.57)	67	86
Atrial fibrillation combined with cardiac insufficiency	26	56	71	64.44 (1.12)	72	87

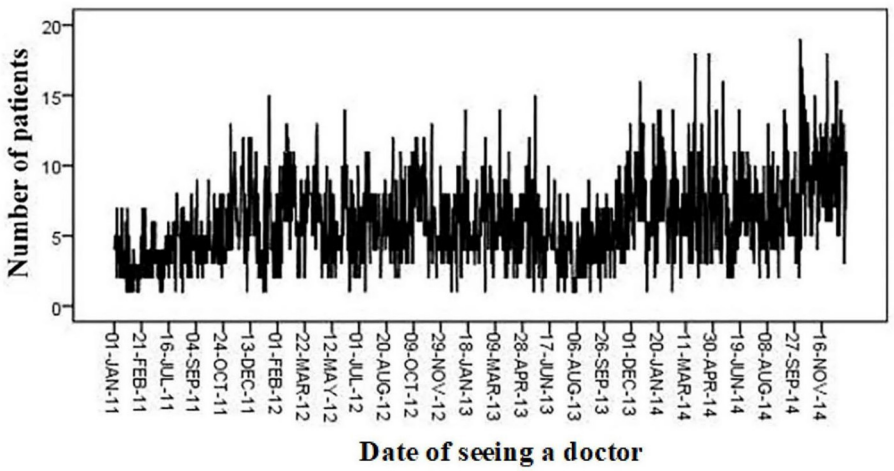

FIGURE 2 | Number of patients with atrial fibrillation seen in emergency departments per day, 2011–2014.

research evidence in China that air pollutant PM2.5 affected the clinical outcomes of CVD (22).

Short-term air pollution exposure may lead to a variety of cardiac arrhythmias, including AF. Several studies suggested that air pollutants may cause cardiac autonomic dysregulation, which may induce atrial electrophysiological changes (23–26). In addition, inflammation and oxidative stress may also cause changes in atrial pressure, leading to episodes of AF.

There is a lack of epidemiological evidence on the effects of air pollution on the incidence, recurrence, and clinical outcomes of AF. To date, only two studies have investigated the association between exposure to air pollution and the incidence of AF. One of these studies examined the association between AF and nitrogen dioxide, whereas the other study examined the relationship between PM2.5 and PM10 and AF (27). These studies suggested an association between air pollution and AF. Consistent with this, we found that the risk of AF episodes increased with poorer air quality, which may be associated with higher PM2.5 concentrations. The risk of AF episodes increased significantly when PM2.5 was >390 $\mu g/m^3$, and the risk of emergency room visits increased abruptly, which was consistent with the results of previous studies.

Several time-series analyses have examined the short-term effects of air pollution on cardiovascular events and found that exposure to air pollution (especially PM2.5 and NO_2) increased the risk of AF (28, 29). In this study, we found a significant positive association between PM2.5 concentration and the risk of emergency room visits in patients with AF by time-series autocorrelation analysis. This result suggested that short- to medium-term exposure to certain PM2.5 concentrations was significantly associated with the risk of emergency room visits for patients with AF. This finding is consistent with the evidence of the arrhythmogenic effects of PM2.5 mentioned above.

In previous epidemiological studies examining the correlation between short-term air pollution exposure and AF, mainly hospitalized patients or individuals with continuous rhythm monitoring (i.e., patients with implanted devices or individuals undergoing ambulatory electrocardiographic monitoring) were included (30). Most studies examined patients with implantable

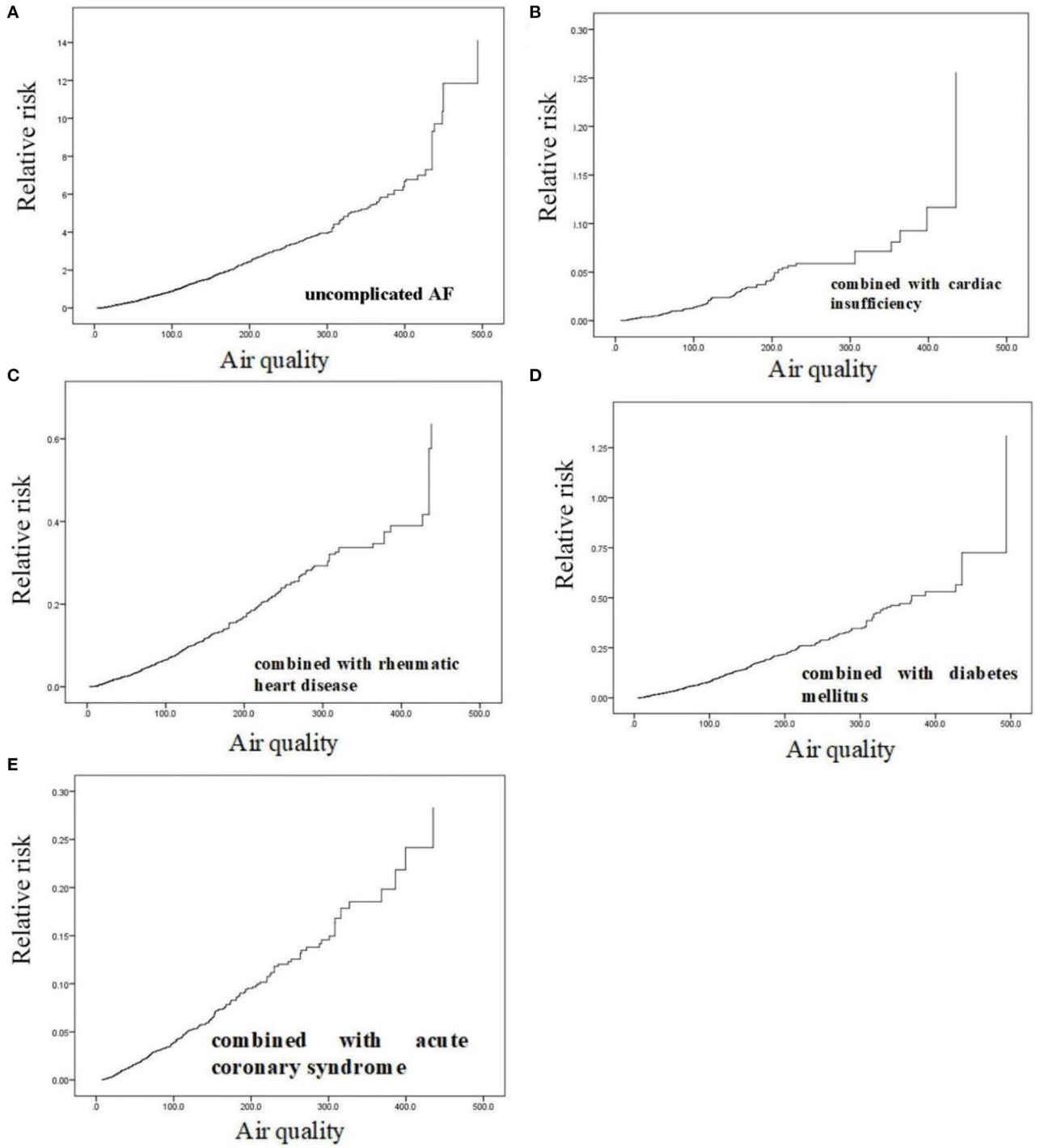

FIGURE 3 | Correlation analysis of particulate matter with aerodynamic diameter <2.5 μm and risk of emergency room visits for patients with atrial fibrillation. **(A)** uncomplicated AF; **(B)** AF combined with cardiac insufficiency; **(C)** AF combined with rheumatic heart disease; **(D)** AF combined with diabetes mellitus; **(E)** AF combined with acute coronary syndrome.

cardioverter defibrillators (ICDs) and revealed an association between PM and non-allergic diseases or episodes of ventricular fibrillation (31). However, because heart failure is one of the most common causes for the implantation of an ICD, the clinical characteristics of patients with ICDs are not representative of those of the general population; therefore, the finding that PM

TABLE 4 | Autocorrelation analysis of the time series of the air quality and risk of emergency room visits for patients with atrial fibrillation.

Number of days lagged	Autocorrelation	Standard error	Value	Significance
1	−0.467	0.027	292.814	0.000
2	−0.028	0.027	293.899	0.000
3	−0.044	0.027	296.449	0.000
4	0.101	0.027	310.117	0.000
5	−0.046	0.027	312.920	0.000
6	0.223	0.027	380.078	0.000

concentration had an effect on the incidence of AF in patients with ICDs in a high-risk susceptible population cannot be extrapolated to the general population. In this study, data from all patients with AF visiting the Emergency Department of Anzhen Hospital during the study period were collected, and the results of the study were more relevant to the actual clinical level.

This study is of great clinical significance; however, it also has limitations. First, information was collected through the Anzhen Hospital patient record database, where only patient records with a clear AF diagnosis by a physician could be obtained, which may have led to missing of undiagnosed cases. Second, the date of AF diagnosis was used, which may not be the exact date of AF onset. Third, considering the spontaneous termination or intervention, the true incidence of paroxysmal AF may have been underestimated. Finally, this study used data related to air pollution only at the patients' place of residence and lacked information on exposure to pollutants in other places, including exposure in occupational settings or while commuting, as well as indoor pollutants; thus, we could not accurately characterize the cumulative exposure to environmental and indoor air pollution. Our next steps will be to thoroughly examine potential susceptible subgroups, understand the impact of environmental exposures on CVDs, and gain a deeper understanding of the overall human cost of economic development by finding the true time of AF onset and collecting information on other air pollution exposures in patients.

Exposure to certain concentrations of PM2.5 significantly increased the risk of emergency room visits in patients with AF. There was a correlation between short- and medium-term exposure to the air pollutant PM2.5 and emergency room visits in patients with AF. These findings highlight the importance of air quality improvement and provide a rationale for developing interventions to reduce CVD risk in the population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

BL and XH: study concept, design, analysis, interpretation of data, and drafting of the article. BL and XL: drafting of the article. XD and CM: critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
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Effect of Uric Acid-Lowering Agents on Patients With Heart Failure: A Systematic Review and Meta-Analysis of Randomised Controlled Trials

Hongxuan Xu[1,2], *Yunqing Liu*[1,2], *Lingbing Meng*[1,3], *Li Wang*[2,4] *and Deping Liu*[1,3,5]*

[1] *Department of Cardiology, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, China,* [2] *The Key Laboratory of Geriatrics, Beijing Institute of Geriatrics, Beijing Hospital National Center of Gerontology, National Health Commission, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, China,* [3] *Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China,* [4] *Departments of Neurology, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, China,* [5] *Peking University Health Science Centre, Peking University Fifth School of Clinical Medicine, Beijing, China*

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****Correspondence:***
Deping Liu
lliudeping@263.net

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Background: Elevated serum uric acid (SUA) level is considered an independent predictor of all-cause mortality and the combined endpoint of death or readmission in cardiovascular disease patients. However, the causal relationship between uric acid-lowering therapies (ULTs) and heart failure is still controversial.

Design: Meta-analyses were performed to systematically compile available evidence to determine the overall effect of ULTs on heart failure patients.

Method: We conducted this systematic review following the PRISMA statement guidelines. Databases were searched to identify randomised controlled trials related to the influence of a ULT intervention in people with heart failure. Data extracted from the included studies were subjected to a meta-analysis to compare the effects of ULTs to a control.

Results: Pooled analysis of left ventricular ejection fraction (LEVF) showed an insignificant result towards the ULT group (MD, 1.63%; 95%CI, −1.61 to 4.88; $p = 0.32$; three studies). Pooled analysis of the 6-Minute Walk Test (6MWT) showed an insignificant result towards the ULT group (MD, 4.59; 95%CI, −12.683 to 22.00; $p = 0.61$; four studies). Pooled analysis of BNP/NT-pro-BNP led to a nearly statistically significant result towards the ULT group (SMD, −0.30; 95%CI, −0.64 to 0.04; $p = 0.08$; five studies). Pooled analysis of all-cause mortality and cardiovascular death between ULTs (all XOIs) and placebo did not show a significant difference (RR, 1.26; 95% CI, 0.74 to 2.15, $p = 0.39$).

Conclusion: ULTs did not improve LVEF, BNP/NT-pro-BNP, 6MWT, all-cause mortality, and CV death in heart failure patients. UA may just be a risk marker of heart failure.

Keywords: uric acid, hyperuricemia (HUA), heart failure, left ventricular ejection fraction, six minute walk test, B type natriuretic peptide, mortality

INTRODUCTION

Since we lack the uricase enzyme, which oxidises uric acid (UA) to allantoin, UA is the final metabolite of purine's degradation by xanthine oxidase (XO) enzyme in humans and some primates. The liver, intestines, muscle, and vascular endothelium produce UA from food digestion and endogenously synthesised purine compounds. Hyperuricemia is defined as having a serum uric acid (SUA) level of >7.0 mg/dl in men and >5.7 mg/dl in women, and the prevalence of hyperuricemia in the USA is above 20% both in men and women (1).

Heart failure (HF) is a lethal chronic condition that affects at least 26 million people worldwide and increases prevalence (2). Despite HF treatments having progressed remarkedly over the past three decades, HF with preserved ejection fraction (HFpEF), where its morbidity and mortality are on par with HF with reduced ejection fraction (HFrEF), still needs treatments proven to be effective (3).

UA has an antioxidant effect that is considered preventive for cardiovascular disease (CVD), such as atherosclerosis (4, 5). Nevertheless, xanthine oxidoreductase (XOR) produces UA and brings out reactive oxygen species (ROS); inhibiting XO activity improves myocardial function in patients with idiopathic dilated cardiomyopathy, suggesting that XO activity may contribute to energy metabolism in human heart failure (6, 7). These findings implicate an intricate role of xanthine oxidase activity involved in the development of CVD.

A meta-analysis involving 12,854 acute heart failure patients indicated that elevated SUA level is an independent predictor of all-cause mortality and the combined endpoint of death or readmission in acute heart failure patients (8). One cohort study showed that UA is a predictor of both short-term and long-term mortality of the elderly admitted to the medical department (9, 10). Although a plethora of evidence supports the association between hyperuricemia and increased mortality risk, it is still controversial whether UA plays a role as a risk marker or risk factor in CVD and cardiometabolic disease.

Synthesised RCT data showed no difference in cardiovascular events among urate-lowering therapies (ULTs) in gout (11). Xanthine oxidase inhibitor (XOI) did not significantly reduce the risk of major adverse cardiovascular events and death (12). Conflicting evidence suggests a reverse causality of the relationship between SUA elevation and CVD (13). The purpose of this meta-analysis is to assess the effect of ULTs on HF patients.

METHODS

We conducted this systematic review under the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement guidelines (14).

We carried out a keyword search using the terms "uric acid," "heart failure," and "randomised controlled trials" and ULTs like "Uricosuric" and "Xanthine oxidase inhibitor." We searched Ovid MEDLINE, Web of Science, EMBASE, PubMed, ClinicalTrials, Google Scholar, and Cochrane Library databases from the start date of September 20, 2020. These databases were searched using a combination of subject headings (such as Medical Subject Headings), filters (such as "RCT"), or PICOS framework when available. We also reviewed the references of included studies to identify additional pertinent studies. We imposed no language or time restriction. A protocol was developed before commencing this review on PROSPERO [CRD42020209883].

Inclusion and Exclusion Criteria

Two reviewers independently assessed the records identified from the search for eligibility. Any discrepancies were resolved by consensus. We included any randomised controlled trials comparing ULTs vs. placebo in adult heart failure patients. The target population was adults (aged 18 years and above) with heart failure (as defined by New York Heart Association). The outcome had to objectively measure cardiac capacity and mortalities. We excluded studies that were not placebo controlled and missing critical data. Studies that followed or treated the patients with XOI for <4 weeks or did not measure heart functions and mortalities were excluded. Studies whose intervention groups received co-interventions were also excluded.

Study Quality

The two authors assessed study quality based on the seven domains defined by the Cochrane Collaboration tool for assessing the risk of bias (15): (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; (5) incomplete outcome data; (6) selective reporting; and (7) other biases, including baseline imbalance, early stopping, and bias due to vested financial interest or academic bias.

Data Extraction

One author (XHX) extracted all data, and both authors (XHX and LYQ) reviewed the data for accuracy. The following data were collected: country, duration of the trial, date of publication; numbers of individuals included, inclusion criteria, exclusion criteria, baseline characteristics, intervention, and control; outcomes: SUA change, left ventricular ejection fraction (LVEF), Six-Minute Walk Test (6MWT), brain natriuretic peptide/N-terminal Brain Natriuretic Peptide (BNP/NT-BNP), all-cause mortality, and cardiovascular death.

We used Origin 2019b (OriginLab Corporation) to extract data from the figures. We used the methods derived from Wan et al. to estimate the sample mean and standard deviation (SD) from the sample size, median, and interquartile range (16).

Data Synthesis

Meta-Analyses

Meta-analysis was performed with Review Manager 5.4.1. We used a random-effect model and calculated the mean difference (MD) to generate pooled estimates of LVEF and 6MWT changes, a random-effect model and standard mean difference (SMD) to calculate the intervention effects of SUA and BNP/NT-BNP across studies. Mortality data were performed by risk ratio (RR) using random-effect Mantel-Haenszel methods (17). We

calculated the standard deviation using an assumption of a 0.5 correlation or deriving correlation from studies with existing data for studies that did not report the standard deviation of the mean of change, following the Cochrane Handbook for Systematic Reviews of Interventions. The I^2 statistic was used to assess the degree of statistical heterogeneity (0–40%: might not be important, 30–60%: moderate heterogeneity, 50–90%: substantial heterogeneity, 75–100%: considerable heterogeneity) (18). *P*-values <0.05 were considered statistically significant.

We conducted subgroup analyses to explore the potential causes of heterogeneity for treatment effect on different ULT methods (XOIs vs. uricosuric drugs).

Trial Sequential Analysis

Trial sequential analysis is a methodology that considers how much information is needed to anticipate a specific required information size (19). We used the TSA program version 0.9.5.10 Beta (Copenhagen Trial Unit) to adjust the confidence intervals due to sparse data and repetitive testing of cumulative data and calculate the required information size. If the cumulative Z-curve crosses a trial sequential monitoring boundary or enters the futility area, the required information size may have reached a sufficient level of evidence. Conversely, the conclusive evidence is insufficient if the Z-curve does not cross any boundaries. The required information size was calculated based on autogenerated empirical data per input data. We performed the trial sequential analysis at the level of an overall 5% risk of type I error and a power of 20%.

Sensitivity Analysis

We conducted a *post-hoc* sensitivity analysis to assess a single study's impact on overall heterogeneity.

RESULTS

Included Studies

Six studies (20–25), with a total of 864 participants, fulfilled the inclusion criterion (**Figure 1**). SUA was assessed in

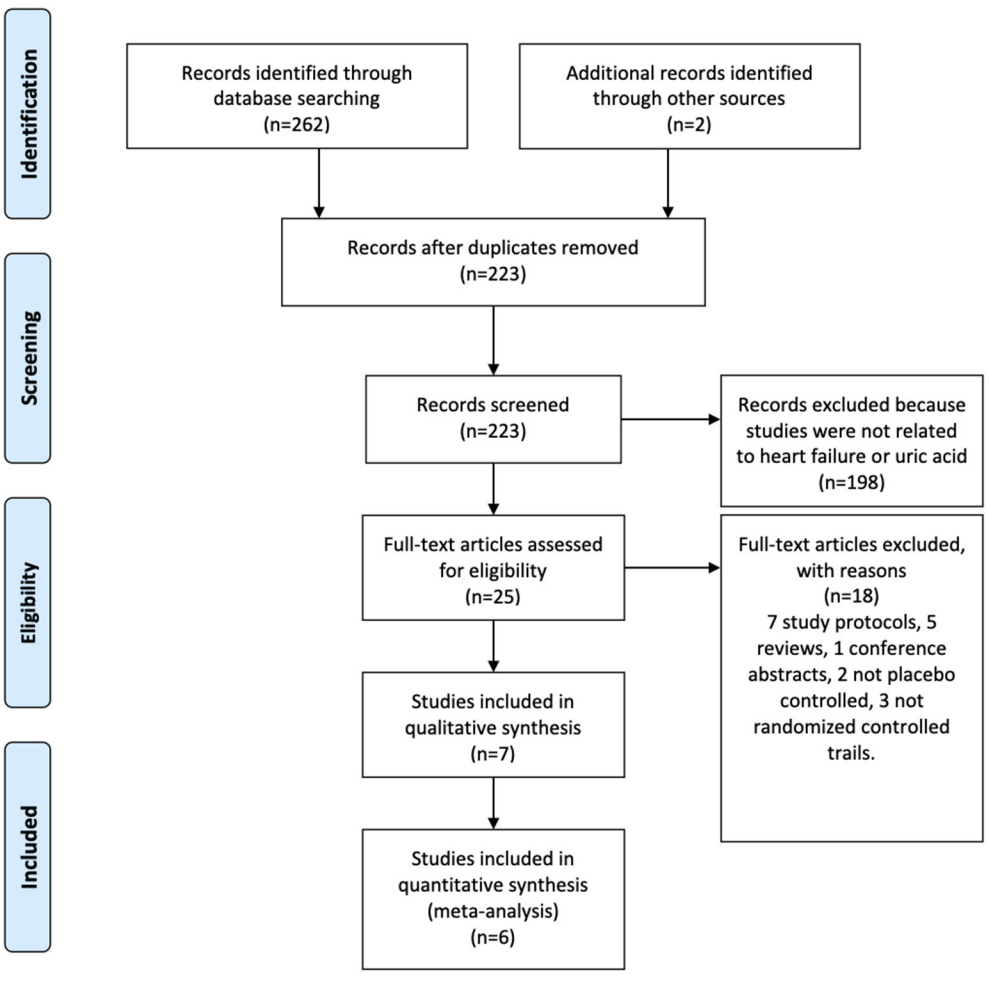

FIGURE 1 | Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

five studies, LVEF in three studies, BNP/NT-BNP in five studies, 6MWT in four studies, and mortality in two studies. **Table 1** summarises the characteristics of the included studies.

The most common SUA-lowering therapy was XOI (four out of six studies). The follow-up duration ranged from 4 to 24 weeks, with a median of 10 weeks. The mean age was 62 years (range, 58–69). Men accounted for 66% (range, 36–82%) of the pooled population. The ULTs were uricosuric drugs (benzbromarone, probenecid) and XOIs (allopurinol, oxypurinol).

Risk of Bias and Grade

Five studies did not provide enough details regarding the method of randomisation other than just stating that it was a randomised trial (20–23, 25). Hence, the random sequence generation and allocation concealment were unclear. One study was deemed to have a high risk of incomplete outcome data (25). **Figure 2** summarised the risk of bias. **Table 2** summarised the GRADE evidence table.

Left Ventricular Ejection Fraction

The LVEF at baseline in the control group (23.4% ± 7.7) was significantly lower than the intervention group (25.6% ± 8.6) in (24). The LVEF at baseline in Ogino et al. (49.5 ± 12.7) is higher than the other two studies due to different inclusion criteria. Pooled analysis of LEVF (**Figure 3**) showed an insignificant result towards the ULT group (MD, 1.63%; 95%CI, −1.61 to 4.88; $p = 0.32$; three studies) with substantial heterogeneity ($I^2 = 82\%$), a test for subgroup heterogeneity showed low heterogeneity ($I^2 = 0\%$).

The effect size was mainly powered by Cingolani et al. (21). After excluding this study, the result was still insignificant (MD, 0.01; 95% CI, −1.86 to 1.88; $p = 0.99$) but with low heterogeneity ($I^2 = 0\%$). A test for subgroup heterogeneity showed low heterogeneity ($I^2 = 0\%$) (**Figure 4**).

TSA using a random-effect model showed that the cumulative Z-curve of LVEF neither crossed a trial sequential monitoring boundary nor entering the futility area (**Figure 5**).

B-Type Natriuretic Peptide and N-Terminal-Pro-B-Type Natriuretic Peptide

Pooled analysis of BNP/NT-pro-BNP (**Figure 6**) showed a nearly statistically significant result towards the ULT group (SMD, −0.30; 95% CI, −0.64 to 0.04; $p = 0.08$; five studies) with substantial heterogeneity ($I^2 = 72\%$), a test for subgroup heterogeneity showed substantial heterogeneity ($I^2 = 81.5\%$).

The difference between subgroups was significant ($p = 0.02$). The XOI group showed an insignificant result (SMD, −0.03; 95% CI, −0.17 to 0.12; $p = 0.78$; three studies) with low heterogeneity ($I^2 = 0\%$). The uricosuric group led to a significant effect (SMD, −1.01; 95% CI, −1.83 to 0.19; $p = 0.02$; two studies) with moderate to substantial heterogeneity ($I^2 = 59\%$).

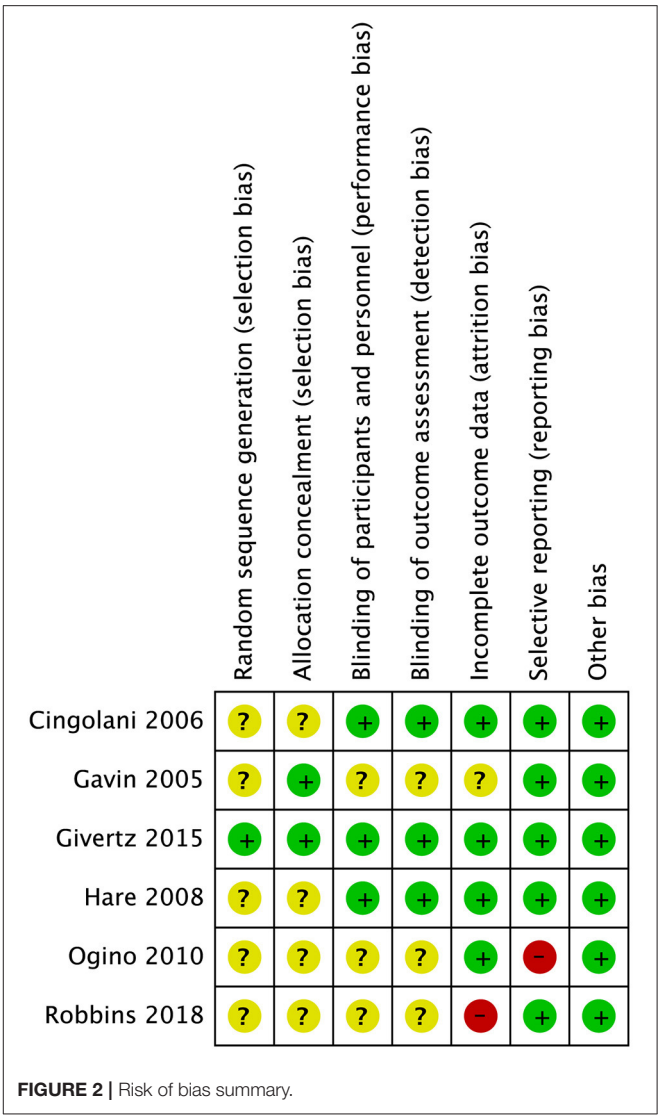

FIGURE 2 | Risk of bias summary.

Six-Minute Walk Test

Pooled analysis of 6MWT (**Figure 7**) showed an insignificant result towards the ULT group (MD, 4.59; 95% CI, −12.683 to 22.00; $p = 0.61$; four studies) with substantial heterogeneity ($I^2 = 76\%$), a test for subgroup heterogeneity showed low heterogeneity ($I^2 = 0\%$).

However, similar to LVEF, after excluding Cingolani et al., the overall heterogeneity drastically reduced to $I^2 = 0\%$. The effect size turned towards the control group (MD, −3.47; 95% CI, −14.84 to 7.90; $p = 0.55$; three studies) (**Figure 8**).

TSA using a random-effect model showed that the cumulative Z-curve of 6MWT neither crossed a trial sequential monitoring boundary nor entering the futility area (**Figure 9**).

TABLE 1 | Characteristics of included studies.

References	Age (control/ intervention)	Male %	Inclusion criteria	Intervention	Control	Duration	Intervention protocol	Outcomes measured
Gavin and Struthers (20)	67	77	NYHA II-III	Allopurinol (n = 44)	Placebo (n = 44)	12 week	Allopurinol 300 mg/day	BNP, 6MWT
Cingolani et al. (21)	66.7/70.6	60/63	NYHA II-III, LVEF ≤40%, 6MWT <425 m	Oxypurinol (n = 21)	Placebo (n = 26)	1 month	Oxypurinol 100 mg/day a week, then 600 mg/day	SUA, LVEF, 6MWT
Hare et al. (22)	65/64	70/76	NYHA III-IV, LVEF ≤40%	Oxypurinol (n = 203)	Placebo (n = 202)	24-week	Oxypurinol 100 mg/day a week, then 600 mg/day	SUA, BNP, mortality
Ogino et al. (23)	59/62	75/74	NYHA I-III, hyperuricemia (UA >7.0 mg/dl)	Benzbromarone (n = 14)	Placebo (n = 14)	8-week	Benzbromarone 50 mg/day	SUA, BNP, LVEF
Givertz et al. (24)	63/63	78/86	LVEF ≤40%, SUA ≥9.5 mg/dl	Allopurinol (n = 128)	Placebo (n = 125)	24-week	Allopurinol 300 mg/day, then 600 mg/day if tolerated	SUA, NT-pro-BNP, LVEF, 6MWT, mortality
Robbins et al. (25)	57.7	80	NYHA II-IV, LVEF ≤40%	Probenecid (n = 20)	Placebo (n = 20)	4-week	Probenecid 2 g/d (twice daily)	NT-pro-BNP, 6MWT

NYHA, New York Heart Association; SUA, serum uric acid; BNP, B-type natriuretic peptide; NT-pro-BNP, N-terminal-pro-B-type natriuretic peptide; LVEF, left ventricular ejection fraction; 6MWT, Six-Minute Walk Test.

All-Cause Mortality and Cardiovascular Death

Overall, the pooled analysis of all-cause mortality and cardiovascular death between ULTs (all XOIs) and placebo did not show a significant difference (RR, 1.26; 95% CI, 0.74 to 2.15; $p = 0.39$) with low heterogeneity ($I^2 = 0\%$) (**Figure 10**).

The all-cause mortality is slightly in favour of the control group (RR, 1.36; 95% CI, 0.68 to 2.73; $p = 0.39$).

DISCUSSION

This meta-analysis examined the relationship between ULTs and all-cause mortality, CV mortality, BNP/NT-pro-BNP, 6MWT, and LVEF in HF patients. We did not find any statistically significant difference between ULTs and placebos. However, it did show a significant effect in favour of the uricosuric group (benzbromarone and probenecid) on BNP/NT-pro-BNP.

A large quantity of experimental and clinical data suggests that oxidative stress contributes to ventricular and vascular remodelling and disease progression in HF. XO is a potent source of oxidative stress, and therefore an intuitive target for therapy.

Probenecid is an agonist of transient receptor potential vanilloid 2 (TRPV2), which may increase myocardial contractility *via* increased calcium cycling on a beat-to-beat basis (26). It increased the force generation and calcium sensitivity of single cardiomyocytes, consistent with improved cardiac function observed in patients with HFrEF (25). Even though the results of Robbins et al. were compatible with most pooled results of included studies, it was a source of underlying heterogeneity. Cingolani et al. (21) had the most significant SUA reduction and the shortest follow-up period; it may indicate a short-term protective effect of XOI.

Previous studies indicated that XOI improves peripheral vasodilator capacity and blood flow, both locally and systemically in hyperuricemia CHF patients (27). Moreover, acute exposure of arteries and aortas isolated from aged WKY rats to a high UA concentration did not provoke changes in endothelial function (28). Allopurinol may improve endothelial function by reducing vascular oxidative stress and not in urate reduction (29). However, similar to our results, several recent meta-analyses, including observational studies, demonstrated that XOI did not have a long-term protective effect regarding all-cause mortality and cardiovascular death (30, 31).

Imbalanced nitric oxide (NO) bioavailability may induce impairment of endothelium-dependent relaxation due to an imbalance between endothelium-derived relaxing factors (EDRFs) and endothelium-derived contracting factors (EDCFs) (32). XOR and UA may decrease endothelial NO bioavailability through multiple mechanisms (33). Despite that ROS is produced during the XOR process, XOR is not the only pathway that contributes to nitroso-redox

TABLE 2 | GRADE evidence table.

Certainty assessment							No of patients		Effect		Certainty	Importance
No of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Uric lowering therapies**	**Placebo**	**Relative (95% CI)**	**Absolute (95% CI)**		
Left ventricular ejection fraction												
3	Randomised trials	Not serious	Not serious	Not serious	Serious[a]	None	163	165	–	MD **1.63 % higher** (1.61 lower to 4.88 higher)	⨁⨁⨁◯ moderate	Important
B-type natriuretic peptide and N-terminal-pro-B-type natriuretic peptide												
5	Randomised trials	Not serious	Serious[b]	Not serious	Not serious	None	387	383	–	SMD **0.3 SD lower** (0.64 lower to 0.04 higher)	⨁⨁⨁◯ moderate	Important
Six-Minute Walk Test												
4	Randomised trials	Not serious	Not serious	Not serious	Not serious	None	170	167	–	MD **3.47 m lower** (14.84 lower to 7.9 higher)	⨁⨁⨁⨁ high	Critical
Cardiovascular death												
2	Randomised trials	Not serious	Not serious	Not serious	Not serious	None	13/331 (3.9%)	11/327 (3.4%)	**RR 1.16** (0.41 to 3.23)	**5 more per 1,000** (from 20 fewer to 75 more)	⨁⨁⨁⨁ high	Critical
All-cause mortality												
2	Randomised trials	Not serious	Not serious	Not serious	Not serious	None	18/331 (5.4%)	13/327 (4.0%)	**RR 1.36** (0.68 to 2.73)	**14 more per 1,000** (from 13 fewer to 69 more)	⨁⨁⨁⨁ high	Critical

[a] *The numbers of studies and participants are relatively low.*

[b] *The results of the two subgroups are inconsistent. RR: relative risk. m: meters.*

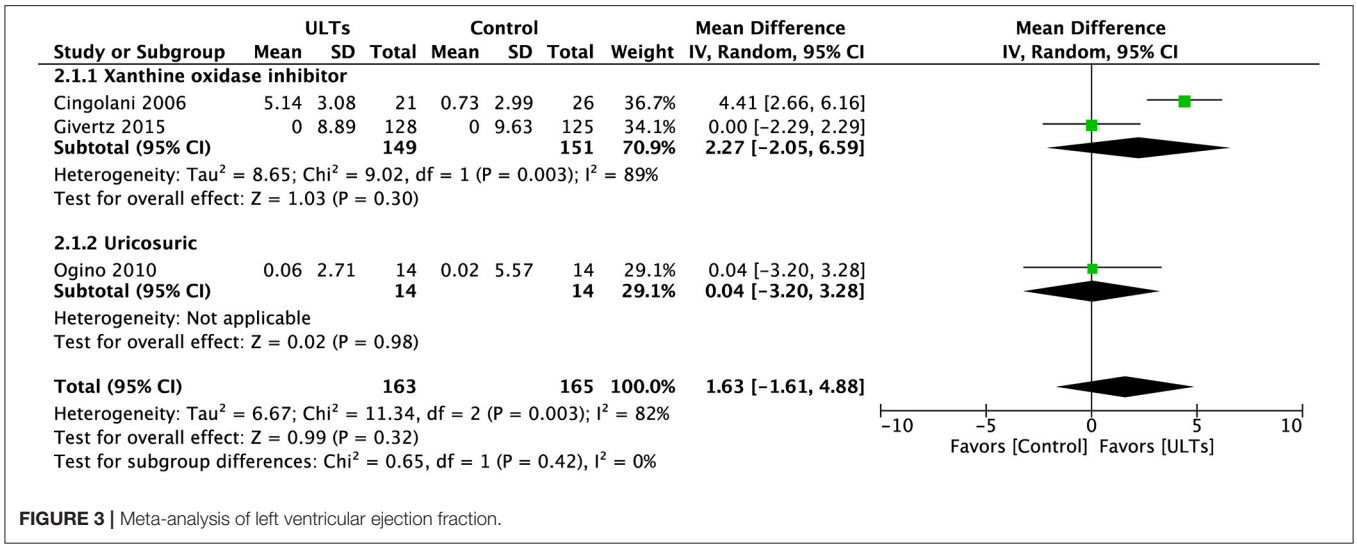

FIGURE 3 | Meta-analysis of left ventricular ejection fraction.

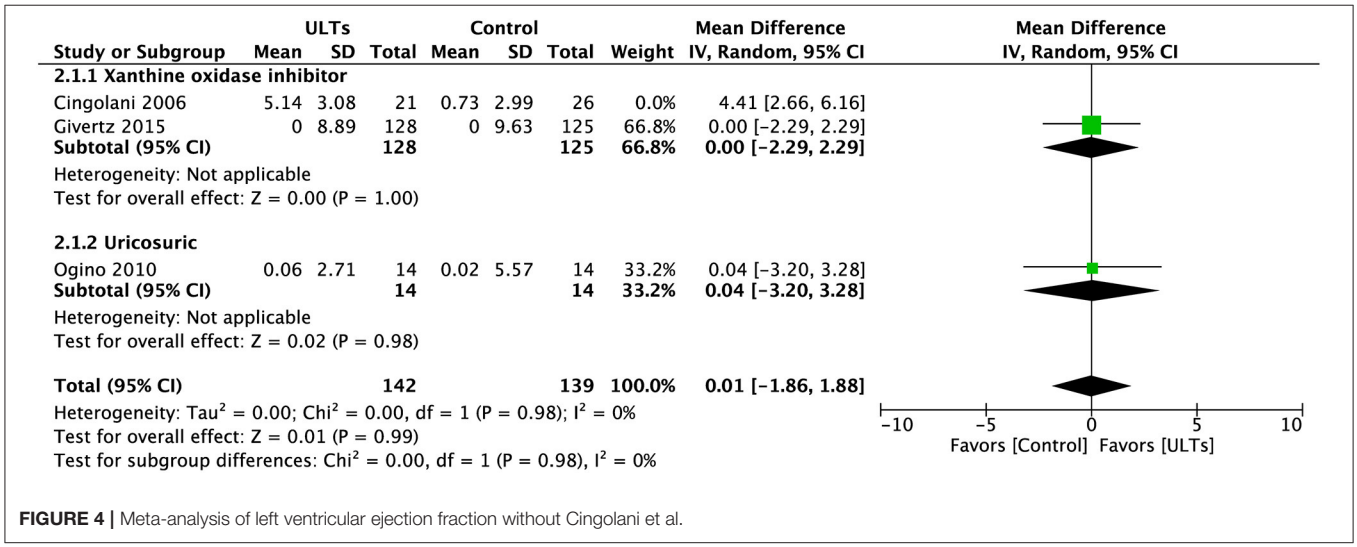

FIGURE 4 | Meta-analysis of left ventricular ejection fraction without Cingolani et al.

imbalance. Other enzymes also have ROS, such as NADPH oxidase enzymes, the respiratory chain in the mitochondria, and superoxide dismutase. XOI might be inadequate to curtail the cascade of ROS accumulated in HF patients.

Along with these pathophysiological assumptions, researchers observed that elevated UA levels are related to HF markers, such as measures of myocardial mechanical and energetic efficiency, LVEF, LV stroke volume, cardiac output, cardiac remodelling, endothelium dysfunction, plasma BNP levels, and CHF disease progression (27). It was also considered an independent predictor of mortality in HF patients and a robust dose-dependent increase of risk parallel to higher UA levels. Several studies showed a U-shaped association between UA and all-cause or CV mortality; SUA $\geq$8 or <4 mg/dl independently predicts higher all-cause and CVD-related mortality in the elderly (34, 35). Accordingly, we pooled several studies with an HF population with reduced ejection fraction and with or without elevated serum UA levels. In the OPT-CHF and EXAT-HF, both trials failed to show a beneficial effect regarding mortality. Notably, OPT-CHF included patients regardless of the UA level at baseline were included, while EXAT-HF only included patients with elevated serum uric acid levels (UA $\geq$9.5 mg/dl). However, the consistency of included studies was high despite the different inclusion criteria. We found that using ULTs, including XOI and uricosuric drugs on HF patients with or without hyperuricemia, did not improve heart function, exercise capacity, all-cause, and CV mortality. Therefore, UA may not be a causative risk factor in HF patients.

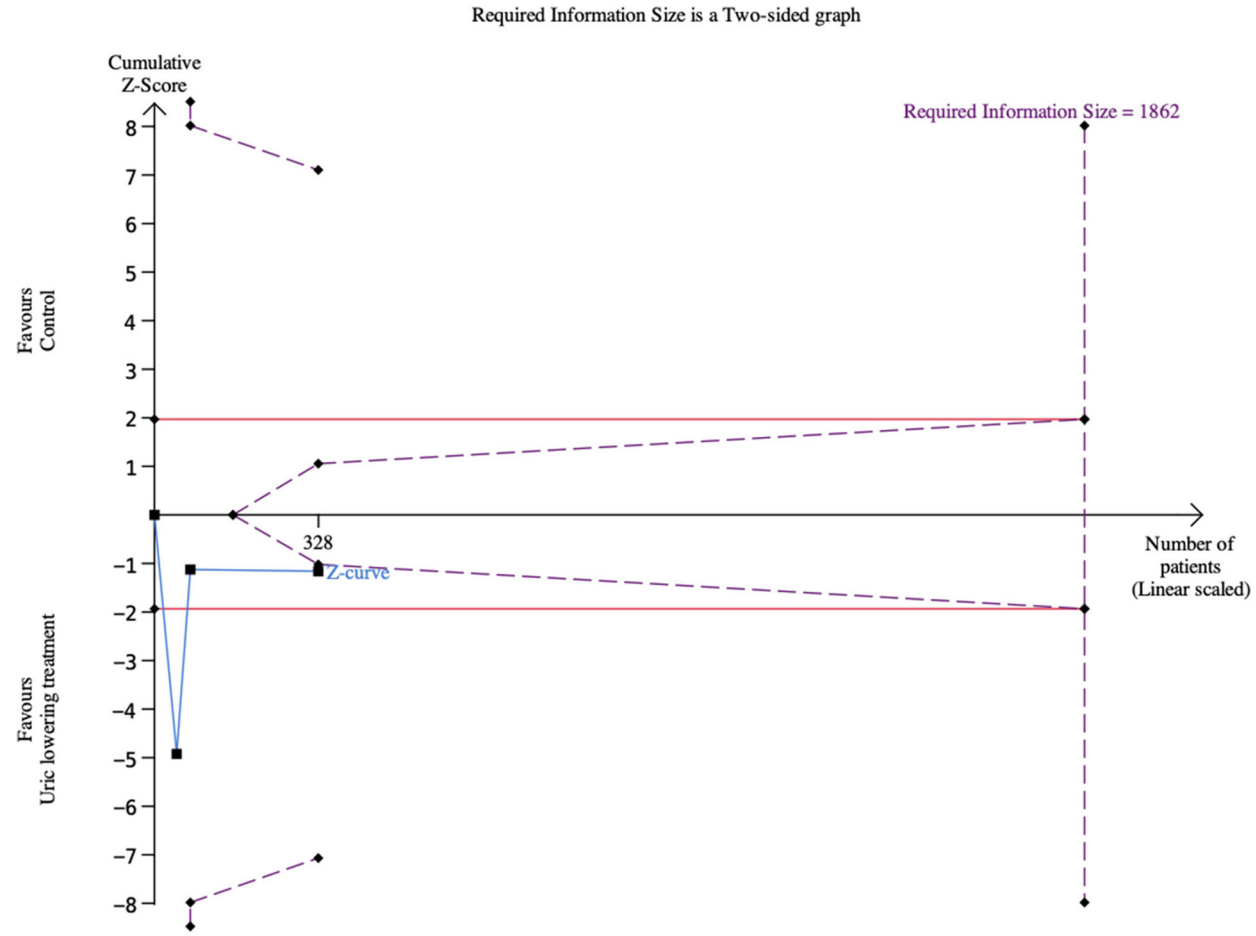

FIGURE 5 | Trial sequential analysis of left ventricular ejection fraction.

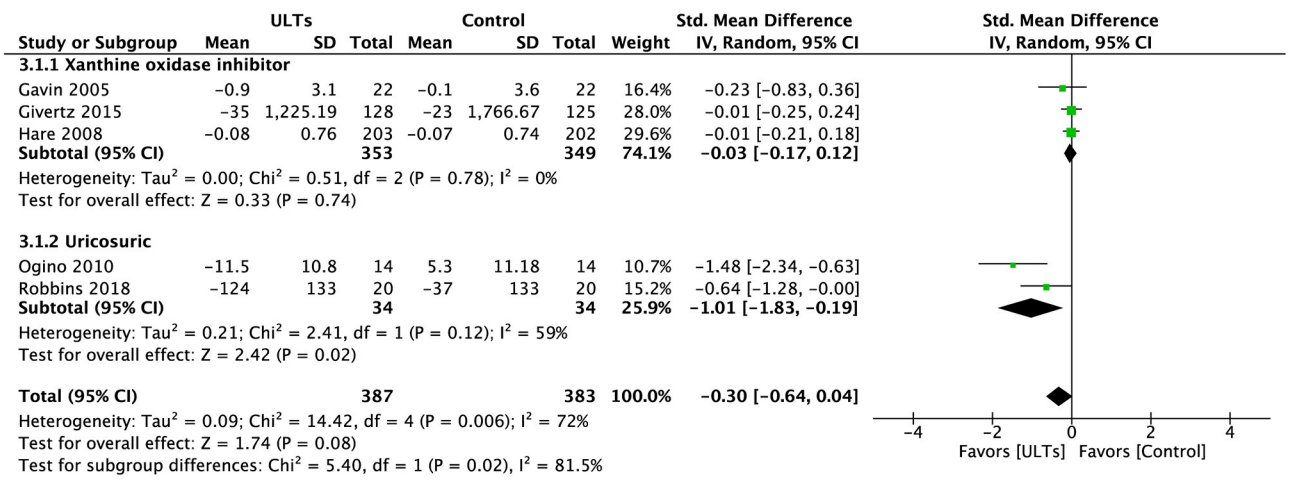

FIGURE 6 | Meta-analysis of B-type natriuretic peptide and N-terminal-pro-B-type natriuretic peptide.

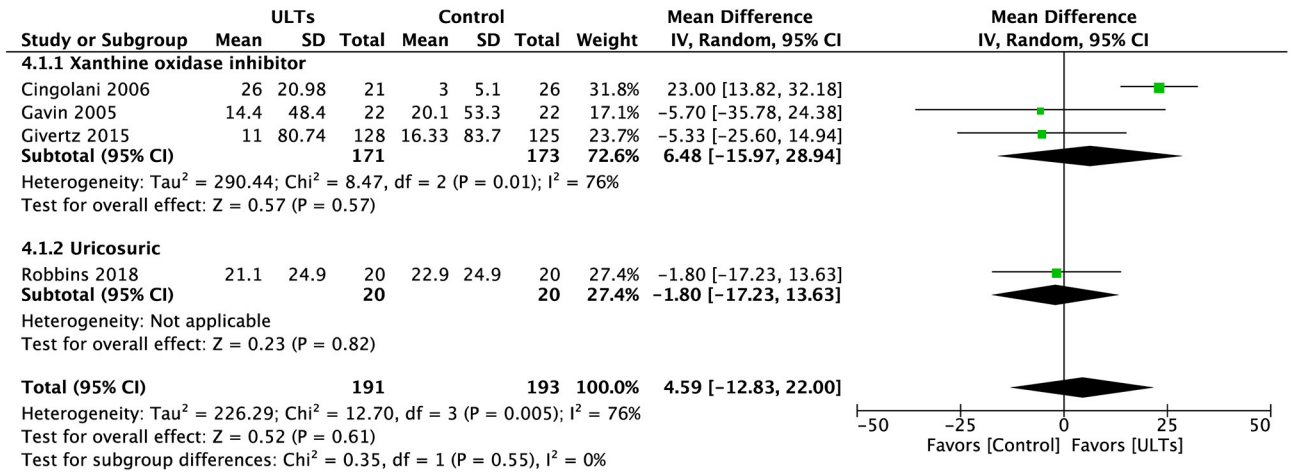

FIGURE 7 | Meta-analysis of 6-Min Walk Test.

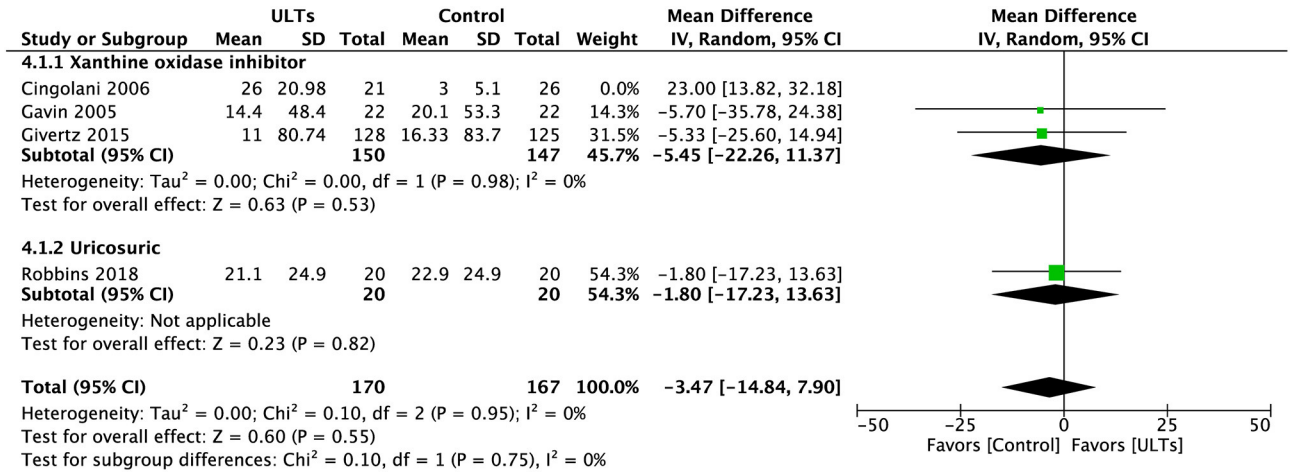

FIGURE 8 | Meta-analysis of 6-Min Walk Test without Cingolani et al.

LIMITATIONS

Even though most studies in this meta-analysis included patients with HFrEF and it is the most common type of HF (36), two studies (21, 23) did not specifically include patients with HFrEF. In HFpEF, the endothelial cells that line small blood vessels in the heart become dysfunctional and fail to synthesise adequate amounts of NO (37). Evidence showed that XOI could decrease NO production (38).

Since there is only one ongoing study (39), we cannot include the XO selective, uric acid-lowering drug febuxostat. However, febuxostat recently showed to increase the risk of future development of cardiovascular disease (40).

The overall sample size was small, and even though we included only randomised, placebo-controlled trials, different patient inclusion, exclusion criteria, and dosing protocol can lead to potential heterogeneity. The duration of included studies was also various. Therefore, the conclusions may be influenced by publication bias and should be regarded as preliminary.

CONCLUSION

ULTs did not improve LVEF, BNP/NT-pro-BNP, 6MWT, all-cause mortality, and CV death in HF patients. UA may just be a risk marker of HF.

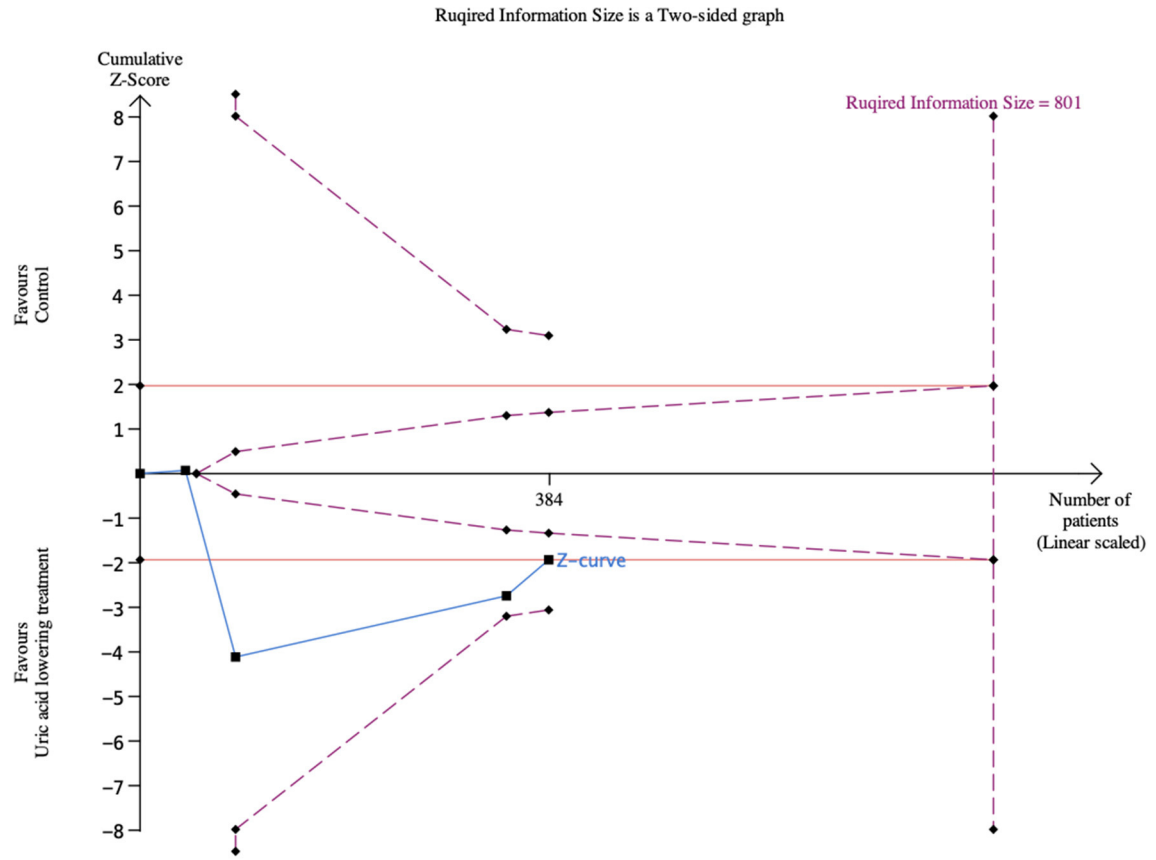

FIGURE 9 | Trial sequential analysis of 6-Min Walk Test.

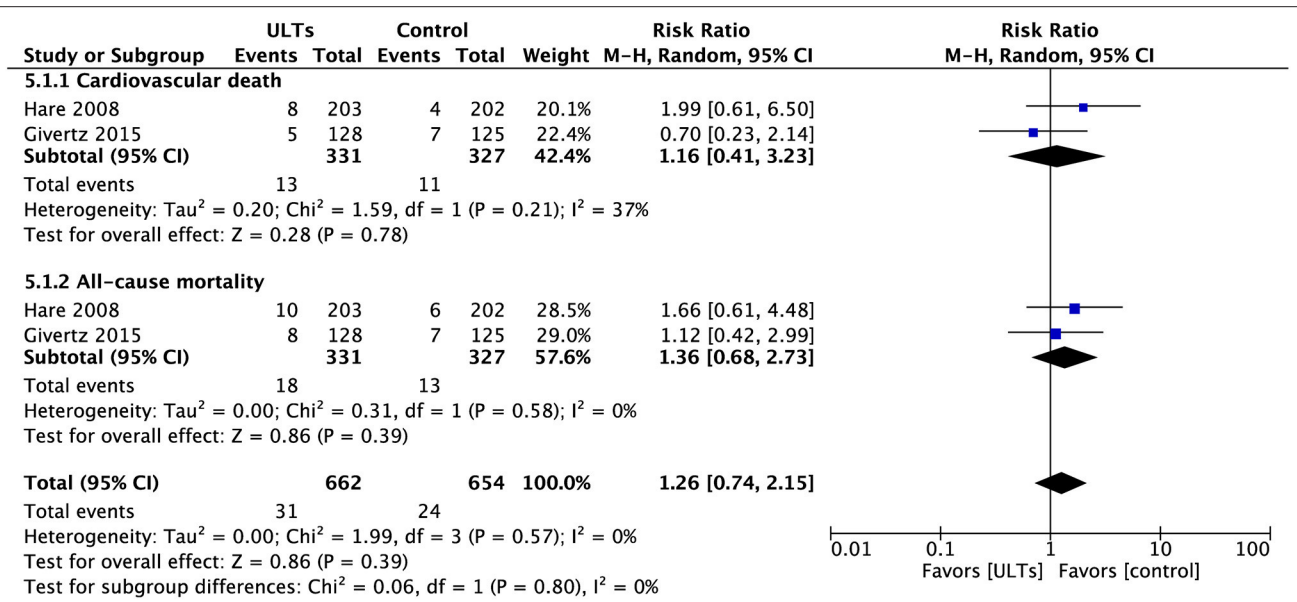

FIGURE 10 | Meta-analysis of all-cause mortality and cardiovascular death.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HX and DL contributed to the study's conception. HX and YL screened studies and extracted data. HX performed the data analyses and wrote the manuscript. DL, LM, LW, and YL helped perform the analysis with constructive discussions. All authors had read and approved the final manuscript.

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Neutrophil to Lymphocyte Ratio Is Increased and Associated With Left Ventricular Diastolic Function in Newly Diagnosed Essential Hypertension Children

*Miao Hou†, Lei Cao†, Yueyue Ding, Ye Chen, Bo Wang, Jie Shen, Wanping Zhou, Jie Huang, Qiuqin Xu, Haitao Lv and Ling Sun**

Department of Cardiology, Children's Hospital of Soochow University, Suzhou, China

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Laura Sabatino, Italian National Research Council, Italy

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Yazhou He, University of Edinburgh, United Kingdom
Serena Del Turco, Italian National Research Council, Italy

***Correspondence:**
Ling Sun
sunny70mail@163.com

†These authors have contributed equally to this work

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Aim: Hypertension is associated with cardiac structural and functional changes, including left ventricular hypertrophy (LVH) and LV systolic dysfunction diastolic dysfunction. Neutrophil-to-lymphocyte ratio (NLR) is a novel inflammatory biomarker associated with cardiovascular diseases. The current study aimed to evaluate NLR in children with newly diagnosed essential hypertension and its relationship between blood pressure and cardiac changes.

Methods and Subjects: Sixty-five children with newly diagnosed essential hypertension and 54 healthy children were included. Clinical characteristics, blood cell counts, and biochemical parameters were collected. LVH was assessed by calculation of LV mass index (LVMI), and LV systolic function was evaluated by measuring LV ejection fraction and fractional shortening. LV diastolic function was primarily assessed with E/E′ ratio by Doppler and echocardiography.

Results: The hypertension children had significantly higher LVMI and E/E′ ratio than the controls, whereas there was no difference in LV systolic function between the two groups. The NLR was significantly higher in the hypertension group than the control group. Moreover, NLR was positively correlated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels in the hypertension group. Additionally, a significantly positive correlation between NLR and E/E′ ratio was found in the hypertension group. However, NLR was not related to LVH and LV systolic function indicators in hypertension children.

Conclusion: NLR is elevated in hypertension children, and it is associated positively with office blood pressure levels. Moreover, NLR may help assess LV diastolic function in hypertension children.

Keywords: hypertension, children, neutrophil-lymphocyte ratio, left ventricular hypertrophy, left ventricular diastolic function

BACKGROUND

Hypertension is the leading risk factor for cardiovascular disease and mortality in adults, with a prevalence of 31.1% worldwide (1). In parallel with the growing prevalence of childhood obesity, it is becoming an increasing problem among children over the last few decades, as a consequence of obese children who are at approximately a three-fold higher risk for hypertension than non-obese children (2). According to the American Heart Association, the prevalence of high blood pressure is 14.2% for US children (3), and the incidence of high blood pressure is 14.13–17.00% for children aged 7–17 years in China (4). Of note, a recent longitudinal study has demonstrated that blood pressure in childhood is the strongest independent predictor of future blood pressure in adulthood (5), emphasizing the importance of blood pressure management in childhood.

Growing evidence shows that hypertension results in target organ damage, even in prehypertension children (6). The increased left ventricular mass (LVM) and cardiac function abnormalities are the early change in target organ damage (7). Therefore, screening for rapid and straightforward indicators to reflect the target organ damage has become a useful strategy in managing childhood hypertension.

Over the last years, comprehensive data have demonstrated the pivotal role of low-grade inflammation in the pathogenesis of essential hypertension and target organ damage in both adults (8) and children (9). The white blood cells (WBCs) and their subtypes with platelets are the essential cells of inflammation. Therefore, blood cell parameters have attracted increasing attention in chronic inflammation disease. The neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte (LMR), and platelet-to-lymphocyte ratio (PLR) were proposed as the inexpensive, easily accessible, and widely available inflammatory markers. They have been shown to be related to cardiovascular diseases in adults, including atherosclerosis (10), heart failure (11), acute coronary syndromes (12), and hypertension (13). Moreover, Skrzypczyk et al. reported that NLR correlated with 24-h ambulatory mean arterial pressure levels in adolescents (14), which suggest that blood cell parameter may also be useful in the pediatrics population. To date, no studies have investigated the possible link between NLR and target organ damage in hypertension children. Therefore, our study aimed to evaluate blood cell count inflammatory markers in children with newly diagnosed essential hypertension and explore the possible link between NLR and cardiac structural and functional changes.

METHODS

Ethics Committee Approval

The local Ethics Committee of Children's Hospital of Soochow University approved the research project (ID: 2020CS076). All procedures performed involving human participants followed the Declaration of Helsinki, and informed consent was obtained from all participants and their parents included in the study.

Study Group

In the current study, we retrospectively studied 65 children (48 boys, 17 girls) with newly diagnosed essential hypertension hospitalized in the cardiology department in the Children's Hospital of Soochow University from January 2016 to December 2020, and 54 age and sex-matched healthy children were recruited from the community-based population as the control group.

Clinical parameters, including age, gender, and body mass index (BMI; kg/m^2) were obtained in all analyzed children. Hypertension was defined as systolic and/or diastolic pressure $\geq$95th percentile for sex, age, and height according to the reference value of the Chinese Child Blood Pressure References Collaborative Group (15). Office blood pressure was measured by an automated oscillometric device (Datascope Accutor Plus) with the appropriate size cuff that had been validated for use in children (16). The appropriate cuff size (with bladder width of about 40–50% of the arm circumference and the bladder length of at least 80% of the arm circumference) was determined by measuring the mid-upper arm circumference. Blood pressure was measured in the non-dominant arm in triplicate at 3-min intervals after a 15 min rest in the sitting position with the arm and back supported. After excluding the first reading, the average of two subsequent readings was calculated for analysis.

To exclude secondary hypertension, a thorough medical history, physical examination, and auxiliary examination was carried out following the guideline of the American Academy of Pediatrics (17). In addition, based on medical history, physical examination, and determined high-sensitivity C-reactive protein (hsCRP) levels, children with active inflammation were excluded in the current study.

Laboratory Assessment

Blood was obtained from an antecubital venous catheter after 10–12 h of night fasting. All specimens were EDTA-K2 anticoagulated and tested within 30 min of collection. The hematological parameters, including white blood cell (WBC), differential WBC counts (neutrophils, lymphocytes, and monocytes), and platelet count (Plt) were measured by an automated hematology analyzer. The neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR) were calculated.

Moreover, plasma glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), hsCRP, alanine aminotransferase (ALT), and creatinine were determined at the Department of Clinical Laboratory of the Children's Hospital of Soochow University.

Echocardiographic Assessment

All echocardiographic parameters were performed using commercially available ultrasound equipment iE33 (Phillips Healthcare, North Andover, Massachusetts, USA).

Left Ventricular Geometry

The M-mode tracing was used to measure the end-diastolic interventricular septal wall thickness (IVSd), left ventricular

end-diastolic diameter (LVIDd), left ventricular end-systolic diameter (LVIDs), and end-diastolic posterior wall thickness (PWTd). The left ventricular mass (LVM) was then calculated using the following formula: LVM = 0.8 × 1.04 × [(IVSd + LVIDd + PWTd)^3 – LVIDd^3] + 0.6, LVM index (LVMI) = LVM/height$^{2.7}$, relative wall thickness (RWT) = (IVSd + PWTd)/LVIDd. LV hypertrophy (LVH) in children and adolescents is defined as the LVMI ≥ 95th percentile on sex-specific normative LVMI data published by Khoury et al. (18).

Left Ventricular Systolic Function

LV systolic function was assessed by the LV ejection fraction (EF) and fractional shortening (FS) (19).

Left Ventricular Diastolic Function

Pulsed Doppler Assessment

Mitral inflow velocities were acquired with pulsed wave Doppler. The velocities during the early transmitral flow (E) and inflow with atrial contraction (A) were measured, and the E/A ratio was calculated.

Tissue Doppler Imaging

Myocardial flow velocities were obtained in the apical four-chamber view. The peak early E′ and late A′ velocities were recorded, then the E′/A′ ratio and E/E′ ratio were calculated (20), and the left ventricular diastolic dysfunction was defined as E/E′ ratio > 14, according to the recommendations of the American Society of Echocardiography (21).

Statistics

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL). Values were expressed as mean and SD. The Shapiro–Wilk-test was used to determine the normality of data. Means were compared using an independent *t*-test between hypertension and control groups. Categorical variables were compared using the chi-square test. Correlations between variables were evaluated using Pearson's tests. Multivariate linear analyses were performed to estimate the association of potential confounding factors between the LV diastolic function indicators. A P-value < 0.05 was considered significant.

RESULTS

Clinical Characteristics and Biochemical Parameter in Hypertension and Control Group

During the study period, 177 children with newly diagnosed hypertension were hospitalized. Among them, 85 cases with secondary hypertension and 16 cases with active inflammation and two cases with missing data were excluded; and nine cases refused to participate. Therefore, 65 children with newly diagnosed essential hypertension were evaluated, and the selection process is shown in **Figure 1**. The clinical characteristics and biochemical parameters of hypertension and control groups are shown in **Table 1**. There was no difference in terms of sex and age between the two groups. SBP, DBP, pulse pressure, and BMI were significantly higher in the hypertension group than in the control groups. Also, the serum uric acid, ALT, and hsCRP levels in children with hypertension were significantly higher than in the control group, respectively. However, there were no differences in lipids and glucose levels between the two groups (**Table 1**).

Echocardiography Parameters in Hypertension and Control Group

The LVM, LVMI, and RWT were higher in the hypertension group compared with the control group, and 8 of 65 children in the hypertension group had LVH (12.31%). Besides, the E/E′ ratio was higher in the hypertension group in comparison with the control group, LV diastolic dysfunction was found in 1 of the 65 hypertensive subjects (1.54%) (**Table 2**). However, there was no difference in LV ejection fraction and FS between the two groups.

Blood Cell Counts in Hypertension and Control Group

The WBC count and neutrophil counts were significantly higher in hypertension children than those in the control group, whereas lymphocyte, monocytes, and platelet counts were similar between the two groups (**Table 3**). Moreover, NLR is higher in the hypertension group than the control group. However, there was no difference in PLR and LMR between hypertension and control groups (**Table 3**).

Correlation Between Blood-Cell Count Inflammatory Markers and Office Blood Pressure With Body Mass Index in Hypertension Children

In the hypertension group, univariate correlation analysis determined a significant positive correlation between NLR with office SBP (r = 0.344, P = 0.005) and DBP (r = 0.310, P = 0.012) levels (**Figure 2**). However, NLR was not associated with BMI levels. Moreover, WBC counts, neutrophil counts, lymphocyte counts, monocytes counts, platelet counts, PLR, and LMR were not correlated with office blood pressure or BMI levels (**Supplementary Table 1**).

Correlation Between Blood-Cell Count Inflammatory Markers and Left Ventricular Diastolic Function With Hypertrophy in Hypertension Children

NLR was negatively correlated with E′ (r = −0.319, P = 0.010) and the E′/A′ ratio (r = −0.463, P < 0.001), and positively correlated with the E/E′ ratio (r = 0.330, P = 0.007) in the hypertension group (**Figure 3**). Neutrophil counts were also found to correlate negatively with E′ (r = −0.427, P = 0.001) and the E′/A′ ratio (r = −0.318, P = 0.036) in the hypertension group. Furthermore, after adjusting age, gender, and BMI, the multivariate regression analyses still detected the significant associations between NLR and E/E′ (β = 0.593, P = 0.003, 95% CI: 0.209–0.978). However, there was no correlation between WBC, lymphocyte, monocytes, platelet counts, and BMI with diastolic function parameters (**Supplementary Table 2**).

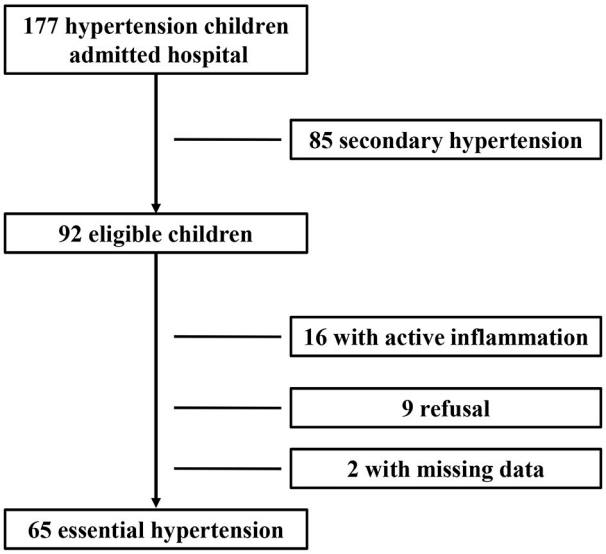

FIGURE 1 | Participant selection process.

TABLE 1 | Clinical characteristics and biochemical parameter in hypertension group and control group.

	Control group	Hypertension group	*P*-value
Clinical characteristics			
Gender (M/F)	43/11	48/17	0.459
Age, years	12.33 ± 2.3	12.37 ± 2.34	0.937
BMI (kg/m^2)	108.52 ± 9.47	141.08 ± 11.95	0.003
SBP (mmHg)	67.75 ± 7.63	85.15 ± 11.66	<0.001
DBP (mmHg)	35.87 ± 15.11	55.05 ± 13.68	<0.001
PP (mmHg)	18.77 ± 2.06	26.62 ± 14.62	<0.001
Biochemical variables			
Uric acid, μmol/L	328.99 ± 83.26	407.11 ± 111.07	<0.001
ALT, μmol/L	15.24 ± 8.76	31.49 ± 41.66	0.004
Creatinine, μmol/L	69.28 ± 17.34	66.15 ± 15.47	0.311
TG, mmol/L	1.14 ± 0.50	1.14 ± 0.49	0.950
TC, mmol/L	3.64 ± 1.12	3.87 ± 0.98	0.296
HDL-c, mmol/L	1.22 ± 0.27	1.26 ± 0.41	0.654
LDL-c, mmol/L	1.94 ± 0.73	2.16 ± 0.97	0.348
Glucose, mmol/L	4.14 ± 1.45	4.27 ± 1.55	0.612
hsCRP, mg/dl	0.32 ± 0.42	2.22 ± 5.03	0.004

SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; ALT, alanine aminotransferase; hsCRP, high-sensitivity C-reactive protein.

