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Infectious diseases and hematology: diagnosis and management, volume II

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Infectious diseases and hematology: diagnosis and management, volume II

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Seroprevalence of Hepatitis B virus surface antigen among African blood donors: a systematic review and meta-analysis

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Background: Transfusion Transmitted Infections (TTIs) are still a growing public health problem in Africa. Studies that synthesize the available evidence on the seroprevalence of Hepatitis B Surface Antigen (HBsAg) among African blood donors are scarce. Therefore, this study aimed to synthesize qualitatively and quantitatively the seroprevalence of Hepatitis B Virus Surface Antigen (HBsAg) among blood donors in Africa.

Methods: We conducted a systematic review and meta-analysis where we included all studies that reported the seroprevalence of HBsAg among blood donors in Africa. The references were searched from electronic databases: PubMed, Web of Science, Cochrane, Scopus, WHO research database-HINARI, Global Index Medicus and ClinicalTrials.gov. We further analyzed the full list of references of all included studies. The pooled seroprevalence was estimated through random effect model. The heterogeneity was assessed through Cochrane's *Q* test and I^2, respectively. Meta-regression, subgroup and sensitivity analyses were conducted.

Results: We obtained 124 studies that met our inclusion criteria, comprising 3,573,211 blood donors tested for HBsAg. The pooled seroprevalence of HBsAg among blood donors in Africa was 6.93% (95% CI: 5.95–7.97%; $I^2 = 100\%$; $p < 0.001$). We found that the heterogeneity was explained by the study performed country and, African region. The higher prevalence was observed in Western 10.09% (95% CI: 8.75–11.50%), Central 7.81% (95% CI: 5.34–10.71%), and Eastern African region 4.87% (95% CI: 3.77–6.11%) and lower prevalence were observed in Southern 2.47% (95% CI: 0.54–5.75%) followed by Northern Africa region with 1.73% (95% CI: 0.45–3.79%). Additionally, based on the date of publication, we found that the highest prevalence was observed in studies published between 2001 and 2010 (9.41, 95% CI: 7.19–11.90) and the lowest prevalence was observed in studies published between 2011 and 2024 (6.26%; 95% CI: 5.19–7.42).

Conclusion: The seroprevalence of HBsAg among blood donors in Africa is still very high and heterogeneous. Therefore, intensifying the screening and vaccination of the population for Hepatitis B is critical to ensure blood safety toward eliminating Hepatitis B in Africa.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=395616, PROSPERO CRD42023395616.

KEYWORDS

blood donors, seroprevalence, serologic tests, Hepatitis B virus, African countries

Introduction

Hepatitis B Virus (HBV) remains one of the most serious public health concerns challenging the world, with an estimated 257–291 million individuals having chronic Hepatitis B (1). Africa is one of the highest-burden regions for Hepatitis B, where it is estimated that nearly 116 million people live with Hepatitis B and 81 million are chronically infected (2). An infected person can transmit HBV through direct contact with blood, unprotected sexual intercourse, use of contaminated needles and syringes, mother to child transmission during delivery, and transfusion of infected blood (3). Transfusion of infected blood is one of the main modes of HBV transmission, particularly in the sub-Saharan Africa region (4). Therefore, the World Health Organization (WHO) recommends that all countries provide access to screening and preventive measures such as vaccination and treatment for Hepatitis B (5).

Blood transfusion can be potentially lifesaving, but the risk of several Transfusions-Transmissible Infections (TTIs) such as Hepatitis B is high. For this reason, screening of blood donors for TTIs is essential for transfusion safety.

Although more sensitive tests are highly recommended for screening Hepatitis B among blood donors, most of lower- and middle-income countries still widely use rapid diagnostic tests. These methods are still indispensable to guarantee blood donation safety in many African countries (6). To maintain a safe supply of blood transfusion and products, the WHO recommends that all blood donations be screened for infections before use (7).

Several systematic reviews and meta-analyses have estimated the prevalence of hepatitis B among blood donors in some specific African countries (8–12). However, comprehensive studies on the prevalence of Hepatitis B among blood donors in Africa are scarce. Therefore, this study aimed to systematically synthesize the available evidence on the seroprevalence of Hepatitis B Virus Surface Antigen (HBsAg) among Blood Donors in Africa.

Abbreviations: AIDS, Acquired immunodeficiency syndrome; Anti-HBc, Hepatitis B virus core antibody; Anti-HCV, Hepatitis C virus antibody; Anti-HCV+, Hepatitis C virus antibody positive; Anti-HIV, Human Immunodeficiency Virus antibody; BD, Blood donors; ELISA, Enzyme-linked immunosorbent assay; FRBD, Family replacement blood donor; HBV, Hepatitis B virus; HBV-OBI, Occult Hepatitis B infection; HBsAg, Hepatitis B virus surface antigen; HBsAg-, Hepatitis B virus surface antigen negative; HBsAg+, Hepatitis B virus surface antigen positive; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HIV-1, Human immunodeficiency virus type 1; HIV-2, Human immunodeficiency virus type 2; QGIS, Quatum geo graphic information system; STDs, Sexually transmitted diseases; TPHA, *Treponema pallidum* hemagglutination assay; *T pallidum*, *Treponema pallidum*; TTIs, Transfusion-transmitted infections; VDRL, Venereal Disease Research Laboratory; VNR, volunteer's non-remunerated; VNRBD, volunteer's non-remunerated blood donors; WHO, World Health Organization.

Methods

Study design

This study is a systematic review and meta-analysis based on The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA Statement Guideline updated in 2020) (13). The study protocol was registered in the PROSPERO with the number CRD42023395616.

Search strategy and study selection

We included primary studies published in any language from inception through March 1st 2024, and having extractable data on seroprevalence of HBsAg among blood donors in Africa aged 16–65. We excluded case series, reviews, comments, editorials, and studies with duplicate data.

All relevant articles were searched in electronic databases, namely: PubMed/Medline, SCOPUS, Web of Science, WHO research database-HINARI, Cochrane database library, Global Index Medicus and Clinicaltrials.gov. The research query is in the Supplementary Table S1. We further analyzed systematically the full list of references of all included studies.

Two reviewers (AEQ, NC) carried out the study selection process independently, and discrepancies were resolved by the third reviewer (LA). This study was part of a more extensive research project that assessed the seroprevalence of Serologic Markers of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), and Syphilis in Blood Donors in Africa.

Due to the considerable volume of results, we decided to split such a study into four separate analyses based on the transmitted blood infection disease (Hepatitis B virus, Hepatitis C Virus, Syphilis, and HIV).

Data extraction

Two reviewers, AEQ and NC independently extracted the data for each included study based on a predefined and agreed-upon data extraction form designed for this study. The differences in extracted data were discussed, and persistent discrepancies were resolved by a third reviewer (LA). For each included study, we extracted the following information: Author name, year of publication, date of participant enrolment, study design, name of the country and African region where the study was performed, the total number of participants for each study, the total number of blood donors who tested positive for HBsAg, age, sex, type of blood donors (VNRBD-Voluntary Non-Remunerated Donors, RD- Replacement or Paid Donors/FD and FD-Family Donors), and the method used for screening and Hepatitis B diagnosis. This data was stored in a

Microsoft Excel 2021 spreadsheet (Microsoft Corporation, Redmond, Washington, USA).

Study's quality assessment

Two reviewers, AEQ and NC, independently assessed the quality of each included study using the risk of bias tool SeroTracker-RoB: a decision rule-based algorithm for reproducible risk of bias assessment of seroprevalence studies (14). The differences in the quality assessment of the included studies were discussed, and persistent disagreements were resolved by the third reviewer (LA). This tool derives from the Joanna Briggs Institute Checklist for Prevalence Studies and asks nine questions to assess the risk of bias. The questions are (a) Was the sample frame appropriate to address the target population? (b) Were study participants recruited in an appropriate way? (c) Was the sample size adequate? (d) Was the data analysis conducted with sufficient coverage of the identified sample? (e) Were valid methods used for the identification of the condition? (f) Was the condition measured in a standard, reliable way for all participants? (g) Was there appropriate adjustment for test characteristics? (h) Was there appropriate adjustment for population characteristics? (i) Was the response rate adequate, and if not, was the low response rate unlikely to introduce bias? And the last was the assessment of the overall risk of bias (lower, moderate, high and unclear) according to the scores from the responses of the previous nine items.

Data analysis

All the data were analyzed through R software version 4.3.2 (2023-10-31) using meta package and the functions for meta-analysis of proportion (15). We used the proportion of blood donors who tested positive for HBsAg as the parameter of interest to be estimated as our effect measure and meta-analyzed. We used the DerSimonian-Laird random effects model to estimate the pooled seroprevalence of HBsAg among blood donors in Africa, and the proportions were estimated based on Freeman-Tukey double arcsine transformation (FTT) (16). The findings were presented with 95% confidence intervals.

We run a Cochrane Q test and I^2 statistic (percentage of total variability due to true heterogeneity, that is, to between-studies variability) to assess the presence of heterogeneity and its relative magnitude, respectively (17). We performed subgroup and sensitivity analysis to investigate the moderator variables of the observed heterogeneity. Because we are analyzing and synthesizing prevalence studies from all of Africa and several different countries, we inherently assumed the presence of heterogeneity, and we mainly focused our analysis and results on subgroups and the assessment of moderators of heterogeneity.

The subgroup analysis studies were stratified by the country, African region, and year of publication. The years of publication were categorized into three categories (before 2000, 2001–2010 and 2011–2024). This cut-off was chosen based on the behavior of the distribution of the number of studies by year. To determine the moderators of heterogeneity, temporal trends and regional differences in our study, we performed meta-regression analyses using the following variables: year of study publication and African region (Western, Northern, Eastern, Central, and Southern), risk of bias, study location (unicentric and multicentric), setting (Urban and Rural), proportion of men, age, type of blood donors and country where the study was performed. In our study, we defined study location as unicentric if the study was carried out in a single center or one hospital. In contrast, a multicentric study means the study was conducted in multiple centers or hospitals. The setting variable refers to whether the study was conducted in an urban or rural area.

The publication bias was assessed through a funnel plot and by Egger's statistics regression test. We mapped the spatial pattern of the pooled estimates of seroprevalence of HBsAg among blood donors in Africa by country. The map was created using Quantum Geographic Information System (QGIS) software (18).

Results

A total of 4,408 were identified through database and manual searching, and 500 duplicate articles were removed. The title and abstract of the remaining 3,908 were screened, and 3,605 articles were removed as they were found to be irrelevant to our study. The remaining 303 references were assessed for eligibility through the complete text examination, and 179 were excluded because they did not meet our inclusion criteria. The remaining 124 studies were considered for qualitative and quantitative synthesis involving 3,573,211 participants.

Among 179 that did not meet our inclusion criteria, 77 did not study the prevalence of Hepatitis B among blood donors, 43 were systematic reviews, 16 studies did not have relevant data, five studies did not have their full text available, 12 studies included population already positive to Hepatitis B, 12 studies included children, 14 studies included pregnant women (See Figure 1; Supplementary Table S3).

Study characteristics

Supplementary Table S2 shows the characteristics of the studies included in this work. Thirty (55.5%) of the 54 African countries are represented in the 124 studies included. Most of the studies were conducted in Western Africa 51 (41.13%), followed by Eastern Africa 32 (25.81%), then by Central 26 (20.97%), and lastly by the Northern 9 (7.26%) and Southern 6 (4.84%) African region.

The year of study publication ranged from 1990 to 2024. The majority, 89 (75%), were published after 2010. The median proportion of men in the included studies was 83.75%.

Regarding the risk of bias, most studies had a moderate risk of bias 70 (56.45%), followed by a low risk of bias 36 (29.03%), and lastly by a high risk of bias 18 (14.52%).

Seroprevalence of hepatitis B surface antigen

We found that the pooled seroprevalence of HBsAg among blood donors in Africa was 6.93% (95% CI: 5.95–7.97%; see the forest plot in Figure 2).

In subgroup analysis, we found statistically significant differences in the seroprevalence of HBsAg among blood donors in Africa

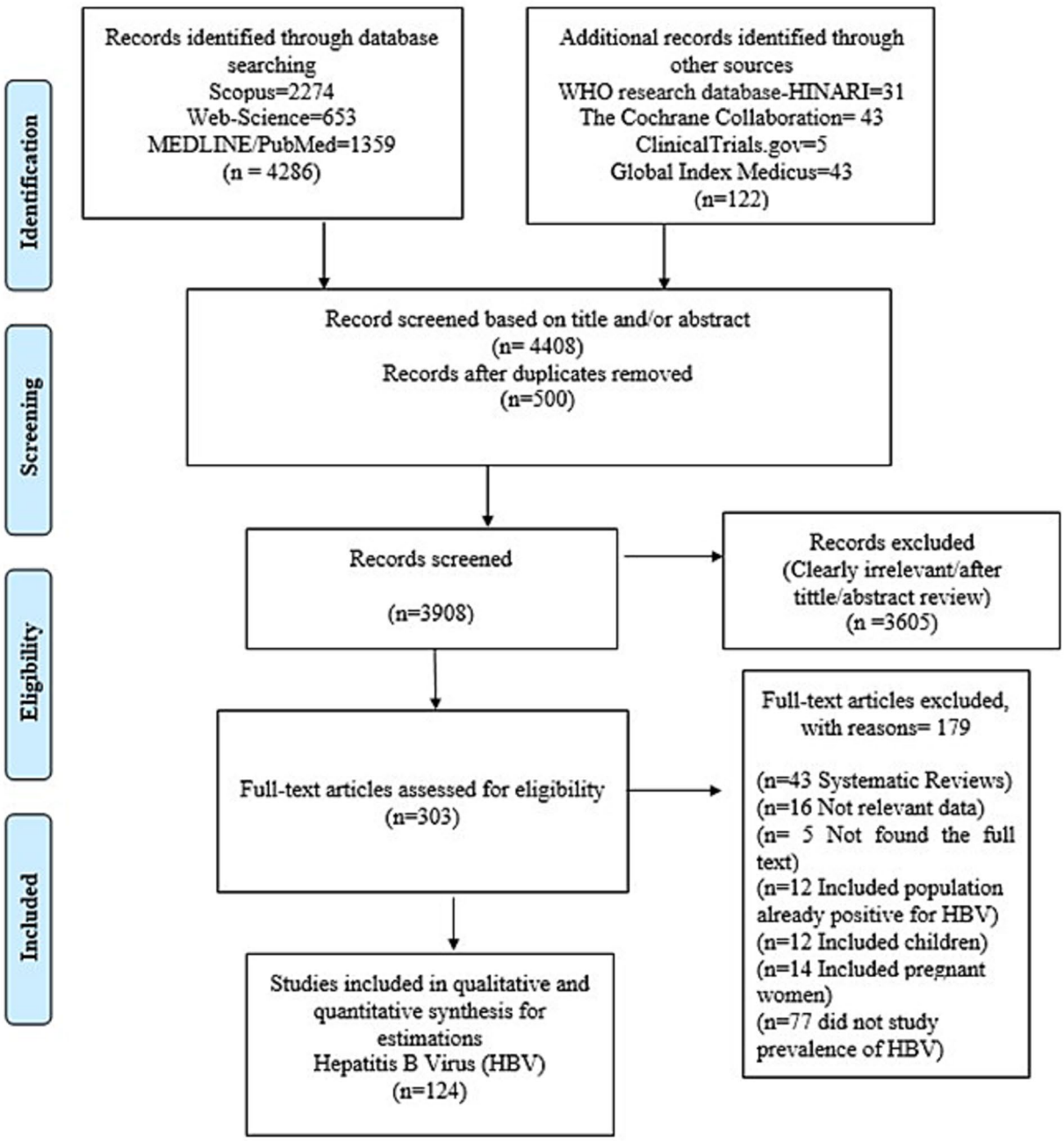


FIGURE 1
PRISMA flow diagram of studies reviewed, screened, and included.

according to the study country ($p < 0.01$), year of study publication ($p < 0.03$) and African region ($p < 0.01$), (Figures 2, 3; Table 1).

Regarding the seroprevalence of HBsAg by African regions, we found that the Western region had the highest prevalence of HBsAg at 10.09% (95% CI: 8.75–11.50%), followed by the Central region with 7.81% (95% CI: 5.34–10.71%), then by the Eastern Africa region with 4.87% (95% CI: 3.77–6.11%) the Southern with 2.47% (95% CI: 0.54–5.75%) and finally, by the Northern African region with 1.73% (95% CI: 0.45–3.79%).

Regarding to the year of study publication, highest prevalence was observed in studies published between 2001 and 2010 (9.41%; 95% CI: 7.19–11.90%) followed by studies published from 1990 to 2000 (8.07%; 95% CI: 3.80–13.73%) and the lowest prevalence was observed in the studies published between 2011 and 2024 (6.26%; 95% CI: 5.19–7.42%) (see Table 1).

We generally found high heterogeneity among pooled studies (Cochran *Q*-test $p < 0.001$ and $I^2 = 100\%$). In the meta-regression analysis, we observed that the heterogeneity was moderated by the African region ($p < 0.01$) and the country where the study was performed ($p < 0.01$) (see Table 2).

Among the studied moderator variables, 44.69% of the heterogeneity was explained by the country where the study was performed ($p < 0.01$), and by the African region 28.60% ($p < 0.01$). We did not find a statistically significant variation in the seroprevalence

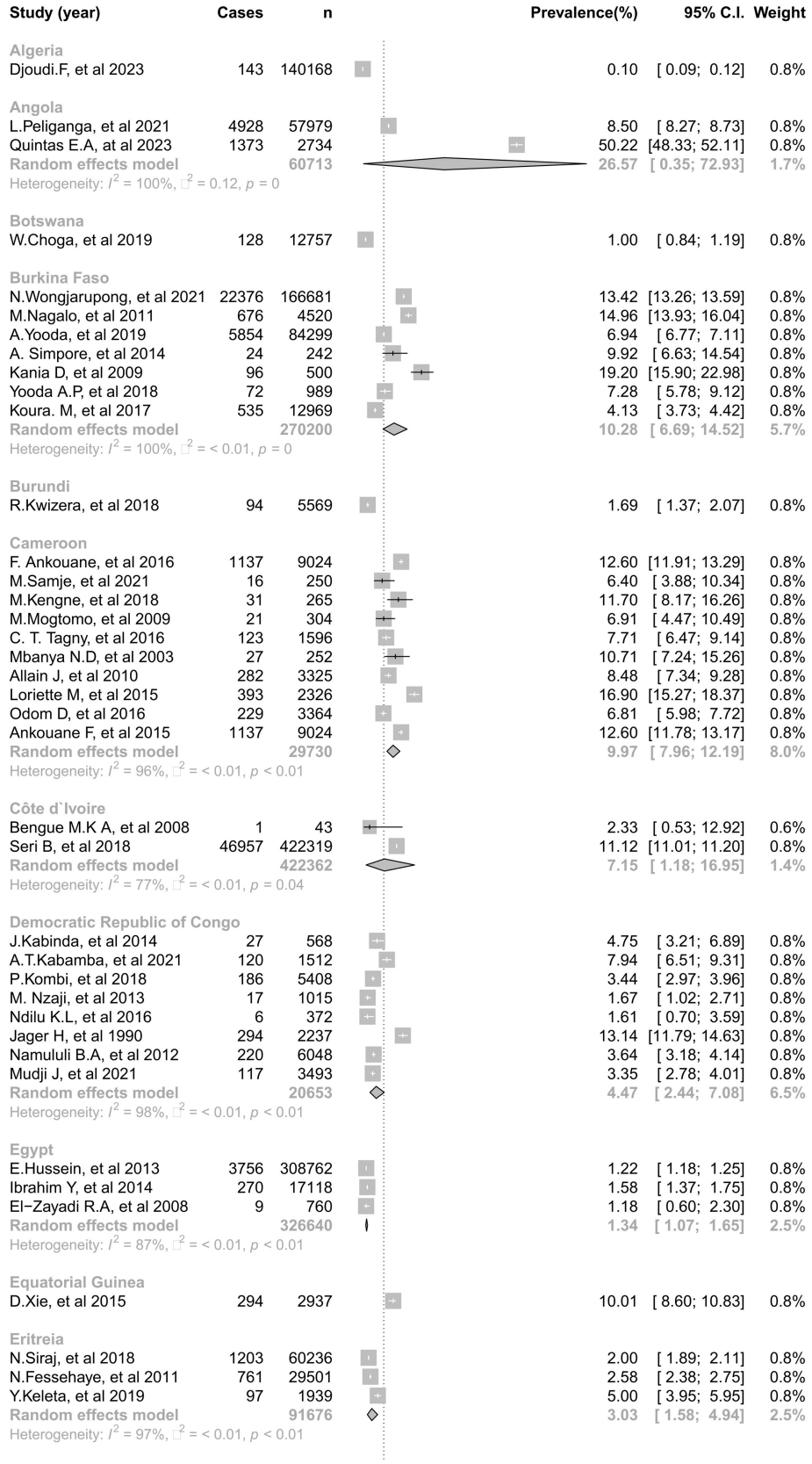
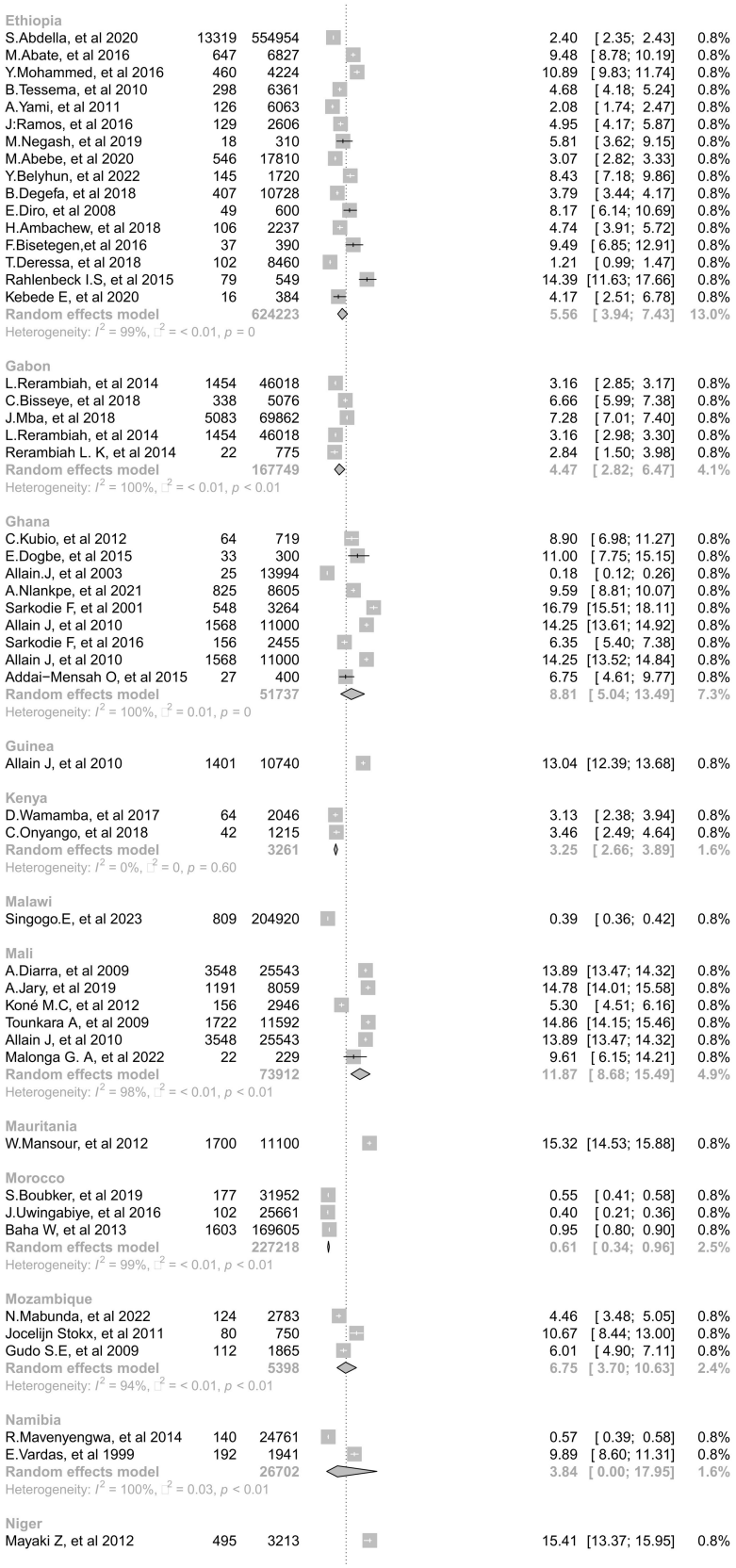


FIGURE 2 (Continued)



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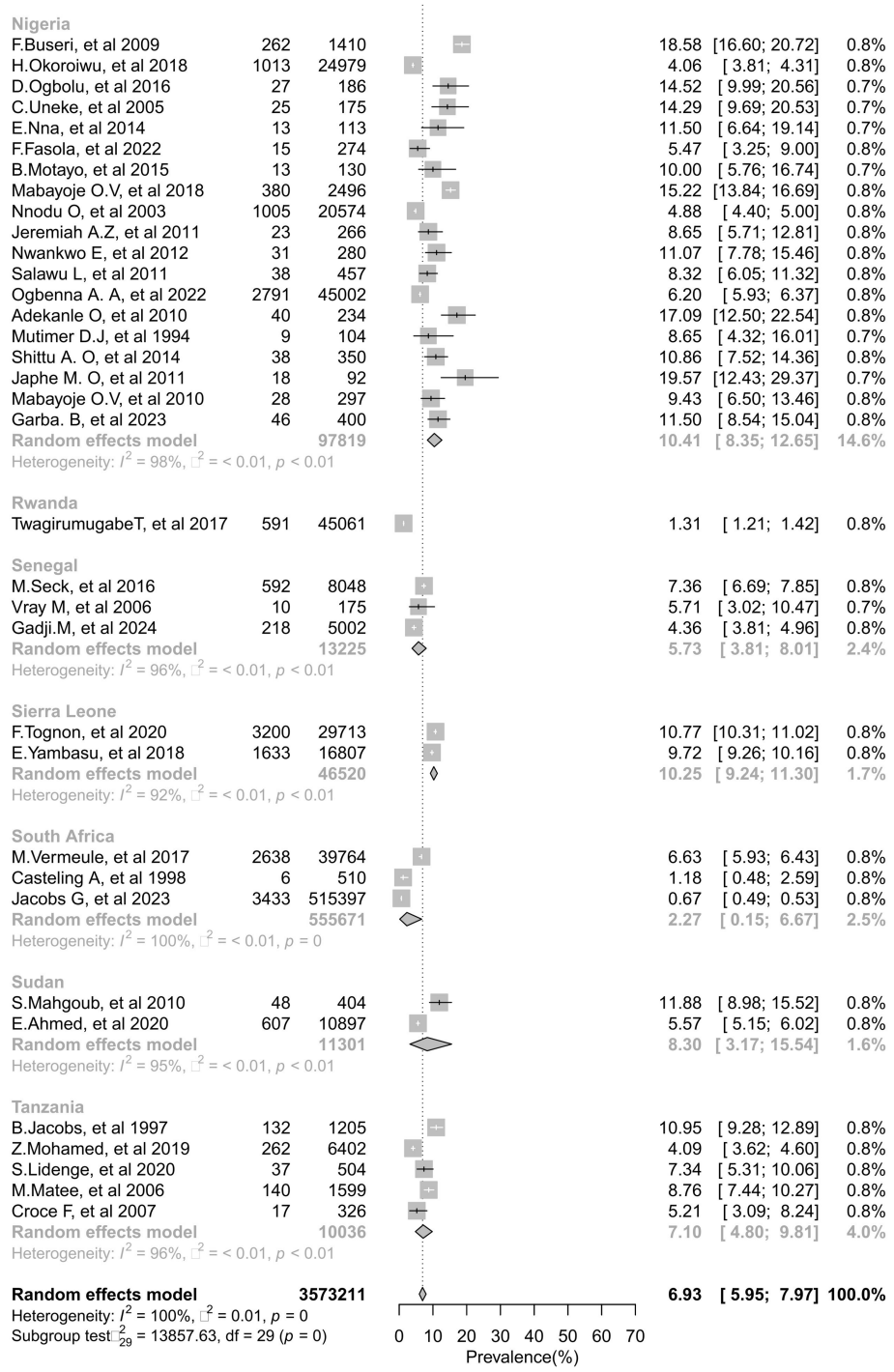

FIGURE 2
Forest plot of the pooled seroprevalence of Hepatitis B Surface Antigen among Blood donors in Africa by country, Random-effect model: subgroup analysis by region. ES estimated prevalence of HBV.

of HBsAg by the risk of bias ($p = 0.92$), study location ($p = 0.05$), setting ($p < 0.69$), year of study publication ($p = 0.07$) type of blood donor ($p = 0.64$), age ($p = 0.89$) and proportion of males ($p = 0.31$) (see Table 2).