On the other hand, these blood cell count inflammatory markers were not associated with left ventricular hypertrophy and systolic function parameters (**Supplementary Table 3**). However, BMI was positively correlated with LVMI in hypertension children ($r = 0.588$, $P < 0.001$).

TABLE 2 | Echocardiography parameters in the hypertension group and control group.

	Control group	Hypertension group	*P*-value
LVM, g	105.61 ± 29.6	139.32 ± 49.1	<0.001
LVMI, g/m$^{2.7}$	29.42 ± 6.56	37.71 ± 10.67	<0.001
RWT (%)	0.30 ± 0.04	0.35 ± 0.06	<0.001
LV hypertrophy, *n* (%)	/	8 (12.31%)	/
E′, cm/s	12.66 ± 1.84	12.62 ± 1.77	0.898
E′/A′ ratio	2.05 ± 0.56	2.25 ± 1.13	0.295
E/A ratio	2.17 ± 0.59	1.92 ± 0.59	0.033
E/E′ ratio	7.77 ± 1.63	8.53 ± 1.79	0.038
Diastolic dysfunction, *n* (%)	/	1 (1.54%)	/
LVEF (%)	72.52 ± 3.85	71.6 ± 6.23	0.389
FS (%)	42.42 ± 4.71	43 ± 6.21	0.606

LVM, left ventricular mass; LVMI, left ventricular mass index; RWT, relative wall thickness; LVEF, left ventricular ejection fraction; FS, fractional shortening.

TABLE 3 | Blood cell count inflammatory markers in hypertension and control group.

	Control group	Hypertension group	*P*-value
WBC (10^9/L)	6.70 ± 1.71	7.65 ± 2.27	0.017
N (10^9/L)	3.77 ± 1.27	4.62 ± 1.72	0.003
L (10^9/L)	2.42 ± 0.76	2.43 ± 0.91	0.931
M (10^9/L)	0.41 ± 0.13	0.46 ± 0.16	0.089
Plt (10^9/L)	280.66 ± 76.35	287.48 ± 77.57	0.110
NLR	1.68 ± 0.75	2.18 ± 1.12	0.005
PLR	122.46 ± 36.71	132.08 ± 57.77	0.287
LMR	6.19 ± 1.74	6.41 ± 7.2	0.832

WBC, white blood cell count; N, neutrophils count; L, lymphocytes count; M, monocytes count; Plt, platelet count; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

DISCUSSION

The incidence of hypertension among children is overgrowing in recent years, and high blood pressure can induce cardiac structural and functional target organ damage (22). Our study demonstrated the elevation of LVMI and the reduction of diastolic function in newly diagnosed essential hypertension children. Moreover, we found elevated NLR in the hypertension group, and it is positively correlated with office blood pressure levels, which might imply the possible link between the inflammation and elevation of blood pressure in the hypertension children. Interestingly, NLR was positively correlated with left ventricular diastolic parameter E/E′ ratio in hypertension children, which suggests that NLR may serve as a useful indicator to reflect diastolic dysfunction in hypertension children. To our knowledge, this is the first study that analyzes the change in NLR and its relationship between LV diastolic function in newly diagnosed essential hypertension children.

It is well-established that LVH is an independent risk factor for cardiovascular morbidity and mortality in adulthood (23,

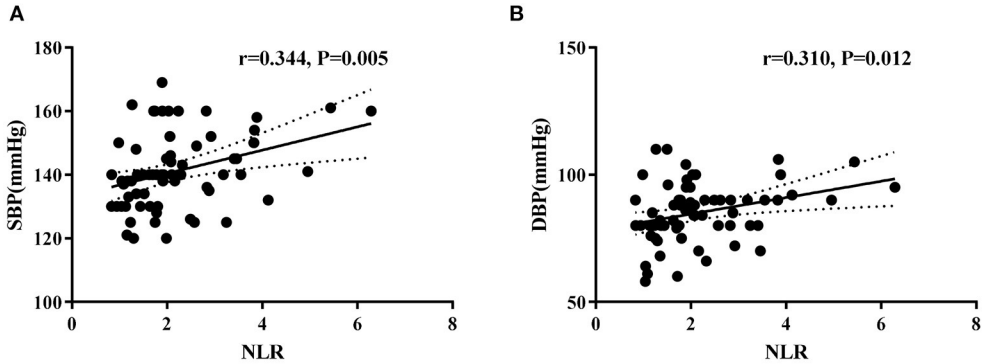

FIGURE 2 | Correlations between neutrophil-to-lymphocyte ratio (NLR) and systolic blood pressure **(A)** and diastolic blood pressure **(B)** levels in hypertension children.

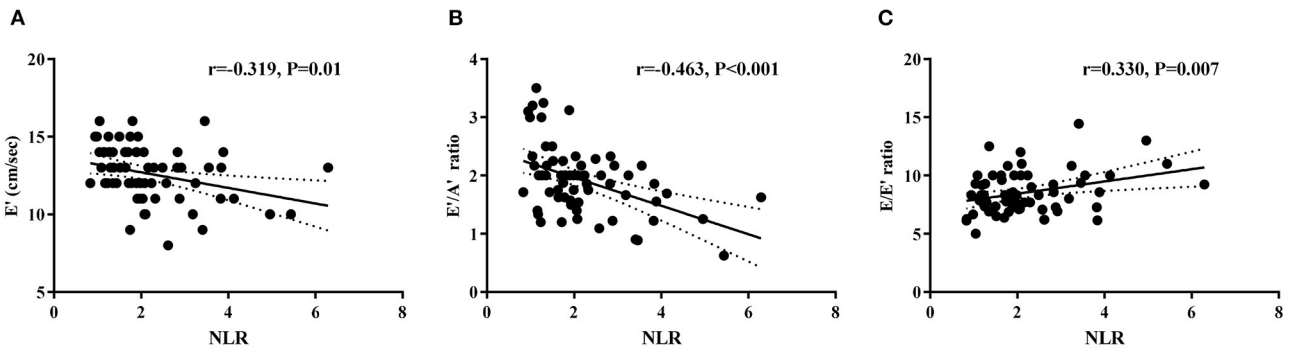

FIGURE 3 | Correlations between NLR and left ventricular diastolic function parameters E′ **(A)**, E′/A′ ratio **(B)**, and E/E′ ratio **(C)** in hypertension children.

24). Previous studies have proved that LVH was common in hypertensive children (25). However, there is limited data available in the Chinese pediatric population. In this study, we found that LVM and LVMI were both higher in hypertension children, and among 65 hypertension adolescents, 12.31% (8/65) had LVH. Similarly, Litwin et al. demonstrated that 10.3% of 44 hypertension children had some form of LV hypertrophy in the USA population (26), and Falkner reported among 35 African-American adolescents, 19% of them had LV hypertrophy (27). A cross-sectional study of 101 primary hypertension children also reported that 34% of them had LV hypertrophy (28). All these studies demonstrated that LVH was common in hypertensive children. However, the prevalence of LVH varies among these studies, which may be explained by the differences in ethnic and hypertension grades of these participants. Interestingly, BMI was positively correlated with LVMI in hypertension children, and these findings are consistent with the direct positive correlation between BMI and LVH among obese adults in a systematic review (29), which indicates the possible additive and/or interactive effects of obesity and blood pressure on LVH (30).

Several studies have shown that NLR is elevated and related to poor clinical outcomes of cardiovascular disease in adults, including acute coronary syndrome, atherosclerosis, and heart failure (31–33). However, few studies have looked at NLR levels in children with newly diagnosed hypertension (14, 34). In the current study, we found that the neutrophil counts and NLR were significantly higher in hypertension children than in healthy children, and the elevated NLR may reflect the upregulation of overall inflammatory and stress status in these children. Likewise, Derya et al. found that NLR is increased in newly diagnosed hypertension adults and associated positively with low-grade inflammation indicator C-reactive protein levels (13), which is consistent with our findings.

Furthermore, we demonstrated that NLR positively correlated with both office SBP and DBP levels in hypertension children. Similarly, Cimen et al. reported that NLR correlated with blood pressure levels in adults (35). The increase in NLR may reflect the activity of two different immune pathways in the process of blood pressure regulation. On the one hand, neutrophils secrete many cytokines that trigger and amplify inflammatory reactions (36), and activated neutrophils' release of various proteolytic enzymes that promote endothelial damage and tissue destruction (37, 38). Also, neutrophils can lead to the release of reactive oxygen species (39), and ROS-induced oxidative stress has been shown to cause vasoconstriction (40) with sodium and water retention in the kidney (41). On the other hand, lymphocytes are the primary cells in the regulatory pathway of the immune system, and T lymphocytes cells have been shown to play a crucial role in the BP elevation caused by angiotensin II response to sodium and volume challenges (42). Therefore, NLR gives more

information than either of the above parameters in hypertension, which indicates that inflammation may play an essential role in the development of hypertension.

The possible links between NLR and cardiac damages in these hypertension children were also demonstrated in this study. LVH and LV diastolic dysfunction are both the early complication of hypertension (43); and eight children had LVH, and one child had diastolic dysfunction among these hypertension participants. As known, the E/E′ ratio seems to be the most reliable parameter to evaluate LV diastolic function in patients with heart disease, and the increase in E/E′ ratio reflects LV diastolic dysfunction (44). Interestingly, we found that NLR positively correlated with E/E′ ratio among hypertension children; after adjusting for the potential confounding covariates, including BMI, gender and age, NLR still correlated with E/E′ ratio. To our best knowledge, this is the first study to report the correlation between NLR and diastolic function in hypertension children. Since no correlation between blood cell count inflammatory markers and LVH parameters was demonstrated in this study, which suggests that the LV diastolic dysfunction in these hypertensive children is probably due to systematic inflammation (45) rather than left ventricular hypertrophy. These results indicate that NLR may serve as a helpful marker to evaluate the LV diastolic function in hypertension children.

There are several limitations to this study. First, this is a single-center retrospective study, and the sample size is relatively small, along with the large burden of multiple analyses, which increases possible type I error. Second, the gender of hypertension children is unbalanced in this study. Recently, no difference in the global prevalence of hypertension in children was found in a meta-analysis (46). However, the prevalence of hypertension among school-age children was higher in boys than in girls (16.1 vs. 12.9%), according to an updated Report on Cardiovascular Health and Diseases in China (47). Therefore, the gender-specific prevalence of child hypertension might vary among different regions. Moreover, these hypertension children were recruited from a hospital-based population, which may also increase the risk of selective bias and resulting in gender discrepancies. Third, since this is an observational study, we cannot make any causal inferences. Fourth, due to the physiological characteristics in the blood cell counts of children under 5 years of age (48), the results cannot be extrapolated to this population.

In conclusion, we demonstrated that NLR is elevated in hypertension children, and it is associated positively with office blood pressure levels and LV diastolic dysfunction parameters. Our results indicate that inflammation may play a crucial role in the development of hypertension, and the higher NLR may indicate the increased risk for the development of hypertension in children. Moreover, NLR can serve as a useful marker to reflect left ventricular diastolic dysfunction in pediatric patients with primary hypertension.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of Children's Hospital of Soochow University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MH, LC, YD, and LS conceived and designed the study and wrote the paper. MH, LC, YD, YC, BW, JS, WZ, JH, QX, HL, and LS performed the study. YC, BW, JS, WZ, JH, QX, and HL reviewed and edited the manuscript. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.576005/full#supplementary-material

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Longitudinal Effect of Hemoglobin Concentration With Incident Ischemic Heart Disease According to Hepatic Steatosis Status Among Koreans

Dong Hyuk Jung[1,2], *Yong Jae Lee*[2,3] *and Byoungjin Park*[1,2]*

[1] Department of Family Medicine, Yongin Severance Hospital, Yongin, South Korea, [2] Department of Family Medicine, Yonsei University College of Medicine, Seoul, South Korea, [3] Department of Family Medicine, Gangnam Severance Hospital, Seoul, South Korea

Background: An increased hemoglobin (Hb) level may have detrimental effects on hepatic steatosis (HS) as well as cardiovascular disease (CVD). We investigated Hb's effect on incident ischemic heart disease (IHD) risk in the context of hepatic steatosis (HS).

Methods: We assessed 17,521 non-diabetic participants and retrospectively screened for IHD using the Korea National Health Insurance data. High Hb was defined as Hb levels ≥16.3 g/dL in men and 13.9 g/dL in women (>75th percentile). The participants were divided into five groups: reference (group 1), mild HS only (group 2), mild HS and high Hb (group 3), severe HS only (group 4), and severe HS and high Hb (group 5). We assessed hazard ratios (HRs) with 95% confidence intervals (CIs) for IHD using multivariate Cox proportional hazards regression models over 50 months from the baseline survey.

Results: During the follow-up period, 330 (1.9%) participants developed IHD (310 angina pectoris and 20 myocardial infarction). Compared with the reference group (group 1), the HRs for IHD were 1.04 (95% CI, 0.75–1.46) in group 2, 1.14 (95% CI, 0.70–1.85) in group 3, 1.58 (95% CI, 1.08–2.32) in group 4, and 1.79 (95% CI, 1.15–2.80) in group 5, after adjusting for IHD risk factors.

Conclusions: We found the combined effect of HS and Hb levels on the incidence of IHD.

Keywords: hemoglobin, hepatic steatosis, cohort study, ischemic heart disease, risk factor, extrahepatic complications

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***Correspondence:**
Byoungjin Park
bjpark96@yuhs.ac

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INTRODUCTION

Hepatic steatosis (HS) is an asymptomatic condition, but it is the most common liver disease in Western and developed Asian countries (1, 2). With an increase in high-calorie diet and obesity, triglyceride accumulates in the liver, resulting in inflammation and hepatic cellular damage, which progresses to steatohepatitis and fibrosis (3, 4).

Recently, extrahepatic complications have attracted growing interest as a risk factor for diabetes, atherosclerosis, and metabolic syndrome (5–7). In particular, HS's role in the pathogenesis of

IHD has gained much interest. Epidemiological studies have reported a relationship between HSand the incidence of CVD events and mortality (8, 9). A previous study also showed that the severity of HS is dose-dependently related to atherosclerosis (10). However, these findings are more definite in patients with both hepatic manifestations and other risk factors such as advanced fibrosis and elevated hepatic enzymes (6). Thus, it is assumed that additional elements could modify the relationship between HS and IHD events. The serum hemoglobin (Hb) quantification can be performed using a simple blood test in a primary clinical setting and is essential when hematologic diseases, including anemia, are suspected (11). Previous studies have shown that Hb levels may be the modifying factors because an increased Hb level has detrimental effects on both IHD and non-alcoholic fatty liver disease (NAFLD). High Hb levels are closely related to body iron and ferritin stores (12). Elevated serum iron levels have been implicated in insulin resistance and cellular oxidative stress (13, 14). Thus, elevated Hb levels may be intricately associated with IHD and NAFLD. Despite the close interrelationship between Hb, HS, and IHD, only a few studies have investigated the interaction among them.

In this regard, we investigated the combined effect of HS and elevated Hb levels within the normal range on incident IHD risk in a large cohort of non-diabetic Korean adults using National Health Insurance data.

MATERIALS AND METHODS

Study Design and Participants

This retrospective study is based on the Health Risk Assessment Study, aiming to explore surrogate markers for CVD among non-diabetic Korean adults. The study cohort consisted of 20,530 individuals who voluntarily visited the Health Promotion Centre of Gangnam Severance Hospital, Yonsei University College of Medicine, for regular health examinations between November 2006 and June 2010. Among the participants initially assessed, we excluded 1,590 (7.7%) subjects with a history of IHD or ischemic stroke, a previous diagnosis of type 2 diabetes, or a fasting plasma glucose level $\geq$126 mg/dL. Participants who met at least one of the following criteria were also excluded: <20 years of age; missing data; positive for hepatitis B surface antigen or hepatitis C antibody; presence of liver cirrhosis on abdominal ultrasonography; Hb levels below 10 g/dL or above 18 g/dL; presence of chronic kidney disease, defined based on either a reduced renal function or renal tissue damage with an eGFR $<$ 60 mL/min/1.73 m^2 or proteinuria of $\geq$1+; current use of aspirin (N = 1,419). Following these exclusions, we included 17,521 participants (8,976 men and 8,545 women) in the final analysis (**Figure 1**).

Data Collection

Each participant completed a questionnaire about their lifestyle and medical history. We obtained cigarette smoking status, alcohol consumption, and physical activity characteristics from the questionnaires. Smoking status was categorized as non-smoker and ex-smoker, or current smoker. Questions regarding alcohol intake included frequency of intake on a weekly basis. Regular alcohol drinking was defined as alcohol consumption $\geq$140 g per week. Participants were asked about the number of times per week they engaged in physical exercise, and regular exercise was defined as physical activity of moderate intensity exercising $\geq$ three times per week. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, in light indoor clothing without footwear. Body mass index (BMI) was calculated as a participant's weight divided by the height squared (kg/m^2). Obesity was defined as BMI $\geq$ 25 kg/m^2 according to Korean guideline. Systolic blood pressure and diastolic blood pressure were measured in the sitting position after 10 min using a standard mercury sphygmomanometer (Baumanometer, W.A. Baum Co Inc., Copiague, NY, USA) on the right arm. All blood samples were obtained from the antecubital vein after overnight fasting for 12 h. Hb levels were quantified using an automated blood cell counter (ADVIA 120, Bayer, NY, USA). Fasting plasma glucose, total cholesterol, triglyceride, HDL-cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured by enzymatic methods using a Hitachi 7600 automated chemistry analyser (Hitachi Co., Tokyo, Japan). High-sensitivity C-reactive protein (hsCRP) concentrations were measured with a Roche/Hitachi 912 System (Roche Diagnostics, Indianapolis, IN, USA). Hypertension was defined as the current use of hypertension medication, a systolic blood pressure $\geq$ 140 mmHg, or diastolic blood pressure $\geq$ 90 mmHg. We calculated the HS index (HSI) score as follows: HSI score = 8 $\times$ ALT/AST ratio + BMI (kg/m^2), (+ 2 for women).

Abdominal Ultrasonography

Liver ultrasonography was performed using a 3.5-MHz transducer (HDI 5000, Philips, Bothell, USA) by experienced radiologists blinded to the laboratory and clinical data. Fatty liver was assessed semi-quantitatively and described as absent (grade 0), mild (grade 1), mild to moderate (grade 2), moderate (grade 3), moderate to severe (grade 4), or severe (grade 5), based on hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring.

Study Outcomes

To define outcomes, we linked a personal 13-digit identification number for each participant that was assigned using the Health Insurance Review and Assessment Data (HIRA), which is derived from the universal coverage system in Korea, from November 1, 2006 to December 31, 2010. The primary outcome was new-onset IHD, which comprised of angina pectoris (ICD-10 code I20) or acute myocardial infarction (ICD-10 code I21) occurring after study enrolment.

Statistical Analysis

According to the fatty liver grade, the 17,521 participants were categorized into three groups: no HS and mild HS (grade 1–2) or severe HS (grade 3–5). High Hb was defined as Hb levels $\geq$ 16.3 g/dL in men and 13.9 g/dL in women (>75th percentile). To assess the combined effect of HS and Hb concentrations on incident IHD, we divided the study participants into five groups: no HS reference group, mild HS only group, mild HS and high Hb group, severe HS

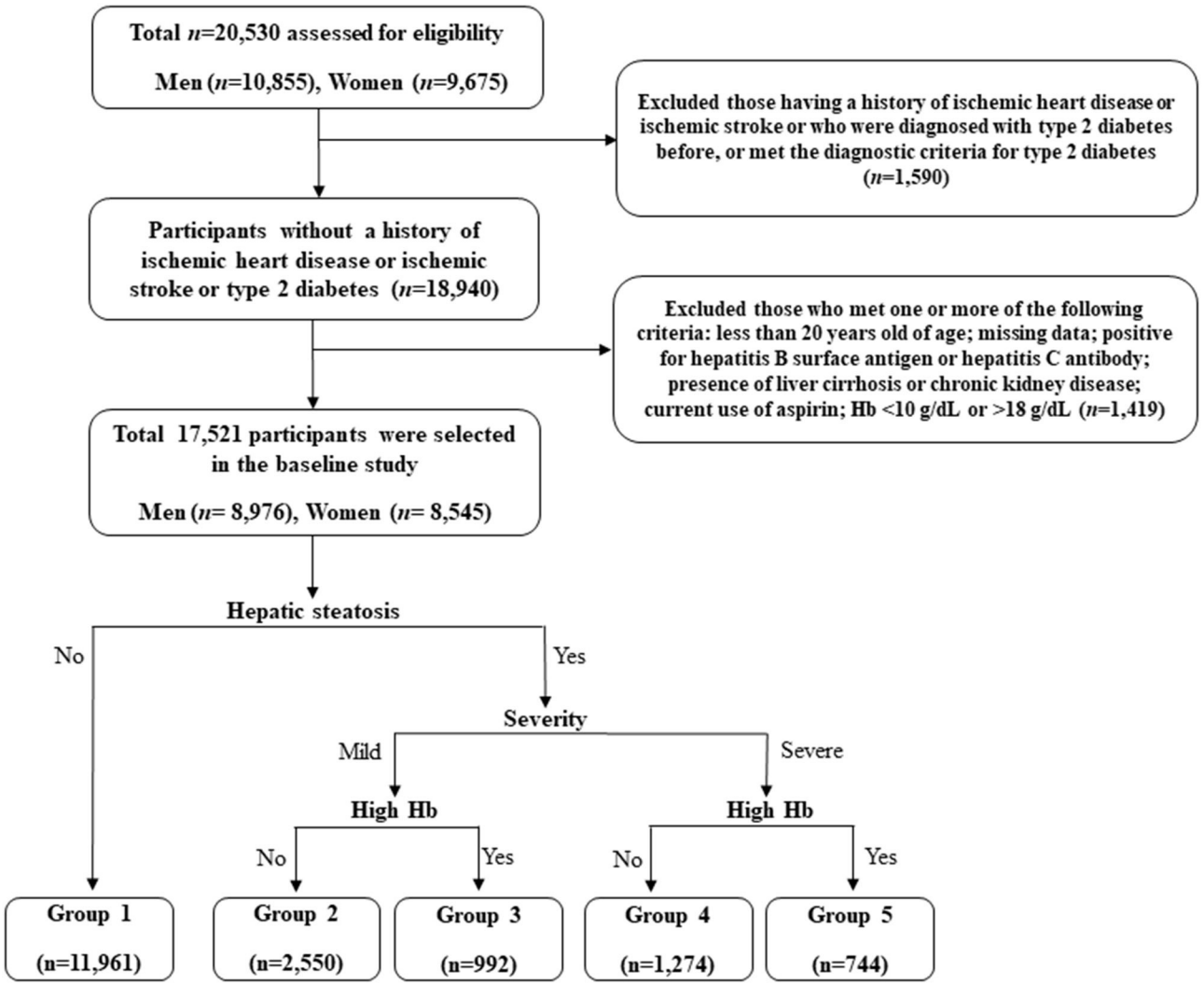

FIGURE 1 | Flowchart for the selection of study participants.

only group, and severe HS and high Hb group. The study population' baseline characteristics were compared among the groups using analysis of variance for continuous variables and chi-squared test for categorical variables. Kaplan–Meier curves were used to assess the cumulative incidence of IHD. The log-rank test was used to determine whether the distributions of the cumulative IHD incidence differed among groups. We used pairwise comparisons of receiver-operating characteristic (ROC) curves and concordance (C) statistic to assess the ability of a risk factor to predict IHD. After setting the first group as the reference group, the hazard ratios (HR) and 95% confidence intervals (CIs) for IHD were calculated using multivariate Cox proportional hazards regression models after adjusting for potential confounding variables. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided, and statistical significance was set at $P < 0.05$.

RESULTS

Table 1 shows the study population's baseline characteristics ($n = 17,521$; 8,976 men and 8,545 women) according to HS and Hb levels. The mean age and BMI were 44.7 ± 10.4 years and 23.3 ± 3.1 kg/m^2. The mean Hb concentration was 14.4 ± 1.5 g/dL. The prevalence of severe HS was 11.5%. The mean values of mean arterial pressure, ALT, total cholesterol, and triglyceride levels were highest in the group with high HS and Hb levels (group 5). The most significant proportion of current smokers, alcohol drinkers, obesity, and hypertension was in group 5, while the proportion of individuals who participated in the regular exercise was lowest in group 4. Furthermore, group 5 showed the highest significant cumulative incidence of IHD over 50 months after the baseline survey (log-rank test, $P < 0.001$) (**Figure 2**).

Table 2 shows the multivariate Cox proportional hazards regression results for the prediction of IHD according to HS

TABLE 1 | Baseline characteristics of the study population.

Characteristics	Overall (n = 17,521)	Group 1 No hepatic steatosis (n = 11,961)	Group 2 Mild hepatic steatosis only (n = 2,550)	Group 3 Mild hepatic steatosis + high Hb (n = 992)	Group 4 Severe hepatic steatosis only (n = 1,274)	Group 5 Severe hepatic steatosis + high Hb (n = 744)	*P*-value[a]	*Post hoc*[b]
Age (years)	44.7 ± 10.4	43.8 ± 10.6	47.2 ± 10.1	45.8 ± 9.5	46.2 ± 9.6	45.8 ± 10.5	<0.001	a,b,c,d,e,g
Male sex (%)	51.2	41.8	67.4	67.0	81.3	74.2	<0.001	–
BMI (kg/m^2)	23.3 ± 3.1	22.2 ± 2.6	24.7 ± 2.5	25.2 ± 2.5	26.4 ± 2.9	26.6 ± 2.8	<0.001	a,b,c,d,e,f,g,h,i
Systolic BP (mmHg)	121.7 ± 15.4	118.8 ± 15.0	124.9 ± 14.5	128.8 ± 13.9	130.1 ± 14.1	132.1 ± 14.6	<0.001	a,b,c,d,e,f,g,i,j
Diastolic BP (mmHg)	75.9 ± 10.1	74.0 ± 9.7	78.0 ± 9.6	81.1 ± 9.2	81.5 ± 9.2	82.8 ± 9.4	<0.001	a,b,c,d,e,f,g,i,j
AST (IU/L)	21.5 ± 11.8	20.0 ± 10.0	21.9 ± 12.5	23.4 ± 8.4	28.3 ± 19.6	29.7 ± 14.9	<0.001	a,b,c,d,e,f,g,h,i
ALT (IU/L)	22.7 ± 21.8	18.5 ± 18.5	25.0 ± 20.1	29.1 ± 16.7	39.9 ± 33.0	43.8 ± 28.3	<0.001	a,b,c,d,e,f,g,h,i,j
GGT (IU/L)	31.2 ± 39.0	25.5 ± 32.2	37.0 ± 37.6	43.7 ± 40.0	50.5 ± 67.4	54.4 ± 49.1	<0.001	a,b,c,d,e,f,g,h,i
Hb (g/dL)	14.4 ± 1.5	14.1 ± 1.5	14.5 ± 1.3	16.0 ± 1.2	14.9 ± 1.1	16.3 ± 1.1	<0.001	a,b,c,d,e,f,g,h,i,j
FPG (mg/dl)	91.1 ± 9.8	89.2 ± 8.9	94.6 ± 9.7	93.9 ± 10.2	96.5 ± 10.6	96.8 ± 10.6	<0.001	a,b,c,d,f,g,h,i
Total cholesterol (mg/dl)	189.0 ± 33.5	183.9 ± 31.8	196.2 ± 34.5	203.0 ± 34.4	199.7 ± 32.7	209.2 ± 35.6	<0.001	a,b,c,d,e,f,g,i,j
Triglyceride (mg/dl)	122.7 ± 85.2	101.3 ± 57.2	151.4 ± 93.6	166.1 ± 90.9	186.7 ± 123.1	201.0 ± 160.0	<0.001	a,b,c,d,e,f,g,h,i,j
HDL-C (mg/dl)	53.6 ± 12.8	56.5 ± 12.8	48.7 ± 10.7	48.5 ± 10.4	44.8 ± 8.9	45.4 ± 8.7	<0.001	a,b,c,d,f,g,h,i
hsCRP (mg/L)	1.3 ± 3.6	1.1 ± 3.0	1.7 ± 5.6	1.7 ± 3.0	2.1 ± 3.7	2.0 ± 3.8	<0.001	a,b,c,d,f
Current smoker (%)	24.9	21.5	27.1	38.7	31.4	41.8	<0.001	–
Alcohol drinking (%)[c]	44.0	41.8	45.9	49.2	49.6	55.5	<0.001	–
Regular exercise (%)[d]	30.5	31.6	29.5	28.2	26.3	26.1	<0.001	–
Hypertension (%)	19.9	14.5	25.7	31.3	35.8	44.1	<0.001	
Obesity (%)	26.8	14.4	43.3	52.1	65.7	70.0	< 0.001	
HSI score	32.2 ± 4.7	30.5 ± 3.7	34.2 ± 3.9	35.6 ± 4.0	37.7 ± 4.9	38.5 ± 4.6	<0.001	a,b,c,d,e,f,g,h,i,j

[a] *P-values were calculated using 1-way ANOVA or Pearson's chi-square test.*

[b] *Post hoc analysis using the Bonferroni method: a, Group 1 vs. Group 2; b, Group 1 vs. Group 3; c, Group 1 vs. Group 4; d, Group 1 vs. Group 5; e, Group 2 vs. Group 3; f, Group 2 vs. Group 4; g, Group 2 vs. Group 5; h, Group 3 vs. Group 4; i, Group 3 vs. Group 5, and j, Group 4 vs. Group 5.*

[c] *Alcohol intake ≥ 140 g/week.*

[d] *Moderate-intensity physical exercise ≥three times/week.*

Hb, hemoglobin; BMI, body mass index; BP, blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; FPG, fasting plasma glucose; HDL-C, high density-lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; HSI, hepatic steatosis index.

and Hb levels. A total of 330 individuals (1.9%, 330/17,521) developed IHD during the follow-up period (310 angina pectoris and 20 myocardial infarction). Compared with the reference group (group 1), the HRs of IHD were 1.11 (95% CI, 0.80–1.98) in group 2, 1.23 (95% CI, 0.76–1.98) in group 3, 1.70 (95% CI, 1.16–2.48) in group 4, and 2.00 (95% CI, 1.29 to 3.10) in group 5 after adjusting for age, sex, BMI, smoking status, alcohol intake, and physical activity (Model 2, **Figure 3**). Similarly, these positive longitudinal associations were found after additionally adjusting for mean arterial pressure, fasting plasma glucose, total cholesterol, and hsCRP (Model 3). The corresponding adjusted HR for group 5 vs. group 1 was 1.79 (95% CI, 1.15–2.80).

Using a pairwise comparison of ROC analyses of incident IHD, the C-index of the groups according to HS and Hb levels was similar to the C-index produced using the fatty liver grades and HSI ($P = 0.123$ and $P = 0.829$, respectively). The specificity of the groups according to HS and Hb levels for classifying IHD was higher than that of the HSI score, which was similar to that of the fatty liver grades (**Table 3**).

DISCUSSION

Among community-dwelling Korean adults without diabetes, we found that patients with HS were more likely to develop IHD than those without steatosis. Hepatic lipid accumulation was associated with dose-dependent IHD in this large-scale, cohort study that included a 50-month follow-up. This study also showed that HS with elevated Hb levels within the normal range is jointly related to the incidence of IHD in the general population. There could be a complex interaction between haematologic risk, metabolic abnormalities, and IHD.

A previous study reported that the presence of NAFLD at baseline, defined clinically using the calculated HSI, can more likely lead to the development of IHD (15). In this study, we calculated the probabilities of new-onset IHD for both HS, defined radiologically, and HSI, and we compared the C-index for each model. There was no statistically significant difference between the two predictive models, suggesting a little difference between imaging methods and the scoring system in grading HS based on the blood test. NAFLD comprises

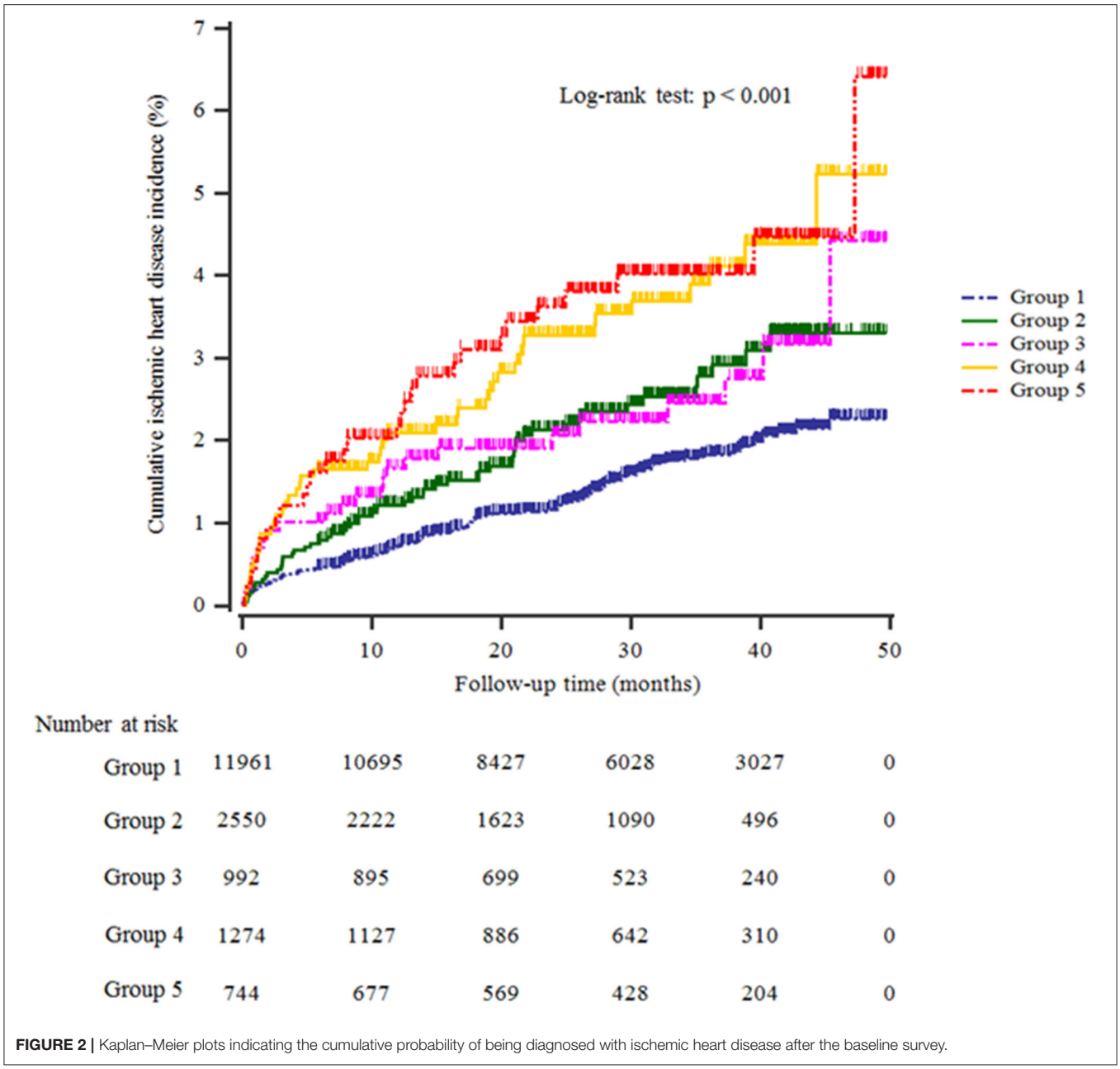

FIGURE 2 | Kaplan–Meier plots indicating the cumulative probability of being diagnosed with ischemic heart disease after the baseline survey.

a spectrum of liver disease, ranging from simple steatosis to steatohepatitis and liver cirrhosis (16). Although liver biopsy is considered the gold standard for NAFLD assessment, it has limited applicability in clinical settings (17). Imaging methods, including ultrasonography and MRI, is widely used as diagnostic tools for HS in clinical practice (18). However, there is no correlation between the histologic and radiologic severity of HS. NAFLD can be characterized by hepatic tissue damage from the inflammation caused by HS (19).

In assessing patients' hepatic condition using radiologic methods, some serologic markers, representing hepatic damage, require particular attention. A previous study reported that ferritin, regarded as a measure of iron storage, correlates with Hb concentration (12) . This means that high Hb levels could be another sign of high iron accumulation in human organs. Elevated ferritin levels are found in up to 30% of NAFLD patients, suggesting an association between elevated Hb levels and liver damage (20). The Korean Heart Study reported that high Hb levels increase the risk of IHD events (21). Iron accumulation, platelet count, and blood viscosity might explain why high Hb levels directly increase the development of IHD (21).

Although we could not determine the exact mechanism responsible for the complex interaction between Hb, HS, and IHD, several explanations for this interaction deserve

TABLE 2 | Hazard ratios and 95% confidence intervals for new-onset ischemic heart diseases according to hepatic steatosis and hemoglobin.

		Group 1 No hepatic steatosis	Group 2 Mild hepatic steatosis only	Group 3 Mild hepatic steatosis + high Hb	Group 4 Severe hepaticsteatosis only	Group 5 Severe HS + high Hb	P for trend
New cases of ischemic heart disease, n		177	56	24	44	29	
Mean follow-up, years		2.4 ± 1.1	2.2 ± 1.1	2.4 ± 1.1	2.4 ± 1.1	2.5 ± 1.1	
Pearson-years of follow-up		28,471	5,593	2,370	2,996	1,889	
Incidence rate/1,000 person -years		6.2	10.0	10.1	14.7	15.4	
Model 1	HR (95% CI)	1.00 (reference)	1.22 (0.90–1.65)	1.46 (0.95–2.23)	1.89 (1.35–2.65)	2.11 (1.42–3.13)	<0.001
	P-value		0.204	0.083	<0.001	<0.001	
Model 2	HR (95% CI)	1.00 (reference)	1.11 (0.80–1.98)	1.23 (0.76–1.98)	1.70 (1.16–2.48)	2.00 (1.29–3.10)	0.008
	P-value		0.534	0.404	0.006	0.001	
Model 3	HR (95% CI)	1.00 (reference)	1.11 (0.80–1.55)	1.23 (0.76–1.98)	1.75 (1.20–2.55)	2.00 (1.29–3.11)	0.005
	P-value		0.544	0.408	0.003	0.002	
Model 4	HR (95% CI)	1.00 (reference)	1.04 (0.75–1.46)	1.14 (0.70–1.85)	1.58 (1.08–2.32)	1.79 (1.15–2.80)	0.039
	P-value		0.806	0.591	0.019	0.010	

Model 1: adjusted for age and sex.
Model 2: adjusted for age, sex, body mass index, smoking status, alcohol intake, and physical activity.
Model 3: adjusted for age, sex, obesity, smoking status, alcohol intake, physical activity, mean arterial blood pressure, fasting plasma glucose, total cholesterol, and high-sensitivity C-reactive protein level.
Model 4: adjusted for age, sex, body mass index, smoking status, alcohol intake, physical activity, mean arterial blood pressure, fasting plasma glucose, total cholesterol, and high-sensitivity C-reactive protein level.

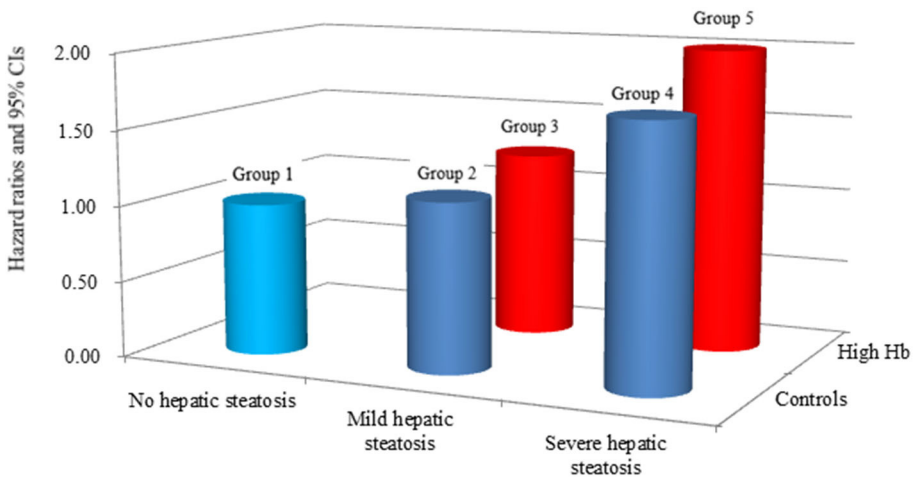

FIGURE 3 | Hazard ratios for incident ischemic heart disease according to hepatic steatosis and hemoglobin after adjusting for age, sex, body mass index, smoking status, alcohol intake, and physical activity.

consideration. Hb is an iron-containing protein, and iron accumulation can increase cardiovascular risk (22). Iron is a redox-active transitional metal and a potential catalyst in diverse cellular reactions resulting in reactive oxygen species (ROS) (23). ROS leads to endothelial tissue damage and metabolic disturbances, which are considered fundamental mechanisms for the development of IHD (24). In addition, ROS catalyzed by iron promotes low-density lipoprotein oxidation, and thus, they easily enter the arterial wall's inflammatory cells and lead to atherogenesis (25). Elevated Hb concentration contributes to increased blood viscosity, which increases peripheral resistance and decreases blood flow and perfusion (26). High Hb and hematocrit levels can induce platelet aggregation by releasing adenosine diphosphate (ADP) (27). A small population study showed that ADP-induced platelet aggregation was related to an increased incidence of coronary artery disease (28).

Previous data suggest that steatosis is related to cardiac arrhythmia and valvular heart disease, partly explaining the increased risk of IHD events in patients with steatosis (29, 30). Although HS with IHD is independent of elevated Hb levels, hepatotoxicity, induced by elevated Hb levels, may synergistically increase the risk of IHD development. A previous study has shown a bidirectional association between HS and elevated ferritin, represented by high Hb

TABLE 3 | Hepatic steatosis with hemoglobin vs. fatty liver grades and HIS score for predicting ischemic heart disease.

	Pairwise comparison of C-index			Ability to classify IHD			
	Difference	95% CI	*P*-value	Sensitivity (%)	Specificity (%)	C-index	*P*-value
Hepatic steatosis with Hb vs. fatty liver grade	0.003	−0.001 to 0.006	0.123				
Hepatic steatosis with Hb vs. HSI score	0.003	−0.025 to 0.031	0.829				
Fatty liver grade vs. HSI score	<0.001	−0.028 to 0.028	0.976				
Hepatic steatosis with Hb				46.4	68.5	0.584	<0.001
Fatty liver grade				46.4	68.5	0.581	<0.001
HSI score				69.1	46.1	0.581	<0.001

Hb, hemoglobin; HSI, hepatic steatosis index; C-index, concordance index; IHD, ischemic heart disease.

levels (31). HS is another clinical feature of insulin resistance. Insulin-induced downregulation of hepcidin increases iron absorption and accumulation in the liver and other organs (32). Conversely, iron accumulation may contribute to insulin resistance and hyperinsulinemia by interfering with hepatic insulin extraction (33). Furthermore, iron leads to highly toxic free radicals, such as the superoxide anion and hydroxide, through the Fenton reaction (23). Collectively, HS and elevated Hb levels are related to ROS and insulin resistance. They may share a common pathologic pathway, suggesting a possible synergistic action to the development of IHD.

Some strengths and limitations require careful consideration and may affect the interpretation of the present study results. A major strength of the work was that we conducted a cohort study using many Korean individuals linked to HIRA data, derived from the universal coverage system in Korea. As a result, there was a meager chance that the data was missing. Furthermore, this is the first study to investigate the combined effect of Hb levels and HS on the development of IHD, although these effects still require further confirmations. Another strength of our research is its large sample of 17,521 participants and its record of 330 IHD events over a median of 50 months follow-up.

This study had some limitations that should also be acknowledged. First, we could not assess the serum iron and ferritin levels from the HERAS-HIRA dataset. Further histochemical studies are needed because the relationship between Hb and tissue iron stores remains controversial. Second, although liver biopsy is the gold standard for diagnosing HS, we used imaging studies to define and grade HS. However, we also used a scoring system for the assessment of hepatic disease to decrease the bias in defining HS. Third, some individuals with diabetes may have been included in the participants because glycated hemoglobin A1c and 2-h oral glucose tolerance tests were not performed at the beginning of the study. Lastly, the HERAS-HIRA dataset assessed only newly developed IHD, not coronary angioplasty, myocardial resuscitation, or sudden death.

CONCLUSIONS

This study showed that HS participants were more likely to develop IHD than those without steatosis, and hepatic lipid and triglyceride accumulation, represented by steatosis severity, are individually associated with dose-dependent IHD. Moreover, we confirmed the combined effects of HS and Hb levels on incident IHD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board of Yonsei University College of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DJ, YL, and BP: study concept and design. YL and BP: acquisition, analysis, and interpretation of data and critical revision of the manuscript for important intellectual content. DJ: drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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The Complementary Relationship Between Echocardiography and Multi-Slice Spiral CT Coronary Angiography in the Diagnosis of Coronary Artery Thrombosis in Children With Kawasaki Disease

*Yun-ming Xu[1], Yan-qiu Chu[1], Xue-mei Li[1], Ce Wang[1], Quan-mei Ma[2], Xiao-na Yu[3], Xian-yi Yu[1], Rui Chen[1], Yan-lin Xing[1], Xue-xin Yu[1], Le Sun[1], Xiao-zhe Cui[1] and Hong Wang[1]**

[1] Department of Pediatrics, Shengjing Hospital of China Medical University, Shenyang, China, [2] Department of Radiology, Shengjing Hospital of China Medical University, Shenyang, China, [3] Department of Ultrasound, Shengjing Hospital of China Medical University, Shenyang, China

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Junjie Xiao,
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All India Institute of Medical Sciences Bhopal, India

***Correspondence:**
Hong Wang
wanghong_64@sina.com

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Aim: To compare the diagnostic values by using transthoracic echocardiography (ECHO) and multi-slice spiral CT coronary angiography (CTCA) for identifying coronary artery thrombosis in children with Kawasaki disease (KD).

Methods: Total 97 KD children with coronary artery dilation complications in our hospital from June 2012 to December 2020 were included in the study. CTCA and ECHO were performed after over 1 month of illness.

Results: Coronary artery thrombosis was found in 14 out of 97 patients. Among them, 10 were identified as positive by CTCA, 9 were identified as positive by ECHO, and 5 were identified as positive by both CTCA and ECHO.

Conclusion: Both CTCA and ECHO can be used to diagnose coronary artery thrombosis. ECHO has advantage in identifying low-density thrombus, and CTCA is better for the clot in distal coronary artery. They can complement each other.

Keywords: children, Kawasaki disease, coronary artery lesion, thrombosis, echocardiography, CTCA

INTRODUCTION

Kawasaki disease (KD) was first reported by Mr. Kawasaki in 1967. It is an acute febrile eruptive disease characterized by systemic vasculitis, which occurs in children under 5 years old (1), and the most frequent complication in the cardiovascular system is coronary artery lesions (CAL). At present, KD has surpassed rheumatic fever and became the most common acquired heart disease in children in developed countries (2). The probability of progressing to coronary artery disease is 15–25% in patients without systematic treatment (3). Coronary artery aneurysm with thrombosis is the most dangerous among various complications of KD in sub-acute and convalescent. Without timely treatment, it can cause acute myocardial infarction (4–6), ischemic cardiomyopathy, and even sudden death. For patients who survived, most of them will be challenged with low quality

of life and shortened life expectancy (7). Methods of monitoring coronary thrombosis include percutaneous echocardiography (ECHO), multi-slice spiral CT coronary angiography (CTCA), like selective coronary angiography performed in adults, and cardiac contrast-enhanced MR (8). In this study, we are the first comparing the advantages and limitations of ECHO and CTCA in the identifying thrombosis in coronary artery in KD patients.

METHODS AND MATERIALS

Patients

A total of 256 KD children were diagnosed with coronary artery dilatation from Dec 2010 to Mar 2020, and they were treated in the pediatric cardiovascular ward of Shengjing hospital, China Medical University. (1) In all, 97 out of 256 cases went through ECHO and CTCA in the same period time 1 month after illness. Data for these 97 patients were retrospectively analyzed. Among them, 68 were males (70.1%) and the average age was 4.9 years old (3 months to 13 years old). (2) In 159 of 256 patients, 132 of them whose CAL were recovered within 1 month of illness and CTC was not performed; 13 of them had severe allergies and were not scheduled for CTCA. CTCA tests failed in the rest 14 of 159 patients due to inadequate sedation or heart rate exceeding 90 beats per minute.

Method of Examination

ECHO

Philips IE33, EPIC-7C color Doppler echocardiography and S12-4 probe were used. The patients were examined in a quiet state. Patients who were not cooperative were treated with rectal administration of 5% chloral hydrate 1 ml/kg (maximum dose 30 ml) in advance. The left main coronary artery (LM), left anterior descending branch (LAD), left circumflex branch (LCX), and right coronary artery (RCA) were investigated. ECHO was performed by the sonographer specializing in the cardiovascular system.

CTCA

Philips Iqon spectral CT and Toshiba Aquilion One CT machines were used. Patients with heart rates over 90 bpm were treated with propranolol 1 mg/kg (maximum dose 20 mg) 1 h before the examination. For those whose heart rates that continued to be over 90 bpm, metoprolol 0.5 mg/kg (maximum dose 25 mg) would be administrated sublingually. For those who were uncooperative with the examination, hibernation mixture (chlorpromazine 1 mg/kg and promethazine 1 mg/kg, maximum dose of 25 mg each) was administrated intramuscular and 5% chloral hydrate 1 ml/kg (maximum dose of 30 ml) was rectal administrated. The CT three-dimensional reconstruction of coronary artery was performed in the supine position with a slice thickness of 1.0 mm at an interval of 1.0 mm. The non-ionic contrast agent Iohexol 350 was injected intravenously through a high-pressure syringe at a rate of 2.5 ml/s, and then spiral scanning was performed. The best cardiac cycle was selected for three-dimensional reconstruction, and the coronary artery was analyzed. Both ECHO and CTCA were performed within 3 days. CTCA was performed in patients who had one of the following conditions: (1) CAL occurred over 1 month before medicine was

TABLE 1 | The comparison of examination by ECHO and CTCA in KD patients with thrombosis.

KD	*n*	Male (%)	Age	LM		LAD		LCX		RCA	
				ECHO	CTCA	ECHO	CTCA	ECHO	CTCA	ECHO	CTCA
Thrombosis	14	11 (78.6)	6.1 ± 1.6	3	0	4	4	0	1	3	7
X^2				3.360		0.000		0.000		2.489	
p				0.222		1.000		1.000		0.115	

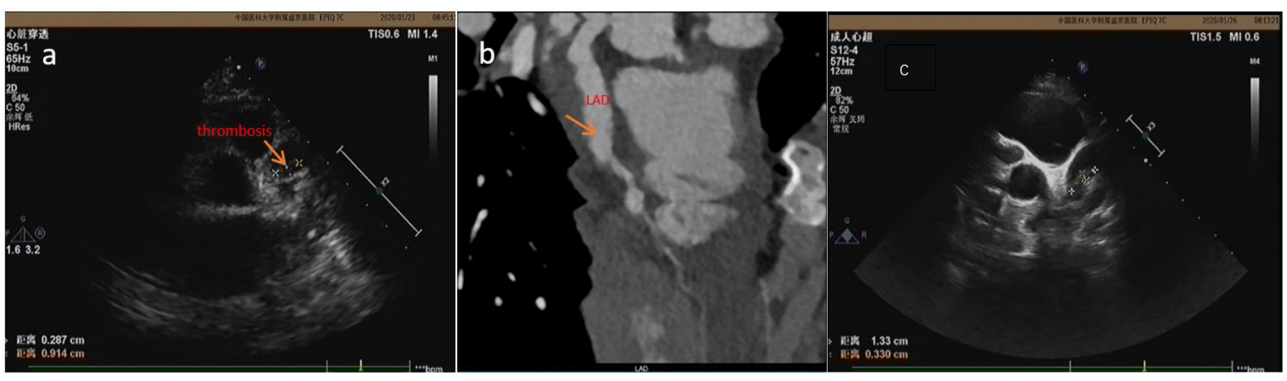

FIGURE 1 | The thrombosis was detected by ECHO at 19 days **(a)** and 22 days **(c)** of illness in case 1 but wasn't detected by CTCA **(b)** at 18 days of illness.

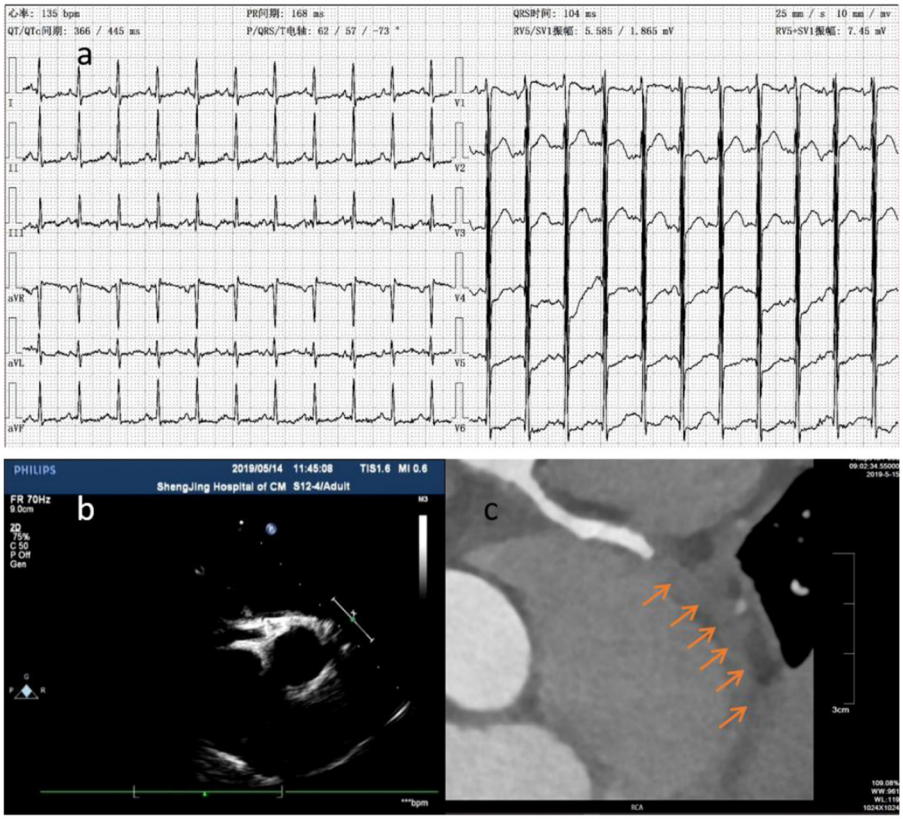

FIGURE 2 | The ECG showed downward ST segment and flat T wave **(a)**. Thrombosis (arrow) was detected at distal RCA by CTCA **(c)** in case 2 at about 6.5 months of illness, whereas it wasn't detected by ECHO 2 days before and 1 day after **(b)** CTCA.

withdrawn; (2) thrombosis was identified in ECHO; (3) giant CAA identified every year or every 2 years.

Diagnostic Criteria

The diagnosis of KD/incomplete Kawasaki disease (IKD) met the criteria of the Kawasaki Disease Research Committee of Japanese Ministry of health and welfare in 2002 (9) and the guidelines issued by American Heart Association (AHA) in 2004 (10). Coronary dilatation met the diagnostic criteria from the Ministry of health and welfare of Japan (11) and from new statement (12) issued by the AHA in 2017.

Outcome Measurement

The diameter of coronary artery in these patients were measured by both ECHO and CTCA methods, which was listed by LM, LAD, LCX, and RCA segments. Calcification and thrombosis in each coronary artery aneurysm (CAA) were defined by ECHO and CTCA.

Statistical Analysis

SPSS 22.0 statistical software was used for statistical processing. The normal distribution of collected data was presented by $\chi^2 \pm s$. Student's *t*-test was used for comparison between the two groups. The skew distribution of collected data was presented by median (m) or interquartile interval (P25 negative P75). The count data was presented in percentage (%). The chi-square test showed that $p < 0.05$ was statistically significant. KM curve analysis between LVEF $\geq$45% in groups with thrombosis or not.

RESULTS

General Information

The imaging data of 97 children with KD were retrospectively analyzed. Positive coronary artery thrombosis was identified in 14 out of 97 patients: 9 of 14 (64.29%) by ECHO, 10 of 14 (71.43%) by CTCA, and 5 of 14 by both ECHO and CTCA. The sensitivity of CTCA was slightly higher than that of ECHO for KD with right coronary artery aneurysm (See **Table 1**).

Case Analysis of Coronary Artery Thrombosis

There were three cases of LM, four cases of LAD, one case of LCX, and nine cases of RCA. Diagnosis of coronary artery thrombosis was missed in four cases by CTCA, including mural thrombosis in three cases (LAD in one case, RCA in two cases) and low-density LM in one case (**Figure 1**). In case 5, CTCA detected thrombosis in LAD and RCA, whereas ECHO only detected thrombosis in LAD. ECHO missed distal thrombosis in

RCA in five cases, and one of them (case 2) had RCA occlusion (**Figure 2**).

Coronary aneurysms have little effect on the blood supply to the myocardium, and the occurrence of ischemic cardiomyopathy depends on whether there is stenosis at both ends of the CAA and whether the blood flow is blocked by thrombus in the CAA. The central thrombus in CAA has the greatest impact on myocardial blood supply. The prognosis of patients with LVEF <45% was poor (the case 3 patient passed away). K-M curve analysis showed that the difference was significant ($p < 0.001$; **Figure 3**) (See **Table 2**).

DISCUSSION

Advantage and Limitation of ECHO

As for the means of examination, the advantage of ECHO is that it is non-invasive, convenient, and repeatable (13–15). The limitation of ECHO includes (i) that because the heart is spherical and surrounded by lungs, ECHO only examines the internal structure of the heart and provides limited evaluation on distal vessels, left circumflex artery, and a poor acoustic window in growing children (16); and (ii) for which the diagnosis of coronary artery calcification and stenosis is frequently missed when using ECHO (17). The sensitivity of ECHO in identifying coronary tumor is 66.7% (18). Furthermore, it is subject to several fallacies and is operator dependent (16). We have screened patients for CAL and thrombus using ECHO at least twice and then performed CTCA within 3 days of ECHO test for missed aneurysm and thrombus. Case 1: The patient had a history of leukemia in the past. Doctors at local hospital suspected that leukemia recurred in the early stage of his fever. Because he had rashes and conjunctival congestion, he was considered for differentiated diagnosis for KD. ECHO was performed at 5 days of illness and showed normal coronary arteries but an enlarged right ventricular and a little pericardial effusion. Then he was not diagnosed with KD and treated with methylprednisolone intravenously from the 5th to 15th day of illness. The fever subsided at 14 days after the disease onset. ECHO was performed in our hospital at 16 days of illness. Giant CAAs were found in bilateral coronary arteries, and thrombus was identified in LAD. Because the thrombus density was lower, there was still blood flowing through the coronary artery. He did not have palpitation, chest tightness, or chest pain symptoms. ECG did not show change in ST-T. However, the left ventricular end diastolic diameter (LVED) was increased to 42 (normal <36 mm). The filling defect (thrombosis) was not detected by CTCA (**Figure 1**). Therefore, subsequent anticoagulant therapy was applied, and the thrombus diminished on the 26th day of illness, shown by ECHO performed every other day. At the 85th day of illness, the thrombus reappeared. After thrombolytic therapy, the thrombus diminished. Our results are similar to those reported before (19); most acute coronary thrombosis in KD occurred in LAD. KD patients with coronary artery thrombosis are at risk of sudden death due to myocardial infarction.

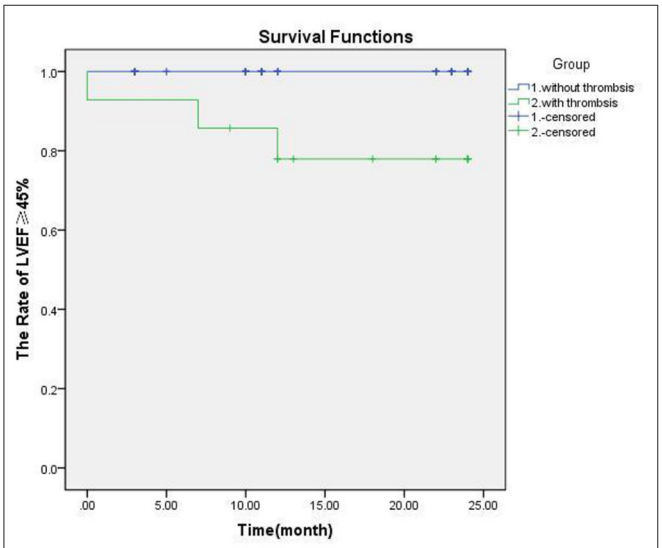

FIGURE 3 | The legend needs modification. The effect of thrombosis on myocardial function and survival in patients with Kawasaki disease. There was significant difference of prognosis in groups with thrombosis or not ($p = 0.000$).

Advantage and Limitation of CTCA

CTCA examines the lumen structure by injecting contrast agent into the coronary artery. This method makes it possible to delineate the coronary artery anatomy with higher temporal resolution and motion-free images at all heart rates with acceptable radiation exposure (19). CTCA can identify the coronary artery calcification (20) and help visualize distal coronary artery (18). Furthermore, CTCA is a useful imaging method for delineating coronary artery in KD patients for long term follow-up, especially in older children with thick chest walls and poor acoustic windows (21). CTCA allows comprehensive evaluation of coronary arteries in children with KD (22). When KD patients with CAA developed chest tightness, chest pain, shortness of breath, edema, and oliguria, clinicians should seriously suspect that patients may have myocardial infarction, even ECHO results are normal. We highly recommend to perform CTCA for these patients. For instance, the case 2 patient was diagnosed with KD and CAL. After taking medicine for 1 month, there was no significant changes observed in the coronary artery, and the patient did not complain any discomforts. His parents stopped his medicine. Six months later, the patient could not lie on his back and had irritative cough, accompanied with edema and oliguria. The level of cTnI was significantly increased by 1.406 (normal value <0.04 μg/L), and the level of Hs-cTnT was 6.15 (normal value <0.014 μg/ml). Although there was no abnormal coronary artery blood flow in both ECHO tests, ECHO identified significantly weakened and uncoordinated motion in the ventricular wall. The left ventricular ejection fraction (LVEF) was significantly reduced to 25%. ECG showed obvious myocardial ischemia (**Figure 2a**). CTCA confirmed RCA occlusion caused by distal thrombosis in RCA and the stenosis at the opening of LCX. The patient's symptoms were relieved by treatment with heparin, warfarin,

TABLE 2 | The location of coronary artery thrombosis identified.

Case	Age(y)	Gender	ECHO					CTCA				
			LM (mm) (*Z* score)	LAD (mm) (*Z* score)	LCX (mm)	RCA (mm) (*Z* score)	Exam time (days after diagnosis)	LM (mm) (*Z* score)	LAD (mm) (*Z* score)	LCX (mm)	RCA (mm) (*Z* score)	Exam time (days after diagnosis)
1	6	M	3.6–7 (1.97–9.81)	11.0 (22.17) (thrombus 3*9)	5.1	5.0–8.3 (5.65–12.96)	9		Middle 6.1 (9.94)	6.3	10.7–5.0 (18.28–6.31)	10
2	8	M	4.2 (3.80)	3.5 (3.83)	3.9	3.1–4.7 (1.80–6.13)	224			Stenosis of opening, proximal dilated 3.4	Middle and distal aneurysms were occlusion by thrombus	225
3	3	M	6.3 (10.57)	25*24 (64.99–62.20) (1–2 mm thrombus in anterior wall)		3–3.4 (2.64–3.65)	180	18			Proximal dilated	182
4	8	M	3.0 (0.66)	6.2 (10.3) (patchy thrombus)	2.6	2.6 (0.39)	2,418		Proximal aneurysm formation, possibly peri-aneurysmal thrombus			2,419
5	5	M		11.1–12.3 (21.71–24.70) thrombus		11–15 (21.43–31.08)	489		Proximal giant aneurysm 18*30 (43.49–75.77) (thrombus)	opening in aneurysm	Multiple giant aneurysms, bigger one 16*26 (33.49–57.59) (thrombus)	492
6	6	M	3.4–4.7 (1.19–4.12)	11.0–11.8 (21.42–23.37) thrombus	2.5	7.5–9.2 (10.65–14.32)	73	3.2	16.2 (34.09) multiple aneurysm (massive thrombus)	10.6*15 multiple aneurysm (few thrombus).	13.5*42.2 (23.60–85.55) multiple aneurysm	73
7	6	M	3.3 (1.71)	6.2–10.3 (10.85–21.42)		3.5–10.1 (2.76–18.45)	25		Middle fusiform aneurysm, maximum diameter 11 (23.23)		Full-range fusiform dilatation, the widest 10 (17.71) (small mural thrombus)	25
8	7	M	4.2 (3.67)	10.8 (22.34) fusiform dilatation	2.5	10.6 (18.71) mural thrombus	79		Proximal fusiform dilatation, 10.1 (20.55)		Fusiform dilatation, maximum diameter 11 (20.09), distal artery irregularly dilated, part of thrombus	79
9	6	F	2.9 (0.11)			3.9–11 (2.71–18.73) local aneurysm	1,862	3 (0.34)	3 (1.96)	1.8	Proximal 3.8 (2.71), local fusiform dilatation in the middle 11.1 (18.94) mural thrombus	1,861
10	4	F	4.4–4.7 (5.26–6.02) thrombus			2.3 (0.62)	364	4.2 (4.76)				366

(Continued)

TABLE 2 | Continued

Case	Age(y)	Gender	ECHO					CTCA				
			LM (mm) (Z score)	LAD (mm) (Z score)	LCX (mm)	RCA (mm) (Z score)	Exam time (days after diagnosis)	LM (mm) (Z score)	LAD (mm) (Z score)	LCX (mm)	RCA (mm) (Z score)	Exam time (days after diagnosis)
11	5	M	3.1–4.5 (1.80–5.29)			4.7–14.2 (6.26–29.18) local aneurysm, multiple mural thrombus	882	Local dilated			Proximal aneurysm 17*12 (35.93–23.87)	885
12	7	F	4.0–11.0 (3–19.28) 7*3 thrombus			3.5–4.2 (2.42–3.99) strong ECHO	2,611	Aneurysm 16*12 (30.9–21.60)	Originated from aneurysms	Originated from aneurysms	Proximal larger aneurysms, with Calcification and thrombus in it	2,614
13	9	M	4.2 (3.17)	2.9 (1.80)	2.5	2.9–3.4 (0.85–1.94)	515				Vessel thickening from proximal 2 cm to distal, thrombus	515
14	5	M	2.3 (−0.98)			2.0 (−0.96)	1,185	3.5 (1.8)	Proximal aneurysm 6.7*5 (11.53–7.27) mural calcification	2.8	Middle aneurysm 9.7 (16.69) with calcification and thrombus	1,187

Plavix, and aspirin, LVEF was increased to 53%, NT Pro-BNP level was decreased, and myocardial ischemia was relieved. The anticoagulant therapy was effective in recovering myocardial blood supply. Due to consideration of decreased kidney function, CTCA was not reperformed at that time. He was transferred to Beijing children's hospital. He was intermittently monitored DIC for two times, (INR 1.1 and 1.2), platelet aggregation function was not monitored, and RCA was still blocked by CTCA 6 months later (**Figure 2c**). After that, his LVED increased continuously, and LVEF was about 45%. As a result, the patient was not permitted to run. Thus, CTCA can find coronary artery stenosis and distal thrombosis.

Performing CTCA requires more procedural preps than performing ECHO: (i) use of an oral beta blocker to ensure heart rate <90 bpm; (ii) fasting for more than 4 h to prevent vomiting and aspiration (contrast agent injection may result in nausea); (iii) keeping the patient stable during examination (KD mostly occurs in children under 5 years old, and parents sometimes do not accept dual sedation required for performing examination); (iv) parents' concern about their children being exposed to X-rays; and (v) application of contrast agent. KD mostly occurs in allergic children (23), and they may also be allergic to iodine and have a potential risk of anaphylactic shock. Repeated use of contrast agent in a short period of time may cause renal damage (24). In addition, the requirements to perform cardiac MRA are higher than CTCA—the roaring noise of the instrument affects the sedative effect, and the examination time is longer than 30 min, which requires prolonged sedation. It also requires the application of radiation and contrast agents. Therefore, it is not frequently used to test for CAA or thrombosis but to test for ischemic cardiomyopathy (such as in case 2). Coronary artery thrombosis is the most serious complication in KD, because it can lead to myocardial infarction, coronary cardiomyopathy, and even sudden death (25) (case 3 was found to have coronary artery thrombosis and ischemic cardiomyopathy. About 2 years later, the patient suddenly died). So timely diagnosis, regular follow-up, and timely treatment are the keys to preventing disease progression. In case 4, the patient was 18 months old, and he was diagnosed with KD in our hospital on the 12th day of illness. Although he was treated with IVIG immediately, he still developed a giant CAA in LM. Because he had allergic rhinitis and used to pick his nose, after taking warfarin, dipyridamole, and aspirin, he often happened to have epistaxis and had nose frequent bleeding. His parents had to discontinue the drug. During DIC monitoring, his INR was always <2 (the standard INR was 2–3 during warfarin treatment). About 5.5 years later, there was no thrombus reported in CAA. However, at 6.5 years of illness, there was a large thrombus detected in LM. After intravenous treatment with heparin for 1 week, followed by warfarin, the thrombus did not shrink significantly (**Figure 4**). At 7.5 years of illness, the patient experienced chest tightness and palpitation after exercise. Prognosis in the long run was not clear. Therefore, it is important to identify thrombus timely and it is essential to execute standardized treatment.