Although the year of study publication was not statistically significant in the meta-regression analysis, we did find a decreased trend in the seroprevalence of hepatitis B among African blood donors over the years (see Figure 4).

The funnel plot showed asymmetry, and the regression Egger's test was statistically significant ($p < 0.01$). Meaning that the evidence of the presence of risk of publication bias was identified (see Figure 5).

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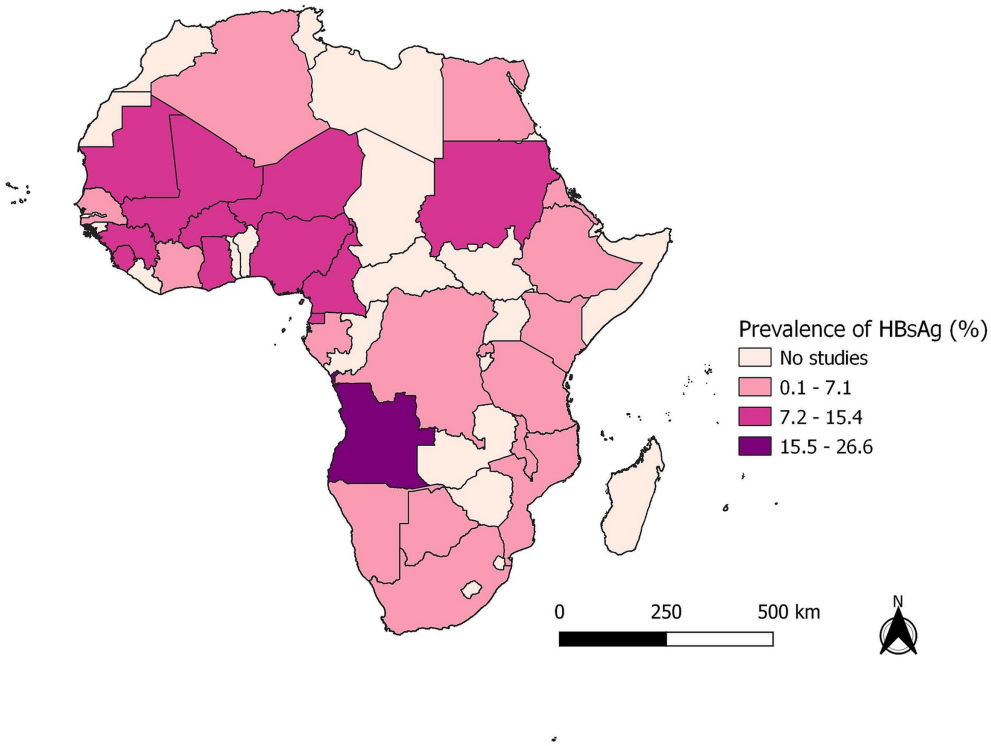

FIGURE 3
Map of the seroprevalence of Hepatitis B among blood donors in Africa.

TABLE 1 Sub-group analysis of the pooled prevalence of HBsAg estimation in African blood donors by regions (1990–2024).

Moderator variables	Category	Number of studies	Aggregate sample size	Prevalence % (95% CI)	I^2 (%)	*p*-value
Africa region	Western	51	1,000,828	10.09 (8.75; 11.50)	99.7	0.01
	Eastern	32	990,144	4.87 (3.77; 6.11)	99.7	
	Central	26	281,782	7.81 (5.34; 10.71)	99.7	
	Northern	9	705,327	1.73 (0.45; 3.79)	99.8	
	Southern	6	595,130	2.47 (0.54; 5.75)	99.9	
Year of publication	1990–2000	5	5,997	8.07 (3.80; 13.73)	96.4	0.03
	2001–2010	26	151,880	9.41 (7.19; 11.90)	99.7	
	2011–2024	93	3,415,334	6.26 (5.19; 7.42)	99.9	

I^2 = Heterogeneity; *p*-value: significance test of subgroup differences.

TABLE 2 Moderators of heterogeneity on the seroprevalence of HBsAg in blood donors in Africa.

Variables	Moderators test *p*-value	R^2 (%)
African region	< 0.01	28.60
Country	< 0.01	44.69
Risk of bias	0.92	0.0
Location	0.05	2.05
Setting	0.69	0.00
Type of Blood donors	0.64	0.00
Age	0.89	0.00
Proportion of male	0.31	0.07
Year of study publication	0.07	2.02

R^2: The amount of heterogeneity accounted for.

Discussion

Our study shows that the seroprevalence of HBsAg among blood donors in African countries was 6.93% (95% CI: 5.95–7.97%). This finding is consistent with a report on the prevalence of HBsAg in the general population in Africa, which is considered to be higher (19). This means the Hepatitis B virus remains an enormous public health problem in Africa (5). These findings are worrisome as there are reports of transmission of the Hepatitis B virus infection by blood transfusion (20, 21). The risk of becoming infected with HBV in sub-Saharan Africa from a blood transfusion is high and around 4.3 per 1,000 units (4).

In our study, the seroprevalence of HBsAg among blood donors was higher compared to data reported from the European Union, which is 1.1% among first-time blood donors (22), China 1.32% (23), Laos (Southeast Asian country) which was around 2.6% (24)

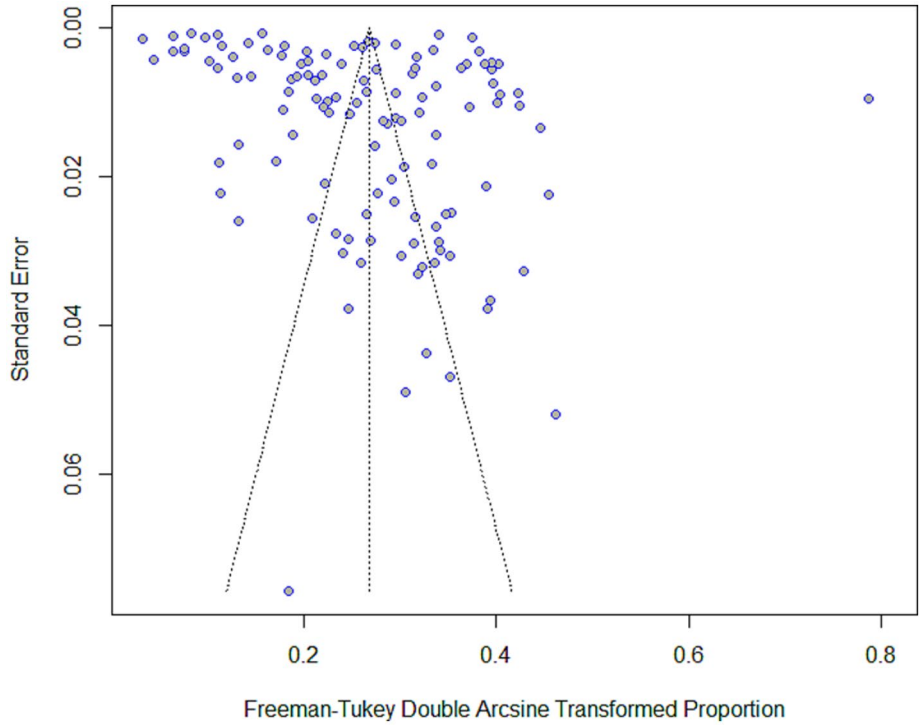

FIGURE 5
Funnel plot of the seroprevalence of HBV in African blood donors from 1990 to 2022.

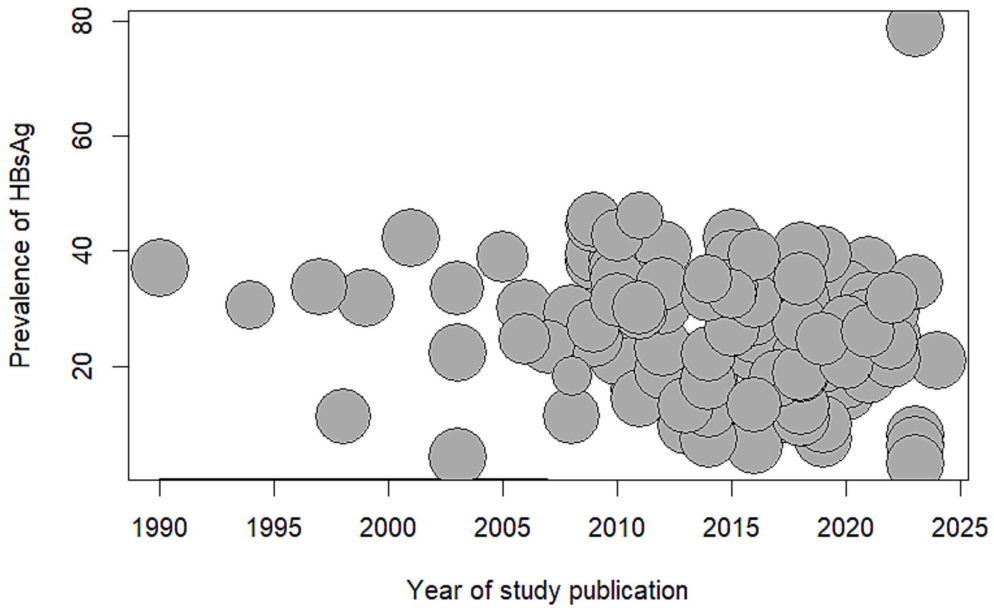

FIGURE 4
Bubble plot meta-regression of seroprevalence of Hepatitis B Surface Antigen among blood donors in Africa and year of study publication. HBsAg: Hepatitis B Surface Antigen.

and in the Eastern Mediterranean and Middle Eastern countries which were 2.03% (25).

We found statistically significant differences in the prevalence of HBsAg based on the African region where the study was performed. The Western Africa region had the highest prevalence of 10.09%, followed by the Central region (7.81%) and Eastern (4.87%), while the Southern (2.47%) and Northern African regions (1.73%) exhibited lower prevalence.

These findings are consistent with the systematic reviews and meta-analyses conducted in countries of the Western region, such as Burkina Faso (26), Kenya (27) in Eastern Africa, and Cameron (28) in the Central region of Africa, which show a higher prevalence of Hepatitis B ranging from 8 to 12%. In contrast, the low prevalence observed in the countries of Northern and Southern Africa is consistent with the epidemiological study on the prevalence of the Hepatitis B virus in Africa, which shows a low endemicity level (<2%) in the Northern region (19, 29).

We found an inverse relationship between the prevalence of Hepatitis B among blood donors in Africa and the year of the study publication, although it was not statistically significant in the meta regression analysis, we did find statistically differences in subgroup analysis splitting the year into three categories. We found that, published studies (after 2010) tended to present lower seroprevalence of Hepatitis B than studies published before 2010. This finding can be explained by the introduction of universal infant and childhood hepatitis B vaccination programs in 1997 (30) and improved screening and treatment of Hepatitis B.

Additionally, we found that the country where the study was carried out was a statistically significant moderator of the heterogeneity of the seroprevalence of HBsAg. These findings can be explained by the existing differences in the access and quality of screening procedures, the social and demographic profile of each country, lifestyle, prevalence of hepatitis B in the general population, and much more importantly, availability of vaccination and treatment services in these countries (19, 31).

Our systematic review has some limitations: The pooled seroprevalence of HBsAg among blood donors that we found cannot be generalized to the whole of Africa as 24 (44%) of African countries did not have any study on the topic. The studies overrepresented countries located in the Western, Central and Eastern regions of Africa and underrepresented those countries in the Northern and Southern regions of the continent. Therefore, further studies are needed concerning underrepresented African areas to complement our findings and to have a good overview of the seroprevalence of HBsAg in Africa. Additionally, we found higher heterogeneity among the included studies ($I^2 = 100\%$). Moreover, we found greater variation in the precision of our estimates due to differences in the total sample sizes of studies across different periods and African regions. Specifically, fewer populations were included in studies conducted in the 1990s compared to the larger number included in studies after 2001. Similarly, smaller sample sizes were observed in the Southern and Northern regions compared to the Western, Eastern, and Central African regions.

Notwithstanding the above limitations, this study has some strengths worth mentioning: to the best of our knowledge, this is the first systematic review and meta-analysis study that analyzed and synthesized the seroprevalence of HBsAg among blood donors in Africa and investigated the reasons for the variability of the prevalence of HBsAg across Africa.

Conclusion: The prevalence of HBsAg among blood donors in Africa is still very high, and it widely varies according to the country, African regions, and year of study publication. Therefore, there is a need for scale-up strategies to intensify the screening of blood donors and extend access to the Hepatitis B vaccine and improve public policy for blood transfusion safety toward Hepatitis B virus elimination.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

AQ: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. NC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. LC: Conceptualization, Supervision, Validation, Writing – review & editing, Investigation. AS: Conceptualization, Investigation, Supervision, Validation, Writing – review & editing. LA: Conceptualization, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing, Formal analysis, Writing – original draft.

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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.

Publisher's note

Supplementary material

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A case report of severe drug-induced immune hemolytic anemia caused by piperacillin

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Piperacillin is a beta-lactamase inhibitor frequently used in the treatment of urinary tract infections. It is a broad-spectrum antibiotic with strong antibacterial action against *Pseudomonas aeruginosa* and *Enterobacter*, especially extended-spectrum beta-lactamase-producing Enterobacteria and *Enterococcus*. Side effects of piperacillin include allergic reactions, rashes such as urticaria, leukopenia, interstitial nephritis, asthma attacks, serological reactions, candida infection, and bleeding with more severe reactions resulting in anaphylactic shock. Anemia and hemolytic anemia are rare adverse reactions to piperacillin, with an incidence of 0.01–0.10%. We report herein the case of a severe postoperative immune hemolytic reaction to piperacillin. Fortunately, we quickly recognized and identified the drug reaction caused by piperacillin, immediately stopped the use of piperacillin, and performed a blood transfusion. The patient recovered and was subsequently discharged from the hospital.

KEYWORDS

piperacillin, drug-induced immune hemolytic anemia, autoimmune hemolytic anemia, human leukocyte antigen, transfusion

Introduction

Piperacillin is the third most common antibiotic to induce hemolytic anemia, after cefotetan and ceftriaxone. Studies have shown that patients treated with piperacillin should be evaluated for drug-induced immune hemolytic anemia (DIIHA) if they develop new anemia or an increase in anemia (1). DIIHA is a secondary form of autoimmune hemolytic anemia (AIHA), which is characterized by the increased destruction of red blood cells (RBCs), triggered by autoantibodies that target antigens on the surface of RBCs (2, 3). DIIHA accounts for approximately 10% of all AIHA cases, with an estimated incidence of 1–3 cases per 100,000 people per year (4). The pathogenesis of AIHA is multi-factorial, including genetic factors, infection, autoimmune diseases, and drugs. DIIHA, however, is rare and difficult to diagnose, with knowledge and clinical experience forming the basis for identifying this phenomenon. Diagnoses are typically obtained by combining a patient's

clinical history with the presentation of hemolytic anemia, relying primarily on the identification of anti-RBC autoantibodies through a direct antiglobulin test (DAT) followed by the exclusion of other potential causes of hemolytic anemia. Mayer et al. (5) reviewed 73 cases of DIIHA from 1996–2005 in a German institute, 13 of which were the result of piperacillin administration. All 13 patients presented with acute hemolysis and had positive DAT results.

We report herein the case of a 27-year-old woman who experienced severe hemolytic anemia due to the use of piperacillin-sulbactam sodium after surgery for endometrial malignancy. Piperacillin-dependent antibodies were detected in her serum; therefore, we ruled out the possibility of isoantibodies. We performed human leukocyte antigen gene complex (HLA) class II antigen typing and found that HLA-DQB1*03-DRB1-09*01 haplotype may potentially mediate DIIHA. This study was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University (2020051).

Case description

A 27-year-old woman was diagnosed with highly differentiated endometrial adenocarcinoma (stage IVc) and underwent surgical treatment 7 years ago. Chemotherapy was administered after the surgery, while platinum drug allergies was discovered during the chemotherapy. Admitted to the hospital due to the discovery of a pelvic and abdominal mass for over 7 months, she was conducted laparotomy for endometrial carcinoma, intestinal resection, intestinal anastomosis, enterostomy, abdominal aorta repair, and left common iliac artery repair under general anesthesia. The intraoperative blood loss was 1700 mL. The patient received a type A, RhD-positive, intraoperative transfusion of 3 units (U) of red leukocyte-depleted suspension containing RBCs and 400 mL of frozen fresh plasma. Postoperatively, low-molecular-weight heparin was used for preventive anticoagulation, and cefuroxime was used to prevent infection. The patient's postoperative Hb was 86 g/dL, she had no obvious bleeding, and routine piperacillin was administered to prevent infection. On the 5th day after surgery, her Hb decreased to 58 g/dL, antibody screening was positive, I(1+), II(1+), III(1+w), DAT (3+) – lactic dehydrogenase (LDH) was 612 IU/L, and unconjugated bilirubin (UCB) was 23.9 uM/L. Therefore, the patient was suspected to have AIHA. Additional clinical information of the patients is presented in Table 1. Her Hb increased to 75 g/dL after a transfusion of 3 U RBCs, indicating effective transfusion.

The patient's Hb dropped to 57 g/dL again on the 9th day after surgery, at which time it was suspected that the autoantibodies had combined with isoantibodies. Her LDH was 915 IU/L, UCB was

TABLE 1 Summary of the patient's clinical information.

Days	Laboratory Results						Symptoms and Signs	Medication Treatment	Transfused RBC (U)	Transfusion Reaction (Y/N)
	Hb (g/L)	PLT (*10^9/L)	WBC (%)	WBC (*10^9/L)	RET (*10^{12}/L)	RET (%)				
0	107	132	64.9	4.3	/	/	Laparotomy for endometrial cancer after chemotherapy	/	3	N
1	/	/	/	/	/	/	Norm	Cefuroxime	/	/
2	86	74	85.9	7.4	/	/	Norm	Piperacillin-tazobactam	/	/
3	88	69	5.9	69.8	/	/	Body temperature increased, with a top of 38°C.	Piperacillin-tazobactam	/	/
4	/	/	/	/	/	/	Norm	Piperacillin-tazobactam	/	/
5	62	60	55.7	3.3	/	/	Norm	Piperacillin-tazobactam	/	/
6	58	53	49.3	2.9	/	/	Norm; Anemic appearance.	Piperacillin-tazobactam	3	Y
7	75	55	61.0	3.8	/	/	Norm; Anemic appearance.	Stop antibiotics	/	/
8	68	50	58.3	3.1	/	/	Norm; Anemic appearance; Fever at night,	Stop antibiotics	/	/

(Continued)

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TABLE 1 Continued

Days	Laboratory Results						Symptoms and Signs	Medication Treatment	Transfused RBC (U)	Transfusion Reaction (Y/N)
	Hb (g/L)	PLT (*10^9/L)	WBC (%)	WBC (*10^9/L)	RET (*10^{12}/L)	RET (%)				
							with a top of 38.5°C.			
9	57	53	55.2	3.4	/	/	Norm; Anemic appearance.	Piperacillin-tazobactam	1.5	Y
10	56	48	76.4	2.9	/	/	Norm; Dizziness and fatigue. Palpebral conjunctiva and nail bed pale, severe anemia appearance.	Piperacillin-tazobactam	3	Y
11	64	56	70.8	4.0	/	/	Norm; Temperature fluctuates between 36.8°C-38°C, poor spirit and sleep, and average appetite. Anemia appearance.	Piperacillin-tazobactam	/	/
12	54	51	54.0	3.1	/	/	Norm; Anemia appearance.	Cefoperazone sodium and sulbactam sodium and Prednisone	/	/
13	/	/	/	/	/	/	Norm; Slightly dizzy; Anemia appearance.	Meropenem and Prednisone	/	/
14	42	56	67.1	4.0	0.0020	0.15	Norm; Dizziness and fatigue, and occasionally flustered. Severe anemia, jaundice.	Meropenem and Prednisone	3	N
15	56	49	76.9	6.7	0.006	0.33	Norm; Dizziness eased.	Meropenem, Prednisone and **IVIG**	/	/
16	55	45	72.7	7.8	0.0034	0.20	Norm; Dizziness eased.	Meropenem, Prednisone and IVIG	/	/
17	52	52	82.0	6.8	0.0094	0.60	Norm; Slightly tired and weak after the activity; Anemia appearance.	Meropenem, Prednisone and IVIG	/	/
18	52	58	71.0	6.8	0.0891	5.57	Norm; Anemia appearance.	Meropenem, Prednisone and IVIG	/	/

(Continued)

TABLE 1 Continued

Days	Laboratory Results						Symptoms and Signs	Medication Treatment	Transfused RBC (U)	Transfusion Reaction (Y/N)
	Hb (g/L)	PLT (*10^9/L)	WBC (%)	WBC (*10^9/L)	RET (*10^{12}/L)	RET (%)				
19	/	/	/	/	/	/	Norm; Anemia appearance.	Prednisone	/	/
20	93	66	76.5	6.4	/	/	Norm; Anemia appearance.	Prednisone	3*	N
21	/	/	/	/	/	/	Norm; Discharge.	Prednisone	/	/

1. The numbers in "Days" column indicate the day after the operation, the number "0 " indicates the day on operation.
2. The "/" indicates not involved. The "Y" indicates occurred transfusion reaction, while "N" indicates not occurred.
3. The "Norm" in "Symptoms and Signs" column indicates Vital signs are stable, and general conditions are good.
4. The "IVIG" indicates Intravenous immunoglobulin.
5. The "*" indicates the antigen of the transfused RBC is Jka(-) and Leb(-).
6. The medication dosages: Piperacillin-tazobactam, 4.5g q8h; Piperacillin-tazobactam, 4.5g q8h; Meropenem, 0.5g q8h; Prednisone, 25mg bid; Intravenous immunoglobulin, 0.4g/kg.d.

21.2 uM/L. The patient experienced fever and chills during the transfusion of 1.5U Jka(-)/Leb(-) RBCs, with the body temperature rose to 39°C half an hour later. Her Hb remained at 56 g/dL, indicating that the blood transfusion had been ineffective. We then evaluated for the presence of drug antibodies, and piperacillin drug antibodies were detected. Piperacillin was discontinued 14 days after surgery, at which time Hb was 42 g/dL. The patient received another 3 U RBC transfusion, as well as simultaneous intravenous immunoglobulin (IVIG) and prednisone therapy. Her Hb increased to 56 g/dL, and the IVIG and prednisone levels were sustained. indicating that the blood transfusion was effective. Her Hb remained at 52 g/dL for 4 days without further change. Another 3U RBC transfusion was administered 18 days after surgery, and her Hb increased from 52 to 93 g/dL, and LDH decreased to 597 IU/L, UCB increased to 14.2 uM/L. Her Hb was re-evaluated 20 days post-discharge, when it was found to be 111 g/dL, and antibody screening was negative. The overall changes in Hb, PLT, and WBC counts are shown in Figure 1. Details of serological information are shown in Table 2. The patient provided written informed consent before enrolment. This study was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University (2020051).

Transfusion-related Serological Test Results

The serological typing reagents used were commercial monoclonal IgM, IgG, and IgA from Sanquin Reagents B. V. (Netherlands). The Microcolumn gel or saline test tube method was used to type the patient's antigen according to the manufacturer's specifications. On the 5th day after surgery, antibody screening was positive, I(1+), II(1+), III(1+w), direct antiglobulin direct test (DAT) (3+) for all IgG, IgG+C3d, and

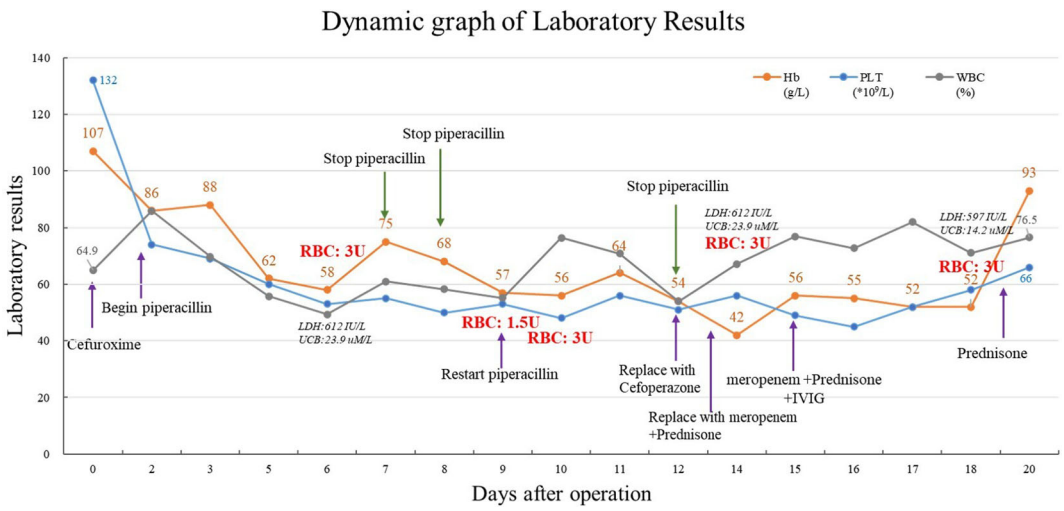

FIGURE 1
Dynamic graph of laboratory results.

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TABLE 2 Transfusion and immunology related information.

Days	Transfusion and Serological Results						Situations
	Transfused RBC (U)	Antibody Screen	DAT	Major Cross	Minor Cross	Transfusion Reaction and Treatment	
0	3	Neg	Neg	Neg	Neg	No adverse transfusion reaction.	Transfused in surgery.
6	3	1+	4+	1+	3+	No adverse transfusion reaction.	Use piperacillin-tazobactam for 5 days
9	1.5	1+	4+	1+	3+	**Symptoms and Signs**: Chills, fever, body temperature: 38.5°C, pulse: 95 beats/min, blood pressure (BP): 117/64mmHg. The temperature rose to 39.0°C half an hour later. **Treatment:** Stopped transfusion immediately and dexamethasone 10mg was administered. Merrill 7.5ml orally for antipyretic.	After 2 days of discontinuation, piperacillin-tazobactam were reapplied on day 1.
10	3	2+	4+	1+	3+	**Symptoms and Signs:** Fever, temperature fluctuation range 36.8°C-39°C;plus: 100 beats/min, BP 97/67mmHg, oxygen saturation 98%. **Treatment:** Closely monitor.	After 2 days of discontinuation piperacillin-tazobactam were reapplied on day 2.
14	3	2+	4+	1+	3+	**Symptoms and signs:** Chest distress; backache. **Treatment:** Intravenous infusion of dexamethasone.	3 days after changing antibiotics.
20	3*	2+	4+	1+	3+	Prophylactic use of dexamethasone, no adverse transfusion reaction.	9 days after antibiotic change, 8 days after hormone therapy, 4 days after IVIG therapy.