The prognosis and quality of life in KD children are related to blood flow in coronary artery. Only stenosis of the coronary artery is rare. The most common event is the occlusion of blood

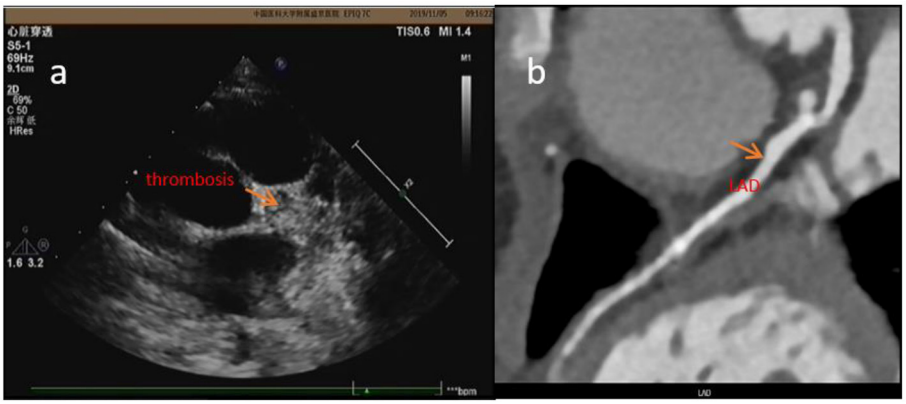

FIGURE 4 | In case 4, the thrombosis was detected by ECHO **(a)** 2 days before CTCA but wasn't detected by CTCA **(b)** at about 6.5 years of illness.

flow after thrombosis in CAA. In our center, because of the lessons of the past, if patients with CAL last over 1 month, even in the absence of myocardial ischemia and with normal ECG, routine CTCA examination is required before stopping the medication (7), in order to find the CAA on the far side. In those with a giant CAA, once thrombosis was found in ECHO, CTCA should be performed in order to find whether another one was missed and determine the degree of ischemia (case 4). For those CAA patients with myocardial ischemic symptoms, even though ECHO presented normal results, CTCA must be done to in case that a distal thrombus (such as in case 2) is missed in ECHO.

In summary, ECHO is a simple, non-invasive and repeatable examination method. At present, it is still the first choice for detecting coronary artery lesions in KD. It can identify the thrombus at the proximal end of coronary artery and even with low density. However, when patients experience chest pain, sweating, fast heartbeat, elevated blood pressure and not able to lie down, combined with laboratory examination, elevated troponin, and brain natriuretic peptide, ST-T change in ECG and other symptoms of myocardial infarction, clinicians should seriously suspect coronary artery thrombosis. If ECHO does not support the diagnosis, CTCA examination must be performed to identify distal lesions (26). CTCA is considered the gold standard for detecting coronary artery lesions with high resolution and sensitivity on distal vessels, and the positive detection rate of thrombosis in our center is higher than that by ECHO. However, it comes with certain risks due to invasive procedures and the usage of contrast agent (27, 28).

Therefore, both echo and CTCA have advantages and disadvantages. They can be combined to improve the diagnosis rate for coronary thrombosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shengjing Hospital, China Medical University, China. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Y-mX: case observation, data collection and analysis, and manuscript editing. Y-qC: case observation and data analysis. X-mL, CW, RC, Y-lX, X-xY, LS, and X-zC: case observation. Q-mM: CTCA image analysis. X-nY: ECHO image analysis. X-yY: some patient diagnosis and treatment. HW: diagnosis of most patients, treatment, all image selection, data analysis, editing the discussion, and conclusion of the manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Cardiovascular Medicine

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Risk Factors of Atrial Arrhythmia in Patients With Liver Cirrhosis: A Retrospective Study

Xiya Lu, Zhijing Wang, Liu Yang, Changqing Yang† and Meiyi Song*†*

Division of Gastroenterology and Hepatology, Digestive Disease Institute, Shanghai Tongji Hospital, Tongji University School of Medicine, Shanghai, China

Background and Objectives: Liver cirrhosis is known to be associated with atrial arrhythmia. However, the risk factors for atrial arrhythmia in patients with liver cirrhosis remain unclear. This retrospective study aimed to investigate the risk factors for atrial arrhythmia in patients with liver cirrhosis.

Methods: In the present study, we collected data from 135 patients with liver cirrhosis who were admitted to the Department of Gastroenterology at Shanghai Tongji Hospital. We examined the clinical information recorded, with the aim of identifying the risk factors for atrial arrhythmia in patients with liver cirrhosis. Multiple logistic regression analysis was used to screen for significant factors differentiating liver cirrhosis patients with atrial arrhythmia from those without atrial arrhythmia.

Results: The data showed that there were seven significantly different factors that distinguished the group with atrial arrhythmia from the group without atrial arrhythmia. The seven factors were age, white blood cell count (WBC), albumin (ALB), serum Na^+, B-type natriuretic peptide (BNP), ascites, and Child-Pugh score. The results of multivariate logistic regression analysis suggested that age ($\beta = 0.094$, OR = 1.098, 95% CI 1.039–1.161, $P = 0.001$) and ascites ($\beta = 1.354$, OR = 3.874, 95% CI 1.202–12.483, $P = 0.023$) were significantly associated with atrial arrhythmia.

Conclusion: In the present study, age and ascites were confirmed to be risk factors associated with atrial arrhythmia in patients with liver cirrhosis.

Keywords: liver cirrhosis, atrial arrhythmia, age, ascites, risk factor

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Jinwei Tian,
The Second Affiliated Hospital of Harbin Medical University, China
Qingchun Zeng,
Southern Medical University, China

***Correspondence:**
Changqing Yang
cqyang@tongji.edu.cn
Meiyi Song
1610688@tongji.edu.cn

†These authors have contributed equally to this work

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INTRODUCTION

The prevalence of liver cirrhosis caused by alcohol consumption, as well as viral, immune, and other potentially pathogenic factors are expected to increase in the coming decades. The interaction between the liver and heart has been described previously (1, 2). First, the heart and liver are common target organs of pathogenic factors, such as alcohol consumption and infection (3, 4). Cardiac complications are not rare during decompensated liver cirrhosis, including diastolic and systolic dysfunction and cardiac electrophysiological remodeling, known as cirrhotic cardiomyopathy (5). Cardiac complications of liver cirrhosis are typically the result of medications, hemodynamic disorders, infections, inflammatory states, and other unknown factors.

As previously reported, patients with liver cirrhosis have an increased risk of atrial arrhythmia even without underlying heart disease (6, 7). This risk does not decrease following liver transplantation. Atrial arrhythmia has been associated with a higher risk of perioperative cardiovascular events and poorer long-term prognoses (8). Atrial fibrillation or flutter, atrial premature beat, and atrial tachycardia are common atrial arrhythmias. Anticoagulation treatment is an important therapeutic element that is used to prevent stroke in patients with atrial fibrillation. However, anticoagulants significantly increase the risk of esophageal and gastric variceal bleeding in patients with liver cirrhosis, concomitantly increasing the risk of death (5, 9). The choice of anticoagulation therapy in patients with liver cirrhosis is more challenging than it is in patients without chronic liver disease (10). Age, obesity, diabetes, hypertension, and cardiovascular disease are known to increase the risk of new-onset atrial arrhythmia (11–14); however, the risk factors for atrial arrhythmia in patients with liver cirrhosis are not fully understood.

In this retrospective study, we examined the medical records of cirrhotic patients with or without atrial arrhythmia to screen for risk factors associated with atrial arrhythmia. Our study aimed to identify the risk factors for new-onset atrial arrhythmia in patients with liver cirrhosis, in order to provide a potential method for predicting atrial arrhythmia in this patient population.

METHODS

Experiment Design and Participants

We enrolled patients with liver cirrhosis who were admitted to the Department of Gastroenterology at Shanghai Tongji Hospital between January 2020 and January 2021, as shown in **Figure 1**. All medical information was obtained from electronic medical records. The exclusion criteria were as follows: (1) pregnancy; (2) structural heart disease or symptoms of heart failure; (3) diagnosis of malignant tumor; (4) cardiogenic liver cirrhosis; (5) severe infection; and (6) underweight and cachexia. A total of 135 patients were included in the study. This study was approved by the Ethics Committee of Tongji Hospital Affiliated to Tongji University.

This study used PASS 15 to calculate the sample size. The minimal significance (α) and statistical power (1 – β) were set at 0.05 and 0.80, respectively. According to the calculation results, the minimum sample size of the control group is 55, the arrhythmia group is 74, and total sample size is 129 as shown in **Table 1**.

Ascites was diagnosed according to the guidelines of the International Ascites Club (15), as follows: mild, only detectable by ultrasound; moderate, evident by moderate symmetrical distension of the abdomen; and severe, with marked abdominal distension.

Hepatic encephalopathy was diagnosed according to AASLD/EASL guidelines (16), as follows: Grade I, mild mental and behavioral changes; Grade II, significant personality change; Grade III, lethargy; and Grade IV, coma.

Gastroesophageal varices were diagnosed by endoscopy and were divided into two subgroups: (1) no variceal bleeding during this admission and (2) with variceal bleeding during this admission.

TABLE 1 | Calculation of sample size using PASS 15.

α	1-β	Control	Atrial arrhythmia	Total
0.05	0.80	74	55	129

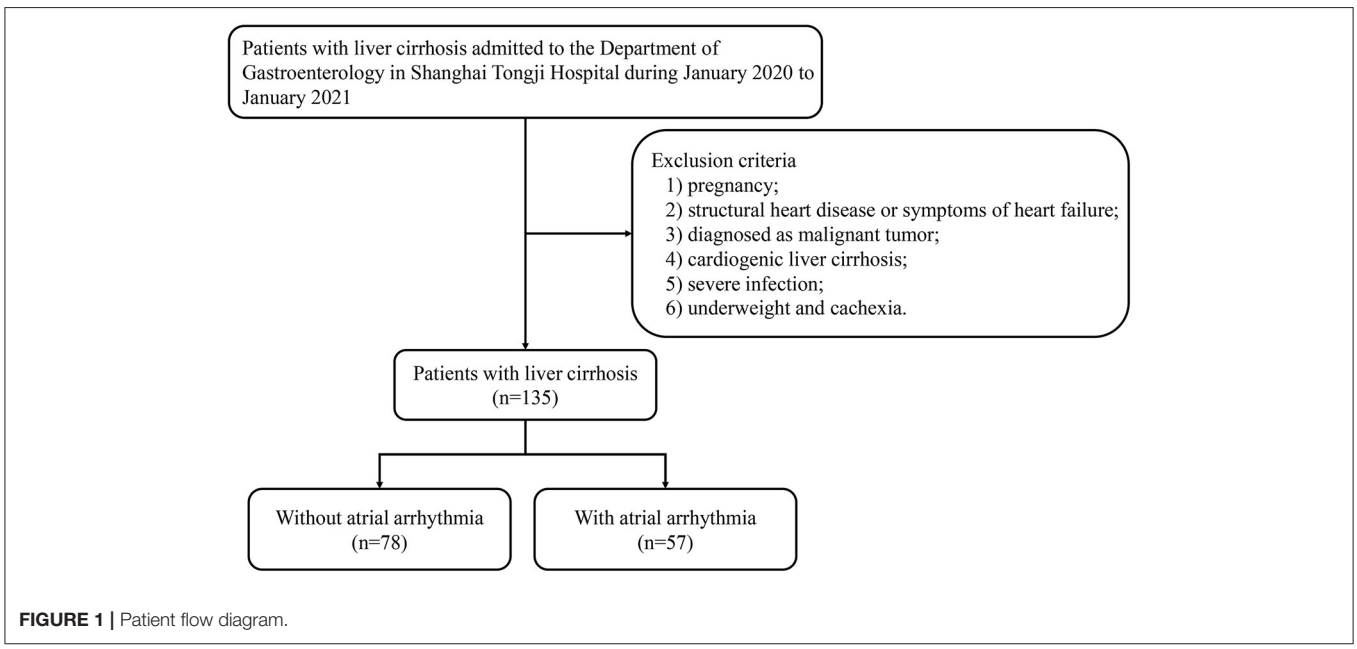

FIGURE 1 | Patient flow diagram.

TABLE 2 | Main characteristics of liver cirrhosis patients with or without atrial arrhythmia.

Variable	Control	Atrial arrhythmia	Total	χ^2	*P*
	(*n* = 78)	(*n* = 57)	(*n* = 135)		
Gender					
Male	36	25	61 (45.19%)	0.070	0.791
Female	42	32	74 (54.81%)		
Body weight (kg)	60.86 ± 8.04	60.14 ± 8.79	60.56 ± 8.34	/	0.623
BMI (kg/m^2)	21.49 ± 1.93	21.55 ± 2.15	21.51 ± 2.01	/	0.852
Age (years)	61.45 ± 11.92	73.54 ± 8.21	66.56 ± 12.07	/	**<0.001**
Etiology of liver cirrhosis					
Alcoholic	9	8	17 (12.59%)	2.295	0.682
Hepatitis B	29	16	45 (33.33%)		
Hepatitis C	6	7	13 (9.63%)		
Immune	19	12	31 (22.96%)		
Others	15	14	29 (22.96%)		
Atrial arrhythmia					
Atrial tachycardia	0	14 (24.56%)	/	/	/
Atrial fibrillation	0	4 (7.02%)	/		
Atrial flutter	0	7 (12.28%)	/		
Atrial premature beats	0	32 (56.14%)	/		

The bold values of "P" represent statistical differences between control and atrial arrhythmia group.

Clinical Information

General information, including age, sex, body weight, body mass index [BMI, weight (kg)/height (m)^2], etiology of liver cirrhosis, blood pressure, pulse pressure, past medical history, and complications of liver cirrhosis were collected from the patient records. The blood pressure of patients was measured using a mercury sphygmomanometer after 5 min in a seated position. Blood pressure was determined as the average of two measurements. The etiology of liver cirrhosis was divided into five classes: alcoholic liver cirrhosis, hepatitis B liver cirrhosis, hepatitis C liver cirrhosis, immune-related liver cirrhosis, and others.

Biochemical characteristics included hemoglobin (Hb), white blood cell count (WBC), platelet count (PLT), prothrombin time (PT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyltransferase (γ-GT), alkaline phosphatase (AKP), total bilirubin, direct bilirubin, albumin (ALB), serum K^+, serum Na^+, serum Cl^-, creatinine, B-type natriuretic peptide (BNP), and troponin. Venous blood was collected from all patients on the morning of the first day after admission, and biochemical characteristics were detected using an automatic biochemical analyzer.

Electrocardiogram Detection

The patients were kept in a horizontal position for 5 min to recover their resting heart rate and underwent ECG detection using a 12-lead synchronous electrocardiograph. The voltage was 10 mm/mV, and the lead wires and electrodes were connected after wiping the skin with 75% ethanol. The paper speed was set to 25 mm/s, and at least five consecutive cardiac cycles were recorded. The ECG diagnosis was performed by two experienced electrocardiologists in a double-blinded manner. A total of 135 patients included in the study were divided into two groups: those without arrhythmia and those with arrhythmia, including atrial tachycardia, atrial fibrillation, atrial flutter, and atrial premature beats.

Portal Vein Doppler Ultrasound Detection

A TOSHIBA Apolio 500 color Doppler ultrasound diagnostic apparatus with a probe frequency of 5 MHz was used for this study. The patients were instructed to fast for 12 h before the ultrasound examination. The tilt probe was adjusted so that the angle between the Doppler sound velocity and the blood vessel path was 60°, and the inner diameter and the blood flow velocity of the main portal vein and the main splenic vein could be measured (17, 18).

Child-Pugh Score

The Child-Pugh score was calculated using ascites, hepatic encephalopathy, total bilirubin, ALB, and PT extension, as previously described (19).

Statistical Analysis

The measurement data are presented as mean ± SD, and the Student's *t*-test was used to compare the characteristic differences between the two groups. Categorical data are recorded as the frequency of categorical variables, and the Chi-square test was used to compare the characteristic differences between the categorical variable groups. *P*-value (P) < 0.05 was considered statistically significant. In the results of the Student's *t*-test and Chi-square test, factors with statistical differences were selected for multiple logistic regression analysis to calculate the odds ratio (OR) and 95% confidence interval (CI).

TABLE 3 | The biochemical characteristics and portal vein doppler ultrasound of liver cirrhosis patients with or without atrial arrhythmia.

Variable	Control	Atrial arrhythmia	Total	*P*
	(*n* = 78)	(*n* = 57)	(*n* = 135)	
Systolic blood pressure (mmHg)	123.82 ± 13.61	123.32 ± 19.35	123.61 ± 16.22	0.866
Diastolic blood pressure (mmHg)	74.33 ± 8.36	72.14 ± 12.57	73.41 ± 10.36	0.255
Pulse Pressure	49.49 ± 13.85	51.18 ± 16.40	50.20 ± 14.94	0.519
Laboratory findings				
HB (g/L)	108.53 ± 27.38	104.44 ± 72.20	106.80 ± 51.12	0.648
WBC ($\times 10^9$/L)	4.59 ± 2.44	6.22 ± 3.25	5.27 ± 2.91	**<0.001**
PLT ($\times 10^9$/L)	84.94 ± 59.54	104.44 ± 63.18	93.17 ± 61.63	0.069
PT (s)	14.16 ± 2.56	14.97 ± 3.56	14.49 ± 3.03	0.154
AST (U/L)	66.31 ± 121.84	54.84 ± 59.33	61.47 ± 100.17	0.513
ALT (U/L)	41.23 ± 71.13	29.82 ± 34.41	36.41 ± 58.60	0.266
γ-GT (U/L)	67.42 ± 68.72	91.21 ± 141.74	76.90 ± 104.22	0.289
AKP (U/L)	135.93 ± 75.68	141.77 ± 130.32	138.25 ± 100.56	0.759
Total bilirubin (μmol/L)	44.02 ± 76.03	48.56 ± 69.86	45.94 ± 73.26	0.724
Direct bilirubin (μmol/L)	17.81 ± 36.91	19.32 ± 32.35	18.45 ± 34.94	0.806
ALB (g/L)	32.56 ± 6.91	28.49 ± 5.16	30.84 ± 6.53	**0.003**
Serum Na^+ (mmol/L)	139.46 ± 4.65	137.22 ± 6.05	138.51 ± 5.38	**0.022**
Serum K^+ (mmol/L)	3.74 ± 0.57	3.99 ± 0.87	3.85 ± 0.72	0.057
Serum Cl^- (mmol/L)	105.32 ± 4.80	103.25 ± 7.09	104.45 ± 5.94	0.059
Serum Creatinine (μmol/L)	94.73 ± 88.94	121.89 ± 76.66	106.20 ± 84.75	0.066
BNP (pg/mL)	97.14 ± 142.51	295.08 ± 308.15	187.27 ± 252.10	**<0.001**
Troponin (ng/mL)	0.02 ± 0.08	0.14 ± 0.67	0.07 ± 0.44	0.190
Portal vein doppler ultrasound findings				
The diameter of the main portal vein (mm)	11.54 ± 2.40	11.43 ± 2.89	11.49 ± 2.59	0.826
The blood flow velocity of the portal vein (cm/s)	16.42 ± 4.46	17.32 ± 5.10	16.77 ± 4.72	0.324
The inner diameter of the main splenic vein (mm)	8.15 ± 2.34	8.02 ± 2.70	8.10 ± 2.48	0.804
The blood flow velocity of the spleen (cm/s)	18.45 ± 7.15	20.95 ± 5.03	19.44 ± 6.48	0.067

The bold values of "P" represent statistical differences between control and atrial arrhythmia group.

TABLE 4 | The complication of liver cirrhosis patients with or without atrial arrhythmia.

Complication	Control	Atrial arrhythmia	Total	χ^2	*P*
	(*n* = 78)	(*n* = 57)	(*n* = 135)		
Ascites					
No	63	19	82 (60.74%)	31.076	**<0.001**
Ascites	15	38	53 (39.26%)		
Hepatic encephalopathy					
No	74	50	124 (91.85%)	2.251	0.202
Hepatic encephalopathy	2	2	4 (2.96%)		
Gastroesophageal varices					
No	13	20	33 (24.44%)	9.949	0.271
No variceal bleeding	60	29	89 (65.93%)		
Variceal bleeding	5	8	13 (9.63%)		

The bold values of "P" represent statistical differences between control and atrial arrhythmia group.

RESULTS

Main Clinical Characteristics of Patients

The main characteristics of patients with liver cirrhosis are presented in **Table 2**. A total of 135 liver cirrhosis patients included [61 men (45.19%) and 74 women (54.81%)], and the age of liver cirrhosis patients was 66.56 ± 12.07 years. Furthermore, body weight of liver cirrhosis patients was 60.56 ± 8.34 kg, and BMI was 21.51 ± 2.01 kg/m^2. Among the liver cirrhosis patients, 17 patients (12.59%) had alcohol-related liver cirrhosis, 45 patients (33.33%) had HBV-related liver cirrhosis, 13 (9.63%) had HCV-related liver cirrhosis, 31 (22.96%) had liver cirrhosis caused by autoimmune diseases, and 29 (22.96%) had liver cirrhosis related to other causes. Among 57 liver cirrhosis patients with atrial arrhythmia, there were 14 (24.56%) patients with atrial tachycardia, 4 (7.02%) patients with atrial fibrillation, 7 (12.28%) patients with atrial flutter, and 32 (56.14%) patients

TABLE 5 | The Child-pugh score of liver cirrhosis patients with or without atrial arrhythmia.

Liver function classification	Control	Atrial arrhythmia	Total	*P*
	(*n* = 78)	(*n* = 57)	(*n* = 135)	
Child-pugh score	7.03 ± 1.84	8.07 ± 2.22	7.49 ± 2.09	**0.003**

The bold values of "P" represent statistical differences between control and atrial arrhythmia group.

TABLE 6 | Multivariate logistic regression analysis of factors associated to atrial arrhythmia in liver cirrhosis patients.

Variable	β	Odds ratio	95% CI		*P*
			Lower	Upper	
Age	0.094	1.098	1.039	1.161	**0.001**
WBC ($\times 10^9$/L)	0.080	1.084	0.866	1.356	0.482
ALB (g/L)	0.026	1.026	0.902	1.168	0.692
Serum Na^+ (mmol/L)	−0.028	0.972	0.879	1.075	0.582
BNP (pg/mL)	0.004	1.004	1.000	1.008	0.063
Child-Pugh score	0.043	1.044	0.689	1.581	0.839
Ascites	1.354	3.874	1.202	12.483	**0.023**

The bold values of "P" represent statistical differences between control and atrial arrhythmia group.

with atrial premature beats. Only age was a significant factor impacting atrial arrhythmia. Liver cirrhosis patients with atrial arrhythmia (73.54 ± 8.21 years) were significantly older than those without atrial arrhythmia (61.45 ± 11.92 years) ($P < 0.001$).

Biochemical Characteristics and Portal Vein Doppler Ultrasound of Liver Cirrhosis Patients With or Without Atrial Arrhythmia

The biochemical characteristics and portal vein Doppler ultrasound results of patients with liver cirrhosis are presented in **Table 3**. The results showed that there were significant differences in WBC, ALB, serum Na^+, and BNP between the two groups. However, no significant differences in the inner diameter and the blood flow velocity of the main portal vein and the main splenic vein were observed. The WBC (6.22 ± 3.25 × 10^9/L) and BNP (295.08 ± 308.15 pg/mL) in liver cirrhosis patients with atrial arrhythmia were significantly higher than those in patients without atrial arrhythmia (WBC: 4.59 ± 2.44 × 10^9/L, BNP: 97.14 ± 142.51 pg/mL). The ALB (6.22 ± 3.25 × 10^9/L) and serum Na^+ (137.22 ± 6.05 mmol/L) in liver cirrhosis patients with atrial arrhythmia were significantly lower than that in the group without atrial arrhythmia (ALB: 32.56 ± 6.91 g/L, serum Na^+: 139.46 ± 4.65 mmol/L).

Complications and Child-Pugh Score of Liver Cirrhosis Patients With or Without Atrial Arrhythmia

The complications of liver cirrhosis (ascites, hepatic encephalopathy, and gastroesophageal varices) are presented in **Table 4**. Among 135 liver cirrhosis patients, 53 (60.74%) had ascites and 4 (2.96%) had hepatic encephalopathy. Additionally, 33 patients (24.44%) had no gastroesophageal varices, 89 patients (65.93%) had gastroesophageal varices but no variceal bleeding, and 13 patients (9.63%) had gastroesophageal varices and variceal bleeding. The results of the Chi-square test suggested that liver cirrhosis patients with ascites were more likely to present with atrial arrhythmia ($\chi^2 = 31.076$, $P < 0.001$), but there were no significant differences related to hepatic encephalopathy or gastroesophageal varices between the two groups.

The Child-Pugh score [calculated on the basis of prolonged PT (PT: 9.8–14.5 s), serum levels of total bilirubin and ALB, severity of ascites, and hepatic encephalopathy] of liver cirrhosis patients with or without atrial arrhythmia are presented in **Table 5**. The results showed that the Child-Pugh scores of liver cirrhosis patients with atrial arrhythmia (8.07 ± 2.22) were significantly higher than those of liver cirrhosis patients without atrial arrhythmia (7.03 ± 1.84) ($P = 0.003$).

Multivariate Logistic Regression Analysis of Factors Predicting Atrial Arrhythmia in Liver Cirrhosis Patients

In this study, multivariate logistic regression analysis was used to identify the risk factors for atrial arrhythmia in patients with liver cirrhosis. The results of multivariate logistic regression analysis of significantly different factors (age, ALB, serum Na^+, BNP, ascites, and Child-Pugh score) are presented in **Table 6**. The results suggested that age (β = 0.094, OR = 1.098, 95% CI 1.039–1.161, $P = 0.001$) and ascites (β = 1.354, OR = 3.874, 95% CI 1.202–12.483, $P = 0.023$) were significantly associated with atrial arrhythmia.

DISCUSSION

In the present study, we aimed to identify the risk factors associated with new-onset atrial arrhythmia in patients with cirrhosis. We enrolled 135 patients with liver cirrhosis and performed a retrospective study. Seven clinical variables were found to be significantly different between liver cirrhosis patients with or without arrhythmia. These factors were age, WBC, ALB, serum Na^+, BNP, ascites, and Child-Pugh score. Once multivariate logistic regression analysis and multivariable adjustment were performed, we found that age and ascites were two risk factors associated with atrial arrhythmia in patients with liver cirrhosis.

In the past few decades, the number of liver cirrhosis-related deaths has steadily increased (20, 21). Globally, significant medical resources are devoted to the treatment and care of patients with liver cirrhosis every year (22–25). Previous studies have demonstrated that liver cirrhosis may cause cirrhotic cardiomyopathy, which is related to atrial arrhythmia, and particularly atrial fibrillation (7, 26).

Our data suggest that patients with liver cirrhosis with atrial arrhythmia are about 10 years older than those without atrial arrhythmia. Multivariate logistic regression analysis showed that atrial arrhythmia was significantly associated with advanced age

in our study. Similarly, Mwalitsa and Gundling found that the occurrence of atrial fibrillation is positively correlated with age (27, 28). Previous studies confirmed that atrial arrhythmia is more likely in elderly patients, and the prevalence of atrial fibrillation is associated with increasing age (29). Additionally, our data suggested that liver cirrhosis patients with ascites were more likely to develop atrial arrhythmia. In general, myocardial diastolic dysfunction occurs before systolic dysfunction in patients with liver cirrhosis and ascites (30). Myocardial diastolic dysfunction is mainly due to myocardial hypertrophy and fibrosis, and consequently induces structural heart disease and arrhythmia (31–34). We hypothesized that ascites induced myocardial diastolic dysfunction due to disorders of peripheral blood circulation, which may be a possible mechanism by which atrial arrhythmia develops. However, a direct correlation between ascites and atrial arrhythmia has not been reported previously. In the present study, we thus identified a novel risk factor for atrial arrhythmia in patients with liver cirrhosis.

In the present study, there were significant differences in ALB, BNP, serum Na^+, and Child-Pugh scores between liver cirrhosis patients with atrial arrhythmia and those without atrial arrhythmia. However, after correction, these factors did not correlate with arrhythmia. Previous studies have been controversial regarding the correlation between the severity of liver disease and cardiac dysfunction (27, 31). In theory, it is considered that the severity of liver cirrhosis correlates with an increased risk of atrial arrhythmia due to the severe complications associated with higher Child-Pugh scores, such as hypoalbuminemia and the disturbance of water and electrolyte balance (29, 35). However, our data suggest that there is no correlation between Child-Pugh scores and the occurrence of atrial arrhythmia in patients with liver cirrhosis. Our data showed that ALB and serum Na^+ levels were significantly decreased in liver patients with atrial arrhythmia, which may be associated with the development of ascites. In patients with liver cirrhosis, the decrease in serum Na^+ caused by water and sodium storage, as well as the inversion of the A/G ratio and the subsequent decrease of plasma colloidal osmotic pressure caused by insufficient synthesis of ALB, are all essential factors in the development of ascites (36, 37). A prospective clinical study found that BNP, the most sensitive biochemical marker, is associated with the MELD score in Child-Pugh C patients (38). It has been reported that BNP is a predictive factor of cardiac decompensation risk in patients with liver cirrhosis after TIPS (39). In the present study, there was no correlation between the significant increase in BNP and the occurrence of atrial arrhythmia, which was probably due to the inclusion of Child-Pugh C patients and Child-Pugh A and B patients.

The occurrence of atrial arrhythmia in patients with liver cirrhosis may be related to the following reasons. In liver cirrhosis, bile acid metabolism disorder may promote the occurrence of atrial arrhythmia (40). In addition, when liver cirrhosis patients are accompanied by ascites, a hyperdynamic circulatory state due to simultaneous splanchnic and peripheral arterial vasodilatation leads to changes in autonomy, excitability, and conductivity of cardiomyocytes (41). The dysfunction of the autonomic nervous system also plays an important role in the occurrence of atrial arrhythmia.

LIMITATIONS

The present study has several limitations. First, the retrospective study had some unavoidable limitations, such as selection bias, which inevitably affect the results (42). Second, the 135 liver cirrhosis patients enrolled were all from a single center, the small sample size was small, and limited clinical information was available. Third, there may be significant differences in factors that were not measured in the present study. As data were collected retrospectively, and patient treatment varied according to individual differences, some clinical information was lacking, particularly the results of portal vein Doppler ultrasound investigations. Related research that draws on data from multiple centers is needed in future studies in order to continue to establish the risk factors for atrial arrhythmia in patients with liver cirrhosis.

CONCLUSION

In conclusion, the present study identified age and ascites as two risk factors associated with atrial arrhythmia in patients with liver cirrhosis. Among patients with liver cirrhosis, elderly patients and patients with ascites are more likely to develop atrial arrhythmia. Patients with liver cirrhosis should undergo regular ECG examinations to detect atrial arrhythmia and active management of both liver cirrhosis and atrial arrhythmia should be practiced, particularly in elderly patients and in patients with complicated ascites.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Tongji Hospital Affiliated to Tongji University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XL collected the clinical information of the enrolled patients, analyzed the data, and drafted the manuscript. ZW and LY collected the clinical information of the enrolled patients. CY and MS supervised the research design and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Hypertension in Children and Adolescents: A Position Statement From a Panel of Multidisciplinary Experts Coordinated by the French Society of Hypertension

Béatrice Bouhanick[1], Philippe Sosner[2,3,4], Karine Brochard[5], Claire Mounier-Véhier[6], Geneviève Plu-Bureau[7], Sébastien Hascoet[8], Bruno Ranchin[9,10], Christine Pietrement[11], Laetitia Martinerie[12], Jean Marc Boivin[13], Jean Pierre Fauvel[14,15] and Justine Bacchetta[9,10]*

[1] Service d'Hypertension Artérielle et Thérapeutique, CHU Rangueil, CERPOP, Université de Toulouse, Inserm, UPS, Toulouse, France, [2] Centre Médico-Sportif MON STADE, Paris, France, [3] Hôpital Hôtel-Dieu, APHP, Centre de Diagnostic et de Thérapeutique, Paris, France, [4] Laboratoire MOVE (EA 6314), Université de Poitiers, Faculté des Sciences du Sport, Poitiers, France, [5] Service de Néphrologie Médecine Interne Pédiatrique, Hôpital des Enfants, CHU Toulouse, Toulouse, France, [6] Institut Cœur-Poumon, Médecine Vasculaire et HTA, CHU, Université Lille, EA 2694 - Santé Publique: Epidémiologie et Qualité des Soins Lille, Lille, France, [7] Unité de Gynécologie Médicale, AP-HP, Hôpital Port-Royal, Université de Paris, Paris, France, [8] Pôle des Cardiopathies Congénitales du Nouveau-Né à L'adulte - Centre Constitutif Cardiopathies Congénitales Complexes M3C, Groupe Hospitalier Paris Saint-Joseph, Hôpital Marie-Lannelongue, Inserm U999, Université Paris-Saclay, Le Plessis-Robinson, France, [9] Centre de Référence des Maladies Rénales Rares, Service de Néphrologie Rhumatologie et Dermatologie Pédiatriques, Filières Maladies Rares ORKID et ERK-Net, Hospices Civils de Lyon, Hôpital Femme Mère Enfant, Bron, France, [10] Faculté de Médecine Lyon Est, Université Lyon 1, Lyon, France, [11] Unité de Néphrologie Pédiatrique, CHU Reims, Reims, France, [12] Centre de Reference des Maladies Rares de la Croissance et du Développement, Université de Paris, Endocrinologie et Diabétologie Pédiatrique, AP-HP, Hôpital Robert-Debré, Paris, France, [13] Département de Médecine Générale, Université de Lorraine, Inserm CIC-P Pierre Drouin Vandœuvre-Lès-Nancy, Vandœuvre-lès-Nancy, France, [14] Service de Néphrologie Hospices Civils, Hôpital Edouard Herriot, Lyon, France, [15] UMR CNRS 5558, Université Claude Bernard Lyon 1, Villeurbanne, France*

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Edited by:
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****Correspondence:***
Béatrice Bouhanick
duly-bouhanick.b@chu-toulouse.fr

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Hypertension is much less common in children than in adults. The group of experts decided to perform a review of the literature to draw up a position statement that could be used in everyday practice. The group rated recommendations using the GRADE approach. All children over the age of 3 years should have their blood pressure measured annually. Due to the lack of data on cardiovascular morbidity and mortality associated with blood pressure values, the definition of hypertension in children is a statistical value based on the normal distribution of blood pressure in the paediatric population, and children and adolescents are considered as having hypertension when their blood pressure is greater than or equal to the 95th percentile. Nevertheless, it is recommended to use normative blood pressure tables developed according to age, height and gender, to define hypertension. Measuring blood pressure in children can be technically challenging and several measurement methods are listed here. Regardless of the age of the child, it is recommended to carefully check for a secondary cause of hypertension as in 2/3 of cases it has a renal or cardiac origin. The care pathway and principles of the therapeutic strategy are described here.

Keywords: high blood pressure, French position statement, adolescents, children, hypertension

INTRODUCTION

Hypertension (HTN) is much less common in children than in adults, but the prevalence of HTN among children and adolescents is negligible: about 2.2% in the United States (US) of America (1). A third of newly diagnosed hypertensive children demonstrate significant target organ damage, left ventricular hypertrophy (LVH) and arterial stiffness in adulthood (2). Several studies demonstrated the evidence of blood pressure (BP) tracking from childhood into adulthood as childhood BP is associated with BP in later life (3).

Although US, Canadian and European guidelines for the management of paediatric HTN have been published for several decades, diagnosis and of the healthcare pathways of the HTN remain heterogeneous among practitioners (4–10).

Discrepancies between several US recommendations exist: The US Preventive Services Task Force stated that the current evidence is insufficient to screen for primary HTN in asymptomatic children and adolescents whereas, according to the potential implications of HTN on cardiovascular disease in adulthood, an early screening has been advocated by other US societies (4–6). At the moment, no French recommendations about the management of children and adolescent HTN were already published. The aim of the group of experts from the French Society of Hypertension was to perform a review of the literature drawing on the latest expert consensuses or international recommendations up to 2020. The aim of the resultant deliberately concise and practical position statement is to enable the document to be used in everyday practise.

METHODOLOGY

The group of experts rated the recommendations using the GRADE approach, which rates both the overall certainty of the scientific evidence (number and quality of studies) and the strength of the resulting recommendations (strength of evidence in favour of/against the recommendation). The recommendations are rated in grades, i.e., Grade A (high scientific certainty in the body of evidence), Grade B (medium scientific certainty in the body of evidence), and Grade C (low scientific certainty in the body of evidence), and classes, i.e., class 1 (recommended), class 2 (suggested), and class 3 (not recommended). The document was read by several members of French scientific societies: the *Société de Cardiopédiatrie* (Society of Paediatric Cardiology), the *Société de Néphrologie pédiatrique* (Society of Paediatric Nephrology), and the *Société Française d'Endocrinologie et de Diabétologie Pédiatrique* (French Society of Paediatric Endocrinology and Diabetology). The following will not be addressed: malignant hypertension and neonatal hypertension. Furthermore, the principles of the therapeutic strategy and treatments will be discussed in general only, not in detail.

Definition of HTN in Children and Adolescents

In the absence of data on cardiovascular morbidity and mortality associated with a certain level of BP, the definition of HTN in children is a statistical value based on the normal distribution of BP in the paediatric population. Children and adolescents are considered as having elevated BP (replaces the term prehypertension) when the measurement is between the 90th and 95th percentiles for age, height, and gender, and as having HTN when their BP is greater than or equal to the 95th percentile. Stage 1 HTN corresponds to a systolic BP (SBP)/diastolic BP (DBP) measurement $\geq$95th percentile and stage 2 HTN corresponds to a systolic or diastolic value $\geq$95th percentile +12 mm Hg. It is important that several measurements of BP be taken over time before HTN is diagnosed and these measurements should be made under correct conditions (see corresponding chapter).

BP curves in children take into account their gender, age and height. The curves used come from two main references: the auscultatory measurements published by the American Task Force in 2004 (11), and the German curves published in 2011 (12). It should be noted that the use of the American values was endorsed in 2016 by the European Society of Hypertension (7). These curves were also updated in 2017 by excluding BP measurements in overweight or obese children in order to avoid the bias related to the frequent association of HTN with overweight children (9). They therefore represent normal BP in children of normal weight, and are therefore more "stringent" than the former ones. However, since our goal here is to provide general paediatricians and general practitioners with practical tools to use in daily life, a simplified table is provided for the easy and daily identification of children in whom it is advisable to check BP several times to screen for possible HTN (**Table 1**). In case of clinical suspicion of HTN, websites that allow calculation of the BP percentile for age, height and gender are easily available, for example: The Baylor College of Medicine site: https://www.bcm.edu/bodycomplab/Flashapps/BPVAgeChartpage.html
or The International Paediatric Hypertension Association site: http://www.iphapediatricHTN.org/resources/calculators/.

Recommendation No. 1

To define HTN, we recommend using the normative BP tables developed according to age, height, and gender, a simplified version of which is provided in **Table 1** (Grade C class 1).

Epidemiology

The prevalence of HTN and prehypertension (more often referred to as elevated BP) in school-aged children (8–17 years) has increased significantly since 1988, as shown notably in the American National Health and Nutrition Examination Survey (NHANES) epidemiological studies (13). It is actually very complex to assess the prevalence of HTN in children and adolescents: based on the normative BP values of 2004 (11), studies in 2007 estimated the prevalence of HTN to be between 2 and 4% in a population of school-aged children and adolescents, but the value is probably overestimated (14, 15). Other studies carried out in American populations gave percentages that were ten times lower, between 0.1 and 0.3%, and probably closer to the values observed in France, even if figures are lacking (16, 17). The prevalence of elevated BP (defined by a BP >90th percentile or >120/80 mm Hg and <95th percentile) appears to waver between 2.2 and 3.5%. The figure

TABLE 1 | Simplified blood pressure table for the screening of potential arterial hypertension.

Age (years)	Blood pressure (mm Hg)			
	Boys		Girls	
	SBP	DBP	SBP	DBP
1	94	49	97	52
2	97	54	98	57
3	100	59	100	61
4	102	62	101	64
5	104	65	103	66
6	105	68	104	68
7	106	70	106	69
8	107	71	108	71
9	109	72	110	72
10	111	73	112	73
11	113	74	114	74
12	115	74	116	75
13	117	75	117	76
14	120	75	119	77
15	122	76	120	78
16	125	78	121	78
17	127	80	122	78

This table provides the 90th percentile of blood pressure for age in a child at the 5th percentile for height. This table is derived from the nomograms of the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (11).

increases in overweight and obese individuals (14, 15). Tu et al. (18) confirmed in a population of 1,111 children that a parallel relationship exists between an increase in body mass index (BMI) and an increase in BP. With a BMI <85th percentile, adiposity has little impact on BP. However, the risk of developing high BP or HTN is increased by a factor of 4 in overweight children, even before the stage of obesity (BMI between the 85th and 94th percentiles), regardless of the child's age. The American Academy of Paediatrics (AAP), backed by the American Heart Association (AHA), revised the 2004 guidelines in 2017 (9). Blood pressure measurements of overweight or obese children were excluded from the previous tables given the close link between being overweight and obesity and elevated BP-HTN, and for adolescents aged 13 years or more, the recommendations of the AAP were aligned with those for adults. Based on these new recommendations, the prevalence of high BP was found to have increased by 1.5% in a recent American study including 22,224 students aged 10–17 years, but the prevalence of HTN remained between 2 and 4% (19). A recent study from Greece highlighted an alarming proportion of almost 25% of school-children aged 9–13 years, the presence of hypertension being positively associated with body mass index and waist circumference in both genders, and with sedentary behaviours only in boys (20); with the recent COVID crisis and shut-down policies inducing more sedentarity and more obesity, this effect may even become more relevant. Consequently, a higher proportion of left ventricular hypertrophy, an independent cardiovascular risk factor, may therefore be detected (21). In a study comparing the impact of two different guidelines (9, 11), the proportion of participants with an abnormal left ventricular mass categorised as hypertensive significantly increased from 20 to 31% as defined in the Fourth Report and CPG, respectively (21). Another study performed in 951 individuals referred to a Paediatric Center for Cardiovascular Risk Prevention showed a 12% increase in the prevalence of children with BP above the 90th percentile using the most recent normograms (9, 22); moreover, the application of more 'physiological' nomograms, based on a population of normal-weight children, did not yield any advantage in identifying individuals with early cardiac organ damage (22). As such, the gain in sensitivity to detect cardiac hypertrophy is counter-balanced by the loss in specificity.

Childhood obesity as well as an increased prevalence of elevated BP are two risk factors for premature cardiovascular disease in adults that should be screened for and treated at an early stage (23, 24).

Frequency of Secondary HTN vs. Essential HTN in Paediatrics

In an American study conducted in Texas between 2005 and 2011 in 275 hypertensive children, 43% had essential HTN and 57% had secondary HTN, but the population was biassed since it consisted of hospitalised patients (25). Primary or essential HTN is the predominant form of HTN in 6–12 year-olds and adolescents, especially in those with a family history of HTN or who are overweight and/or obese. Given the prevalence of obesity in the USA, these American data probably overestimate the frequency of essential HTN in French children and adolescents. In general populations, it is estimated that <10% of all paediatric patients display secondary HTN (26), but secondary HTN is more prevalent in younger children, especially in those under 6 years of age (25, 27). In these cases, secondary HTN is primarily caused by renal and/or renovascular disorders, which account for 63 to 74% of the cases.

When to Measure BP

In children under the age of 3 years, BP should be checked regularly in the following cases: history of low birth weight <2,500 grammes, kidney disease or uropathy, congenital heart disease (aortic coarctation the most), solid organ or bone marrow transplantation, intracranial hypertension, treatment with medicines known to cause HTN, systemic diseases which may be complicated by HTN (neurofibromatosis, tuberous sclerosis, etc.), and syndromes that may be associated with HTN (Williams-Beuren syndrome, etc.) (11). It should be noted that measuring BP in children <3 years of age can be technically very challenging because of cuff size constraints and the restlessness of children (that is frequent…): it can sometimes take up to 30 or 40 min to obtain an accurate BP measurement.

From the age of 3 years, HTN is often asymptomatic in children like in adults and, therefore, it appears justified to measure BP systematically on an annual basis in the same way as weight, height and BMI, or at each medical visit if the child has a personal history of nephropathy, diabetes mellitus,

dyslipidaemia, being overweight/obesity, taking treatments that may induce HTN or a parental history of HTN or early-onset coronary artery disease (men <55 years old and women <65 years old) (9, 11).

Recommendation No. 2

a) Before the age of 3 years, we recommend to measure BP systematically in the following cases:

- History of low birth weight <2,500 grammes;
- Kidney disease or urological abnormality;
- Congenital heart disease;
- Solid organ or bone marrow transplantation;
- Intracranial HTN;
- Exposure to medicines known to cause HTN;
- Systemic disease which may be complicated by HTN (neurofibromatosis, tuberous sclerosis, etc.) (Grade C class 1).

b) After the age of 3 years, we recommend to measure BP systematically at least once a year in the same way as weight, height and BMI as HTN is most often asymptomatic (Grade C class 1).

Method of BP Measurement: Office or Clinical BP, Ambulatory Blood Pressure Monitoring (ABPM), Home Blood Pressure Monitoring (HBPM)

BP is measured differently according to the age of the child:

In new-borns (0–1 month), the gold standard is intra-arterial measurement (28).

After 1 month and up to 3 years of age, the method of reference is non-invasive measurement using an aneroid sphygmomanometer (alternative to mercury, which is not authorised), with palpation of the radial pulse and auscultation of the antecubital fossa (elbow crease).

From 3 to 12 years of age, European (7, 29) and North-American (9, 30) guidelines agree on BP measurement in the office or a so-called "clinical" setting, using the auscultatory manual method and an aneroid sphygmomanometer (Grade C). In the event of an abnormal BP measurement using an automated oscillometric device (which tends to overestimate BP), the BP measurement should be checked using the auscultatory manual method (Grade C) (7, 9, 28–30).

From 13 to 17 years, European (7, 29) and North-American (9, 30) guidelines agree that BP monitoring should be similar to that of adults with a BP measurement being taken in a clinical setting and completed by 24-h ambulatory BP monitoring (ABPM) or home BP monitoring (HBPM), here too with a cuff adapted to the size of the arm and using a validated automatic device (7, 9, 29, 30).

The following conditions must be respected as much as possible when clinically measuring BP:

The environment: it must be calm, with no talking [talking during measurement may cause a deviation of +10 mm Hg in SBP and DBP (31)]; the effect of external factors must be minimised by patients getting enough sleep and avoiding consumption in the previous 24 h of very salty foods or drinks with a high caffeine content, and even tobacco;

Position of the child: seated for 5 min with feet placed on the ground and not suspended (32); the child's legs must not be crossed, his/her arm must be bared, and his/her back and arm must be supported with the antecubital fossa at the level of the heart (30, 33) [a cuff placed on a garment can cause a SBP deviation of +5 to 50 mm Hg, an unsupported back a deviation of +6 to 10 mm Hg, and an unsupported arm a deviation of + 1–7 mm Hg for SBP and 5–11 mm Hg for DBP (31)];

Equipment: a suitably sized cuff must be used [cuff bladder width at least 40% of the circumference of the arm and length covering 80 to 100% of circumference of the arm (30); too small a cuff may result in falsely high readings (11): +10 mm Hg for SBP, +2 to 8 mm Hg for DBP (31), and too wide cuff, falsely low readings (11)].

Measurement: the right arm is the preferred site [site spared in the event of coarctation of the aorta which would result in underestimation of the measurement in the left arm depending on its location (11, 30)]; the stethoscope must be placed over the antecubital fossa, the cuff must not be excessively inflated [limit: 30 mm Hg above the level of disappearance of the radial pulse) (30); a cuff that is too tight can cause restlessness in children (31)]; SBP corresponds to the reappearance of blood flow (Korotkoff phase I); DBP usually corresponds to Korotkoff phase IV (muffling of sounds) which is used instead of phase V (disappearance of sounds) because often in children, Korotkoff sounds are perceived up to 0 mm Hg; The auscultatory method produces measurements with an accuracy of 2 mm Hg, while automatic devices give results with an accuracy of 1 mm Hg (30).

Aneroid sphygmomanometers should be calibrated on a semi-annual basis (11), and automatic devices according to manufacturer recommendations (34). In all cases, the BP monitors must have been validated in children (Grade C). Few automatic oscillometric devices have been validated in children; they cannot distinguish between Korotkoff phases IV and V for DBP. Regarding wrist monitors, they have not been validated in children in any large studies and should therefore be avoided (32). A list of validated devices has been drawn up by Stergiou et al. (29). For the diagnosis of HTN in the event of elevated BP during a 1st visit in asymptomatic patients, measurements must be repeated during 2 other visits 1 month apart (Grade C), or closer together in high-risk cases (Grade B) (30). In some cases, HTN in children is a medical emergency (catecholamine associated hypertension, acute kidney injury, intracranial haemorrhage, neuroblastoma…) and in case of severe HTN, measurements do not need to be repeated.

Even though they are useful tools, ABPM or HBPM [which reference values in paediatrics derive from a single study in a relatively small population (35)] should be used only in selected situations by experts in paediatric HTN, on a case-by-case basis:

Twenty four hour ABPM may be prescribed by experts for paediatric HTN, preferably in children aged 5 years or older and measuring at least 120 cm as it is interpreted with appropriate paediatric normative data for children 5 years of age or height 120 cm (34), and performed with a validated device at their homes (Grade C). This type of monitoring can be indicated to

confirm the diagnosis of HTN, before treatment is initiated (29), and all the more so if the cardiovascular risk is high (Grade B), to analyse the day/night rhythm, or if a white-coat effect is suspected (9). It may also be indicated when the response to treatment is insufficient (Grade B) (9). However, 24 h ABPM may not be well-tolerated and requires the active cooperation of the whole family (36). Its reproducibility is higher than HBPM, but references are lacking for children <120 cm in height (28). A schema for the classification of HTN in children is presented in **Table 2** (37).

HBPM, requires more studies before it can be used for the diagnosis of HTN in children (38), but it could be a useful backup tool for the monitoring of known HTN (Grade C) (9), particularly in older children and adolescents.

The list of oscillometric devices validated in paediatric age are available online (www.dableducational.org).

Recommendation No. 3

a) In children, we recommend to measure BP using an auscultatory method and an aneroid sphygmomanometer (Grade C, class 1).
b) In the event of an abnormal BP reading with an oscillometric BP monitor (which tends to overestimate BP), we recommend to check the measurement using an auscultatory manual method (Grade C, class 1).
c) We recommend to measure BP in a calm place in a child who has been sitting for 5 min with his/her feet on the floor (not suspended), whose back and arms are supported, and whose antecubital fossa is at heart level. An appropriately sized cuff and a BP monitor that has been validated in children must be used (Grade C, class 1).
d) We recommend to measure BP in the right arm (site spared in case of coarctation of the aorta) (Grade C, class 1).
e) In the event of elevated BP during a 1st visit, we recommend in asymptomatic patients to repeat measurements during 2 other visits 1 month apart (Grade C, class 1) or closer together in high-risk cases (Grade B, class 1).
f) 24-h Ambulatory Blood Pressure Monitoring (ABPM) can be performed in selected situations by experts in paediatric HTN, on a case-by-case basis (Grade B, class 1) for children >120 cm in height, but it may not be well-tolerated; ABPM is not recommended in children who measure <120 cm.
g) We recommend to only use Home Blood Pressure Monitoring (HBPM) for the monitoring of known HTN (Grade C, class 1) given the lack of reference values for the diagnosis of HTN.

Clinical Examination

The diagnostic assessment of HTN in a child must be carried out in two stages including the recording of the child's medical past and the performance of a clinical examination followed by laboratory tests and a radiological evaluation.

The aims of a complete physical examination are to provide clues to potential secondary causes of HTN and assess possible hypertensive end organ damage (9). Then, the purpose of the clinical interview is to try and determine whether the HTN is recent or long-standing, to describe the neonatal period (IUGR, prematurity, history of umbilical artery catheterization which increases the risk of secondary vascular stenosis), and to check for a family or personal history of neurological diseases (neurocutaneous disorders), acquired or hereditary kidney disorders, an inherited form of HTN (Liddle's syndrome), and hereditary paraganglioma-pheochromocytoma. A comprehensive list must also be drawn up of current or recent treatments (sympathomimetic drugs, prednisone, calcineurin inhibitors, fludrocortisone, oral contraceptives, etc.). Enquiries should be made about possible excessive consumption of liquorice, mercury poisoning, sudden and brief changes in skin colour (pallor or redness), sudden agitation, throbbing headaches associated with tachycardia (palpitations), profuse sweating suggestive of pheochromocytoma, or exertional dyspnoea suggestive of heart failure and of coarctation of the aorta.

The clinical examination should begin with the taking of measurements, i.e., weight, height and BMI with analysis of the growth curves of the French Auxology Group, and an assessment of the Tanner pubertal stage. Cardiac examination (murmurs, heart failure using Ross/NYHA functional class), abdominal examination (tumour, renal arteries bruits, and pulses of aorta) and neurological examination (intracranial hypertension) must be checked. The superficial arteries should then be palpated and auscultated for pulse discrepancies and an increase in the differential BP value between the arms and legs suggestive of coarctation of the aorta. The examiner must then check whether the patient presents absent or weak superficial pulses, and carotid, aortic abdominal, renal or femoral bruits suggestive of vascular stenosis, and cardiac erethism suggestive of hyperthyroidism or pheochromocytoma. The patient's skin must be examined for café au lait spots (neurofibromatosis type 1), achromic naevus with sebaceous adenomas (tuberous sclerosis), angiomas (Von Hippel-Lindau disease), pseudoxanthoma elasticum in the large flexor surfaces, or signs of acrodynia. Dysmorphic features should be noted: the moon face of Cushing's syndrome, the elfin-like facial features of Williams-Beuren syndrome, the stunted growth and facial dysmorphia of Turner's syndrome, the arachnid morphology associated with Marfan syndrome. Abnormal tallness may be suggestive of acromegaly. Large palpable kidneys may be suggestive of autosomal recessive or dominant polycystic kidney disease. A goitre, especially if associated with a thrill (vibration in hand on palpation) and a murmur on auscultation and/or exophthalmos suggests hyperthyroidism. Neuroblastomas and nephroblastomas may be revealed by HTN caused by secretion or compression. **Table 3** summarises the diagnostic clinical approach when elevated BP or HTN is observed.

Recommendation No. 4

In children and adolescents being evaluated for high BP, the practitioner should perform a physical examination to identify findings suggestive of secondary causes of HTN listed in **Table 3** (Grade B, class 1).

Additional Tests

The recommendation to perform additional tests is not based on any studies but on expert opinions in consensus guidelines, the most recent of which are listed here (9).

TABLE 2 | Suggested schema to classify blood pressure in children (34).

Classification	Office BP	Mean ambulatory SBP or DBP during awake or sleep period, or both	SBP or DBP load (%)
White coat hypertension	≥95th percentile	<95th percentile	<25
Masked hypertension	<95th percentile	≥95th percentile	≥25
Ambulatory hypertension	≥95th percentile	≥95th percentile	25–50
Severe ambulatory hypertension	≥95th percentile	≥95th percentile	>50

BP, blood pressure; DBP, diastolic BP; SBP, systolic BP.

TABLE 3 | Clinical examination in a child or adolescent with elevated blood pressure or hypertension.

- **Height, weight and BMI**
- **Morphological examination**:
  - Check for dysmorphic facial features (Turner's syndrome, elfin-like facial features of Williams-Beuren syndrome, Alagille syndrome, etc.);
  - Café au lait spots (neurofibromatosis), sebaceous adenomas (tuberous sclerosis), angiomas (Von Hippel-Lindau disease);
  - Check for arachnid morphology and joint hypermobility (Marfan syndrome) and/or pseudoxanthoma elasticum;
  - Check for exophthalmos, for a goitre; (hyperthyroidism);
  - Check for the moon face of Cushing's syndrome, stretch marks (hypercortisolism)
- **Cardiovascular examination:**
  - Measure BP in the 4 limbs;
  - Check for and auscultate superficial pulses (coarctation of the aorta);
  - Check for a heart murmur and abdominal (renal artery stenosis), carotid and femoral bruits;
  - Check for signs of heart failure.
- **Abdominal examination**
  - Check for masses (Wilms' tumour, neuroblastoma, autosomal dominant or recessive polycystic kidney disease);
  - Check for hepatosplenomegaly (autosomal recessive polycystic kidney disease).
- **Neurological examination** with an ocular fundus (Alagille syndrome).

BP, blood pressure.

The following tests should be performed in all children and adolescents regardless of the results of the clinical examination: blood electrolytes (serum potassium), serum creatinine (calculation of estimated glomerular filtration rate using the 2009 bedside Schwartz formula); urine sediment examination of the first morning urine; quantitative estimation of cells in urine (haematuria); urine protein to creatinine ratio (normal: <50 mg/mmol before 2 years of age and <20 mg/mmol after 2 years of age).

The following tests should also be requested in overweight or obese children or adolescents (BMI >95th percentile) and in those with a family history of dyslipidaemia: AST, ALT; fasting total cholesterol, HDL, LDL and triglycerides; fasting blood sugar.

Some tests remain optional and depend on the individual's medical history, the physical exam and the initial additional tests. They may be requested by a paediatric cardiologist, paediatric nephrologist or paediatric endocrinologist: TSH, drug screening (in adolescents), blood count, serum calcium, plasma renin and aldosterone assay between 8 and 10 a.m. without interfering treatments, methoxyamine in 24-h urine or plasma (plasma not reimbursed in France in non-hospital care), 24-h cortisol urine test, serum toxin assays: lead, cadmium and mercury.

Toxic causes may be suspected in certain specific situations, particularly lead poisoning. A correlation was found between high blood lead levels and increased BP in a study on 122 children from underprivileged populations, but without matching for low socioeconomic status which was a confounding factor (39). Cadmium is potentially nephrotoxic in children but the relationship with the BP level has not been demonstrated (40). Acute mercury poisoning in children is associated with BP surges like those observed in pheochromocytoma (41, 42). Finally, neonatal exposure to phthalates may be associated with higher BP values in childhood, but not with the onset of HTN in adulthood, and several of their components are currently being studied (43). Assaying them is therefore premature (9).

Testing for microalbuminuria in case of essential HTN is probably not helpful. Its value is not as backed as in adults in the literature because it may be a marker of kidney injury, or be linked to insulin resistance or obesity, and can even occur in the absence of HTN, for example in children having undertaken physical activity. Its prognostic significance is not known. Nevertheless, in paediatrics, there are many knowledge gaps and the care is often based on adult experience. The existence of albuminuria in children, although not proven, suggests choosing angiotensin-converting-enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARBs) as first line treatment in the absence of contraindications. In European and Canadian recommendations, microalbuminuria or albumin-to-creatinine ratio (first-morning measurement) is recommended for routine clinical use for the assessment of target organ damage in all children with HTN whereas for US recommendations, routine testing for microalbuminuria is not recommended for children and adolescents with primary HTN (7, 9, 37).

Testing for endocrine HTN such as that linked to a block (enzyme deficiency, the most frequent of which is 11β hydroxylase), an adrenal adenoma, or pituitary hypersecretion requires the knowledge of a specialist. Endocrine causes account for 0.05 to 6% of cases of secondary HTN (25, 27, 44, 45). They call for a careful clinical interview and examination.

Other Tests

ECG is used fairly systematically after 12 years of age as, before that age, it requires interpretation by an experienced paediatric cardiologist (physiological T-wave inversion in children…). It can be used to eliminate arrhythmia in case of hypercalcaemia or abnormal potassium levels. It should be noted that ECG is a poor diagnostic tool for the detection of LVH with a

very low positive predictive value. Cardiac ultrasound should therefore be the method of choice for the detection of LVH in children (9, 46).

Echocardiography should be performed systematically to screen for coarctation of the aorta, check for LVH as, unlike ECG, it's predictive value for identifying LVH is high, to measure the left ventricular mass index (although indexation based on a study of normotensive children may be questionable) and interventricular septal thickness correlated with BP; to measure fractional shortening which can be altered in acute hypertensive episodes. Testing for LVH is indicated in children in the assessment of target organ damage and in case of pharmacological treatment of HTN (9). Repeated testing as part of monitoring of an abnormality or pharmacological treatment should be approved by a paediatric cardiologist or cardiologist.

An exercise test should be considered in the child to evaluate symptoms that are triggered or aggravated by exercise; to assess the response to exercise when a cardiac pathology exists (congenital or acquired), including ischemia or arrhythmia; or to evaluate the efficacy of medical or surgical treatments (47).

A cardiopulmonary exercise test should be considered in the child with a congenital heart disease: to assess prognosis; to evaluate the patient's functional capacity and prescribe an adapted physical activity programme; or before a cardiac rehabilitation programme (47).

Kidney ultrasound must be performed systematically to screen for underlying uropathy, renal hypoplasia, or discrepant kidney sizes. The size of the kidneys should be mentioned on the report (kidney size charts according to age), as well as the ultrasound appearance of the parenchyma and cortical thickness.

Doppler ultrasound of the renal arteries may be requested as part of a specialist assessment and must be performed by a paediatric radiologist or an experienced angiologist in children over 8 years of age who will cooperate with the procedure and on a case-by-case basis in younger children who are suspected of having renovascular HTN.

The tests for identification of renovascular HTN need to be prescribed by a specialist. There are no precise criteria for its identification: some experts suggest it should be looked for in children and adolescents with stage 2 HTN, in those with predominantly diastolic HTN, particularly in ambulatory measurements, in those with hypokalaemia, abnormal renal function; urine sediment abnormalities or those with discrepant kidney sizes on ultrasound (9). The sensitivity and specificity of renal artery Doppler ultrasound was found to be, respectively, 64–90 and 68–89% in 2 series, with the best results being obtained in non-obese and cooperative children over 8 years of age (48, 49). Doppler ultrasound of the renal arteries is fairly systematically requested in usual practise because it is non-invasive and non-irradiating.

Deciding whether renal artery computerised tomography (CT-scan) or renal magnetic resonance imaging (MRI scan) is indicated requires specialist assessment and test feasibility (children who can't keep still), irradiation and renal function must all be put in the balance (9, 49). A discussion in a multidisciplinary team meeting on the diagnostic strategy is sometimes required.

There is practically no indication for ACE-inhibited renography that has only been incompletely assessed in the field for the screening of renovascular HTN in children and adolescents. It should be avoided except for specific indications targeted by a paediatric nephrologist (9).

Tests dedicated to evaluation of vascular structure or function (pulse wave velocity, carotid intima-media thickness) are still in the research stage and have not been sufficiently assessed to be recommended in routine clinical practise (7, 9).

Vascular ultrasound of the aorta may be requested as part of a specialist assessment. Coarctation may be suggested in cases of a pressure gradient and treatment-resistant HTN, whether or not associated with syndromes such as Turner syndrome, neurofibromatosis type 1, Takayasu disease, Williams-Beuren syndrome, or Alagille syndrome.

Children with snoring ($\geq$3 nights per week), daytime fatigue, sleep enuresis (especially secondary enuresis), tonsillar hypertrophy or deficit/hyperactivity disorder may have obstructive sleep apnoea syndrome (OSAS) with consequent HTN (50). They should undergo polysomnography and the ABPM is the recommended method for assessing BP as both nighttime and daytime BP is affected by it. However, whether OSAS treatment results in improved BP in children is not known (51).

Genetic testing may be requested as part of a specialist assessment, for example in case of pheochromocytoma, but also in case of suspicion of Liddle's syndrome (**Table 3**).

Recommendation No. 5

a) We recommend to perform the following tests in all children and adolescents regardless of the results of the clinical examinations: blood electrolytes (serum potassium), serum creatinine, assessment of glomerular filtration (using the Schwartz formula in children), urine sediment examination of the first morning urine (haematuria), urine protein to creatinine ratio (normal <50 mg/mmol before 2 years of age and <20 mg/mmol after 2 years of age) (Grade A, class 1).

b) The following tests should also be requested in overweight or obese children or adolescents (BMI >95th percentile) and in those with a family history of dyslipidaemia: fasting blood sugar, fasting lipid profile including total cholesterol, HDL and LDL, triglycerides, AST, and ALT (Grade A, class 1).

c) Once these initial examinations have been requested, we recommend to seek the opinion of a paediatric cardiologist and/or nephrologist and/or endocrinologist (Grade C, class 1).

d) We recommend to seek the expertise of a skilled paediatric cardiologist to interpret ECGs in children under 12 years of age (Grade B, class 1).

e) We recommend to systematically perform cardiac echocardiography to screen for LVH and isthmic coarctation of the aorta (Grade A, class 1).

TABLE 4 | Main causes of secondary hypertension in paediatric patients.

Renin excess (note that renin levels may be normal in patient sera)
Nephropathies
Renovascular diseases with or without midaortic involvement
Isthmic coarctation of the aorta
Reninoma
Primary catecholamine excess
Paragangliomas and pheochromocytomas
Neuroblastomas
Hyperthyroidism
Mercury poisoning
Primary mineralocorticoid (aldosterone, DOC) excess
Conn's syndrome
Glucocorticoid-suppressible HTN
Adrenal enzyme block (11β-hydroxylase, 17α-hydroxylase)
Treatment with 9-α-fluorocortisol
Increased tubular reabsorption of sodium
Liddle's syndrome (epithelial sodium channel)
Gordon's syndrome (With No K kinase 1)
Excessive activation of mineralocorticoid receptor by glucocorticoids
Apparent mineralocorticoid excess syndrome (11β-hydroxysteroid dehydrogenase type 2)
Liquorice poisoning
Glucocorticoid resistance (mutation of the glucocorticoid receptor)
Primary glucocorticoid excess
Treatment with prednisone
Adrenal carcinoma
ACTH (adrenocorticotrophin) secreting adenoma
Miscellaneous
Neurological diseases: intracranial HTN, familial dysautonomia
Metabolic disorders: hypercapnia, hypercalcaemia
Medications (calcineurin inhibitors, oral contraceptives, etc.) and psychotropic drugs (amphetamine, cocaine)

f) We recommend to systematically perform Doppler ultrasound and kidney ultrasound to determine whether HTN can be attributed to a renal cause (asymmetry, renal hypoplasia) (Grade A, class 1).

Secondary Causes of Hypertension

In young children (<6 years of age), HTN is most often secondary to another medical condition (27). In such cases, it is often symptomatic, but it may also be the clinical expression of other diseases (52) (**Table 4**).

The most frequent causes in 2/3 of cases are renal parenchymal diseases (especially glomerulopathies) and isolated renovascular diseases (fibromuscular dysplasia, diffuse coronary artery calcification); other causes include various syndromes (Williams-Beuren syndrome, Marfan syndrome, Alagille syndrome, Turner syndrome), neurocutaneous disorders (neurofibromatosis, Von Hippel-Lindau disease, tuberous sclerosis), pseudoxanthoma elasticum or vasculitis (Takayasu, periarteritis nodosa) and, in newborns, thrombosis of the renal artery (following umbilical catheter placement).

Coarctation of the aorta is the most common cause of HTN in new-borns (0–1 months) and infants (1–12 months). It sometimes occurs as part of a syndrome (Turner syndrome, Williams-Beuren syndrome). It is manifested by pulse and BP discrepancies between the arms and legs of more than 20 mm Hg, and by systolic murmur along the left sternal border with dorsal irradiation. In new-borns, coarctation of the aorta can manifest as cardiogenic shock when the ductus arteriosus closes. Antenatal echocardiography can suggest a risk of neonatal aortic coarctation, in particular as a result of the asymmetrical size of the heart chambers.

Endocrine causes, reported with a frequency of 0.05 to 6% according to the authors, are mainly represented by paragangliomas and pheochromocytomas, manifested by vasomotor episodes (sweating, headaches and palpitations); a genetic cause is identified in 25% of cases (multiple endocrine neoplasia type 2A or 2B, neurofibromatosis type 1, Von Hippel-Lindau disease, hereditary paragangliomas).

Mercury and lead poisoning may give rise to HTN as may some medicinal products such as corticosteroids, calcineurin inhibitors, oral contraceptives and even vasoconstrictors, but also psychotropic drugs such as cocaine and amphetamines. Overconsumption of liquorice should also be considered. Careful questioning should focus on obtaining a description of the hypertensive child's environment and medications.

Neurological (encephalitis and intracranial hypertension) and metabolic (hypercalcaemia, porphyria) causes are rarer.

Monogenic HTN is even rarer. It should be suspected in cases of collapsed plasma renin activity and a high aldosterone/renin ratio, especially if there is a family history of early-onset HTN (53).

The aetiologies can also be described according to their pathophysiological mechanisms (54): renin excess, primary catecholamine excess, primary mineralocorticoid (aldosterone) excess, excessive tubular reabsorption of sodium, primary glucocorticoid excess.

Recommendation No. 6

a) Regardless of the age of the child, we recommend to carefully check for a secondary cause of HTN (Grade B, class 1).
b) We suggest focusing the search for secondary HTN on renal or cardiac causes as they account for 2/3 of the causes of secondary HTN (Grade B, class 2).

The Healthcare Pathway

We remind here that urgent care of hypertensive crisis are not included in this review. Hypertension in children and adolescents is usually first diagnosed by the attending primary care physician. Even if all subspecialists should be able to perform the first line check-up for paediatric hypertension, recourse to a second line specialist could be required and it depends on local resources. As much as possible, patients should be referred to a paediatrician, whether a paediatric nephrologist, a paediatric cardiologist or a paediatric endocrinologist, or to corresponding adult specialists for adolescents, even if

the patient may subsequently be referred from one specialist to another.

Who Should Be Addressed Indifferently to a Cardiologist or Paediatric Cardiologist or a Nephrologist or Paediatric Nephrologist or an Endocrinologist or Paediatric Endocrinologist?

Children or adolescents with a BP surge; those suspected of having a secreting pheochromocytoma or paraganglioma; those with hypokalaemia (primary or secondary hyperaldosteronism); those with a concomitant medical condition: neurofibromatosis type 1, dysplasia, Williams-Beuren syndrome, Alagille syndrome; those requiring pharmacological treatment.

Who Should Be Addressed More Specifically to a Nephrologist or Paediatric Nephrologist?

Children or adolescents with a family history of kidney disease (kidney failure, renovascular dysplasia, acute pyelonephritis), a history of uropathy, tubulopathy, kidney failure (evaluation of glomerular filtration using the Schwartz formula), urine sediment disorders; proteinuria, vascular murmur in the renal area; asymmetric kidney size or a single kidney.

Who Should Be Addressed More Specifically to a Cardiologist or Paediatric Cardiologist?

Children or adolescents with clinical signs such as tachycardia, malaise, or heart murmur on auscultation; absent or weak pulse in the legs which can point to aortic coarctation; heart failure; a family history of heart disease; Williams-Beuren syndrome or Turner or Alagille syndromes which predispose to the risk of aortic coarctation.