1. The numbers in "Days" column indicate the day after the operation, the number "0 " indicates the day on operation.
2. The "Neg" indicates a negative reactive result; the "+" indicates a positive reactive result, and the number before "+" indicate the serologic reaction strength.
3. The "*" indicate the antigen of the transfused RBC is Jka(-) and Leb(-).
4. The medication treatment: the changed antibiotics is meropenem; the hormone therapy is prednisone; IVIG indicates Intravenous immunoglobulin.

C3d. The patient's antigen typing results were as follows: DccEe, S-s+, Jk(a-b+), M+N+, Le(a+b-), Fy(a+b+), Wra+, Mia+, Lu(a-b+), Dia+, and P1+. Binding antibody identification results indicated that anti-Jk(a) combined with anti-Le(b) mediated the delayed hemolytic transfusion reaction (Tables 3–5).

Drug antibody test results

Antibody test reagents were purchased from Zhongjiwantai Company (China). The microcolumn gel method was performed according to the manufacturer's protocol. Before the use of piperacillin, no antibody to piperacillin was detected in the plasma and RBCs of patients; when piperacillin was discontinued, antibody to piperacillin was detected in both plasma and RBCs. When discharged, only antibody to piperacillin was detected on RBCs, the titer was 16, and the titer was lower than that when piperacillin was discontinued, indicating that the amount of drug antibody in the body was significantly reduced. Twenty days after discharge, plasma and RBCs were negative for drug antibodies (Table 6).

HLA typing

HLA-DRB1 and -DQB1 were detected after deoxyribonucleic acid (DNA) extraction from peripheral blood, following the protocol as previously described (6, 7). The results showed a haplotype of HLA-DQB1*03:03-DRB1-09*01, and DQB1*03:03-DRB1*14:01.

Discussion

We have reported herein a case of a severe postoperative immune hemolytic reaction caused by the administration of piperacillin. Fortunately, we quickly recognized and identified the drug reaction to piperacillin and were able to immediately stop the administration of piperacillin. We were also able to administer the appropriate treatment, which included blood transfusions, IVIG, and glucocorticoids. This case highlights that doctors must remain vigilant about the potential adverse effects of piperacillin when administering the drug in the clinic. Furthermore, once an adverse reaction occurs, the timely discontinuation of piperacillin and

TABLE 3 Summary of *Immunohematology* of present case.

Blood types	ABO	Rh	Duffy	Kidd	Lewis	P1	MN	Ss	Wra	Mia	Dia	Luth
Results	A	DccEe	Fy(a+b+)	Jk(a-b+)	Le(a+b-)	+	M+N+	S-s+	+	+	+	Lu(a-b+)

TABLE 4 Results of antibody screening test in the plasma and eluate.

Antibody Screening Cells	Rh-hr					Kell			Duffy			Kidd		Lewis		P	MNS				Luth	Colt	Xg	Experimental Results	
	C	D	E	c	e	K	k	Kpa	Jsa	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Cob	Xga	Plasma	Eluate
I	+	+	0	0	+	0	+	0	0	+	0	+	0	0	+	+	0	+	0	+	+	0	+	1+	1+
II	+	0	+	+	0	0	+	0	nt	+	+	+	+	+	0	0	+	0	+	0	0	0	+	1+	1+
III	0	0	0	+	+	+	+	0	0	0	+	0	+	0	0	+	0	+	0	+	0	0	+	w	w

TABLE 5 Reaction results with spectrum cells of patient in plasma and eluate.

	Rh-Hr					Kell						Duffy		Kidd		Lewis		P	MNS				Luther		Xg	Experimental Results		
	C	D	E	c	e	K	k	Kpa	Kp^b	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	IgG+C3		
																										Plasma	Elute	Papain
1	+	+	0	0	+	0	+	0	+	/	+	+	0	+	0	0	+	+	+	0	0	+	0	+	+	1+	w	3+
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	/	w	0	3+
3	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	0	+	+	+	0	w	/	w	0	3+
4	0	+	0	+	+	0	+	0	+	/	+	+	0	+	0	0	+	+	+	0	+	0	+	+	+	1+	1+	3+
5	+	0	0	0	+	0	+	0	+	/	+	+	0	+	+	0	+	+	0	+	+	+	0	+	+	w	0	3+
6	0	0	+	+	0	0	+	0	+	/	+	0	+	+	+	0	+	+	+	0	+	0	0	+	+	1+	1+	3+
7	0	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	0	0	+	+	0	+	+	+	/	w	1+	3+
8	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	0	+	+	+	+	0	+	0	+	/	w	1+	3+
9	0	0	0	+	+	+	0	0	+	/	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	w	0	3+

(Continued)

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TABLE 5 Continued

	Rh-Hr					Kell						Duffy		Kidd		Lewis		P	MNS				Luther		Xg	Experimental Results		
																										IgG+C3		
	C	D	E	c	e	K	k	Kpa	Kp^b	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	Plasma	Elute	Papain
10	0	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	+	+	0	0	+	/	w+	0	3+
11	+	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	+	0	+	/	0	0	3+
12	+	+	+	+	0	0	+	0	+	/	+	+	0	+	+	0	+	+	+	+	0	+	0	+	/	1+	w	3+
13	0	0	0	+	+	0	+	0	+	/	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	1+	1+	3+
14	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	w	0	3+
15	0	+	+	+	+	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	+	+	0	+	1+	1+	3+
16	+	0	0	+	+	0	+	0	+	/	+	+	+	+	+	0	+	+	+	+	0	+	0	+	/	2+	1+	3+

appropriate drug and blood transfusion interventions are key to achieving a good outcome.

Piperacillin is the third most common antibiotic to cause serious adverse drug reactions, as previously reported, among which acute immune hemolysis reaction is one of the more serious reactions (8). Acute piperacillin-induced DIIHA is a complex process mediated primarily by immune mechanisms, which occurs through two main mechanisms: non-drug- and drug-dependent antibodies. Non-drug-dependent antibodies behave similarly to autoantibodies and can cause AIHA, while drug-dependent antibodies react with RBCs only in the presence of drugs (9). The most common mechanism by which piperacillin induces DIIHA is from drug-dependent antibodies, which occurs through three different mechanisms. In the first mechanism, piperacillin acts as a hapten, binding to erythrocyte membranes and triggering the production of high-titer anti-drug antibodies. These antibodies attach to the erythrocyte membrane, causing DAT positivity and subsequent hemolysis. The second is the non-specific absorption of piperacillin on the erythrocyte membrane, leading to membrane modification and promoting immune-mediated hemolysis. DAT is also positive in this mechanism, however, it does not always lead to hemolytic anemia. The third mechanism is attributed to the binding of the preformed immune complex of piperacillin and antibodies to the erythrocyte membrane, which activates the complement and causes severe intravascular hemolysis (10). Both IgG and C3d antibodies were present in this case, indicating persistent intravascular and extravascular hemolysis.

In our case, the patient's Hb level dropped sharply to 42 g/dL, although they no longer declined once the piperacillin was discontinued. After the administration of piperacillin, the RBC count decreased significantly, from 2.7 to 1.8 × 1,012/L, and due to serious RBC damage, the transfusion could not inhibit the antibody-mediated hemolysis. The lowest RBC level was 1.3 × 1,012/L before the cessation of piperacillin, by which time 7.5 U (1.5 L whole blood preparation) had been transfused. As the hemoglobin decreased from 89 to 42 g/dL, approximately 3–4 L of RBCs were destroyed. Additionally, the patient's DAT was positive, indicating the possibility of continuous RBC destruction. The detection of piperacillin drug antibodies provides the most direct evidence for clinical diagnosis and treatment. Furthermore, drug-dependent antibodies can also target platelets, resulting in thrombocytopenia (11). The binding sites of drug-dependent antibodies can be localized to GPIIb/IIIa and GPIb/IX in platelets. In our patient, the platelets decreased from 74 to 49 × 109/L (the lowest point) after surgery, but were 66 × 109/L at the time of the patient's discharge, and 110 × 109/L at her follow-up 20 days post-discharge, indicating that in this case, piperacillin primarily resulted in the immune hemolysis of RBCs and the effect on platelets was less obvious than that of RBCs. Additionally, drug-associated trace metabolites or degradation products can also induce severe or life-threatening DIIHA, suggesting that attention must be paid to the possibility of inducing immune hemolysis when administering piperacillin. Moreover, the possibility of immune hemolysis should be monitored even after the discontinuation of piperacillin (12).

The antibody screening test, which screened positive for antibodies, was the biggest serological difference between this case

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TABLE 6 Results of piperacillin antibody test in the red blood cell and plasma.

Results	DAT			Patient Plasma + Piperacillin treated red blood cells	Positive Ctl + Piperacillin treated red blood cells	Negative Ctl + Piperacillin treated red blood cells	Patient Plasma + Non-Piperacillin treated red blood cells
	IgG	C3d	Ctl	I	II	III	IV
Before piperacillin treatment	0	0	0	0	2+	0	0
Stop piperacillin treatment	1+	2+	0	1+	2+	0	1+
Discharge	1+	1+	0	1+	2+	0	0
20 days after discharge	0	0	0	0	2+	0	0

and previous piperacillin-induced hemolysis. The reaction pattern between the patient's plasma and reagent red blood cells with different antigens showed different intensity, suggesting the possible presence of irregular antibodies, which often leads to ineffective transfusions and hemolysis. The antibody identification results suggested that anti-Jk(a) combined with anti-Le(b) mediated a delayed hemolytic transfusion reaction. However, after a transfusion of red blood cells matching Jk(a) and Le(b) antigens, the anemia of the patient remained unimproved. We became aware and validate whether the hemolytic anemia was caused by drug antibodies, combined with blood transfusion inefficacy and Naranjo's assessment scoring method. However, antibody screening has delayed the identification of drug antibodies. Although antibodies to piperacillin caused fewer cases of antibody screening positive, a previous study in a 50-year-old woman exposed to piperacillin, serological reactivity (DAT and IAT) increased significantly, and autoantibodies with Rhesus-e specificity (autoanti-e) were detectable in the patient's plasma on day 12 of antibiotic treatment. These results suggest that irregular antibodies may delay the diagnosis of drug antibodies and that drug-induced hemolytic anemia transfusion should also consider irregular-like antibody specificity to avoid more severe hemolysis.

Class II HLA molecules, including HLA-DR, -DQ, and -DP, are expressed on the surface of antigen-presenting cells (APCs), including B cells, dendritic cells, and monocytes, presenting endogenous antigens that are recognized by T-cell receptors as part of the adaptive immune response. APCs combine with antigenic peptides to bind CD4+ T helper cells, activating B cells and subsequently producing antibodies against the corresponding epitopes (13). HLA-DRB1 and -DQB1 molecules account for > 90% of all HLA class II molecules (14). As previously reported, the HLA-DRB1-09*01-DQB1*03:03 loci and linked haplotypes are prone to produce isoimmune anti-E antibodies in the Chinese population (15, 16). In a prior study, the HLA typing of a patient with ceftriaxone-induced hemolytic anemia showed DRB1*04:05 and DQB1*04:01 haplotypes, which are more common among patients with autoimmune hepatitis (16–19), and anti-Fya-mediated delayed hemolytic transfusion reactions related to HLA-DRB1*04:03 (20), suggesting that people carrying specific HLA risk alleles may be prone to producing drug-mediated antibodies. In the future, special attention should be paid to the hemolytic response of drug antibodies in patients with these HLA alleles.

The immune hemolysis response has immune memory; therefore, initial exposure to piperacillin stimulates the immune system to produce antibodies against the drug or its complex with RBCs. Once antibodies are produced, the memory B cells remain in the body (21), allowing a quicker and increased production of antibodies against the drug-RBC complex when exposed to the same drug again (18). This accelerated immune response, however, can lead to a more rapid and severe hemolytic reaction. A history of past development of antibodies to related drugs, and consideration of alternative antibiotics where appropriate, is a key step in controlling the risk of developing severe immune hemolysis with the repeat administration of piperacillin in the future (3). Our titer of the drug-dependent antibodies was 16, with patients (8, 22) showing titer of 2, 128, and 256 with piperacillin-induced severe immune hemolytic anemia. Low antibody titer can also induce severe drug-induced hemolysis by piperacillin. After the development of piperacillin drug antibodies, treatment with IVIG and hormone therapy may be necessary to alleviate further hemolysis; however, the specific role of IVIG in this success is unclear. After the patient was identified as having an antibody reaction, she successfully recovered and was discharged from the hospital through a combination of drug cessation, blood transfusion, IVIG, corticosteroids, oxygen inhalation, and close vital sign monitoring.

Conclusion

Piperacillin administration may trigger a severe and potentially fatal hemolytic reaction in some patients, and HLA-DQB1*03-DRB1-09*01 may be a susceptible gene target to the induction of piperacillin antibodies. Effective treatment includes blood transfusion, IVIG therapy, corticosteroids, and related vital sign monitoring. In patients with positive antibody screening in particular, antibody information should be followed up after the cessation of piperacillin to avoid delayed hemolytic transfusion reactions mediated by alloimmunity in the future.

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 was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University (2020051). The studies were conducted in accordance with the local legislation and institutional requirements. The 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

HZ: Writing – original draft, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization. JC: Conceptualization, Investigation, Writing – original draft. GO: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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.

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Characteristics of patients with non-severe infections of different SARS-CoV-2 omicron subvariants in China

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Objective: The aim of this study was to explore the clinical characteristics of patients infected with different Omicron subvariants presenting non-severe disease, evaluate the safety and efficacy of Azvudine for treatment of COVID-19, in order to broaden understanding of Omicron subvariant infections.

Method: A total of 244 individuals with Omicron subvariant (BA.2.76, n = 158; BA.5.1, n = 86) were included in the study. Demographic, clinical, and laboratory data of the study participants were collected and analyzed.

Result: Patients infected with BA.5.1 exhibited a higher incidence of clinical symptoms like fatigue (25.58% vs. 2.53%, $p < 0.001$), headache/dizziness (12.79% vs. 4.43%, $p = 0.017$), nausea/vomiting (10.47% vs. 1.27%, $p = 0.002$), viral loads and inflammatory factors, and shorter virus shedding time than those with BA.2.76. There are 28.1% patients reporting mild adverse events following Azvudine administration. After treatment, the levels of anti-SARS-CoV-2 IgG/IgM, white blood cell, and lymphocyte obviously increased, while C-reactive protein, procalcitonin, and D-dimer reduced. Azvudine speeded up the time for virus clearance compared to control treatment (10 vs. 11 days, $p = 0.032$). Low lymphocyte counts (odd ratio (OR) = 0.607, $p = 0.001$) and anti-SARS-CoV-2 IgG titer (OR = 0.990, $p = 0.028$) were the independent risk factors for long nucleic acid negativization duration after infection. Patients with pneumonia were often accompanied by dyspnea, fatigue and high level of D-dimer. Dyspnea (OR = 10.176, $p = 0.019$) could be used to identify the occurrence of pneumonia in patients infected with Omicron.

Conclusion: The study demonstrated the difference in clinical and laboratory parameters between patients infected with Omicron BA.2.76 and BA.5.1, as well as the safety and efficacy of Azvudine therapy. Our study linked patient manifestations to Omicron subvariant, treatment, and clinical outcomes, which is conducive to healthcare providers/policymakers to revise and implement appropriate countermeasures, facilitating appropriately advise for individuals with Omicron subvariant infections.

KEYWORDS

omicron subvariant, BA.2.76, BA.5.1, clinical features, Azvudine, nucleic acid negativization, pneumonia

1 Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has undergone several evolutionary changes. The incidence of infections with Variants of Concern including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) has increased worldwide (1). Compared with the other predecessors of SARS-CoV-2, Omicron showed decreased lung infectivity and became less pathogenic (2).The mortality rate of hospitalized COVID-19 patients decreased from 15.1% during Delta epidemic to 4.9% during the Omicron epidemic (3).

Furthermore, variations in symptom prevalence and treatment were observed among different Omicron subvariants (4) A prior study indicated that the hospitalization rate for febrile seizure in children was significantly higher during BA.5 predominance than during BA.1/BA.2 (5). Notably, BA.1 patients required glucocorticoids for COVID-19-associated hyperinflammatory syndrome, whereas BA.2 patients did not (6). This disparity may be attributed to the weaker induction of IFN-β secretion by BA.1 and BA.5 sub-lineages compared to BA.2, resulting in delayed antiviral signaling activation (7).

Additionally, the BF.7.14 and BA.5.2.48 subvariants shared identical mutation sites in the spike protein, except for R346T and C1243F (8). No significant differences in clinical manifestations, duration of illness, healthcare-seeking behaviors, or treatment were observed between these two subvariants (9). Compared with BA.2, BA.5 possesses three additional mutations: L452R, F486 V, and R493Q. Among these, R493Q enhances the infectivity of BA.5 (10). Patients infected with Omicron BA.5 exhibited faster infectivity and viral clearance as well as apparent immune escape but experienced less severe disease than those infected with BA.2 subvariant (11, 12). However, Kang's study exhibited different results that Omicron BA.5 variant was associated with more severe and frequent systemic symptoms (13), while no difference of clinical manifestation, hospital admission, or other severe endpoints, was observed between BA.2 and BA.4/5 groups (14). Therefore, the comparison of clinical characteristics between Omicron subvariant BA.2 and BA.5 infections needs further investigation.

Azvudine could integrate RNA synthesis of SARS-COV-2 and inhibit related polymerases, ultimately terminating finally RNA replication (15). On July 25, 2022, the National Medical Products Administration approved the use of Azvudine for teh treatment of COVID-19 in adult patients, making it as the first domestic oral antiviral agent approved in China (15–17). Due to the increased transmissibility and global spread, Omicron variant has led to an outbreak in China (18). Consequently, there has been a notably increase in the number of patients seeking treatment after infection. However, there remains a scarcity of studies evaluating the effectiveness and safety of Azvudine in treating patients infected with Omicron subvariants in real-world settings.

Therefore, we conducted a retrospective study to comprehensively explore the clinical characteristics and viral kinetics between patients infected with two Omicron subvariants (BA.2.76 and BA.5.1), assessed the safety and efficacy of Azvudine, and identified the factors associated with viral clearance time and the incidence of pneumonia. This study sought to gain a broadened understanding of Omicron subvariants and provide information for designing effective treatment strategies.

2 Methods

2.1 Participant inclusion and grouping

Patients infected with Omicron subvariant BA.2.76 or BA.5.1 were retrospectively recruited into this study from the Fifth Hospital of Shijiazhuang [designated hospital for patients with Coronavirus disease 2019 (COVID-19)] between August 10, 2022, and October 9, 2022. The inclusion criteria for participants as follows: (1) aged 18 years or older; (2) positive for SARS-CoV-2 by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) from nasopharyngeal and/or oropharyngeal swabs; (3) source patients of the epidemic outbreak were diagnosed with Omicron BA.2.76 or BA.5.1 infection by gene sequencing; (4) no previous infection with other SARS-CoV-2 strains from self-report and medical records; (5) was transferred to the Fifth Hospital of Shijiazhuang according to the requirements of the COVID-19 prevention and control protocol[1] and (6) willing to participate in the study and sign the informed consent. Exclusion criteria were as follows: (1) patients with not detailed medical records and laboratory examination results; (2) patients receiving Azvudine treatment for HIV; (3) pregnant women or lactating mothers. A total of 244 patients were included in this study. We divided the patients into two groups according to the Omicron strain: BA.2.76 group (n = 158) and BA.5.1 group (n = 86).

The clinical characteristics of the enrolled patients, including COVID-19 symptoms, underlying diseases, and vaccination status, were obtained from the hospital information system at admission and collected before treatment. According to the 9th edition of COVID-19 protocols for diagnosis and treatment (19), the patients recruited in the present study had non-severe (asymptomatic, mild, and moderate) illness. All participants had a clear clinical diagnosis of infection severity on the first day after admission. Nucleic acid testing was

Abbreviations: AEs, Adverse events; COVID-19, Coronavirus disease 2019; CRP, C-reactive protein; CT, Cycle threshold; FC, Fold changes; IL, Interleukin; IQR, Interquartile range; NAN, Nucleic acid negativization; RT-PCR, Real-time reverse transcriptase-polymerase chain reaction; SD, Standard deviation; PCT, Procalcitonin; PLT, Platelets; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; TNF-α, Tumor necrosis factor α; WBC, White cell counts.

1 https://www.gov.cn/xinwen/2022-06/28/content_5698168.htm

performed every one to two days for each patient during hospitalization.

Based on clinical symptoms, nucleic acid cycle threshold (CT), and lung infection, 121 patients in the study received Azvudine drug (5 mg/day for 7–14 days, Henan Genuine Biotech) and two types of self-developed Chinese medicines, including Qingre Kangdu or Lanxiang Jiedu herbal extracts (once daily). The patients in Azvudine group received Azivudine from the second (interquartile range: 1, 2) day after they tested positive for SARS-CoV-2, and the course of treatment lasted a maximum of 14 days. The non-Azvudine treatment group comprised 123 patients who only received either Qingre Kangdu or Lanxiang Jiedu herbal extracts (once daily) during hospitalization.

All patients provided informed consent, and the Institutional Review Board of the Fifth Hospital of Shijiazhuang approved this study (2022001).

2.2 Laboratory tests

The data on routine laboratory indices that reflect inflammation, coagulation, blood cell parameters and immunology were derived from the laboratory information system. C-reactive protein (CRP), procalcitonin (PCT), interleukin (IL), and other indexes were performed on Canon TOSHIBA-FX8 biochemistry analyzer. Blood cell counts were determined using Sysmex XN-1000 Pure Hematology Analyzer. Levels of anti-SARS-CoV-2 IgG and IgM were evaluated using YHLO iFlash 3,000 immunoassay analyzer. RT-PCR for SARS-CoV-2 was performed on Applied Biosystems 7,500 Real-Time PCR system. Except for IL, which was not assessed before discharge, all other indicators were detected on the first day of admission after infection (before treatment) and the day before discharge.

2.3 Statistical analysis

Statistical analysis was performed using R version 4.1.3 software, IBM SPSS Statistics version 26.0 (IBM Corp, USA), and Prism 8.0 (GraphPad, San Diego, California, USA). Normality testing was conducted using the Kolmogorov–Smirnov test. Quantitative data with a normal or non-normal distribution were expressed as mean (standard deviation, SD) and median (interquartile range, IQR), respectively. Categorical variables were presented as numbers and percentages. Independent sample t-test and Wilcoxon rank-sum test were, respectively, applied to analyze normally and non-normally distributed data. For categorical variables, the χ^2 test was performed.

Binary logistic regression analysis was performed using SPSS software. Taking the median NAN durations of (10 days) patients in this study as threshold, binary logistic regression was initially performed to define the clinical (Azvudine treatment, BA.5.1 subvariant, fever symptom) and laboratory (lymphocyte counts and anti-SARS-CoV-2 IgG before treatment) risk factors associated with the prolonged virus clearance (>10 days) of patients infected with Omicron subvariant. The models included the adjusted Model 1 (intaking clinical parameters) and adjusted Model 2 (intaking clinical and laboratory factors).

Besides, indexes with statistical differences between patients with and without pneumonia were included in a univariable analysis, which were used to calculate odds ratios (OR) and 95% confidence intervals (CI) to explore factors associated with the incidence of pneumonia. The following factors were evaluated in the univariable analyses: fatigue (yes vs. no), dyspnea (yes vs. no), and levels of D-dimer. Moreover, statistically significant variables associated with pneumonia in the univariable analyses were further subjected to multivariable logistic regression analyses. A variance inflation factor of below 5 and tolerance of above 0.2 indicated insignificant collinearity among independent variables. Correlation analysis of the non-normally distributed data was done by Spearman's correlation coefficients. $p < 0.05$ was considered statistically significant.

3 Results

3.1 Patients infected with subvariant BA.5.1 showed more clinical symptoms and inflammation feature

The median ages of subjects infected with Omicron subvariant BA.2.76 and BA.5.1 were 50 (35, 60.75) and 38 (34, 50) years ($p = 0.001$), respectively. Gender ratio (female to male) was similar between two groups ($p = 0.394$). Due to the higher unvaccinated rates in BA.2.76 group (8.86% vs. 1.16%, $p = 0.022$) and proved efficacy of SARS-CoV-2 vaccination on symptomatic infection (20, 21), we first analyzed the impact of vaccination status on clinical features of patients infected with BA.2.76 or BA.5.1 and observed comparable COVID-19 severity and symptoms between unvaccinated and vaccinated participants (Supplementary Table S1). Of note, patients infected with BA.5.1 had higher incidence of clinical manifestations including fatigue (25.58% vs. 2.53%, $p < 0.001$), dyspnea (5.81% vs. 0%, $p = 0.005$), abdominal pain (3.49% vs. 0%, $p = 0.049$), headache or dizziness (12.79% vs. 4.43%, $p = 0.017$), nausea or vomiting (10.47% vs. 1.27%, $p = 0.002$), and higher viral load (CT of nucleocapsid protein (CT-N): 18.16 vs. 19.82, $p = 0.001$; CT of open reading frame (CT-O): 18.14 vs. 19.43, $p = 0.010$), but shorter period for SARS-CoV-2 clearance (10 vs. 11 days, $p = 0.028$) compared with patients with BA.2.76 infection. The differences in clinical features at onset may result from the different Omicron subvariants infection.

Additionally, the absolute value of lymphocytes ($p < 0.001$), and the levels of tumor necrosis factor (TNF)-α ($p = 0.024$), and IL-6 ($p = 0.026$) were higher in BA.5.1 group than BA.2.76 group, while the titers of anti-SARS-CoV-2 IgG and IgM titers was comparable between the two groups at baseline (Table 1). Other laboratory indexes with no significant difference were displayed in Supplementary Table S2.

3.2 Azvudine was safe and effective in the treatment of patients infected with the omicron subvariants

The safety of Azvudine was evaluated in this current study. Adverse events had occurred in 28.1% (34/121) of the patients following Azvudine administration, such as nausea (13/121, 10.74%), diarrhea (7/121, 5.76%), vomiting (3/121, 2.47%), and headache (1/121, 0.83%). Notably, no serious adverse events (AEs) were observed for the group that received Azvudine (Table 2). Additionally,

TABLE 1 Baseline characteristics of patients infected with different Omicron subvariants on admission to hospital.

Characteristics	BA.2.76	BA.5.1	*p* value
Number	158	86	/
Age (years)	50 (35, 60.75)	38 (34, 50)	**0.001**
Gender (F/M)	70/88	43/43	0.394
COVID-19 severity, *n* (%)			
Asymptomatic	63 (39.87%)	25 (29.07%)	0.093
Mild	78 (49.37%)	53 (61.63%)	**0.041**
Moderate	17 (10.76%)	8 (9.30%)	0.827
Symptoms, *n* (%)			
Fever	51 (32.28%)	30 (34.88%)	0.680
Fatigue	4 (2.53%)	22 (25.58%)	**<0.001**
Cough	73 (46.20%)	29 (33.72%)	0.059
Dyspnea	0 (0%)	5 (5.81%)	**0.005**
Expectoration	33 (20.89%)	19 (22.09%)	0.826
Sore throat/dry throat	43 (27.22%)	19 (22.09%)	0.380
Abdominal pain	0 (0%)	3 (3.49%)	**0.049**
Diarrhea	1 (0.63%)	2 (2.33%)	0.284
Headache/dizziness	7 (4.43%)	11 (12.79%)	**0.017**
Nausea/vomit	2 (1.27%)	9 (10.47%)	**0.002**
Myalgia	5 (3.16%)	6 (6.98%)	0.202
Vaccination status, *n* (%)			
Unvaccinated	14 (8.86%)	1 (1.16%)	**0.022**
1 dose of vaccine	1 (0.63%)	1 (1.16%)	>0.9999
2 doses of vaccine	4 (2.53%)	3 (3.49%)	0.7015
3 doses of vaccine	139 (87.97%)	81 (94.19%)	0.5105
Time after last vaccine (days)	281 (240, 388)	364 (317.5, 380.5)	**0.004**
Underlying disease, *n* (%)	40 (25.32%)	26 (30.23%)	0.409
Hypertension	27 (17.09%)	7 (8.14%)	0.054
Diabetes	8 (5.06%)	4 (4.65%)	>0.999
Cardiovascular disease	8 (5.06%)	0 (0%)	0.053
Cerebrovascular disease	4 (2.53%)	1 (1.16%)	0.659
Chronic lung disease	4 (2.53%)	0 (0%)	0.300
Chronic kidney disease	1 (0.63%)	2 (2.33%)	0.284
Chronic liver disease	2 (1.27%)	3 (3.49%)	0.348
Hematopathy	2 (1.27%)	3 (3.49%)	0.348
Malignant tumor	4 (2.53%)	15 (17.44%)	**<0.001**
Laboratory characteristics			
CT-N	19.82 (17.04, 23.52)	18.155 (15.09, 21.46)	**0.001**
CT-ORF	19.43 (17.01, 22.40)	18.145 (15.99, 20.74)	**0.010**
Time for SARS-CoV-2 nucleic acid to turn negative (days)	11 (9, 14)	10 (8,11.75)	**0.028**
Anti-SARS-CoV-2 IgG (AU/mL)	12.2 (2.8, 34.56)	8.72 (2.73, 24.09)	0.315
Anti-SARS-CoV-2 IgM (AU/mL)	0.11 (0.02, 0.25)	0.07 (0.03, 0.24)	0.755
Lymphocyte ($\times 10^9$/L)	1.32 (1.02, 1.76)	3.81 (1.49, 4.79)	**<0.001**
Interleukin-6 (pg/mL)	2.81 (1.65, 4.9)	4.16 (2, 12.83)	**0.026**
D-dimer (μg/L)	320 (190, 462.29)	426.28 (250, 482.66)	0.051

Vaccination status refers to the vaccination status of the patient before infection. CT-N, cycle threshold of nucleocapsid protein; CT-O, cycle threshold open read frame. Bold values is that $p < 0.05$.

TABLE 2 Summary of adverse events following taking Azvudine in patients with Omicron infection.

Adverse events	Number
None	87 (71.90%)
Nausea	13 (10.74%)
Diarrhea	7 (5.76%)
Upset stomach	5 (4.13%)
Liver injury	4 (3.31%)
Abdominal bloating	4 (3.31%)
Vomit	3 (2.47%)
Rash	2 (1.65%)
Loss of appetite	2 (1.65%)
Headache	1 (0.83%)
Constipation	1 (0.83%)
Insomnia	1 (0.83%)

Data are displayed as number (%), representing the total number of patients who had adverse reactions (adverse events related to take Azvudine).

AE was found to be closely related to IL -1β levels before treatment, age, and gender (Supplementary Figure S1A). Patients with AEs, or AEs of vomit exhibited higher levels of IL-1β before treatment than those without (Supplementary Figures S1B,C). Older patients were more likely to have AEs of upset upset stomach (Supplementary Figure S1D). Nausea and vomit after Azvudine administration were more common in female patients, while liver injury was the opposite (Supplementary Figures S1E–G).

All patients exhibited favorable prognosis, who were discharged after treatment. Patients in Azvudine group had lower proportion of asymptomatic disease (25.62% vs. 46.34%, $p = 0.001$) and higher moderate severity (18.18% vs. 2.44%, $p < 0.001$) than those in control group (Supplementary Table S3). Azvudine drug still exhibited great benefits for virus clearance, the enhancement of anti-SARS-CoV-2 antibodies and immune cells, as well as the regulation of inflammatory factors. Shorter nucleic acid negativization (NAN) durations was observed in Azvudine rather than control group (10 vs. 11 days, $p = 0.032$) (Figure 1A). The levels of anti-SARS-CoV-2 IgG and IgM, white cell counts (WBC), lymphocyte counts, and platelets (PLT) were elevated to varying degrees, whose fold changes (FC) in Azvudine group were greater than that in control group (Figures 1B,C,G–I). Meanwhile, among patients receiving Azvudine, the FC of CRP, PCT and D-dimer markedly decrease compared with patients in control group (Figures 1D–F).

Given that patients infected with BA.2.76 or BA.5.1 exhibited varying periods for SARS-COV-2 clearance (Table 1), we conducted a detailed analysis of viral clearance times, as well as alterations in anti-SARS-CoV-2 antibodies, inflammatory factors, and immune cells, specifically comparing patients who received Azvudine treatment to those in the control group within both BA.2.76 and BA.5.1 cohorts (Supplementary Figures S2, S3). Although the differences were not statistically significant, patients treated with Azvudine had shorter NAN durations compared to those in the control group, both in the BA.2.76 cohort (11.07 vs.11.83 days) and the BA.5.1 cohort (10.14 vs. 10.91 days) (Supplementary Figures S2A–S3A). In BA.5.1-infected patients who received Azvudine, there were higher increases in anti-SARSCoV-2 lgG and lgM, WBC, lymphocyte counts, PLT, and greater reductions in CRP, PCT, and D-dimer levels compared to those in the control group (Supplementary Figures S3B–I). Patients infected with BA.2.76 showed similar trends except for WBC (Supplementary Figures S2B–I).

3.3 Low levels of lymphocyte and anti-SARS-CoV-2 IgG before treatment prolonged SARS-CoV-2 nucleic acid negativization

The correlation between the clinical and laboratory indexes and the duration of NAN was performed using Spearman's correlation test (Figure 2A). Patients receiving Azvudine treatment ($r = -0.14$, $p = 0.032$), infected with BA.5.1 subvariant ($r = -0.14$, $p = 0.027$), without fever symptom ($r = -0.13$, $p = 0.050$) were negatively associated with SARS-CoV-2 clearance, and so is low lymphocyte counts ($r = -0.27$, $p < 0.001$) and anti-SARS-CoV-2 IgG titers ($r = -0.20$, $p = 0.002$) before treatment (Figures 1A, 2B–D). The shorter virus clearance time in the BA.5.1 group may be due to the Azvudine treatment they received.

Binary logistic regression analysis using model 1 showed that fever (OR = 1.942, 95% CI: 1.115–3.380; $p = 0.019$) was associated with >10 days NAN duration. Model 2 showed that low lymphocyte counts (OR = 0.607, 95% CI: 0.457–0.806; $p = 0.001$) and anti-SARS-CoV-2 IgG level (OR = 0.990, 95% CI: 0.990–0.999; $p = 0.028$) before treatment were independent risk factors for a prolonged NAN duration (Figures 2E–F).

3.4 Dyspnea was related to the incidence of pneumonia in patients infected with omicron subvariants

In this study, 10.25% (25/244) individuals developed pneumonia. Patients with pneumonia exhibited a higher risk of dyspnea (12% vs. 0.91%, $p = 0.008$), fatigue (24% vs. 9.13%, $p = 0.022$), and a higher level of D-dimer (397 vs. 307 mg/L, $p = 0.029$) than patients without pneumonia (Figures 3A–C; Supplementary Table S3).

Then above three indicators were included in univariate logistic regression. Dyspnea (OR = 14.795, 95% CI: 2.345–93.361; $p = 0.004$) and fatigue (OR = 3.142, 95% CI: 1.126–9.771; $p = 0.029$) were associated with pneumonia. Risk factors with statistically significant differences in the univariate logistic regression were further analyzed in multivariate logistic regression, which further proved that dyspnea (OR = 10.176, 95% CI: 1.475–70.214; $p = 0.019$) was independent factors for the occurrence of pneumonia in patients with Omicron infection (Figures 3D–E).

4 Discussion

With the high incidence of Omicron infections but lower disease severity, Chinese authorities implemented "reopening in an orderly and effective manner" policy on December 7, 2022, before this, the city of Shijiazhuang, as a pilot area, led to large patients infected with Omicron variant. A challenge for healthcare providers and public

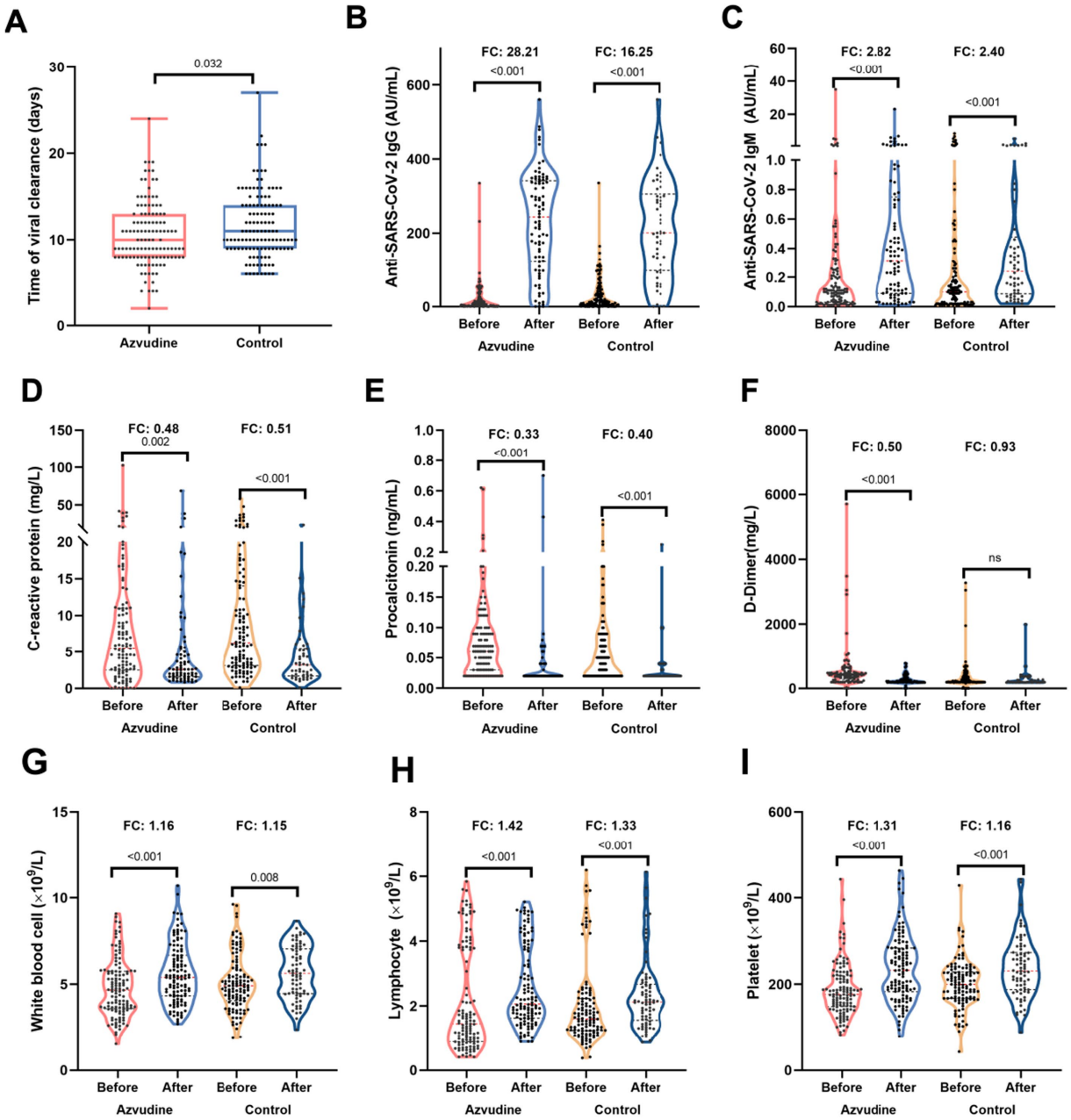

FIGURE 1
Azvudine improved the immune and inflamatory status of patients infected with Omicron variant. **(A)** The time of viral clearance was shorter in Azvudine group than control group. **(B,C)** Azvudine treatment significantly enhanced the titers of anti-SARS-CoV-2 IgG **(B)** and IgM **(C)** compared to control treatment. **(D–F)** The reduction of C-reactive protein **(D)**, procalcitonin **(E)**, and D-dimer **(F)** in azvudine group was greater than that in control group. G-H. After treatment, immune cells including white blood cell counts **(G)**, lymphocyte counts **(H)**, and platelet **(I)** of patients receiving azvudine increased more than those in control group. Statistical analysis was performed by Wilcoxon test **(A)** and paired Wilcoxon test **(B–I)**. FC, fold change.

health officials is appropriately advising individuals with different subvariants infections and implementing healthcare strategies to response Omicron pandemic. However, there is available limited information on patients infected with different Omicron subvariants. Based on this, our study (i) comprehensively exhibited the clinical and laboratory characteristics of non-severe patients infected with Omicron BA.2.76 and BA.5.1, which could help clinicians timely recognize the alterations in clinical spectrum of different subvatiants; (ii) proved the safety and efficacy of Azvudine in the treatment of non-severe COVID-19 patients to maximize their utility and guide appropriate healthcare strategies; (iii) identified the independent risk factors indicating prolonged viral clearance and pneumonia so healthcare providers can give appropriate treatment at an early stage.

Patients infected with the BA.5.1 subvariant had more prominent clinical symptoms such (fatigue, dyspnea, abdominal pain, headache, nausea) and higher viral loads in comparison with infection with BA.2.76 subvariant, which might be due to waning vaccine-induced immunity. Before diagnosis of COVID-19, a longer time after last vaccine was observed in the BA.5.1 group (364 vs. 281 days). Our results are consistent with the findings of prior research that

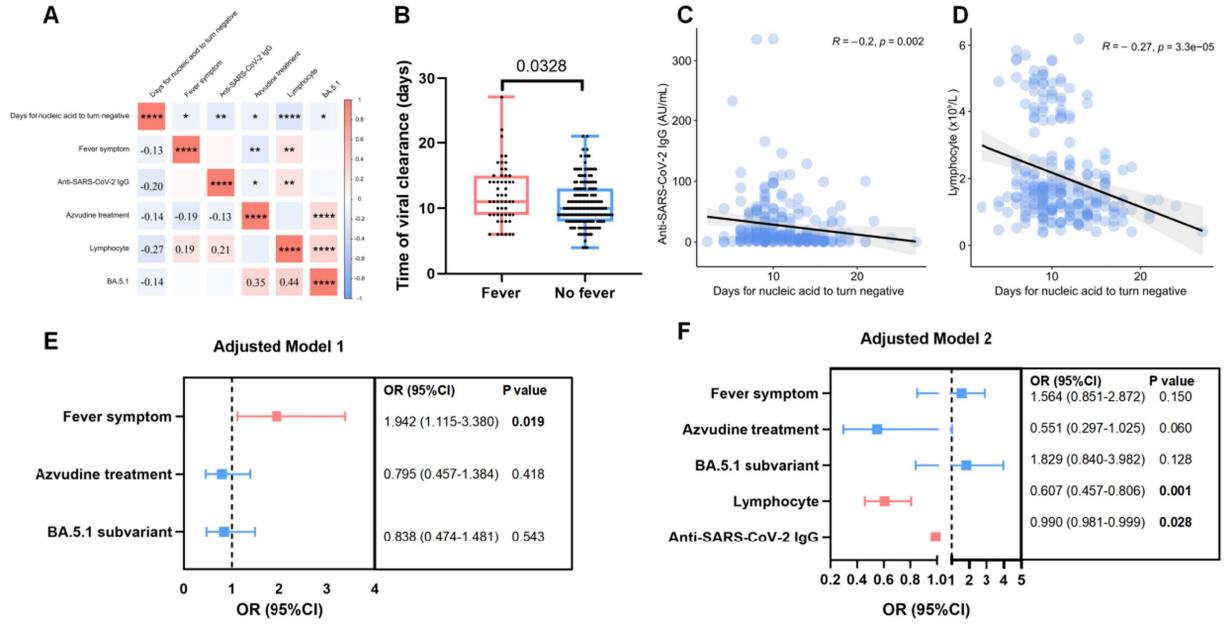

FIGURE 2
Factor associated with virus clearance in patients infected with Omicron subvariants. **(A)** The heatmap showed the factors correlated with the days for nucleic acid to turn negative after infection. **(B)** Box plot showed the difference of virul clearancr time between Omicron infected patients with or without fever symptom. **(C,D)** The association between virul clearance time (days) and lymphocytes counts **(C)** as well as anti-SARS-CoV-2 IgG **(D)**. **(E,F)** Risk factors associated with long days of nucleic acid to turn negative in model 1 **(E)** and model 2 **(F)**. Lymphocytes counts and anti-SARS-CoV-2 IgG titers were evaluated before treatment.

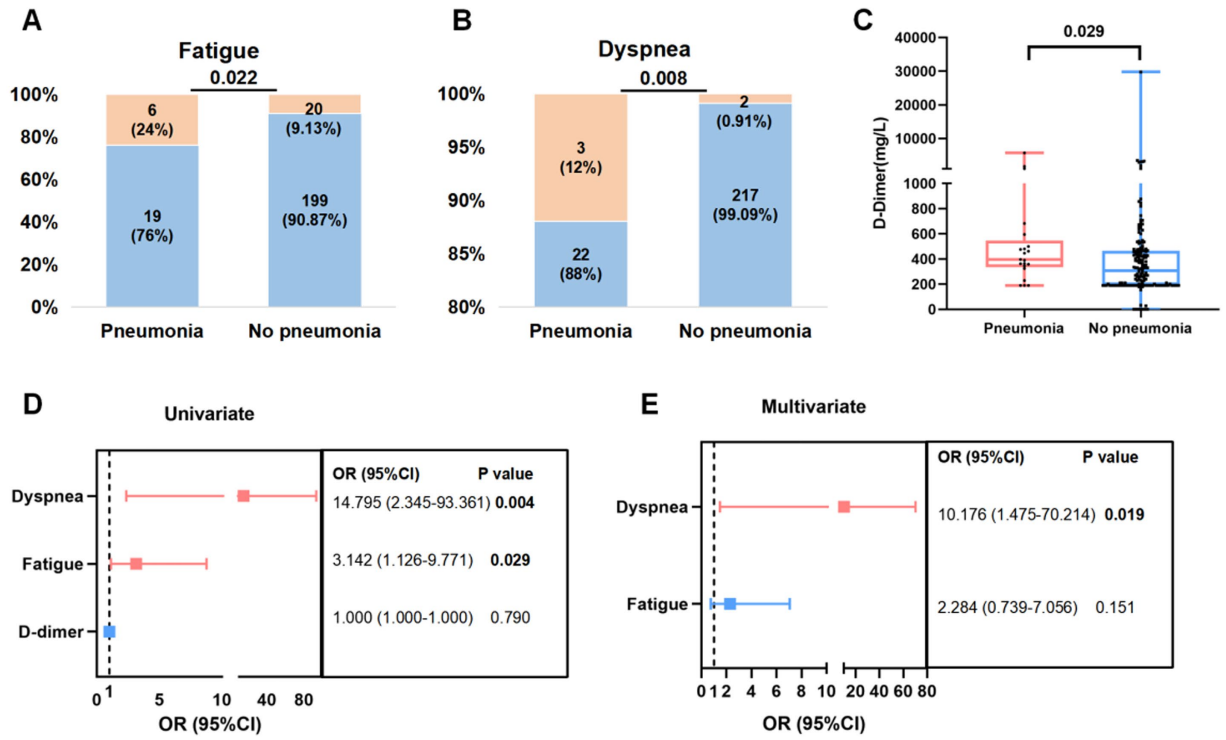

FIGURE 3
Factor associated with pneumonia in patients infected with Omicron subvariants. **(A,B)** Proportion (orange) of fatigue **(A)** and dyspnea **(B)** symptoms in Omicron infected patients with or without pneumonia. **(C)** Levels of D-dimer in patients with or without pneumonia before treatment. **(D,E)** Risk factors related to pneumonia using univariate **(D)** and multivariate logistic regression **(E)**.

demonstrate a decline in vaccine effectiveness over time regardless of Omicron subvariant type (11, 13). Further studies on this field are needed.

The present results showed that Azvudine was safe and effective for treatment of patients presenting with non-severe symptoms. This study reported 28.1% of the patients appeared general drug-related AEs, most of them were gastrointestinal symptoms, like nausea, diarrhea and upset stomach, which is similar to the total AEs rates (29.50%, 272/922) of Azvudine in a meta-analysis including six studies (22). Azvudine treatment effectively reduced the levels of inflammatory factors and increased the levels of anti-SARS-CoV-2 antibodies and immune cells compared with the control. These changes indicate improved immunity, which consequently shortened the virus shedding time and enhanced the recovery from SARS-CoV-2 infection. Recently, several research displayed that Azvudine significantly improved clinical symptoms and reduced the risk of severity in COVID-19 patients (22–25). Meanwhile, it also has good therapeutic effect in patients with compromised immune systems such as hemodialysis patients (26). Compared with other antiviral drugs, research on Azvudine fighting against SARS-CoV-2 was not enough. Further studies are needed to compare of Azvudine and other antiviral drugs, and application of Azvudine in specific populations.

The present study revealed that fever, Azvudine, lymphocyte counts, and anti-SARS-CoV-2 IgG titer were negatively correlated with NAN time. Among them, low levels of lymphocyte and anti-SARS-CoV-2 IgG were identified as independent risk factors for prolonged virus clearance time, consistent with findings from other studies on patients infected with the other Omicron variants (27) and Delta variants (28). Study by He (29) and Yin (30) reported that reduced level of lymphocytes is a potential indicator of the disease progression in patients infected with Omicron. As the COVID-19 becomes more severe, the lymphocyte count decreases, and the lung damage is exacerbated (31–35). Anti-SARS-CoV-2 IgG titer is negatively associated with the viral load. The levels of antibodies against SARS-CoV-2 decrease over time following vaccination, indicating the importance of routinely monitoring of the anti-SARS-CoV-2 antibodies kinetics and consistently administering COVID-19 vaccinations (36).

The present study has some limitations. First, the Omicron subvariant was defined based on the period of predominance, and not all cases were subjected to sequencing. Secondly, it should be considered that we only evaluated the safety and effectiveness of Azvudine in treating Omicron-infected patients in our study, further studies should focus on the comparing Azvudine with other antiviral treatments, and investigate the factors associated with non-response to Azvudine administration in multi-center or larger cohorts. Moreover, this study was conducted when COVID-19 was under control in China, patients infected with other Omicron substrains were not included, limiting the study's external validity.

5 Conclusion

The present findings showed that patients infected with Omicron BA.5.1 were associated with frequent systemic symptoms and exhibited better profiles of laboratory parameters than patients infected with BA.2.76 subvariant. In addition, the results showed that Azvudine is an effective and safe drug for treating non-severe COVID-19 cases. Future studies could compare Azvudine with other antiviral treatments, and explore the factors associated with non-response to antiviral administration, in multi-center or larger cohort with different disease severity. Clinicians can use lymphocyte counts and anti-SARS-CoV-2 IgG titer to predict patients with a longer NAN duration and utilize dyspnea symptom to identify subjects with pneumonia. Our study linked clinical symptoms to different subvariant, treatment, and clinical outcomes, in patients infected Omicron. These findings offer valuable insights for healthcare providers and policymakers, aiding in the formulation of appropriate health strategies to combat future infections and enhance patient prognosis during the ongoing Omicron wave.

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 study was approved by the Ethics Committee of the Fifth Hospital of Shijiazhuang (2022001). Informed consent was obtained from all participants. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

WY: Data curation, Investigation, Project administration, Resources, Writing – review & editing. YLiu: Conceptualization, Data curation, Investigation, Methodology, Project administration, Visualization, Writing – original draft. HZ: Investigation, Methodology, Supervision, Visualization, Writing – review & editing. FW: Data curation, Investigation, Resources, Writing – review & editing. QZ: Data curation, Investigation, Resources, Writing – review & editing. HG: Data curation, Funding acquisition, Project administration, Writing – review & editing. HY: Data curation, Project administration, Resources, Writing – review & editing. TH: Data curation, Investigation, Resources, Writing – review & editing. YLi: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. ED: Conceptualization, Data curation, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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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.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1511227/full#supplementary-material

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Prognostic value of HSP27 in 28-day mortality in septic ICU patients: a retrospective cohort study

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Background: This study aimed to investigate the association between serum heat shock protein 27 (HSP27) levels and 28-day mortality in patients with sepsis.

Methods: This retrospective study analyzed the clinical data of 76 septic patients admitted to the intensive care unit (ICU). Fifty non-septic ICU patients and 50 healthy individuals served as control groups. Serum HSP27 levels were measured on the day of ICU admission and compared to sepsis severity and survival outcomes.

Results: Median serum HSP27 levels in septic patients (4.70 ng/mL, IQR: 2.10–13.48 ng/mL) were significantly higher than those in both non-septic ICU controls and healthy controls (all $p < 0.05$). Moreover, non-survivors exhibited significantly higher median HSP27 levels (9.30 ng/mL, IQR: 3.62–25.91 ng/mL) compared to survivors (3.03 ng/mL, IQR: 1.48–7.39 ng/mL, $p < 0.05$). Multivariate logistic regression analysis confirmed the association between HSP27 levels and 28-day mortality in sepsis patients. Receiver operating characteristic (ROC) curve analysis revealed an area under the curve (AUC) of 0.720 (95% CI: 0.605–0.817, $p < 0.001$) for HSP27 in predicting sepsis prognosis. Survival analysis demonstrated that patients with high serum HSP27 levels (≥2.61 ng/mL) had a worse prognosis than those with low levels (<2.61 ng/mL).

Conclusion: HSP27 shows potential as a biomarker for the diagnosis and prognosis of sepsis, however, further research is necessary to solidify its clinical utility.

KEYWORDS

HSP27, sepsis, prognosis, infection, inflammatory

1 Introduction

As a systemic inflammatory response syndrome triggered by infection, the definition and diagnostic criteria of sepsis have undergone several revisions in recent years (1). The latest definition, proposed by Sepsis-3 in 2016, describes sepsis as a "life-threatening organ dysfunction caused by a dysregulated host response to infection" (2). A global cohort study estimated that approximately 49 million cases of sepsis occurred worldwide in 2017, resulting in 11 million deaths related to sepsis, which accounted for 19.7% of all global deaths (3). The

high incidence and mortality rate of sepsis pose a significant burden on society, necessitating further measures to improve sepsis prevention and treatment. Existing diagnostic indicators have significant limitations in the early detection of sepsis (4). Traditional biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) are widely used, but their limited sensitivity and specificity hinder their clinical utility (5). Consequently, there is an urgent need to identify novel biomarkers to improve the accuracy of sepsis diagnosis and facilitate early detection.