Who Should Be Addressed More Specifically to an Endocrinologist or Paediatric Endocrinologist?

Children or adolescents with clinical signs of hypercortisolism (weight gain and growth failure, facial and truncal obesity, proximal amyotrophy, vertical purple stretch marks, facial erythrosis and hirsutism); short stature (Turner syndrome) or abnormal tallness (acromegaly); a goitre; severe obesity.

Recommendation No. 7

a) After a first diagnosis of HTN generally made by the attending physician or the paediatrician, we suggest to refer children or adolescents more specifically to a paediatric nephrologist or nephrologist when they present with (Grade C, class 2):

- Family history of kidney disease (kidney failure, renovascular dysplasia, acute pyelonephritis);
- History of uropathy, tubulopathy;
- Kidney failure, urine sediment disorders; proteinuria
- Vascular murmur in the renal area;
- Asymmetric kidney size or a single kidney.

b) We suggest to refer children or adolescents more specifically to a paediatric cardiologist or cardiologist when they present with (Grade B, class 2):

- Clinical signs such as tachycardia, malaise, or heart murmur on auscultation;
- Absence or weak pulse in the legs which suggests aortic coarctation;
- Family history of heart disease;
- Williams-Beuren syndrome or Turner or Alagille syndromes which predispose to the risk of aortic coarctation.

c) We suggest to refer children or adolescents more specifically to a paediatric endocrinologist or endocrinologist when they present with (Grade B, class 2):

- Clinical signs of hypercortisolism (weight gain and growth failure, facial and truncal obesity, proximal amyotrophy, vertical purple stretch marks, facial erythrosis +/– hirsutism);
- Short stature (Turner syndrome) or abnormal tallness (acromegaly);
- Goitre;
- Severe obesity.

Principles of the Therapeutic Strategy

Lifestyle Recommendations to Reduce High BP Values

Changing lifestyle and dietary habits is effective for all cases of HTN in children, even severe HTN. The early control of cardiovascular risk factors has been shown to be beneficial in terms of morbidity and mortality in adulthood. Overweight is probably the most important of the conditions associated with elevated BP in childhood (55). According to ESH recommendations, the goal is to maintain or achieve BMI <85th and life style measures should not only precede but also accompany pharmacological treatment (7).

Many paediatric guidelines have been emphasised the benefit of physical activity on aerobic fitness and mental health, physical rehabilitation and patient therapeutic education and a summary of the goals is recorded here. Moderate to vigorous physical aerobic activity, 40 min, 3–5 days/week is recommended avoiding more than 2 h daily of sedentary activities. Competitive sports should be limited only in the presence of uncontrolled stage 2 hypertension.

Fruits, vegetables and grain products should be preferred to sugar, soft drinks in excess and saturated fats. In the event of a water/salt overload, a strict low-sodium diet (0.3–0.5 mmol/kg/day) should be adopted. Less strict restriction (1 mmol/kg/day) is required in the event of severe HTN (severe glomerulonephritis, polycystic kidney disease). Once BP is controlled, sodium intake should remain moderate (2–3 mmol/kg/d).

Exposure to tobacco is a major risk factor for cardiovascular morbidity and prevention should begin during pregnancy (maternal smoking should be discouraged) and continued at all ages with patients being advised not to start smoking and encouraged to quit if necessary. Specific paediatric studies have already demonstrated the deleterious effect of tobacco exposure on vascular parameters as early as during teenage (56).

This behavioural change process involves parents and families and realistic goals are needed to improve adherence to the advices.

Pharmacological Treatment

Pharmacological treatment is indicated, regardless of the cause, in case of persistent HTN despite a change in lifestyle and dietary habits, and immediately in symptomatic patients or in those with stage 2 HTN, chronic kidney disease (CKD) or diabetes. The objective is to obtain a controlled BP lower than the 90th percentile for age (American recommendations) bearing in mind that European recommendations are even more stringent, with a controlled BP objective below the 75th percentile in the absence of kidney failure without proteinuria and below the 50th percentile for age in case of kidney failure and/or concomitant proteinuria (7).

Pharmacological intervention in children <6 years of age should be managed by experts in paediatric HTN and it is a paediatrician or a physician with experience treating children with HTN who should initiate the treatment. In children, medications need to be dosed on a milligramme per kilogramme basis and dosing ranges might differ from those in adults because of differences in drug metabolism and body composition (7). Two only drugs with paediatric liquid formulations: valsartan (3 mg/ml) and acebutolol (40 mg/ml) are approved by European health authorities and available in private pharmacy for paediatric hypertension. They can be useful in younger children.

The European Medical Agency (EMA) has promoted an investigation paediatric plan to improve evidences-based medicine in paediatric drugs but not all classes of drugs are studied in children.

We should keep in mind that the benefits and harms of long-term pharmacologic treatment is not known as trial duration is generally limited to 2 to 4 weeks. Several randomised controlled trials of ACE inhibitors (among them enalapril, lisinopril) and of ARBs (losartan, valsartan, candesartan) in children with HTN have shown a good BP response to the medication (57–62). Safety and tolerability of valsartan in children 6 to 17 years of age with HTN have been recently evaluated by EMA and by the French *Haute Autorité de Santé* (HAS) (63, 64). In children with HTN and renal failure, proteinuria, or diabetes mellitus, an ACE inhibitor or ARB is recommended as the initial antihypertensive agent unless there is an absolute contraindication (9). ACEi and ARBs are contraindicated during pregnancy and warrant contraception/abstinence in female patients of child-bearing age and alternative medications (e.g., calcium channel blocker, β-blocker) can be considered when appropriate (9). Caution should be exercised in situations where there is a risk of dehydration (acute gastroenteritis), especially in younger patients receiving ACEi or ARBs, and laboratory tests should be performed in any doubt. The concomitant use of racecadotril, indicated for acute diarrhoea, and ACEi may increase the risk of angioedema. Therefore, the benefit/risk balance should be carefully assessed before initiating racecadotril treatment in patients taking ACEi.

Other medications studied for paediatric HTN have less consistent results. Trials of the long-acting dihydropyridine calcium channel blockers (amlodipine and felodipine) have shown for amlodipine a difference from placebo in SBP response vs. placebo in 268 children aged from 6 to 16 years (65, 66). ACEi, ARBs, and calcium channel blockers reduced BP similarly (67).

Paediatric experience for thiazide diuretics has been reported but data about their efficacy are missing on monotherapy; Loop diuretics are used in case of cardiac or renal failure but unlike in adult medicine, they are very rarely used for HTN in children even if they are included in the US recommendations in first line therapy or in combination (9). According to US guidelines, beta-blockers are less well-studied in children with HTN (except metoprolol) (68) and are not recommended for first-line treatment, due to the potential side effect profile (9).

Table 5 below lists the main drug classes used for the chronic treatment of HTN in children. The prescribing principles for these therapeutic classes in children and adolescents are the same as for adults. Pharmacologic treatment of HTN should be initiated with an ACEi, ARB, long-acting calcium channel blocker, or a thiazide diuretic (even if in France, thiazide diuretics are very rarely used) (9). The use of a combination product as initial treatment has not been studied and cannot be recommended in children. For good treatment compliance, monotherapy should be favoured with as few as possible daily doses, at times compatible with family life. The choice of the initial molecule should be guided by the aetiology and initiated at the lowest dosage strength, then gradually increased to reach the target BP. If BP is not controlled with the highest dose of the treatment, a 2nd molecule should be introduced with, if possible, rapid switching to fixed-dose combination therapy to promote compliance. The reader should know that data on combined therapies in children are scarce (69), and after initiation of treatment, BP should be checked every 2 to 4 weeks until the HTN is under control, then every 3 to 4 months.

Recommendation No. 8

a) We recommend a change in lifestyle and dietary habits in all cases of childhood HTN (Grade C, class 1).
b) We recommend that a paediatrician or a physician with experience in the treatment of HTN in children and adolescents initiates it (Grade C, class 1).
c) Pharmacological treatment should be initiated in cases of (Grade A, class 1):
   - Symptomatic or stage 2 HTN
   - Secondary HTN
   - Damage to target organs (heart, eyes)
   - Kidney failure
   - Concomitant type 1 or type 2 diabetes
   - Persistence of HTN despite a change in lifestyle and dietary habits, regardless of the cause as the symptoms have an impact on target organs.
d) In children, we recommend to use long-acting calcium channel blockers or angiotensin-converting-enzyme inhibitors (ACEi) / angiotensin receptor blockers (ARBs) (Grade B, class 1).
e) We recommend to target a BP under the 75th age percentile, and even below the 50th percentile in cases of kidney failure and/or concomitant proteinuria (Grade B, class 1).

TABLE 5 | Main drug classes that can be used in children.

Class	Drug	Initial dose	Dose per 24 h	No. doses/24 h	Undesirable effects, precautions
Calcium channel blockers	Amlodipine	0.1–0.2 mg/kg Max 5 mg	0.6 mg/kg/d Max 10 mg/d	1	Preparation of a 1 mg/mL suspension of amlodipine otherwise tablets of 2.5, 5 or 10 mg Tachycardia, flushing, headache, possible peripheral oedema, gingival hypertrophy
	Nicardipine LP	0.25–0.5 mg/kg/d	1–3 mg/kg/d Max 120 mg/d	2	
	Felodipine	5 mg	10 mg	1	
ACE inhibitors	Captopril	Liquid formulation: 5 mg/5 ml and 25 mg/5 ml Newborns: 0.01–0.03 mg/kg/dose Infants: 0.1 mg/kg/dose Older children: 0.3–0.5 mg/kg/dose	6 mg/kg/d Max 150 mg	1 to 3	Monitoring of electrolytes after introduction or increase in dosage (hyperkalaemia, kidney failure) Cough and angioedema under ACE inhibitors Contraindicated during pregnancy (fetotoxicity) Treatment should be interrupted in case of gastroenteritis/dehydration
	Enalapril	Newborns: 0.05 mg/kg/dose Infants: 0.05 mg/kg/dose Older children: 0.08 mg/kg/dose max 5 mg	0.8 mg/kg/d Max 40 mg/d	1	
	Lisinopril	0.08 mg/kg Max 5 mg/d	0.6 mg/kg/d Max 40 mg/d	1	
ARBs	Losartan*	0.7 mg/kg/d Max 50 mg/d	1.4 mg/kg/d Max 100 mg/d	1	
	Irbesartan	2 mg/kg/d	6–12 years <35 kg: 75–150 mg/d ≥13 years >35 kg: 150–300 mg/d	1	
	Valsartan	3 mg/ml drug with liquid formulation	– 1–6 years: 1 mg/kg/d and <4 mg/kg/d – 6–18 years: <35 kg: 20 mg/d and <40 mg/ d >35 kg: 40 mg/d and <80 mg/d		
Beta blockers	Acebutolol	1.5–3 mg/kg/d	5–15 mg/kg/d	1 to 2	Contraindications: AV block not cardioselective (Propranolol); contraindicated in case of asthma and heart failure Limit certain athletic performances
	Acebutolol	40 mg/ml in liquid formulation	10–20 mg/kg/d	1 to 2	
	Propranolol	1 mg/kg/d	4 mg/kg/d Max 640 mg/d	2 to 3	
	Atenolol	0.1–1 mg/kg/d	2 mg/kg/d Max 100 mg/d	1 to 2	
Alpha and beta blocker	Labetolol	1–3 mg/kg/d	10–15 mg/kg/d Max 1,200 mg/d	2	Contraindications: AV block, asthma, heart failure Limit certain athletic performances
Alpha blockers	Prazosin	0.05–0.1 mg/kg/d Max 0.5 mg × 2 per d	0.5 mg/kg/d Max 20 mg per d	2 to 3	Risk of orthostatic hypotension after the 1st dose Fatigue, concentration difficulties
	Clonidine	5 μg/kg/d	30 μg/kg/d max 1.05 mg/d	2 to 3	
Diuretics	Hydrochlorothiazide	0.5–1 mg/kg/d	3 mg/kg/d Max 50 mg/d	1	Monitoring of electrolytes Furosemide is useful for the complementary treatment of HTN in case of kidney failure
	Furosemide	0.5–2 mg/kg/dose	6 mg/kg/d	1 to 2	
	Spironolactone*	1 mg/kg/d	3.3 mg/kg/d Max 100 mg/d	1 to 2	

**Tablets can be crushed doses are given as examples, and the prescription of the drug should remain the physician's own responsibility.*

Special Cases

Hypertensive Emergency: From Which Value and What to Do

Chronic untreated HTN can cause growth retardation, so it is essential to follow growth on a dedicated growth curve. Clinically, HTN may manifest as headaches, epistaxis, ringing in the ears, and malaise during physical exertion. Severe HTN can cause abdominal pain, vomiting, anorexia, a break in the growth curve, polydipsia and polyuria, and recurrent peripheral facial paralysis. Hospitalisation in a specialised paediatric unit is required in the event of symptomatic HTN for rapid institution of treatment (and an aetiological assessment).

HTN may also be revealed by a complication: heart failure with acute pulmonary oedema, reduced visual acuity, convulsions, coma and, possibly, signs of vascular insufficiency resulting in oedema and/or cerebral haemorrhage. In newborns (0–1 months) and infants (1–12 months), HTN may present as vasomotor disturbances or malaise, but is most often revealed by heart failure. In such cases, the condition may be life-threatening due to multiple organ or heart failure, or hypertensive encephalopathy.

Malignant HTN is a life-threatening emergency and includes HTN with organ dysfunction, i.e., neurological, renal or cardiac impairment. Children presenting a hypertensive emergency should be taken care of in a continuing care/intensive care unit so they can be closely monitored and receive organ support. It is recommended to lower BP by 25% in the first 6–8 h, then more gradually over the following 24–48 h. Too rapid normalisation of BP can lead to more serious side effects than the HTN itself.

The reference treatment is intravenous treatment with continuous modes being preferred to boluses to avoid arterial hypotension (risk of organ hypoperfusion and neurological sequelae such as loss of vision). Nicardipine (calcium channel blocker, vasodilator) is the first-line treatment because it allows gradual and controlled decrease in BP with no risk of sudden hypotension. In addition, it is one of the only emergency treatments that is not contraindicated in case of stenosis (renal, carotid or cerebral arteries). The treatment permits a gradual and adjustable decrease in BP. Labetalol (alpha and beta blocker) can also be used in hypertensive emergencies. Diuretics (furosemide and bumetanide) are indicated in hypertensive emergencies with volume overload (**Table 6**). In all cases, the patient must be managed in a specialised paediatric setting, possibly after transfer by the emergency medical service (SAM) to a dedicated unit.

Recommendation No. 9

We recommend that children presenting with a hypertensive emergency be managed in a specialised continuous care/intensive care unit with paediatric experience (Grade A, class 1).

Hypertension and Contraception in Adolescents

The various methods of contraception and the time required to implement contraception in adolescents, detailed in other works (70, 71), will not be addressed in this paper. However, two separate situations can be distinguished: onset of HTN in an adolescent using a combined hormonal contraceptive and contraception prescription in hypertensive adolescents.

Onset of Hypertension in an Adolescent Using a Combined Hormonal Contraceptive (CHC)

The literature presents no arguments for the prescription of a specific type of contraception in adolescents except in the event of definite contraindications. Combined hormonal contraceptives (CHC) and condoms are by far the methods the most used by adolescent girls.

HTN can occur regardless of how long the CHC has been taken and its route of administration. It depends on the dose of ethinylestradiol (EE), especially for the high-dose pills (50 μg EE). Its severity varies, ranging from mild HTN to malignant HTN. Its frequency of onset after the introduction of a CHC is between 0.6 and 8.5% (72).

In the context of use of a CHC, onset of HTN can be explained by increased production of liver angiotensinogen and by water and sodium retention following an action on the mineralocorticoid receptors. CHC modify endocrine parameters (plasma renin activity and aldosterone levels) for 4 to 6 weeks.

Factors that predispose adolescents using CHCs to HTN include being overweight and obesity as well as a family history of HTN (73–75).

In adolescents, as in older women, it is essential to measure BP along with weight and BMI before the first initiation of a CHC. These measurements should be repeated at each follow-up visit to screen for asymptomatic HTN. In addition, patients suffering from with headaches, whether migraine or non-migrainous headaches, should be screened for HTN during visits and on an outpatient basis, especially overweight, obese or diabetic adolescents (masked HTN) (74–76).

The type of CHCs that should be prescribed first-line to adolescents choosing this type of birth control are so-called second generation CHCs (containing 20 or 30 μg of EE and levonorgestrel). None of the other CHCs (except for the one containing norgestimate) should be used first-line due to the higher risk of venous thromboembolism as compared with second generation CHCs both in adolescents and older women. When the CHC is prescribed, the patient should also be advised to use condoms for effective prevention of sexually transmitted infections.

Blood pressure should be reassessed at 3, 6 months, and then every year. If a significant increase in BP is observed during a visit (>140/90 mm Hg), HBPM or 24 h ABPM should be implemented to avoid an alarm reaction and to rule out a white coat effect. In the event of HTN confirmed during a visit, the CHC should be discontinued and replaced by a so-called progestin-only contraceptive (containing only small doses of progestins and that may be administered by various routes [oral route (pill), subcutaneous route (implant), intrauterine route (IUD)]. The effect of progestin-only contraceptives on BP appears to be neutral. If BP doesn't drop to normal levels within 3 months, the cause of the HTN must be investigated and pharmacological treatment initiated along with lifestyle changes.

It should be noted that in adolescents (as in older women), first-line prescription of CHCs delivered by alternative routes [transdermal (patch) or vaginal (ring)] should be avoided as they contain 3rd generation progestins which are associated with a higher thrombogenic risk as compared with second generation

TABLE 6 | Management of hypertensive emergencies.

Drug (brand name)	Therapeutic class	Administration route	Dosage	Time to effect	Duration of effect	Contraindications	Side effects
Nicardipine (Loxen)	Calcium channel blocker	IV	1–3 μg/kg per min	A few minutes	30 to 60 min	/	Reflex tachycardia Headache, flushing, nausea, inflammation at the injection site
Labetolol (Trandate)	Alpha and beta blocker	IV	2–20 mg/kg/day	5 to 10 min	3 to 24 h	Heart failure, Atrioventricular block, Asthma	Bradycardia, hypotension, nausea
Furosemide (Lasilix)	Loop-acting diuretic	IV, IM, or PO	Slow IV 30 min 0.5 to 3 mg/kg/dose every 3 to 4 h. Up to 10 mg/kg/day	5 min	2 to 3 h		Hypokalaemia Ototoxicity Increased blood glucose levels
Bumetanide (Burinex)	Diuretic	IV	0.02 mg/kg/injection up to 1 mg/kg/day	5 min	2 to 3 h		Hypokalaemia Increased blood glucose levels

Doses are given as examples, and the prescription of the drug should remain the physician's own responsibility.

CHCs and also expose patients to an increase in BP. The alternative transdermal and vaginal administration routes should not be used to replace an orally administered CHC: in the event of HTN with a CHC pill requiring interruption of this birth control method, it is not recommended to replace the pill with a CHC delivered by an alternative route.

Recommendation No. 10

a) We recommend measuring BP at the initiation of CHC treatment, then periodically, at 3 and 6 months, and then annually during follow-up visits (Grade B, class 1).
b) We recommend measuring BP in patients presenting with headaches, whether migraine headaches or not, at the initiation of CHC treatment to screen for HTN (Grade B, class 1).
c) In case of confirmed HTN or stage 3 HTN during a visit (>180/110 mm Hg), we recommend to replace the CHC by a progestin-only birth control method (pill, implant, or IUD) (Grade A, class 1).
d) In adolescents, we suggest to avoid the first-line prescription of CHCs delivered by alternative routes [transdermal (patch) or vaginal (ring)] (Grade C, class 2).
e) When an oral contraceptive is prescribed, patients should be advised to also use condoms (Grade C, class 2).

Contraception Prescription in Adolescents With Hypertension

Use of a CHC in hypertensive patients leads to an increase in systolic and diastolic BP, while discontinuation is associated with a significant drop in these values (72). Thus, in the SPCs of CHCs, their prescription is absolutely contraindicated in the event of essential or secondary HTN. The risk related to the use of CHCs is indeed unacceptable and their prescription is contraindicated in stage 2 or 3 HTN, HTN complicated by target organ damage, or HTN associated with other uncontrolled CV risk factors (obesity, diabetes, smoking, etc.). Consequently, the French HAS recommends avoiding the use of CHCs in hypertensive patients and contraindicates their use in stage 2 or 3 HTN or in case of concomitant cardiovascular disorders (77).

The French Society of Hypertension has developed an expert consensus decision pathway on "HTN, Hormones and women/GRADE method," but it does not address the specific case of adolescents (78). The consensus recommends not prescribing a CHC to hypertensive women (controlled or uncontrolled), regardless of the route of administration (oral route, transdermal patch, vaginal ring), because these forms of contraception are associated with a risk of an increase in BP; it is recommended to prescribe hypertensive women seeking a birth control method with an effective form of contraception, i.e., a progestin-only contraceptive regardless of the route of administration (oral, subcutaneous or intrauterine route), or a copper IU, providing there are no gynaecological contraindications.

Finally, if the adolescent prefers a copper IUD, it is possible to implant the devices in adolescents, providing certain precautions are taken and the adolescent is fully informed about the device before its implantation (70). Not having had a child is not a barrier to the use of this type of birth control.

In hypertensive adolescents with incurable causes of HTN, the group proposes not to prescribe any form of CHC and to use only the options applicable to hypertensive women.

When adolescents are prescribed with contraceptives, they should also be advised to use condoms for effective prevention of sexually transmitted infections.

It should be noted that regardless of the type of antihypertensive treatment taken, progestin-only contraceptives pose no drug-drug interaction problems, contrary to certain other treatments (antiepileptics for example).

Furthermore, it is important to ensure that the methods of contraception used by hypertensive adolescents are very effective

TABLE 7 | Summary of the statements.

Definition of HTN in children and adolescents	To define HTN, we recommend using the normative BP tables developed according to age, height and gender, a simplified version of which is provided in **Table 1** (Grade C class 1)
Epidemiology	a) Before the age of 3 years, we recommend to measure BP systematically in the following cases: - History of low birth weight <2,500 grams; - Kidney disease or urological abnormality; - Congenital heart disease; - Solid organ or bone marrow transplantation; - Intracranial HTN; - Exposure to medicines known to cause HTN; - Systemic disease which may be complicated by HTN (neurofibromatosis, tuberous sclerosis, etc.) (Grade C class 1). b) After the age of 3 years, we recommend to measure BP systematically at least once a year in the same way as weight, height and BMI as HTN is most often asymptomatic (Grade C class 1)
Method of BP measurement	a) In children, we recommend to measure BP using an auscultatory method and an aneroid sphygmomanometer (Grade C, class 1) b) In the event of an abnormal BP reading with an oscillometric BP monitor (which tends to overestimate BP), we recommend to check the measurement using an auscultatory manual method (Grade C, class 1) c) We recommend to measure BP in a calm place in a child who has been sitting for 5 min with his/her feet on the floor (not suspended), whose back and arms are supported, and whose antecubital fossa is at heart level. An appropriately sized cuff and a BP monitor that has been validated in children must be used (Grade C, class 1) d) We recommend to measure BP in the right arm (site spared in case of coarctation of the aorta) (Grade C, class 1) e) In the event of elevated BP during a 1st visit, we recommend in asymptomatic patients to repeat measurements during 2 other visits 1 month apart (Grade C, class 1) or closer together in high-risk cases (Grade B, class 1) f) 24 h Ambulatory Blood Pressure Monitoring (ABPM) can be performed in selected situations by experts in paediatric HTN, on a case-by-case basis (Grade B, class 1) for children >120 cm in height, but it may not be well-tolerated; ABPM is not recommended in children who measure <120 cm g) We recommend to only use Home Blood Pressure Monitoring (HBPM) for the monitoring of known HTN (Grade C, class 1) given the lack of reference values for the diagnosis of HTN
Clinical examination	In children and adolescents being evaluated for high BP, the practitioner should perform a physical examination to identify findings suggestive of secondary causes of HTN listed in **Table 3** (Grade B, class 1)
Additional tests	a) We recommend to perform the following tests in all children and adolescents regardless of the results of the clinical examinations: blood electrolytes (serum potassium), serum creatinine, assessment of glomerular filtration (using the Schwartz formula in children), urine sediment examination of the first morning urine (haematuria), urine protein to creatinine ratio (normal <50 mg/mmol before 2 years of age and <20 mg/mmol after 2 years of age) (Grade A, class 1) b) The following tests should also be requested in overweight or obese children or adolescents (BMI >95th percentile) and in those with a family history of dyslipidaemia: fasting blood sugar, fasting lipid profile including total cholesterol, HDL and LDL, triglycerides, AST, and ALT (Grade A, class 1) c) Once these initial examinations have been requested, we recommend to seek the opinion of a paediatric cardiologist and/or nephrologist and/or endocrinologist (Grade C, class 1) d) We recommend to seek the expertise of a skilled paediatric cardiologist to interpret ECGs in children under 12 years of age (Grade B, class 1) e) We recommend to systematically perform cardiac echocardiography to screen for LVH and isthmic coarctation of the aorta (Grade A, class 1) f) We recommend to systematically perform Doppler ultrasound and kidney ultrasound to determine whether HTN can be attributed to a renal cause (asymmetry, renal hypoplasia) (Grade A, class 1)
Secondary causes of HTN	a) Regardless of the age of the child, we recommend to carefully check for a secondary cause of HTN (Grade B, class 1) b) We suggest focusing the search for secondary HTN on renal or cardiac causes as they account for 2/3 of the causes of secondary HTN (Grade B, class 2)
Healthcare pathway	a) After a first diagnosis of HTN generally made by the attending physician or the paediatrician, we suggest to refer children or adolescents more specifically to a paediatric nephrologist or nephrologist when they present with (Grade C, class 2): - Family history of kidney disease (kidney failure, renovascular dysplasia, acute pyelonephritis); - History of uropathy, tubulopathy; - Kidney failure, urine sediment disorders; proteinuria - Vascular murmur in the renal area; - Asymmetric kidney size or a single kidney b) We suggest to refer children or adolescents more specifically to a paediatric cardiologist or cardiologist when they present with (Grade B, class 2): - Clinical signs such as tachycardia, malaise, or heart murmur on auscultation; - Absence or weak pulse in the legs which suggests aortic coarctation; - Family history of heart disease; - Williams-Beuren syndrome or Turner or Alagille syndromes which predispose to the risk of aortic coarctation c) We suggest to refer children or adolescents more specifically to a paediatric endocrinologist or endocrinologist when they present with (Grade B, class 2): - Clinical signs of hypercortisolism (weight gain and growth failure, facial and truncal obesity, proximal amyotrophy, vertical purple stretch marks, facial erythrosis +/– hirsutism); - Short stature (Turner syndrome) or abnormal tallness (acromegaly); - Goitre; - Severe obesity

(Continued)

TABLE 7 | Continued

Principles of therapeutic strategy	a) We recommend a change in lifestyle and dietary habits in all cases of childhood HTN (Grade C, class 1) b) We recommend that a paediatrician or a physician with experience in the treatment of HTN in children and adolescents initiates it (Grade C, class 1) c) Pharmacological treatment should be initiated in cases of (Grade A, class 1): * Symptomatic or stage 2 HTN * Secondary HTN * Damage to target organs (heart, eyes) * Kidney failure * Concomitant type 1 or type 2 diabetes * Persistence of HTN despite a change in lifestyle and dietary habits, regardless of the cause as the symptoms have an impact on target organs d) In children, we recommend to use long-acting calcium channel blockers or angiotensin-converting-enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARBs) (Grade B, class 1) e) We recommend to target a BP under the 75th age percentile, and even below the 50th percentile in cases of kidney failure and/or concomitant proteinuria (Grade B, class 1)
Special cases	We recommend that children presenting with a hypertensive emergency be managed in a specialised continuous care/intensive care unit with paediatric experience (Grade A, class 1)
Contraception in teenagers	a) We recommend measuring BP at the initiation of CHC treatment, then periodically, at 3 months and 6 months, and then annually during follow-up visits (Grade B, class 1) b) We recommend measuring BP in patients presenting with headaches, whether migraine headaches or not, at the initiation of CHC treatment to screen for HTN (Grade B, class 1) c) In case of confirmed HTN or stage 3 HTN during a visit (>180/110 mm Hg), we recommend to replace the CHC by a progestin-only birth control method (pill, implant, or IUD) (Grade A, class 1) d) In adolescents, we suggest to avoid the first-line prescription of CHCs delivered by alternative routes [transdermal (patch) or vaginal (ring)] (Grade C, class 2) e) When an oral contraceptive is prescribed, patients should be advised to also use condoms (Grade C, class 2)
Contraception in adolescents with HTN	a) We recommend not to prescribe combined hormonal contraceptives, regardless of the route of administration (oral, vaginal or transdermal), to adolescents with uncomplicated mild HTN or severe stage 2 or 3 HTN that may/may not be complicated by target organ damage and/or concomitant cardiovascular disease (Grade B, class 1) b) We recommend to offer hypertensive adolescents an effective progestin-only contraceptive that can be administered by various routes (oral, subcutaneous or intrauterine routes) or a copper IUD, providing there are no gynaecological contraindications (Grade C, class 2)

to avoid unwanted pregnancies. Practitioners need to remind an adolescent girl of childbearing age that ACEi/ARBs are contraindicated during pregnancy and warrant contraception. The patients also need to be informed that they will need to plan any future pregnancy because their antihypertensive treatment may need to be modified as some treatments are incompatible with pregnancy (78).

Recommendation No. 11

a) We recommend not to prescribe combined hormonal contraceptives, regardless of the route of administration (oral, vaginal or transdermal), to adolescents with uncomplicated mild HTN or severe stage 2 or 3 HTN that may/may not be complicated by target organ damage and/or concomitant cardiovascular disease (Grade B, class 1).
b) We recommend to offer hypertensive adolescents an effective progestin-only contraceptive that can be administered by various routes (oral, subcutaneous or intrauterine routes) or a copper IUD, providing there are no gynaecological contraindications (Grade C, class 2).

CONCLUSION

Hypertension in children and adolescents, which is less common than in adults, requires the involvement of several medical professionals including paediatricians as the definition of childhood HTN is very different from that of adult HTN. Confirming a diagnosis of HTN is not easy, particularly in childhood. After a first diagnosis of HTN, generally made by the attending physician or paediatrician, it is suggested that children and adolescents be more specifically referred to a paediatric or adult nephrologist, cardiologist or endocrinologist for further examinations and treatment. In this manuscript, we propose clinical practise points to help general physicians and paediatricians to improve the diagnosis and general management of paediatric HTN in daily practise, as summarised in **Table 7**.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

BB coordinated the activities of the group, the preparation of the article, and its submission. BB, PS, KB, CM-V, GP-B, and JB drafted the manuscript. SH, CP, BR, LM, JMB, and JF critically revised the manuscript. All authors approved the final article.

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GROUP OF READERS

From the French Society of Hypertension: Laurence Amar; Theodora Angoulvant; Michel Azizi; Jacques Blacher; Romain Boulestreau; Pierre-Yves Courand; Thierry Denolle; Pierre Fesler; Xavier Girerd; Jean-Michel Halimi; Olivier Hanon; Pierre Lantelme; Sylvain Le Jeune; Benoît Lequeux; Marilucy Lopez-Sublet; Jean Jacques Mourad; Atul Pathak; François Silhol; Pierre-Louis Tharaux; Emmanuelle Vidal Petiot.

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CASE REPORT
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Massive Right Atrial Thrombosis: Are You Brave Enough to Start Anticoagulation? A Case Report

Marco Bergonti[1], *Ciro Ascione*[1]*, *Piergiuseppe Agostoni*[2,3], *Roberto Castelli*[4] and *Carlo Vignati*[2,3]

[1] Cardiovascular Disease Fellowship Program, University of Milan, Milan, Italy, [2] Monzino Cardiology Center, Istituto di Ricovero e Cura a Carattere Scientifico, Milan, Italy, [3] Department of Clinical Sciences and Community Health, Cardiovascular Section, University of Milan, Milan, Italy, [4] Department of Biomedical and Clinical Sciences, Luigi Sacco Hospital, University of Milan, Milan, Italy

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell disorder with overlapping myelodysplastic and myeloproliferative features. The disease is generally characterized by blood monocytosis, bone marrow dysplasia, cytopenia, and hepatosplenomegaly. While malignant blood diseases are frequently associated with a high risk of thromboembolism, CMML is often accompanied by immune-mediated hemorrhagic diathesis. Indeed, very few reports in literature report thrombotic complications of CMML patients. We will briefly present here the case of a patient with CMML who developed a massive right atrial thrombus. We aim to highlight the non-negligible thrombotic burden of the disease, and we will get through the differential diagnosis of right atrial masses and the management of right atrial thrombi, which are a rare and poorly known entity.

Keywords: right atrial thrombosis, pulmonary thromboembolism, thrombus, cardio-oncology, coagulation, right atrium mass

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Edited by:
Junjie Xiao,
Shanghai University, China

Reviewed by:
Jahaira Lopez-Pastrana,
Temple University, United States
Alessandro Iadanza,
University of Siena, Italy

***Correspondence:**
Ciro Ascione
ciroascione92@gmail.com

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INTRODUCTION

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell disorder with myelodysplastic and myeloproliferative overlap features. The disease is generally characterized by blood monocytosis, bone marrow dysplasia, cytopenias, and hepato-splenomegaly (1–4).

Of note, CMML is often accompanied by immune-mediated hemorrhagic diathesis while thrombotic manifestations are rare (1, 4), especially involving massive spontaneous thrombosis in the right atrium. Indeed, this condition has only been described once in the literature (5). We report below the second of such cases.

CASE DESCRIPTION

A 78-year-old male was admitted to our hospital for increasing fatigue, multiple episodes of syncope, and paroxysmal episodes of dyspnoea. He was known for CMML diagnosed in 2016 and currently treated with supportive care. He had markedly enlarged spleen (20 cm of diameter) and severely reduced hemoglobin and platelet level. Chemotherapy had not been deemed suitable due to comorbidity.

His past medical history also comprised heart failure with mid-range ejection fraction (HFmEF) secondary to anterior myocardial infarction. He had previously underwent double-chamber pacemaker implantation for critical bradycardia in 2010. He had severe aortic stenosis and stage III chronic kidney disease (CKD). He did not have persistent or paroxysmal atrial fibrillation, a history of thromboembolism, or a family history of coagulopathy. He was on therapy with Clopidogrel 75 mg q.d., Levo-tiroxin 150 mcg q.d., tamsulosin 0.4 mg q.d., pantoprazole 40 mg q.d., furosemide 25 mg q.d., spironolactone 25 mg q.d., sacubitril/valsartan 24/26 mg b.i.d., silodosin 8 mg q.d., allopurinol 150 mg q.d., calcitriol 0.5 mcg q.d., and erythropoietin 4,000 IU twice per week.

Upon arrival in the emergency department (ED), he appeared pale and diaphoretic. His blood pressure was 100/60 mmHg, heart rate was 80 bpm, and oxygen saturation was 96%. His physical examination was remarkable only for a harsh systolic murmur radiated to both carotids. His lungs were clear and no lower limb edema was evident. His labs upon admission in the ED were as follows: white blood cell count 11,300/μl with 32% monocyte, platelets 47,000/μl, hemoglobin 9.3 g/dl, PT 16.8 s, INR 1.44, aPTT 37 s, fibrinogen 123 mg/dl [150–450], antithrombin III 58.0% [80–120], D-dimer 1,732 ng/ml, and creatinine 3.08 mg/dl (eGFR 18 ml/min).

In the ED, he underwent transthoracic echocardiography (TTE) showing a dilated right atrium containing a 5.57 × 3.30 × 2.69 cm pedunculated mass, with irregular heterogeneous surface, attached to the superior atrial wall, partially crossing and nearly occluding the tricuspid valve (**Figures 1A,B**). The superior and inferior vena cava were not involved. Contrast CT described an intraluminal hypodense filling defect, with irregular profile, sparing the superior and inferior vena cava and being apparently attached to the atrial catheter of the pacemaker and the atrial portion of the ventricular catheter (**Figure 1C**). Mass density was compatible with thrombus apposition. Venous color Doppler ultrasound of the lower extremity was negative.

Based on echocardiography and tomography characteristics, the hypothesis of spontaneous thrombus in the right atrium, although extremely rare, was favored as the most probable diagnosis for this giant atrial mass. After Heart Team discussion, all open heart surgical options were excluded due to the prohibitive risk associated with his comorbidities. In light of stable hemodynamic conditions, fibrinolytic therapy was ruled out as well. Subcutaneous anticoagulation with unfractionated heparin (UH) was thus started. Two days later, respiratory insufficiency and metabolic acidosis suddenly developed. Pulmonary embolism secondary to the detachment of thrombi debris from the right atrium was quickly suspected. Emergency CT angiography showed a complete detachment of the atrial thrombus into the pulmonary arteries (**Figures 2A,B**). The same exam showed the presence of small residual thrombotic apposition across the atrial catheter (**Figure 2C**). The patient was slowly stabilized. He remained in the intensive care unit (ICU) for 25 days and was discharged home after 38 days of hospitalization on oral anticoagulation with warfarin plus clopidogrel 75 mg.

TIMELINE

2016	The patient was diagnosed with chronic myelomonocytic leukemia
July 2019 (Day of the admission)	The patient was admitted to the hospital for increasing fatigue and syncopal episodes
Day of the admission: 11.00 a.m.	Diagnosis of right atrial mass at transthoracic echocardiography
Day of the admission: 2.00 p.m.	Contrast CT confirmed the presence of right atrial thrombus. Anticoagulation is started.
2 days after the admission	Detachment of thrombi debris from the right atrium caused massive pulmonary embolization
25 days after the admission	The patient was discharged from the ICU
37 days after the admission	The patient was discharged at home.

DISCUSSION

The case here reported shows an extremely rare complication of CMML, which has only been described once in the medical literature (5).

Oncologic patients are notoriously predisposed to thromboembolic events, and this holds true even for hematologic conditions. Indeed, venous thromboembolism (VTE) is a frequent complication found in ∼11% of patients with acute leukemia (3). However CMML confers the patient a significant bleeding risk, compared to a poorly recognized thrombotic risk (1, 4).

Among the different conditions predisposing to bleeding risk, we have to cite the fact that dysplastic macrophages tend to attach factor X leading to an acquired factor X deficiency (1). Autoimmune acquired hemophilia is another rare but remarkable condition, which has been described (6). Indeed case reports of thromboembolic complications in CMML patients are scarcely reported in the literature, especially when dealing with extremely rare sites of thrombus formation, such as the right atrium. Only recently has the pro-coagulant effect of pathologically activated monocytes been demonstrated (7).

Right atrial (RA) masses are a rare entity. The differential diagnosis of RA masses must include three conditions: benign or malignant neoplasm, tricuspid valve vegetation, and thrombus (8).

While on the left side, thrombi are predominantly located into the appendage, the RA presents some peculiar features. Indeed, the right appendage is broad-based and less deep. Because of this, thrombus formation is less common. In addition, on the right side, thrombi usually originate in patients with atrial fibrillation or pro-thrombotic state. Other conditions that predispose to right atrial thrombus include tricuspid prosthesis or stenosis of the tricuspid valve, atrial septal closure devices, and central venous lines. Right-sided pacemaker leads confer a mildly higher risk of spontaneous thrombi, often in association

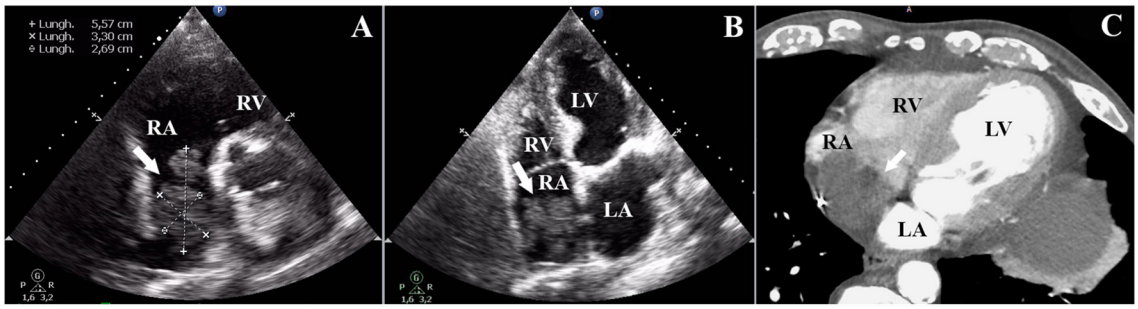

FIGURE 1 | (A,B) Modified short-axis view and four-chamber view showing irregular thrombotic mass pointing toward the tricuspid valve, into the right ventricle. **(C)** Venous phase, contrast CT scan with evidence of intraluminal hypo-dense filling defect, with irregular profile, nearly occluding an enlarged right atrium.

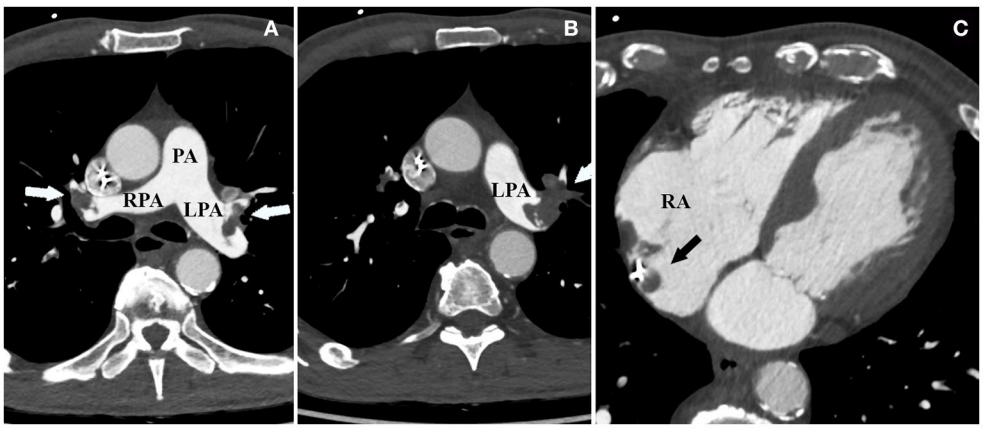

FIGURE 2 | (A,B) Bilateral thrombotic apposition nearly occluding the pulmonary arteries and causing bilateral massive pulmonary embolism. **(C)** Nearly complete detachment of the thrombus from the right atrium, which is now only in part visible in proximity to the atrial catheter.

with other risk factors (9). Thromboembolic events are possible adverse effects of erythropoietin administration; however, to our knowledge, it was never associated with atrial thrombosis (10). No other medications taken by the patients are associated with thrombotic risk.

When dealing with RA thrombi, one has to bear in mind that they can be divided into two categories: type A thrombi originate in deep peripheral veins and transit the RA (extremely mobile, worm-shape); type B thrombi, originating into the right atrium, are attached to the atrial wall and immobile. The distinction carries clinical significance as Type A patients are a high-risk group that warrants surgery or fibrinolysis. Type B thrombi are much more benign and vitamin K antagonists seem to be sufficient (9).

Our patient presented with a spontaneous right atrial thrombus, a rare condition that is made nearly unique by the concomitant presence of CMML. Indeed, our patient had no other potential risk factors for developing right atrial thrombus except for pacemakers leads. Genetic tests for hypercoagulable disorders were negative. No tricuspid valve disease or atrial septal defect closure device was present. Thrombophilia screening, including factor V Leiden, MTHFR, and the G20210A mutation of thrombin, was negative.

In light of his history and these findings, we can infer that his lymphoproliferative disease may be the cause of the thrombotic event, and this is the second of such cases ever reported in the medical literature.

In the literature, there are different recommendations for right heart thrombosis treatment: surgical removal, the administration of thrombolytic agents, or anticoagulation therapy with heparin, with similar mortality rates (38, 38, and 30%, respectively); a significantly lower probability of short-term survival is described in untreated patients (19% in patients with pulmonary emboli and 53% in those without pulmonary emboli) (11). Given the high bleeding risk of patients with CMML, anticoagulation seems to be the best alternative.

Another treatment possibility is the AngioVac thrombectomy system (AngioDynamics, Inc, Latham, New York), a venovenous filtration apparatus used for aspiration of thrombi and/or vegetations, which could be used with clinical benefit in patients with right atrial thrombi; however, this system is used only in a small number of centers and there is limited literature about its efficacy (12).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MB investigated the patient, collected and interpreted the data, and participated in manuscript preparation. CA collected and interpreted the data and participated in manuscript preparation. PA responsible for critical revising for intellectual content. RC interpreted the data and participated in manuscript preparation. CV investigated the patient, interpreted the data, and participated in manuscript preparation. All the authors critically revised the manuscript and approved the final version.

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frontiers in Endocrinology

ORIGINAL RESEARCH
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Association Between C-Peptide Level and Subclinical Myocardial Injury

Ziwei Chen[1], *Jing He*[2], *Qiang Ma*[3*] and *Mingbing Xiao*[4*]

[1] Department of Cardiology, Affiliated Hospital of Nantong University, Nantong, China, [2] Department of Oncology, Affiliated Hospital of Nantong University, Nantong, China, [3] School of Public Health, Nantong University, Nantong, China, [4] Research Center of Clinical Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong, China

Background: Previous studies have confirmed an association between C-peptide levels with the risk of cardiometabolic diseases. However, whether circulating C-peptide was related to subclinical myocardial injury (SC-MI) remains unknown.

Methods: A total of 3,752 participants without a history of cardiovascular diseases were included in our study from National Health and Nutrition Examination Survey III (NHANES III). Multivariable linear regression was performed to explore the correlation between C-peptide and cardiac injury score (CIIS). Multivariate logistic regression was used to examine the association between C-peptide quartile and SC-MI.

Results: Circulating C-peptide was significantly associated with CIIS (β:0.09, 95% confidence interval [CI]: 0.00–0.17; $p = 0.041$). Compared with the lowest quartile, the highest quartile of circulating C-peptide increased a 1.48-fold risk of SC-MI (Odds ratio = 1.66, 95% CI: 1.18–1.87; $p = 0.001$).

Conclusions: The level of C-peptide was independently associated with CIIS and SC-MI, which could serve as a new risk factor of SC-MI.

Keywords: C-peptide, subclinical cardiac injury, NHANES III, association, cross sectional study

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Laura Sabatino, Italian National Research Council, Italy

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Dongfeng Zhang, Qingdao University, China
Cristina Vassalle, Gabriele Monasterio Tuscany Foundation (CNR), Italy

***Correspondence:**
Mingbing Xiao
xiaomingbing@ntu.edu.cn
Qiang Ma
qiangma@ntu.edu.cn

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BACKGROUND

Subclinical myocardial injury (SC-MI) is an early cardiac injury without clinically evident coronary heart disease or heart failure (1, 2). SC-MI is defined by an electrocardiographic-based scoring system, namely, cardiac infarction/injury score (CIIS) >10 (3). SC-MI was reported to be associated with the progression of coronary heart disease (1) and cardiovascular and all-cause mortality (4).

Abbreviations: SC-MI, subclinical myocardial injury; CIIS, cardiac injury score; NHANES III, National Health and Nutrition Examination Survey III; WBC, white blood cell; RBC, red blood cell; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate transaminase glucose; BMI, body mass index.

Previous studies have reported that physical activity (5), obesity (6), diastolic blood pressure (7), Vitamin D (8) and TyG index (9) were associated with SC-MI.

C-peptide is a small peptide with 31 amino acids and is released upon insulin secretion to ensure the correct folding of proinsulin (10, 11). It is known that C-peptide has been widely used as a biomarker of diabetes diagnosis in clinical practice. Observational studies have found the association between C-peptide and cardiovascular diseases (12, 13). High levels of C-peptide could increase the risks of atherosclerosis and myocardial infarction (14, 15). C-peptide was reported to increase the level of triglyceride and to decrease HDL-C (16). However, C-peptide also inhibited oxidative stress and endothelial apoptosis (17), showing a cardioprotective role. Therefore, it remains unknown that circulating C-peptide level was associated with SC-MI.

In our study, we examined the association between levels of serum C-peptide and SC-MI based on a cross-sectional study.

METHODS

Study Population

All participants were included from the US National Health and Nutrition Examination Survey (NHANES III). The NHANES is nationwide multistage survey designed to assess the health and nutritional status of adults and children in the United States (https://www.cdc.gov/nchs/nhanes/index.htm) by the Centers for Disease Control and Prevention (CDC). After excluding individuals with missing circulating C-peptide data, we included 3,752 participants without a history of cardiovascular diseases (**Figure 1**). The survey protocol was approved by the Institutional Review Board of the CDC.

Covariate Assessments

The list of covariates includes baseline demographics, the risk factor of cardiovascular diseases, or factors influencing C-peptide levels. Sociodemographic variables, including age, gender, and

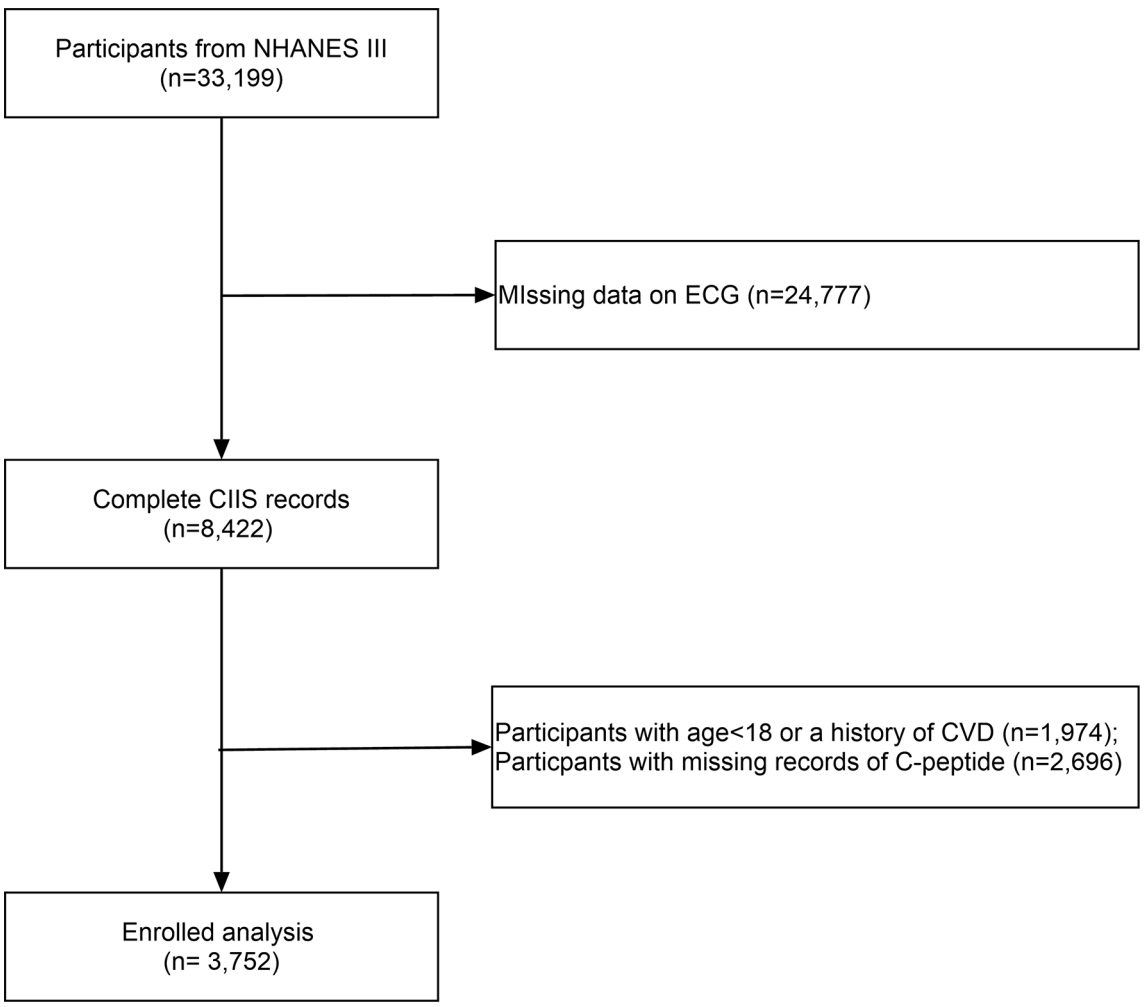

FIGURE 1 | The flow chart of the selection process.

race, were collected by using standardized questionnaires. The systolic blood pressure, diastolic blood pressure, pulse rate, height, and weight of each participant were obtained from the physical examinations. White blood cell (WBC), red blood cell (RBC), and hemoglobin were obtained by whole blood cell count test. Triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP), creatinine, alanine aminotransferase (ALT), aspartate transaminase (AST), glucose, glycated hemoglobin, and insulin were measured by standard biochemistry assays. Body mass index (BMI, kg/m^2) was calculated as weight divided by height squared. Race was classified as non-Hispanic white, non-Hispanic black, Mexican American, and other. Smokers were defined as those who self-reported smoking more than 100 cigarettes during their lifetime, and alcohol users were those who had at least 12 drinks in the last 12 months (18). Physical activity was defined as taking vigorous or moderate activities. Multiple imputation was performed for covariates with missing values.

Circulating C-Peptide Measurement

Serum C-peptide was measured using a radioimmunoassay (RIA), where the 125I-labeled C-peptide completes with C-peptide in the specimen for antibody site. Bound and free C-peptide is separated by adding a second PEG-accelerated double antibody. The antibody-bound fraction is precipitated and counted. The radioactivity is inversely proportional to the quantity of C-peptide in the specimen. Circulating C-peptide was treated as a continuous and a quartile variable to examine its association with CIIS and SC-MI, respectively. The quartile of C-peptide levels was categorized into four groups: Q1 (<0.452 nmol/L), Q2 (0452–0.737 nmol/L), Q3 (0.737–1.082 nmol/L), and Q4 (>1.082 nmol/L).

Outcome Definition

Resting 12-lead electrocardiograms were recorded by experienced technicians with a Marquette MAC 12 system. Analysis of electrocardiograms was achieved through a computerized automated process and visual inspection by a trained technician located in a centralized core laboratory. Briefly, the SC-MI defined from the CIIS rests on a weighted scoring system taking several objective electrocardiographic waveform components related to myocardial injury and ischemia, both discrete and continuous, and generating a risk-stratified scoring system (6, 7). A combination of 11 discrete and 4 continuous variables are counted to define the final score to evaluate the disease severity levels: CIIS>20 for probable injury; CIIS>15 for possible injury; CIIS>10 for borderline abnormality. SC-MI was defined as a total of CIIS>10 (3).

Statistical Analysis

Sample weights and stratification were incorporated in all analyses because of the complex sampling design of the NHANES data. Categorical variables were expressed as frequencies and percentages. Continuous variables were reported as mean ± standard deviation or median (interquartile range) if skewed distribution. The difference between groups were compared using one-way ANOVA for continuous variables or Kruskal–Wallis test if not normalized distributed and chi-square test for categorical variables. The multivariable linear regression was used to explore the association between C-peptide and CIIS (log2-transformed for normality) while the multivariable logistic regression was used to explore the association between C-peptide and SC-MI. To rule out the cofounding factors, we adjusted for age and gender in Model 1. In model 2, we adjusted for age, gender, race, smoking, drinking, smoking, physical activity, and BMI. In model 3, we adjusted for age, gender, race, smoking, drinking, smoking, BMI, TC, TG, CRP, creatinine, AST, ALT, glucose, glycated hemoglobin, and insulin. The variables that were adjusted for were based on the p value <0.05 in univariate analysis or the risk factors of cardiometabolic diseases. The restricted cubic spline models with knots at 10th, 50th, and 90th percentage were used for the dose–response analysis. Data were analyzed using IBM SPSS 25.0 and R software 3.6. A two-tailed p-value <0.05 was considered as statistically significant.

RESULTS

Baseline Characteristics

A total of 3,752 participants were enrolled in our study with an average age of 60.2 ± 13.1, of which 1,778 (47.3%) were males. **Table 1** showed the baseline characteristics grouped by quartiles of circulating C-peptide levels. Participants with high C-peptide levels tend to be old, and male, as well as having higher levels of BMI, TC, TG, LDL-C, CRP, glucose, and glycated hemoglobin.

Association Between C-Peptide Levels and CIIS

The CIIS was higher with the increased quartile of C-peptide. Multivariable linear regression analysis was used to explore the association between C-peptide and log2-transformed CIIS (**Table 2**). After adjusting for age and sex, circulating C-peptide was positively related to CIIS (β = 0.13, 95% CI: 0.06–0.19; p < 0.001). The linear relationship still existed after adjusting for lifestyles in Model 2 (β = 0.09, 95% CI: 0.00–0.17; p = 0.039) and laboratory examinations in Model 3 (β = 0.09, 95% CI: 0.00-0.17; p = 0.041). Compared with the lowest quartile, the highest quartile of C-peptide was positively associated with CIIS across three models.

Association Between C-Peptide Levels and SC-MI

The prevalence of SC-MI was 31.6%, 31.3%, 38.6%, and 43.6% across quartiles, respectively. **Table 3** summarizes the results of multivariable logistic regression between C-peptide quartiles and SC-MI. Compared to the lowest quartile, the highest quartile was significantly associated with SC-MI in model 1 adjusted for sociodemographics (OR = 1.59, 95% CI:1.31–1.92; p < 0.001), and this association remained statistically significant in Model 2 (OR = 1.48, 95% CI:1.18–1.86; p = 0.001) and Model 3 (OR = 1.48, 95% CI:1.18–1.87; p = 0.001). In addition, one-unit increase of C-peptide was associated with 1.27-fold higher risk of SC-MI (OR = 1.27, 95% CI:1.08–1.50; p = 0.004).

TABLE 1 | Baseline characteristics of study participants across serum C-peptide categories.

	Q1 (≤0.452)	Q2 (0.452–0.737)	Q3 (0.737–1.082)	Q4 (≥1.082)	*p*-value
n	941	938	936	937	
Age (years)	58.4 ± 13.3	59.6 ± 12.9	61.3 ± 13.4	61.4 ± 12.8	<0.001
Male (%)	377 (40.1)	447 (47.7)	472 (50.4)	480 (51.2)	<0.001
Race (%)					<0.001
Non-Hispanic white	521 (55.4)	527 (56.2)	484 (51.7)	476 (50.8)	
Non-Hispanic black	249 (26.5)	198 (21.1)	200 (21.4)	172 (18.4)	
Mexican-American	142 (15.1)	179 (19.1)	220 (23.5)	252 (26.9)	
Other	29 (3.1)	34 (3.6)	32 (3.4)	37 (3.9)	
Smoking (%)	312 (33.2)	303 (32.3)	265 (28.3)	202 (21.6)	<0.001
Drinking (%)	511 (54.3)	523 (55.8)	549 (58.7)	523 (55.8)	0.467
Physical activity (%)	580 (61.7)	513 (54.7)	478 (51.1)	461 (49.2)	<0.001
BMI (kg/m^2)	24.0 ± 4.2	26.5 ± 4.2	28.5 ± 5.2	31.2 ± 6.1	<0.001
sBP (mmHg)	127.6 ± 19.8	130.9 ± 18.6	134.9 ± 19.1	136.2 ± 18.3	<0.001
dBP (mmHg)	58.3 ± 6.8	58.1 ± 7.1	58.1 ± 7.2	57.9 ± 7.4	0.680
Pulse (bpm)	73.3 ± 11.4	74.7 ± 11.9	76.0 ± 12.3	79.0 ± 13.1	<0.001
WBC, 10^9/L	6.6 ± 2.0	6.9 ± 2.2	7.3 ± 3.1	7.8 ± 2.6	<0.001
RBC, 10^{12}/L	4.5 ± 0.4	4.6 ± 0.4	4.7 ± 0.4	4.8 ± 0.5	<0.001
Hemoglobin (g/dl)	13.5 ± 1.4	14.0 ± 1.4	14.1 ± 1.4	14.4 ± 1.5	<0.001
Total cholesterol (mg/dl)	206 (53)	216 (51)	216 (52)	214 (52)	<0.001
Triglycerides (mg/dl)	86 (49)	113 (61)	139 (86)	166 (91)	<0.001
LDL-C (mg/dl)	127 (49)	140 (44)	138 (47)	136 (47)	<0.001
HDL-C (mg/dl)	57 (23)	51 (20)	46 (16)	42 (14)	<0.001
C-reactive protein (mg/dl)	0.21 (0.1)	0.21 (0.2)	0.21 (0.3)	0.33 (0.5)	<0.001
Creatinine (mg/dl)	1.05 ± 0.18	1.08 ± 0.22	1.11 ± 0.27	1.18 ± 0.55	<0.001
AST (U/L)	21.7 ± 13.8	21.2 ± 11.6	22.3 ± 16.9	24.6 ± 18.7	<0.001
ALT (U/L)	13.6 ± 9.9	15.1 ± 9.5	17.6 ± 14.2	21.7 ± 17.8	<0.001
Glucose (mg/dl)	90 (13)	96 (14)	101 (17)	108 (24)	<0.001
Glycated hemoglobin (%)	5.5 ± 1.0	5.7 ± 1.1	5.9 ± 1.3	6.2 ± 1.5	<0.001
Insulin (μU/ml)	5.6 (2.5)	7.9 (2.9)	11 (4.4)	17.8 (9.3)	<0.001
CIIS	6.2 (8.7)	6.6 (7.8)	7.2 (9.6)	8.5 (11.2)	<0.001
SC-MI	297 (31.6)	646 (31.1)	361 (38.6)	528 (43.6)	<0.001

TABLE 2 | Multivariable linear regression between C-peptide and log2-CIIS.

	Model 1		Model 2		Model 3	
	***β* (95% CI)**	***p***	***β* (95% CI)**	***p***	***β* (95% CI)**	***p***
Q1	Ref		Ref		Ref	
Q2	0.01 [–0.10, 0.11]	0.892	–0.01 [–0.11, 0.10]	0.863	–0.01 [–0.11, 0.10]	0.856
Q3	0.13 [0.02, 0.23]	0.015	0.10 [–0.01, 0.21]	0.074	0.10 [–0.01, 0.21]	0.083
Q4	0.21 [0.10, 0.31]	<0.001	0.16 [0.04, 0.28]	0.011	0.16 [0.03, 0.28]	0.015
Per one-unit	0.13 [0.06, 0.19]	<0.001	0.09 [0.00, 0.17]	0.039	0.09 [0.00, 0.17]	0.041

OR, odds ratio; CI, confidence interval; BMI, body mass index; TC, total cholesterol; TG, triglyceride, CRP, c-reactive protein.
Model 1 was adjusted for age and gender.
Model 2 was adjusted for age, gender, race, smoking, drinking, physical activity, BMI, TC, TG, CRP, and creatinine.
Model 3 was adjusted for age, gender, race, smoking, drinking, physical activity, BMI, TC, TG, CRP, creatinine, glucose, glycated hemoglobin, and insulin.

To explore the nonlinear relationship, we performed dose–response analysis based on restricted cubic spline models (**Figure 2**). It suggested that C-peptide was linearly and positively related to SC-MI (p for nonlinearity = 0.860).

Subgroup Analysis

Subgroup analysis was performed to explore the potential factors modifying the association between C-peptide and SC-MI. The association of C-peptide with SC-MI was consistent across age and gender (**Table 4**). Besides, we found that a higher level of C-peptide increased the risk of SC-MI across the disease severity (**Table 5**). However, it was only significant in the borderline abnormality group (p = 0.031).

DISCUSSION

Our study confirmed that circulating C-peptide level was independently associated with electrocardiographic subclinical myocardial injury after adjusting for cardiovascular risk factors and glucose metabolism-related biomarkers.

Previous studies have shown the association between C-peptide and cardiovascular disease (19). Michelle et al. examined the effect of C-peptide on atherosclerosis and found that high C-peptide levels were related with increased lipid deposits and smooth muscle cell proliferation in the vessel wall, contributing to atherosclerosis (12). Antonio Cabrera de Leon et al. found that elevated C-peptide was

TABLE 3 | Multivariable logistic regression between C-peptide categories and subclinical myocardial injury.

	Model 1		Model 2		Model 3	
	OR (95% CI)	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
Q1	Ref		Ref		Ref	
Q2	0.95 [0.78, 1.16]	0.619	0.92 [0.75, 1.13]	0.426	0.92 [0.75, 1.12	0.414
Q3	1.29 [1.06, 1.56]	0.010	1.23 [1.00, 1.51]	0.053	1.22 [0.99, 1.51]	0.056
Q4	1.59 [1.31, 1.92]	<0.001	1.48 [1.18, 1.86]	0.001	1.48 [1.18, 1.87]	0.001
Per one-unit	1.35 [1.18, 1.54]	<0.001	1.25 [1.07, 1.47]	0.005	1.27 [1.08, 1.50]	0.004

Model 1 was adjusted for age and gender.

Model 2 was adjusted for age, gender, race, smoking, drinking, physical activity, BMI, TC, TG, CRP, and creatinine.

Model 3 was adjusted for age, gender, race, smoking, drinking, physical activity, BMI, TC, TG, CRP, creatinine, glucose, glycated hemoglobin, and insulin.

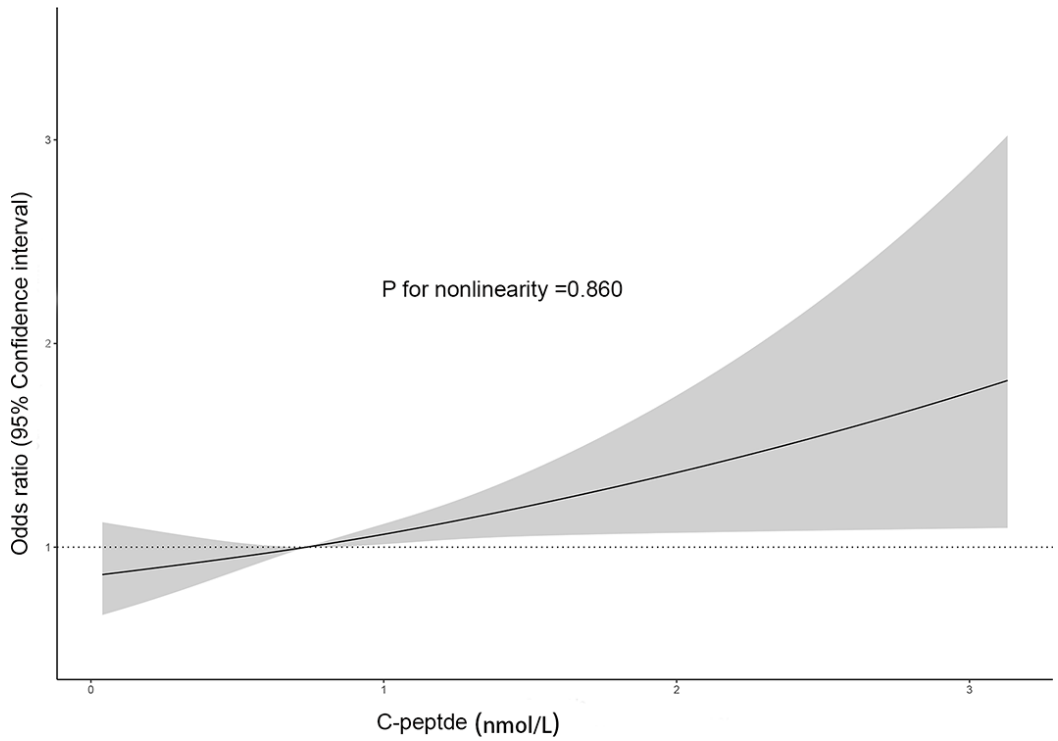

FIGURE 2 | The dose–response relation between C-peptide and SC-MI.

TABLE 4 | Subgroup analysis between C-peptide and SC-MI.

	OR (95% CI)	***p***	***p* for interaction**
Gender			0.153
Male	1.26 [1.00, 1.62]	0.062	
Female	1.21 [0.95, 1.53]	0.121	
Age			0.262
≤65	1.43 [1.12, 1.84]	0.005	
>65	1.21 [0.97, 1.57]	0.113	

TABLE 5 | The association between C-peptide and severity of SC-MI.

Disease severity	**Cases**	**OR (95%CI)**	**P**
Borderline abnormality; 10<CIIS≤15	671	1.24 [1.02, 1.51]	0.031
Possible injury; 15<CIIS≤20	391	1.12 [0.90, 1.37]	0.293
Probable injury; CIIS>20	273	1.15 [0.89, 1.45]	0.259

associated with the incidence of myocardial infarction and coronary artery disease in the general population (20). What is more, Min et al. showed an association between serum C-peptide levels and all-cause and cause-specific mortality among adults without diabetes (21). Furthermore, we have observed a strong association of C-peptide levels with CIIS and SC-MI in our study, which could contribute to early diagnosis and intervention.

The underlying mechanisms for this association were complicated. The C-peptide has been related to increasing triglyceride levels while decreasing HDL-C levels, which were negatively correlated to cardiovascular death (16). However, evidence also indicated that C-peptide has an anti-inflammatory role (22) and anti-oxidative role (23). So, it needs more prospective clinical trials to determine whether the increased C-peptide in the SC-MI was causal or compensatory.

Certain limitations need to be taken into consideration in the interpretation of our study. Our study was designed by a cross-sectional scheme, only revealing the correlation between C-peptide and SC-MI. Besides, only baseline C-peptide was included in our study, and it may be more meaningful to examine the change of C-peptide.

CONCLUSION

We observed an association between high C-peptide levels and SC-MI in the general population.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NHANES III.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Affiliated Hospital of Nantong University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XMB and MQ designed this study, ZC wrote the manuscript. JH performed the experiments. All authors contributed to the article and approved the submitted version.

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Gallbladder Polyps Increase the Risk of Ischaemic Heart Disease Among Korean Adults

Yong-Jae Lee[1†], *Byoungjin Park*[2†], *Kyung-Won Hong*[3] *and Dong-Hyuk Jung*[2*]

[1] Department of Family Medicine, Gangnam Severance Hospital, Seoul, South Korea, [2] Department of Family Medicine, Yongin Severance Hospital, Yongin, South Korea, [3] Theragen Bio Co. Ltd., Suwon-si, South Korea

Background: Gallbladder (GB) polyps and ischaemic heart disease (IHD) share some common risk factors. We investigated the longitudinal effects of gallbladder (GB) polyps, as a surrogate metabolic indicator, on IHD.