HSP27, also known as heat shock protein family B (small) member 1 (HSPB1) (6), is a critical stress protein that protects cells from damage (7). It is now understood that the molecular structure of HSP27 exists in both phosphorylated and polymerized states (8). During cellular stress responses, HSP27 plays a key protective role. When cells are exposed to heat, oxidative stress, or inflammatory factors, HSP27 levels are rapidly upregulated, inhibiting protein aggregation and misfolding, thus protecting cells from damage (9).

As a crucial stress protein, HSP27 has demonstrated significant diagnostic and prognostic utility in various diseases (10–13). While studies have shown correlations between other heat shock proteins, such as HSP70, HSP60, and HSP90α, and sepsis severity (14–18), the relationship between HSP27 and sepsis mortality remains uncertain. Therefore, this study aims to assess the prognostic value of HSP27 as a biomarker for sepsis-related mortality.

2 Materials and methods

2.1 Ethics statement

Ethical approval for this study was obtained from the Ethics Committee of the Second Affiliated Hospital of Nanchang University. All patients provided written informed consent.

2.2 Study design

This retrospective case–control study enrolled septic patients admitted to the Second Affiliated Hospital of Nanchang University between September 2022 and September 2023 who met the Sepsis 3.0 diagnostic criteria (19). Patients were categorized into survival and death groups based on their 28-day survival outcomes. Exclusion criteria included HIV infection, immune system disorders, immunosuppressive or cytotoxic drug use, pregnancy, breastfeeding, refusal of informed consent, age under 18 or over 80 years, acute cerebrovascular or cardiovascular incidents, malignant tumors, severe hematological disorders, and ambiguous medical histories affecting SOFA score assessment. Fifty healthy individuals and 50 non-septic intensive care unit (ICU) patients, matched for age and gender with the septic ICU patients, served as the health control and ICU control groups, respectively. The primary reasons for ICU admission among the 76 septic patients were shock, respiratory failure, high-risk surgery, renal failure, acute pancreatitis, and other critical conditions. The 50 non-septic ICU patients were admitted primarily due to respiratory failure ($n = 27$), disturbance of consciousness ($n = 12$), shock ($n = 7$), and other critical conditions ($n = 4$).

2.3 Data collection

A retrospective case–control study was conducted using the Hospital Information System (HIS) to retrieve clinical data from patients fulfilling predefined inclusion and exclusion criteria. Access to this data was granted for research purposes. While the authors had access to personally identifiable information during or post-data collection, all data were handled confidentially. General patient demographics, such as age, gender, infection source, comorbidities, length of hospital stay, and vital signs were recorded. Laboratory investigations included peripheral venous blood samples drawn within 48 h of admission to measure complete blood count, liver and kidney function tests, CRP, PCT, and SOFA score. Additionally, venous blood specimens were collected within 2 days of admission to assess complete blood count, liver and kidney function tests, CRP, interleukin-1 beta (IL-1β), PCT, and SOFA score.

2.4 Measurement of HSP27 levels

From all participants, a 5 mL fasting blood sample was drawn within 48 h of admission to the ICU. The serum was isolated and stored at −80°C until analysis. Serum HSP27 concentrations were quantified using a human HSP-27/HSPB1 ELISA Kit (Sangon Biotech). Standards, controls, and serum samples were diluted with sample buffer and added to a 96-well microplate. A horseradish peroxidase-conjugated antibody was subsequently added and incubated at 37°C for 60 min. Color development was induced with tetramethylbenzidine (TMB), and the optical density (OD) at 450 nm was measured using a microplate reader. The concentration of HSP27 in each sample was determined by interpolation from a standard curve generated from the OD values of the standard samples.

2.5 Statistical analysis

Data entry and statistical analysis were conducted utilizing SPSS software version 25.0 (IBM Corp., Armonk, NY, United States). Categorical variables were assessed with the χ^2 or Fisher's exact test, depending on sample size considerations. Continuous data were evaluated for normality using appropriate tests. Non-normally distributed data were presented as median and interquartile range (IQR), while normally distributed data were presented as mean and standard deviation. Group comparisons for continuous data were performed using either the Mann–Whitney *U* test for two independent groups or one-way analysis of variance (ANOVA) with *post hoc* tests for multiple comparisons, depending on the presence of equal variances among groups. Receiver operating characteristic (ROC) curves were generated to assess the sensitivity and specificity of HSP27 in diagnosing sepsis. The area under the ROC curve (AUC) with its corresponding 95% confidence interval (CI) was used to evaluate the prognostic value of HSP27. Survival curves were constructed using the Kaplan–Meier method to compare survival times between patient groups. Data visualizations were created using the online tool Hiplot Pro.[1] A *p*-value of less than 0.05 was considered statistically significant.

1 https://hiplot.com.cn/

3 Results

3.1 Characteristics of the study population

This study enrolled a total of 176 participants, comprising 76 sepsis patients, 50 ICU controls, and 50 healthy controls. Table 1 provides a detailed overview of the demographic and clinical characteristics of the study. Upon admission to the ICU, sepsis patients exhibited significantly elevated heart rate and blood pressure compared to the ICU control group ($p < 0.05$). The median HSP27 level in sepsis patients at admission was 4.70 ng/mL (IQR: 2.10, 13.48), which was significantly higher than that observed in healthy controls (1.06 ng/mL [IQR: 0.22, 2.06]) and non-septic ICU patients (2.6 ng/mL [IQR: 1.12, 6.08]) (all $p < 0.05$). Additionally, compared to the healthy control group, sepsis patients displayed significant alterations in various hematological parameters, including elevated levels of creatinine, blood urea nitrogen, monocytes, and neutrophils, as well as decreased levels of platelets, hematocrit, and lymphocytes (all $p < 0.05$). Compared to the ICU control group, sepsis patients exhibited significantly higher levels of lymphocytes, platelets, and transferrin (all

TABLE 1 Characteristics of healthy controls, ICU controls, and septic patients.

Parameters	Healthy controls (n = 50)	ICU controls (n = 50)	Septic patients (n = 76)
Patient characteristics			
Age, years	52.5 (46, 61)	57.5 (47, 70.5)	64.5 (52, 71.75)
Male, female	30 (60%), 20 (40%)	34 (68%), 16 (32%)	53 (69.7%), 23 (30.3%)
Underlying disease (n, %)			
Hypertension	/	11 (22.0%)	28 (36.8%)[b]
Diabetes	/	3 (6.0%)	14 (87.5%)[b]
COPD	/	7 (14.0%)	24 (31.6%)[b]
Constants			
Temperature	/	36.49 (36.42, 36.56)	36.52 (36.20, 36.85)
Breath_rate, beats/min	/	19.91 (19.28, 20.48)	19.53 (18.28, 21.17)
Heart_rate, beats/min	/	89 (85, 94)	95 (84, 106)[b]
Systolic blood pressure, mmHg	/	110 (107, 116)	90 (83, 96)[b]
Diastolic blood pressure, mmHg	/	70 (64, 76)	60 (53, 67)[b]
Laboratory values			
White blood cells ($\times 10^9$/L)	5.89 (5.29, 6.66)	11.21 (9.42, 15.67)	10.85 (7.76, 14.45)[a]
Neutrophils ($\times 10^9$/L)	3.11 (2.69, 3.77)	8.89 (8.02, 12.76)	9.31 (6.55, 12.59)[a]
Lymphocytes ($\times 10^9$/L)	2.05 (1.80, 2.50)	0.92 (0.68, 1.52)	0.83 (0.53, 1.18)[a, b]
Monocytes ($\times 10^9$/L)	0.40 (0.33, 0.50)	0.59 (0.40, 0.99)	0.63 (0.33, 0.97)[a]
Hematocrit (%)	44.55 (42.13, 49.25)	36.55 (28.45, 41.83)	34.85 (30.25, 40.43)[a]
Platelets ($\times 10^9$/L)	252 (197.75, 286)	192.5 (148.75, 290)	105 (68, 178.25)[a, b]
Blood urea nitrogen (mmol/L)	4.81 (4.10, 5.69)	5.75 (4.12, 8.45)	7.77 (5.19, 15.01)[a, b]
Creatinine (μmol/L)	69.0 (56.5, 84.25)	60.5 (51, 76)	109.5 (66, 219.5)[a, b]
Total bilirubin (μmol/L)	11.9 (9.68, 15.90)	12.4 (8.1, 17.9)	15.15 (9.63, 28.13)[a]
Ferritin (μg/L)	/	752 (448.75, 1434.5)	797 (427, 2008.25)
Transferrin (g/L)	/	1.44 (0.99, 1.72)	1.14 (0.96, 1.42)[b]
Serum iron (μmol/L)	/	5.55 (3.10, 9.40)	5.80 (4.20, 10.50)
Transferrin saturation (%)	/	27.70 (21.05, 33.98)	27.50 (21.60, 33.40)
TIBC (μmol/L)	/	35.6 (26.45, 41.2)	34.2 (29.2, 41.2)
CRP (mg/L)	/	115.83 (66.61, 165.75)	81.86 (39.24, 147.42)
PCT (ng/mL)	/	1.34 (0.71, 2.37)	1.87 (0.78, 8.16)
HSP27 (ng/mL)	1.06 (0.22, 2.06)	2.6 (1.12, 6.08)	4.70 (2.10, 13.48)[a, b]
SOFA score	/	1 (1, 2)	4 (3, 7)[b]
ICU stay (days)	/	10 (6, 19)	11 (7, 17)

"/" represents that the corresponding data is not obtained; "a" represents that the comparison of healthy controls with septic patients, $p < 0.05$; "b" represents that the comparison of ICU controls with septic patients, $p < 0.05$.

ICU, Intensive Care Unit; COPD, chronic obstructive pulmonary disease; TIBC, total iron binding capacity; CRP, C-reactive protein; PCT, procalcitonin; HSP27, heat shock protein 27; SOFA, sequential organ failure assessment.

$p < 0.05$). Furthermore, the SOFA score was significantly higher in sepsis patients compared to ICU controls ($p < 0.05$), indicating a greater degree of organ dysfunction in the sepsis group.

3.2 Serum HSP27 levels as a potential diagnostic biomarker for sepsis patients

To assess the diagnostic performance of HSP27, SOFA score, and PCT in differentiating ICU sepsis patients from ICU controls, we employed ROC curve analysis. HSP27 exhibited an AUC of 0.706 (95% CI: 0.616–0.795, $p < 0.001$), with a sensitivity of 72.0% and a specificity of 61.8%. While this performance was inferior to the SOFA score (AUC: 0.962, 95% CI: 0.940–0.984, sensitivity: 76.3%, specificity: 100%, $p < 0.001$), it surpassed that of PCT (AUC: 0.578, 95% CI: 0.477–0.679, sensitivity: 40.5%, specificity: 86.0%, $p = 0.088$) (Figure 1). These findings suggest that HSP27 may have potential as a diagnostic biomarker for sepsis.

3.3 Elevated serum HSP 27 levels are associated with poorer survival outcomes in ICU sepsis patients

Of the 76 sepsis patients, 39.47% ($n = 30$) succumbed to sepsis within 28 days of ICU admission. Table 2 presents a comparative analysis of the baseline characteristics and laboratory parameters between the surviving and non-surviving patient groups. Notably, non-survivors exhibited significantly elevated levels of creatinine, PCT, and IL-1β compared to survivors (all $p < 0.05$). Furthermore, the median HSP27 level was significantly higher in non-survivors [9.30 ng/mL (IQR: 3.62, 25.91)] compared to survivors [3.03 ng/mL (IQR: 1.48, 7.39)] ($p < 0.001$). Besides, non-survivors had significantly higher SOFA scores, indicating a greater degree of organ dysfunction.

3.4 HSP27 levels related to the severity of ICU sepsis patients

To investigate the association between HSP27 levels and sepsis severity, we performed a risk factor analysis. The results indicated that higher HSP27 levels were associated with elevated levels of PCT and SOFA scores (Figure 2). Univariate and multivariate logistic regression models were employed to identify independent predictors of 28-day mortality among septic patients. Univariate analysis revealed a significant positive correlation between higher HSP27 levels and increased 28-day mortality (odds ratio [OR] = 1.035, 95% confidence interval [CI]: 1.005–1.067, $p = 0.022$). Similarly, PCT and SOFA scores were significantly associated with increased mortality (OR = 1.160, 95% CI: 1.046–1.265, $p = 0.001$; and OR = 1.348, 95% CI: 1.130–1.608, $p = 0.001$, respectively). In contrast, creatinine levels were not significantly associated with 28-day mortality (OR = 1.002, 95% CI: 0.999–1.005, $p = 0.122$).

To further elucidate the independent prognostic value of HSP27, multiple logistic regression models were constructed (Table 3). After adjusting for potential confounders such as creatinine, PCT, and IL-1β, HSP27 remained significantly associated with 28-day mortality in sepsis (Model 2: OR = 1.038, 95% CI: 1.007–1.071, $p = 0.017$; Model 3: OR = 1.039, 95% CI: 1.006–1.073, $p = 0.021$; Model 4: OR = 1.036, 95% CI: 1.003–1.071, $p = 0.035$). However, upon further adjustment for the SOFA score (Model 5: OR = 1.034, 95% CI: 0.998–1.072, $p = 0.064$), the association between HSP27 and mortality lost statistical significance. These findings suggest that HSP27 levels are related to the 28-day mortality of ICU sepsis, but the SOFA score remains the established gold standard in clinical practice.

3.5 The ability of HSP27 to predict 28-day mortality in sepsis patients

To evaluate the prognostic significance of HSP27 in predicting 28-day survival, ROC curve analysis was performed. The AUC for HSP27 was 0.720 (95% CI: 0.605–0.817, $p < 0.001$), indicating good predictive accuracy. The optimal cutoff value for HSP27 was 2.61 ng/mL, yielding a sensitivity of 90.0% and a specificity of 50.0% in predicting 28-day mortality (Table 4). A combination of HSP27 and the SOFA score further improved prognostic accuracy, with an AUC of 0.767 (95% CI: 0.656–0.856, $p < 0.001$) and a specificity of 91.3%. This combined approach outperformed the use of either SOFA score (AUC: 0.702, 95% CI: 0.607–0.819, specificity: 86.6%) or HSP27 alone. Kaplan–Meier survival curves were constructed to compare the 28-day mortality rates between patients with high and low HSP27

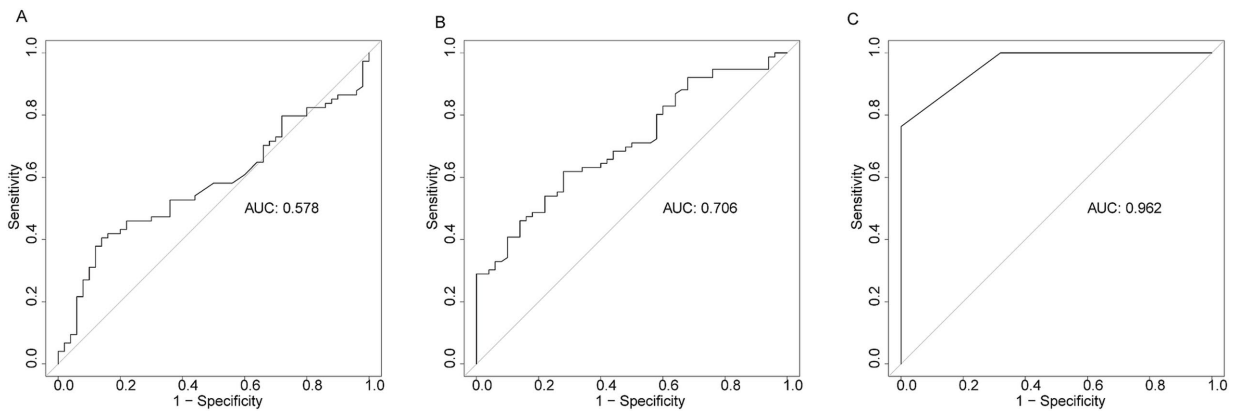

FIGURE 1
Receiver operating characteristic curve (ROC) of PCT **(A)**, HSP27 **(B)**, and SOFA score **(C)** for diagnosis of sepsis.

TABLE 2 Index comparison between the survival and non-survival groups of septic patients.

Parameters	Survival (n = 46)	Non-survival (n = 30)	P-value
Patient characteristics			
Age, years	64 (49.75, 71)	66 (54.75, 72.25)	0.500
Male/Female	34/12	19/11	0.326
Underlying disease (n, %)			
Hypertension	17 (37.0%)	11 (36.7%)	0.980
Diabetes	8 (17.4%)	6 (20.0%)	0.774
COPD	13 (28.3%)	11 (36.7%)	0.441
Laboratory values			
White blood cells ($\times10^9$/L)	9.79 (5.83, 14.30)	11.53 (10.17, 16.57)	0.079
Neutrophils ($\times10^9$/L)	8.16 (4.19, 12.58)	10.25 (8.45, 14.04)	0.064
Lymphocytes ($\times10^9$/L)	0.90 (0.63, 1.26)	0.81 (0.43, 1.14)	0.338
Monocytes ($\times10^9$/L)	0.58 (0.29, 0.85)	0.78 (0.41, 1.08)	0.118
Hematocrit (%)	34.70 (29.75, 39.90)	35.90 (31.98, 41.48)	0.506
Platelets ($\times10^9$/L)	105 (77.75, 182)	108 (54, 195.75)	0.780
Blood urea nitrogen (mmol/L)	6.91 (5.13, 11.67)	10.82 (6.09, 19.11)	0.107
Creatinine (μmol/L)	87 (60.25, 174.25)	166.5 (72.75, 279)	0.039
Uric acid (μmol/L)	269 (180, 470)	349 (143.75, 536.5)	0.592
Total bilirubin (μmol/L)	14.85 (9.88, 29.05)	15.65 (9.40, 27.65)	0.927
Ferritin (μg/L)	944 (413.75, 2015.5)	544 (407.75, 1811)	0.476
Transferrin (g/L)	1.14 (0.99, 1.41)	1.15 (0.87, 1.45)	0.596
Serum iron (μmol/L)	5.8 (4.2, 9.75)	5.6 (3.75, 12)	0.864
Transferrin saturation (%)	27.8 (23.5, 33.35)	26.95 (16.9, 35.6)	0.632
TIBC (μmol/L)	34.7 (29.25, 42.15)	32.75 (27.65, 40.15)	0.628
PaO_2 (mmHg)	75 (52, 98.05)	74.5 (57, 93)	0.863
$PaCO_2$ (mmHg)	34 (28.8, 37.5)	35.5 (30.0, 40.1)	0.396
CRP (mg/L)	69.20 (31.26, 126.85)	92.55 (44.00, 203.78)	0.162
PCT (ng/mL)	1.47 (0.75, 4.45)	10.25 (8.45, 10.04)	0.039
HSP27 (ng/mL)	3.03 (1.48, 7.39)	9.30 (3.62, 25.91)	0.001
IL-1β (pg/mL)	2.17 (0.91, 4.60)	4.56 (1.52, 8.05)	0.035
SOFA score	4 (2, 5.25)	7 (3, 10)	<0.001
ICU stay (days)	12 (7, 18)	9 (6, 15.5)	0.183

COPD, chronic obstructive pulmonary disease; TIBC, total iron binding capacity; PaO_2, pressure of oxygen; $PaCO_2$, partial pressure of carbon dioxide; CRP, C-reactive protein; PCT, procalcitonin; HSP27, heat shock protein 27; IL-1β, interleukin 1 beta; SOFA, sequential organ failure assessment; ICU, Intensive Care Unit.

levels based on the established cutoff value. The 28-day survival rate was significantly lower for patients with HSP27 levels above the cutoff than those with lower levels (log-rank test, $p = 0.013$) (Figure 3).

4 Discussion

As a systemic inflammatory response syndrome, sepsis often presents with non-specific symptoms in its early stages, leading to delayed diagnosis (20). However, the underlying pathological processes of sepsis initiate early, gradually causing systemic damage (21, 22). In its advanced stages, sepsis rapidly progresses, characterized by systemic immune dysregulation, uncontrolled inflammation, and tissue injury. Patients with advanced sepsis may develop multiple organ dysfunction syndrome (MODS), a life-threatening condition that is difficult to manage and associated with poor outcomes (23). Early intervention is critical to implement timely and effective therapeutic strategies before the condition becomes irreversible (24). Therefore, there is an urgent need for a biomarker that can facilitate early diagnosis and prognostication of sepsis.

The SOFA score, a widely recognized standard for assessing organ dysfunction, is a crucial tool in the diagnosis and prognostication of sepsis. Despite its complexity, which involves multiple variables, the SOFA score remains the primary method used in clinical practice to evaluate the severity and predict the outcome of sepsis (25). In recent years, an increasing number of biomarkers, including CRP, PCT, and IL-1β, are being utilized in clinical settings to enhance, have been explored for their potential to improve the predictive accuracy of

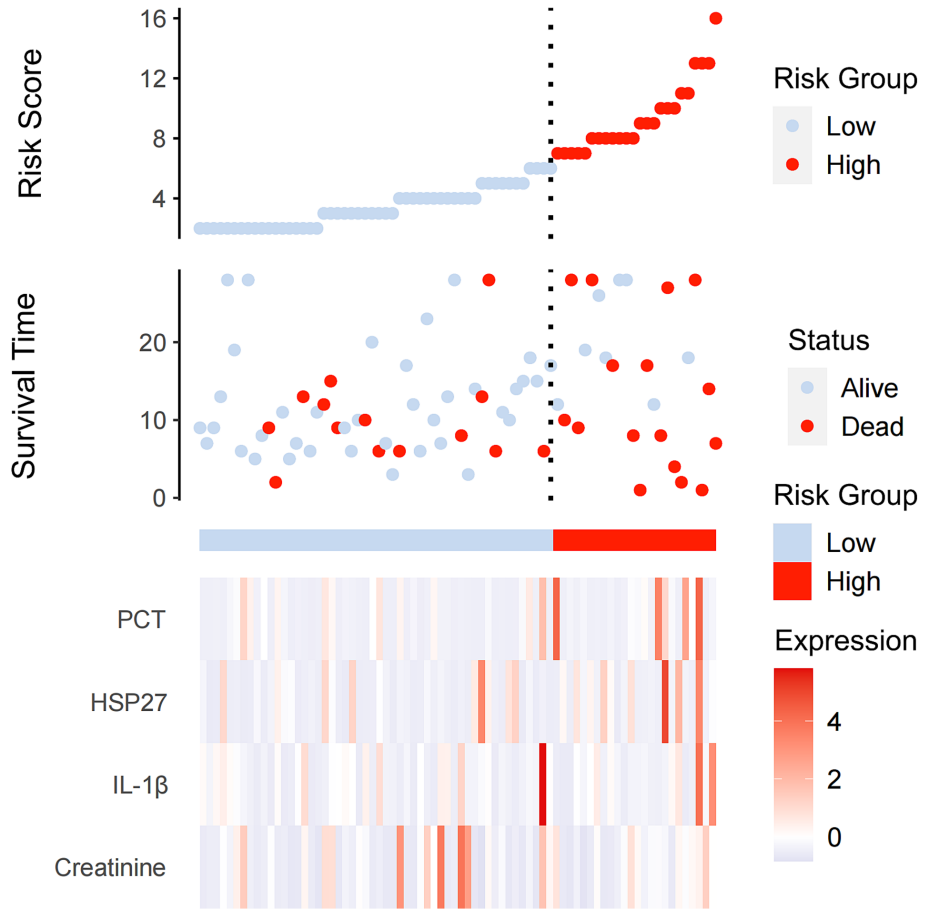

FIGURE 2
Serum PCT, HSP27, IL-1β, and creatinine levels at admission correlated with disease severity (SOFA score) in septic patients.

TABLE 3 Multivariate logistic regression analysis for sepsis after adjusting effects of confounders.

Multivariate logistic regression analysis	*B*	S. E.	Wald	*P*-value	Odds ratio	95%CI
Model 1	0.035	0.015	5.239	0.022	1.035	1.005–1.067
Model 2	0.037	0.016	5.657	0.017	1.038	1.007–1.071
Model 3	0.038	0.016	5.364	0.021	1.039	1.006–1.073
Model 4	0.036	0.017	4.463	0.035	1.036	1.003–1.071
Model 5	0.034	0.018	3.435	0.064	1.034	0.998–1.072

Model 1 included only HSP27. In Model 2, a multivariate analysis was conducted incorporating variables HSP27 and Creatinine (CREA). A multivariate analysis was performed incorporating variables HSP27, CREA and procalcitonin (PCT) in Model 3. Model 4 included HSP27, CREA, PCT and IL-1β. Finally, in Model 5, HSP27, CREA, PCT and IL-1β, and the SOFA score were included for multivariate analysis.

TABLE 4 Receiver operating characteristic (ROC) analysis for laboratory indexes on sepsis prognosis.

Indexes	AUC (95% CI)	Cut-off	*z*-value	Youden index *J*	*P*-value	Sensitivity	Specificity
Creatinine (μmol/L)	0.643 (0.525–0.749)	205	2.136	0.281	0.033	43.3%	84.8%
PCT (ng/mL)	0.613 (0.494–0.722)	3.06	1.619	0.296	0.106	60.0%	69.6%
HSP27 (ng/mL)	0.720 (0.605–0.817)	2.61	3.767	0.400	<0.001	90.0%	50.0%
IL-1β (pg/mL)	0.643 (0.525–0.750)	3.00	2.064	0.317	0.039	60.0%	71.7%
SOFA score	0.722 (0.607–0.819)	6.0	3.631	0.359	<0.001	53.3%	86.6%
HSP27 + SOFA score	0.767 (0.656–0.856)	/	4.748	0.446	<0.001	53.3%	91.3%

AUC, area under curve; CI, confidence interval; PCT, procalcitonin; HSP27, heat shock protein 27; SOFA, sequential organ failure assessment.

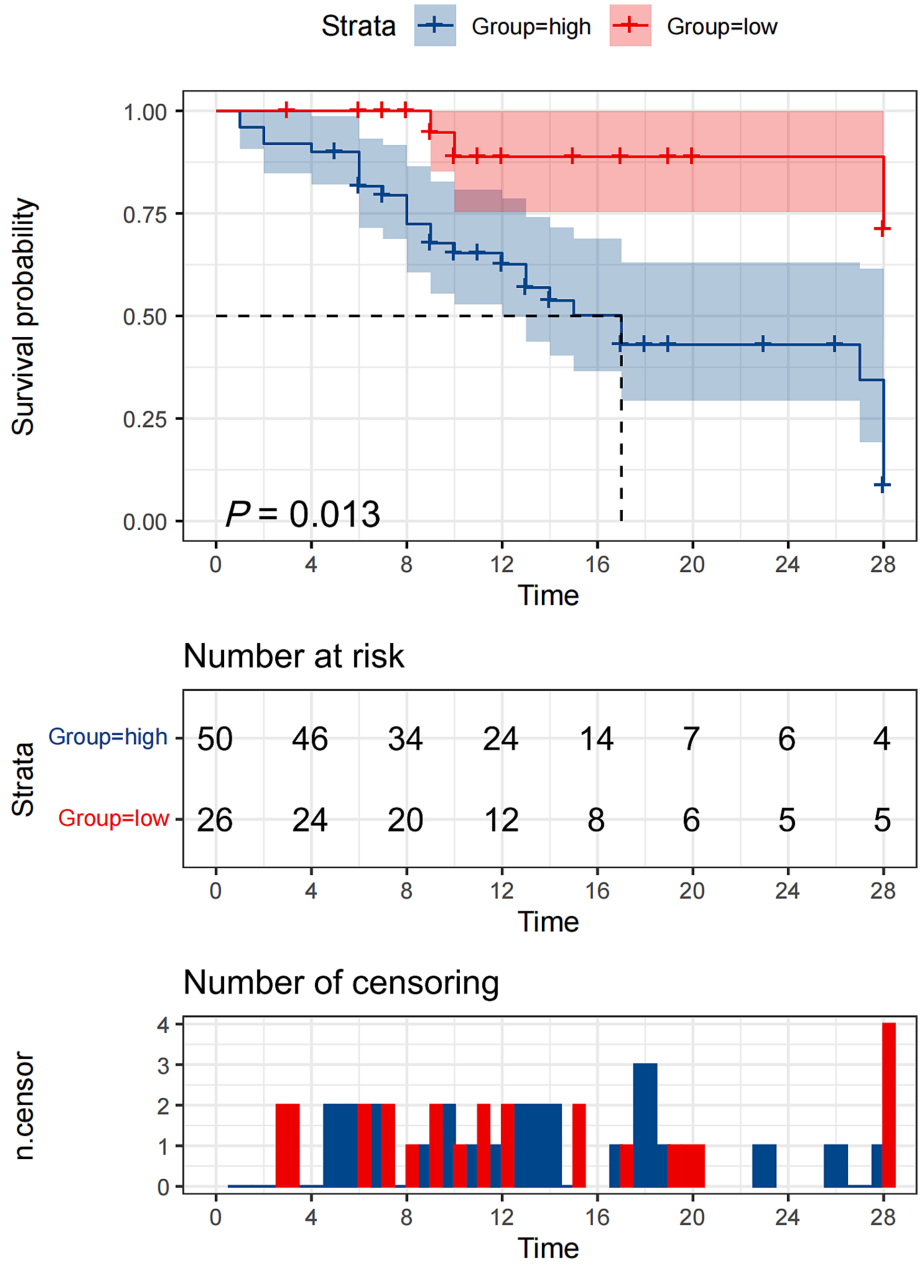

FIGURE 3
Kaplan–Meier survival curves of 76 adult patients with sepsis based on the HSP27 cutoff value (2.61 ng/mL) on day of ICU admission.

sepsis prognosis (26–28). However, their limitations in terms of sensitivity and specificity hinder their widespread clinical application in the diagnosis and prognostication of sepsis.

Our study revealed that ICU sepsis patients exhibited significantly elevated serum HSP27 levels within 48 h of ICU admission compared to a control group. As an anti-inflammatory molecule (29), elevated HSP27 plays a crucial protective role in mitigating organ dysfunction induced by sepsis (30–32). To further assess the diagnostic utility of HSP27 in ICU sepsis, ROC curve analysis demonstrated a significantly higher AUC for HSP27 compared to PCT. Consequently, HSP27 emerges as a promising biomarker for ICU sepsis. Moreover, sepsis patients within the mortality group exhibited significantly higher serum HSP27 levels compared to the survival group. Additionally, sepsis patients with elevated serum HSP27 levels at admission exhibited a higher 28-day mortality rate. By combining HSP27 levels with SOFA scores, the specificity of ROC-based diagnosis increased from 86.6 to 91.3% ($p < 0.001$, Table 4). Finally, Kaplan–Meier survival analysis indicated a correlation between elevated serum HSP27 levels and reduced patient survival rates. These findings collectively demonstrate the prognostic value of HSP27 levels in ICU patients with sepsis.

More importantly, while the AUC for HSP27 was slightly lower than that for the SOFA score, the early changes in HSP27 expression levels during the onset of sepsis could facilitate earlier diagnosis and prognostic assessment. Early identification of sepsis patients is crucial for improving patient outcomes, and the early

diagnostic potential of HSP27 may contribute to more timely clinical interventions. Moreover, the level of HSP27 correlates with sepsis severity, with higher levels associated with more severe disease and poorer prognosis. This suggests that HSP27 may serve as a valuable prognostic biomarker. Additionally, the quantification of serum HSP27 levels using ELISA technology is a convenient and highly accurate method, making it suitable for widespread implementation in hospital laboratories. By closely monitoring changes in HSP27 levels, clinicians can assess the therapeutic response of sepsis patients and make timely adjustments to their treatment plans.

In conclusion, our findings suggest that HSP27 may serve as a valuable biomarker for both the diagnosis and prognosis of sepsis.

4.1 Limitations

Although this retrospective, single-center study offers valuable insights into the role of HSP27 in sepsis, several limitations inherent to its design and scope must be acknowledged. Firstly, the retrospective nature of the study and its single-center design may introduce selection bias, potentially overestimating the diagnostic and prognostic utility of HSP27. To address this, future large-scale, multicenter, prospective studies are imperative. Secondly, the relatively small sample size and the inclusion of predominantly critically ill patients limit the generalizability of the findings to other patient populations, such as those with postoperative or non-critical sepsis. Expanding the sample size and incorporating a broader spectrum of patient conditions would enhance the reliability and applicability of the results. Thirdly, despite efforts to account for known confounders, the possibility of unmeasured or unrecognized confounding factors that may influence the observed associations cannot be entirely excluded. Finally, the lack of serial monitoring of serum HSP27 levels at multiple time points during the course of sepsis precludes a comprehensive understanding of the temporal relationship between HSP27 fluctuations and disease progression. Addressing these limitations through future studies will provide a more comprehensive understanding of the potential clinical utility of HSP27 in sepsis management.

5 Conclusion

In summary, this study demonstrates that serum HSP27 levels are significantly elevated in patients with sepsis, particularly in those who do not survive the 28-day period. These findings suggest a strong association between elevated HSP27 levels and increased mortality risk in sepsis patients. Our results highlight the potential utility of HSP27 as a valuable biomarker for the diagnosis, prognosis, and potentially the therapeutic management of sepsis.

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 humans were approved by The Second Affiliated Hospital of Nanchang University Medical Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

LY: Writing – original draft, Methodology. ZF: Data curation, Writing – original draft. FY: Writing – review & editing. XW: Funding acquisition, Writing – review & editing.

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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.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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A case report of acute promyelocytic leukemia with myeloid sarcoma of the lumbar spine and literature review

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Acute promyelocytic leukemia (APL) presenting solely as myeloid sarcoma (MS) is extremely rare. This report describes a 53-year-old male who presented with low back pain and a movement disorder in his lower limbs. MRI and PET/CT scans of the lumbar spine revealed an intraspinal mass. Pathological analysis of the surgically resected mass identified it as myeloid in origin. Routine blood tests were unremarkable, and bone marrow smears and immunophenotyping showed no evidence of abnormal myeloblasts or promyelocytes. However, bone marrow aspirates testing for acute leukemia fusion genes by qPCR revealed the presence of the *PML::RARA* fusion. Further investigation via FISH confirmed the fusion in both the bone marrow and the extramedullary mass. The patient was ultimately diagnosed with isolated promyelocytic extramedullary sarcoma (MS/APL). Treatment with all-trans retinoic acid and arsenic trioxide alleviated the back pain and restored the patient's mobility. After 1 year of consolidation therapy, bone marrow smears confirmed sustained remission, and the *PML::RARA* fusion gene was undetectable. In addition to this case, we review 41 other APL patients with extramedullary sarcoma as their first symptom (MS/APL) at the time of diagnosis and provide an analysis of these cases.

KEYWORDS

acute promyelocytic leukemia, myeloid sarcoma, extramedullary infiltration, literature review, treatment

Introduction

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) defined by the genetic translocation that forms the *PML::RARA* fusion gene between chromosomes 15 and 17 (1). This fusion disrupts gene transcription, halting myeloid differentiation at the promyelocytic stage (1, 2). APL accounts for approximately 10%–15% of all AML cases and is typically diagnosed through abnormal blood tests, along with coagulation and fibrinolytic dysfunction (3, 4). The incorporation of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) into treatment protocols has dramatically improved outcomes for APL patients, achieving a complete remission (CR) rate exceeding 90% (5, 6).

Myeloid sarcoma (MS), also known as granulocytic sarcoma or chloroma, is characterized by the extramedullary accumulation of myeloid blasts (7). It can occur as an isolated condition or in association with myeloid malignancies, particularly AML, and often signifies relapse following AML remission (7, 8). Although MS can affect individuals of all ages, it is more common in children than adults, with a male-to-female ratio

of approximately 1.2:1 (7, 9, 10). MS/extramedullary infiltration is a rare complication of APL, affecting only 3%–5% of patients, typically during disease relapse post-remission (11, 12). The central nervous system and skin are the most common sites of extramedullary involvement, while other areas such as lymph nodes, the gastrointestinal tract, bones, soft tissues, and testes are less frequently affected (12, 13). Rare cases of APL-related EM infiltration at unusual sites have also been reported. Key factors associated with extramedullary involvement in APL include being under 45 years of age, elevated white blood cell count, and the presence of the bcr3 subtype of the *PML::RARA* fusion gene (14). The occurrence of APL with MS or EM infiltration as the sole initial presentation is extremely rare. Here, we present a case of APL-related MS manifesting as a lumbar epidural mass.

Case presentation

A 53-year-old male presented with 8 months of low back pain and weakness in both lower limbs. A CT scan at a local hospital revealed soft tissue shadows at the right posterior margin of the L2/3 intervertebral disc and in the spinal canal at the same level. MRI showed abnormal signals in the T12, L2, and S1 vertebral bodies, along with intraspinal soft tissue masses at the L2 pyramidal plane. Neoplastic lesions were suspected, and the patient received treatment with traditional Chinese medicine. Although there was initial improvement, his condition progressively worsened, leading to an inability to walk. PET/CT scans revealed uneven density in several vertebrae, with soft tissue shadows in the right portion of the L2 vertebra, extending into the right intervertebral foramen and inward into the spinal canal. There was slightly increased FDG uptake in the vertebral bodies and appendages, and active FDG metabolism was also noted in the spinal cord cavity from the T12-L2 segment (Figure 1). A follow-up MRI 1 month later showed multiple areas of bone destruction in the T2, L1, L2, and S1-3 vertebrae, suggesting metastatic involvement. Additionally, heterogeneous signal intensity in the spinal canal at the L1-3 level indicated possible involvement. The patient underwent surgical resection of the L2 vertebral body and the epidural mass, along with spinal fixation. Preoperative blood tests, including routine examinations and coagulation studies, were normal. Histopathological analysis of the resected tissue suggested a neoplastic tumor.

The case was referred to our pathology department for further consultation. Immunophenotyping results were as follows: CD34 (−), CD117 (+), MPO (+), CD20 (−), CD79a (−), CD3 (−), CD138 (−), CD38 (−), Mum-1 (−), CD56 (−), IgK (−), Igλ (−), and Ki-67 (+, approximately 60%). *In situ* hybridization for EBV showed no EBER1/2 expression. Gene rearrangement analysis by PCR and GENESCAN revealed no clonal amplification peaks for IgH or IgK. Based on these results, along with the morphological and immunophenotypic findings, MS was strongly considered. Postoperatively, the patient showed some improvement in low back pain and lower limb weakness, but remained unable to stand or walk. One month after surgery, the patient sought treatment at our hematology clinic. A bone marrow smear revealed significantly active marrow hyperplasia, but no blasts or abnormal promyelocytes were detected (Figure 2A). Flow cytometry showed abnormal promyelocytes with approximately 0.5% of nucleated cells, positivity for CD123, CD9, CD117, CD64, and CD33, but negativity for HLA-DR, CD11b, CD15, and CD56. These findings raised strong suspicion for APL-associated MS. Further tests confirmed our suspicion: PCR of peripheral blood was positive for *PML::RARA*, with a *PML::RARA/ABL* ratio of 0.9305%. Chromosomal analysis revealed 46,XY,t(15;17)(q24;q21)[2]/46,XY[18] (Figure 2B). Multiplex real-time PCR of bone marrow also showed positivity for *PML::RARA* (bcr-1). FISH analysis of the MS tissues revealed a 94% positivity rate for *PML::RARA* fusion signals (Figure 2C), while bone marrow FISH showed a 8% positivity rate for *PML::RARA* fusion at the 17q21/15q22-24 site, including 4% atypical signals (Figure 2D). Genetic testing revealed no mutations typically associated with AML prognosis at diagnosis. Routine blood tests, coagulation, and fibrinolysis remained normal, with no hepatosplenomegaly or systemic lymphadenopathy observed. Given these findings, the patient was diagnosed with acute promyelocytic extramedullary sarcoma (MS/APL).

The patient began treatment on the 48th day post-surgery, consisting of ATRA 10 mg three times daily and ATO 10 mg intravenously once a day for 30 days. During treatment, the patient developed mild ATRA syndrome, including fever, facial edema, and weight gain, which were managed with dexamethasone and furosemide. In addition, the expression level of the *PML::RARA* fusion gene reached the highest value on day 24 of induction treatment with a *PML::RARA/ABL* ratio of 16.3309%. After the first cycle of chemotherapy, the patient's condition improved significantly. He was able to stand and walk independently with a brace, and his lumbar pain was greatly reduced. Bone marrow smears revealed no blasts, with promyelocytes comprising 1% of nuclear cells. Both peripheral white blood cell and blood cell counts normalized, indicating CR. However, the *PML::RARA* fusion gene remained detectable, with a *PML::RARA/ABL* ratio of 0.9669%. Following discharge, the patient continued ATRA at 10 mg three times daily, alternating with 2 weeks of rest, followed by 2 weeks of Realgar-Indigo naturalis formula (RIF, 5 tablets three times daily). This consolidation therapy was planned for two cycles. Two months later, a bone marrow smear confirmed sustained CR, and the *PML::RARA* fusion gene was no longer detectable. CT scans of the lumbar spine showed no mass (Figure 3). The patient's back pain had significantly improved, and he was able to walk freely. The consolidation regimen of ATRA and RIF was maintained for 6 months, with regular monitoring of bone marrow cytology, genetic tests, and spinal imaging every 2 months, all of which showed normal results. Eight months after the first induction, the patient received radiation therapy. He tolerated the treatment well with no major complications. One year after treatment, the patient remained in remission, with molecular analyses of bone marrow and peripheral blood showing no evidence of *PML::RARA* fusion transcripts (Figure 4).

Literature review

Our review identified 41 cases of APL where MS was the initial presenting symptom. Key details of these cases, including onset locations, clinical features, and treatment responses, are

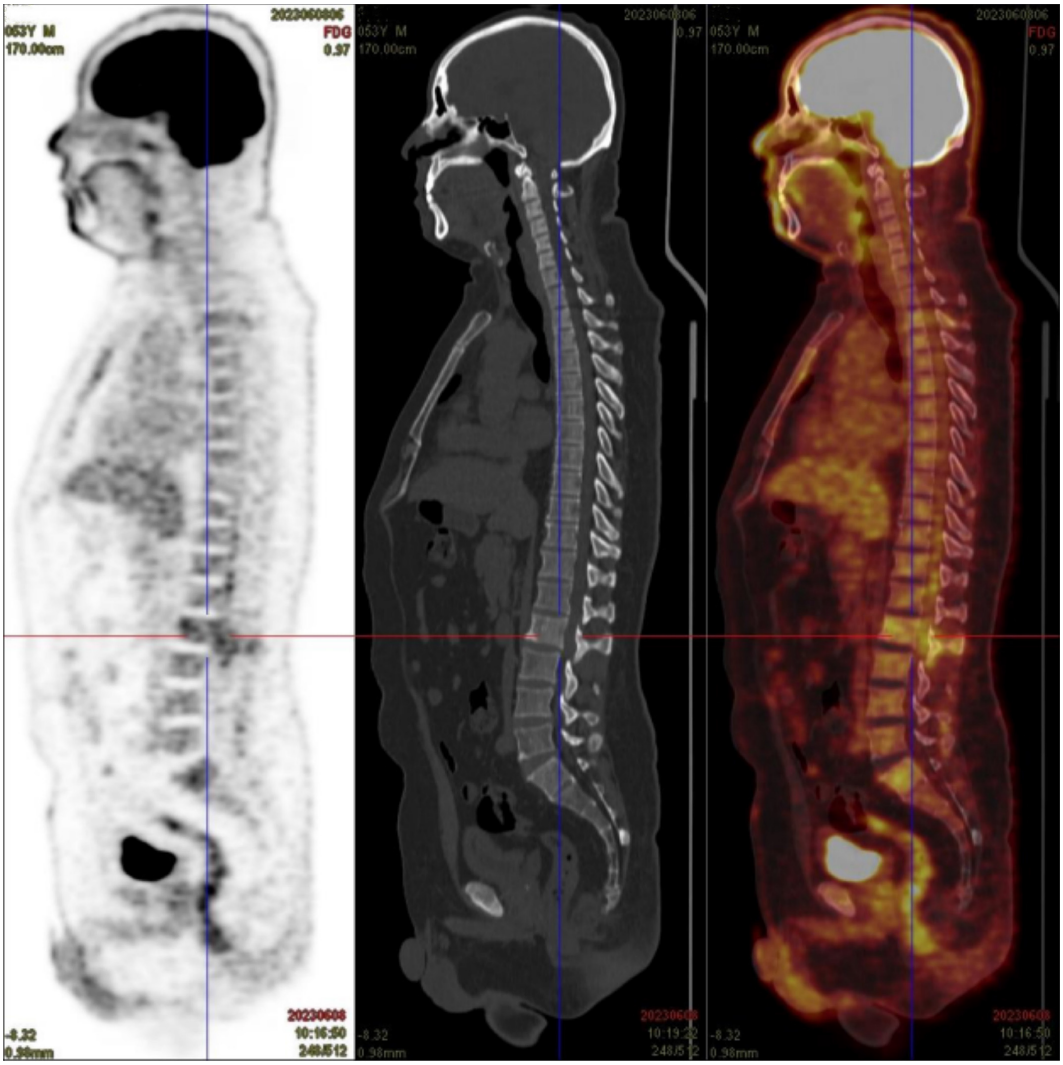

FIGURE 1
The PET/CT scan of the patient showed areas of uneven density in several vertebrae. Slightly increased FDG uptake was observed in the vertebral bodies and appendages, with active FDG metabolism also detected in the spinal cord cavity between the T12 and L2 segments.

summarized in Table 1 (15–54). The patients were predominantly young, with a median age of 39.5 years (range: 1–77 years), and only 15% were aged ≥60 years. The male-to-female ratio was 24:17, showing no significant sex differences. The spine was the most common site of extramedullary infiltration (12/41 cases) (15, 18, 27, 28, 33, 35, 37, 43, 49–51, 54), followed by the skin (4/41) (19, 29, 42, 52), pleura (3/41) (19, 40, 53), and ovary (2/41) (25, 49). Other less common sites included the intracranial region (2/41) (22, 48), tongue (2/41) (24, 44), humerus (2/41) (23, 39), and colon (2/41) (31, 46), among others. Notably, one patient developed sarcoma in a donor kidney after renal transplantation, not in their own kidney (45). Most MS cases were confined to a single site (78%, 32/41), with multiple-site (9 cases) and multi-organ (7 cases) involvement occurring less frequently. Immunophenotyping of extramedullary masses typically showed MPO positivity. Other markers included CD68 (20%, 8/41), CD43, CD33, and CD117 (15%, 6/41), and CD13 (10%, 4/41). Bone marrow infiltration was observed in 59% (24/41) of patients, while 42% (17 patients) (17, 19, 21, 23, 25, 26, 32, 33, 37–39, 41, 43, 45, 49, 53, 54) had no blasts or promyelocytes in the bone marrow and circulating blood, or did not meet the diagnostic criteria for APL. Six patients had elevated white blood cell counts (16, 22, 24, 41, 42, 44), and three presented with disseminated intravascular coagulation (DIC) (15, 22, 53). Based on white blood cell counts, patients were classified into high-risk (6 patients) (16, 22, 24, 41, 42, 44) and low-risk (27 patients) (15, 17–20, 25–31, 34–40, 46–53) groups, while the remaining cases (21, 23, 32, 33, 43, 54) could not be classified. Chromosome 15 and 17 translocations (t(15;17)) were detected in 54% (22/41) of cases. Seven cases (17%) (22, 25, 33, 41, 47, 49, 52) had a normal karyotype, and 5% (2/41) (48, 54) had complex karyotypes. The common *PML::RARA* fusion was present in 59% (24/41) of patients, while rare fusion signals involving *RARA* [fused with *NPM1* (35), *FIP1L1* (48), *ZBTB16* (51), and *TTMV* (54)] were detected in four cases. One case lacked *RARA* rearrangement, but RT-PCR testing revealed an in-frame fusion between *CPSF6* exon 4 and *RARG* exon 4 (*CPSF6::RARG*) (47). Six patients (15%) had concurrent gene mutations, with *FLT3* mutations being the most common (7%, 3/41) (37, 41, 50). Other mutations included *KARS* (47, 48) and *WT1* (47, 54)

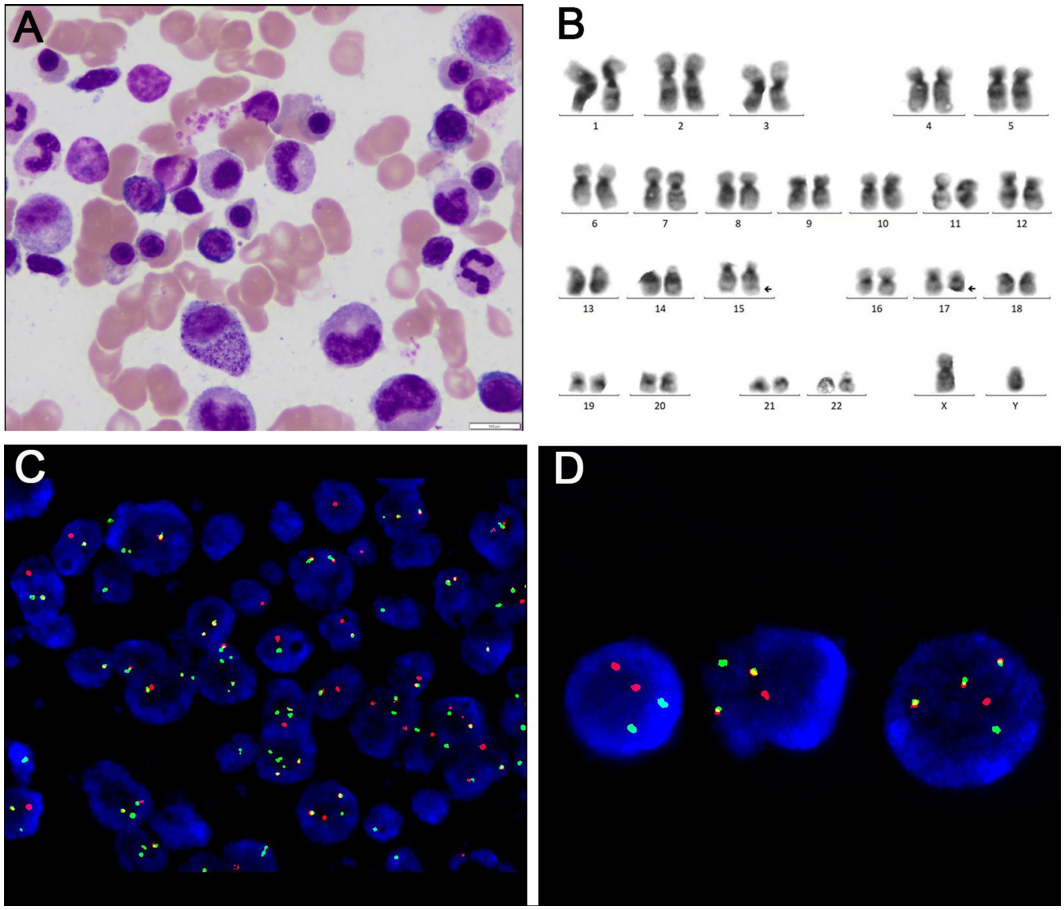

FIGURE 2
(A) Bone marrow cytomorphology by bone marrow aspirate smear. **(B)** Karyotype of bone marrow. **(C)** FISH probe detection of myeloid sarcoma biopsies. **(D)** FISH probe detection of bone marrow aspirate.

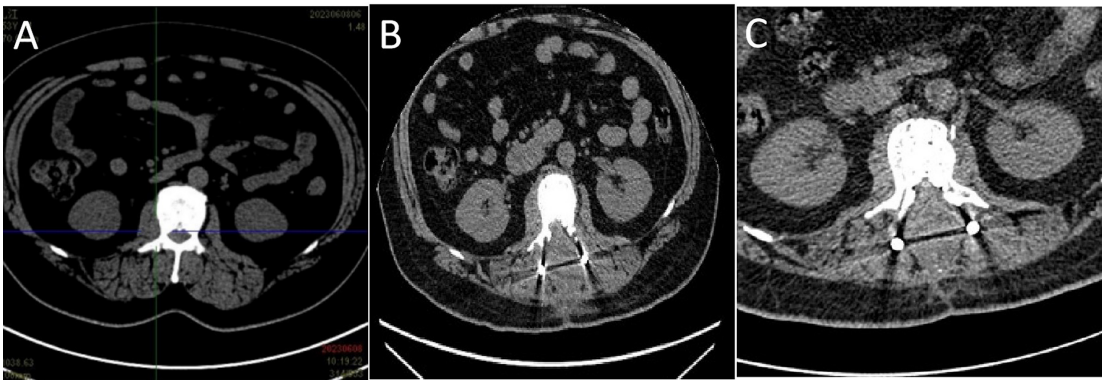

FIGURE 3
(A) PET/CT scan at the L2 level before surgery. **(B)** CT scan at the L2 level after surgery. **(C)** CT scan at the L2 level after one cycle of retinoic acid combined with arsenic trioxide induction therapy.

(each in two patients), as well as *EZH2* (47), *KMT2C* (50), and *SMAD9* (54) mutations.

A total of 40 patients received treatment, with 28 achieving remission, resulting in an overall response rate of 70%. Among low-risk patients, the remission rate was 70% (19 out of 27), while high-risk patients had a slightly higher remission rate of 83% (5 out of 6). Thirty patients were treated with ATRA combined with chemotherapy, and 83% (25 out of 30) achieved remission. One patient did not respond to treatment, two died from multiple organ failure, and two succumbed to intracranial hemorrhage. Additionally, three patients who received only chemotherapy also responded to treatment. Follow-up duration varied widely

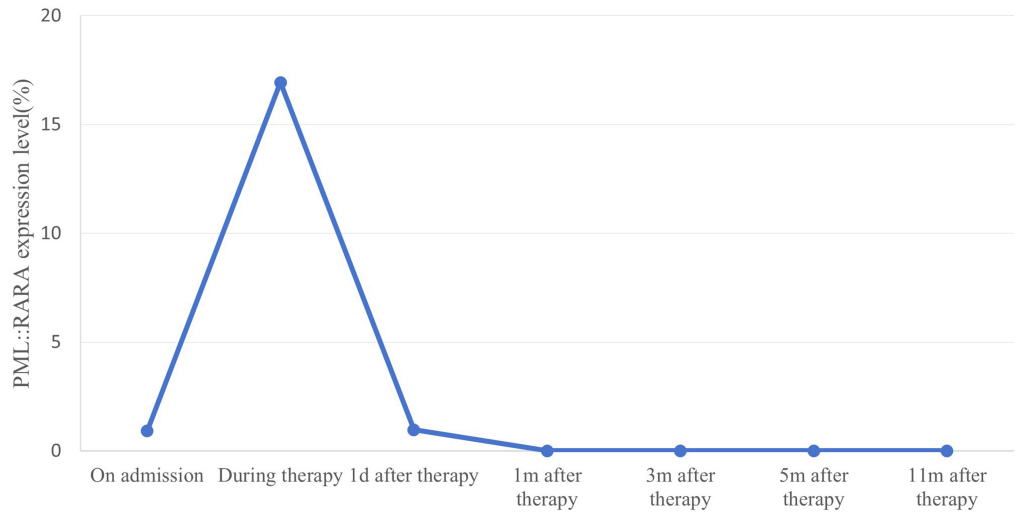

FIGURE 4
Quantitative changes in expression of *PML::RARA* fusion gene monitored by real-time PCR. *PML::RARA* fusion gene levels in peripheral blood of patients were dynamically monitored by real-time PCR. The detected value of *PML::RARA* level (*PML::RARA/ABL*) = *PML::RARA* copy number/*ABL* copy number × 100%. When the detected value showed negative, it indicated that there was no fusion gene expression or the fusion gene expression level in the submitted samples was lower than the lower limit of detection (100 copies/ml) of this method. The *PML::RARA* fusion gene decreased after chemotherapy and remained negative during maintenance therapy.

across studies, ranging from less than 1 week (20, 22) to as long as 288 months (49). This variation is attributed to several factors, including severe exhaustion and bleeding in some patients, either untreated or occurring during the myelosuppressive phase following surgery or chemotherapy. Notably, long-term survival was observed in patients who underwent surgery with careful monitoring and received ATRA combined with chemotherapy. Interestingly, the longest-followed patients experienced recurrent relapses of MS in various locations, despite no abnormalities being detected in the bone marrow or peripheral blood. These patients maintained long-term survival and good quality of life through surgery and chemotherapy. In addition to relapse at other extramedullary sites, MS/APL can progress to non-M3 AML. One patient, for instance, progressed to AML with the t(8;21)/RUNX1:RUNX1T1 translocation after more than 2 years of remission following ATRA and chemotherapy (25).