Methods: We enrolled 19,612 participants from the health risk assessment study (HERAS) and Korean Health Insurance Review and Assessment Service (HIRA) database. The primary outcome was IHD, which consisted of angina pectoris (ICD-10 code I20) or acute myocardial infarction (ICD-10 code I21) that occurred after enrolment into the study. We calculated hazard ratios (HRs) with 95% confidence intervals (CIs) for IHD according to the presence of GB polyps using multivariate Cox proportional hazards regression models.

Results: The median follow-up period was 29.9 months and a total of 473 individuals (2.4%, 473/19,612) developed IHD. Individuals with GB polyps had an increased risk of IHD compared with the control group after adjusting for potential confounding variables (HR = 1.425; 95% CI, 1.028–1.975). Furthermore, the coexistence of hypertension or dyslipidaemia resulted in an increased risk (HR = 2.14, 95% CI, 1.34–3.44 or HR = 2.09, 95% CI, 1.32–3.31, respectively) of new-onset IHD in the GB polyp group.

Conclusions: GB polyps was an independent risk factor of IHD. Awareness of these associations will inform clinicians on the need to include cardiovascular risk management as part of the routine management of patients with GB polyps.

Keywords: gallbladder, polyps, coronary disease, comorbidity, cohort study

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***Correspondence:**
Dong-Hyuk Jung
balsan2@yuhs.ac

[†]*These authors have contributed equally to this work*

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INTRODUCTION

Ischaemic heart disease (IHD) is the leading cause of morbidity and mortality among middle-aged and older individuals globally (1). The incidence of cardiovascular disease (CVD) has increased in developed Asian countries because life style and eating habits have become more westernized (2, 3). Also, IHD carries the 2nd leading death rate following cancer in South Korea, while the recent trend has been gradually rising over the last decade (4). Therefore, it is crucial for physicians to assess the presence of IHD-related risk factors for early prevention of IHD (5).

Gallbladder (GB) polyps are defined based on the presence of polypoidal lesions in the GB mucosa, and ultrasonography (USG) is generally used in clinical settings to detect these polyps (6). USG is a non-invasive tool with >90% sensitivity and specificity for diagnosing GB polyps (7).

The prevalence of GB polyps in Korea is estimated at 2.2–8.5%, which is higher than that for Western countries but lower than the prevalence in China (8–10). Although, most GB polyps are cholesterol polyps and benign lesions, the presence of GB polyps is used for the early detection of malignancy and to determine the appropriate time to undergo GB resection surgery (11).

Previous studies suggested that the presence of GB polyps is closely associated with insulin resistance, obesity, and CVD (12, 13). Hence, GB polyps and IHD may share common risk factors; however, few studies have explored the relationship between these two conditions (14).

We conducted a regional and community-based cohort study to investigate the association between GB polyps found by USG and the development of IHD. We included data from the health risk assessment study (HERAS) and Korea Health Insurance Review and Assessment Service (HIRA) database.

MATERIALS AND METHODS

Data and Study Cohort

This retrospective study was derived from the HERAS, which has been previously described in details on design and methodology (15). In brief, the cohort consisted of 20,530 sequential subjects

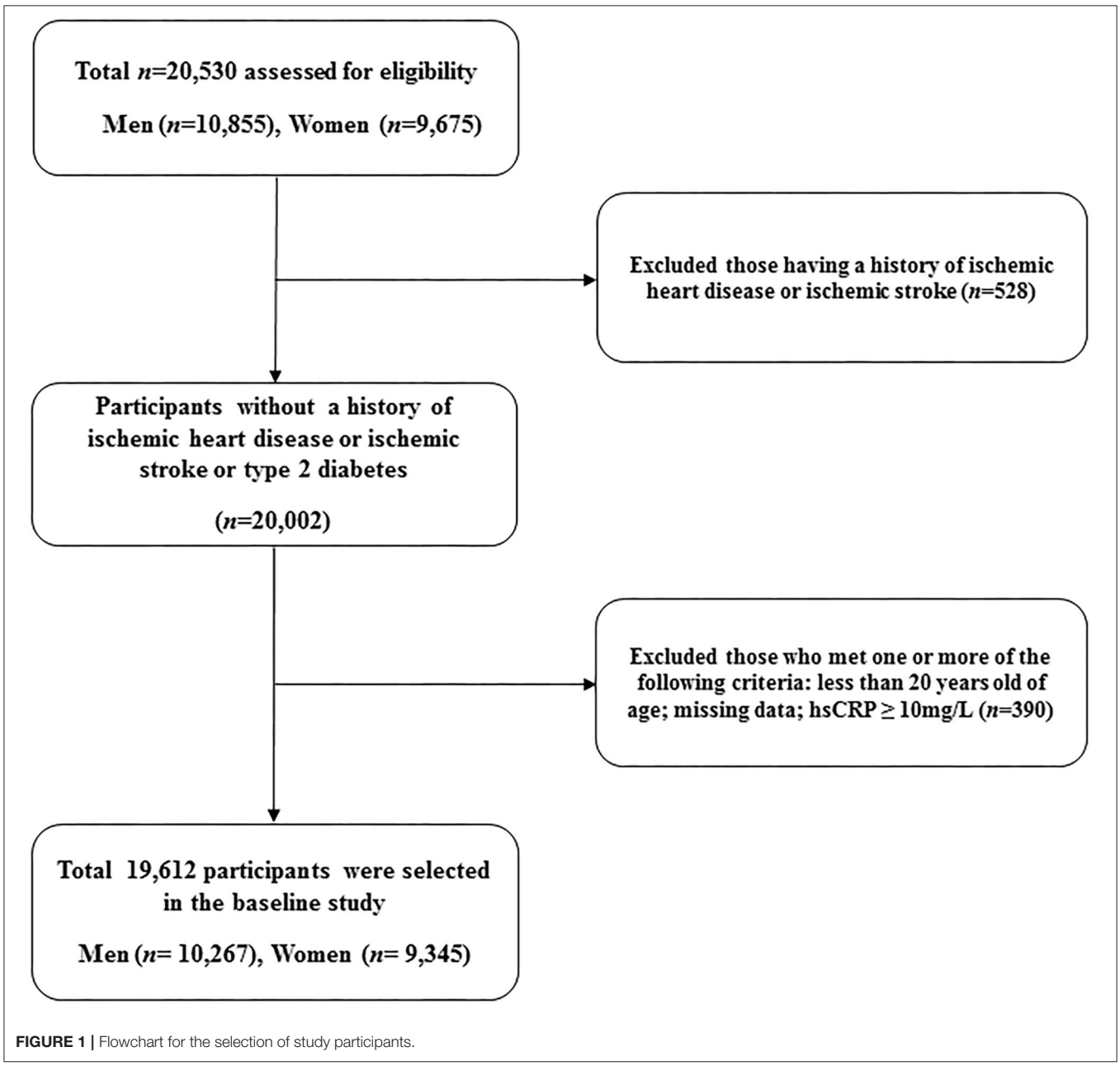

FIGURE 1 | Flowchart for the selection of study participants.

TABLE 1 | Clinical and biochemical characteristics of the study population.

	Total (n = 19,612)	Controls (n = 18,413)	Gallbladder polyp (n = 1,119)	P-value[a]
Age, years	45.7 ± 10.9	45.7 ± 10.8	46.8 ± 10.7	<0.001
Male sex, %	52.4	51.9	59.0	<0.001
Body mass index, kg/m^2	23.4 ± 3.1	23.4 ± 3.1	23.4 ± 3.0	0.939
Systolic blood pressure, mmHg	122.4 ± 15.7	122.4 ± 15.7	122.6 ± 15.5	0.674
Diastolic blood pressure, mmHg	76.3 ± 10.2	76.3 ± 10.2	76.5 ± 10.0	0.668
Mean arterial pressure, mmHg	91.7 ± 11.6	91.7 ± 11.7	91.8 ± 11.4	0.661
Fasting plasma glucose, mg/dL	94.4 ± 18.9	94.3 ± 19.0	95.1 ± 18.4	0.145
Total cholesterol, mg/dL	189.3 ± 34.1	189.3 ± 34.1	190.3 ± 34.2	0.325
Triglyceride, mg/dL	126.3 ± 90.9	126.4 ± 89.8	125.2 ± 106.7	0.715
HDL-cholesterol, mg/dL	53.2 ± 12.7	53.2 ± 12.8	52.0 ± 12.1	<0.001
Aspartate aminotransferase, IU/L	21.9 ± 12.1	21.9 ± 12.3	21.0 ± 8.6	<0.001
Alanine aminotransferase, IU/L	23.3 ± 21.8	23.4 ± 22.1	22.2 ± 15.7	0.015
γ-glutamyltransferase, IU/L	32.3 ± 40.8	32.3 ± 41.3	32.2 ± 31.8	0.894
C-reactive protein, mg/L	1.1 ± 1.4	1.1 ± 1.4	1.0 ± 1.2	0.104
Current smoker, %	24.6	24.6	25.0	0.009
Alcohol drinking, %	43.6	43.8	40.5	0.031
Regular exercise, %	31.7	31.6	33.2	0.270
Hypertension, %	23.2	23.2	24.0	0.495
Type 2 diabetes, %	5.3	5.3	5.1	0.773
Dyslipidaemia, %	38.7	38.8	38.2	0.693
Medication for comorbidities, %				
Hypertension	10.1	10.0	12.3	0.008
Type 2 diabetes	3.0	2.9	3.6	0.176
Dyslipidaemia	3.0	3.0	2.6	0.403
Impaired fasting glucose, %	16.9	16.7	19.9	0.004

[a] *P-values were calculated using t-test (age, body mass index, systolic blood pressure, diastolic blood pressure, mean arterial pressure, fasting plasma glucose, total cholesterol, triglyceride, HDL-cholesterol, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, and C-reactive protein) or the chi-squared test (sex, current smoker, alcohol drinking, regular exercise, hypertension, type 2 diabetes, dyslipidaemia, and impaired fasting glucose).*
HDL, high density-lipoprotein cholesterol.

who visited the Health Promotion Center at the Yonsei University Gangnam Severance Hospital for health examinations between November 2006 and June 2010. We excluded 528 participants who had previously been diagnosed with IHD or ischaemic stroke. In addition, patients who met at least one of the following criteria were excluded: <20 years of age, missing data, or high-sensitivity C-reactive protein (hsCRP) levels ≥ 10 mg/L (n = 390). Informed consent was obtained from each participant.

Each participant completed a questionnaire that described lifestyle and medical history. The questionnaire on smoking status consists of the following categories: never smoked, current smoker, and former smoker. A regular alcohol drinker was defined as a person who consumed more than 140 g of alcohol per week. Regular exercise was defined as moderate physical activity three or more times per week. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, in light indoor clothing without footwear. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the sitting position after 10 min of rest using a standard mercury sphygmomanometer (Baumanometer, W.A. Baum Co Inc., Copiague, NY, USA). Mean arterial pressure was calculated from SBP and DBP. Dyslipidaemia was defined as total cholesterol ≥ 240 mg/dL, triglycerides ≥ 150 mg/dL, high density-lipoprotein (HDL) cholesterol < 40 mg/dL for men and < 50 mg/dL for women, or the use of lipid-lowering medication. Diabetes was defined as a fasting plasma glucose (FPG) greater or equal to 126 mg/dL, a self-reported history of diabetes, or current use of diabetes medication. Among individuals without diabetes, impaired fasting glucose was defined as FPG levels between 100 and 126 mg/dL.

TABLE 2 | Overall incidence of ischaemic heart disease according to the presence of gallbladder polyp.

	Controls	Gallbladder polyp
New cases of ischaemic heart disease, n	430	43
Mean follow-up, years	2.4 ± 1.1	2.2 ± 1.2
Pearson-years of follow-up	43,539	2,613
Incidence rate/1,000 person-years	9.9	16.5

Exposures and Outcomes

Experienced radiologists performed abdominal USG using a 3.5-MHz transducer (HDI 5000, Philips, Bothell, WA, USA) and were blinded to laboratory and clinical data. GB polyps were assessed by well-described radiological criteria. A GB polyp was diagnosed if ultrasonography showed any size of hyperechoic mass protruding from the GB wall, and there was no acoustic shadowing or movement with a postural change. The control group without GB polyps consisted of 18,413 participants, and the GB polyp group comprised 1,119 patients. The primary outcome, previously described, was IHD, which consisted of angina pectoris (ICD-10 code I20) or acute myocardial infarction (ICD-10 code I21) that occurred after enrolment into the study (15). To define baseline and post-survey outcomes, we linked a personal 13-digit identification number that was assigned to each participant by the Korean HIRA between November 1, 2006 and December 31, 2010.

Statistical Analysis

We compared the baseline characteristics according to the presence of GB polyps using Student's *t*-tests for continuous variables and chi-squared tests for categorical variables. We have used box plots and the Kolmogorov-Smirnov test to evaluate the distribution of the variables. Adjusted survival curves were used to estimate the cumulative incidence of IHD for each group. Using the Cox proportional hazards regression model, we calculated hazard ratios (HRs) and 95 % confidence intervals (CIs) for new-onset IHD after adjusting for potential confounding variables. Furthermore, we evaluated whether the presence of GB polyps affected the incidence of IHD when combined with metabolic comorbidities. All analyses were performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided, and statistical significance was set at $P < 0.05$. The data are presented as numbers ± standard deviation or percentage.

RESULTS

A total of 19,612 participants (10,267 men and 9,345 women) were included in the final analysis (**Figure 1**). **Table 1** presents the baseline characteristics of the HERAS-HIRA cohorts according to the presence of GP polyps. There were no differences in BMI, total cholesterol, FPG, TG, or hsCRP between the two groups. The prevalence of comorbidities, including hypertension, type 2 diabetes mellitus (T2DM), and dyslipidaemia, was not significantly different between the two groups.

Table 2 shows the incidence of and difference in IHD between the control and GB polyp groups. A total of 473 individuals (2.4%, 473/19,612) developed IHD during the follow-up and the patients with GB polyps had a higher risk of developing IHD. Furthermore, the GB polyp group showed a higher cumulative incidence of IHD over 50 months after adjusting for age, sex, hypertension, diabetes, and dyslipidaemia ($P = 0.021$) (**Figure 2**).

Table 3 presents the univariate and multivariate HRs of IHD categorized by age, sex, alcohol intake, blood tests including lipid profiles, and baseline comorbidities. The increase in IHD risk in the multivariate model was dependent upon greater age, male sex, increased BMI, total cholesterol, or alanine aminotransferase, and GB polyps. **Table 4** shows Cox proportional hazard regression analyses for the risk of IHD in patients with GB polyps in the presence of different comorbidities. The coexistence of GB polyps and hypertension or dyslipidaemia resulted in greater risk of IHD compared with GB polyps alone. However, this cumulative effect was observed only in patients with impaired FPG, not in participants with diabetes.

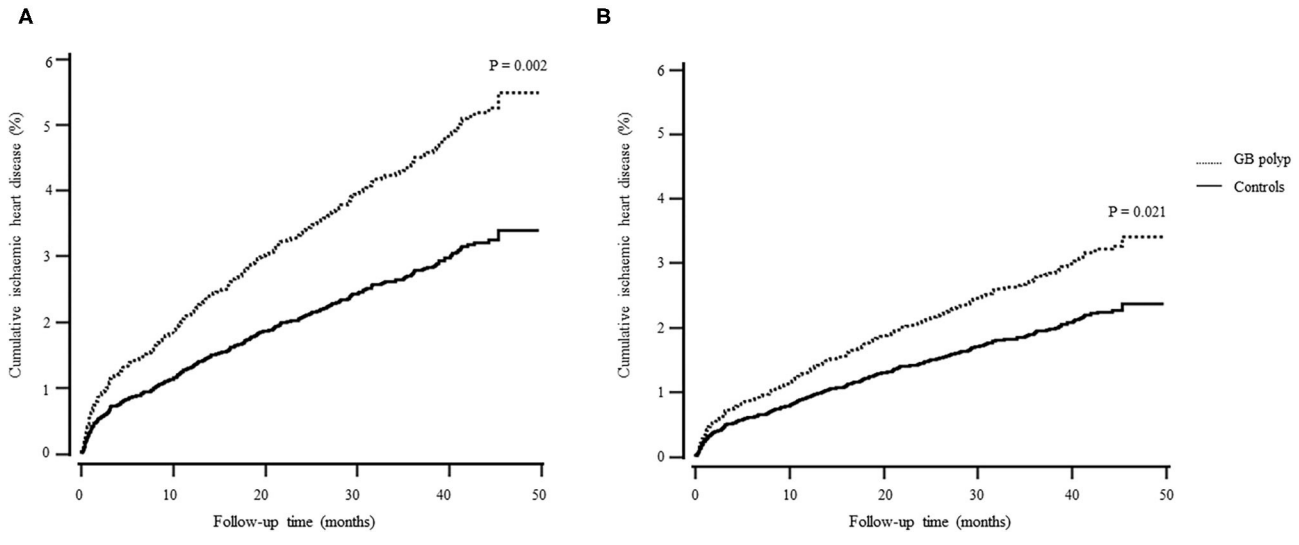

FIGURE 2 | Cox regression survival curve. **(A)** unadjusted; **(B)** adjusted for age, sex, hypertension, diabetes, and dyslipidaemia.

TABLE 3 | Univariate and multivariate Cox proportional-hazards regression models for incident ischaemic heart disease.

Variables	Univariate			Multivariate		
	HRs	95% CIs	*P*-value	HRs	95% CIs	*P*-value
Age, years	1.071	1.063–1.080	<0.001	1.057	1.047–1.067	<0.001
Male sex, yes or no	1.759	1.453–2.130	<0.001	1.502	1.122–2.010	0.006
Body mass index, kg/m^2	1.104	1.075–1.135	<0.001	1.046	1.011–1.083	0.010
Current smoking, yes or no	1.264	0.992–1.610	0.058	1.278	0.937–1.741	0.121
Alcohol drinking, yes or no	0.722	0.596–0.876	<0.001	0.766	0.618–0.949	0.014
Regular exercise, yes or no	1.599	1.325–1.930	<0.001	1.163	0.955–1.415	0.132
Mean arterial pressure, mmHg	1.023	1.016–1.031	<0.001	0.996	0.987–1.005	0.354
Fasting plasma glucose, mg/dL	1.011	1.008–1.013	<0.001	1.004	0.999–1.008	0.103
Total cholesterol, mg/dL	1.006	1.003–1.008	<0.001	1.003	1.001–1.006	0.018
Alanine aminotransferase, IU	1.003	1.002–1.004	<0.001	1.004	1.002–1.006	<0.001
C-reactive protein, mg/L	1.116	1.059–1.177	<0.001	1.004	0.939–1.074	0.904
Hypertension medication, yes or no	3.766	3.091–4.588	<0.001	1.714	1.350–2.175	<0.001
Diabetes medication, yes or no	3.163	2.288–4.373	<0.001	1.064	0.715–1.583	0.758
Dyslipidaemia medication, yes or no	4.139	3.103–5.521	<0.001	1.930	1.412–2.638	<0.001
Gallbladder polyp, yes or no	1.632	1.192–2.233	0.002	1.425	1.028–1.975	0.033

TABLE 4 | Hazard ratios and 95% confidence intervals for ischaemic heart disease according to gallbladder polyp in the context of metabolic diseases.

Comorbidities	Model 1				Model 2			
	Controls		GB polyp		Controls		GB polyp	
	HRs (95% CIs)	*P*-value	HRs (95% CIs)	*P*-value	HRs (95% CIs)	*P*-value	HRs (95% CIs)	*P*-value
Hypertension								
No	1.00 (reference)		1.44 (0.94–2.19)	0.090	1.00 (reference)		1.45 (0.93–2.24)	0.100
Yes	1.49 (1.22–1.83)	<0.001	2.14 (1.34–3.44)	0.001	1.52 (1.23–1.88)	<0.001	2.14 (1.32–3.48)	0.002
Type 2 diabetes								
No	1.00 (reference)		1.49 (1.07–2.07)	0.018	1.00 (reference)		1.50 (1.06–2.11)	0.021
Yes	1.46 (1.09–1.94)	0.013	1.59 (0.59–4.26)	0.358	1.50 (1.12–2.02)	0.006	1.58 (0.59–4.23)	0.367
Dyslipidaemia								
No	1.00 (reference)		1.51 (0.98–2.33)	0.059	1.00 (reference)		1.48 (0.94–2.32)	0.089
Yes	1.55 (1.28–1.87)	<0.001	2.09 (1.32–3.31)	0.001	1.54 (1.26–1.88)	<0.001	2.14 (1.33–3.43)	0.001
IFG								
No	1.00 (reference)		1.42 (0.98–2.07)	0.066	1.00 (reference)		1.45 (0.98–2.13)	0.062
Yes	1.35 (1.07–1.70)	0.011	1.90 (1.08–3.32)	0.025	1.41 (1.11–1.80)	0.004	1.87 (1.04–3.35)	0.035

Model 1: adjusted for age and sex (+ diabetes if IFG).
Model 2: adjusted for age, sex, smoking status, alcohol intake, and physical activity (+ diabetes if IFG).
IFG, impaired fasting glucose.

DISCUSSION

We found that the presence of GB polyps alone was associated with a 42.5% increase in the risk of developing IHD compared with a non-polyp control group. By including IHD risk factors, we discovered that the coexistence of hypertension or dyslipidaemia resulted in an increased risk of IHD in the GB polyp group by approximately 2-fold. However, this cumulative effect was observed only in patients with impaired fasting blood glucose, but not in those with type 2 diabetes mellitus, most of whom are on glucose-lowering medications.

To our knowledge, only one other report has described a relationship between GB polyps and CVD (13). However, in this latter study, the authors did not fully assess IHD-related risk factors to sufficiently show a causal relationship between GB polyps and IHD. In our study, we evaluated multiple risk factors that influence the development of IHD, including blood glucose levels, lipid profiles, blood

pressure, liver function, alcohol intake, smoking status, and self-reported diseases, such as T2DM, hypertension, and dyslipidaemia.

It is generally believed that GB disease is positively correlated with metabolic syndrome, obesity, and T2DM because these conditions share common risk factors, such as sedentary life styles, dyslipidaemia, and fat rich diets (12, 16). Our results imply that GB polyps are closely related to the development of IHD, although this association may be an epiphenomenon and not a causal effect. However, our findings suggest that GB polyps may be a risk factor for IHD that is independent of traditional risk factors.

In the present study, patients who presented with GB polyps and the comorbidity hypertension or dyslipidaemia developed an increased risk of IHD that was greater than patients with GB polyps alone. This interaction was observed in patients with impaired FPG, but not in those with T2DM. Considering that the prevalence of metabolic diseases is not different between the GB polyp group and controls at the baseline, both metabolic and non-metabolic factors may contribute to the progression of IHD. Epidemiological studies have reported that the development of IHD is enhanced when CVD risk factors are combined with obesity and metabolic syndrome, but not T2DM, in the general population (17–20). This finding may be explained in part by the medications used (e.g., metformin) and life style modifications that may occur after a diagnosis of T2DM. Metformin has been shown to protect the heart from fibrosis and remodeling after myocardial infarction and decrease inflammation and oxidative stress (21). As a result, patients with T2DM taking metformin may have a weakened risk of developing IHD (22). In addition, our study showed that hypertension had a more obvious effect on IHD development than mean blood pressure at the baseline assessment. This may be related to a follow-up period of <5 years.

Recent epidemiological and experimental studies have reported possible mechanisms by which GB polyps influence the development of IHD. GB polyps are tumor or tumor-like protrusions arising from the GB mucosa and are divided into true polyps and pseudopolyps (23). True polyps are classified as adenomas or adenocarcinomas, and pseudopolyps, which represent over 90% of GB polyps, consist mainly of cholesterol and inflammatory polyps (24, 25). As the names suggest, the growth and development of the majority of GB polyps are closely related with cholesterol metabolism and inflammation (25). Acetyl-CoA acetyltransferase 2 (ACAT2) is a key enzyme in the biogenesis of lipid bodies, which may facilitate the pinocytosis of cholesterol and papillary hyperplasia in the GB mucosa (26). Additionally, this enzyme decreases GB contractility leading to cholesterol deposition in the GB wall (27). ACAT2 is also responsible for incorporation of cholesteryl ester in apoprotein B-containing lipoproteins that leads to increased very low-density lipoprotein (VLDL) secretion and coronary artery atherosclerosis (28). It has been reported that inflammation is closely related to ACAT2 activity and downregulating ACAT2 is associated with lowering cholesterol and preventing atherosclerosis (29). Collectively, the interactive linkage between ACAT activity, inflammation, and dyslipidaemia may have served as a shared mechanism for GB polyps and IHD, an essential starting point for prevention and treatment (30).

Previous cross-sectional studies have consistently found that patients with metabolic syndrome have a high prevalence of GB polyps, suggesting that insulin resistance may be a potential cause (16, 31). An epidemiological study reported that hyperinsulinemia increased the incidence of GB polyp in Korean men (32). Therefore, we propose that screening patients with GB polyps for metabolic disturbances will be important for early detection and prevention of IHD.

The present study had several strengths. First, potential IHD-related confounding factors were assessed by blood tests along with traditional risk factors. Second, the identification of GB polyps occurred during the process of screening the general population rather than relying on the diagnosis by doctors based on symptoms of GB disease. Also, we minimized the misclassification of asymptomatic patients into the control group in our study and decreased cohort bias. USG by experienced radiologists has over 90% of accuracy for the diagnosis of GB polyps (33, 34). Third, this study was carried out using data from a large scale, cohort study to ascertain IHD risk factors and included analyses of multiple physiological tests.

Nevertheless, this study also has some limitations. For the majority of patients with GB polyps, there was no information regarding a pathological condition because the data were obtained from asymptomatic patients who underwent health check-ups. Additionally, this study was a retrospective cohort study and USG was performed only at baseline. Therefore, we were unable to discover new-onset GB polyps during the follow-up period, which may have led to a selection bias. The study cohort was composed of volunteers sequentially visiting for health examination screenings conducted at a single hospital; participants appeared healthier than most community-based cohorts. The number of participants with both GB polyps and comorbidities is small, 1.4% for hypertension, 0.3% for diabetes, and 2.3% for dyslipidaemia. Further research is needed on the risk of IHD according to GB polyps in patients with each cardiometabolic comorbidity. Lastly, individuals with diabetes may have been underestimated because hemoglobin A1c and 2-h oral glucose tolerance tests were not measured at the baseline. Despite these limitations, this is the first study to assess a role for conventional IHD risk factors in the association between GB polyps and development of IHD.

CONCLUSIONS

This study showed that the presence of GB polyps is associated with the development of IHD. Metabolic comorbidities are significant factors that increase the risk of gallbladder polyps associated with IHD. Also, early intervention of GB polyps may reduce IHD risk. These findings may guide the prevention and therapy of patients with both GB polyps and IHD and warrant further studies on effective treatment options.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Yonsei University College of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-JL, BP, and D-HJ: study concept and design. Y-JL, BP, and K-WH: acquisition, analysis, and interpretation of data. BP and D-HJ: drafting of the manuscript. Y-JL and D-HJ: critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: K-WH was employed by Theragen Bio Co. Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Hyperuricemia Predicts Adverse Outcomes After Myocardial Infarction With Non-obstructive Coronary Arteries

Abdul-Quddus Mohammed[1], Fuad A. Abdu[1], Lu Liu[1], Wen Zhang[1], Guoqing Yin[1], Yawei Xu[1] and Wenliang Che[1,2]**

[1] Department of Cardiology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China,
[2] Department of Cardiology, Shanghai Tenth People's Hospital Chongming Branch, Shanghai, China

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***Correspondence:**
Fuad A. Abdu
1691026@tongji.edu.cn
Wenliang Che
chewenliang@tongji.edu.cn

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Background: Serum uric acid (SUA) is a well-known predictor of adverse outcomes in patients with various clinical conditions. However, the impact of SUA on patients with myocardial infarction with non-obstructive coronary arteries (MINOCA) remains unclear. Here, we aimed at investigating the potential association between hyperuricemia and the adverse outcomes in MINOCA patients.

Methods: Overall, 249 MINOCA patients were enrolled in the present study. Clinical characteristics and laboratory data, were measured in all patients. Based on SUA levels, patients were classified into two groups; the hyperuricemia group [SUA level $>$ 6 mg/dL (360 μmol/L) in women and $>$ 7 mg/dL (420 μmol/L) in men], and the normuricemia group. The primary endpoint of our study was major adverse cardiac events (MACE), defined as cardiovascular death, stroke, heart failure, non-fatal MI, and angina rehospitalization.

Results: Seventy-two patients were in hyperuricemia group and 177 in normuricemia group. Fifty-two MACE events were recorded after 30 months of follow-up period. The incidence of MACE was higher in hyperuricemia group compared with normuricemia group (31.9 vs. 16.3%, $P = 0.006$). Kaplan-Meier survival curves illustrated a significantly increased risk of MACE in hyperuricemia group (log-rank $P = 0.006$). The multivariable logistic analysis demonstrated that hyperuricemia was independently associated with a high risk of MACE after 30 months of follow-up (OR, 2.234; 95% CI, 1.054–4.737, $P = 0.036$).

Conclusion: Hyperuricemia is associated with adverse outcomes and appears to be an independent predictor of MACE in MINOCA patients. This finding suggests that the SUA levels may serve as a surrogate biomarker related to risk prediction and adverse outcomes of MINOCA patients.

Keywords: myocardial infarction, MINOCA, serum uric acid, hyperuricemia, outcome

INTRODUCTION

Despite great advances in our undertanding of cardiovascular diseases, myocardial infarction with non-obstructive coronary arteries (MINOCA) remains an intriguing clinical entity which has gained increased attention over the recent years, comprises 5–15% of overall acute myocardial infarction (AMI) cases (1, 2). MINOCA is not a benign entity with multiple underlying pathological mechanisms, and it is associated with a higher risk of a major adverse cardiac event (MACE) leading to early rehospitalization, with an in-hospital mortality and 5 years mortality rate of 4.57 and 10.9%, respectively (3–6). Therefore, identifying new biomarkers for adverse outcome associated to patients with MINOCA would be of potential interest, as they represent a heterogenous group and could be monitored with caution and treated with an appropriate preventive strategy.

Serum uric acid (SUA) represents the end-product of purine catabolism (7). Elevated SUA is now considered to have a potential relationship with multiple clinical conditions (8) particularly, cardiovascular diseases (9, 10). Emerging research also correlates hyperuricemia with enhanced intracellular oxidative stress, inflammation and vasoconstriction, as well as endothelial dysfunction (7, 11, 12) as a result, atherosclerosis and cardiovascular disease progression (13). In addition, elevated SUA is implicated as a marker of poor outcomes in patients with arterial hypertension (14), metabolic syndrome (15), coronary artery disease (CAD) (16), cerebrovascular disease (17), myocardial infarction (MI) (18), heart failure (HF) (19), renal disease (10, 20) and in general population (21, 22). However, the role of hyperuricemia in patients with MINOCA, as well as whether hyperuricemia is associated with adverse outcomes has not been studied.

Therefore, the present study sought to explore the association between hyperuricemia and adverse outcomes in MINOCA patients.

METHODS

Study Population

This was an observational study which was carried out at the department of cardiology, Shanghai Tenth People's Hospital, between 2014 and 2018. We enrolled 249 consecutive patients who underwent coronary angiography (CAG) and were diagnosed with MINOCA.

MINOCA was identified as patients who had evidence of AMI with non-obstructive coronary artery disease (defined as no stenosis; or stenosis < 50%) as recommended by the current position statement from the European Society of Cardiology (ESC) working group and the fourth Universal Definition of MI (1, 2). We excluded patients who had a prior history of MI or coronary intervention, severe liver disease, gout, and a malignant tumor; patients with typical myocarditis or Takotsobu syndrome; patients who were unable to obtain SUA determination; and patients who do not have SUA baseline results.

After admission, demographic and baseline clinical data [such as age, sex, smoking history, blood pressure, heart rate, diabetes, hypertension, BMI, hyperlipidemia, chronic kidney disease (CKD) and atrial fibrillation], and diuretic were collected. In addition, all patients following their entry underwent an electrocardiogram, echocardiography, and CAG procedure.

Fasting blood was obtained within 24 h of hospitalization. The serum uric acid (SUA) levels, serum cardiac biomarkers [such as troponin-T (Tn-T), myoglobin, N-terminal-pro-brain natriuretic peptide (NTproBNP), and creatine kinase-isoenzyme MB (CK-MB)], lipid panel measures [such as total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)] and estimated glomerular filtration rate (eGFR) were measured in all patients.

SUA levels were measured by Olympus AU4500 automatic chemistry analyzer (Olympus Corporation, Tokyo, Japan). GFR was estimated using a Modification of Diet in Renal Disease (MDRD) method, and CKD is defined as eGFR < 60 ml/min/1.73 m^2 (23). Comorbidities such as hypertension, dyslipidemia and diabetes were described as either previously known or if patients are on specific therapy. NTproBNP levels were measured by the Eleusis electro-chemiluminescent immunoassay (Roche Diagnostics Ltd. Rotkreuz, Switzerland).

Left ventriculography and echocardiography have been used to evaluate wall motion, and intravascular ultrasound or optical coherence tomography were utilized to assess atherosclerotic plaque disruption or plaque erosion, coronary angiography derived flow fractional reserve was performed in selected patients to evaluate the functional significance of coronary artery lesions in the present study to investigate the ultimate causes of MINOCA patients.

Based on SUA levels, patients were divided into two groups: the normuricemia group and the hyperuricemia group. Hyperuricemia was defined as SUA levels > 6 mg/dL (360 μmol/L) in women and > 7 mg/dL (420 μmol/L) in men, as previously published (24, 25).

The study was approved by the institutional ethics committee (Shanghai Tenth People's Hospital, Tongji University, Shanghai, China) and was complied in accordance with the Helsinki Declaration. Each enrolled participant in this study signed an informed consent form.

Follow-Up

Follow-up was carried out for 30 months after discharge through outpatient visits, telephone calls, reviewing electronic medical records, and clinical notes by two experienced cardiologists to obtain the patient's clinical status and outcome events or the first reported outcome case at the Shanghai Tenth People's Hospital.

The study primary endpoint was MACE, defined as cardiovascular death, stroke, heart failure, non-fatal MI, and angina rehospitalization. Cardiovascular death was described as death due to the cardiac origin, including acute coronary

Abbreviations: SUA, Serum uric acid; AMI, acute myocardial infarction; MINOCA, myocardial infarction with non-obstructive coronary arteries; ACS, acute coronary syndrome; MACE, major adverse cardiac events; eGFR, estimated Glomerular filtration rate; CAG, coronary angiography; CK-MB, creatine kinase-isoenzyme, Tn-T, troponin T; NTproBNP, N-terminal-pro-brain natriuretic peptide; BMI, body mass index; LVEF, left ventricular ejection fraction; CI, confidence interval; OR, odds ratio; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

syndrome (ACS), severe arrhythmias, refractory congestive heart failure, or sudden death with no obvious cause. Non-fatal MI was defined as characteristic signs and symptoms of myocardial ischemia in the presence of new ECG changes or increased levels of cardiac biomarkers of myocardial damage (2). Stroke was defined as evidence of ischemic infarct in any cerebral artery caused by either thrombotic or an embolic occlusion (26). The definition of heart failure was in accordance with the previous HF guidelines (27). Angina rehospitalization was defined as any rehospitalization or readmission to emergency department due to recurrent ischemic discomfort with objective evidence recorded by the physician.

Statistical Analysis

Data were processed using the Statistical Package for Social Sciences (SPSS) v.22. The categorical variables are expressed as percentages (%), and numbers were expressed as the mean ± SE. To compare the categorical variables, the chi-square test and the Fisher's exact tests were performed. An independent sample *t*-test was performed when numerical variables were compared between the groups. Multivariable logistic regression analysis was performed to explore the adjusted odds ratio (OR) for MACE events to determine the predictors of clinical endpoints. Traditional cardiovascular risk factors (e.g., age, sex, hypertension, diabetes, BMI, atrial fibrillation, hyperlipidemia, CKD, and LVEF), biochemical parameters [serum levels of uric acid (categorical variables), Tn-T, myoglobin, NTproBNP, TG, TC, LDL-C and HDL-C], and diuretic use were considered as covariates in the univariate models. Univariate predictors (when $P < 0.10$) were variables in covariates for multivariable models. Kaplan-Meier analysis was used to assess MACE-free survival rates, and the log-rank test was performed to identify differences between groups. Furthermore, the association between SUA levels and the risk of MACE was also evaluated using restricted cubic spline Cox regression. To assess potential relationship between hyperuricemia and MACE, subgroup analyses were performed, and interactions between hyperuricemia and each subgroup including age, sex, hypertension, diabetes, dyslipidemia, smoking, atrial fibrillation, LVEF, and eGFR levels were evaluated by a Cox proportional hazards regression model. All statistical analyses were performed two-sided and required a statistical significance of P-value < 0.05.

RESULTS

Baseline Characteristics of the Study Population

Baseline characteristics and angiographic data of the study population stratified by the presence of hyperuricemia are

TABLE 1 | Baseline characteristics of the study population.

	Hyperuricemia (*n* =72)	Normuricemia (*n* =177)	*P*-value
Age (years)	64.31 ± 14.97	62.00 ± 12.96	0.226
Female, *n* (%)	27 (37.5)	95 (53.7)	0.021
Hypertension, *n* (%)	44 (61.1)	77 (43.5)	0.012
Diabetes, *n* (%)	14 (19.4)	27 (15.3)	0.419
Smoking history, *n* (%)	35 (48.6)	65 (36.7)	0.083
BMI (kg/m^2)	25.08 ± 4.24	23.68 ± 3.61	0.012
Atrial fibrillation, *n* (%)	13 (18.1)	14 (7.9)	0.020
Chronic kidney disease	30 (41.7)	17 (9.6)	<0.001
Hyperlipidaemia, *n* (%)	16 (22.2)	23 (13.0)	0.069
eGFR, ml/min	70.38 ± 28.66	94.41 ± 26.42	<0.001
LVEF (%)	51.03 ± 13.91	56.21 ± 10.46	0.002
STEMI, *n* (%)	30 (41.7)	71 (40.1)	0.821
Systolic blood pressure (mmHg)	138.04 ± 25.29	141.69 ± 22.12	0.259
Diastolic blood pressure (mmHg)	79.83 ± 14.48	80.54 ± 12.45	0.698
Heart rate, beats per minute	84.31 ± 20.95	80.75 ± 16.55	0.157
Diuretic	35 (48.6)	35 (19.8)	<0.001
Angiographic data			
Normal coronary arteries (0% stenosis), *n* (%)	21 (29.2)	97 (54.8)	<0.001
Mild coronary stenosis (stenosis <50%), *n* (%)	51 (70.8)	80 (45.2)	<0.001

BMI, body mass index; LVEF, left ventricular ejection fraction; eGFR, estimated Glomerular filtration rate; STEMI, ST-segment elevation myocardial infarction.

TABLE 2 | Laboratory findings of the study population.

	Hyperuricemia (*n* =72)	Normuricemia (*n* =177)	*P*-value
Uric acid, μmol/L	489.17 ± 99.25	296.84 ± 72.33	<0.001
Tn-T (ng/mL)	0.48 ± 1.27	0.50 ± 1.06	0.895
CK-MB (ng/mL)	23.96 ± 67.02	17.94 ± 34.77	0.354
Myoglobin (ng/ml)	211.57 ± 411.52	113.59 ± 190.81	0.011
NTproBNP (pg/mL)	3,796.49 ± 6,378.48	1,576.58 ± 3,773.46	0.001
TC (mmol/L)	4.33 ± 1.25	4.22 ± 0.99	0.462
TG (mmol/L)	1.73 ± 1.21	1.35 ± 0.71	0.004
HDL-C (mmol/L)	1.02 ± 0.49	1.05 ± 0.49	0.657
LDL-C (mmol/L)	2.47 ± 1.06	2.47 ± 0.87	0.977

CK-MB, creatine kinase-isoenzyme; Tn-T, troponin T; NTproBNP, N-terminal-pro-brain natriuretic peptide; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

TABLE 3 | Patients outcomes.

	Hyperuricemia (n =72)	Normuricemia (n =177)	*P*-value
MACE	23 (31.9)	29 (16.3)	0.006
Cardiovascular death	5 (6.9)	9 (5.1)	0.554
Nonfatal MI	1 (1.4)	1 (0.5)	0.496
Heart failure	1 (1.4)	2 (1.1)	1.000
Stroke	2 (2.8)	1 (0.5)	0.201
Angina rehospitalization	14 (19.4)	16 (9.0)	0.022

MACE, major adverse cardiovascular events; MI, myocardial infarction.

summarized in **Table 1**. Overall, 249 patients were eligible and identified for the diagnostic criteria of MINOCA (mean age, 62.67 ± 13.58 years). Seventy-two patients (28.9%) were in the hyperuricemia group and 177 patients (71.1%) in the normuricemia group. Compared with normuricemia group, hypertension, CKD and atrial fibrillation were more frequent in hyperuricemia group, BMI and diuretic use were also higher in hyperuricemia group, whereas eGFR and LVEF values were lower. By comparison, the normuricemia group had a higher rate of females than hyperuricemia group. Coronary angiography data revealed that patients in hyperuricemia group had a higher rate of mild coronary stenosis than normuricemia group.

Laboratory analysis of the study population is summarized in **Table 2**. Hyperuricemia group had higher levels of TG and myocardial biomarkers such as myoglobin and NTproBNP compared with the normuricemia group (all $P < 0.05$). In contrast, other comorbidities such as age, smoking history, hyperlipidemia, diabetes, heart rate, systolic and diastolic blood pressure, and other laboratory findings appeared to be similar between the two groups (all $P > 0.05$).

Hyperuricemia and Clinical Outcomes

The average follow-up period was 30 months. All patients were available with follow-up data, 52 MACE were recorded. Hyperuricemia group was associated with 23 MACE, whereas, 29 MACE occurred in the normuricemia group. The hyperuricemia group had a higher incidence of MACE and angina rehospitalization compared with normuricemia group (31.9 vs. 16.3% and 19.4 vs. 9.0%, respectively; all $P < 0.05$) (**Table 3**). Increased risk of MACE in hyperuricemia group is

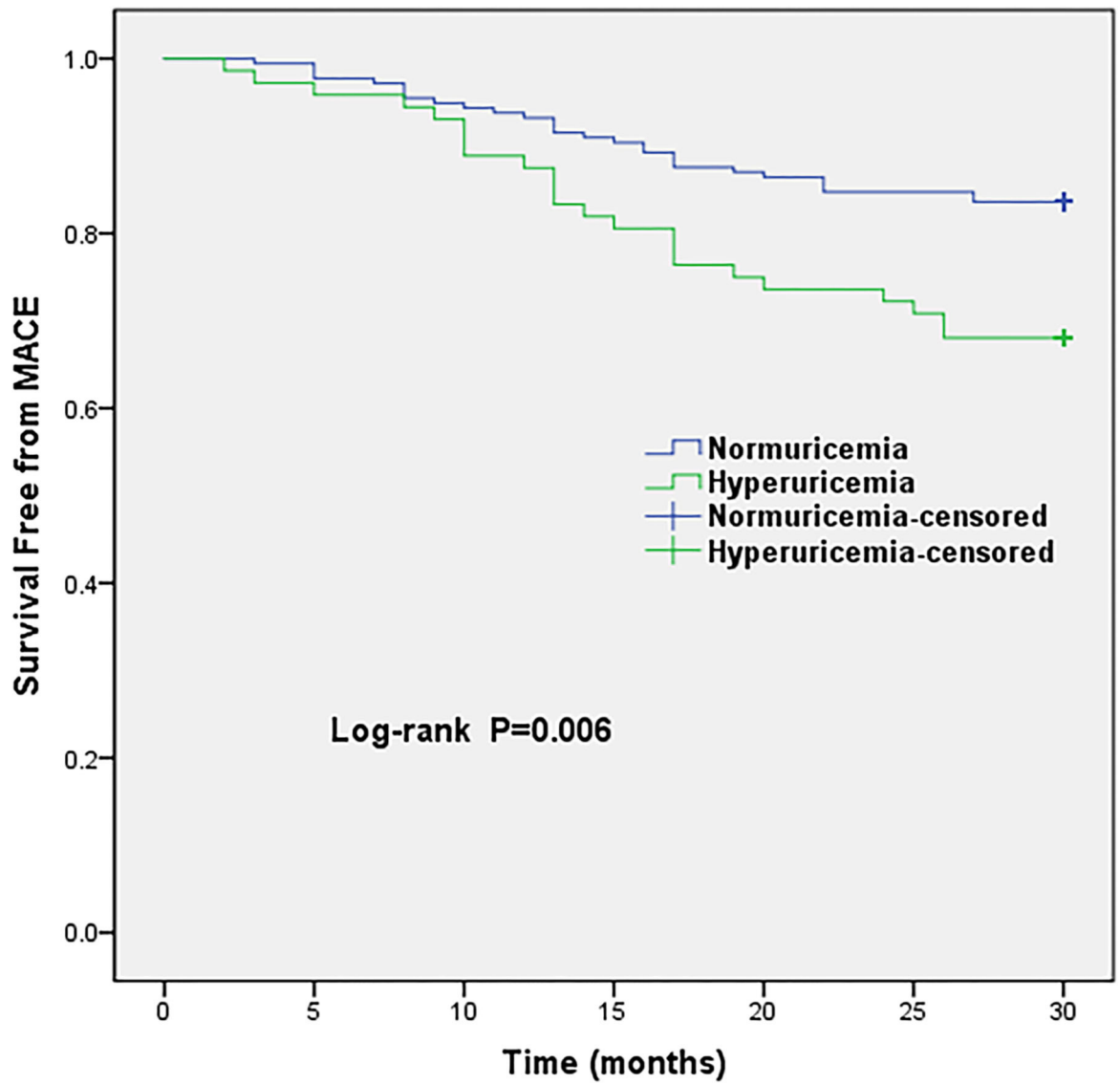

FIGURE 1 | Kaplan-Meier survival curves of MACE in MINOCA patients with hyperuricemia vs. normuricemia. MACE, major adverse cardiovascular events.

TABLE 4 | Univariate and multivariable analysis of predictors of MACE.

Variables	Univariate analysis		Multivariable analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Hyperuricemia	2.395 (1.269–4.523)	0.007	2.234 (1.054–4.737)	0.036
Hypertension	2.151 (1.145–4.041)	0.017		
Diabetes	2.012 (0.956–4.235)	0.066		
Age	1.038 (1.013–1.064)	0.003	1.029 (1.000–1.058)	0.048
LVEF	0.963 (0.940–0.987)	0.002	0.966 (0.938–0.995)	0.022
CKD	2.078 (1.022–4.224)	0.043		
Diuretic use	1.838 (0.965–3.499)	0.064		

LVEF, left ventricular ejection fraction; CKD, chronic kidney disease; CI, confidence interval; OR, odds ratio.

shown in Kaplan-Meier survival curves, which demonstrated that MINOCA patients within the hyperuricemia group were clearly separated from the normuricemia group, and the significant statistical difference is noted (log-rank $P = 0.006$) (**Figure 1**). Additionally, the restricted cubic splines revealed a linear relationship between SUA levels and MACE, indicating that the risk of MACE occurrence increased gradually with continuous increase in SUA levels (P for non-linearity = 0.806) (**Supplementary Figure 1**).

The predictors of MACE from the univariate and multivariable analysis are shown in **Table 4**. Univariate logistic analysis showed that hyperuricemia was independent predictor of MACE in MINOCA patients (OR, 2.395; 95% CI, 1.269–4.523, $P = 0.007$). Furthermore, after adjusting for potential confounders, multivariable logistic analysis persistently demonstrated that hyperuricemia was associated with an increased risk of MACE over the follow-up period of 30 months (OR, 2.234; 95% CI, 1.054–4.737, $P = 0.036$).

The associations between hyperuricemia and MACE stratified by sex, age, hypertension, diabetes, dyslipidemia, smoking, atrial fibrillation, LVEF, and eGFR levels are shown in **Table 5**, which demonstrated that there were no interactions between hyperuricemia and clinically related variables except for an interaction with hypertension ($P = 0.048$); HR of 1.026 (95% CI, 0.379–2.773, $P = 0.960$) with hypertension, and HR of 4.314 (95% CI, 1.564–11.906, $P = 0.005$) without hypertension.

TABLE 5 | Subgroup analysis of the association between hyperuricemia and adverse outcomes.

Factors	Subgroup	HR (95% CI)	Interaction *P*-value
Age	<65 years	2.396 (0.865–6.582)	0.677
	≥65 years	1.769 (0.673–4.648)	
Sex	Male	2.963 (1.055–8.327)	0.412
	Female	1.624 (0.571–4.609)	
Hypertension	Yes	1.026 (0.379–2.773)	0.048
	No	4.314 (1.564–11.906)	
Diabetes	Yes	1.646 (0.275–9.863)	0.784
	No	2.155 (1.008–4.603)	
Smoking	Yes	3.238 (0.913–11.481)	0.408
	No	1.741 (0.731–4.152)	
Atrial fibrillation	Yes	0.431 (0.045–4.145)	0.137
	No	2.538 (1.208–5.335)	
Hyperlipidaemia	Yes	0.503 (0.052–4.839)	0.175
	No	2.596 (1.230–5.435)	
LVEF	<50	2.147 (0.744–6.191)	0.636
	≥50	1.519 (0.570–4.049)	
eGFR	<60	2.784 (0.591–13.119)	0.488
	≥60	1.466 (0.574–3.747)	

BMI, body mass index; LVEF, left ventricular ejection fraction; eGFR, estimated Glomerular filtration rate; HR, hazard ratio; CI, confidence interval.

DISCUSSION

The present study evaluated whether hyperuricemia was associated with adverse outcomes in patients with MINOCA. Our study found that hyperuricemia is associated with adverse outcomes and appears to be an independent predictor of MACE in MINOCA patients, which suggests that quantification of SUA is an appropriate measure of predicting adverse outcomes following MINOCA.

MINOCA is a distinct type of MI that has received a considerable amount of interest as it was recently introduced in the ESC and the fourth Universal Definition of MI (2018) guidelines (1, 2). MINOCA is described as a non-benign entity with multiple underlying pathological etiologies leading to management inconsistency. The in-hospital mortality rate was 4.6% in a Japanese study of 13022 MINOCA patients (5). A recent multi-center cohort study in 16849 MINOCA patients reported an 18.7% rate of MACE events over 12 months, one out of every five MINOCA patients suffered a major adverse event (28). Therefore, additional risk stratification to further refine new clinical predictive factors is essential to identify MINOCA patients who are at increased risk of new MACE events.

During the past decade, there has been an increasing awareness that hyperuricemia is associated with potential cardiovascular diseases (9). Noteworthy, the relation to its prevalence varied among the studies due to diverse definitions of hyperuricemia. In patients with ACS, the prevalence of

hyperuricemia was found to be 34.4 and 29.3% (29, 30). Consistent with previous studies, the present study found a 28.9% prevalence rate of hyperuricemia in MINOCA patients.

Data among short and long-term prognosis in ACS patients demonstrated an independent association between hyperuricemia and adverse outcomes, and indicated that hyperuricemia can predict in-hospital adverse outcomes, as well as cardiovascular and all-cause mortality (29–33). Consistently, the potential role of hyperuricemia in predicting adverse prognosis is also supported by findings from AMI studies (34, 35). SUA was a strong predictor for in-hospital mortality among ST elevation myocardial infarction (STEMI) patients submitted to primary PCI (18). In a long-term follow-up of 8.4 years, the Rotterdam study found major adverse events were independently associated with hyperuricemia (36). Hyperuricemia also independently predicted the risk of mortality and MACE in patients who underwent cardiac revascularization and cardiac valve surgery (37). Two recent MINOCA studies reported that renal impairment was associated with mortality in MINOCA patients (38, 39), the latter study also found that every third patient with MINOCA is diagnosed with impaired kidney function, which implies that the uric acid may play a significant role related to the extent of impaired cardiovascular and/or renal hemodynamics in patients with MINOCA. To our knowledge, no prior study has evaluated the association between hyperuricemia and MINOCA and its impact on clinical outcomes. Our study provides new insights into the relationship between hyperuricemia and the adverse outcomes of MINOCA patients. In the present study, we observed that MINOCA patients in hyperuricemia group had a higher incidence of MACE and angina rehospitalization compared with normuricemia group. Accordingly, the Kaplan-Meier survival curves illustrated that the hyperuricemia group had a significantly higher risk of total MACE. Moreover, multivariable logistic regression showed that MINOCA patients in the hyperuricemia group demonstrated a significant association with adverse outcome, which implies that SUA levels may serve as an important index of poor prognosis in high-risk MINOCA patients.

In addition, hyperuricemia group had higher prevalence of hypertension, hypertriglyceridemia, BMI, and AF which has been correlated with elevated SUA level by earlier studies (15, 40). A potential relationship between hyperuricemia and CAD incidence and adverse events has been reported earlier (41), moreover, SUA was significantly associated with cardiovascular and all-cause mortality and revealed a "J-shaped" pattern in patients with CAD (42). In early-onset CAD, hyperuricemia was linked to the severity of CAD among patients with unstable angina and MI (43). Compared with low uric acid levels, hyperuricemia was independently associated with a 6-fold higher incidence of HF in the general population (19), as well as abnormal LVEF (44). Thus, hyperuricemia exerts a potential role in developing atherosclerotic diseases and their risk factors leading to adverse cardiovascular events (7, 12, 13). Our findings are consistent with these studies indicating that MINOCA patients in hyperuricemia group are associated with low LVEF levels and higher NT-proBNP and myoglobin levels compared to the normuricemia group; moreover, coronary arteries with mild stenosis was more frequent in hyperuricemia group, suggesting that hyperuricemia is correlated with the degree of myocardial injury and severity of MINOCA patients. Nevertheless, concomitant use of diuretics in patients with clinical conditions such as heart failure and/or renal impairment may alter serum uric acid levels, consequently affecting clinical outcomes. In the present study, even after adjustment for diuretics intake, multivariate logistic regression analysis still stated that hyperuricemia was associated with increased risk of MACE in MINOCA patients, large prospective studies are required to confirm this finding.

Taken together, the findings in the present study highlight the potential clinical significance of SUA on cardiovascular risk in MINOCA patients, if added in the risk stratification of MINOCA patients at an early stage, may prevent MACE and improve treatment options and overall outcomes in high-risk individuals. In addition, SUA levels are shown to be clinically important estimates of predicting poor prognosis. Therefore, SUA may serve as a surrogate biomarker to improve clinical risk classification in patients following MINOCA. Furthermore, hyperuricemia is a potentially treatable factor, uric acid is collected routinely in clinical practice and is inexpensive to obtain. However, it remains unclear whether modulating SUA and improving cardiovascular outcomes has clinical benefits.

Few potential limitations were related to this study. First, this was a single-centered, small retrospective-observational study with a short follow-up period. Second, data regarding SUA was available only at the admission; as a result, we are unable to provide any details on the impact of SUA level change over the follow-up period. Third, treatment with Xanthine Oxidase (XO) inhibitors which may affect the patients clinical outcomes were lacking; nevertheless, additional research warrants the effects of XO inhibitors in lowering SUA and improving cardiovascular outcomes as previous reports implicated mixed results; therefore, lowering SUA to improving cardiovascular outcomes in MINOCA patients needs further verification. Furhermore, although hyperuricemia was associated with adverse clinical outcomes after adjusting for multiple potential confounding factors, the unmeasured confounding exposure effects still cannot be entirely omitted. In addition, our results provided from one center cannot be extrapolated to different populations for general medical practice. Further research with a larger cohort and multi-center prospective studies may warrant establishing the role of uric acid and cardiovascular risk among MINOCA patients who represent a diverse phenotype and further elucidate the precise mechanisms.

CONCLUSION

This is the first study investigating the impact of SUA on MACE in MINOCA patients. Our study demonstrated that hyperuricemia is associated with adverse outcomes and appears to be an independent predictor of MACE in MINOCA patients. This finding suggests that the SUA levels may serve as a surrogate biomarker related to risk prediction and adverse outcomes of

MINOCA patients. Whether modulating UA in MINOCA may benefit clinically requires further elucidation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shanghai Tenth People's Hospital, Tongji University, Shanghai, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

A-QM, FA, and WC contributed to the conception and designed of the research. Data acquisition, analysis and interpretation were performed by LL, GY, A-QM, and FA collected the data. WZ was involved in data cleaning, follow-up, and verification. A-QM, FA, YX, and WC drafted and revised the manuscript critically for important intellectual content. WC and FA approved the final version of the manuscript. The final version to be published was approved by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.716840/full#supplementary-material

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Prevalence and Predictors of Left Ventricular Diastolic Dysfunction in Malaysian Patients With Type 2 Diabetes Mellitus Without Prior Known Cardiovascular Disease

Kok Han Chee[1], *Kok Leng Tan*[2]*, *Ibrahim Luqman*[1], *Shahrizal Shudim Saiful*[1], *Yee Yean Chew*[1], *Karuthan Chinna*[1] and *Alexander Tong Boon Tan*[1]

[1] Medical Department, University Malaya Medical Centre, Kuala Lumpur, Malaysia, [2] Regenerative Medicine Cluster, Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia, Kepala Batas, Malaysia

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***Correspondence:**
Kok Leng Tan
tankl@usm.my

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Objective: Existing data showed that left ventricular diastolic dysfunction is common in individuals with type 2 diabetes mellitus (T2DM). However, most of the studies included diabetic patients who have prior cardiovascular disease, which might be the compounding factor for ventricular dysfunction. This study aimed to determine the prevalence and predictors of left ventricular diastolic dysfunction in an Asian population with T2DM without prior cardiovascular disease using the latest recommended echocardiographic assessment for left ventricular diastolic dysfunction.

Design and Participants: This is a cross-sectional study in which eligible patients with T2DM without history of coronary artery disease, heart failure, or valvular heart disease were recruited. Demographic data, diabetic control, comorbidities, microvascular/macrovascular complications, and medications prescribed were recorded. Venous blood was sent to test for B-type natriuretic peptide, and transthoracic echocardiography was performed to assess left ventricular dysfunction.

Setting: This study was performed in a tertiary healthcare center located in Kuala Lumpur, Malaysia.

Results: Of the 301 patients, 83.1% have had T2DM for >10 years, with 45.8% being poorly controlled. Comorbidities include hypertension (77.1%), hyperlipidemia (91.0%), and pre-obesity/obesity (72.9%). Majority had absence of microvascular (albuminuria, retinopathy, and neuropathy) and macrovascular (peripheral vascular disease and stroke) complications. None had raised B-type natriuretic peptide levels, and 93.7% had no symptoms of heart failure. On echocardiographic assessment, 70.1% had left ventricular diastolic dysfunction, and 90.5% had Grade 1/mild severity. Age, ethnicity, insulin therapy, presence of hypertension, and hyperlipidemia were significantly associated with left ventricular diastolic dysfunction. Older T2DM patients of Chinese ethnicity and on insulin are about two times more likely to develop left ventricular diastolic dysfunction.

Conclusion: There was a high prevalence of asymptomatic left ventricular diastolic dysfunction among patients with T2DM without prior known cardiovascular disease. Older age, insulin therapy, and Chinese ethnicity were risk factors for left ventricular diastolic dysfunction in T2DM.

Keywords: diastolic dysfunction, diabetes mellitus, left ventricular dysfunction, prevalence, Asian

STRENGTHS AND LIMITATIONS

- This was the first study reporting the prevalence and predictors of left ventricular diastolic dysfunction (LVDD) in a multiethnic Southeast Asian type 2 diabetes mellitus cohort without prior known cardiovascular disease.
- Two-dimensional echocardiographic assessment for LVDD using the latest ASE/EACVI 2016 recommendations.
- This study did not definitively exclude coronary artery disease using coronary angiogram.

INTRODUCTION

Heart failure (HF) is an important cause of morbidity and mortality in type 2 diabetes mellitus (T2DM) (1). The Framingham Heart Study revealed that HF is more prevalent in individuals with diabetes when compared to those without, with a five-fold increase in women and a two-fold increase in men (2). The presence of T2DM is also associated with worse clinical status and higher all-cause and cardiovascular (CV) mortality in both reduced and preserved ejection fraction HF (3). Unlike left ventricular systolic dysfunction (LVSD), left ventricular diastolic dysfunction (LVDD) is often underdiagnosed in diabetes. LVDD is itself associated with poor prognosis and can progress to LVSD (4, 5) and is thought to predate the onset of LVSD in patients with diabetes.

The American Society of Echocardiography (ASE)/European Association of Cardiovascular Imaging (EACVI) 2016 (6) recommendations introduced an uncomplicated method to diagnose and grade the severity of LVDD. Echocardiographic parameters such as left atrium volume index, tricuspid regurgitation velocity, E/e′, and septal or lateral e′ velocity were used for the diagnosis of LVDD in the presence of normal left ventricular ejection fraction (LVEF), whereas the diagnosis of HF with preserved ejection fraction (HFpEF) incorporates clinical signs and symptoms of HF, presence of LVDD on echocardiography (ECHO), and rise in biomarkers [B-type natriuretic peptide (BNP)].

The Strong Heart Study demonstrated T2DM to be independently associated with asymptomatic LVDD (1). However, it is important to note that many previous studies were conducted in a CV diabetic cohort and before the widespread use of renin–angiotensin system (RAS) blockers and statins or the advent of glucose-lowering drugs such as sodium-glucose co-transporter-2 inhibitors (SGLT2-i) or glucagon-like peptide-1 receptor analog (GLP1-RA) that modify risk of ischemic heart disease (IHD) and HF. Therefore, the prevalence and predictors of LVDD in populations using the latest guideline-directed medical therapy may differ.

Most data on HF in individuals with T2DM have been derived from Caucasian populations with limited studies in Asian patients. There may be differences in the clinical features, prevalence, and predictors of HF in Asian ethnicities. It is well-known that migrant South Asians are at higher risk of coronary artery disease compared with European Caucasians living in the same environment (7). Therefore, it is possible that Asian ethnicities may have differences in risk of HF as well. The few published studies evaluating Asian ethnic groups involve migrant populations living in the West who are potentially exposed to diets, degrees of physical activity, and socioeconomic factors that differ from those in their countries of origin. Also, these analyses sometimes failed to view Asians as a heterogeneous group and analyzed different Asian ethnic subgroups as a single entity. Emerging evidence shows that Singapore Asians (63% Chinese, 26% Malay, and 11% Indian) with HF, when compared to New Zealand Caucasians, are at lower risk of atrial fibrillation, especially if they have T2DM (8). Comparing data from population-based HF cohorts, Bank et al. (9) found that Southeast Asians with HF have a three-fold higher prevalence of diabetes compared with European Caucasians despite being younger and less obese. Asian patients with T2DM with HF also had poorer outcomes such as increased all-cause mortality compared to their Caucasian counterparts (9). While these findings are of great interest, that study analyzed three Asian ethnicities (Chinese, Malay, and Indian) as a single "Asian entity."

Early detection of LVDD can lead to the institution of preventive measures to halt disease progression. There is a need to delineate the prevalence and predictors of diastolic dysfunction to enable systematically targeted intervention aimed at reducing morbidity and mortality in patients with T2DM. We designed this study to determine the prevalence of LVDD in the current landscape of T2DM management, where RAS blocker and statin use is more widespread. LVDD was diagnosed using echocardiographic parameters based on the latest ASE/EACVI 2016 recommendations in a multiethnic population of Malaysians with T2DM without known HF, coronary artery disease, or valvular heart disease. We also evaluated the strength of association of clinical predictors, in particular glycemic control, and ethnicity with the presence of LVDD.

MATERIALS AND METHODS

Study Design and Participants

This was a cross-sectional study conducted at the University Malaya Medical Center (UMMC), a tertiary healthcare center located in the city of Kuala Lumpur, Malaysia. The prevalence

of LVDD was estimated to be 50% in a diabetic population (10). A sample size of 271 was required with 5% absolute precision and 90% confidence for single-proportion estimation. The study was conducted between January and December 2018. The study protocol was approved by the institutional ethical review board (UMREC ID NO 20171126-5850) and fully conformed to the principles of the Helsinki Declaration.

All patients with T2DM who visited the diabetic outpatient clinic during the study period were assessed for eligibility in participating in this study. Written informed consent was obtained from all participants. Patients with known history of coronary artery disease, HF, valvular heart disease, and end-stage renal failure requiring dialysis were excluded. Ineligibility also extended to those with malignancy, acute infection, or inflammatory disease.

Demographic data included age, gender, ethnicity, weight, and height, and the presence of comorbidities such as hypertension and hyperlipidemia was recorded. Body mass index (BMI) categorization was done based on the World Health Organization Expert Consultation on Asian BMI report. The evaluation of diabetes and its complications included the duration of T2DM, HbA_{1c} status, renal function [presence of albuminuria and estimated glomerular filtration rate (eGFR)], and presence of other macrovascular and microvascular complications (stroke, peripheral vascular disease, retinopathy, and neuropathy). Current prescribed medications for all patients were recorded.

Evaluation of Left Ventricular Dysfunction

Evaluation of HF in all patients was done by taking a detailed history and performing clinical examination, ECHO, and venous blood sampling for BNP level.

The history of HF symptoms as defined by the New York Heart Association (NYHA) Functional Classification (11) was elicited from all patients. Symptoms elucidated include level of breathlessness, fatigue, and palpitation related to activity; presence of orthopnea; and occurrence of paroxysmal nocturnal dyspnea (PND). Clinical examination of the CV system including presence of pedal edema, raised jugular venous pressure (JVP), and the respiratory system for signs of HF.

Transthoracic ECHO was performed by a senior sonographer who was blinded to the study outcome. Echocardiographic parameters such as left atrium volume index, tricuspid regurgitation velocity, E/e′, and septal or lateral e′ velocity were recorded. LVDD was classified and graded according to the latest recommendations of the ASE/EACVI 2016. LVSD was defined as an ejection fraction of <50%. Those with structural abnormalities (previously undiagnosed) were excluded from the study.

The evaluation of BNP levels was done by analyzing plasma BNP using Siemens ADVIA® Centaur BNP assay, which is an automated two-site sandwich chemiluminescent immunoassay.

Statistical Method

Data were analyzed using SPSS version 22. Descriptive statistics were used for analysis of demographic characteristics. Continuous variables were expressed as mean ± SD. The differences between normally distributed numeric variables were evaluated by *t*-test or one-way analysis of variance, while non-normally distributed variables were analyzed by Mann–Whitney *U*-test or Kruskal–Wallis variance analysis, as appropriate. Multivariable logistic regression analysis was done to identify the association of independent variables.

TABLE 1 | Demographic characteristics.

Variable	*n* (%)
Age	
Mean (min, max)	61 (26, 86)
Gender	
Male	106 (35.22)
Female	195 (64.78)
Ethnicity	
Malay	85 (28.24)
Chinese	87 (28.90)
Indian	123 (40.86)
Others	6 (2.00)
Obesity	
Underweight	7 (2.34)
Normal	34 (11.37)
Overweight	40 (13.38)
Pre-obese	122 (40.80)
Obese	96 (32.11)
Comorbidities	
Hypertension	
Present	232 (77.08)
Absent	69 (22.92)
Hyperlipidemia	
Present	274 (91.03)
Absent	26 (8.64)
No data	1 (0.33)

Total number of patients evaluated were n = 301 except for obesity, n = 299. Obesity was categorized based on the World Health Organization Expert Consultation on Asian Body Mass Index (12) (kg/m^2): underweight, <18.5; normal, 18.5–22.9; overweight, 23.0–24.9; pre-obese, 25.0–29.9; and obese, ≥30.0.

Patient and Public Involvement

There was no patient and public involvement in the design, conduct, reporting, or dissemination plans of our research.

RESULTS

Patient Population

Three hundred and fifty patients with T2DM were screened during the study duration, and 315 were included in the study. However, 14 patients were excluded from the final analysis, as they did not proceed with blood investigations. The remaining 301 patients had blood investigations and ECHO assessment for LVD. The demographic characteristics are listed in **Table 1**.

Evaluation of Diabetic Status

The diabetic status of the patients is listed in **Table 2**. Most patients (83.1%) had T2DM for >10 years. The mean HbA_{1c} was

TABLE 2 | Evaluation of diabetic status including diabetic complications.

Variable	*n* (%)
Duration of diabetes (years)	
<5	21 (6.98)
5–10	30 (9.97)
>10	250 (83.05)
HbA_{1c} (%)	
Mean (min, max)	8.3 (5.4, 15.5)
<7	61 (20.27)
7–8	102 (33.89)
>8	138 (45.84)
Renal function	
Albuminuria	
Present	86 (28.57)
Absent	215 (71.43)
eGFR (ml/min/1.73 m^2)	
Mean (min, max)	78 (24, 110)
Normal (≥90)	156 (51.83)
Mild (60–89)	100 (33.22)
Moderate (30–59)	44 (14.62)
Severe (<30)	1 (0.33)
Retinopathy	
Present	67 (22.26)
Absent	234 (77.74)
Neuropathy	
Present	58 (19.27)
Absent	243 (80.73)
Peripheral vascular disease	
Present	6 (2.01)
Absent	293 (97.99)
Stroke	
Present	16 (5.32)
Absent	285 (94.68)

Total number of patients evaluated were n = 301 except for peripheral vascular disease, n = 299; eGFR, estimated glomerular filtration rate.

8.3% (±1.7%) with almost half (45.8%) being poorly controlled (HbA_{1c} > 8.0%). Majority had absence of microvascular (albuminuria, retinopathy, and neuropathy) and macrovascular (peripheral vascular disease, and stroke) complications.

Assessment of LVD

Of 301 patients, 19 (6.3%) presented with symptoms of HF (**Table 3**). Most patients were classified into New York Heart Association (NYHA) Class 1. Only 3.7% of patients had dyspnea on exertion while 0.3% complained of orthopnea. None had dyspnea at rest or PND. On clinical examination, only nine (3.0%) had pedal edema, and none had raised JVP.

On ECHO assessment, 211 patients (70.1%) had LVDD according to the ASE/EACVI 2016 recommendations. LVDD severity was mostly Grade 1 (90.5%). None of the patients exhibited LVSD (results not shown). The mean LVEF using the modified Simpson method was 68%.

TABLE 3 | Assessment for LVD.

Variable	*n* (%)
Symptoms of HF	
Present	19 (6.33)
Absent	281 (93.67)
LVDD	
Present	211 (70.10)
Absent	90 (29.90)
Severity of diastolic dysfunction	
Grade 1	191 (90.52)
Grade 2	18 (8.53)
Grade 3	2 (0.95)
LVEF (%)	
Mean (min, max)	68 (50, 78)
BNP (pg/ml)	
Mean (min, max)	25.81 (2, 206)

n = 301 except for severity of diastolic dysfunction, n = 211; LVEF, left ventricular ejection fraction; BNP, B-type natriuretic peptide.

Mean BNP was 25.81 pg/ml (±27.87) (range 2–206 pg/ml), and none of the patients had abnormal BNP level (results not shown).

Medicines Prescribed

Metformin was the most prescribed glucose-lowering drug followed by insulin (**Table 4**). A similar proportion of patients was prescribed with SGLT2-i and dipeptidyl peptidase 4 inhibitors (DPP4-i), while the alpha-glucosidase inhibitor was the least used. Angiotensin-converting enzyme inhibitors (ACE-i) and angiotensin II receptor blockers (ARB) for hypertension were prescribed to 75.4% of the patients, while hyperlipidemia was treated mainly with statins (90.4%).

Presence of LVDD Based on Clinical and Laboratory Characteristics

The mean age of patients with LVDD was 63 years (±9), older than patients without LVDD (**Table 5**). Indian (37.9%) and Chinese (34.1%) patients were slightly more affected. The proportion of patients with hypertension and LVDD was significantly higher ($p = 0.006$). Similarly, the number of patients with hyperlipidemia and LVDD was significantly higher than those without LVDD (93.84 vs. 86.67%; $p = 0.039$). Significantly more patients on insulin had LVDD (78.70 vs. 16.67%; $p = 0.044$).

Risk Association for LVDD

Age, ethnicity, insulin therapy, and presence of hypertension and hyperlipidemia were significantly associated with LVDD in our study. On univariate analysis, every unit of increase in age increased the risk of LVDD by 1.05 [95% confidence interval (CI) 1.03, 1.08; $p < 0.001$]. It also showed that hypertension increased the risk of LVDD by 2.21 (95% CI 1.26, 3.86; $p = 0.006$), but the duration and number of hypertensive medications did not. Besides, use of insulin increased the risk of LVDD by 2.41 (95% CI 1.27, 4.56; $p = 0.007$). On multivariate analysis, however, the

TABLE 4 | Medications prescribed.

Drugs	Yes, *n* (%)	No, *n* (%)
Glucose-Lowering agent		
Metformin	276 (91.69)	25 (8.31)
Sulfonylurea	93 (30.90)	208 (69.10)
Insulin	181 (60.13)	120 (39.87)
SGLT2-i	126 (41.86)	175 (58.14)
DPP4-i	121 (40.20)	180 (59.80)
GLP1-RA	25 (8.31)	276 (91.69)
Alpha-glucosidase inhibitor	6 (1.99)	295 (98.01)
Anti-hypertensives		
ACE-i/ARB	227 (75.42)	74 (24.58)
Calcium channel blockers	137 (45.51)	164 (54.49)
Diuretics	53 (17.61)	248 (82.39)
β-Blockers	42 (13.95)	259 (86.05)
α-Blockers	15 (4.98)	286 (95.02)
Others	2 (0.66)	
Lipid-Lowering agent		
Statins	272 (90.37)	29 (9.63)
Fibrates	38 (12.62)	263 (87.38)
Ezetimibe	3 (1.00)	298 (99.00)
Anti-platelets	117 (38.87)	184 (61.13)
Aspirin	99 (32.89)	202 (67.11)
Clopidogrel	9 (2.99)	292 (97.01)
Ticlopidine	9 (2.99)	292 (97.01)
Anti-coagulants		
Novel oral anti-coagulants	1 (0.33)	300 (99.67)
Warfarin	0	301 (100)

n = 301; SGLT2-i, sodium-glucose co-transporter 2 inhibitors; DPP4-i, dipeptidyl peptidase 4 inhibitors; GLP1-RA, glucagon-like peptide-1 receptor analog; ACE-i/ARB, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers.

TABLE 5 | Presence of LVDD based on clinical and laboratory characteristics.

Parameters	With LVDD, *n* = 211 (%)	Without LVDD, *n* = 90 (%)	*p*-value
Age, years ± SD	63 ± 9	57 ± 11	<0.001
Ethnicity			0.018
Malay	56 (26.54)	29 (32.22)	
Chinese	72 (34.12)	15 (16.66)	
Indian	80 (37.91)	43 (47.77)	
Others	3 (1.42)	3 (3.33)	
Gender			0.731
Male	73 (34.59)	33 (36.66)	
Female	138 (65.40)	57 (63.33)	
BMI, kg/m^2 ± SD	27.37 ± 4.59	27.69 ± 5.35	0.596
Normal	68 (32.22)	21 (23.33)	0.302
Overweight	91 (43.12)	44 (48.88)	
Pre-obese and obese	52 (24.64)	25 (27.77)	
Smoking	17 (8.06)	6 (6.66)	0.914
Hypertension	172 (81.52)	60 (66.66)	0.006
Duration of hypertension			0.604
<5 years	5 (2.37)	1 (1.11)	
5–10 years	23 (10.90)	5 (5.56)	
>10 years	135 (63.98)	47 (52.22)	
Duration of T2DM			0.315
<5 years	12 (5.69)	9 (10.00)	
5–10 years	23 (10.90)	7 (7.78)	
>10 years	176 (83.41)	74 (82.22)	
Hyperlipidemia	198 (93.84)	78 (86.67)	0.039
Stroke	10 (4.74)	6 (6.67)	0.500
PVD	4 (1.90)	2 (2.22)	
Retinopathy	49 (23.22)	18 (20.00)	0.538
Neuropathy	41 (19.43)	17 (18.89)	0.913
Albuminuria	85 (40.28)	35 (38.89)	0.821
Microalbuminuria	53 (25.12)	19 (21.11)	
Macroalbuminuria	32 (15.17)	16 (17.78)	
Insulin therapy	166 (78.70)	15 (16.67)	0.044

LVDD, left ventricular diastolic dysfunction; SD, standard deviation; BMI, body mass index; T2DM, type 2 diabetes mellitus; PVD; peripheral vascular disease.

presence of hypertension and hyperlipidemia lost its significance. Older T2DM patients of Chinese ethnicity and on insulin are about two times more likely to develop LVDD.

DISCUSSION

We found that none of our multiethnic high-risk Asian cohort with long-standing T2DM had evidence of LVSD on echocardiography. However, more than two-thirds of the patients had evidence of LVDD according to the ASE/EACVI 2016 recommendations (6), mostly at Grade 1/mild severity. Such low prevalence of LVSD, asymptomatic of HF cohort, is unexpected given that 86.3% of our patients were overweight/pre-obese/obese, 83.1% had been diagnosed with T2DM for more than a decade (60.1% insulin requiring), 77.1% had hypertension, and 91.0% dyslipidemia.

The remarkable absence of LVSD or higher grades of LVDD may be attributed to many of our patients being treated with statins (90.3%) and RAS blockers (75.4%), with as many as 41.9% receiving SGLT2-i. All these agents are known to modulate CV disease and HF. Our exclusion of patients with known heart disease, including coronary artery disease, which is the most important cause of HF in T2DM may account for such low prevalence. The widespread use of statins and RAS blockers may also explain the relatively low prevalence of retinopathy (22.3%) and albuminuria (28.6%) in our sample population.

We managed our patients at a government-funded tertiary healthcare center providing affordable care; it is uncertain whether our results could be extrapolated to primary and secondary healthcare settings. Nevertheless, compliance with international guidelines on standards of care and prescription of RAS blockers, statins, and SGLT2-i (13) can prevent or delay the progression of cardiac disease.

The prevalence of HF in patients with T2DM is reported to be 12–57% (14) while reports on the prevalence of LVDD in these patients vary from 23 to 75% (15). This variability accounted

for the differences in diagnostic methods and patient cohorts (demographics, inclusion/exclusion criteria, and medications prescribed). A recent meta-analysis published in 2018 found the prevalence of LVDD to be 35.0% in the general T2DM population (16). There are only a handful of small studies (patient population <150) reporting a prevalence of LVD ranging 54.3–65.0% in Asian populations (17–19). Our observation that 70.1% of Malaysian patients with established T2DM on treatment have asymptomatic, mainly Grade 1/mild LVDD is not dissimilar to these reports. One study (18) reported that 92.2% of its LVDD patients had mild dysfunction. On the other hand, a study by Chaudhary et al. (20) on normotensive newly diagnosed T2DM patients before treatment initiation found an alarming 41.0% prevalence of LVDD, majority (87.8%) with Grade 1 dysfunction. Like these Asian studies, we found no patients with undiagnosed HF.

Some Western studies also found LVDD to be more prevalent than LVSD in patients without known coronary artery disease. In a cohort of 386 Italian patients (SHORTWAVE study) (1), 42% had confirmed LVDD (the majority with Grade 1 dysfunction) while 3.6% had EF < 50%. The mean age in this population was >60 years, with T2DM of short duration (mean of 4–5 years). Seventy-two percent were on RAS blockers and 45% on statins. The low prevalence of LVSD and LVDD in this study can be explained by the exclusion of inducible ischemia by stress echocardiography, short mean duration of T2DM, and widespread use of RAS blockers. However, in a cohort of Danish T2DM patients without known coronary artery disease or overt heart disease (21), the prevalence of Grade 2 LVDD was slightly higher (18%) and total LVDD lower (40%) than our findings. These patients had a mean age like ours but a shorter mean duration of T2DM (4.5 years) that may account for its lower prevalence compared to our cohort. Only 9% of the Danish cohort had LVSD. There were also no details of therapy provided. In a French cohort of T2DM patients (15) (mean duration of 11 years), which excluded patients with EF < 55% and coronary artery disease (diagnosed by stress testing/myocardial perfusion studies within 1 month of enrolment), the prevalence of LVDD was 47% (33% Grade 1 and 14% Grade 2). The lower prevalence of LVDD compared to our cohort can be explained by younger patients and the exclusion of those with coronary artery disease using functional and imaging modalities. However, it is surprising that despite the long duration of T2DM and lack of RAS blockers use, there was a relatively low prevalence of LVDD.

Population characteristics and healthcare system practices can determine prevalence of HF and LVDD. In a cross-sectional study (22) of 581 Dutch patients with T2DM without known HF, 27.7% were diagnosed as having HF during the study duration (22.9% with HFpEF and 4.8% with HFrEF). One in four had asymptomatic LVDD and 0.7% asymptomatic LVSD. The alarmingly high rates of HFpEF in this population may be accounted for by an older cohort (as age is associated with LVDD), the low usage of RAS blockers, and the inclusion of patients with IHD. These patients (unlike ours) were recruited from a primary care setting, which may have influenced medication use; e.g., only 52.7% were on RAS blockers although 65.6% had hypertension.

Our patients were not known to have and were asymptomatic of CV disease. Absence of regional wall motion abnormalities in all study patients suggested they did not have coronary artery disease; however, we did not perform coronary angiogram to ascertain it. This coupled with tertiary healthcare where almost three-fourths of our patients were on RAS blockers, >90% on statins, and 41.9% on SGLT2-i may account for the low prevalence of HF, LVSD, and asymptomatic high-grade LVDD. Unlike our work, none of the forementioned studies, Asian or Western, were carried out in the current T2DM management landscape where SGLT2-i use is widespread.

Reported associations of LVDD in patients with T2DM are age, female gender, duration of T2DM, HbA_{1c} status, obesity, higher systolic blood pressure, and presence of albuminuria, CKD ≥ Stage 3, and retinopathy (1, 15, 17, 19, 21, 23–26). Upon univariate analysis, we found that age, hypertension, hyperlipidemia, insulin therapy, and ethnicity were correlated with presence of asymptomatic LVDD. However, upon multiple logistic regression, only older age, insulin therapy, and Chinese ethnicity were confirmed to be independently, positively associated. Duration of T2DM was not an independent predictor of LVDD. This may be due to skewing of our tertiary healthcare center population toward those with long-standing T2DM, with >80% having been diagnosed for >10 years. On the other hand, insulin therapy was independently correlated with presence of LVDD. T2DM has a long preclinical asymptomatic stage; hence, the point of diagnosis does not truly reflect the true onset of disease. The natural history of T2DM is characterized by progressive loss of β-cell function over the years, leading to secondary sulfonylurea failure and the eventual need for insulin therapy (27, 28). Therefore, the need for insulin therapy in T2DM may be a more accurate surrogate marker of long-duration T2DM. Our findings are similar to those of Nichols et al. who also found insulin therapy to be independently associated with HF in patients with T2DM (29).

We found patients of Chinese ethnicity to be at higher risk of LVDD. Subgroup analysis of these patients did not find any difference in glycemic control, hypertension, retinopathy, albuminuria, renal function, and SGLT2-i or antihypertensive therapy. These patients however were significantly older, with a lower BMI and a lower proportion of insulin use. Indians, who originate from South Asia, are known to have higher risk for atherosclerotic CV disease, while East Asians have a lower risk (7). Our study excluded patients with known CV disease, which probably led to Indian ethnicity being not a risk for LVDD. East Asians may be at higher risk of LVDD potentially due to unelucidated genetic factors. Our findings need to be validated and further evaluated in larger sample populations that include Chinese and other East Asian ethnic groups.

Similar to the SHORTWAVE study, we did not find glycemic control to be independently associated with asymptomatic LVDD (1). This is in contrast with findings from previous studies which found a positive correlation between HbA_{1c} and echocardiographic parameters of LVDD (19) and incident HF (30, 31). Iribarren et al. observed that a 1% increase in HbA_{1c} was associated with an 8% increased risk of HF in a large cohort of 50,000 patients with diabetes (30). When interpreting our results

in the light of these past studies, we should consider that the positive associations between HbA_{1c} and incident symptomatic HF may not be applicable in our patients with asymptomatic LVDD. These studies were also conducted in populations that were managed prior the usage of RAS blockers and statins as standard of care. The analysis by Iribarren et al. was conducted in a cohort where 11% of those with HF had coronary artery disease at diagnosis (30). Also, the evaluated cohort had only 15 and 26% of its population treated with insulin and ACE-I, respectively, and 23% with $HbA_{1c} < 7\%$, while 71% did not have low-density lipoprotein cholesterol (LDL-C) levels reported. Therefore, a comparison with our cohort of patients who did not have coronary artery disease and were managed with a vastly different standard of care protocol cannot be equivalent. In another prospective population-based study (31), a cohort of 1,827 participants of the Atherosclerosis Risk in Communities study found similarly strong association between baseline HbA_{1c} and incident HF after a mean of 9.9 years' follow-up. No specific data on ACE-i and statin use were reported in this paper, and risk of HF was not adjusted for use of these medications. Neither BMI nor obesity was associated with presence of LVDD on univariate or multivariate analysis in our sample population, which is similar to the findings from the SHOCKWAVE cohort (1).

Asymptomatic LVDD in patients with T2DM is likely due to microangiopathy, interstitial fibrosis, extracellular collagen deposition, calcium transport abnormalities, and neurohormonal alterations and is the earliest manifestation of diabetic cardiomyopathy (2, 15). Presence of asymptomatic LVDD has been associated with higher risk of progression to atrial fibrillation, HF, and all-cause mortality in patients with T2DM (2, 23, 24, 32). Although presence of Grade 1 LVDD in the majority of our cohort may not seem very alarming, evidence from prospective cohort studies in Western populations (2, 33) indicates that even patients with Grade 1 LVDD can deteriorate with time, with the main predictors being aging, retinopathy, and increase in blood pressure. In patients with T2DM and asymptomatic LVDD, robust analyses have shown that adjusted risk of progression to HF is increased by 61% compared with those without LVDD, with a 5-year cumulative probability of developing HF of 36.9% (2).

Given that the presence of LVDD and its potential for progression have important prognostic implications, our findings of high prevalence of mild LVDD indicate the need for strategies to screen for and monitor progression of LVDD with regular ECHO, clinical examination, and appropriate preventive measures to control modifiable risk factors such as hypertension, hyperlipidemia, retinopathy, progressive nephropathy, and improvement of glycemic control.

CONCLUSION

This cross-sectional study demonstrated a high prevalence (70.1%) of asymptomatic LVDD in a high-risk cohort of patients with T2DM treated with RAS blockers, statins, and SGLT2-I, without prior known CV disease. Most of the LVDD patients were at Grade 1/mild severity. Older age, insulin therapy, and Chinese ethnicity were risk factors for diastolic dysfunction. Such findings emphasize the need for regular screening and monitoring for progression of cardiac dysfunction as well as appropriate therapeutic risk mitigation measures given the long-term prognostic implications of LVDD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Malaya Research Ethics Committee (UMREC). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KHC, ATBT, and KLT conceived the original study concept and contributed to the study design. KLT, IL, SSS, YYC, and KC participated in data collection and analysis. KHC and KLT drafted the manuscript with input from all authors. All authors met the International Committee of Medical Journal Editors criteria for authorship and have read and approved the final manuscript.

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Correlation Analysis of Anti-Cardiolipin Antibody/D Dimer/C-Reactive Protein and Coronary Artery Lesions/Multiple-Organ Damage in Children With Kawasaki Disease

*Yun-ming Xu, Yan-qiu Chu and Hong Wang**

Pediatric Department of Shengjing Hospital, China Medical University, Shenyang, China

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Cristina Vassalle, Gabriele Monasterio Tuscany Foundation (CNR), Italy

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Maria Cristina Maggio, University of Palermo, Italy
Rakesh Kumar Pilania, Post Graduate Institute of Medical Education and Research (PGIMER), India

***Correspondence:**
Hong Wang
wanghong_64@sina.com

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Aim: Kawasaki disease (KD) is a systemic vasculitis with unknown etiology. In addition to cardiovascular system involvement, it can also have other multiple organs involved. This study is aimed at investigating the correlation between anti-cardiolipin antibody (ACA)/D dimer/C reactive protein (CRP) and coronary artery lesions (CAL)/multiple-organ lesions in children with KD.

Methods: Retrospective analysis was performed in 284 KD/IKD patients from May 2015 to April 2016. Among them, 175 were males (61.6%), with average age of 2 years and 5 months old. Patients were divided into ACA+ group and ACA- group, elevated D dimer group (DDE) and normal D dimer group (DDN), and coronary artery injury (CAL) group and non-coronary artery injury (NCAL) group.

Results: ACA was most likely tested positive in younger KD children ($p < 0.05$). ACA+ and hypoproteinemia were correlated with CAL, thrombocytosis, and granulocytopenia ($p < 0.05$–0.01). Levels of cTnI and CK in the CAL group were significantly higher than those in the NCAL group ($p < 0.05$). CAL was more frequently detected in younger patients and patients with prolonged fever, later IVIG treatment, and elevated CRP over 100 mg/l, but there was no statistically significant difference (all $p > 0.05$). In the KD with DDE group, the incidence of granulopenia, thrombocytosis, myocardial damage, cholestasis, hypoproteinemia, and aseptic urethritis was significantly higher than that in the KD with DDN group ($p < 0.05$–0.01). However, elevated D dimer was not associated with CAL. CRP elevation was highly correlated with D dimer, but not with CAL.

Conclusion: Higher incidence of CAL and myocardial damage occurred in KD patients with positive ACA and hypoproteinemia. In the current study, ACA was only tested for positive and negative, which is a limitation to this study. To further elucidate the association, ACA titers would establish its significance in drawing a conclusion for the

significance of ACA in CAL and myocardial damages. In addition, higher incidence of CAL occurred in younger patients. The higher D dimer was associated with increased multiple-organ damage (MOD). CRP was closely correlated with D dimer, but not correlated with ACA and CAL.

Keywords: anticardiolipin antibody (ACA), D dimer, C reactive protein (CRP), coronary artery lesions (CALs), multiple organ damage, Kawasaki disease (KD), children

INTRODUCTION

Kawasaki disease (KD) is a systemic vasculitis with unknown etiology. It is one of the common connective tissue diseases in children. Currently, KD has emerged as a major pediatric disorder throughout the developed world (1). In developing countries, KD is currently being diagnosed and reported in both China and India (2–4). Multiple organs/systems can be involved in KD. Coronary artery aneurysm (CAA) is the most significant complication that affects the quality of life in the long term. Even in patients receiving IVIG treatment within 10 days of disease onset, the incidence of CAL is still about 5% (5). Prediction of the occurrence of CAL and MOD by utilizing laboratory examination has become a priority for pediatric cardiologists.

In 2014, four KD children with fever but without identified underlying pathogenesis were hospitalized at the department of rheumatism. Results of the ANA\ANCA\ANA titer test, cytokine test (interferon + interleukin), and anticardiolipin antibody (ACA) test indicated that all the four patients had elevated interleukin and two of them were tested positive for ACA and negative for other tests (6, 7). Meanwhile, two KD patients with KD shock syndrome (KDSS) and macrophage activation syndrome (MAS) were transferred to a pediatric intensive care unit (PICU) where the D dimer test was performed (8). Both had significantly elevated D dimer. These clinical aspects prompted us to review literature on ACA and D dimer. As reported in literature, ACA is positive in infection (9), myocardial infarction (10), infectious endocarditis (11), recurrent abortion (12), KD complications (13), systemic lupus erythematosus (14), and stroke (15). Elevated D dimer is associated with vascular endothelial damage (16), and it has been a supporting tool in early diagnosis of KD (17). Furthermore, it is correlated with CAL (18) and so on. We hypothesized that, since KD in children is systemic vasculitis with a high risk in blood clot, ACA+ and D dimer may be associated with disease progression and be used as indications in KD-related complications. Therefore, we added the ACA/D dimer test to the newly admitted children with KD/IKD.

METHODS

Patients

A total of 284 IKD and KD cases were collected from the pediatric cardiovascular ward of Shengjing Hospital, China Medical University, from May 2015 to April 2016. Among them, there were 175 males (61.62%). The average age of patients was 2 years and 5 months (2 months to 11 years) old. All data were retrospectively analyzed.

Multiple-Organ Injuries

When two or more than two organs are impacted in the same child with KD around the acute and/or subacute stages.

Inclusion Criteria

Patients met diagnostic criteria of KD and IKD (19). The diagnostic criteria for CAL were based on the guidelines of the Japanese Circulator Association (20).

Exclusion Criteria

(1) Incomplete clinical data records; (2) Without ACA and D-dimer tests; and (3) Without IVIG treatment.

Data Collection

All children diagnosed with KD/IKD had routine tests done at admission: blood routine, liver function, CK, CKMB, hs-cTnT, cTnI, NT pro-BNP, and urine routine. The ACA-IgG antibody (ACA antibody tests were done in 2015) was measured before IVIG in 2 ml of blood using enzyme-linked immunoadsorption assay (ELISA), following the manufacturer's instruction (Beijing Beier Company), and the test is qualitative (the cutoff is 0.1+ negative control OD value, ACA IgG positive $\geq$ 0.1+ negative control OD value). D dimer was measured in blood with EDTA anticoagulant using immunoturbidimetry for the routine disseminated intravascular coagulation (DIC) test following instructions (Instrumentation Laboratory Company), and the normal range is $<$252 μg/l. CRP was measured in blood using the radical immunodiffusion method following the manufacturer's instructions (Beckman Coulter, Inc.). Albumin was tested following the manufacturer's instruction (Abbott's Diagnosis, Inc.), using the bromocresol green (BCG) method. CRP/D dimer and albumin were measured several times within 2 weeks of onset. The highest value of CRP/D dimer and the lowest value of albumin were included in the table for statistical analysis.

Groups

(1) Based on ACA-IgG results, 284 KD/IKD patients were divided into ACA-positive (ACA+) and ACA-negative (ACA–) groups. (2) Based on D dimer results, 280 KD/IKD (4 children without DIC analysis were excluded) were divided into D dimer-elevated (DDE) and D dimer normal (DDN) groups. (3) According to ECHO results, 284 KD/IKD were divided into coronary artery injury (CAL) and no-coronary artery injury (NO-CAL) groups.

Statistical Analysis

SPSS 22.0 statistical software was used for statistical analysis. The *t* test was used for data in normal distribution. Statistical differences were measured by t test. Median (M) or quaternary

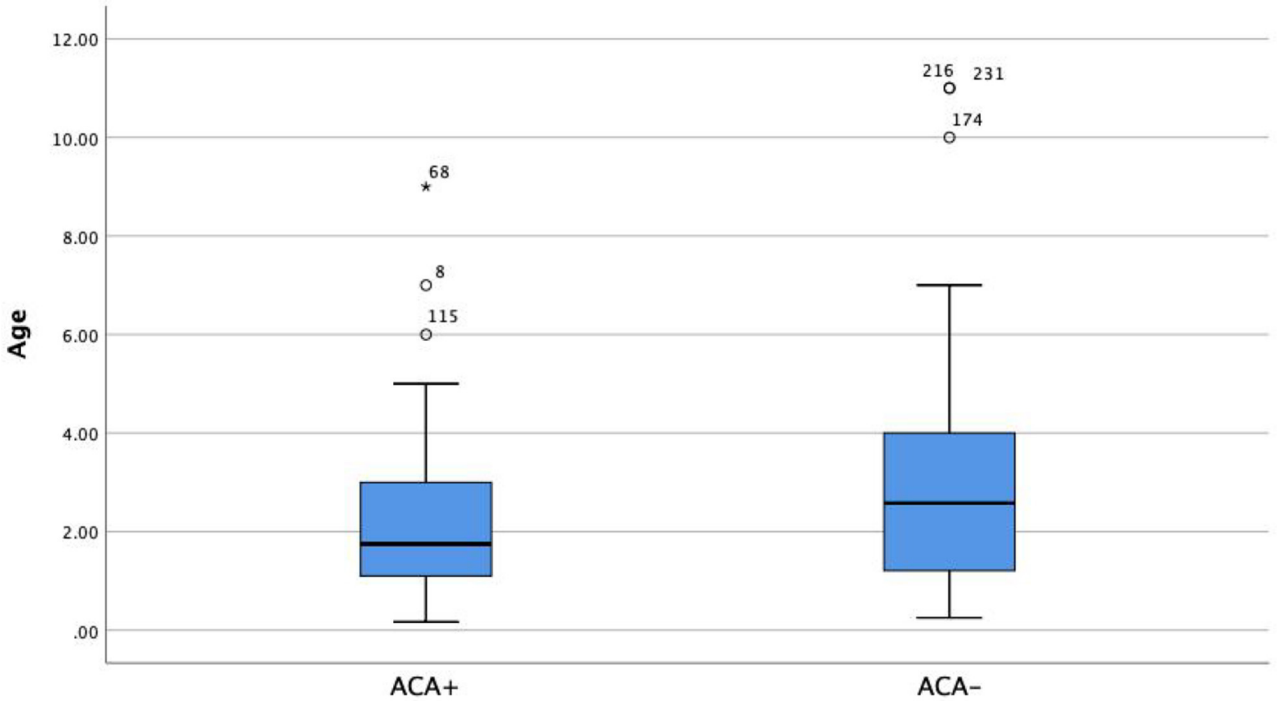

FIGURE 1 | Age difference for ACA+ group vs. ACA– group.

TABLE 1 | General information.

Groups	*n*	*M* (%)	Age (Y)	Fever (d)	First IVIG (d)	IVIG -R (%)	CRP ≥ 100 mg/L (%)	CRP ≥ 80 mg/L (%)	CRP ≥ 50 mg/L (%)	CRP ≥ 30 mg/L (%)
ACA+	168	105(62.5)	2.1 (1.0, 2.9)	8.1	8.0	10	38 (22.6)	52 (31.0)	95 (56.5)	124 (73.8)
ACA–	116	70 (60.3)	2.8 (1.1, 4.0)	8.0	8.0	12	33 (28.4)	48 (41.4)	70 (60.3)	85 (73.3)
P		0.714	0.002	0.763	0.581	0.173	0.265	0.071	0.524	0.920
DDE	192	113 (58.9)	1.8 (1.2, 3.0)	8 (7, 9)	7 (6, 8)	14 (7.3)	57 (29.7)	82 (42.7)	135 (70.3)	165 (85.9)
DDN	88	59 (67.0)	2.4 (1.2, 4.0)	8 (6, 9)	8 (6, 10)	7 (8.0)	14 (15.9)	18 (20.5)	30 (34.1)	44 (50.0)
P		0.191	0.222	0.274	0.002	0.845	0.014	0.000	0.000	0.000
CAL	17	11 (64.7)	2.1 ± 1.3	10.2 ± 5.2	9.2 ± 4.4	3 (17.6)	7 (41.18)	7 (41.18)	10 (58.82)	14 (82.35)
NO-CAL	267	164 (61.4)	2.4 ± 1.8	8.0 ± 2.3	7.7 ± 2.0	19 (7.1)	66 (24.72)	93 (25.34)	154 (57.68)	190 (51.77)
P		0.787	0.433	0.114	0.186	0.134	0.153	0.595	0.926	0.413

DDE, D-dimer elevated; DDN, D-dimer normal; IVIG-R, IVIG resistance; CRP, C reactive protein.

interval (p25–p75) was used for data in non-normal distribution. Enumeration data were shown as rate (%), and the chi-square test was used for comparison. The ROC curve was drawn to analyze the predictive value of relevant laboratory indicators on coronary artery injury in KD. $P < 0.05$ indicates statistically significant difference.

RESULTS

General Information

Patients in the ACA+ group were significantly younger (2.1 years old) than those in the ACA- group (2.8 years old) (**Figure 1**, $Z = -2.516$, $p = 0.002$).

The average time of the first IVIG treatment in the DDE group was 1 day earlier than that in the DDN group (both groups had fever≥ 5 days) ($p < 0.05$). CRP was highly associated with D dimer despite the cutoff value of CRP (all $p < 0.001$), There was no significant correlation between D dimer and ACA (**Table 1**).

The Correlation Between ACA/D-Dimer and Myocardial/Liver Damage in KD/IKD Children

There were no significant differences in myocardial and liver damages between CAL and NO-CAL groups ($p > 0.05$).

TABLE 2 | The correlation between ACA/D-dimer and myocardial/liver damage in KD/IKD children.

Groups	*n*	CKMB (>24U/L)	CTNI (>0.04 μg/L)	Hs-CTNT (>0.014 ng/mL)	NT-pro BNP (>300 pg/mL)	TBA (> 10 umol/L)	ALT (>40 U/L)	AST (>35 U/L)	ALB (<30 g/L)	AAR
ACA+	168	20 (17, 27)	0.005 (0, 0.011)	0.005 (0, 0.007)	358 (169,1049)	5.7 (2.9,9.9)	20 (11.7,43.5)	25 (20,37)	33.5 (29.9,36.3)	1.42 (0.68,1.91)
ACA–	116	20 (17, 25)	0.009 (0, 0.003)	0.005 (0,0.007)	500 (150,1378)	6.3 (3.8,12.1)	17.5 (11,65)	24 (17,34.8)	33 (29.5,37.2)	1.41 (0.77,2.01)
P		0.556	0.137	0.291	0.152	0.698	0.815	0.061	0.771	0.961
DDE	192	(190) 20 (17, 24)	(187) 0.010 (0.010, 0.012)	(188) 0.005 (0.003, 0.007)	(188) 543.6 (228.5, 1377)	(169) 6.4 (3.4,11.3)	20 (11, 66.5)	25.5 (19, 35.5)	32.3 (29.3, 35.7)	1.24 (0.58, 1.91)
DDN	88	(87) 22 (17.5, 28)	(86) 0.010 (0.010, 0.010)	(85) 0.004 (0.003, 0.007)	(84) 185.4 (66.2, 484.7)	(80) 4.9 (3.2, 8.4)	16 (11, 23)	23 (18, 34)	36.2 (33.1, 39.1)	1.61 (1.15, 2.08)
P		0.160	0.002	0.145	0.000	0.002	0.002	0.035	0.000	0.006
CAL	17	17 (15.5, 21.5)	0.01 (0, 0.024)	0.004 (0, 0.007)	358 (130, 1323)	3 (23.1)	5 (31.3)	3 (18.8)	32.6 (28.0,35.1)	1.41 (0.78,1.68)
NO-CAL	267	20 (17, 26)	0 (0, 0.01)	0.005 (0, 0.007)	435.5 (153, 1116)	65 (27.8)	72 (27.3)	67 (25.4)	33.9 (30.2,36.9)	1.43 (0.75,2.00)
P		0.110	0.013	0.698	0.838	1.000	0.775	0.768	0.364	0.933

CKMB, creatine kinase isoenzyme MB; CTNI, cardiac troponin I; Hs-CTNT, high sensitive cardiac troponin T; NT-proBNP, N-terminal brain naturetic peptide premise; TBA, total bile acid; ALT, alanine transaminase; AST, aspartate transaminase; DDE, D-dimer elevated; DDN, D-dimer normal; AAR, AST/ALT ratio; the average of AAR is 0.8.

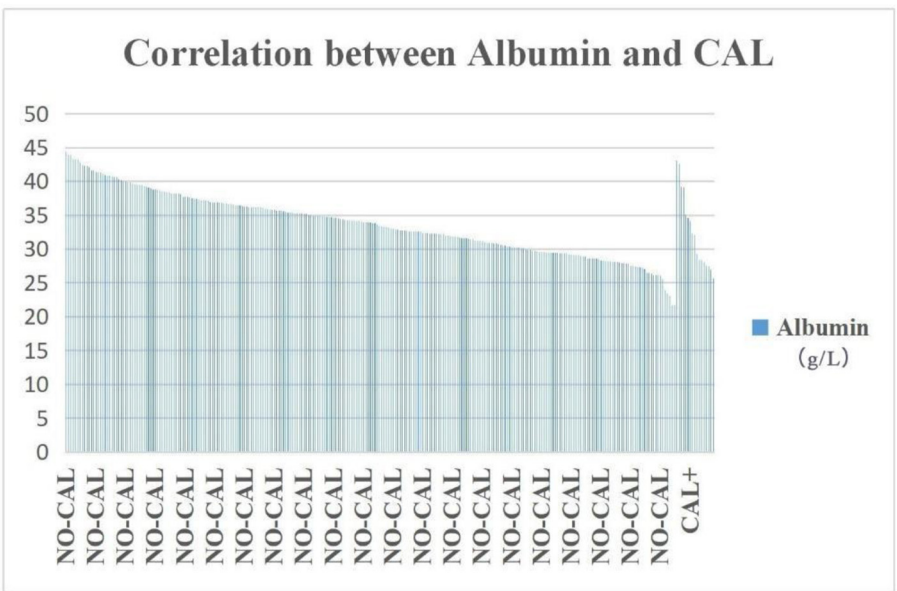

FIGURE 2 | The incidence of hypoproteinemia was significantly higher in the CAL group than in the NO-CAL group ($p < 0.05$). The X-axis indicates with or without presence of CAL. The Y-axis represents the albumin (g/L).

Based on the reported average of AAR at 1.1 by Wang et al. (21), 10 patients in the CAL group had lower AAR ($p = 0.855$).

NT pro-BNP, ALT, AST, and TBA were significantly higher in the DDE group than those in the DDN group ($p = 0.002$, 0.035, and 0.002, respectively), whereas ALB and AAR were significantly lower in the DDE group than in DDN ($p = 0.006$ and 0.000, respectively) (**Table 2**). The incidence of hypoproteinemia was significantly higher in the CAL group than in the NO-CAL group ($p < 0.05$) (**Figure 2**).

The Correlation Between ACA/D Dimer and the Incidence of Multiple-Organ Injuries

The incidence of granulocytopenia, thrombocytosis, and CAL in the ACA+ group was significantly higher than in the ACA- group ($p < 0.01$–0.05).

The incidence of elevated NT pro-BNP and ALT, hypoproteinemia, sterile urethritis, and thrombocytosis was significantly higher in the DDE group than in the DDN group ($p < 0.01$–0.05) (**Table 3**).

TABLE 3 | The correlation between ACA/D-dimer and the incidence of multiple-organ involvement.

Groups	*n*	CAL (%)	Granulopenia (N < 1.0 × 10^9/l)	Anemia (Hb < 90 g/l)	Thrombocytosis (PLT > 450 × 10^9/l)	CTnI (>0.04 μg/l)(%)	NT pro-BNP (>300 pg/ml)(%)	ALT (>40 U/l) (%)	Hypoproteinemia (ALB < 30 g/L) (%)	Cholestasis (TAS>10 umol/l) (%)	SM	Pneumonia	SU	
ACA+	168	14 (8.3)	75 (44.6)	22 (13.1)	111 (66.1)	2/164 (1.2)	88/164 (53.7)	45 (26.8)	72/168 (42.9)	25/142 (17.6)	10 (6.0)	58 (34.5)	21 (12.5)	
ACA–	116	3 (2.6)	31 (26.7)	13 (11.2)	61 (52.6)	5 (4.3)	71/110 (64.5)	33 (28.4)	32/116 (28.3)	27/108 (27.6)	11 (9.5)	34 (29.3)	20 (17.2)	
P		0.045	0.002	0.634	0.022	0.130	0.073	0.758	0.540	0.009	0.264	0.356	0.264	
														ACA+
DDE	192	12 (6.3)	53 (27.6)	24 (12.5)	125 (65.1)	6 (3.1)	128/187 (68.4)	63 (32.8)	59 (30.7)	32 (16.7)	16 (8.3)	66 (34.4)	31 (16.1)	113 (59.2)
DDN	88	4 (4.5)	19 (21.6)	10 (11.4)	44 (50)	0 (0)	28/83 (33.7)	14 (5.9)	4 (4.5)	11 (12.5)	4 (4.5)	26 (29.5)	2 (2.3)	30 (34.1)
P		0.568	0.942	0.787	0.016	0.182	0.000	0.003	0.000	0.732	0.514	0.424	0.004	0.000

DDE, D-dimer elevated; DDN, D-dimer normal; SM, sterile meningitis; SU, sterile urethritis.

In the DDE group, there were 113 patients who were ACA+ (59.2%), which was significantly higher than in the DDN group ($p < 0.000$), but the incidence of DDE was not significantly higher than DDN in the CAL group (**Figure 3**).

The Correlation Between CAL and Myocardial/Liver Damages

CAL group presented with significantly higher incidence of hypoproteinemia and cTnI, ($p < 0.05$). CAL occurred frequently in patients who were younger, with prolonged fever, later IVIG treatment, CRP elevated over 100 mg/L, and lower AAR. However, there were no significant difference ($p > 0.05$; **Table 4**).

The Receiver Operating Characteristics (ROC) of cTnI Used in Predicting Coronary Artery Damage in Children With KD

The AUC of the area under cTnI curve was 0.657, 95% CI was 0.508–0.805 ($p < 0.05$). When 0.0175 was used as the cutoff value, the sensitivity and specificity for predicting CAL were 0.412 and 0.876 (**Figure 4**).

DISCUSSION

The Current Clinical Application of ACA Test

ACA are anticardiolipin antibodies. They were first detected using radioimmunoassay (22). Under normal conditions, the immune system does not recognize cardiolipin that lies at the inner mitochondrial membrane. However, once cardiolipin is exposed, it will trigger an immune response and produce the autoimmune antibody ACA. Immunophenotypes include IgG, IgM, and IgA. Among them, IgG is a pathogenic autoantibody, and its high titer and long half-life make it an important player in immune response. In a study including 34 Italian KD children, anticardiolipin (aCL) was detected in 30% of KD patients (IgG aCL antibodies were found in 14 patients, IgM aCL in 1, and 1 had both) (23). The target antigen of ACA is negatively charged cardiolipin on the platelet and endothelial cell membranes. ACA can stimulate immune response to the phospholipids of its own cell membranes and damage the cell membranes and thus release a large amount of inflammatory cytokines and chemokines and further trigger vascular inflammation. ACA is commonly found in the following diseases: (1) thrombotic diseases, such as myocardial infarction (24), stroke (25), and habitual abortion. There is marked activation of the endothelium and immune system in KD. Anticardiolipin antibodies (aCL) can cause activation of the endothelium. An increase in IgA anticardiolipin antibodies in KD patients suggests that the degree of increase in these antibodies correlates with the degree of systemic inflammation (26). Low IgG was not a risk factor for CAAs in this study. However, KD patients with relatively high IgG prior to treatment may have an increased risk of resistance to initial IVIG therapy (27). Studies have suggested that (13, 28) ACA, as one of laboratory indications for thrombosis, can cause damage in vascular endothelial cells, interfere with the coagulation system, affect platelet function, and reduce

TABLE 4 | The correlation between CAL and CRP/D-Dimer/myocardial damage/Hypoproteinemia.

Groups	Gender M (%)	Age (years)	Fever (days)	First IVIG (days)	CRP≥100 mg/l (%)	D-Dimer (ug/L)	CTnI (μg/L)	Hs-cTnT (ng/mL)	NT-proBNP (pg/mL)	Hypoproteinemia (%)
CAL ($n = 17$)	11 (64.7)	2.1 ± 1.3	10.2 ± 5.2	9.2 ± 4.4	7 (41.2)	622.9 ± 509.3	0.01 (0, 0.024)	0.004 (0, 0.007)	358 (130, 1323)	8 (47.06)
NO-CAL ($n = 267$)	164 (61.4)	2.4 ± 1.8	8.0 ± 2.3	7.7 ± 2.0	64 (24.0)	726.3 ± 1835.3	0 (0, 0.01)	0.005 (0, 0.007)	435.5 (153, 1116)	65 (17.71)
P	0.787	0.433	0.114	0.186	0.146	0.823	0.013	0.698	0.838	0.047

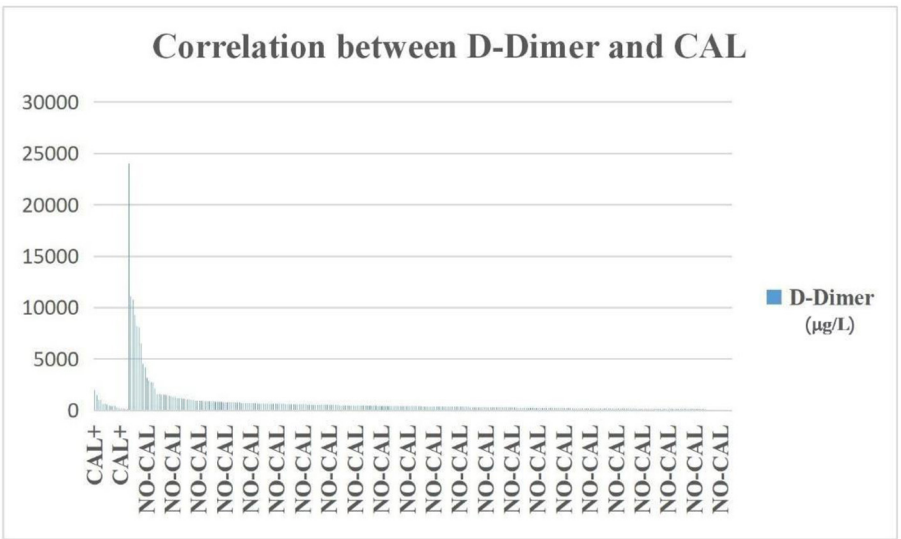

FIGURE 3 | The incidence of D-dimer was not significantly higher in the CAL group than in the NO-CAL group ($p > 0.05$). The X-axis indicates with or without presence of CAL. The Y-axis represents D-dimer in μg/L.

fibrinolytic activity. Finnazzi et al. (29) performed a blind study on 360 ACA-positive patients and concluded that a high level of ACA was a meaningful indicator for predicting thrombosis. Sueoka et al. (30) raised an alarm in patients who had developed thrombosis in the presence of positive ACA after percutaneous thoracoscopic surgery. There have been discussions on the clinical significance of positive ACA antibodies. In healthy controls, aCL antibodies were found in five patients (22%) (23). In order to differentiate from false-positive interference, many medical institutions have implemented testing ACA antibody titers (31) (our hospital is currently using semi-quantified). (1) ACA is also found in connective tissue diseases, such as systemic lupus erythematosus and KD. (2) ACA can be detected in patients infected with syphilis, AIDS, hepatitis C, tuberculosis, parvovirus, and cytomegalovirus. Thus, there is a limitation in evaluating the association of ACA-IgG with vasculitis in Kawasaki disease. ACA titer may stratify the association between ACA and Kawasaki disease. In our study, the incidence of CAL and the proportion of thrombocytosis in children with elevated ACA were significantly increased, suggesting a possible mechanism that inflammatory mediators and the activation of negatively charged cardiolipin in platelets caused damages in the vascular endothelium, which increased the risk of thrombosis. Our results showed that CAL was more common in ACA+ patients. This is consistent with literature reports (32). In contrast to the correlation between CAL and low AAR reported by Wang et al. (21), our results showed that CAL was not associated with AAR. On the other hand, elevated D dimer was correlated with low AAR and NT pro-BNP, but not with CAL. Since the COVID-19 outbreak, there have been various complications related to thrombosis, and they are accompanied by DIC and an increase in ACA (33, 34). These results suggest that COVID-19 virus can activate vascular endothelium and cause endothelial damage and intravascular coagulation.

The Significance of Elevated D Dimer

The D dimer test is often used in clinical diagnosis of diffuse intravascular coagulation (DIC), deep vein thrombosis (DVT), pulmonary embolism, myocardial infarction, cerebral infarction, etc. This index can not only be used for the diagnosis of thrombotic diseases but also be used as an index for monitoring the dose of thrombolytic drugs for therapeutic effect. KD itself is an inflammation-mediated vasculitis that activates the vascular endothelium and causes endothelial damage. Symptoms are similar to multisystem inflammatory syndrome in children

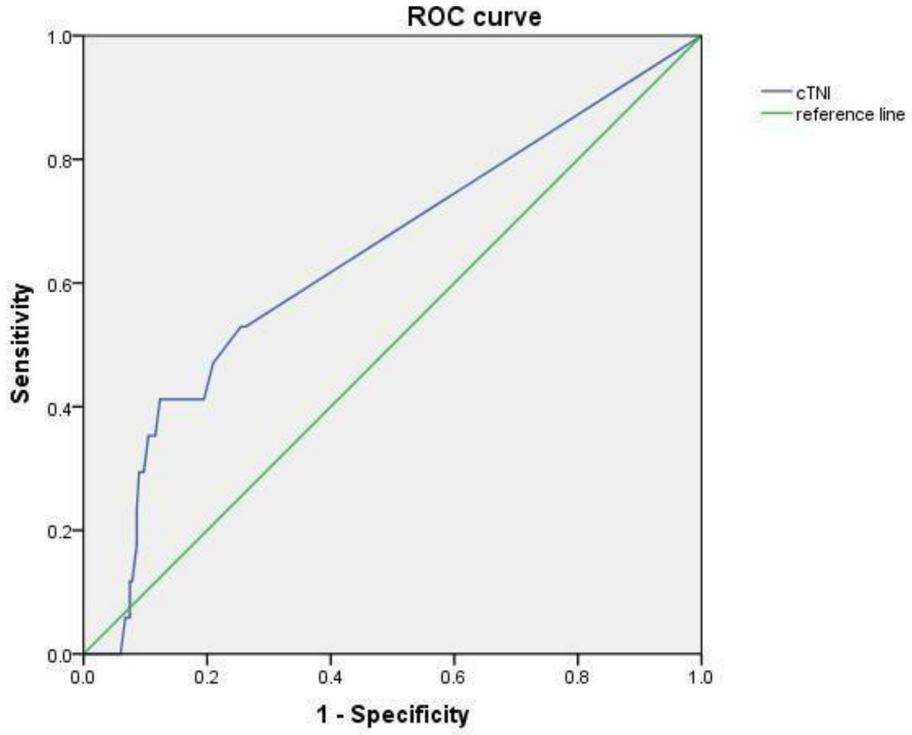

FIGURE 4 | The ROC curve of cTnI levels in predicting CAL in children with KD.

with COVID-19. Elevated D dimer is associated with DIC hypercoagulation and macrophage activation and aggravates tissue disintegration (32). In addition, elevated D dimer has been reported to be correlated with CAL (35) and complications in multiple organs. Our study showed that elevated DD was only correlated with complications in MDO but not with CAL. Kong et al. (36) reported that elevated D dimer was also correlated with IVIG resistance in KD children (18). However, our results showed that D dimer elevation was not associated with IVIG resistance. We also found that KD with MAS complication and necrotizing pneumonia was not associated with CAL, although patients had fever which lasted for more than 2 weeks. CRP increased surprisingly (7). The conclusion drawn from our study disagrees with other reports in literature (37). In this study, there were two KD children with elevated D dimer over 10,000 μg/L (D dimer at 24,007 μg/l in one 19-month-old boy with pneumonia and aseptic meningitis and at 10,773 μg/l in the other 17-month-old girl with hemophagocytic syndrome, hyponatremia, pneumonia, bilateral pleural effusion, liver function damage, hypoproteinemia, pancreatic injury, and aseptic meningitis). Both patients did not have CAL. It is consistent with the report by Ming-Tsan et al. (38). Data at our center showed that there was no relationship between increased D dimer and IVIG resistance either, which is conflicted with reported studies (39). This may be associated with earlier IVIG treatment by 1 day in DDE, or it may be related to the differential expression of immune responsive genes during the KD occurrence and development (40).

D Dimer in Children With Multisystem Inflammatory Syndromes

Last year, the coronavirus disease 2019 (COVID-19) hit the world, and a new manifestation emerged as a multisystem inflammatory syndrome in children (MIS-C) which carries similar clinical symptoms of KD, including toxic shock syndrome and severe sepsis (41). The severity is significantly correlated with D dimer, even KDSS (42). COVID-19 linked with thrombotic microangiopathy triggers multiple vasculitis along with arteriole thrombosis, and medium and large venous and arterial vessels mediate the disseminated intravascular coagulation (DIC) (43). Additionally, 52% of MIS-C has elevated D dimer (44). Although some children with KD in our center last year had very similar symptoms to COVID-19 with MIS-C (45), we did not find any evidence of COVID-19 infection in these patients. However, the ultimate treatments for these patients rely on IVIG and glucocorticoid, which are similar to treatments for rheumatic immune diseases such as KD (46).

CONCLUSION

Our study indicated that ACA+ and hypoproteinemia were correlated with CAL, granulocytopenia, and thrombocytopenia in KD children. In the current study, ACA was only tested for positive and negative, which is a limitation to this study. To further elucidate the association, ACA titers would establish its significance in drawing a conclusion for the significance of

ACA in CAL and myocardial damages. Elevated D dimer was correlated with MOD but not with CAL; CRP was correlated with D dimer, but not with ACA and CAL.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

YX: patient's observation, data collection and analysis, and the editing of manuscript. YC: patient's observation and data analysis. HW: patient's diagnosis, treatment, data analysis, and the editing of manuscript about discussion. All authors contributed to the article and approved the submitted version.

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Cardiovascular Risk Awareness and Calculated 10-Year Risk Among Female Employees at Taibah University 2019

Amal M. Qasem Surrati [1], Walaa Mohammedsaeed [2] and Ahlam B. El Shikieri [3]*

[1] Family and Community Medicine Department, College of Medicine, Taibah University, Medina, Saudi Arabia, [2] Medical Laboratories Technology Department, College of Applied Medical Sciences, Taibah University, Medina, Saudi Arabia, [3] Clinical Nutrition Department, College of Applied Medical Science, Taibah University, Medina, Saudi Arabia

Cardiovascular diseases (CVD) are the most common cause of death and disability worldwide. Saudi Arabia, one of the middle-income countries has a proportional CVD mortality rate of 37%. Knowledge about CVD and its modifiable risk factors is a vital pre-requisite to change the health attitudes, behaviors, and lifestyle practices of individuals. Therefore, we intended to assess the employee knowledge about risk of CVD, symptoms of heart attacks, and stroke, and to calculate their future 10-years CVD risk. An epidemiological, cross-sectional, community-facility based study was conducted. The women aged ≥40 years who are employees of Taibah University, Al-Madinah Al-Munawarah were recruited. A screening self-administrative questionnaire was distributed to the women to exclude those who are not eligible. In total, 222 women met the inclusion criteria and were invited for the next step for the determination of CVD risk factors by using WHO STEPS questionnaire: It is used for the surveillance of non-communicable disease risk factor, such as CVD. In addition, the anthropometric measurements and biochemical measurements were done. Based on the identified atherosclerotic cardiovascular disease (ASCVD) risk factors and laboratory testing results, risk calculated used the Framingham Study Cardiovascular Disease (10-year) Risk Assessment. Data were analyzed using GraphPad Prism 7 software (GraphPad Software, CA, USA). The result showed the mean age of study sample was 55.6 ± 9.0 years. There was elevated percentage of obesity and rise in abdominal circumference among the women. Hypertension (HTN) was a considerable chronic disease among the participants where more than half of the sample had it, i.e., 53%. According to the ASCVD risk estimator, the study participants were distributed into four groups: 63.1% at low risk, 20.2% at borderline risk, 13.5% at intermediate risk, and 3.2% at high risk. A comparison between these categories based on the CVD 10-year risk estimator indicated that there were significant variations between the low-risk group and the intermediate and high-risk groups ($P = 0.02$ and $P = 0.001$, respectively). The multivariate analysis detected factors related to CVD risk for women who have an intermediate or high risk of CVD, such as age, smoking, body mass index (BMI),

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***Correspondence:**
Amal M. Qasem Surrati
asurrati@taibahu.edu.sa;
dr-aamaal@hotmail.com

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unhealthy diet, blood pressure (BP) measurements, and family history of CVD ($P < 0.05$). The present study reports limited knowledge and awareness of CVD was 8.6 that is considered as low knowledge. In conclusion, the present study among the university sample in Madinah reported limited knowledge and awareness of CVD risk. These findings support the need for an educational program to enhance the awareness of risk factors and prevention of CVD.

Keywords: cardiovascular disease, knowledge, awareness, risk factors, Madinah-KSA, calculated 10 year risk

INTRODUCTION

Cardiovascular disease (CVD) is coronary heart disease (CHD), heart failure, ischemic stroke, peripheral vascular disorder, and atherosclerosis of the aorta and its branches. The lifetime risk of CVD reaches 50% for those aged 30 years without known CVD (1). Many countries in the Gulf Cooperation Council have suffered a higher number of deaths from non-communicable diseases which are estimated to comprise 65–78% of the total adult loss (2) and ∼73% of all death in Saudi Arabia (2016) (3). The main changeable risk factors for CVD are dyslipidemia, type 2 diabetes (T2DM), increased blood pressure (BP), obesity, and smoking, and these are claimed to be responsible for more than 50% of cardiovascular mortality (4). Using data from 52 countries, the INTERHEART study found that nine changeable factors accounted for more than 90% of the risk of new-onset myocardial ischemia: tobacco, dyslipidemia, hypertension (HTN), diabetes, central obesity, psychosocial factors, daily intake of fruits and vegetables, regular alcohol consumption, and frequent physical exercise (5). Persistent exposure to harmful factors causes a greater increase of atherosclerotic plaque.

Risk calculation for CVD is an important method to predict the future impact of atherosclerotic cardiovascular disease (ASCVD) and to enhance the adherence to healthy lifestyle measures and therapies (6). The use of risk calculators is intended to predict total CVD risk depending on the presence of independent factors in a mathematical equation, which concludes that a percentage depends on the absolute risk for other outcomes. This allows the physician to calculate the risk to prescribe therapy of each client. More than 100 scores were available in the literature. CVD risk calculation measures are approved to use for adults to guide therapy for hyperlipemia, HTN, and diabetes. For example, the Framingham Risk Assessment is an easy and validated method for recognizing persons at risk for ASCVD (7).

The result of the Framingham research showed that for individuals of 50 years of age with no cardiovascular problems, the risk of harmful effects happening later in life was 51.7% in men and 39.2% in women, with a median survival of 30 and 36 years, respectively. Estimation of future risk is important in adulthood because, if attention is paid only to short-term risk, fewer people will modify their daily habits by obeying a management plan (8). Most of the risk factors for CVD and stroke are changeable with protective strategies, such as therapeutic lifestyle changes (TLCs) and the introduction of therapy with proven benefits (9). With primary prevention, the modification of multiple major risk factors will further reduce the risk of coronary artery disease (CAD) and stroke (10). The high-risk category is defined as adults with a 10-year risk of ASCVD >7.5% or a lifetime risk >30%, as well as those with very high low-density lipoprotein (LDL) cholesterol (LDL-C) levels or primary genetic hyperlipidemias for whom equations may not correctly reflect the risk. The 2013 American Heart Association (AHA)/American College of Cardiology (ACC) guidelines recommended the treatment with a statin for patients with a 10-year risk of ≥7.5%, although the statin treatment can be considered even at a risk of 5% (11). Primary prevention, healthy lifestyle strategies to help high-risk individuals decrease their CVD risk include: stopping, reducing, or avoiding smoking; maintaining a healthy diet; being physically active; keeping a body mass index (BMI) <25 kg/m^2 and a waist-hip ratio <0.8 in women and <0.9 in men; lowering BP to <140/90 mmHg; lowering TC to <190 mg/dl; decreasing LDL-C to <115 mg/dl; targeting the glucose levels in patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or diabetes; and prescribing aspirin at 75 mg/d if BP is controlled.

Many countries have adapted primary prevention measures to decrease the later costs of complicated cases requiring tertiary care. The key aspect leading to the proven effectiveness of this approach is the perception of the risk of an individual for certain health diseases (12). In any sector considered an important representative sample, workers' quality of life, health beliefs, and ability to adapt healthy measures are predicted to affect their productivity and likelihood of avoiding chronic disease. By decreasing the healthcare demand, the financial status of any institution will improve (13). Awareness of CVD and its modifiable risk factors is an important step toward modulating the health beliefs, behaviors, and daily habits of individuals (14, 15). It also helps them correctly calculate their future risk and motivates them to follow prevention-seeking actions and adapt behavior to decrease harm (16–18). Furthermore, estimating the current level of information about CVD available in a community has positive public health effects as it helps in creating focused educational materials as well as planning (19).

Abbreviations: FBG, fasting blood glucose; TLCs, therapeutic lifestyle changes; CVD, cardiovascular disease; CAD, coronary artery disease; ASCVD, atherosclerotic cardiovascular disease; LDL, low density lipoprotein; TC, total cholesterol; BMI, body mass index; NCD, non-communicable disease; BP, blood pressure; CHD, coronary heart disease; IFG, impaired fasting glucose; CVA, cerebrovascular accident; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; CVA, cerebrovascular accident.

Knowledge about CVD and its modifiable risk factors is a vital prerequisite to the changing health attitudes, behaviors, and lifestyle practices of individuals. As far as we know, there is limited quantitative data focusing on awareness and knowledge of CVD risk factors in Madinah city. Therefore, we aimed to determine the cardiovascular risk awareness and calculated 10-year risk among the women aged ≥40 years.

MATERIALS AND METHODS

A descriptive, cross-sectional, community-based study was conducted between March 1 and August 30, 2019, among the women aged 40 years and above who are current employees at Taibah University.

Data Collection Method and Tools

The required sample size was calculated to be 385 participants. The calculation was done using the sample size software online (available at http://sampsize.sourceforge.net/iface/#prev) for prevalence studies assuming unknown exactly prevalence of CVD risk awareness 50% at *CI* 95% and power of test 80%.

A self-administered screening questionnaire developed by the researchers was distributed to the women to eliminate those who were not eligible. Those women who fulfilled the inclusion criteria were selected, and data were collected from them in a systematic, non-invasive way. Participants with complete data and had their blood samples drawn were included in the study ($n = 222$). The exclusion criteria covered individuals who had been diagnosed with CVD, such as myocardial ischemia, stroke, unstable angina, revascularization surgery, disease of the aorta, coronary angioplasty, and peripheral arterial disease, as well as pregnant women.

They were told the objective of the study and were free to choose not to participate. Confidentiality was guaranteed, and they were given an electronic agreement to sign. The questionnaire included the selected demographic and health information, such as the age of the participant and the presence of any health problems. They were then invited to participate in the next step when CVD risk factors were determined. The tools used in this stage are outlined below. The questionnaire was pre-tested before the start of the study to ensure their accuracy and precision. The tools were pretested for clarity and understanding on 10 volunteers and adjustments were carried out accordingly.

A modified WHO STEPS instrument, which is used for the surveillance of non-communicable disease risk factors that include the risk factors associated with CVD, was employed. The questionnaire was accessed via the weblink www.who.int/ncds/steps. The sociodemographic characteristics (e.g., age and education level) and behavioral measurements (e.g., tobacco use; dietary pattern; physical activity level; a history of diabetes, HTN, hyperlipidemia, and CVD; lifestyle advice; and cervical cancer screening) of the participants were determined. Information such as marital status, education level, and occupation was recorded.

Dietary data were assessed using 2-weekdays 24-h recall records. Foods were converted into nutrients using the Diet Organizer application version 3.1 (MulberySoft, Thailand), and results were compared with recommended dietary allowance (RDA) standardized based on 40–51 and 52–63 years. The intake of certain foods that were previously reported to be associated with CVDs risk was also assessed. These included foods, such as the intake of whole grain cereals, high fiber foods, fruit, and vegetables. In this study, the number of servings for fruits, vegetables, and water intake were obtained from the Saudi Healthy Food Palm (fruit serving = 2–4/day, vegetables serving = 3–5/day, and water at least 6 cups/day).

The physical evaluations were performed, measuring BP ≥140 systolic and /or ≥90 diastolic consider hypertensive, pulse, and anthropometric analysis (such as weight, height, and waist circumference [WC]) calculated as below.

Weight was measured two times following published protocols using OMRON—Body Fat Scales (BF508l, China) after being calibrated. The WC was measured two times using a non-stretchable measuring tape, and for accuracy, the readings were taken from the right side of the body (two fingers above the navel). The risk of CVD was increased in women with WC >88 cm. BMI was calculated using standard formulas (weight in kilogram and height in meters square) for those ≥30 consider obese.

The biochemical measurements were analyzed following the published protocols. These included lipid profile and fasting blood glucose. All tests were performed in clinical biochemistry labs in the National Guard Hospital (the kits and lab items were supported by Taibah University), using an automated machine (ARCHITECT c4000, Abbott Laboratories, IL, USA).

Build on the recognized ASCVD risk factors and laboratory testing results, ASCVD risk was approximated using the Framingham Study Cardiovascular Disease (10-year) Risk Assessment (9) (validated in the age of 30+ population) and the ASCVD Risk Estimator for both 10-year and lifetime ASCVD risk (validated in the age 40–79 years and age 20–60 years populations, respectively). Using an online risk calculator (https://tools.acc.org/ldl/ascvdriskestimator/index.html/calculate/estimator), the participants were categorized into four risk groups based on the 10-year ASCVD risk percentages: low risk [<5%], borderline risk [5%–<7.5%], intermediate-risk [>7.5%–<20%], and high risk [>20%].

The total CVD knowledge score was calculated as a continuous variable by summing the scores of the respondents concerning CVD types, heart attack symptoms, stroke symptoms, and CVD risk factors. The total knowledge score was 24, and the levels of CVD knowledge were categorized as low knowledge [≤12], moderate knowledge (13–19), and high knowledge [≥20] (20). Each correct response was given a score of 1, and a wrong response was scored as 0, making the total score 24 with each subdivision having a score of between 4 and 9. Knowledge results were classified for each part as follows:

- Stroke symptoms (Score = 5) Factors of CVD (Score = 9)
- CVD symptoms (Score = 6) Heart attack symptoms (Score = 4)

Ethical permission was sought from the Ethical Clearance Committee at the Faculty of Applied Medical Sciences. SREC/AMS 2019/42/CND on 22-Oct-2019 Permission was also obtained from the colleges, committees, and sectors from where the samples were recruited. The written consent forms were signed by all the participants before the start of the study, affirming that all data would be treated with confidentiality and that they have the right to withdraw from the study at any time they wished.

Operational Definitions of the Variables Used in Study

1- Body mass index is the ratio between weight in kilograms to height in meters squared. Based on the National Institutes of Health (NIH), BMI of the participants was classified as underweight (BMI $\leq$18.0), normal (BMI = 18.5–24.9), overweight (BMI = 25.0–29.9), or obese (BMI $\geq$30.0).
2- Blood pressure was measured by an automatic device. The measurements were taken in the right upper limb, after 5 min of rest, with the subject seated and the arm supported. BP was recorded as the average of three measurements taken 5 min apart during rest. The average of the three BP measurements was calculated. Normal systolic blood pressure is defined as $<$140 mmHg and diastolic $<$90 mmHg.
3- Waist circumference was measured two times using a non-stretchable measuring tape, and for accuracy, the readings were taken from the right side of the body (two fingers above the navel). The CVDs risk was increased in women with WC $>$88 cm.
4- Lipid panel measurement an elevated cholesterol is defined as a total cholesterol $\geq$200 mg/dl and a low HDL level as $<$40 mg/dl an elevated low-density lipoprotein (LDL) is defined per ACC/AHA guidelines as a primary elevation $\geq$190 mg/dl or an LDL of $>$70 mg/dl with the presence of diabetes in a subject 40–75 years of age, and patients with a ASCVD predicted risk $>$7.5% with an LDL $>$70 mg/dl. Triglyceride levels is defined as $\geq$200 mg/dl.

Statistical Analysis

Data were analyzed using the GraphPad Prism 7 software (GraphPad Software, CA, USA). Quantitative data were expressed as frequency (percentages) and mean $\pm$ SD. The Student's *t*-test was used to compare four categories of calculated CVD risk. A multivariable statistical analysis (multiple logistic regression model) was performed to study the association between the 10-year CVD risk factors and knowledge of CVD based on the chosen factors. Data were presented with a 95% *CI* and an odds ratio (*OR*).

RESULTS

Study Population: Demographic and Clinical Characteristics of Study Participants

The demographic and clinical characteristics of the 222 women included in the study were as follows: their mean age was 55.6 $\pm$ 9.0 years, 81% of them were married, and 47.7% had a bachelor's degree. The BMI mean of the study population was 29.9 $\pm$ 5 kg/m^2, and 34% of them were overweight with 44.6% considered obese. Abnormal waist circumference $>$88 cm was seen in 63% of them, and 33.8% had a high and 28.3% very high percentage of body fat. More than half of the women, 58%, had an appropriate sleep duration of 6–8 h/day while a short sleep duration of fewer than 5 h/day was reported by 37% of the samples (assessed from a self-administered questionnaire in WHO STEPS). Unhealthy dietary habits were seen in 85% of the participants. HTN was the most common chronic problem, present in 53%, followed by diabetes in 7%. The most frequently reported problems in the family history of the samples were hypercholesterolemia in 23% and HTN in 18% (all data evaluated from the self-administered questionnaire in WHO STEPS) (**Table 1**).

Calculated 10-Year Cardiovascular Risk Among Women at Taibah University

According to the online ASCVD risk estimator, the study participants were divided into four categories: 63.1% at low risk, 20.2% at borderline risk, 13.5% at intermediate risk, and 3.2% at high risk (**Table 2**). A comparison among these categories according to the CVD 10-year risk estimator indicated that there were significant differences between the low-risk group and the intermediate and high-risk groups ($P = 0.02$ and $P = 0.001$, respectively).

Respondents' Knowledge of CVD Types, Heart Attack, and Stroke Symptoms, and CVD Risk Factors for Women

Table 3 indicates that the most frequent type of CVD reported was CHD [39.6%] followed by rheumatic heart disease [28.8%] and congenital heart disease [27.5%]. The most common stroke symptoms appearing in collected data were sudden dizziness/difficulty walking [62%], sudden lack of focus/difficulty speaking [60%], sudden numbness/weakness in the face or arms [55%], and sudden visual impairment [43%]. For heart attack symptoms, the respondents reported chest pain/discomfort [78%], shortness of breath [74%], and pain in the arms or shoulders [55.7%]. Participants indicated knowledge of the following CVD risk factors: unhealthy diet [85%], hypertension [80%], obesity [79%], smoking [77%], hypercholesterolemia [75%], lack of exercise [71%], stress/anxiety [67%], and diabetes [61%]. Each correct response was given a score of 1, and a wrong response was scored as 0. The total possible score was 24, the knowledge scores were categorized as follows: stroke symptoms (Score = 5), heart attack symptoms (Score = 4), risk factors of CVD (Score = 9), and CVD diseases (Score = 6). Calculated scores indicated that the overall knowledge of the participants was 8.6 which was considered low knowledge as presented in **Table 3**.

Factors Associated With 10-Year CVD Risk Estimates Among Women

The multivariate analysis identified factors associated with CVD risk for women who have an intermediate or high risk of CVD

TABLE 1 | Demographic and clinical characteristic of women at Taibah University ($n = 222$).

Variables	Number of women (%)
Age	
Mean ± SD	55.6 ± 9.0
40–51 years	184 (82.9%)
52–63 years	38 (17.1%)
Marital status	
Single	18 (8.1%)
Married	179 (80.6%)
Divorced	20 (9.1%)
Widow	5 (2.3%)
Education level	
Primary complete and below	33 (14.9%)
Secondary and higher schooling	38 (17.1%)
Complete university or college	106 (47.7%)
Post-graduate studies	45 (24.6%)
Smoking status	
Smoker	22 (9.9%)
Non-smoker	200 (90.1%)
BMI classification	
Normal	47 (21.2%)
Overweight	76 (34.3%)
Obesity grade I	63 (28.4%)
Obesity grade II	25 (11.3%)
Obesity grade III	11 (4.9%)
Average waist circumference (cm)	
Mean ± SD	90.1 ± 43.3
<88.0 cm	82 (36.3%)
>88.0 cm	140 (63.7%)
Classification of percent body fat	
Low <15%	15 (6.8%)
Normal (18–22%)	25 (11.3%)
Moderately (22–30%)	44 (19.8%)
High (30–40%)	75 (33.8%)
Very high >40%	63 (28.3%)
Sleeping pattern (hours/day)	128 (57.7%)
Appropriate sleep (6–8 h/d)	
Short sleep (≤5 h/d)	82 (36.9%)
Long sleep (≥9h/d)	12 (5.4%)
Categories of blood pressure	
Normal	89 (40.1%)
Elevated	16 (7.2%)
Stage 1	74 (33.3%)
Stage 2	42 (18.9%)
Hypertensive crisis	1 (0.5%)
An unhealthy diet	
Unhealthy diet	189 (85.1%)
Healthy diet	33 (14.9%)
Family's history of disease	
Diabetes	13 (5.9%)
Die at an early age due to CVD	32 (14.4%)
CVD	23 (10.4%)
Hypertension	42 (18.9%)
Hypercholesterolemia	52 (23.4%)
Chronic disease	
Diabetes	16 (7.2%)
Hypertension	117 (52.7)
Hyperlipemia	5 (2.3%)
Chronic renal disease	2 (0.9%)
Lupus	2 (0.9%)
Rheumatoid arthritis	2 (0.9%)
Psoriasis	4 (1.8%)
Blood analysis	
FBG (mmol/L)	4.9 ± 1.19
Total cholesterol (mmol/L)	**6.2 ± 1.1***
LDL-cholesterol (mmol/L)	**3.1 ± 0.84***
HDL-cholesterol (mmol/L)	1.4 ± 0.37
Triglycerides (mmol/L)	**1.91 ± 1.1***

The results presented as frequency (percentage %). The high levels of certain biomarkers, such as cholesterol, LDL, and triglycerides significantly beyond the normal range in healthy women are marked with bold and an asterisk (*) on the table and the data presented as the mean concentrations ± SD. FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein.