For patients with solitary MS and no bone marrow infiltration, the most common sites of infiltration were the spine (5 out of 17) and other bony sites (5 out of 17), followed by the pleura (2 out of 17). Singular cases were observed in the mediastinum, testis, ovary, and breast (1 each). Notably, no reports of involvement in digestive tract organs were found. Similar to other MS/APL cases, these patients were predominantly young, with a median age of 29 years, and there was no significant sex difference (male-to-female ratio of 10:7). The treatment response rate in this group was 70% (12 out of 17), with 91% (10 out of 11) of patients treated with ATRA achieving remission, while the remaining patients succumbed to severe bleeding. Five cases progressed to bone marrow infiltration. Three of these cases were thought to reflect disease development before treatment, while the remaining two cases developed bone marrow blasts or promyelocytes months after treatment initiation. This progression was considered to indicate a combination of disease relapse and progression.

Discussion

Myeloid sarcoma typically manifests in patients with APL during relapse, with extramedullary involvement being relatively uncommon. It occurs in approximately 3%–5% of APL patients (12, 55). In the European multicenter PETHEMA trial, only 10 of 169 relapse cases showed extramedullary involvement, predominantly in the central nervous system and skin (14). Instances where MS presents as the initial symptom, without significant bone marrow or peripheral blood abnormalities, or where APL diagnostic criteria are not met, are exceedingly rare. Recent reports have identified new cases and additional sites of extramedullary infiltration. Among these, spinal extramedullary masses are the most frequently observed, followed by skin and pleura. Other tissues, including rarely transplanted tissue, have also been implicated. In MS/APL patients without evidence of disease in the bone marrow or peripheral blood, the extramedullary masses predominantly involve bony structures such as the spine, sternum, and humerus. However masses located in the digestive system are rare in these patients compared to other MS/APL cases. Therefore, isolated MS located in the skeleton is even more important to evaluate thoroughly and the possibility of promyelocytic sarcoma should be considered.

An important consideration for clinicians is the need for a comprehensive understanding and systematic evaluation of donor health in patients undergoing organ transplantation, to exclude blood-related diseases. In cases of MS/APL in transplanted organs, it is crucial to not only examine the patient's bone marrow but also conduct *PML::RARA* testing on bone marrow and peripheral blood from both the donor and other recipients. This strategy ensures prompt detection and management of potential complications. The timing of detection post-transplant remains an unresolved issue that requires further investigation.

Clinicians often face significant challenges in diagnosing APL with MS, particularly when the presentation involves solitary

TABLE 1 Cases of acute promyelocytic leukemia with MS as the first symptom.

Reference	Age/ sex	MS site	Type	Immuno-phenotype	High WBC count	DIC	Blasts/ promy-elocyte in BM (%)	Auer rods	Karyo type	Fusion gene	Gene mutation	Risk level	Treat-ment	Response	PFS
Blesco et al. (16)	4/M	Pelvis	Single	NA	+	–	–/59	NA	NA	NA	NA	Adverse	Vincristine + prednisone + adriamycin, radiotherapy	NR	-(CR after 14 m)
Kubonishi et al. (17)	23/M	Mediastinum	Single	MPO	–	–	NA/NA (2 m later: 3/63)	–	NA	NA	NA	Favorable	Radiotherapy, mediastinal tumor resection,	NR	-(14 m later died of heart failure)
Zuiable et al. (18)	31/M	(T12∼L3/4) extradural space	Multiple	NA	–	–	2/NA NA/90	NA	NA	NA	NA	Favorable	Laminectomy, radiotherapy, DA, atuo-HSCT	CR	>18 m
Tosi et al. (15)	27/M	(L3∼4) extradural space	Multiple	MPO, CD43, Lys	–	+	–/NA (many of promyelocyte cells)	+	t(15;17) (q22;q11)	NA	NA	Favorable	Laminectomy, ATRA + DA	NR	–
Bobbio-Pallavicini et al. (19)	–/M	Pleura, fronto-parietal scalp and lumbar region	Multiple	MPO, CD43	–	–	–/–	–	NA	*PML::RARA* (bcr1)	NA	Favorable	Chemotherapy (involve ATRA)	CR	13 m
Takeh et al. (20)	66/M	Ilem	Single	NA (infiltration by giant promyelocytes)	–	–	NA/infiltration by giant promyelocytes	NA	NA	NA	NA	Favorable	Limited intestinal resection and anastomosis	NA	Died 14 h after surgery
Gopal et al. (21)	27/M	Left testicle	Single	MPO, CD43, CD117, CD33, CD34	NA	NA	–/–	NA	46,XY,t(15;17) (q22.3;q21.1) [15]	NA	NA	NA	Radical orchiectomy	NA	12 m (and relapsed in the contrala-teral testicle)

(Continued)

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TABLE 1 (Continued)

Reference	Age/sex	MS site	Type	Immuno-phenotype	High WBC count	DIC	Blasts/promy-elocyte in BM (%)	Auer rods	Karyo type	Fusion gene	Gene mutation	Risk level	Treat-ment	Response	PFS
Fukushima et al. (22)	39/F	Left cerebellar hemisphere	Single	LCA, CD13, CD33	+	+	NA/NA (with proliferation of abnormal promyelocytes)	+	Normal	*PML::RARA*	NA	Adverse	IA, Posterior fossa decompression	NA	1 m (died 4 days after surgery)
Worch et al. (23)	16/F	Right humerus, right proximal femur, and distal tibia	Multiple	MPO, CD13, CD15, CD33, CD117	NA	NA	−/−	−	t(15;17)	*PML::RARA*	NA	NA	ATRA	CR	NA
Mohamedbhai et al. (24)	45/M	Tongue	Single	MPO, CD45, CD68	+	−	NA/NA (diffuse infiltration)	NA	t(15;17) (q22;q12)	NA	NA	Adverse	ATRA + DA	CR	>1 m
Wang et al. (25)	26/F	Right ovary	Single	MPO, TdT, CD13, CD33, CD99, CD45 (LCA), CD20, CD3, CK, Vim, INH, PLAP	−	−	−/−	−	Normal	*PML::RARA*	NA	Favorable	IA, MA, 6-MP + MTX + ATRA	CR	27 m and then progressed to AML with t(8;21) (q22;q22)/ RUNX1:: RUNX1T1 (FAB type:M2)
Thomas and Chelghoum (26)	19/M	Sternum	Single	NA	−	−	−/−	−	t(15;17) (q22;q21-22)	*PML::RARA*	NA	Favorable	Tumor resection, ATRA + IA, radiotherapy	CR	>24 m
Kyaw et al. (27)	26/M	(T2∼4, T12∼L2) extradural space	Multiple	NA	−	−	NA/NA (diffuse infiltration)	NA	NA	*PML::RARA* (bcr1)	NA	Favorable	Radiotherapy, ATRA + DNR	CR	>5 m
Bittencourt et al. (28)	53/M	(T6∼T8) extradural space	Multiple	NA	−	−	NA/NA (diffuse infiltration)	+	46,XY,t(15;17) (q22;q12)	*PML::RARA*	NA	Favorable	ATRA + DNR, radiotherapy	CR	NA (soon after hematological remission) and died of sepsis

(Continued)

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TABLE 1 (Continued)

Reference	Age/ sex	MS site	Type	Immuno-phenotype	High WBC count	DIC	Blasts/ promy-elocyte in BM (%)	Auer rods	Karyo type	Fusion gene	Gene mutation	Risk level	Treat-ment	Response	PFS
Shvartsbeyn et al. (29)	46/M	Abdominal skin	Multiple	NA (myeloid nature)	–	–	~95/NA	+	t(15;17)	*PML::RARA*	NA	Favorable	ATRA + IDA + dexamethasone	Dead	Died of multi-organ failure
Benjazia et al.(30)	17/F	Rectum	Single	MPO, Lys, CD43	–	–	80/NA	+	46,XX,t(15;17) (q22;q21)	*PML::RARA*	NA	Favorable	ATRA + IDA	CR	>48 m
Damodar et al. (31)	29/M	Colon	Single	MPO, CD43, CD3, Ki67 (70%)	–	–	NA/NA (reported as AML)	NA	t(15;17) (q24;q21)	*PML::RARA*		Favorable	ATRA + DNR	CR	NA
Yamashita et al. (32)	1/M	Mandible	Single	CD45	NA	–	NA/NA	NA	NA	*PML::RARA*	NA		ATRA + anthracycline antitumor agent	CR	>12 m
Piñán et al. (33)	61/F	(T12~L1) extradural space	Multiple	MPO, CD43, CD68, Ki67	NA	NA	–/–	–	Normal	–	NA	NA	Laminectomy, radiotherapy	NA	Progre-ssion to APL after 9 months
Li et al. (34)	44/M 31/F	(Left 3rd, right 4th) costal cartilage; Perianal	Multiple	MPO, MPO, Vim, LCA, CD3, CD5, CD20, Actin, CD2, Kappa, Lambda, S-100, Ki67 (~50%)	– –	– –	23/55; 3/92	NA	NA NA	*PML::RARA* (bcr3) *PML::RARA* (bcr1)	NA NA	Favorable Favorable	ATRA + ATO + THP; ATRA + ATO + THP + AraC	CR CR	>24 m >24 m
Kikuma et al. (35)	52/M	(The 7th thoracic) vertebra	Single	MPO, CD68, Lys	–	–	89.2/NA	–	46,XY,t(5;17) (q35;q12)	*NPM1::RARA*	NA	Favorable	Steroid, radiotherapy, IA + ATRA	CR	NA
Rodriguez et al. (36)	43/F	Appendix	Single	MPO, CD68	–	–	90/NA	NA	t(15;17) (q22;q12)	*PML::RARA*	–	Favorable	Laparoscopic appendectomy, ATRA + DA	CR	NA
Shah et al. (37)	56/M	Extradural space	Multiple	MPO, CD43, CD45, CD68, CD117	–	–	NA/NA	–	46,XY,t(15;17) (q24;q21) [16]/46,XY[5]	*PML::RARA*	FLT3-ITD	Favorable	T5-T9 decompressive laminectomy with fusion and resection of the epidural mass, ATRA + IDA	CR	12 m (and developed relapse periphe-rally)

(Continued)

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TABLE 1 (Continued)

Reference	Age/sex	MS site	Type	Immuno-phenotype	High WBC count	DIC	Blasts/promy-elocyte in BM (%)	Auer rods	Karyo type	Fusion gene	Gene mutation	Risk level	Treat-ment	Response	PFS
de Andrade et al. (38)	24/F	Oral cavity	Single	MPO, CD99, Ki67 (60%)	–	–	NA/NA	NA	t(15;17)	NA	NA	Favorable	ATRA + IA	NA	1 m (and died of hemorrh-age)
Sawhney et al. (39)	52/F	Right humerus	Single	MPO, CD33, CD117, CD71, CD34	–	–	–/–	NA	t(15;17)	*PML::RARA*	NA	Favorable	ATRA + ATO	CR	8 m
Hwang et al. (40)	52/M	Pleural effusion	Multiple	NA	–	–	NA/56.3	+	47,XY, + add(5) (q11.2)x2,der(5;8) (q10;p10),del(7) (q32), t(15;17) (q22;q21)	*PML::RARA*	–	Favorable	ATRA + IDA	Dead	Died of shock and multi-organ failure
Oravcova et al. (41)	34/F	Left breast	multiple	MPO, CD34, Ki67 (60%∼70%)	+	–	3/NA	NA	Normal	*PML::RARA* (bcr3)	FLT3-ITD	Adverse	IDA + ATRA, intrathecal chemotherapy and CNS radiotherapy	CR	5 m and died of CNS failure
Collinge et al. (42)	49/F	Abdominal skin? purulent change	Multiple	MPO, CD68, CD163	+	–	NA/80	+	t(15;17)	*PML::RARA* (bcr1)	NA	Adverse	ATRA + ATO	CR	>6 m
Yamashita et al. (43)	50/M	(L2∼L4) extradural space? right rib? bones throughout body	Multiple	NA	NA	NA	–/NA	+	47,XY,+8, der(11;22) (q10;q10), add(14) (q32), der(15)t(15;17) (q22;q12), ider(17) (q10)t(15;17)*	*PML::RARA**	NA		Before diagnosis of APL: radiotherapy, DA, HDAC, MA After diagnosis of APL: ATRA + DA (induction chemotherapy)? ATRA + ATO, GO + tamibarotene	CR	3 m and died of cerebral hemorrh-age

(Continued)

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TABLE 1 (Continued)

Reference	Age/sex	MS site	Type	Immuno-phenotype	High WBC count	DIC	Blasts/promy-elocyte in BM (%)	Auer rods	Karyo type	Fusion gene	Gene mutation	Risk level	Treat-ment	Response	PFS
Ignacio-Cconchoy et al. (44)	35/M	Tongue	Single	MPO, CD68, CD15, Ki67 (88%)	+		NA/90	+	t(15;17) (q22;q21)	*PML::RARA*	NA	Adverse	ATRA + DNR	CR	NA
Wong et al. (45)	65/M	Heterogeneous allograft kidney	Single	MPO	NA	NA	0.02/NA	+	t(15;17) (q24;q21)	*PML::RARA*	NA	NA	–	–	Dead of cardiac arrest caused by coronary artery stenosis
Wang et al. (46)	77/F	Colon	Single	MPO, CD117, CD68, CK, CgA, Ki67 (65%)	–	–	68/NA	NA	t(15;17) (q22;q21)	*PML::RARA*	NA	Favorable	ATRA + ATO	CR	
Han et al. (47)	67/F	Right obturator internus, obturator externus and some lymph nodes	Multiple	NA	–	–	NA/72	NA	Normal	*CPSF6::RARG*	WT1, KRAS, EZH2	Favorable	ATRA + HA	NR	1 m and died of intracranial hemorrhage
Wang et al. (48)	2/F	Posterior fossa	Single	NA	–	–	NA/74.5	+	46,XX,t(4;17) (q12;q22)[9]/46, idem,del(16) (q22)[3]/45,idem,-x,-4,-9,-15,del(16) (q22), + marl, + mar2, + mar3[7]/46,xx[3]	*FIP1L1::RARA*	KRAS	Adverse	ATRA + DA	CR	5 m

(Continued)

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TABLE 1 (Continued)

Reference	Age/ sex	MS site	Type	Immuno-phenotype	High WBC count	DIC	Blasts/ promy-elocyte in BM (%)	Auer rods	Karyo type	Fusion gene	Gene mutation	Risk level	Treat-ment	Response	PFS
Zhou and Li (49)	40/F	Right ovary (T9∼10) extradural space	Multiple	MPO, CD34, Lys	–	–	–/–	NA	46,XX[20]	*PML::RARA*	NA	Favorable	ATRA, right breast tumor excision, laminectomy	CR	288 m (but with relapse of MS at different sites)
Shu et al.(50)	50/F	(C6∼C7) extradural space	Multiple	MPO, TDT, CD56, CD43, Ki67 (60%)	–	–	–/50	–	t(15;17) (q24;q21)	*PML::RARA*	RUNX1, FLT3, KMT2C gene SNV and InDel	Favorable	Intraspinal tumor resection and spinal Galveston fixation, ATRA + ATO + DNR	CR	>10 m
Cho et al. (51)	56/M	extradural space	Multiple	MPO						*ZBTB16:: RARA*					
Harrer et al. (52)	67/M	Right hemilarynx and skin	Multiple	CD45, MPO	–	–	50/NA	NA	Normal	*PML::RARA*	NA	Favorable	DA? ATRA, DA + ATRA, MA + ATRA	CR	>24 m
Loyaux et al. (53)	38/F	Pleural effusion	Multiple	CD45 dim, CD117, CD33, CD13	–	+	NA/NA	+	t(15;17) (q24;q21)	*PML::RARA* (bcr2) **	–	Favorable	ATRA + ATO	CR	NA (>2 m)
Chen et al. (54)	7/M	(L1) extradural space	Multiple	MPO, CD33	NA	NA	–/–	–	46,XY,dup(17) (q23q25)[15]/45,X,-Y,der(16)t(Y;16) (q12;q22), dup(17) (q23q25)[4]/45,X,-Y,del(4) (p14),der(16) t(Y;16) (q12; q22),dup(17) (q23q25)[1]	*TTMV:: RARA*	WT1, SMAD9	NA	DAE (×6 cycles) + Ara-C (×4 cycles)	CR	6 m (and then MS relapse and BM infiltra-tion)

MS, myeloid sarcoma; WBC, white blood cell; DIC, disseminated intravascular coagulation; BM, bone marrow; PFS, progression-free survival; M, male; F, female; NA, not available; NR, non-remission; CR, complete remission; m, month(s); DA, daunorubicin and cytarabine; ATRA, all-trans-retinoic acid; HSCT, hematopoietic stem cell transplantation; IA or IDA, idarubicin and cytarabine; MA, melphalan and adriamycin; 6-MP, 6-mercaptopurine; MTX, methotrexate; DNR, daunorubicin; THP, pirarubicin; ATO, arsenic trioxide; HDAC, high-dose cytarabine; APL, acute promyelocytic leukemia; GO, gemtuzumab ozogamicin; HA, homoharringtonine and cytarabine; DAE, dexamethasone, cytarabine and etoposide. *Detected at second relapse of MS, not at initial diagnosis. **The fusion gene was detected only in pleural fluid and was negative in both blood and bone marrow.

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promyelocytic sarcoma. When a mass is detected in any part of the body, fine needle aspiration often fails to provide sufficient diagnostic evidence of myeloid malignancy. In cases without coagulation abnormalities or other contraindications to surgery, a local pathological biopsy followed by immunohistochemical examination of the mass is essential to determine its origin. For suspected myeloid-origin tumors, it is critical to perform a bone marrow aspirate to rule out APL or other forms of non-M3 AML. Even when blood and bone marrow smears and flow cytometry do not show abnormalities, molecular testing is crucial. Both qPCR and FISH should be performed to detect *PML::RARA* fusion gene positivity. Although molecular analysis and FISH of MS biopsy tissue can be technically challenging, they are important for accurate diagnosis and should be performed whenever possible. For patients without atypical promyelocytes in the peripheral blood and bone marrow, and with no cytogenetic abnormalities, the detection of *PML::RARA* transcripts or *RARA* rearrangements in MS tissues via qPCR or FISH becomes the key diagnostic criterion. Additionally, karyotype analysis of the bone marrow, showing translocations involving chromosomes 15 and 17, can further strengthen diagnostic confidence in cases of solitary MS. Thus, the presence of *PML::RARA* is considered a critical marker for both the early diagnosis of solitary promyelocytic sarcoma and the monitoring of treatment efficacy and recurrence. An intriguing observation in some cases is the identification of rare fusion genes, although their association with MS/APL remains unclear. This highlights the need for further research to understand the significance of these rare fusions. Moreover, the absence of *PML::RARA* does not reliably exclude APL, emphasizing the importance of comprehensive testing. Next-generation sequencing and RT-PCR for other rare fusion transcripts could reveal unexpected findings, potentially offering new insights into MS/APL diagnostics.

This case is similar to previously reported MS/APL with a spinal intradural mass as the first manifestation, and the patient usually presents with low back pain and difficulty walking. These symptoms may occur with or without abnormal blood counts and coagulation. In this case, induction chemotherapy with ATRA combined with ATO was initiated after local lumpectomy. ATRA treatment continued to maintain *PML::RARA* negativity, followed by local radiotherapy. The patient achieved remission and maintained a good quality of life.

Patients with MS/APL, particularly those with spinal intraspinal masses at onset, often have a poor prognosis, highlighting the need for effective treatment strategies to improve outcomes. Treatment for these cases is similar to that for extramedullary relapses of APL, involving surgical decompression, local radiotherapy, and leukemia chemotherapy. Surgical resection is essential for reducing tumor volume, alleviating tissue compression, and preventing further spread. If coagulopathy is not significant, surgery should be performed promptly to relieve pain and improve mobility. Systemic therapy for the underlying leukemia is always necessary, regardless of bone marrow involvement or isolated MS/APL (56). ATRA, while effective, poorly penetrates the blood-brain barrier and is associated with relapses in the central nervous system (CNS) (57). Additionally, ATRA has been shown to increase tumor cell adhesion molecule expression (58–60), which could promote extramedullary metastasis and invasion. However, a higher incidence of extramedullary recurrence has not been observed in APL patients receiving ATRA compared to those treated with chemotherapy alone, though CNS recurrence is slightly more common, yet not statistically significant (55). Real-world data indicate that two-drug induction therapy combining ATRA and ATO offers longer disease-free survival compared to ATRA combined with chemotherapy (AIDA) (61–63). Thus, the combination of ATRA and ATO is recommended for treating *PML::RARA*-positive MS/APL. For rare *RARA* rearrangements, the specific fusion partners should be considered to determine whether ATRA is appropriate. The role of radiotherapy in treating APL-related extramedullary sarcoma remains debated. Some researchers view it as an effective strategy for eliminating residual tumor tissue and reducing recurrence risk after surgery (64, 65). However, others caution that local radiotherapy may increase the patient's overall burden, leading to infections, treatment failure, or delays in chemotherapy (66). In some cases, patients intolerant to therapy have died from severe infections unrelated to chemotherapy. Furthermore, the potential for bone marrow infiltration by leukemic cells following radiotherapy, either from disease progression or radiotherapy-induced malignancy, remains a contentious issue. Given these considerations, we propose that a combination of ATRA and ATO be considered the optimal approach for treating *PML::RARA*-positive MS/APL. Local radiotherapy could be administered after consolidation therapy, weighing its potential benefits against its risks. New studies have explored the use of gilteritinib for extramedullary recurrence of APL with FLT3 mutations, showing rapid and sustained regression of the sarcoma (67). For patients with isolated MS/APL at initial diagnosis, whether targeted agents can improve remission and disease-free survival in the presence of specific gene mutations warrants further investigation. Additionally, hyperthermia, which shows synergistic effects with ATO in destabilizing *PML::RARA* fusion proteins both *in vivo* and *in vitro*, may offer a promising new therapeutic strategy (68).

Conclusion

In conclusion, we describe the rare presentation of APL solely as MS in a patient, which ultimately led to the diagnosis of MS/APL. Additionally, we provide a comprehensive review of similar cases to further elucidate this uncommon clinical manifestation of APL. The case and literature review contribute to the growing body of knowledge regarding the presentation, diagnosis, and treatment of MS/APL, potentially guiding future clinical practice in similar cases.

Author contributions

YD: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. KY: Supervision, Writing – review & editing. YL: Data curation, Investigation, Supervision, Writing – review & editing. YZ: Supervision, Visualization, Writing – review & editing. YG: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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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.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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Risk factors and clinical outcomes of cytomegalovirus infection following haploidentical hematopoietic stem cell transplantation in patients with aplastic anemia: a single-center retrospective study

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Background: Cytomegalovirus (CMV) infection remains a critical cause of mortality after allogeneic hematopoietic stem cell transplantation, despite significant advancements in CMV prevention and treatment with the introduction and widespread use of letermovir. However, in China, due to limitations in the availability and cost of medications, some patients still face challenges in accessing letermovir. For this subset of the population, exploring the risk factors for CMV infection remains significant in predicting its occurrence.

Methods: Therefore, a retrospective analysis was conducted on 88 haploidentical hematopoietic stem cell transplant recipients over 4 years.

Results: Our study results indicate that chronic graft-versus-host disease (cGVHD) is an independent risk factor for CMV infection following haploidentical hematopoietic stem cell transplantation (Haplo-HSCT). Survival analysis reveals lower survival rates in the refractory CMV infection (RCI) group compared to the non-RCI group, with patients having lower viral loads demonstrating higher rates of seroconversion and improved survival under the same treatment regimen.

Conclusion: Strengthening the monitoring of CMV-DNA in post-transplant patients, actively promoting hematopoietic recovery, preventing the occurrence of CMV infection, and controlling the development of CMV infection can lead to better survival outcomes for patients with aplastic anemia undergoing Haplo-HSCT.

KEYWORDS

cytomegalovirus infection, aplastic anemia, haploidentical hematopoietic stem cell transplantation, chronic graft-versus-host disease, immunology

1 Introduction

Cytomegalovirus (CMV) is a common and crucial viral infection following allogeneic hematopoietic stem cell transplantation (allo-HSCT) (1). Depending on the type of transplantation and geographical region, the incidence of CMV infection ranges from approximately 40%–70% (2, 3), significantly impacting both the survival and quality of life of affected patients. Despite significant advancements in CMV prevention and treatment with the introduction and widespread use of letermovir, in China, some patients still face challenges in accessing letermovir due to limitations in the availability and cost of medications. For these individuals, CMV infection remains associated with an increased risk of mortality (4), particularly in the case of refractory CMV infection (RCI) (4, 5), which can result in a mortality rate exceeding 80% (6), with CMV pneumonia being the most lethal manifestation. Additionally, the Chinese population resides in a high-risk zone for CMV infection, with an adult CMV serum positivity rate ranging from 80% to 93.7% (7, 8). This significantly increases the likelihood of CMV infection (9). For this subset of the population, exploring the risk factors for CMV infection remains meaningful in predicting its occurrence.

In the current study, we conducted a retrospective analysis of clinical data from 88 patients with aplastic anemia (AA) who underwent haploidentical hematopoietic stem cell transplantation (Haplo-HSCT). The aim was to investigate the incidence of CMV infection and its associated risk factors in haploidentical transplant recipients.

2 Materials and methods

2.1 Patients

In this study, we retrospectively analyzed the clinical data of 88 patients diagnosed with AA who underwent Haplo-HSCT at the Department of Hematology, Zhejiang Provincial Hospital of Traditional Chinese Medicine, from September 2018 to November 2022. The patients were actively followed up until July 2023. Among the 88 patients (44 males, 44 females), with a median age of 32 years (range: 9–55), there were 70 cases of severe aplastic anemia (SAA) and 18 cases of non-severe aplastic anemia (NSAA), all meeting the diagnostic criteria for AA (10). This study has been approved by the Institutional Review Board of the hospital. The baseline characteristics of the patients are presented in Table 1.