TABLE 2 | Cardiovascular calculated 10-year risk among female at Taibah University.

CVD calculated risk categories	Frequency (%)	Mean ± SD	*P*-value
Low (<5%)	140 (63.1%)	3.9 ± 0.67	
Borderline (5–7.4%)	45 (20.2%)	6.82 ± 0.88	0.06
Intermediate (7.5–19.9%)	30 (13.5%)	15.5 ± 1.42	0.02*
High (>20%)	7 (3.2%)	21.3 ± 2.4	0.001**

*Data presented as frequency (percentage %) and mean ± SD. The t-test was used to compare between low group and the other groups. Statically significant at *P ≤ 0.05 or **P ≤ 0.001.*

($n = 37$). As demonstrated in **Table 4**, factors independently related to CVD risk were age, smoking, BMI, unhealthy diet, blood pressure measurements, and family history of CVD ($P < 0.05$). The CVD risk was significantly greater among the women aged 52–63 years compared with other age groups ($P = 0.02$). The study participants were found to be at a higher CVD risk if they reported having a high BMI, having a high BP level ($OR = 6.8$), regularly eating an unhealthy diet ($OR = 3.8$), and having a family history of CVD ($OR = 3.7$). There was also a strong association between increased risk of CVD and the levels of lipids, such as cholesterol ($OR = 7.8$, $P = 0.001$) and LDL ($OR = 6.9$, $P = 0.003$, **Table 4**).

The results of the multivariate analysis for factors associated with total knowledge of CVD scores and the characteristics of respondents. In the multivariate logistic analysis, factors significantly associated with CVD knowledge included are age,

TABLE 3 | Percentage of women giving correct response to CVD risk factors and their response scores.

Variables	Percentage of women (%)	Mean score
Stroke symptoms (Score = 5)		
Severe headache without a known cause	37.8%	1.4
Sudden dizziness, difficulty walking, or loss of balance	62.6%	2.3
Sudden visual impairment in one or both eyes	42.8%	1.5
Sudden lack of focus, difficulty speaking, or understanding others	60.4%	2.2
Sudden numbness/weakness in the face, arm, or leg	55.4%	2.1
The total score		9.5/5 = 1.9
Heart attack (Score = 4)		
Shortness of breath	73.9%	2.7
Pain in the arms or shoulder	55.7%	2.1
Chest pain or discomfort	78.4%	2.9
Pain in the neck, jaw, or back	40.4%	1.5
The total score		9.2/4 = 2.3
Factors of CVD (Score = 9)		
An unhealthy diet	85.1%	3.2
Obesity	78.8%	2.9
Stress and anxiety	67.6%	2.5
Lack of exercise	70.7%	2.6
Harmful increase in cholesterol	75.3%	2.8
Hypertension	79.7%	3
Smoking	77.1%	2.8
A family history of cardiovascular disease	49.5%	1.8
Diabetes	61.3%	2.3
The total score		23.9/9 = 2.7
Considered as CVD diseases symptoms (Score = 6)		
Lung arterial thrombosis	26.6%	1.9
Congenital heart disease (defects from birth)	27.5%	1.0
Heart rheumatism	28.8%	1.1
Vascular stenosis disease	18.9%	1.6
Brain arterial disease	8.6%	2.9
Coronary heart disease	39.6%	1.5
The total score		10/6 = 1.7
The total knowledge score = 24		8.6

Data presented as percentage (%). The overall cardiovascular disease (CVD) knowledge score was calculated as a continuous variable by summing the respondent's scores of CVD risk factors. The maximum overall knowledge score was 24: low knowledge ≤12, moderate knowledge = 13–19, and high knowledge ≥20.

education, BP measurements, healthy diet, BMI, and family history of CVD ($P < 0.05$). Knowledge about CVD was markedly greater among the women aged 40–51 years in contrast to other age groups ($P = 0.001$, data not shown). The study participants were found to be more knowledgeable about CVD if they had reported high education levels ($P < 0.05$), a consistently healthy diet ($P = 0.003$), a normal BMI ($P = 0.03$), and a family history of CVD ($P = 0.01$).

DISCUSSION

Our study aimed to determine the level of knowledge about CVD risk factors, heart attacks, and stroke symptoms among the women and to calculate their risk, meaning the probability of an individual experiencing a CVD event over a given period. Our study results identified the knowledge gaps among our community regarding the CVD risk, and therefore would help in planning future health educational programs while addressing the prevalent needs.

Although people usually underestimate their CVD risk, the possibility of realizing that they are at a higher risk increases when they are aware of the presence of a risk factor (21). Anticipating future risks helps physicians apply primary preventive measures, such as providing health education for the person and community at risk before complications occur. The WHO member states have committed to providing counseling and therapy for at least 50% of the people aged 40 years

TABLE 4 | Factors associated with 10-year CVD risk estimate among women with intermediate and high risk ($n = 37$).

Variables	Number of women (%)	OR	95% CI	*P*-value
Age				
40–51 years	20 (54.1%)		Reference	0.02*
52–63 years	17 (45.9%)	3.81	1.533–4.712	
Marital status				
Single	10 (27%)	1.8	0.876–1.987	>0.05
Married	27 (73%)		Reference	
Education level				
Secondary and higher schooling	11 (29.7%)		Reference	>0.05
Complete university or college	26 (70.3%)	1.5	1.992–1.765	
Smoking status				
Smoker	12 (32.4%)	4.6	0.998–5.871	0.01*
Non-smoker	25 (67.6%)		Reference	
BMI classification				
Normal	5 (13.5%)		Reference	
Overweight	13 (35.2%)	4.7	2.651–6.876	0.03*
Obesity	19 (51.3%)	5.8		0.002**
Categories of blood pressure				
Normal	16 (43.2%)		Reference	
Elevated	16 (43.2%)	1.9	0.654–1.987	>0.05
Stage 1	5 (15.6%)	6.8	2.651–7.976	0.01*
An unhealthy diet				
Unhealthy diet	27 (72.9%)	3.8	1.761–4.951	0.04*
Healthy diet	10 (27.1%)		Reference	
Family's history of disease				
Yes	21 (56.8%)	3.7	1.555–4.982	0.05*
No	16 (43.2%)		Reference	
Blood analysis				
FBG (mmol/L)	3.8 ± 1.33	1.9	0.896–1.996	>0.05
Total cholesterol (mmol/L)	6.92 ± 1.8*	7.8	2.621–9.811	0.001**
LDL-cholesterol (mmol/L)	3.7 ± 0.97*	6.9	1.842–7.861	0.003**
HDL-cholesterol (mmol/L)	1.7 ± 0.67	1.7	0.643–0.898	>0.05
Triglycerides (mmol/L)	1.88 ± 1.31	1.5	0.652–1.943	>0.05

*Multiple logistic regression showed odd ratios (OR), with a 95% CI values for the factors associated with CVD risk. All differences were statistically significant at a level of *P ≤ 0.05 or **P ≤ 0.001. The analysis was done to evaluate the most risk factors between two groups.*

or older who have a high risk of CVD by 2025 (22). Most individuals in our sample were obese 45%, and 34% were overweight. In comparison, 29.4% of Lebanese, 38.7% of Bahraini are obese. Whereas, the proportion of overweight is 46.5% among the Lebanese (23) and 39.7% among the Bahraini (24). This trend of increased obesity and overweight in our study is mostly attributable to modern lifestyle changes, such as easy transportation, long office hours, and hot weather conditions, in addition to many other social factors that together inhibit healthy physical activities of walking, jogging, or outdoor playing among the Saudi population.

The prevalence of obesity as reported in the two studies in systematic Review among women in Saudi Arabia was 57.1 and 46.7%, respectively (25). Based on the data from the Framingham Offspring project, obesity, as calculated by BMI markedly and independently, influences the incidence of CHD and CVA after adjusting for the traditional risk factors (26). The higher WC of more than 88 cm among 78% of them may be due to the unhealthy diet, lack of exercise, and maintenance of gestational weight gain. This number was more than the reported percentage among the female employees in King Faisal University in Al Hassa, Saudi Arabia, where the mean WC was 91.7 ± 16.5 cm ($CI = 89.4–101.8$), 46.5% had a WC >88 cm, and 38.4% were overweight (27).

In this study, the most reported chronic disease was HTN, i.e., 53%, and that is higher than that of Lebanese 29.8% (23), Bahrainis 36.9% (24), Kuwaitis 8.9%(28), and out of a systematic review among the Saudi was 21.8% (25). This higher prevalence of HTN may be due to the inclusion of older participants in our study.

Unfortunately, HTN is a well-established risk factor for adverse cardiovascular outcomes, such as deaths from CHD and stroke (29). In a cohort of over 1.25 million patients aged 30 years and above without baseline CVD, including 20% with baseline

treated HTN, the hypertensive patients had a 63.3% lifetime risk of developing CVD compared with a 46.1% risk for those with normal baseline BP (1). Regarding diabetes among our sample, the percentage was 7.2% compared with 6.6% of Kuwaitis (28), 22.8% in a study of Lebanese (23), and a range between 4 and 5.2% among the Saudi women, according to a systematic review (25). Patients with T2DM have 2–4 times increase in the risk of incident CHD and ischemic stroke and 1.5 to 3.6-fold increase in mortality (30). Regardless of the estimated 10-year ASCVD risk, it is recommended to treat T2DM patients who are between 40 and 75 years of age with a moderate-intensity statin drug. For patients with various ASCVD risk factors, the high-intensity statin drugs are favorable for decreasing the LDL-C levels by 50% or more.

As we expected based on the data regarding BMIs and abdominal obesity of our participants, 85% of them regularly consumed an unhealthy diet. Overall knowledge of study participants was found to be 8.6, that is considered as low knowledge. Subsequently, that would lead to delay in seeking medical care, impacting negatively to the patients' outcomes. This is in line with the problem of other studies conducting in different populations worldwide, the majority of Jordanian participants were found to have poor to moderate knowledge of CVDs (31), and Kuwaiti respondents had poor knowledge of CVD types, i.e., both stroke and heart attack symptoms, but moderate knowledge of CVD risk factors (28). Muhamad et al. found limited knowledge and practice among the female patients in primary care facilities in Malaysia (32), while Fahs et al. found good knowledge of CVD risk factors among the Lebanese population (23). Concerning stroke risk factors and warning symptoms, a study of Saudis by Alreshidi et al. found that 63.8% of their participants had insufficient knowledge, attitudes, and practice (33). A second study by Alhazzani also showed a deficit in knowledge about strokes (34). The perception of individual oneself at higher risk increases when the existence of a risk factor is already known. The implementation of awareness programs targeting the population in attractive and convincing ways is a must to increase knowledge and awareness. Furthermore, the most reported stroke symptoms in this study were sudden dizziness and difficulty walking/loss of balance, reported by 63%, whereas in Awad's study (28), sudden confusion and trouble speaking or understanding others was most prevalent. In our study, like the Jordanian study (30), chest pain or discomfort was the most reported heart attack symptom 78%, and 82% reported that higher than the percentage in the Kuwaiti study 50% (28).

The results showed a higher awareness of unhealthy diet [85%], HTN [80%], obesity [79%], smoking [77%], dyslipidemia [75%], and lack of exercise [71%] as CVD risk factors. This is mainly because of the high prevalence of obesity and HTN among Saudi population who receive frequent non-pharmacological advice by healthcare providers to link these factors as contributors to CVD morbidity and mortality in their daily clinic.

Among the Kuwaiti cohort (28), tobacco, obesity, unhealthy food, and physical inactivity were commonly reported, while hypercholesterolemia, HTN, diabetes mellitus, stress, and a family history of CVD were less frequently identified as the CHD risk factors. Smoking was the most commonly reported risk factor [75.7%], followed by obesity [71.2%] and a high-fat diet [62.0%], among the Jordanian population (31). The study of the Lebanese population showed that they were most aware of smoking as a CVD risk factor and least aware of diabetes (23). This may be due to countries' variations in the prevalence of public health problems and information provided by the mass media.

In addition, in this study, the elements independently associated with CVD knowledge were age, education, smoking, consuming healthy food, and family history of CVD ($p < 0.05$).

Knowledge of CVD was considerably greater among those aged 40–51 years compared with other age groups. The study participants were observed to be more knowledgeable of CVD if they reported high education levels, healthy daily diets, and a family history of CVD. So, education is considered as an important element to increase the public health and improving the quality of life.

In the Kuwaiti study, the independent factors related to a good level of CVD knowledge were, among women aged 50–59 years, a high degree of learning, consumption of healthy food, and a family history of CVD (28). The evidence from the studies showed that the degree of education is a potent predictor of CVD knowledge as those who had obtained higher education had better CVD knowledge scores (31). Good attitude and practice were significantly associated with younger age groups and highly educated participants (33). Our study found that the women who reported having a family history of CVD had a good knowledge percentage compared with the participants without such a history, which is similar to the findings of Mukattash et al. (31) and Al Hamarneh et al. (35). This is because their awareness increases through sharing of the experiences by their family members.

The results of our research have revealed that in 13.5 and 3.2% of women with intermediate and high risks of CVD, there is a strong association between the increase in the risk of CVD and the increase in age, BMI, blood pressure, and cholesterol levels. One-quarter of the 4,500 participants in Alzeidan's study were found to have a >10% risk of suffering CVD within the next 10 years and so not only increasing morbidity and mortality but also increasing demands of tertiary healthcare (36). Another figure from Saudi research shows that among 4,932 individuals, 55% of whom were female, the risk of coronary disease within the next 10 years was low in 92.6%, intermediate in 3.2%, and high in 4.1% of the subjects (37). In a study by Saeedi et al., in which 65% of study participants were female, 69% of the participants were in a low- risk group, while 31% were in the moderate and high-risk groups[1]. Additionally, among 2,029 women in the AlQuaiz study, 83% were low risk (Score <10), and 17% had intermediate-to-high Framingham risk scores (38). Lastly, the CVD risk factors must be well-known at national level for entire population to decrease curve of death rate related to CVD.

[1]https://journalissues.org/wp-content/uploads/2018/03/Saeedi-et-al.pdf

Limitations and Strengths

A smaller sample size and inclusion of only female participants limit the generalizability of results. The precision of the study might be low because of the relatively small sample size, thus generalizability of the findings to the larger Saudi population was difficult. In addition, limited comparison with data from studies conducted in the Arab population with specific dietary habits and lifestyles that may consider expansion and preparing a separate paper in our future study on the CVD risk and lifestyles habit. Furthermore, the awareness and knowledge of risk factors are investigated, but not perception. On the other hand, this study addresses a pertinent issue of CVDs and their risk factors in Madinah city will add to the current knowledge of CVD in KSA and the Gulf countries, carried among an educated sample that will help later to raise the awareness of CVD risk factors in our community. The epidemiological investigation tools used are valid and reliable. The relation between dietary habits and CVD risk factors will be studied in a separate paper.

CONCLUSIONS

Low levels of knowledge regarding CVD risk factors were reported among the participants. There was a high percentage of obesity and increased abdominal circumference among them. HTN was a significant chronic disease as more than half of the sample reported it. Frequently recognized risk factors were unhealthy diet, HTN, obesity, smoking, dyslipidemia, and lack of exercise. In this study, the factors independently associated with CVD knowledge were age, education, smoking, eating a healthy diet, and family history of CVD.

Health stakeholders may use these results for future planning of educational programs to promote the awareness of risk factors and prevention of CVD in Madinah. Primary prevention strategies can positively reduce the ASCVD risk if certain healthy behaviors are adopted early and continued throughout life, and these strategies will help people avoid developing established ASCVD risk factors, such as high BP, obesity, and hyperlipidemia. However, more studies that include larger samples of both gender from different region in Saudi kingdom are needed to validate the findings of this study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s. The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AQ conceptualized the idea of research based on the importance and relevance of the topic and contributed to literature search, provided research materials, collected and organized data and references, and provided logistic support. WM identified the appropriate methods of analysis, interpreted the results of the study, and wrote the final draft of the article. AS helped in enriching references and writing discussion of the study. All authors have contributed to writing, designing, critically review and so approved the final draft, and are responsible for the content included here.

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REVIEW
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Relationship Between Sarcopenia and Cardiovascular Diseases in the Elderly: An Overview

Nana He[1,2,3], *Yuelin Zhang*[4], *Lu Zhang*[1], *Shun Zhang*[2,3] *and Honghua Ye*[1]*

[1] Department of Cardiology, HwaMei Hospital (Previously Named Ningbo No. 2 Hospital), University of Chinese Academy of Sciences, Ningbo, China, [2] Department of Experimental Medical Science, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China, [3] Key Laboratory of Diagnosis and Treatment of Digestive System Tumors of Zhejiang Province, Ningbo, China, [4] Department of Medicine, University of Ningbo, Ningbo, China

With the advent of population aging, aging-related diseases have become a challenge for governments worldwide. Sarcopenia has defined as a clinical syndrome associated with age-related loss such as skeletal muscle mass, strength, function, and physical performance. It is commonly seen in elderly patients with chronic diseases. Changes in lean mass are common critical determinants in the pathophysiology and progression of cardiovascular diseases (CVDs). Sarcopenia may be one of the most important causes of poor physical function and decreased cardiopulmonary function in elderly patients with CVDs. Sarcopenia may induce CVDs through common pathogenic pathways such as malnutrition, physical inactivity, insulin resistance, inflammation; these mechanisms interact. In this study, we aimed to investigate the relationship between sarcopenia and CVDs in the elderly. Further research is urgently needed to understand better the relationship, pathophysiology, clinical presentation, diagnostic criteria, and mechanisms of sarcopenia and CVDs, which may shed light on potential interventions to improve clinical outcomes and provide greater insight into the disorders above.

Keywords: sarcopenia, cardiovascular diseases, elderly people, comorbidity, aging

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***Correspondence:**
Honghua Ye
lindayenbzjch@163.com

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INTRODUCTION

Sarcopenia is a progressive and widespread decline in skeletal muscle mass and function, leading to the loss of workforce and mobility in the elderly. The onset and progression of sarcopenia are closely associated with old age, skeletal muscle disuse, malnutrition, chronic systemic inflammation, and anabolic disorder. Sarcopenia puts a great deal of pressure on society by significantly increasing hospitalization and mortality rate in elderly patients (1, 2). Sarcopenia is a relatively common disease that causes impaired exercise capacity in the elderly, strongly associated with CVDs. Aging and dysfunction of the locomotor system limit exercise in the elderly, increasing the risk of CVDs. CVDs and sarcopenia can coexist, further reducing exercise tolerance and quality of life and increasing mortality. Sarcopenia and CVDs interact to accelerate the disease process (3, 4). As the global population ages, the number of patients with CVDs and sarcopenia increases, and the resulting health problems such as loss of work capacity and mobility in the elderly are of concern. Exploring the mechanism between sarcopenia and CVDs could provide a scientific basis for clinical diagnosis and treatment.

SARCOPENIA

Definition

Derived from the Greek words sarx (muscle) and penia (deficiency), sarcopenia is a chronic degenerative senile syndrome similar to osteoporosis. It has a significant impact on the quality of life of the elderly. Sarcopenia was first introduced by Rosenberg in 1989 and refers to age-related muscle loss and muscle strength decline (5). Initially, the understanding of "sarcopenia" was limited to reducing skeletal muscle mass with age in older people. Subsequently, it was discovered that the muscle mass of skeletal muscles is not linearly related to muscle strength and function and that a decline in pure muscle mass is not significantly associated with functional status in older people. For a long period of time, research on sarcopenia did not make much progress. It was not until 2010 (6) that the European Working Group on Sarcopenia in Older People (EWGSOP) first proposed a clinical definition of sarcopenia. It is recommended that both skeletal muscle mass and function (muscle strength or mobility) be reduced for a diagnosis of sarcopenia to be made. The definition of sarcopenia also changed from the initial decades of focusing only on the muscle mass of skeletal muscle to focus on both a decrease in muscle mass and muscle strength and more on changes in function. In 2011 (7), the International Working Group on Sarcopenia (IWGS) provided a similar definition of sarcopenia, emphasizing physical function evaluation, including the ability to sit up from a chair or a pace test. In 2014 (8), the Asia Working Group for Sarcopenia (AWGS) and the Foundation for the National Institutes of Health (FNIH) also launched their respective expert consensus on sarcopenia. In 2018 (9), the EWGSOP, based on the latest results of basic and clinical research on sarcopenia in the past 10 years, renewed its consensus. EWGSOP defines sarcopenia as a syndrome of progressive and generalized decline in skeletal muscle mass associated with low muscle strength or low physical performance. In addition, the EWGSOP newly identifies subcategories of sarcopenia as acute and chronic. In addition, sarcopenia lasting for <6 months is considered an acute disease, while those with an illness for more than six months are considered a chronic disease. In 2019 (10), the AWGS also updated its consensus on sarcopenia. Sarcopenia is age-related loss of muscle mass associated with low muscle strength and or low physical performance. The EWGSOP definition of sarcopenia is now widely used internationally. Sarcopenia is associated with various adverse outcomes, including falls, dysfunction, weakness, and death. Sarcopenia is now officially recognized as a muscle disorder with the diagnosis code ICD10-MC, suggesting that it will receive widespread attention from the medical community as a disorder with unique characteristics and a more accurate understanding of screening, diagnosis, intervention, and treatment of the condition (11).

Diagnosis

Due to the lack of specific clinical manifestations of sarcopenia and the fact that human muscle mass is affected by various factors such as race, region, age, and gender, there is currently no unified standard for diagnosing sarcopenia at home and abroad.

TABLE 1 | The specific latest diagnostic criteria for sarcopenia.

Criterion	Muscle mass	Muscle strength	Muscle function
EWGSOP2	ASM/height^2 <7.0 kg/m^2 in men ASM/height^2 <6.0 kg/m^2 in women	Handgrip<27 Kg in men Handgrip<16 Kg in women	Walking speed≤0.8 m/s
AWGS2	ASM/height^2 <7.0 kg/m^2 in men ASM/height^2 <5.4 kg/m^2 in women	Handgrip<28 Kg in men Handgrip<18 Kg in women	Walking speed<1.0 m/s
IWGS	ASM/height^2 <7.23 kg/m^2 in men ASM/height^2 <5.67 kg/m^2 in women	- -	Walking speed<1.0 m/s
FNIH	ASM/BMI <0.789 in men ASM/BMI <0.512 in women	Handgrip<26 Kg in men Handgrip<16 Kg in women	Walking speed≤0.8 m/s

EWGSOP (9), AWGs (10), IWGS (7), FNIH (12) all state that the diagnosis of sarcopenia should take into account a combination of muscle mass and muscle function, with the leading indicators including muscle mass, muscle strength, and muscle function. Specific updated diagnostic criteria for sarcopenia are shown in **Table 1.** The prevalence of sarcopenia varies with different diagnostic criteria and different measures of muscle mass. The prevalence of sarcopenia in the elderly in the community ranges from 8.7 to 28.5% under different diagnostic criteria (13).

Epidemiological Characteristics

Epidemiological studies have found that skeletal muscle begins to degenerate after the 40 and accelerates with age. The quantity and quality of skeletal muscle are declining at a rate of ∼8% per year (14). Due to differences in study populations, research methods and diagnostic criteria, the prevalence of sarcopenia varies greatly between different studies. The prevalence of sarcopenia in people over 50 years of age ranges from 1–33% (11). In particular, the prevalence of sarcopenia ranges from 5 to 13% in people aged 60 to 70 years and up to 50% in people aged 80 years and older (15). There are also significant differences in the incidence of sarcopenia in different regions. The prevalence of sarcopenia is 38.9% in males and 17.8% in females in Canada (16). In Australia, the prevalence of sarcopenia is <20% in the population aged 70 years or older (17). In the UK, the prevalence of sarcopenia in men and women is 4.6 and 7.9%, respectively (18). Research on sarcopenia in the Asian population is still in its infancy. In Asia, the incidence of sarcopenia in Thailand was 35.33% in men and 34.74% in women, respectively; in Japan, it was 6.7∼11.3% in men and 6.3∼11.7% in women; in South Korea, it was 6.3∼21.8% in men and 4.1∼22.1% in women; in China, it was 12.3% in men and 7.6% in women (8). The study included 200 elderly inpatients with an average age of 74.49 ± 6.32 years. Sarcopenia was detected in 28 (14%) of the patients. Among them, the in-hospital mortality rate of patients with sarcopenia was 28.6%, and that of patients without sarcopenia was 11.0%. There is

an increased length of stay and mortality in older inpatients with sarcopenia (1). Differences in the prevalence of sarcopenia may be related to ethnicity, lifestyle, exercise habits, and the use of quantitative diagnostic criteria adopted by different research institutes. It is estimated that by 2050, there will be more than 200 million elderly patients with sarcopenia in the world (6). Sarcopenia can increase the risk of weakness, falls and fractures, decrease the quality of life, even the ability to live independently, and increase the infection rate, and mortality rate in patients. Sarcopenia is associated with CVDs, diabetes, renal insufficiency, cancer, cognitive impairment, and even with the prognosis of some diseases (19–22).

SARCOPENIA AND CVDS

The main feature of sarcopenia is skeletal muscle disorders such as loss of muscle mass, quality, strength, and physical performance. It is commonly seen in elderly patients with chronic diseases. Sarcopenia may be considered one of the most important causes of poor physical performance and reduced cardiorespiratory fitness in older patients with CVDs. CVDs may induce sarcopenia through common pathogenetic pathways such as hormonal changes, malnutrition, and physical inactivity, mechanisms that influence each other. Sarcopenia is also an age-related disease closely related to CVDs, and there are similarities between the two in terms of risk factors and pathogenesis.

Sarcopenia and Heart Failure (HF)

The prevalence of sarcopenia is high in older HF patients, with sarcopenia also predictor of HF prognosis. Patients with HF are often associated with decreased muscle and strength. The ubiquitin protease system, myogenic protein signaling pathways, apoptosis, malnutrition due to gastrointestinal edema, and inflammatory factors may all contribute to sarcopenia (23, 24). The impaired exercise tolerance of HF patients is related to the changes of failed cardiomyocytes and skeletal muscle cells. The onset, development, and progression of sarcopenia follow the same clinical course as HF, with the two interacting. One SICA-HF study covered 200 HF patients, with the average age of the patients was (70.8 $\pm$ 8.3) years. It was showed a prevalence of sarcopenia of 19.5% in patients with HF (25). Another study reported that 19.7% of HF patients with preserved ejection fraction have sarcopenia (26).

The coexistence of sarcopenia and HF may be the result of their common pathophysiological pathways. Skeletal muscle in patients with HF has multiple histological abnormalities, and 2/3 of patients with chronic heart failure (CHF) have myofibrillar atrophy and decreased muscle capillary density (27). Moreover, few studies have shown that the observable skeletal muscle fiber atrophy rate observable in patients with CHF is ∼68% (28). The imbalance of muscle protein synthesis and decomposition is a major factor in the development of sarcopenia (29). Oxidative stress can accelerate skeletal muscle degeneration and increase muscle protein decomposition. Levels of inflammatory markers tend to be elevated in patients with HF. Studies have shown that high levels of inflammatory cytokines are negatively associated with muscle strength and mass (30). In patients with heart failure and sarcopenia, the level of growth hormone (GH) is increased, while the level of insulin-like growth factor-1(IGF-1) is significantly reduced, suggesting that there may be GH resistance, leading to an inhibition of skeletal muscle formation. In patients with HF, the PI3K/Akt/mTOR signaling pathway involved in regulating protein synthesis is inhibited, while the ubiquitin-protease system that promotes protein breakdown, autophagy, and apoptosis are overactivated, and the dynamic balance between skeletal muscle production and destruction is broken, and then sarcopenia (31). In addition, patients with HF may suffer from poor appetite and malabsorption due to urinary difficulties, nausea, adverse drug reactions, which leads to inadequate or excessive nutrient loss and gastrointestinal symptoms and is associated with the pathogenesis of sarcopenia. Reduced peripheral perfusion due to left ventricular insufficiency from HF and reduced physical activity, which limits daily activities, can also cause a reduction in skeletal muscle, leading to the development of sarcopenia (30). Paradoxically, sarcopenia is not associated with a sarcopenia cardiac muscle, but the cardiac muscle shows hypertrophy which seems to be "not-functional." Physiological cardiac hypertrophy usually occurs during pregnancy and in athletes, while pathological hypertrophy induces by factors such as prolonged and abnormal hemodynamic stress (i.e., hypertensive state), which can lead to cardiac dysfunction. The cardiac mass modification and dysfunction process, called "cardiac sarcopenia," is similar to what happens in skeletal muscle, but few current studies exist. In a FLEAR study of elderly hospitalized patients, it was found that 19.4% of patients had sarcopenia and HF; in the absence of sarcopenia, the prevalence of HF was 16.3%. Through an echocardiographic study, it was discovered a correlation between sarcopenia and cardiac hypertrophy (32). Previous studies have shown a negative correlation between grip strength and heart mass in patients at risk of sarcopenia. More importantly, the decrease of muscle strength is associated with the increase of ventricular mass and the reduction of ejection fraction, resulting in "not-functional cardiac hypertrophy" (33–35). Heart failure with preserved ejection fraction (HFpEF) represents an important cardiovascular entity with increasing prevalence and relatively high mortality. Therefore, the earliest description of HFpEF is mainly conceptualized as a diastolic filling disorder. Only later inflammation and multimorbidity, which play a key role in the development of sarcopenia, are considered the main factors in developing HFpEF (36, 37). In this case, a sarcopenia heart characterized by a "not-functional hypertrophy" may be considered as an intriguing hypothesis. Thus it can be seen that the interaction and mechanism between sarcopenia and HF are very complicated.

Sarcopenia and Hypertension

At present, there are few studies on the relationship between sarcopenia and hypertension. A total of 1,611 Chinese elderly people aged $\geq$60 years, who were diagnosed and assessed according to the AWGS recommended algorithm, had been included in a study on the relationship between sarcopenia and cardiovascular risk factors (CVRF), including diabetes, hypertension, and dyslipidemia, was analyzed. The results

showed that the high prevalence of sarcopenia in the Chinese elderly population is related to CVRF. In addition, diabetes and hypertension, rather than dyslipidemia, were significantly associated with sarcopenia. It indicated that CVRF, especially diabetes and hypertension, may help predict the risk of sarcopenia in the elderly (4). Some studies put forward the concept of "sarcopenia obesity," specifically referring to the coexistence of sarcopenia and fat accumulation. A follow-up study of 3,320 people in Korea found that the 10-year risk of CVDs in obese patients with sarcopenia was higher than those with non-obesity and non-sarcopenia. In contrast, the 10-year risk of CVDs in patients with simple obesity or sarcopenia was not significantly increased. A British study followed up 4,252 older men and found that patients with sarcopenia obesity highly correlated with CVD mortality. Patients with sarcopenia obesity have a higher mortality rate, but the study did not observe an increase in CVD incidence. Studies have also shown that patients with sarcopenia obesity are at high risk of developing type 2 diabetes mellitus, hypertension, and hyperlipidemia (38–40). In the early stage, it was considered that sarcopenia caused by aging is related to an increased incidence of hypertensive retinopathy and hypertensive kidney damage. More recently, it has been found that hypertension is related to the decrease in the number of capillaries around muscle cells (41). Therefore, it has been hypothesized that blood pressure-induced changes in the capillary network of muscle tissue are one of the risk factors for the occurrence of sarcopenia in elderly patients.

Sarcopenia and Atherosclerosis

Atherosclerotic cardiovascular diseases (ACVDs), such as coronary atherosclerotic heart disease, atherogenic stroke or transient ischemic attack, transient ischemic attack, and peripheral artery disease, are acute diseases that affect the health of older people. They are also a direct cause of death, disability, and high medical costs. Exploring the related comorbidities and risk factors, looking for reliable prognostic markers has become a current research hotspot. A study of 335 Japanese subjects (mean age 64.9 years) found that risk factors for atherosclerosis (blood pressure, cholesterol) were significantly higher in the sarcopenia group than in the control group ($P < 0.05$), after controlling for age, gender, and body mass index (BMI) (42). A large-scale cohort study showed that early atherosclerosis index carotid-femoral pulse wave velocity (PWV) independently and negatively correlated with skeletal muscle mass in Americans aged 70 to 79 (43). The decline in muscle mass and muscle strength was associated with endothelial dysfunction in another study of 208 Brazilian people over 80 years of age, with a 3.6-fold increased risk of atherosclerosis due to a decrease in muscle mass, suggesting that sarcopenia is strongly associated with atherosclerosis. This correlation was also found in middle-aged people, and the results were more precise in middle-aged men (44). A cross-sectional study of 31,108 middle-aged Koreans found that relative limb skeletal muscle index (RSMI) linearly correlated with the prevalence of coronary heart disease (CHD) and coronary artery calcification (CAC) score. After excluding insulin resistance or lack of physical activity, low muscle mass remained an independent risk factor for CHD (45). In addition, another study used ultrasound to measure the intima-media thickness (IMT) in 321 patients with ischemic heart disease to determine arteriosclerosis and used an index of muscle function as a criterion to determine sarcopenia. The carotid artery thickness is divided into two groups (IMT $\leq$ 2.6 mm) and low (MT > 2.6 mm). The results show a significant correlation between the lower limb muscle function index (step speed) and the isometric strength of the quadriceps in patients with ischemic heart disease and arteriosclerosis. In contrast, the grip strength does not correlate with it (46). Therefore, it can be concluded that reducing skeletal muscle is an independent risk factor for arteriosclerosis vascular disease and is closely related to other risk factors of arteriosclerosis.

Atherosclerosis develops from cellular and molecular inflammation, a potential factor in sarcopenia (47). Inflammatory factors such as tumor necrosis factor (TNF) and interleukin-6 (IL-6) are essential catabolic factors, which stimulate protein catabolism, inhibit muscle synthesis, and promote muscle atrophy. TNF-α promotes early atherosclerosis by increasing the transcytosis of low-density lipoprotein (LDL) across endothelial cells (48). IL-6 is an upstream regulator and plays a central role in promoting downstream inflammatory response, the leading cause of atherosclerosis. The circulating levels of IL-6 have been independently associated with subclinical atherosclerosis in several studies (49). While other studies have shown that multiple inflammatory factors are negatively associated with muscle mass or muscle strength, elevated cytokines, particularly IL-6, may be a confounding factor in the underlying pathology. This study also found blood pressure, total serum cholesterol, LDL-cholesterol, and high-sensitivity C-reactive protein (hs-CRP) levels in community populations with sarcopenia. Multifactorial analysis showed that independent risk factors for sarcopenia caused serum hs-CRP levels. As a predictor of cardiovascular events, hs-CRP may be related to physical function, and experiments have shown that high CRP levels are related to the risk of losing muscle strength. One of the mechanisms is that high LDL levels in atherosclerosis induce endothelial cells to express CRP, which increases the expression of the endothelial receptor for oxidized low-density lipoprotein (LOX-1) and promotes the occurrence of arteriosclerosis (50). It is speculated that the determination of serum CRP level could be used to assess the risk of arteriosclerosis and screen for skeletal muscle reduction. Oxidative stress is crucial to the pathogenesis of atherosclerosis, and it also plays a vital role in sarcopenia. It was found that disruption of redox homeostasis in motor neurons of Cu/Zn superoxide dismutase knockout mice triggered disruption of neuromuscular junctions, which combats skeletal muscle mitochondrial function and increases the production of reactive oxygen species. When the production of reactive oxygen species in skeletal muscle grows through muscle feedback, the retrograde response is triggered, further impingement of the neuromuscular junction. This vicious circle eventually leads to the breakdown of the neuromuscular junction, denervation and muscle fiber loss, and the occurrence of sarcopenia (51). It can be seen that inflammation and oxidative stress are the common pathogenesis of sarcopenia and atherosclerosis.

Sarcopenia and CHD

Sarcopenia is closely related to the onset and prognosis of CHD in the elderly and is an independent risk factor for the onset and poor prognosis of CHD in the elderly. At the same time, it is also a risk factor for atherosclerosis in elderly patients and a predictor of poor prognosis for elderly patients with percutaneous coronary intervention (PCI). Studies have extensively shown that sarcopenia may be involved in the development and progression of CHD (3, 30, 52). The study included a total of 475 elderly patients with coronary artery disease who underwent successful PCI to assess sarcopenia by measuring the cross-sectional area of skeletal muscle at the first lumbar vertebra (L1) and exploring the impact of low skeletal muscle mass on the prognosis of patients with coronary artery disease who underwent successful PCI. The results suggest that 29.7% of patients have low L1 skeletal muscle index (SMI). A low L1 SMI is an independent predictor of all-cause mortality and major adverse cardiovascular events (53). A study included a total of 345 Chinese older patients with CHD, with a median age of 74 years. Among the patients, 78 (22.6%) were diagnosed with sarcopenia, according to AWGS. The purpose of this study was to explore the prevalence and prognostic significance of sarcopenia in elderly patients with CHD. The results show that the prevalence of sarcopenia was very high in hospitalized elderly patients with CHD. The incidence of unscheduled follow-up visits in elderly CHD patients with sarcopenia was higher than that in patients without sarcopenia (54). A study used AWGS criteria to assess sarcopenia in 354 elderly patients with CHD over 65 years of age and found that 22.6% were patients with sarcopenia. Its follow-up found that elderly CHD patients with sarcopenia were significantly more than non-sarcopenia patients. The time of no adverse cardiac and brain events was significantly shorter than that of non-sarcopenia patients (54). A study also included 99 patients with acute myocardial infarction with a mean age of 71.6 years. Patients were diagnosed and evaluated for sarcopenia regarding the diagnostic indicators of EWGSOP. The results showed that the prevalence of sarcopenia was 64.6%, which was higher than that of normal people. The prevalence of sarcopenia was much higher in male patients than in female patients, and sarcopenia was associated with thrombolysis-related myocardial infarction scores (55). The study assessed skeletal muscle mass in 378 patients with ST-segment elevation acute myocardial infarction. All-cause death, non-fatal myocardial infarction, non-fatal ischemic stroke, hospitalization for congestive heart failure, and unplanned revascularization were used as the endpoints of long-term follow-up. The results showed that the lower limb skeletal muscle index was still independently associated with the high risk of primary complex events (56). In addition, some studies have shown that muscle mass and muscle strength are negatively correlated with the increase of coronary artery calcification score. Furthermore, muscle mass is positively correlated with coronary artery diastolic ability, suggesting that sarcopenia is related to subclinical coronary atherosclerosis (44). Loss of muscle mass correlates with coronary artery calcification, an independent risk factor for CHD. Decreased skeletal muscle mass also increases the risk of death in patients with CHD (57). Reduced muscle mass diagnosed by CT is a strong predictor of poor prognosis in patients with CHD who undergo percutaneous coronary intervention (53).

The mechanism of the role of sarcopenia in CHD is currently unclear. Studies have shown that sarcopenia and obesity form a vicious circle in the body and then participate in the occurrence and development of CVDs led by CHD through a series of mechanisms such as insulin resistance, mitochondrial dysfunction, oxidative stress, adipokines, and inflammatory factors (58).

THE PATHOGENESIS OF SARCOPENIA AND CVDS

Sarcopenia is a multi-cause disease with risk factors including lifestyle, changes or imbalances of hormones and inflammatory factors, imbalances of protein synthesis and decomposition, motor unit reconstruction, development, and evolution. Sarcopenia often coexists with CVDs, tumors, chronic non-obstructive disease, chronic kidney disease, endocrine disease, and rheumatic immune disease (59). Various causes of inhibition of skeletal muscle cell proliferation signaling pathways and excessive activation of apoptotic signaling pathways can disrupt the dynamic balance between muscle production and destruction, ultimately leading to diseases (60).

Malnutrition, physical inactivity, insulin resistance, inflammation, hormonal changes, autophagy, apoptosis, and oxidative stress are involved in the occurrence of CVDs and sarcopenia (61). Sarcopenia and CVDs are closely related and interact to influence the course of the disease. In addition, CVDs aggravate sarcopenia's adverse outcomes, including falls, fractures, frailty, cachexia, hospitalization, and mortality. At the same time, the prevalence of CVDs in sarcopenia patients is significantly increased, such as HF, hypertension, atherosclerosis, and CHD (**Figure 1**). HF leads to peripheral ischemia and hypoxia, induces skeletal muscle cell apoptosis, even necrosis, muscle atrophy, and decreases exercise ability; the reduced or lost exercise capacity caused by sarcopenia leads to obesity, dyslipidemia, inflammatory reaction, insulin resistance, and then promotes CVDs (3, 30, 62). The pathophysiological mechanism underlying sarcopenia and CVDs is unclear and progressively focused on and explored by researchers. It was suggested that mechanisms such as inflammation, oxidative stress, and insulin resistance might also be involved in the occurrence and development of CVDs and sarcopenia in the elderly. The mechanism between sarcopenia and CVDs can be understood from the following aspects.

Inflammation

Studies have shown that long-term systemic chronic inflammation seems to be involved in the whole process of CVDs, and sarcopenia in the elderly (63–65). Senescence-associated secretory phenotype (SASP) is one of the key factors in chronic inflammation-induced atherosclerotic plaque instability, part of the pathogenesis of atherosclerosis (63) and an independent risk factor for myocardial infarction

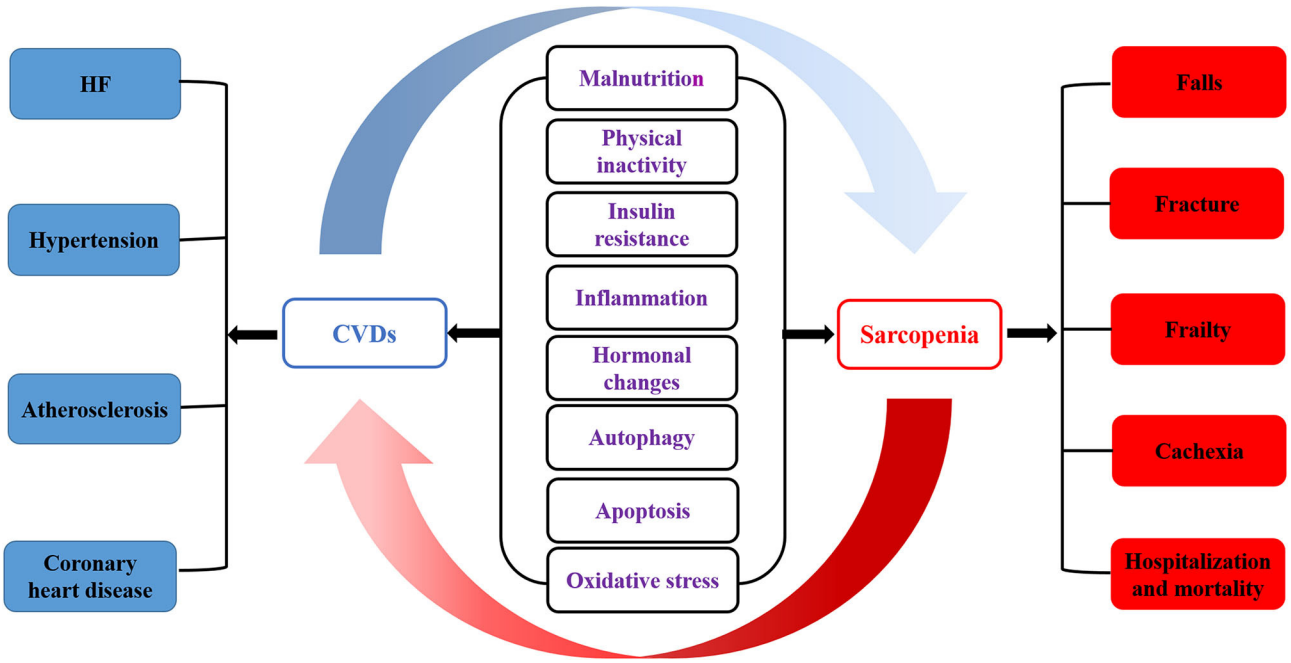

FIGURE 1 | The pathogenesis of sarcopenia and CVDs. Malnutrition, physical inactivity, insulin resistance, inflammation, hormonal changes, autophagy, apoptosis and oxidative stress are involved in the occurrence of CVDs and sarcopenia. Sarcopenia is closely related to cardiovascular disease, which affects each other's course of disease. In addition, CVDs aggravates the adverse outcomes of sarcopenia, including falls, fracture, frailty, cachexia, hospitalization and mortality. At the same time, the prevalence of CVDs in sarcopenia patients is significantly increased, such as HF, hypertension, atherosclerosis and CHD.

and cardiovascular death (66). As an upstream factor in the inflammatory response, IL-6 reflects the level of systemic inflammation and can prompt the level of systemic catabolism and promote the downstream inflammatory response (67). Studies have confirmed that the long-term activation of the IL-6 signaling pathway is significantly related to the degree of atherosclerosis in elderly patients (49). Selective inhibition of the IL-6 signaling pathway and reduction of systemic inflammatory levels can substantially reduce the incidence of cardiovascular events (68). As individuals age, the body's adipose tissue tends to increase, and levels of free cholesterol and fatty acids rise, which can induce a rise in chronic systemic inflammation by converting M2 macrophages into pro-inflammatory M1 macrophages that produce pro-inflammatory factors such as IL-6 (47). In addition, it was found that the level of IL-6 in patients with sarcopenia is independently related to the occurrence of sarcopenia (47). IL-6 can promote the catabolism of skeletal muscle and cause muscle atrophy. The increase of IL-6 concentration in the blood circulation is related to the severity of HF and the activation of the sympathetic system (69). Inflammation in heart failure patients may promote the development of sarcopenia. The SICA-HF study observed that in patients with HF, IL-6 was significantly higher in the sarcopenia group than in the non-sarcopenia group, but IL-1β and tumor necrosis factor-α did not differ significantly between the two groups (25, 70). Studies have also shown that inflammation activates the body's catabolic pathways, promotes the hydrolysis of muscle protein, leads to an imbalance between protein synthesis and catabolism, and contributes to sarcopenia development (71).

Oxidative Stress

During the aging process, the body produces large amounts of reactive oxygen species (ROS) due to changes in the function of the respiratory chain; and as the defense function of antioxidant cells is impaired, the ROS produced are not cleared in time and accumulate in the body (72). This is when the body is in a state of oxidative stress. The increased level of oxidative stress in the body can lead to various CVDs such as hypertension, atherosclerosis, myocardial infarction, HF, and arrhythmia (73). The main reasons are as follows: (a) the increase of ROS in the body causes vasoconstriction and promotes arterial hypertension; (b) ROS can negatively affect cardiac calcium processing, cause arrhythmia, and induce hypertrophic signaling and apoptosis to increase cardiac remodeling; (c) ROS has been shown to promote the formation of atherosclerotic plaques; (d) ROS can cause vascular endothelial dysfunction in patients with CVDs and cause adverse cardiovascular events (74–76). Oxidative stress is a common mechanism in many age-related diseases. As we age, the body's antioxidant capacity decreases significantly. The accumulation of ROS in the body will affect the nitrification, nitrosation, carbonylation, and glycation of proteins, thereby affecting muscle protein synthesis (77). At the same time, ROS can also mediate and enhance the hydrolysis of muscle protein, leading to sarcopenia (77). Furthermore, obese patients with sarcopenia have significantly increased levels of circulating

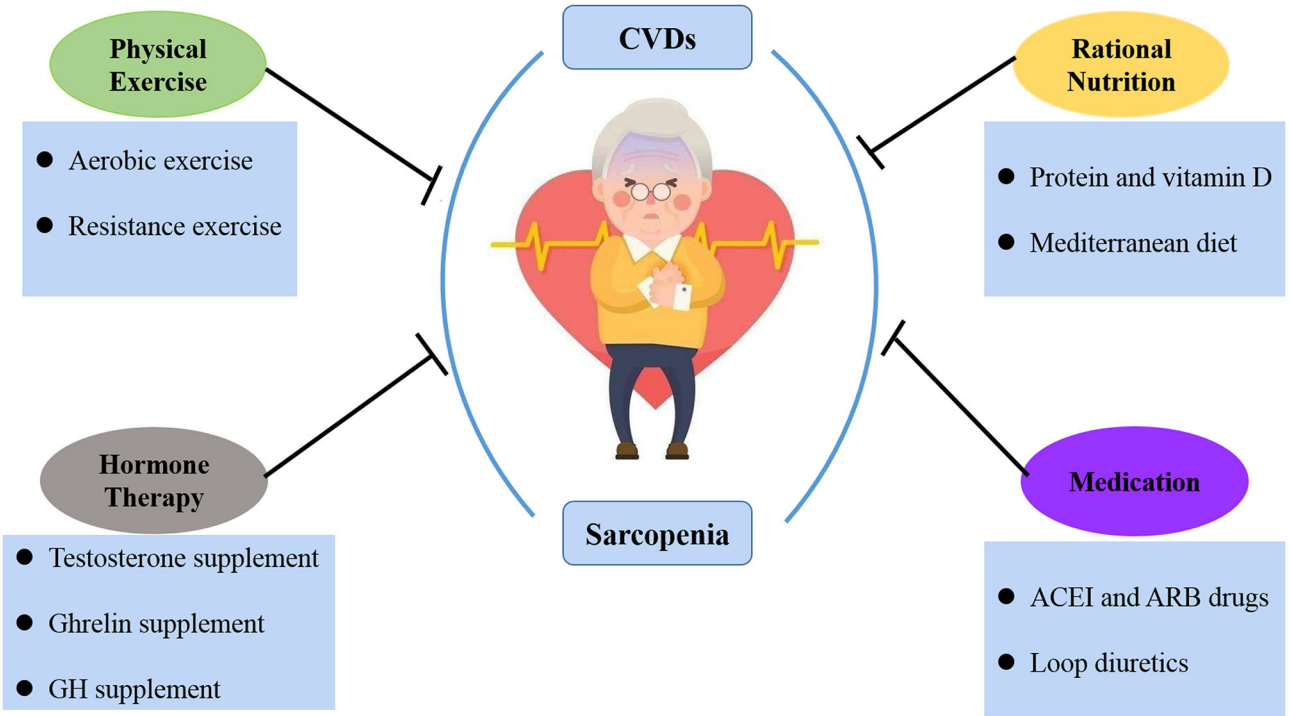

FIGURE 2 | The treatment of sarcopenia and CVDs. At present, the joint intervention of sarcopenia and CVDs is mainly from physical exercise, rational nutrition, hormone therapy and medication.

oxidative stress and are significantly associated with CVDs risk in such patients (78).

Insulin Resistance

In recent years, factors related to metabolism have been extensively studied. Insulin resistance is the most representative pathway, and it seems to be related to sarcopenia and CVDs. Many studies have provided reliable clinical evidence, suggesting that insulin resistance is a major cardiovascular risk factor independent of other risk factors in CVDs in older adults in community populations and patients with type I and type II diabetes (79). In patients with ischemic stroke, insulin resistance is independently associated with poor functional prognosis after acute ischemic stroke (80, 81). Skeletal muscle is the leading site of glucose uptake, deposition, and actin secretion, which protect insulin resistance. A reduction in muscle mass can lead to insulin resistance. When the body becomes insulin resistant, on the one hand, insulin secretion in the body is reduced. Glucose homeostasis is disrupted, leading to glucose utilization disorders, while the muscle is an essential organ for the body to absorb and utilize glucose, making its energy supply to muscle significantly reduced; On the other hand, the metabolism of skeletal muscle in limbs of the body increases significantly, and the dysfunction of muscle microvascular function will substantially affect the function and state of skeletal muscles, which will lead to the decrease of skeletal muscle content and sarcopenia (82–84).

JOINT INTERVENTION OF SARCOPENIA AND CVDS

Currently, the central combined interventions for sarcopenia and CVDs come from physical exercise, proper nutrition, hormone therapy, and medication (**Figure 2**).

Physical Exercise

Physical exercise is an economical, safe, and effective intervention for both CVDs and sarcopenia. Physical exercise methods include active and passive exercise and aerobic and resistance exercise, increasing muscle mass and strength, improving exercise and balance ability, and reducing falls and fractures (52, 85–88). Aerobic exercise can be carried out with simple equipment such as swimming, jogging, and push-ups or with the aid of elastic bands. These exercises effectively prevent CVDs that may be caused by an immediate increase in heart rate and blood pressure (89). Resistance exercise can increase muscle volume and muscle contractility and improve submaximal exercise endurance in patients with heart failure. Studies have shown that a combination of aerobic and resistance exercise can increase the effectiveness of exercise rehabilitation. Medium to long-term resistance training, aerobic exercise, and other forms of exercise or a mixture of exercise can significantly improve muscle strength, increase muscle mass, slow the progression of HF and inhibit skeletal muscle breakdown in some patients with sarcopenia (90). In one study, patients with chronic heart failure

were found to have significantly improved endurance and 6-min walking distance in all muscle groups after 10 weeks of high-intensity progressive resistance exercise (91). Recent studies have further confirmed the importance of exercise training in suppressing inflammatory factors, reducing oxidative stress, reducing myostatin expression, and inhibiting the ubiquitin protease system in patients with HF (92).

Rational Nutrition

Nutritional intervention is currently the primary prevention and treatment method for sarcopenia, including supplementation of protein and amino acids (β-Hydroxy-β-Methyl butyrate (HMB), antioxidants, long-chain fatty acids, vitamin D, and creatine) (93, 94). Protein and vitamin D intake is crucial to the prevention and treatment of sarcopenia. Protein is essential for muscle metabolism in the body. Essential amino acids such as leucine and isoleucine are necessary to promote muscle protein synthesis (95, 96). There are still controversies regarding vitamin D supplementation for the prevention and treatment of sarcopenia. The correlation between vitamin D level and muscle mass is poor, but it can slightly improve muscle strength (97, 98). In addition, HMB is the active metabolite of the essential amino acid leucine and has a critical interventional effect on sarcopenia. It has been shown to inhibit muscle proteolysis, promote muscle protein synthesis, inhibit muscle protein decomposition, maintain cell membrane integrity, improve immunity and reduce inflammation. A meta-analyses systematic study showed that nutritional supplementation with HMB can enhance lean muscle mass and preserve muscle strength and function in the elderly with sarcopenia or frailty (99). A review of Clinical Trials showed that HMB supplementation is essential for the maintenance of muscle mass in the elderly over 65 years old, especially the elderly who are bedridden or sedentary, and contributes to the reduction of muscle metabolism. Many studies have shown that HMB increased muscle mass and strength in older people with reduced lean body mass (100–102). In addition, studies have shown that HMB supplementation has a positive effect on lowering plasma cholesterol and blood pressure, thereby reducing the risk of cardiovascular disease (103–105). It was shown that HMB slows HF progression by maintaining lean body mass and limiting the effects of cachexia. Therefore, HMB is likely to be crucial for the nutritional management of patients with HF-induced cachexia (106). Therefore, nutritional support is essential for the recovery of sarcopenia patients. The Mediterranean diet is an ideal diet for patients with CHD, rich in nutrients and balanced. The Mediterranean diet also helps delay muscle wasting in the elderly and reduces the risk of sarcopenia (107). As with exercise, patients need to adhere to an appropriate diet for a long time to achieve good outcomes.

Hormone Therapy

Some studies have shown that supplementing testosterone is beneficial to muscle and skeletal tissues (108, 109), particularly in increasing muscle strength, improving mobility, and reducing the hospitalization rate of elderly patients with sarcopenia (110). Decreased testosterone can cause fatigue and weakened exercise capacity, while testosterone supplementation can increase muscle strength and improve exercise capacity (111). At lower doses, testosterone increases protein synthesis, thus increasing muscle mass (112). In comparison, testosterone activates the recruitment of satellite cells at higher doses and reduces adipose-derived stem cells, thereby increasing myogenesis and reducing adipogenesis (110). Testosterone replacement therapy can improve metabolism and exercise tolerance in patients with chronic heart failure. Results showed an increase in peak oxygen uptake, 6-min walk distance, and body weight in the treatment group compared to the control group, directly related to the serum testosterone concentration (113). However, testosterone therapy may increase the risk of benign prostatic hyperplasia and tumor in male patients and masculinize female patients, limiting its wide clinical application.

Ghrelin exerts protective effects in skeletal muscle by regulating autophagy, apoptosis, insulin resistance, and inflammation (114). Ghrelin can also inhibit atherosclerosis, ischemia-reperfusion injury, ventricular remodeling, and improve cardiac function and endothelial function (115). As ghrelin is highly expressed in tumor tissue, its clinical application needs careful evaluation. However, attention should be paid to the side effects of testosterone therapy, such as benign prostatic hyperplasia, prostate cancer, polycythemia, and sleep apnea syndrome. Moreover, the intramuscular injection has higher safety than oral treatment.

Growth hormone (GH) is an essential endogenous hormone that can promote the growth of organs and tissues, promote protein synthesis, and affect fat and mineral metabolism. GH is involved in the regulation of skeletal muscle growth mainly through insulin growth factors. It can increase skeletal muscle mass but has no noticeable effect on muscle strength (116). Notably, GH can increase the risk of fluid retention and insulin resistance and adversely affect the cardiovascular system.

Medication

Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) have multiple cardiovascular protective effects, and their anti-inflammatory and antioxidant effects also benefit muscle tissue (117). Early studies have found that ACEI drugs can delay the decline of muscle mass. Recent studies have negated its effect on muscle mass and muscle strength. However, ARB can effectively improve the muscle strength of hemodialysis patients (118). In addition, recent studies have found that loop diuretics can increase the risk of sarcopenia in non-dialysis patients with chronic kidney disease. In patients with HF, spironolactone can prevent skeletal muscle loss and improve muscle strength, possibly due to improved cardiac function (119).

SHORTCOMINGS AND PROSPECTS

Globally, t the incidence of sarcopenia is gradually increasing, and it has received full attention from European and American countries. However, for the Asian region, the research on sarcopenia is still in its infancy. As for sarcopenia, from the initial focus on muscle mass to the latest 2018 EWGSOP2,

muscle strength is the primary diagnostic element, indicating that the understanding of its essence is constantly deepening. However, many areas still need to be further explored, including the pathophysiological processes such as the occurrence, development, and outcome of sarcopenia, sarcopenia-related biomarkers, screening, and preventive measures for high-risk people. In terms of the diagnosis of sarcopenia, there are some subjective diagnosis critical values at present. More objective and reasonable diagnosis critical value needs to be determined by standardized clinical research big data and gender and regional specificity. In terms of treatment, it is considered that nutrition and exercise are two treatment methods that can be implemented clinically to delay sarcopenia. However, the specific application, usage, dosage, and effectiveness of related nutritional supplements in nutritional therapy still need more research data to support. The exercise therapy method, frequency, and intensity also need clinical research to further confirm and refine. Although many studies have shown that nutritional s supplements combined with exercise are effective in treating sarcopenia, more research is also needed to standardize the treatment plan. At present, in terms of drug treatment, there is still a lack of clinical first-line drugs, and a small number of drugs for the treatment of sarcopenia are expected to enter phase III clinical trials in the next few years. However, the preliminary research of many drugs will face significant challenges.

Sarcopenia needs more basic and clinical research to explore its risk factors, pathogenesis, and intervention measures. At present, there is no unified conclusion on the mechanism of the relationship between sarcopenia and CVDs. However, according to the existing research, it can be determined that there are many similar pathophysiological mechanisms between sarcopenia and CVDs. Furthermore, sarcopenia has a specific correlation with the poor prognosis of CVDs. Therefore, it is necessary to pay attention to the common pathway of the two diseases, carry out systematic, basic, and large sample clinical research, and look for reliable biomarkers, so as to provide new ideas for the prediction and diagnosis of sarcopenia and cardiovascular diseases, as well as the early intervention of adverse prognosis.

CONCLUSION

In summary, sarcopenia and CVDs are highly prevalent in the elderly and share common pathogenesis and interactions. Understanding their relationship is still in its initial stages, and more clinical and experimental data are needed. A large number of studies have shown that the progression of CVDs and the decline in muscle function will further worsen the patient's condition. By screening patients for sarcopenia at an early stage, establishing effective early detection methods and evaluation methods, and providing early and comprehensive interventions, the progression of the disease can be effectively delayed. Nevertheless more importantly, patients with CVDs should be rehabilitated as soon as possible to break the vicious cycle of sarcopenia and CVDs through scientific nutritional programs and training guidance. Effective treatment of either sarcopenia or CVDs can have a positive impact on another disease. However, some drugs have acted as a double-edged role in the treatment of the two diseases. A healthy lifestyle and proper drug treatment have become necessary means for preventing and treating CVDs and sarcopenia. In the future, more high-quality research is still needed to provide a basis for optimal treatment options for people with specific diseases, such as CVDs co-morbid with sarcopenia.

AUTHOR CONTRIBUTIONS

NH drafted the manuscript of this review article. SZ and HY conceived and supervised the manuscript. YZ and LZ also collected and organized the information and prepared the table and figures for the manuscript. All authors contributed to the article and approved the submitted version.

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Clinical Characteristics of Cryoglobulinemia With Cardiac Involvement in a Single Center

Kun He[1], *Yun Zhang*[2]*, *Wei Wang*[3], *Yu Wang*[2], *Yue Sha*[2] *and Xuejun Zeng*[2]*

[1] *Department of Gastroenterology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China,* [2] *Department of Family Medicine & Division of General Internal Medicine, Department of Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, State Key Laboratory of Complex Severe and Rare Diseases (Peking Union Medical College Hospital), Beijing, China,* [3] *Department of Hematology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*

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***Correspondence:**
Yun Zhang
zykmpl@163.com
Xuejun Zeng
zxjpumch@126.com

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Background: Cryoglobulinemia is a syndrome characterized by the presence of cryoglobulins (CGs) in serum, and cardiac involvement is a rare occurrence that can affect treatment and prognosis. This study aimed to explore the clinical characteristics of cryoglobulinemia with cardiac involvement.

Methods: 108 patients diagnosed with cryoglobulinemia who were admitted and treated in Peking Union Medical College Hospital (PUMCH) between June 1985 and June 2019 were enrolled in the present study. Clinical characteristics, therapy, and prognosis of patients with cardiac involvement were retrospectively analyzed.

Results: The cryoglobulinemia with cardiac involvement was found in 7 patients, thus reaching the incidence of 6.5%. Heart failure was the main cardiac manifestation found in these patients, all with the involvement of external cardiac organs. Laboratory examinations showed significant elevation of N-terminal brain natriuretic peptide precursor (NT-proBNP) and brain natriuretic peptide (BNP) with negative troponin (cTnI). Electrocardiogram (ECG) was generally normal or only showed low-flat and biphasic multi-lead T waves. Echocardiography was performed in 6 patients, all of whom showed enlargement of heart cavity. Five patients had reduced left ventricular myocardial contractible motion with decreased ejection fraction, 3 patients had pericardial effusion, and 1 patient had left ventricular hypertrophy or severe aortic insufficiency. Cardiac magnetic resonance imaging showed delayed myocardial enhancement in 2 patients. One patient underwent a myocardial biopsy, which showed perivasculitis. Condition in 6 patients who received active treatment targeting improved in the early stage. Three patients (3/7, 42.9%) died due to disease progression during follow-up period.

Conclusions: Cryoglobulinemia with cardiac involvement is a rare but serious condition that has relatively high risk of death. When patients with cryoglobulinemia without underlying heart disease experience heart failure, chest pain, or elevation of asymptomatic NT-proBNP and BNP, there is a high possibility of cardiac involvement, even if the electrocardiogram and troponin are negative. Further examinations such

as echocardiography, cardiac magnetic resonance imaging, and myocardial biopsy examination could contribute to the diagnosis. Cardiac manifestations could be timely reversed after active targeted treatment. NT-proBNP and echocardiography could be used for the monitoring of disease efficacy.

Keywords: cryoglobulinemia, cardiac involvement, clinical characteristics, treatment outcome, retrospective study

INTRODUCTION

Cryoglobulinemia is a syndrome characterized by the presence of cryoglobulins (CGs) in the blood. Its pathological mechanism includes small vessel vasculitis caused by the deposition of immune complexes on the blood vessel wall and activation of complement. According to the composition of immunoglobulins, cryoglobulins can be divided into three types: Type I, a monoclonal immunoglobulin (usually IgG or IgM, rarely IgA or free light chain) that can be seen in malignant tumors with B cell lineage; Type II, which is a mixed type of monoclonal IgM and polyclonal IgG that can be seen in hepatitis C and other infections, connective tissue diseases (CTDs) and lymphoproliferative diseases; Type III, which is polyclonal IgM and polyclonal IgG, and usually appears secondary to CTDs or infection. Type II and III CGs are also known as mixed cryoglobulinemia. Cryoglobulinemia can cause multi-system diseases, including skin and mucosal injury, glomerulonephritis, peripheral nerve, joint pain, etc. Laboratory tests usually reveal positive monoclonal immunoglobulins, unexplained high titer rheumatoid factor (RF), and persistent hypocomplementemia. Therefore, the determination of blood cryoglobulinemia contributes to diagnosis. Cardiac involvement is a rare occurrence that can affect treatment and prognosis (1). To improve the understanding of cryoglobulinemia with heart involvement, the clinical data of 7 patients with cryoglobulinemia with heart involvement treated in our hospital were retrospectively analyzed and summarized. Relevant literature was also reviewed.

MATERIALS AND METHODS

Patients

We reviewed medical records from all inpatients treated in Peking Union Medical College Hospital (PUMCH) between June 1985 and June 2019. A total of 108 patients diagnosed with cryoglobulinemia were found. The inclusion criteria were: (1) cryoglobulin positivity (cyocrit $>$ 1.0%) and a clinical diagnosis of cryoglobulinemia based on comprehensive clinical, physical and chemical, imaging and pathological data (2, 3); (2) age $>$ 18 years old (male or female), and admission to the ward with complete inpatients medical records; (3) cardiac involvement defined as abnormal structure or function of the heart caused by cryoglobulinemia based on clinical symptoms, physcial examinations, laboratory tests, radiology examinations, biopsy, and exclusion of heart failure caused by non-cardiac diseases like renal insufficiency and other secondary heart diseases such as hypertension, coronary heart disease (4). The exclusion criteria were: the missing inpatient medical records; availability of only outpatient clinic medical records.

Methods

We recorded general data, the clinical manifestations, laboratory tests, imaging and pathology data, therapy, outcomes of patients with heart involvement by retrospective analysis. Clinical response was defined by analyzing the course of cardiac involvement (clinical, biologic, and radiologic improvement) and referring to the Bermingham vasculitis activity score (BVAS) of systemic vasculitis. Relapse was defined as reappearing of clinical signs of active vasculitis in any organ after remission (5, 6). Follow-up was conducted by outpatient or telephone contact. The last follow-up was in May 2020.

Statistical Analysis

Normal distribution of data was established by the Kolmogorov-Smirnov test, and data were presented as mean $\pm$ standard deviation (Mean $\pm$ SD). Descriptive statistical analysis was carried out by SPSS 20.0 statistical package (SPSS, Chicago, IL, USA).

RESULTS

General Data

There were 7 patients (7/108, 6.5%) with cryoglobulinemia with cardiac involvement who were treated in different departments, including the department of Nephrology (3/7), the department of Hematology (3/7), and the Department of General Medicine (1/7, who was later transferred to the Department of Hematology for treatment). There were 4 male and 3 female patients, with the mean age of cryoglobulinemia onset of 45.6 $\pm$ 13.1 years old and the mean age of cardiac involvement of 46.1 $\pm$ 12.8 years old. General data are shown in **Table 1**.

Types and Etiology of Cryoglobulin

Type I cryoglobulin was found in 2 patients, including one secondary to B-cell lymphoma and the other without a definite secondary factor. Type II cryoglobulin was found in 4 patients, including 1 secondary to chronic viral hepatitis B, 2 to B-cell lymphoma, and 1 without a definite secondary factor. One patient had type III cryoglobulin and no definite secondary factor.

Heart Manifestation

Heart manifestations are shown in **Table 2**. Among the 7 patients, only 1 patient had a history of hypertension. The main clinical manifestations were chest tightness, suffocation, and edema; 2

TABLE 1 | General data and clinical features of 7 patients in cryoglobulinemia with cardiac involvement.

Patients	Gender	Time at diagnosis (y)	Age at diagnosis (y)	The onset age of cardiac involvement (y)	Inpatient department	Previous heart disease	Involved organs other than the heart						
							Skin	Peripheral nerve	Kidney		Gastrointestinal tract	Lung	Others
									Clinical syndrome	Renal biopsy			
1	M	2015	25	25	Nephrology	–	–	–	RI; NS	EPGN	–	+	–
2	M	2015	39	41	Nephrology	–	Purpura	–	RI; NS	–	–	–	–
3	M	2014	66	66	Nephrology	Hypertension	Purpura	–	RI; CNS	EPGN	–	–	Pancytopenia
4	F	2016	52	52	Hematology	–	Purpura	–	RI; CNS	–	–	–	Two bloodlines decreased; multiple lymph nodes enlargement; splenomegaly
5	F	2016	41	43	Hematology	–	Purpura	–	RI; NS	–	–	–	Two bloodlines decreased; multiple lymph nodes enlargement; splenomegaly
6	F	2019	54	54	Internal medicine of general medicine	–	Purpura	+	RI; CNS	–	+	–	–
7	M	2016	42	42	Hematology	–	Purpura	–	RI; Proteinuria	–	–	–	–

Patients	Secondary causes	Laboratory examination					Renal biopsy
		Cryoglobulin	M-Ig	C3 (0.73–1.46 g/L)	C4 (0.1–0.4 g/L)	Rheumatoid factor (IU/mL)	
1	HBV	II	IgMκ	0.636	0.013	254	EPGN
2	–	II	NM	0.373	0.059	138	ND
3	–	III	–	0.358	0.004	228	EPGN
4	SBL	I	IgMκ	0.232	0.003	463	ND
5	MZBL	II	IgMκ	0.711	0.043	11,405	ND
6	IBL	III	IgMκ	0.944	0.001	366	ND
7	–	I	IgG k	N	N	NM	ND

y, year; M, male; F, female; "–", negative; "+", positive; RI, Renal insufficiency; NS, Nephrotic syndrome; CNS, Chronic nephritis syndrome; EPGN, Endocapillary proliferative glomerulonephritis. HBV, Hepatitis B Virus; "–", negative; SBL, Small B-cell lymphoma; MZBL, marginal zone B-cell lymphoma; IBL, Indolent B cell lymphoma not otherwise specified; M-Ig, Monoclonal immunoglobulin; RF, Rheumatoid factor; EPGN, Endocapillary proliferative glomerulonephritis; N, Normal but without specific value; NM, Not mentioned; ND, Not done.

TABLE 2 | Cardiac related manifestations, treatment, and prognosis in 7 patients with cryoglobulinemia.

Patients	Before treatment								Treatment	1-2 months after treatment				Prognosis
	cTnI (0–0.056 ug/L)	NT-ProBNP (0–125 ng/L)	BNP (0–100 ng/l)	ECG	Echocardiography		MRI	Cardiac biopsy		Cardiac manifestations	NT-ProBNP (0-125ng/L)	Echocardiography		
					LVEF	Others						LVEF	Others	
1	0.023	32,826	ND	Low and flat T- waves	37%	LV enlargement; Decreased LV systolic motion; Moderate PCE	ND	ND	Prednisone; Entecavir	Better	6354	49%	LV enlargement; Decreased LV systolic motion; Micro-PCE	Echocardiography was close to normal 2 years after treatment
2	0.05	>35,000	>5,000	N	37%	Enlargement of LV and RH; The decreased systolic motion of LV and RV; Severe aortic and tricuspid insufficiency; Mild PCE	Patchy delayed enhancement of ventricular septum	ND	Prednisone; Cyclophosphamide;	Worsen	ND	ND	ND	Death
3	0.01	7,710	302	Low and flat T- waves	59%	LV hypertrophy; Whole heart enlargement; Moderate PCE	ND	ND	Prednisone; Cyclophosphamide; Plasma exchange	Better	ND	59%	Whole heart enlargement; Mild-moderate PCE	Recurrence and death
4	0.003	2,049	190	Low and flat T- waves	48%	LA enlargement; Decreased LV systolic motion; Moderate PCE	ND	ND	Rituximab; Prednisone	Better	808	49%	LA enlargement; Decreased LV systolic motion; Mild PCE	Recurrence and death
5	0.01	7,424	369	N	ND	ND	Linear delayed enhancement of basal segment of the ventricular septum	ND	Rituximab; Cyclophosphamide Prednisone (RCP chemotherapy)	Better	100	ND	ND	No recurrence
6	0.045	>35,000	>5,000	Bipolar T-waves	48%	LA enlargement; Decreased LV systolic motion; Mild PCE	ND	ND	Rituximab; Cyclophosphamide; Dexamethasone (DRC chemotherapy)	Better	906	ND	ND	No recurrence
7	Normal	13,000	ND	N	37%	LV enlargement Decreased LV systolic motion	No delayed enhancement	Perivascular monocyte infiltration	Recurrence after glucocorticoid shock → Bortezomib; Cyclophosphamide; Dexamethasone (BCD chemotherapy)	Better	1010	56%	N	No recurrence

ECG, Electrocardiogram; LVEF, Left ventricular ejection fraction; LV, Left ventricular; PCE, pericardial effusion; RV, right ventricles; RH, right heart; LA, Left atrial; MRI, magnetic resonance imaging; N, normal; NM, Not mentioned; ND, Not done.

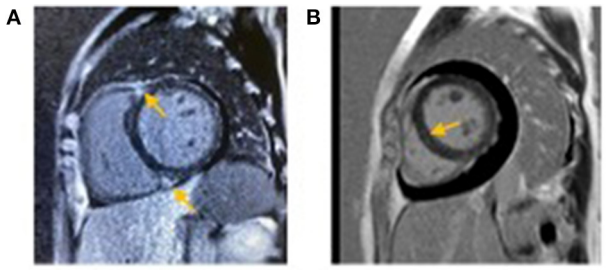

FIGURE 1 | (A) Case 2 (Yellow arrow): Cardiac magnetic resonance imaging showing patchy delayed enhancement of the insertion at both ends of the ventricular septum. **(B)** Case 4 (Yellow arrow): Cardiac magnetic resonance imaging showing linear delayed enhancement in the basal segment of the ventricular septum.

patients had heart symptoms as the first manifestation, and the remaining 4 patients experienced heart-related symptoms in the process of disease progression or recurrence. The values of cTnI in 7 cases were all within the normal range. The values of NT-proBNP and BNP were significantly increased, especially in 2 cases with values higher than the upper limit of normal detection.

Six cases underwent echocardiography, and the results were as follows: 6 cases had enlarged cardiac chambers, including left ventricular enlargement, with left and right ventricular enlargement and left atrial enlargement found in 2 cases; 5 cases had reduced left ventricular systolic motion and ejection fraction; 3 cases had pericardial effusion; 1 case had severe aortic insufficiency; 1 case had left ventricular hypertrophy with a history of hypertension. Three patients underwent cardiac magnetic resonance imaging. In addition to the corresponding findings of echocardiography, two patients had delayed myocardial enhancement in the ventricular septum (**Figure 1**). A myocardial biopsy was performed in 1 patient, revealing perivascular monocyte infiltration.

Extracardiac Manifestations

In addition to cardiac involvement, renal involvement and renal insufficiency were present in all patients, including 3 cases of nephrotic syndrome, 3 cases of chronic nephritis syndrome, and 1 case with proteinuria in the nephrotic range. Renal biopsy was conducted in 1 case of nephrotic syndrome and the other case of chronic nephritis syndrome. Pathological results in both cases showed proliferative glomerulonephritis in capillaries, which could be consistent with renal involvement of cryoglobulinemia. The skin manifestations were purpura-like rash in all 6 cases. The lung, peripheral nerve, and gastrointestinal tract involvement were found in one case. There were 3 cases with decreased hemogram, including 1 case with unknown cause and the other 2 cases complicated with multiple lymph node enlargement and splenomegaly, which were considered secondary to lymphoma bone marrow involvement.

Laboratory Tests

Increased rheumatoid factor and reduced complement were found in 6 cases. In 1 case, the complement was normal, and the result of a rheumatoid factor was unknown. Monoclonal immunoglobulin was detected in 6 cases, where 1 case was type III cryoglobulin negative, 4 cases had IgMk, and 1 case had IgGk.

Treatment Plan and Efficacy

Two patients secondary to B-cell lymphoma received combined chemotherapy containing rituximab. One patient with type I cryoglobulin without definite secondary factors relapsed after receiving glucocorticoid pulse therapy in another hospital, and was later given chemotherapy containing bortezomib in our hospital. One patient with chronic hepatitis B was given high-dose glucocorticoid and antiviral therapy. The performance of heart and value of NT-proBNP in these 4 patients were significantly improved after receiving treatment. Early reexamination of echocardiography suggested that the heart structure and function were recovered; 1 patient showed normal echocardiography after 2 years of follow-up.

Four patients were followed up to the cut-off date without recurrence. One patient secondary to B-cell lymphoma (case 4) was treated with rituximab and high-dose glucocorticoid due to infection as a complication. As the above 4 patients, the cardiac indexes of this patient improved in the early stage. Nevertheless, the patient (case 4) died of recurrence after half a year later. The remaining 2 patients without definite secondary factors received immunosuppressive treatment with high-dose glucocorticoid and cyclophosphamide, where 1 patient received plasma exchange while the other did not. The former relapsed and died after experiencing a temporary improvement, while the latter died following the deterioration of the disease. Therefore, 3 patients (3/7, 42.9%) died due to disease progression during follow-up period.

DISCUSSION

Cryoglobulin is globulin that precipitates at the temperature below 37°C and re-dissolves at 37°C. This phenomenon was first observed in 1933, following which cryoglobulin was first defined in 1947. Afterward, it was gradually reported in many diseases (7, 8). Cryoglobulinemia, which is an uncommon condition, rarely includes cardiac involvement; however, it has been associated with a higher risk of death. Up to now, the cardiac involvement of cryoglobulinemia was mostly reported in individual cases, few of whom are from China. Herein, we retrospectively analyzed the data of cryoglobulinemia with heart involvement treated in our center to further understand this disease.

In a retrospective study of 165 patients with mixed cryoglobulinemia associated with hepatitis C conducted in 2013, Terrier et al. (4) found that 7 of patients had heart involvement (4.2%), with a median onset age of 61 years old (40–76), while the onset age of heart involvement was not mentioned. Currently, there is a lack of related research reports from China. This study found that the prevalence of cardiac involvement with cryoglobulinemia was approximately 6.5%, the mean onset age of cryoglobulinemia was 45.6 $\pm$ 13.1 years old, and the cardiac involvement was 46.1 $\pm$ 12.8 years old.

According to previous literature reports, almost all heart involvement in cryoglobulinemia reported so far were mixed

type CGs. There was 1 case with heart involvement in type I cryoglobulinemia found in the hematology department of our center (9). Retrospective analysis of cases in our center revealed another case of type I cryoglobulinemia with cardiac involvement besides the previously mentioned one. The cryoglobulin of the remaining 5 cases with cardiac involvement was mixed type, including 4 cases of type II and 1 case of type III.

Among the 7 cases in this study, 6 had no underlying heart disease, and 1 had pre-existing hypertension. All patients presented with congestive heart failure, including acute pulmonary edema and edema around the body. Laboratory examinations indicated significantly increased NT-proBNP and BNP. The echocardiographic findings included decreased left ventricular myocardial systolic movement and left ventricular ejection fraction, cardiac cavity enlargement to varying degrees, left ventricular hypertrophy, pericardial effusion, and severe aortic valve insufficiency. Cardiac magnetic resonance imaging was performed in 3 cases, while 2 showed delayed myocardial enhancement, thus suggesting cardiomyopathy. No delayed myocardial enhancement was found on cardiac magnetic resonance imaging in the last case, which may be related to the previous application of high-dose glucocorticoids in other hospitals. One patient underwent a myocardial biopsy, which revealed perivasculitis. The clinical manifestations, laboratory indexes, and echocardiography of the heart all improved after treatment of cryoglobulinemia and secondary causes, which further supported the premise that the damage caused by heart diseases contributed to cryoglobulinemia. As previously mentioned in the study by Terrier et al. (4), there were 7 cases with heart failure and 4 cases with chest pain. Electrocardiogram showed T wave changes in 6 cases, ST-segment changes in 1 case, roughly normal condition in 1 case, while elevated troponin was found in 5 cases. Echocardiography revealed left ventricular hypertrophy in 1 case, pericardial effusion in 2 cases, myocardial contraction movement in 5 cases, and cardiac lumen enlargement in 6 cases. Myocardial MRI was performed in 5 patients, including 3 cases with delayed myocardial enhancement. In the present study, we found no chest pain, ST-segment abnormality, or elevated troponin. Although coronary artery was not evaluated in our study due to renal insufficiency, high risks of coronary atherosclerosis were not found in these cases which suggested cardiac damage was not considered to be caused by coronary lesions in a traditional way. Electrocardiogram results (close to normality or only multi-lead T wave change), negative troponin, and echocardiography without segmental ventricular wall motion abnormality further supported this assumption, which was also consistent with previous studies. For example, in Terrier's study (4), patients with ST-segment changes and troponin elevation underwent coronary angiography

TABLE 3 | Cardiac involvement related to cryoglobulinemia reported in the literature.

References	Age (y)	Gender	Clinical manifestations	Cardiac MRI	Coronary artery CTA	Myocardial biopsy	Cryoglobulin	Secondary causes	Treatment	After treatment
Tulio et al. (10)	79	F	Dyspnea, Edema	ND	ND	ND	II	HCV and intestinal TB	Recurrence after glucocorticoid shock → Rituximab; Antiviral therapy	Better
Ali et al. (11)	45	M	Dyspnea, Edema	Pericarditis and myocarditis	ND	ND	(Cryoglobulinemia was confirmed by clinical and renal puncture)	HCV	Rituximab; Plasma exchange; Antiviral therapy	Better
Cavalli et al. (12)	65	M	Dyspnea, Edema	Decreased LV systolic motion; LV hypertrophy; Delayed enhancement of inferior and lateral walls of LV	–	ND	ND	HCV	Rituximab; Antiviral therapy	Better
Karras et al. (13)	63	F	Dyspnea, Edema	Decreased LV systolic motion and enlarged LV	–	ND	II	HCV	Rituximab; Antiviral therapy	Better
Ghijsels et al. (14)	44	M	Dyspnea, Edema	ND	–	ND	II	–	Glucocorticoid; Cyclophosphamide; Plasma exchange; Rituximab	Better
Culclasure et al. (15)	65	M	Dyspnea, Edema	ND	ND	ND	II	–	Glucocorticoid; Immunosuppressant (Unknown); Plasma exchange	Better

y, years; F, female; M, male; MRI, magnetic resonance imaging; LV, Left ventricular; ND, Not done; "–", negative; HCV, Hepatitis C Virus; TB, tuberculosis; Electrocardiogram and echocardiography data were not mentioned in the above literature.

(CAG), which showed no obvious abnormality. Moreover, we found negative results for coronary evaluation of 3 patients in other foreign case reports (**Table 3**). Besides, Maestroni *et al.* conducted a study in (16), reporting on coronary artery vasculitis found after autopsy in two deceased patients with cryoglobulinemia with heart involvement. Therefore, we speculated that cryoglobulin-associated vasculitis might cause heart disease by mediating coronary microcirculation disturbance. In the present study, there was 1 case with left ventricular hypertrophy on echocardiography, which is consistent with previous studies mentioned above. Although the case in our study with left ventricular hypertrophy had pre-existing hypertension, it was still necessary to consider the possibility of left ventricular hypertrophy being secondary to cryoglobulinemia as it improved and disappeared after the treatment of the primary disease. This mechanism might be associated with inflammatory edema in myocardial involvement caused by cryoglobulinemia, which needed to be confirmed by myocardial biopsy. As for the severe valve disease mentioned in this study, the correlation between valve disease and cryoglobulinemia remains unclear as there were no follow-up echocardiography data (the patient died following the deterioration of the disease), and no related reports were recorded in previous studies. Yet, we assume that heart valve lesions observed in the present study might be related to cryoglobulinemia as the patient had no pre-existing heart disease and previous studies reported heart valve lesions in ANCA-related vasculitis, which equally belong to the category of small vessel vasculitis. A heart biopsy was also needed for definite confirmation (17).

In the present study, skin and renal lesions were the most common extracardiac manifestations in patients with cardiac involvement. Laboratory examinations showed increased RF and decreased complement, while 1 case with normal complement may be due to the previous high-dose glucocorticoid treatment. These results were roughly consistent with previous studies (18, 19). In addition, Terrior et al. (4) compared patients with cryoglobulinemia to those without cardiac involvement and found 6-month, 1-, and 2-year survival rates of 86% vs. 99%, 71% vs. 96%, and 48% vs. 90%, respectively (HR 5.01, $P = 0.003$). This suggested that patients of cryoglobulinemia with cardiac involvement had a higher risk of death. This study also showed relatively high risk of death in patients with cardiac involvement as 3 patients (3/7, 42.9%) died due to disease progression during follow-up period. Therefore, clinicians need to pay greater attention to patients with cryoglobulinemia with cardiac manifestations. In a retrospective study of 54 patients with HCV-associated mixed cryoglobulinemia conducted in 2010, Antonelli et al. (20) found that elevated NT-proBNP in laboratory examination for patients with no cardiac manifestations may indicate the presence of subclinical cardiac damage. This seems to suggest that NT-proBNP may be a potential indicator for early identification of cardiac involvement, being cost-effective and convenient to detect. In addition, our results revealed that NT-proBNP and echocardiogram were also improved with the improvement of the cardiac clinical manifestations after treatment, thus suggesting that both of them could be used for monitoring the efficacy of diseases. By the way, BNP may have the same effect for monitoring as Nt-proBNP because they have the same origin, which needs dynamic data of it before and after treatment to confirm in the further study. And BNP may make more sense than NT-proBNP in patients with cardiac involvement and kidney disease at the same time as it was less affected by renal insufficiency.

Given the rarity of this disease and the lack of mass evidence-based medical evidence for the cardiac involvement of cryoglobulinemia, currently, there is no recognized treatment plan. Patients are mainly treated by hematologists or immunologists according to their personal experience, and the treatment philosophy may vary for each individual case. In addition to treating secondary causes of cryoglobulinemia, immunosuppressive therapy should be selected according to the scope and severity of target organ involvement. The main therapeutic modalities include high-dose glucocorticoids, cyclophosphamide, rituximab, and plasma exchange (21, 22), which is also roughly consistent with the treatment regimens received by the patients in the present study. Since the heart is an important target organ, active treatment should be given once the involvement of the heart is confirmed. In this study, patients were mainly treated in the Department of Nephrology and Department of Hematology. The ones treated at the former department mainly received glucocorticoids and immunosuppressive agents, while some patients needed combined plasmapheresis. The treatment at the latter department mainly focused on combination therapy with rituximab, which may be related to past treatment experience and secondary disease due to lymphoma. In terms of efficacy, available case reports and case series analyses, including the study conducted in our center, all suggested that the clinical symptoms, biochemical indexes, and imaging changes of cardiac lesions of the patients receiving regular and standardized treatment all significantly improved after early treatment, while the long-term prognosis still requires longer follow up. Notably, the condition of a heart-involvement patient with type I cryoglobulinemia without clear secondary etiology who was treated in our center by bortezomib-containing chemotherapy after relapse of high-dose glucocorticoid therapy significantly improved. To the best of our knowledge, this was the first case receiving bortezomib treatment for cryoglobulinemic cardiac involvement. From the perspective of mechanism, rituximab mainly targets B cells, while bortezomib mainly targets plasma cells. Both of them seem to have good efficacy in treating heart invvement with cryoglobulinemia, which may provide new ideas and references for the treatment of such patients.

The present study has the following limitations: firstly, this was a retrospective study; thus, there may be information bias. Secondly, as a single-center study, the number of included cases was small, and the follow-up time was short, so it was difficult to fully reflect the characteristics and prognosis of this disease. However, it is also difficult to obtain large-scale clinical data and

conduct prospective studies due to the rarity of heart involvement with cryoglobulinemia.

CONCLUSIONS

In this study, we retrospectively analyzed the clinical data of patients with cryoglobulinemia with heart involvement treated in our center and reviewed relevant literature, thus aiming to improve the understanding of clinicians on cryoglobulinemia with heart involvement as well as advance the early diagnosis and treatment of this kind of disease. Heart involvement in cryoglobulinemia is a rare occurrence that carries a relatively high risk of death. For patients with cryoglobulinemia, cardiac involvement should be considered when there are cardiac-related clinical manifestations or asymptomatic elevation of NT-proBNP and BNP. Electrocardiogram changes may be insignificant, and troponin can be negative in these patients. Echocardiography, cardiac magnetic resonance imaging, and myocardial biopsy can help detect the disease, and the heart condition can be reversed with early and targeted treatment. In addition, subsequent multi-center, prospective and large-sample-size study are needed to confirm the conclusions in this study, further clarify the viscera lesions, pathogenesis, and risk factors of cryoglobulinemia with cardiac involvement and conduct early detection, diagnosis, and early treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Committee of Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YZ: study conception and design. KH: literature review and data extraction. WW and XZ: quality control. KH: statistical analysis. KH, YZ, WW, YW, YS, and XZ: manuscript preparation. YZ: manuscript review. All authors contributed to the article and approved the submitted version.

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The Relative Contribution of Plasma Homocysteine Levels vs. Traditional Risk Factors to the First Stroke: A Nested Case-Control Study in Rural China

Feng Zhou[1,2†], Chengzhang Liu[3,4,5†], Lijing Ye[6†], Yukai Wang[1], Yan Shao[1], Guohua Zhang[1], Zhenpeng Duan[1], Jingjuan Chen[1], Jingyun Kuang[1], Jingyi Li[6], Yun Song[7], Lishun Liu[7], Pierre Zalloua[8], Xiaobin Wang[9], Xiping Xu[7,10,11] and Chengguo Zhang[1*]*

[1] Department of Neurology, First People's Hospital of Foshan, Foshan, China, [2] Research Center of Clinical Medicine, Nanfang Hospital, Southern Medical University, Guangzhou, China, [3] Shenzhen Evergreen Medical Institute, Shenzhen, China, [4] Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, China, [5] Institute of Biomedicine, Anhui Medical University, Hefei, China, [6] State Key Laboratory of Natural Medicines, Research Center of Biostatistics and Computational Pharmacy, China Pharmaceutical University, Nanjing, China, [7] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China, [8] School of Medicine, Lebanese American University, Byblos, Lebanon, [9] Department of Population, Family and Reproductive Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [10] The State Key Laboratory for Organ Failure Research, National Clinical Research Study Center for Kidney Disease, Guangzhou, China, [11] Renal Division, Nanfang Hospital, Southern Medical University, Guangzhou, China

Background: Approximately 75% of Chinese hypertensive patients have elevated homocysteine (Hcy). Its implication in risk assessment and prevention of the first stroke remains an important clinical and public health question.

Methods: This study was based on a community cohort recruited from 2016 to 2018 in the rural China. To maximize cost efficiency, we used a nested case-control design, including 3,533 first stroke cases and 3,533 controls matched for age ±1 years, sex, and village. Individual associations of tHcy and traditional risk factors with the first stroke were examined, and their population-attributable risks (PARs) were estimated.

Results: There was a significant dose-response association between first stroke and total Hcy (tHcy) levels, with adjusted odds ratios of 1.11 (95% CI: 0.97, 1.26) for tHcy 10–15 μmol/L and 1.44 (1.22, 1.69) for tHcy ≥ 15 μmol/L, all compared to tHcy < 10 μmol/L. A similar trend was found for ischemic and hemorrhagic stroke. tHcy and systolic blood pressure (SBP) were independently and additively associated with the risk of first stroke (tHcy: 1.06 [1.02, 1.1]; SBP: 1.13 [1.1, 1.16]; *P*-interaction, 0.889). Among the ten main risk factors examined, the top two contributors to the first stroke were SBP and tHcy, with PARs of 25.73 and 11.24%, respectively.

Conclusions: Elevated tHcy is the second most important contributor and acts additively with SBP to increase the risk of the first stroke. This finding underscores the importance of screening and treating elevated tHcy along with traditional risk factors to further reduce the burden of the first stroke in the high-risk populations.

Keywords: homocysteine, systolic blood pressure, first stroke, ischemic stroke, population attributable risk

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Midwestern University, United States
Tieci Yi,
Peking University First Hospital, China

***Correspondence:**
Xiping Xu
xipingxu126@126.com
Chengguo Zhang
drzhangcg@163.com

[†] These authors have contributed equally to this work

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INTRODUCTION

Stroke is the second leading cause of death and disability worldwide and the leading cause of death in China (1). China has the highest stroke burden in the world (2), and this burden has been increasing over the past 4 decades, particularly in the rural areas (3, 4). While stroke in China share traditional risk factors, the high and escalating stroke rate urges us to investigate distinctive feature of stroke epidemiology in China and identify additional intervention targets (5).

Hypertension is the most important modifiable risk factor for stroke risk (6). In addition, a unique and well-observed clinical feature is that approximately 75% of Chinese hypertensive patients have hyperhomocysteinemia (HHcy) (7). The high prevalence of HHcy in China is due to several reasons. Unlike the US, China is a country without mandatory folic acid fortification. The Chinese diet plus cooking methods result in a low intake of folate and folic acid. Chinese population has a high rate of C677T mutation in the methylenetetrahydrofolate reductase (*MTHFR)* gene encoding a homocysteine (Hcy) metabolism-related enzyme (8). Although the independent and interactive impacts of HHcy and hypertension on cardiovascular diseases have been previously reported (9, 10), few recent studies have been conducted to quantify the relative effect of HHcy, in the context of traditional risk factors, on the first stroke, ischemic stroke, and hemorrhagic stroke, especially among rural Chinese community populations who have a disproportionately high burden of stroke and its severe health and economic sequala.

Population-attributable risk (PAR) provides insight into the relative significance of a given risk factor on stroke risk in the general population and can be used to predict the impact of public health interventions on adverse outcomes (11). To date, the PARs of tHcy and potentially modifiable etiological factors for the first stroke in the rural Chinese population remain mostly unknown.

Therefore, we conducted this nested case-control study, estimated to what extent HHcy alone and in conjunction with systolic blood pressure (SBP) can contribute to the risk of the first stroke. We also computed PARs for HHcy and other individual risk factors to estimate the proportion of the first stroke that could be prevented by the elimination of etiological factors from the population.

METHODS

Study Design and Population

Our present study was a subset of the "H-type Hypertension and Stroke Prevention and Control Project", which is a community-based, observational, multicenter, real-world registry study, and was conducted in the rural areas of Rongcheng County, and Lianyungang County, China. The detailed inclusion and exclusion criteria, follow-up, and outcomes of the study have been described in a previous publication (12). Briefly, eligible participants were local residents aged $\geq$ 35 years with essential hypertension, defined as a mean seated SBP $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg at the screening visit. The exclusion criteria were as follows: confirmed stroke at the time of screening; diagnosed secondary hypertension; and a history of cancer, myocardial infarction, or severe mental diseases. In the first stage, participants were screened and recruited, and the baseline data were collected; in the second stage of the 3-year observation, they were scheduled for follow-up every 3 months.

We implemented this nested case-control study design because it was economical and extrapolates the data well and matching lowers the interference of confounding factors to a certain degree. In the second stage, patients with stroke data from the Rongcheng Center for Disease Control and Prevention (CDC) and the Lianyungang CDC who had complete records that were selected as cases. First stroke cases and nonstroke controls were 1:1 matched by age $\pm$1 years, sex, and village. The initial sample consisted of 3,546 pairs. Next, we excluded the participants with missing values of blood pressure ($n = 6$) and tHcy ($n = 7$) and unpaired individuals ($n = 13$). Based on the inclusion and exclusion criteria, 3,533 stroke cases and 3,533 matched controls with complete Hcy measurements were selected for the final data analysis. The detailed procedure is presented in **Figure 1**.

Baseline Assessments and Definitions

Baseline data collection was conducted in the first stage by trained research staff using uniform standard operating procedures. Questionnaires were administered to collect information on demographics, life habits, and the physical intensity of the job. Body mass index (BMI) was calculated as the bodyweight divided by the square of the height (kg/m^2). The participants were classified as never, former, or current smokers *via* a self-reported survey. The physical intensity of the job was measured *via* a validated scale and classified as low, moderate, or high (13). Clinical and medical information was obtained from medical records. Diabetes was defined as fasting plasma glucose $\geq$ 7 mmol/L or taking diabetes medications. Coronary heart disease (CHD) was defined as previously documented myocardial infarction, coronary revascularization, or patients with symptoms of electrocardiographic modifications (14).

Venous blood was drawn after overnight fasting during the baseline visit. Plasma and serum samples were collected and subsequently stored at -80°C until laboratory tests were conducted. Plasma tHcy, fasting glucose, creatinine, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL) were measured by the automated analyzer. Low-density lipoprotein (LDL) was calculated by the Friedewald formula (15). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (16). HHcy was defined in various ways: (1) plasma total homocysteine (tHcy) levels $\geq$ 15 μmol/L by American guidelines and subsequently adjusted to $\geq$ 10 μmol/L by the American Heart Association/American Stroke Association guidelines on the primary prevention of stroke (17, 18), (2) $>$ 12 μmol/L by German, Austrian and Swiss Homocysteine Society guidelines (19), and (3) $\geq$ 10 μmol/L by the Chinese guidelines (20). In this study, we defined the categories of tHcy as $<$ 10, 10 to $<$ 15, and $\geq$ 15 μmol/L.

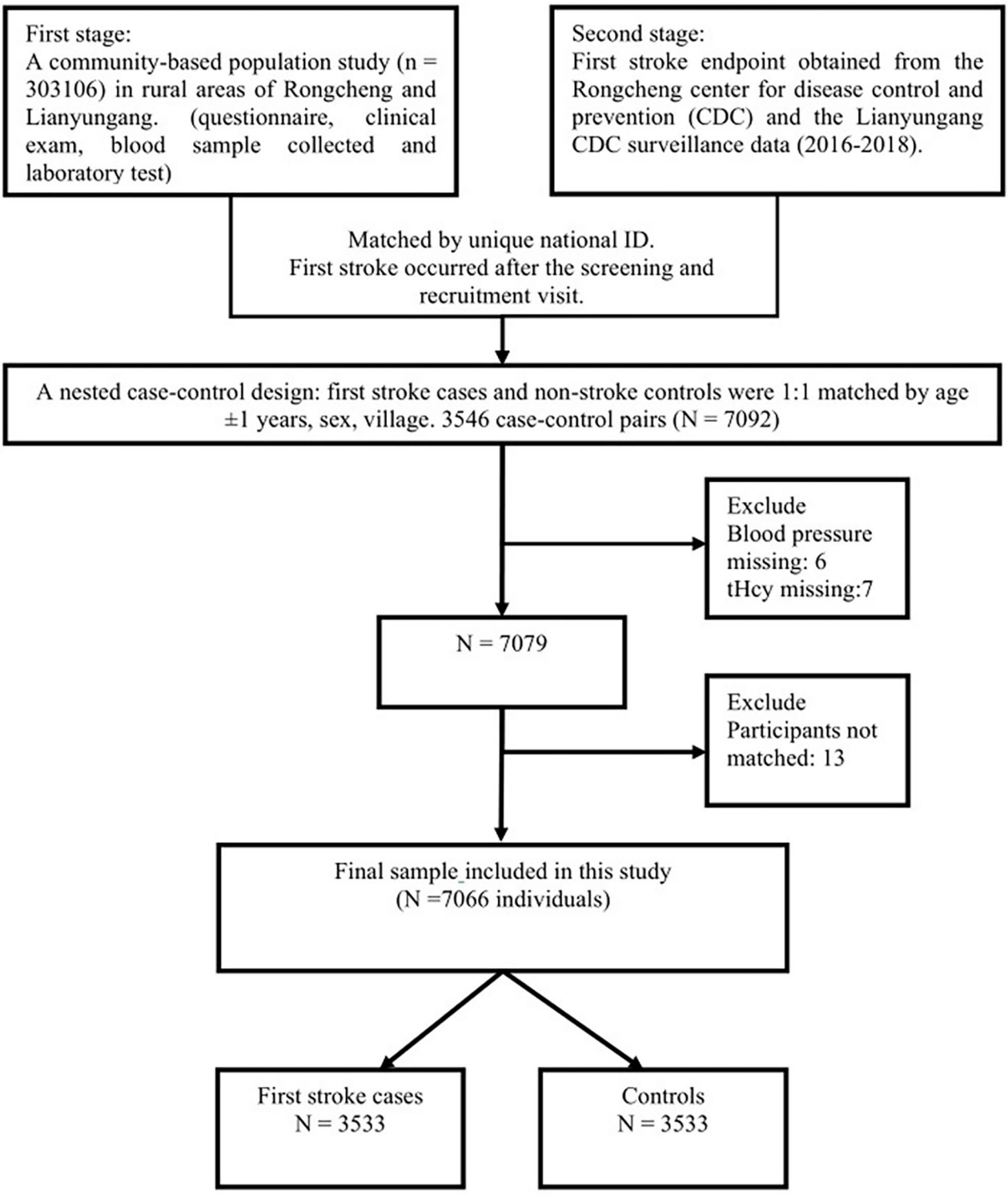

FIGURE 1 | Flow chart of the study design and participants. A total of 3,533 cases were individually matched to 3,533 controls by age ±1 years, sex, and village.

Outcomes

The primary outcome of interest was a first, nonfatal or fatal symptomatic stroke (ischemic and hemorrhagic). Silent stroke and subarachnoid hemorrhage were excluded. The first stroke was confirmed by the CT and/or MRI and was diagnosed based on the presence of the International Classification of Diseases

TABLE 1 | Baseline characteristics of the study participants.

	First stroke		Ischemic stroke		Hemorrhagic stroke		Mixed stroke	
	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases
N	3,533	3,533	3,070	3,070	426	426	37	37
Age, years	67.8 (9.3)	67.9 (9.3)	68.0 (9.2)	68.0 (9.2)	66.7 (10.0)	66.8 (10.0)	66.1 (8.7)	66.1 (8.8)
Female	1,953 (55.3)	1,953 (55.3)	1,705 (55.5)	1,705 (55.5)	223 (52.3)	223 (52.3)	25 (67.6)	25 (67.6)
Body mass index, kg/m^2	25.6 (3.7)	26.1 (4.1)	25.6 (3.7)	26.1 (4.1)	25.7 (3.8)	26.1 (4.4)	26.5 (4.9)	25.4 (3.7)
Systolic blood pressure, mmHg	148.2 (20.9)	153.8 (21.9)	148.2 (20.9)	153.2 (21.8)	148.4 (20.9)	157.2 (21.8)	151.2 (20.4)	159.8 (25.6)
Diastolic blood pressure, mmHg	86.5 (12.8)	90.3 (13.7)	86.5 (12.8)	89.8 (13.4)	86.5 (12.3)	93.2 (15.4)	90.5 (12.7)	94.1 (14.6)
Smoking status								
never	2,561 (72.5)	2,514 (71.2)	2,230 (72.6)	2, 191 (71.4)	301 (70.7)	292 (68.5)	30 (81.1)	31 (83.8)
former	286 (8.1)	271 (7.7)	250 (8.1)	221 (7.2)	36 (8.5)	49 (11.5)	0 (0.0)	1 (2.7)
current	686 (19.4)	748 (21.2)	590 (19.2)	658 (21.4)	89 (20.9)	85 (20.0)	7 (18.9)	5 (13.5)
Physical intensity of job								
low	2, 170 (61.4)	2,301 (65.1)	1,894 (61.7)	2,013 (65.6)	260 (61.0)	272 (63.8)	16 (43.2)	16 (43.2)
moderate	989 (28.0)	888 (25.1)	854 (27.8)	758 (24.7)	117 (27.5)	112 (26.3)	18 (48.6)	18 (48.6)
high	374 (10.6)	344 (9.7)	322 (10.5)	299 (9.7)	49 (11.5)	42 (9.9)	3 (8.1)	3 (8.1)
Disease History								
Hypertension	2,798 (79.2)	3, 101 (87.8)	2,430 (79.2)	2,677 (87.2)	335 (78.6)	388 (91.1)	33 (89.2)	36 (97.3)
Coronary heart disease	281 (8.0)	365 (10.3)	239 (7.8)	319 (10.4)	40 (9.4)	40 (9.4)	2 (5.4)	6 (16.2)
Diabetes	625 (17.7)	893 (25.3)	535 (17.4)	810 (26.4)	78 (18.3)	77 (18.1)	12 (32.4)	6 (16.2)
Medication used								
Antihypertensive drugs	1,372 (38.8)	1,744 (49.4)	1,206 (39.3)	1,528 (49.8)	152 (35.7)	207 (48.6)	14 (37.8)	9 (24.3)
Glucose-lowering drugs	174 (4.9)	285 (8.1)	151 (4.9)	273 (8.9)	21 (4.9)	12 (2.8)	2 (5.4)	0 (0.0)
Laboratory tests								
Fasting glucose, mmol/L	6.0 (2.0)	6.5 (2.5)	6.0 (2.0)	6.5 (2.5)	6.0 (1.9)	6.2 (2.3)	7.0 (3.3)	6.7 (4.1)
Homocysteine, μmol/L	13.4 (7.9)	14.1 (8.0)	13.4 (8.2)	14.1 (7.8)	13.1 (6.0)	14.4 (9.4)	13.9 (7.1)	12.6 (4.9)
Total cholesterol, mmol/L	5.5 (1.2)	5.5 (1.2)	5.5 (1.2)	5.5 (1.2)	5.4 (1.2)	5.6 (1.2)	5.4 (1.0)	5.5 (1.4)
Triglyceride, mmol/L	1.4 (0.9)	1.6 (1.0)	1.4 (1.0)	1.6 (1.1)	1.4 (0.9)	1.5 (0.9)	1.5 (0.8)	1.7 (0.9)
High-density lipoprotein, mmol/L	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)	1.5 (0.4)	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)
Low-density lipoprotein, mmol/L	3.3 (0.8)	3.3 (0.9)	3.3 (0.8)	3.3 (0.9)	3.2 (0.9)	3.3 (0.8)	3.1 (0.7)	3.2 (0.9)
eGFR, ml/min/1.73 m^2	94.9 (13.7)	93.4 (15.1)	94.6 (13.6)	93.3 (14.9)	96.5 (13.8)	94.3 (16.3)	98.5 (13.3)	96.2 (13.0)

Continuous variables are presented as mean (SD), categorical variables are presented as n (%).
eGFR, estimated glomerular filtration rate.

(ICD)-10 codes. Secondary outcomes included ischemic stroke and hemorrhagic stroke. Using a standardized form, we collected data from eligible consenting first-stroke patients who were enrolled in the CDC surveillance.

Statistical Analysis

All of the analyses were conducted using R software (version 3.5.3; http://www.R-project.org) and Empower (version 2.17.9; www.empowerstats.com). A two-tailed $P < 0.05$ was considered to be statistical significant in all analyses.

Baseline characteristics are presented as the mean (SD) for continuous variables and as proportions (%) for categorical variables. Statistical differences between cases and controls were calculated by paired *t*-test for continuous variables and by chi-squared tests for the categorical variables. Conditional logistic regression analysis was performed to assess the odds ratio (OR) and 95% CI for the association between HHcy, traditional risk factors, and the risk of the first stroke, ischemic and hemorrhagic strokes, other than for stratified analyses, for which we used unconditional logistic regression. We used smoothing curve fitting to further characterize the shape of the relationship between tHcy, SBP, and first stroke and its subtypes. All the regression analyses were adjusted for pertinent covariates. The categories of covariates are as follows: center (Rongcheng, Lianyungang), age (< 65, ≥ 65 years), sex (male, female), BMI (< 28, ≥ 28 kg/m^2), smoking status (never, former, and current), physical intensity of job (low, moderate, and high), tHcy (< 10, 10 to < 15, ≥ 15 μmol/L), SBP (< 140, 140 to < 160, ≥ 160 mmHg), intake of antihypertensive drugs (no, yes), diabetes (no, yes), CHD (no, yes), TC (< 5.2, ≥ 5.2 mmol/L), TG (< 1.7, ≥ 1.7 mmol/L), HDL (male ≥ 1.03/female ≥ 1.3, male < 1.03/female < 1.3 mmol/L), LDL (< 3.4, ≥ 3.4 mmol/L), eGFR (≥ 90, < 90 ml/min/1.732 m^2).

Adjusted PARs with respective 95% CIs for individual risk factors and their combinations for the first stroke and its subtypes were calculated by logistic regression models and

TABLE 2 | The associations of tHcy and traditional risk factors with the risk of first stroke, ischemic, and hemorrhagic stroke.

	First stroke (3,533 pairs)		Ischemic stroke (3,070 pairs)		Hemorrhagic stroke (426 pairs)	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
tHcy, μmol/L						
<10	ref	ref	Ref	ref	ref	ref
10<15	1.11 (0.98, 1.26)	1.11 (0.97, 1.26)	1.08 (0.94, 1.24)	1.08 (0.94, 1.25)	1.30 (0.90, 1.89)	1.32 (0.88, 1.98)
≥15	1.53 (1.31, 1.79)	1.44 (1.22, 1.69)	1.46 (1.24, 1.73)	1.39 (1.16, 1.66)	2.11 (1.33,3.34)	1.81 (1.09,3.02)
SBP, mmHg						
<140	ref	ref	Ref	ref	ref	ref
140-<160	1.44 (1.28, 1.63)	1.38 (1.22, 1.57)	1.37 (1.21, 1.57)	1.31 (1.15, 1.51)	2.37 (1.56,3.58)	2.21 (1.44,3.40)
≥160	2.10 (1.82,2.41)	1.85 (1.60,2.14)	1.95 (1.68,2.27)	1.72 (1.47,2.02)	3.79 (2.44,5.91)	3.39 (2.13,5.40)
Body mass index, kg/m^2						
<28	ref	ref	Ref	ref	ref	ref
≥28	1.18 (1.05, 1.31)	1.01 (0.90, 1.13)	1.19 (1.06, 1.34)	1.02 (0.90, 1.16)	1.19 (0.88, 1.61)	0.95 (0.68, 1.34)
Smoking status						
Never	ref	ref	Ref	ref	ref	ref
Ever	1.12 (0.98, 1.28)	1.16 (1.01, 1.34)	1.11 (0.96, 1.29)	1.17 (1.00, 1.36)	1.17 (0.81, 1.68)	1.01 (0.67, 1.51)
Diabetes						
No	ref	ref	Ref	ref	ref	ref
Yes	1.57 (1.40, 1.76)	1.44 (1.28, 1.63)	1.70 (1.50, 1.93)	1.56 (1.37, 1.78)	0.98 (0.70, 1.39)	0.96 (0.66, 1.41)
Total cholesterol, mmol/L						
<5.2	ref	ref	Ref	ref	ref	ref
≥5.2	1.00 (0.91, 1.11)	0.99 (0.88, 1.10)	0.97 (0.87, 1.08)	0.96 (0.85, 1.08)	1.26 (0.94, 1.69)	1.22 (0.87, 1.70)
Triglycerides, mmol/L						
<1.7	ref	ref	Ref	ref	ref	ref
≥1.7	1.29 (1.16, 1.44)	1.11 (0.99, 1.25)	1.33 (1.19, 1.49)	1.14 (1.00, 1.30)	1.02 (0.76, 1.38)	0.93 (0.65, 1.32)
High-density lipoprotein, mmol/L						
male≥1.03/female≥1.3	ref	ref	Ref	ref	ref	ref
male<1.03/female<1.3	1.17 (1.03, 1.34)	1.08 (0.93, 1.25)	1.23 (1.07, 1.42)	1.11 (0.94, 1.30)	0.76 (0.51, 1.11)	0.86 (0.54, 1.39)
eGFR, ml/min/1.73m^2						
≥90	ref	ref	ref	ref	ref	ref
<90	1.35 (1.19, 1.54)	1.19 (1.03, 1.36)	1.36 (1.19, 1.57)	1.21 (1.04, 1.41)	1.39 (0.96, 2.00)	1.13 (0.75, 1.70)

tHcy, total homocysteine; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate.
Adjusted for homocysteine, systolic blood pressure, body mass index, smoking status, diabetes, estimated glomerular filtration rate, triglyceride, high-density lipoprotein, physical intensity of job, anti-hypertensive drug, coronary heart disease.

adjusted for confounding. The method was introduced in study of Bruzzi (21). The PAR point estimator is implemented in the R package attribrisk.

RESULTS

Baseline Characteristics

Among 3,533 first stroke cases, 3,070 (86.9%) had an ischemic stroke, 426 (12.1%) had a hemorrhagic stroke, and 37 (1.0%) had mixed lesions of both ischemia and hemorrhage. The mean age at blood sample collection was 67.9 (SD, 9.3) years for first stroke cases and 67.8 (SD, 9.3) years for controls; females made up 55.3%. Compared with controls, cases tended to have a higher BMI; higher levels of SBP, DBP, fasting glucose, tHcy, and TG; and lower levels of eGFR at baseline ($P < 0.05$). In addition, cases had higher proportions of medication use than controls ($P < 0.05$). Cases with mixed strokes were excluded from stroke subtype analyses. Similar trends were found in ischemic and hemorrhagic strokes (**Table 1**).

Effects of tHcy and Traditional Risk Factors on the First Stroke

Overall, there were significant associations between tHcy, traditional risk factors, and first stroke in the general population (**Table 2**), in sex subgroups (**Table A1** in Supplementary Material), in age subgroups (**Table A2** in Supplementary Material), and in center subgroups (**Table A3** in Supplementary Material). In the general population, tHcy [10–15 vs. < 10 μmol/L, OR: 1.11 (95% CI: 0.97, 1.26); ≥ 15 vs. < 10 μmol/L, 1.44 (1.22, 1.69)], SBP [140–160 vs. < 140 mmHg, 1.38 (1.22, 1.57); ≥ 160 vs. < 140 mmHg, 1.85 (1.60, 2.14)], smoking status [ever vs. never, 1.16 (1.01, 1.34)], diabetes [yes vs. no, 1.44 (1.28, 1.63)], and eGFR [< 90 vs. ≥ 90 ml/min/1.73 m^2, 1.19 (1.03, 1.36)] were significantly associated

TABLE 3 | Independent and combined effects of systolic blood pressure and homocysteine on first stroke, ischemic and hemorrhagic stroke.

SBP, mmHg/tHcy, μmol/L	*N*	Events (%)	Crude	Adjusted	
			OR (95% CI)	OR (95% CI)	*P*
First stroke					
SBP, per 10 increment*	7,066	3,533 (50)	1.16 (1.13, 1.19)	1.13 (1.1, 1.16)	<0.001
tHcy, per 5 increment†	7,066	3,533 (50)	1.08 (1.04, 1.12)	1.06 (1.02, 1.10)	0.003
P for interaction			0.880	0.889	
Joint effcts					
SBP <140					
tHcy <10	498	194 (39)	ref	ref	
10≤ tHcy <15	1,040	432 (41.5)	1.23 (0.98, 1.55)	1.20 (0.95, 1.52)	0.134
tHcy ≥15	505	247 (48.9)	1.77 (1.35,2.34)	1.56 (1.17,2.08)	0.002
140≤ SBP <160					
tHcy <10	752	357 (47.5)	1.59 (1.25,2.04)	1.47 (1.14, 1.90)	0.003
10≤ tHcy <15	1,545	746 (48.3)	1.77 (1.41,2.22)	1.61 (1.27,2.04)	<0.001
tHcy ≥15	683	379 (55.5)	2.54 (1.95,3.30)	2.10 (1.58,2.77)	<0.001
SBP ≥160					
tHcy <10	423	240 (56.7)	2.48 (1.87,3.29)	2.19 (1.64,2.93)	<0.001
10≤ tHcy <15	1,018	574 (56.4)	2.60 (2.04,3.32)	2.13 (1.65,2.74)	<0.001
tHcy ≥15	602	364 (60.5)	3.27 (2.49,4.30)	2.64 (1.98,3.53)	<0.001
Ischemic stroke					
SBP, per 10 increment*	6,140	3,070 (50)	1.14 (1.11, 1.17)	1.11 (1.08, 1.15)	<0.001
tHcy, per 5 increment†	6,140	3,070 (50)	1.07 (1.03, 1.11)	1.06 (1.01, 1.10)	0.010
P for interaction			0.925	0.821	
Joint effects					
SBP <140					
tHcy <10	442	176 (39.8)	ref	ref	
10≤ tHcy <15	916	387 (42.2)	1.22 (0.96, 1.56)	1.22 (0.95, 1.57)	0.121
tHcy ≥15	459	230 (50.1)	1.79 (1.34,2.38)	1.60 (1.18,2.16)	0.002
140≤ SBP <160					
tHcy <10	641	315 (49.1)	1.64 (1.26,2.13)	1.52 (1.16,2.00)	0.002
10≤ tHcy <15	1,357	651 (48)	1.66 (1.30,2.11)	1.53 (1.19, 1.97)	<0.001
tHcy ≥15	598	325 (54.3)	2.29 (1.73,3.03)	1.93 (1.43,2.60)	<0.001
SBP ≥160					
tHcy <10	343	189 (55.1)	2.25 (1.66,3.06)	1.99 (1.45,2.73)	<0.001
10≤ tHcy <15	884	496 (56.1)	2.43 (1.88,3.15)	2.01 (1.53,2.63)	<0.001
tHcy ≥15	500	301 (60.2)	3.08 (2.29,4.13)	2.56 (1.88,3.51)	<0.001
Hemorrhagic stroke					
SBP, per 10 increment*	852	426 (50)	1.29 (1.19, 1.40)	1.26 (1.15, 1.37)	<0.001
tHcy, per 5 increment†	852	426 (50)	1.16 (1.03, 1.31)	1.09 (0.98, 1.22)	0.113
P for interaction			0.911	0.938	
Joint effects					
SBP <140					
tHcy <10	52	17 (32.7)	ref	ref	
10≤ tHcy <15	115	40 (34.8)	1.11 (0.51, 2.42)	0.96 (0.43,2.15)	0.930
tHcy ≥15	45	17 (37.8)	1.43 (0.55, 3.75)	1.10 (0.41,2.99)	0.849
140≤ SBP <160					
tHcy <10	100	38 (38)	1.30 (0.59, 2.88)	1.16 (0.52,2.60)	0.719
10≤ tHcy <15	173	88 (50.9)	2.81 (1.34, 5.86)	2.36 (1.10,5.04)	0.027
tHcy ≥15	83	53 (63.9)	5.52 (2.36, 12.92)	3.99 (1.61,9.92)	0.003
SBP ≥160					
tHcy <10	74	47 (63.5)	4.33 (1.91, 9.82)	3.68 (1.59,8.52)	0.002

(Continued)

TABLE 3 | Continued

SBP, mmHg/tHcy, μmol/L	*N*	Events (%)	Crude	Adjusted	
			OR (95% CI)	OR (95% CI)	*P*
10≤ tHcy <15	122	70 (57.4)	4.05 (1.83, 8.99)	3.31 (1.44,7.59)	0.005
tHcy ≥15	88	56 (63.6)	5.11 (2.23, 11.69)	3.77 (1.56,9.12)	0.003

SBP, systolic blood pressure; tHcy, total homocysteine.
Conditioned on the matching factors of age, sex, and study site, and adjusted for body mass index, smoking status, diabetes, estimated glomerular filtration rate, triglyceride, high-density lipoprotein, physical intensity of job, antihypertensive drugs, coronary heart disease.
**Adjusted models included tHcy.*
†Adjusted models included SBP.

with adjusted first stroke risk. Regarding ischemic stroke, tHcy, SBP, smoker, diabetes, and TG were significantly associated with adjusted ischemic stroke risk. Nevertheless, only tHcy and SBP were significantly associated with the adjusted hemorrhagic stroke risk.

The associations between SBP, tHcy, and stroke risks are plotted in **Figures A1, A2** in Supplementary Material. Further analyses confirmed the dose-relationship between SBP, tHcy levels, and the risk of the first stroke, ischemic and hemorrhagic strokes, either in the general population, or in age, sex, or center subgroups after adjusting for pertinent covariates.

Stratified Analyses by Important Covariates

In the stratified analyses, diabetes (*P*-interaction = 0.02), HDL (*P*-interaction = 0.022), and LDL (*P*-interaction = 0.039) modified the association between tHcy and first stroke. Nevertheless, age, sex, BMI, smoking status, SBP, TG, physical intensity of the job, and history of CHD did not significantly modify the association between tHcy and first stroke or ischemic and hemorrhagic strokes (**Table A4** in Supplementary Material).

Additive Effects of tHcy and SBP on the First Stroke

The tHcy concentrations (per 5 μmol/L increment) were positively correlated with the risk of first stroke [1.06 (1.02, 1.10)], ischemic stroke [1.06 (1.01, 1.10)], but not hemorrhagic stroke [1.09 (0.98, 1.22)]. SBP levels (per 10 mmHg increment) were positively correlated with the risk of first stroke [1.13 (1.10, 1.16)], ischemic stroke [1.11 (1.08, 1.15)] and hemorrhagic stroke [1.26 (1.15, 1.37)]. However, homocysteine and SBP did not interact for the first stroke, ischemic or hemorrhagic strokes (**Table 3**).

Table 3 and **Figure 2** show that first stroke risk increased with both tHcy and SBP levels. The lowest first stroke risk was among subjects with the coexistence of tHcy < 10 μmol/L and SBP < 140 mmHg, while the highest risk of the first stroke was found among subjects with the coexistence of tHcy ≥ 15 μmol/L and SBP ≥ 160 mmHg after adjustments for potential covariates. Similar trends were found in ischemic and hemorrhagic strokes. SBP and tHcy had additive effects on the risk of first stroke and ischemic and hemorrhagic strokes.

PARs of tHcy and Traditional Risk Factors for the First Stroke

When we studied the first stroke, SBP was the most important risk factor (PAR, 25.73%), followed by tHcy (11.24%). In addition, a low-physical intensity job (9.17%), diabetes (8.01%), eGFR (6.57%), smoker (4.04%), TG (3.68%), and CHD (1.88%) contributed significantly to the stroke burden. The combined PAR of SBP and tHcy for the first stroke was 34.08%. The total proportion of the first stroke that could be attributed to a combination of these 11 examined risk factors was 54.88% (**Table 4**, **Figure 3**).

When we restricted the analyses to ischemic stroke, similar contributions of individual and combined risk factors were found (**Table 4**). SBP explained 22.21% of ischemic stroke, followed by tHcy (PAR, 9.89%). SBP and tHcy combined to explain 29.83% of the ischemic strokes. The combination of all 11 involved risk factors explained 53.79% of ischemic strokes. Compared with ischemic stroke, hemorrhagic stroke was to a greater extent explained by SBP (PAR, 51.15%). SBP and tHcy combined to explain 60.91% of hemorrhagic stroke, and all involved eleven risk factors combined to explain 65.24% of hemorrhagic stroke (**Table 4**).

We summarized 11 studies on PARs of risk factors for the first stroke in **Table A5** in Supplementary Material. Participants were recruited from different regions, most of them were hospital based, with different study designs and different risk factors. The major factors affecting the risk of the first stroke can be divided into conventional cardiovascular factors, family history, lifestyle, dietary habits, education, and other aspects. The reported PAR of hypertension for the first stroke in different studies varies widely.

DISCUSSION

To our knowledge, this is by far one of the largest prospective nested case-control studies of this kind to confirm that

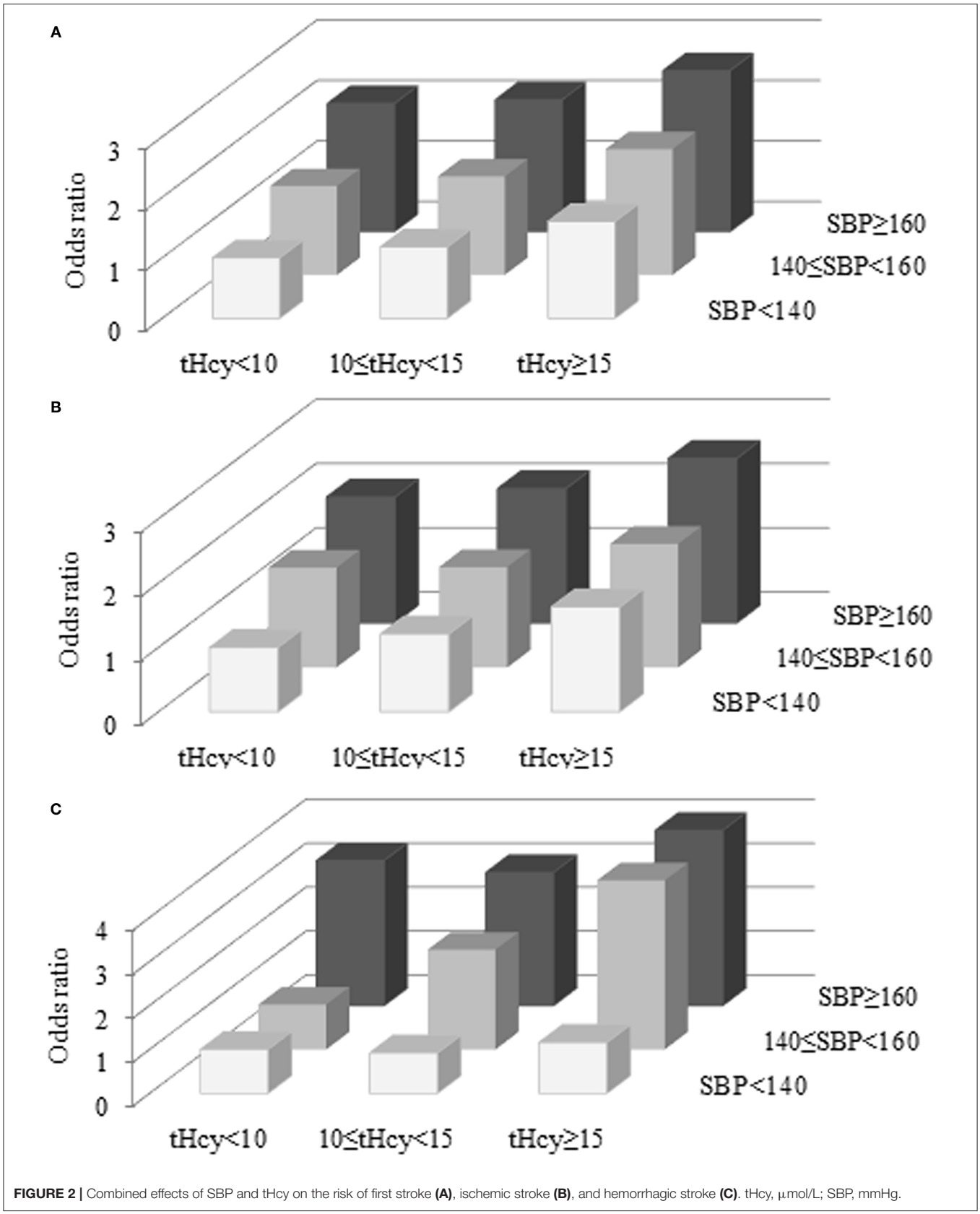

FIGURE 2 | Combined effects of SBP and tHcy on the risk of first stroke **(A)**, ischemic stroke **(B)**, and hemorrhagic stroke **(C)**. tHcy, μmol/L; SBP, mmHg.

TABLE 4 | Adjusted odds ratio and population attributable risks for first stroke, ischemic stroke, and hemorrhagic stroke.

	First stroke		Ischemic stroke		Hemorrhagic stroke	
	OR (95% CI)	PAR% (95% CI)	OR (95% CI)	PAR% (95% CI)	OR (95% CI)	PAR% (95% CI)
Individual risk factors						
tHcy ≥10 μmol/L	1.17 (1.03, 1.33)	11.24% (1.58, 18.93)	1.14 (1.00, 1.31)	9.8% (-0.62, 18.46)	1.35 (0.91,2.01)	19.92% (-8.31,40.86)
SBP ≥140 mmHg	1.52 (1.34, 1.71)	25.73% (19.18,32.54)	1.43 (1.25, 1.62)	22.21% (14.23,28.53)	2.63 (1.75,3.94)	51.15% (36.45,65.21)
Body mass index ≥28 kg/m^2	1.01 (0.90, 1.14)	0.33% (−3.22, 3.45)	1.02 (0.90, 1.16)	0.66% (−2.76, 3.61)	0.99 (0.71, 1.37)	−0.46% (−12.57, 10.2)
Smoker	1.16 (1.01, 1.34)	4.04% (0.11, 7.38)	1.17 (1.00, 1.36)	4.08% (0.13,7.8)	1.03 (0.69, 1.53)	0.87% (−13.7, 12.06)
Diabetes	1.47 (1.30, 1.65)	8.01% (5.46, 10.67)	1.58 (1.39, 1.80)	9.63% (7.03, 12.34)	1.02 (0.70, 1.48)	0.35% (−7.22, 7.24)
Triglyceride ≥1.7 mmol/L	1.13 (1.01, 1.27)	3.68% (0.29, 7.03)	1.16 (1.02, 1.32)	4.39% (0.71, 8.19)	0.93 (0.65, 1.32)	−2.31% (−13.06, 7.72)
High-density lipoprotein (male<1.03/female<1.3 mmo/L)	1.07 (0.92, 1.24)	1.05% (−1.5, 3.39)	1.10 (0.94, 1.30)	1.6% (−0.97, 3.97)	0.83 (0.52, 1.33)	−2.64% (−10.85, 3.53)
Low-density lipoprotein ≥3.4 mmol/L	1.08 (0.97, 1.20)	3.12% (−1.64, 7.35)	1.05 (0.93, 1.18)	1.94% (−2.7, 7.24)	1.29 (0.94, 1.79)	9.66% (−2.01, 20.9)
eGFR <90 ml/min/1.73 m^2	1.269 (1.10, 1.44)	6.57% (2.49, 10.34)	1.28 (1.11, 1.48)	7.17% (2.89, 11.19)	1.22 (0.81, 1.82)	5.23% (−6.56, 15.44)
Low-physical intensity of job	1.16 (1.04, 1.30)	9.17% (2.73, 15.82)	1.18 (1.04, 1.33)	9.86% (2.73, 16.7)	1.05 (0.74, 1.48)	2.95% (−22.7, 23.33)
History of coronary heart disease	1.22 (1.02, 1.46)	1.88% (0.12, 3.41)	1.25 (1.04, 1.52)	2.1% (0.35, 3.99)	0.93 (0.56, 1.54)	−0.72% (−5.77, 4.06)
Combinations of risk factors						
SBP ≥140 mmHg and tHcy ≥10 μmol/L		34.08% (24.81, 41.98)		29.83% (17.62, 38.79)		60.91% (40.39, 76.54)
All risk factors above		54.88% (46.94, 61.56)		53.79% (45.53, 61.37)		65.24% (45.35, 79.16)
All risk factors but without tHcy		49.29% (42.27, 55.70)		48.87% (41.20, 55.16)		56.76% (34.98, 72.62)

SBP, systolic blood pressure; tHcy, total homocysteine; eGFR, estimated glomerular filtration rate.

Adjusted for body mass index, smoking status, diabetes, estimated glomerular filtration rate, triglyceride, high-density lipoprotein, low-density lipoprotein, physical intensity of job, anti-hypertensive drug, coronary heart disease.

with increasing tHcy and SBP levels, the risks of the first stroke, ischemic and hemorrhagic strokes significantly increase. We found that more than half of the first strokes in this study population were attributable to established modifiable risk factors. Baseline SBP and tHcy were two of the most important risk factors and can independently and additively increase the risk of the first stroke. However, the contribution of modifiable risk factors differed between ischemic and hemorrhagic strokes.

Consistent with previous studies, (5, 8, 13, 22) our findings showed that SBP and tHcy were positively correlated with the first stroke. It is universally acknowledged that high SBP is the leading risk factor for all stroke types (1, 5, 23). Among different populations, however, data on the association of tHcy with the risk of stroke subtypes remain limited. Huo et al. (8) noted a strong association of HHcy with the risk of first stroke and the ischemic subtype, but the analysis was underpowered for assessing hemorrhagic stroke among the Chinese population. A nested case-control study of Japanese adults showed positive relationships between HHcy and the risks of first stroke and ischemic stroke (22). However, it did not investigate the interaction between tHcy and blood pressure. For hemorrhagic stroke in that study, despite the lack of statistical significance between hemorrhagic stroke and HHcy, a trend in higher quartiles of tHcy with an increased risk of hemorrhagic stroke was observed. We found a significant association of tHcy ≥ 15 μmol/L and SBP ≥ 140 mmHg with hemorrhagic stroke. Nevertheless, studies on the association between HHcy and hemorrhagic stroke are inconsistent (18, 24–28). Overall, studies suggest positive associations of HHcy with the risk of the first stroke.

*****Furthermore, we reported that HHcy and high SBP additively increased the risks of the first stroke, ischemic and hemorrhagic stroke. We speculate that the effect of HHcy and SBP on stroke may stem from their multifaceted biological pathways. Pathophysiologically, HHcy damages the vascular structure through different isoforms of oxidative stress, inflammation, and apoptosis promotes atherosclerotic properties and atherosclerotic plaque rupture, and subsequently increases stroke risk (29). In addition, the adverse effects of HHcy in stroke are caused by the upregulation of angiotensin II, subsequently leading to hypertension (30). Hypertension promotes stroke through activation of angiotensin II and angiotensin type 1 receptor in the blood vessels and increases vasoconstriction, causing vascular wall damage and blood–brain barrier disruption (31). In addition, HHcy upregulates pathogenic genes *via* DNA demethylation to increase vascular remodeling and hypertension (32). Interestingly, mild and moderate HHcy levels primarily affect the epigenetic regulation of gene expression through the interference of transmethylation reactions, while severe HHcy might be more destructive through oxidative stress, inflammation, and apoptosis (29, 33). The multiple pathogenesis involved in the association of tHcy with systolic hypertension may explain their additive effects for stroke.

Our study provides further evidence that SBP and HHcy are two of the most important risk factors for the first stroke in Chinese adults in the rural areas. Consistent with other studies (5, 13), high SBP was the strongest contributor for

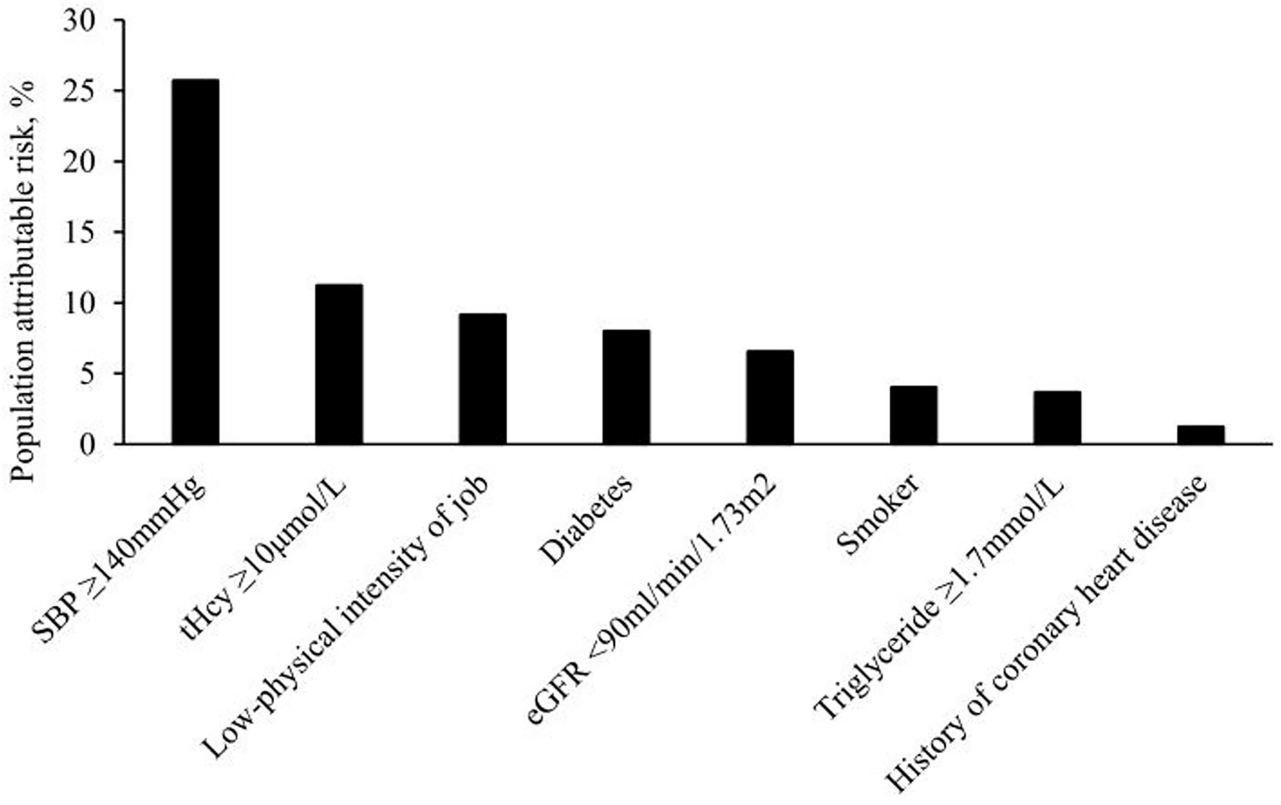

FIGURE 3 | Population-attributable risks of traditional risk factors and tHcy for first stroke.

the first stroke and its aetiologic subtypes. HHcy was the second most important contributor for the first stroke in our study, while it was not recruited to the panel of risk factors in the previous studies. The prevalence of HHcy was high in China (7), and preventive control of HHcy is an effective approach to decrease the burden of stroke (8), therefore, we should pay additional attention to HHcy, the leading risk factor for the first stroke. The PARs of risk factors in our study were considerably lower than those reported previously. Several explanations are possible. First, the risk factors included in different studies are not completely consistent, and the criteria for defining risk factors are different. Second, there were differences regarding whether risk factors were assessed before or after the occurrence of stroke. Risk factors might be raised in the acute stroke phase, whereas before the stroke onset might be lower. Therefore, previous case-control studies may have overestimated the PARs. Third, we highlighted the difference in population characteristics. Previous studies were mostly hospital-based Western populations, while our study focused on the first stroke in the community-based Chinese population. Finally, the lack of data on the psychosocial factors and socioeconomic status probably did not materially influence our total PAR because causality for these factors has not been established.

The strengths of our study include its large real-world sample, economical nature, nested case-control design, and ability to adjust for the traditional risk factors for stroke. Several potential limitations should be addressed. First, our findings cannot establish causality. Moreover, the study population was Chinese adults aged 35 years and over in the rural areas, therefore, our findings cannot be extrapolated to other populations. Second, this study did not evaluate the associations of stroke with diet, hypertensive medication use, or supplementation with B vitamins in the follow-up period that may affect the outcome of stroke (34, 35). Third, we were unable to distinguish subtypes of ischemic or hemorrhagic stroke owing to the lack of imaging data. The associations of tHcy with arterial territories of stroke are worth further study.

In conclusion, consistent associations between tHcy and first stroke and ischemic and hemorrhagic stroke were observed after adjustment for potential covariates. This study emphasizes that SBP and tHcy are two of the most important risk factors and have independent and additive effects on the risk of the first stroke, ischemic and hemorrhagic stroke. The findings underscore the importance of screening and controlling high SBP and HHcy among the Chinese population in a rural area in order to further reduce the population burden of the first stroke.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XW, XX, and CZ gave the conception and methodology. FZ, CL, LY, YW, YSh, GZ, ZD, JC, and JK helped in the investigation. CL, LY, and JL were involved in data curation and analysis. YSo and LL helped in the validation. FZ, PZ, and CZ wrote the manuscript. XX and CZ helped in the supervision and with the financial support. All the authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.727418/full#supplementary-material

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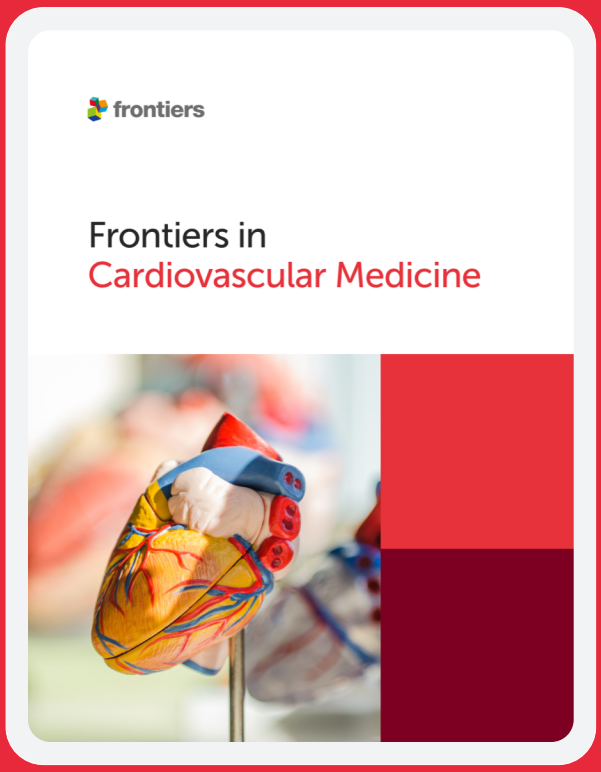

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