All patients underwent myeloablative conditioning, with 22 patients receiving the BUCY (busulfan/cyclophosphamide) conditioning regimen, and 66 patients undergoing the FCA (fludarabine/cyclophosphamide/antithymocyte globulin) conditioning regimen. A combination of antithymocyte globulin (ATG), mycophenolate mofetil (MMF), cyclosporine (CSA), and short-term methotrexate (MTX) was employed for graft-versus-host disease (GVHD) prophylaxis in all patients. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed according to standard references (11–13). For patients experiencing aGVHD, immediate first-line treatment involved administering methylprednisolone at a dose of 1–2 $mg \cdot kg^{1} \cdot d^{1}$. In cases where methylprednisolone was ineffective or dependency occurred, second-line therapies such as ruxolitinib, anti-CD25 monoclonal antibodies, MMF, among others, were administered. The primary treatment for cGVHD involved the use of methylprednisolone and/or CSA as the first-line approach.

Follow-up for all 88 patients was conducted through methods such as phone interviews and hospital registration systems, with the follow-up deadline set at July 2023. Neutrophil engraftment was defined as a consecutive 3-day absolute neutrophil count (ANC) $> 0.5 \times 10^9$/L, while platelet engraftment was defined as a consecutive 7-day platelet count (PLT) $> 20 \times 10^9$/L without requiring platelet transfusions. Primary graft failure was defined according to established literature (14). Overall survival (OS) time post-transplantation was defined as the period from transplantation to either patient death or the last follow-up date.

2.2 CMV monitoring, definitions, and antiviral therapy

According to our internal standards, blood CMV-DNA positivity is defined as a quantitative PCR result with a CMV viral load $> 1 \times 10^2$ copies/ml (15). CMV viremia is defined as two consecutive CMV-DNA tests showing levels exceeding 500 copies/ml, or a single CMV-DNA test result exceeding 1,000 copies/ml (16). In this study, the occurrence of viremia in patients was considered a confirmed CMV infection. RCI is defined as a situation where, after receiving reasonable anti-CMV treatment for 2 weeks, the CMV viral load remains unchanged or increases (17, 18). The definition of CMV-related diseases follows the literature reference (15), while the definitions of Epstein-Barr virus (EBV) viremia and related diseases adhere to the literature reference (19). Prophylaxis for CMV infection with ganciclovir was administered from day 9 to 2 during the pre-transplant period, and acyclovir prophylaxis for herpes virus infection was given from day 1 to 1-year post-transplant.

All patients underwent quantitative PCR monitoring of peripheral blood CMV-DNA and EBV-DNA twice a week from the initiation of pre-transplant conditioning until day +90. From day +90 onward, monitoring was conducted every 1–2 weeks until day +180. After day +180, in the presence of symptoms suggestive of a possible viral infection, simultaneous retesting of CMV-DNA and EBV-DNA was performed. If positivity occurred during this period, the monitoring frequency increased to twice a week until viral clearance. The first-line treatment options for CMV infection included either ganciclovir or sodium phosphonoformate. For RCI, drugs not used in the first-line regimen were selected for monotherapy or combination therapy. Once CMV-DNA became negative for two consecutive tests, acyclovir was administered orally for prophylaxis.

2.3 Statistical analyses

Inter-group continuous variables were subjected to two-tailed *t*-tests or Kruskal–Wallis tests, while categorical variables were analyzed using Chi-square tests or Fisher's exact tests. Logistic

TABLE 1 Patient and transplant characteristics according to post-transplant CMV infection.

Factors	*N* (%)	No CMV infection (*n*, %)	CMV infection (*n*, %)	Statistics	*p*-Value
Total	88 (100)	52 (59.1)	36 (40.9)		-
Patient age at transplantation					
≤40 years	65 (73.9)	25 (69.4)	40 (76.9)	$\chi^2 = 0.616$	0.432
40 years	23 (26.1)	11 (30.6)	12 (23.1)		
Sex					
Male	44 (50.0)	16 (44.4)	28 (53.8)	$\chi^2 = 0.752$	0.386
Female	44 (50.0)	20 (55.6)	24 (46.2)		
Donor sex					
Male	50 (56.8)	20 (55.6)	30 (57.7)	$\chi^2 = 0.040$	0.842
Female	38 (43.2)	16 (44.4)	22 (42.3)		
Donor/recipient sex combination					
Female to male	18 (20.5)	5 (13.9)	13 (25.0)	$\chi^2 = 1.614$	0.204
Others	70 (79.5)	31 (86.1)	39 (75.0)		
Diagnosis					
NSAA	18 (20.5)	7 (19.4)	11 (21.2)	$\chi^2 = 0.038$	0.845
SAA	70 (79.5)	29 (80.6)	41 (78.8)		
The blood type of the donor and the recipient					
Incompatible	34 (38.6)	12 (33.3)	22 (42.3)	$\chi^2 = 0.723$	0.395
Compatible	54 (61.4)	24 (66.7)	30 (57.7)		
Stem cell source					
PBSCs	10 (11.4)	5 (13.9)	5 (9.6)	$\chi^2 = 0.386$	0.535
PBSCs + BM	78 (88.6)	31 (86.1)	47 (90.4)		
Conditioning regimen					
Bu + Cy	22 (25.0)	7 (19.4)	15 (28.8)	$\chi^2 = 1.003$	0.317
Flu + Cy + ATG	66 (75.0)	29 (80.6)	37 (71.2)		
UC-BSC assisted reinfusion					
No	40 (45.5)	18 (50.0)	22 (42.3)	$\chi^2 = 0.508$	0.476
Yes	48 (54.5)	18 (50.0)	30 (57.7)		
MSC assisted reinfusion					
No	38 (43.2)	15 (41.7)	23 (44.2)	$\chi^2 = 0.057$	0.811
Yes	50 (56.8)	21 (58.3)	29 (55.8)		
NE 28-day engraftment					
No	6 (6.8)	5 (13.9)	1 (1.9)	$\chi^2 = 4.740$	0.004
Yes	82 (93.2)	31 (86.1)	51 (98.1)		
PLT 28-day engraftment					
No	22 (25.0)	7 (19.4)	15 (28.8)	$\chi^2 = 1.003$	0.317
Yes	66 (75.0)	29 (80.6)	37 (71.2)		
aGVHD					
No	48 (54.5)	21 (58.3)	27 (51.9)	$\chi^2 = 0.353$	0.553
Yes	40 (45.5)	15 (41.7)	25 (48.1)		
cGVHD					
No	62 (70.5)	31 (86.1)	31 (59.6)	$\chi^2 = 7.174$	0.007
Yes	26 (29.5)	5 (13.9)	21 (40.4)		
EBV infection					
No	33 (37.5)	18 (50.0)	15 (28.8)	$\chi^2 = 4.062$	0.044
Yes	55 (62.5)	18 (50.0)	37 (71.2)		

SAA, severe aplastic anemia; NSAA, non-severe aplastic anemia; Bu, busulfan; Cy, cyclophosphamide; FCA, fludarabine, cyclophosphamide, antithymocyte globulin; NE, neutrophils; PLT, platelet; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host; EBV, Epstein-Barr virus.

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TABLE 2 Analysis of risk factors associated with CMV infection after allogeneic hematopoietic stem cell transplantation.

Factors	CMV infection (*n*, %)	Univariate analysis		Multivariate analysis	
		p-Value	OR value (95% CI)	*p*-Value	OR value (95% CI)
Patient age at transplantation					
≤40 years	40 (76.9)	0.432	0.682 (0.261~1.778)	–	–
40 years	12 (23.1)				
Sex					
Male	28 (53.8)	0.386	0.686 (0.292~1.611)	–	–
Female	24 (46.2)				
Donor sex					
Male	30 (57.7)	0.842	0.917 (0.389~2.160)	–	–
Female	22 (42.3)				
Donor/recipient sex combination					
Female to male	13 (25.0)	0.204	0.484 (0.156~1.504)	–	–
Others	39 (75.0)				
Diagnosis					
NSAA	11 (21.2)	0.845	0.900 (0.312~2.598)	–	–
SAA	41 (78.8)				
The blood type of the donor and the recipient					
Incompatible	22 (42.3)	0.395	0.682 (0.281~1.652)	–	–
Compatible	30 (57.7)				
Stem cell source					
PBSCs	5 (9.6)	0.535	1.516 (0.405~5.675)	–	–
PBSCs + BM	47 (90.4)				
Conditioning regimen					
Bu + Cy	15 (28.8)	0.317	0.595 (0.215~1.652)	–	–
Flu + Cy + ATG	37 (71.2)				
UC-BSC assisted reinfusion					
No	22 (42.3)	0.476	1.364 (0.580~3.204)	–	–
Yes	30 (57.7)				
MSC assisted reinfusion					
No	23 (44.2)	0.811	0.901 (0.381~2.127)	–	–
Yes	29 (55.8)				
NE 28-day engraftment					
No	1 (1.9)	0.004	8.226 (0.918~73.716)	0.169	4.831 (0.513~45.498)
Yes	51 (98.1)				
PLT 28-day engraftment					
No	15 (28.8)	0.317	0.595 (0.215~1.652)	–	–
Yes	37 (71.2)				
aGVHD					
No	27 (51.9)	0.553	1.296 (0.550~3.055)	–	–
Yes	25 (48.1)				
cGVHD					
No	31 (59.6)	0.007	4.200 (1.405~12.555)	0.043	3.244 (1.035~10.042)
Yes	21 (40.4)				
EBV infection					
No	15 (28.8)	0.044	2.467 (1.0126~5.989)	0.341	1.597 (0.609~4.185)
Yes	37 (71.2)				

SAA, severe aplastic anemia; NSAA, non-severe aplastic anemia; Bu, busulfan; Cy, cyclophosphamide; FCA, fludarabine, cyclophosphamide, antithymocyte globulin; NE, neutrophils; PLT, platelet; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host; EBV, Epstein-Barr virus; CMV, cytomegalovirus; –, multivariate analyses were not included.

TABLE 3 Univariate and multivariate analysis of factors influencing overall survival after allogeneic hematopoietic stem cell transplantation.

Factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age	3.280	1.148∼9.371	0.017	1.273	0.359∼4.520	0.709
Sex	0.975	0.342∼2.781	0.962	–	–	–
Donor sex	0.337	0.094∼1.207	0.075	–	–	–
Donor/recipient sex combination	3.575	0.468∼27.335	0.183	–	–	–
Diagnosis	0.934	0.260∼3.347	0.915	–	–	–
The blood type of the donor and the recipient	1.144	0.383∼3.414	0.806	–	–	–
Stem cell source	0.459	0.128∼1.645	0.213	–	–	–
Conditioning regimen	1.214	0.339∼4.351	0.762	–	–	–
UC-BSC assisted reinfusion	0.591	0.205∼1.703	0.317	–	–	–
MSC assisted reinfusion	0.277	0.087∼0.885	0.019	0.320	0.092∼1.116	0.074
NE 28-day engraftment	0.194	0.054∼0.703	0.005	1.101	0.251∼4.821	0.899
PLT 28-day engraftment	0.105	0.033∼0.336	0.000	0.132	0.036∼0.481	0.002
aGVHD	1.185	0.416∼3.379	0.747	–	–	–
cGVHD	0.602	0.168∼2.159	0.424	–	–	–
EBV infection	0.290	0.097∼0.867	0.017	1.393	0.345∼5.626	0.642
CMV infection	1.203	0.403∼3.590	0.736	–	–	–

SAA, severe aplastic anemia; NSAA, non-severe aplastic anemia; Bu, busulfan; Cy, cyclophosphamide; FCA, fludarabine, cyclophosphamide, antithymocyte globulin; NE, neutrophils; PLT, platelet; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host; EBV, Epstein-Barr virus; CMV, cytomegalovirus; –, multivariate analyses were not included.

regression models for binary variables were employed for both univariate and multivariate analyses, with the latter incorporating all factors from the univariate analysis with a p-value < 0.10. The cumulative incidence of CMV infection was computed using a competing risk model. Kaplan–Meier methodology was employed to determine the probability of OS, and comparisons were made using the log-rank test. A p-value < 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS 26.0 software, and graphical representations were created using GraphPad (Supplementary Table 1).

3 Results

3.1 Patient clinical characteristics and hematopoietic recovery

This study included a total of 88 patients with AA who underwent haploidentical transplantation, comprising 44 males and 44 females. The median age at the time of transplantation was 32 years (range: 9–55). Disease classification was as follows: NSAA in 18 cases, SAA in 65 cases, and very severe aplastic anemia (VSAA) in 5 cases. The median time for neutrophil engraftment in 85 patients was 12 days (range: 9–48), with 82 achieving engraftment within 28 days. For platelet engraftment, the median time for 81 patients was 16 days (range: 7–92), with 15 achieving engraftment within 28 days. Ultimately, hematopoietic recovery was achieved in 81 patients, while the remaining 7 patients experienced graft failure, adverse events, or early mortality (Table 1).

3.2 Overview of CMV infection, treatment, and outcome

Before transplantation, both donor and recipient CMV-DNA quantification levels were below the detection range ($<1 \times 10^2$ copies/ml). Among the patients, 70 were CMV-IgG positive, and the remaining 18 were not assessed. By the end of the follow-up period, CMV infection occurred in 52 out of the 88 patients (59.1%). The median time to the first occurrence of CMV infection was 36.5 days (range: 11–189).

After the first-line treatment, 40 patients (76.9%) achieved CMV-DNA negativity, while the remaining patients experienced RCI. Among the 12 RCI patients, 5 (41.7%) achieved viral clearance after receiving second-line treatment, while 6 died with persistent CMV-DNA positivity. The overall rate of viral clearance after CMV infection treatment was 86.5% (45/52).

Among CMV-infected patients, there were 29 cases in the group with the highest viral load below 1×10^4 copies/ml, and the clearance rate was 96.6% (28/29). In the group with a viral load exceeding 1×10^4 copies/ml, there were 23 cases, and the clearance rate was 73.9% (17/23). The difference in clearance outcomes between the two groups was statistically significant ($p = 0.035$).

3.3 Risk factors for CMV infection

The results are shown in Table 2. Univariate analysis indicated that neutrophil engraftment beyond 28 days ($p = 0.004$), cGVHD ($p = 0.007$), and EBV infection ($p = 0.044$) were clinical risk factors for CMV infection in AA patients undergoing Haplo-HSCT. Multivariate analysis further identified cGVHD ($p = 0.043$) as an

independent risk factor for the occurrence of CMV infection after Haplo-HSCT.

3.4 Overall prognosis and survival analysis of patients with CMV infection

Until the follow-up endpoint, a total of 14 patients had died, with the specific causes as follows: 4 died from sepsis, 4 from severe pneumonia, 1 from cerebrovascular accident, 2 from aGVHD, 2 from post-transplant lymphoproliferative disorder (PTLD), and 1 from acute heart failure. The 4-year OS rate for all 88 patients was 84.1% (Figure 1). The survival rate for the non-CMV infection group was 86.1% (31/36), and for the CMV infection group, it was 82.7% (43/52), with no statistically significant difference in survival time between the two groups ($p = 0.736$) (Figure 2).

Among the 52 patients with CMV infection, the OS rate was 50% (6/12) in the RCI group and 92.5% (37/40) in the non-RCI group, with a statistically significant difference in survival outcomes between the two groups ($p = 0.000$). For the group with the highest viral load above 1.0×10^4 copies/ml, the survival rate was 73.9%, while for the group with a load below 1.0×10^4 copies/ml, the survival rate was 89.5%, with no statistically significant difference in survival time between the two groups ($p = 0.130$) (Figure 3).

3.5 Analysis of factors influencing survival

A univariate and multivariate Cox regression analysis was conducted to explore potential factors influencing the survival time of patients post-transplantation. The results are presented in Table 3. Univariate analysis indicated that recipient age >40 years ($p = 0.017$), unassisted infusion of mesenchymal stem cells ($p = 0.019$), neutrophil engraftment beyond 28 days ($p = 0.005$), platelet engraftment beyond 28 days ($p = 0.000$), and non-EBV infection ($p = 0.017$) were risk factors affecting patient survival. Multivariate Cox regression analysis identified platelet engraftment beyond 28 days (HR = 0.132, 95% CI 0.036∼0.481, $p = 0.002$) as an independent risk factor influencing the survival time of patients.

4 Discussion

Following the initial infection, CMV establishes a lifelong latent infection in the host under the control of the immune response. Reactivation of CMV is a common event in recipients of allo-HSCT. In this study, 59.1% (52/88) of patients experienced CMV infection during the postoperative follow-up period, with 23.1% (12/52) of CMV-infected patients developing RCI. The incidence of CMV infection and RCI in this study is similar to previous reports (4, 5). Without the use of letermovir, exploring risk factors for CMV infection may provide new insights into treatment strategies.

In previous studies, recipient seropositive status, graft source, transplantation type, HLA compatibility, and GVHD have been identified as common risk factors for CMV infection (20–22). We observed that among the 26 patients who developed cGVHD, there was an 81% incidence of CMV infection. This observation leads us

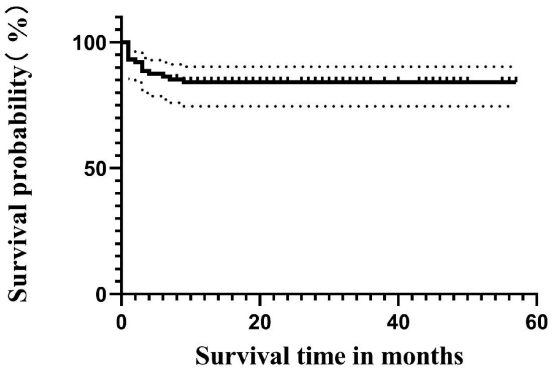

FIGURE 1
Survival curves up to 48 months.

to infer that cGVHD is a significant risk factor for CMV infection, and our study confirms this hypothesis. Our data indicate that cGVHD is an independent risk factor for CMV infection. This finding is not entirely consistent with previous research results (22). On the one hand, there may be a reciprocal interaction between CMV virus and cGVHD. This could be related to the type of disease, as the CMV virus is more likely to infect when T cells are deficient or impaired. In the case of AA transplantation, long-term use of immunosuppressive agents is required, leading to a slower immune reconstitution compared to other hematological malignancies after transplantation, thus providing opportunities for extended periods of immune reconstitution recovery, which may increase the risk of infection. On the other hand, the EBV may contribute to CMV infection by influencing aGVHD and cGVHD.

The main pathophysiological process of cGVHD is immune-mediated inflammatory response. Chronic inflammation causes thymus damage and B cell and T cell immune disorder, which eventually leads to tissue fibrosis. T lymphocytes can cause tissue damage and fibrosis through direct cytolysis and cytokine secretion (23, 24), especially CD4+ T lymphocytes, whose interaction with B cells promotes B cell differentiation and the production of autoantibodies. These include antibodies against cytoskeletal intermediate filaments, cytoplasmic squamous epithelial cells, and nucleolar B23, These antibodies participate in inflammation and activate signal transduction pathways, leading to increased expression of type I collagen genes, promoting fibroblast activation, and inducing typical cGVHD clinical symptoms such as skin sclerosis and pulmonary fibrosis. In addition, T cell subsets play a crucial role in the immune regulation of cGVHD. Activation of the NOTCH2 signaling pathway in B cells has a profound effect on T cell subsets, including helper T cells (Th) and regulatory T cells (Treg) (25–27). This results in delayed immune reconstitution after allo-HSCT, increased risk of death and cGVHD, and increased risk of CMV reactivation. In addition, since patients with AA use immunosuppressants longer after transplantation than those with other hematological malignancies, it is more likely to cause delayed immune reconstitution after transplantation.

There is limited research on the correlation between cGVHD and CMV infection, but the relationship between cGVHD and CMV infection is not absent. Olkinuora et al. (28) found that mild aGVHD and cGVHD can promote the recovery of cellular

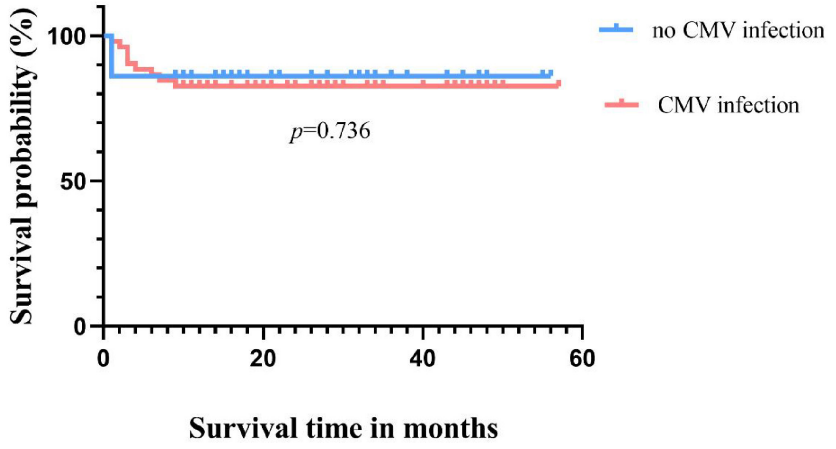

FIGURE 2
Overall survival with and without CMV infection.

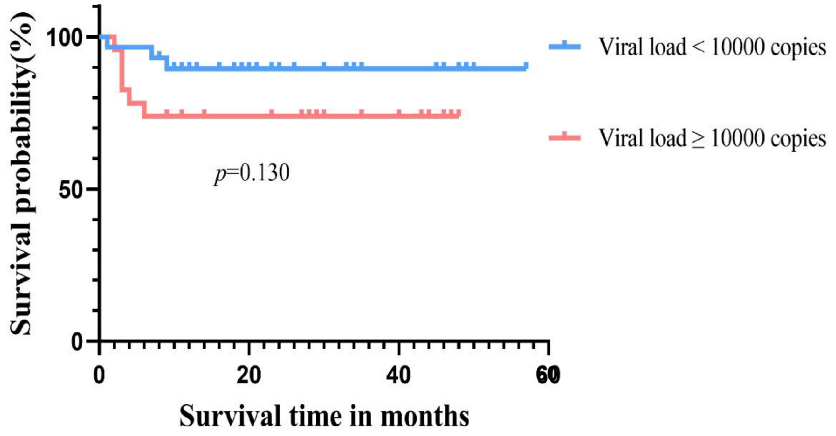

FIGURE 3
Overall survival between with low viral load group and the high viral load group.

and humoral immunity, while moderate to severe cGVHD hurts immune recovery after transplantation. Furthermore, active CMV infection can contribute to the occurrence and exacerbation of cGVHD by increasing levels of IL-2 and tumor necrosis factor-alpha in peripheral blood (29).

Previous research results indicate that the influence of CMV infection on aGVHD is affirmative (30, 31). The study by Styczynski (32) indicates that the incidence of CMV infection in patients with aGVHD is almost twice that of patients without aGVHD [$p < 0.0001$, 60.1% (885/1,472) vs. 32.1% (892/2,780)]. Cantoni et al. (33) found that GVHD and its treatment can induce CMV replication, and CMV replication increases the risk of GVHD occurrence (34, 35). It is noteworthy that in our study, there was no significant difference in the incidence of CMV infection between patients with and without aGVHD.

As is well-known, EBV, as one of the common viral infections after allo-HSCT, is also a routine monitoring indicator. Previous studies have suggested that EBV infection increases the incidence of II–IV degree aGVHD and cGVHD (34). Since CMV infection is influenced by aGVHD, EBV infection may indirectly affect CMV infection by influencing post-transplant immune reconstitution. There is a complex interrelationship between CMV infection, EBV infection, and the occurrence of GVHD. However, current research on the impact of EBV infection on CMV infection is limited, and the relationship among these three factors is not yet clear. Interestingly, in this study, univariate analysis found an association between the occurrence of CMV infection and cGVHD as well as EBV infection, which warrants further in-depth investigation.

Cytomegalovirus infection has a significant impact on the prognosis of patients, especially with a higher mortality rate in CMV disease and RCI, significantly affecting patient survival (36, 37). In our study, although there was no significant difference in survival rates between CMV-infected and uninfected patients (82.7% vs. 86.1%, $p = 0.736$), the occurrence of RCI was associated with shorter OS compared to the non-RCI group (50% vs. 96.6%, $p = 0.000$), consistent with previous reports (4, 5). The direct and indirect effects of CMV in this study may negatively influence patient prognosis in different ways. On the other hand, consistent with Green et al., a higher CMV viral load after transplantation

was associated with an increased risk of death (adjusted hazard ratio [HR] 19.8, 95% CI 9.6–41.1) (38). However, in our cohort, the peak viral load of CMV reactivation in transplant recipients was used as a qualitative parameter. The CMV-infected patients were divided into two groups based on the highest viral load, with a threshold of 1.0×10^4 copies/ml. Regarding the final survival rate, no significant difference was observed between the low viral load group and the high viral load group (89.5% vs. 73.9%, $p = 0.130$). This may be influenced by sample size and other factors. However, under the same treatment, the low viral load group had a higher rate of turning negative compared to the high viral load group (96.6% vs. 73.9%, $p = 0.035$). This suggests that patients with lower viral loads are more likely to turn negative and have a higher survival rate under the same treatment regimen, thereby improving the prognosis. This also emphasizes the importance of closely monitoring CMV, and with the advent and clinical application of letermovir (39). These patients may benefit from letermovir. Therefore, early intervention, especially after discontinuing prophylaxis, may be considered if necessary. On the other hand, the quantitative definition of pre-transplant CMV serostatus, rather than qualitative, influences the 3-year survival rate after allo-HSCT. This provides new insights into the negative prognostic impact of CMV on transplant recipients (35). However, the lack of pre-transplant serostatus in some patients in this cohort is a limitation of this study. The CMV seropositivity rate of Chinese HSCT patients is as high as 80%–93.7%, which is much higher than that in European and American countries. Therefore, although some patients in this study lack serological status, we can still speculate that they are at risk of CMV reactivation.

Furthermore, we attempted survival analysis, indicating that CMV infection was not statistically significant. Factors such as hematopoietic reconstruction and age may influence patient OS, and failure of platelet engraftment within 28 days ($p = 0.002$) emerged as an independent risk factor affecting patient OS. This differs from previous studies reporting CMV infection as an independent prognostic factor. It is considered that this discrepancy may be due to the combined influence of other factors, and further studies with an expanded sample size are needed to validate these findings.

5 Conclusion

In summary, further emphasis on monitoring CMV-DNA in transplant recipients is warranted, particularly in patients developing cGVHD, necessitating proactive prevention of CMV infection. High viral load patients should receive more aggressive treatment to prevent RCI occurrence, early combination therapy when necessary. Once CMV infection progresses to RCI, the prognosis is poor. Actively promoting hematopoietic reconstruction, preventing the occurrence of CMV infection, and controlling the development of CMV infection can lead to improved survival in AA patients undergoing Haplo-HSCT. In addition, we should pay close attention to the level of T lymphocyte subsets to evaluate cellular immune reconstitution, and rationally adjust immunosuppressants to further reduce CMV reactivation. For patients who use letermovir to prevent CMV infection, we can also further study its effect on the level of T lymphocyte subsets and cellular immune reconstitution.

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 humans were approved by Ethics Committee of the First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

JF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. XZ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. ZT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. YZ: Resources, Supervision, Validation, Writing – review & editing. HH: Resources, Supervision, Validation, Writing – review & editing. JC: Resources, Supervision, Validation, Writing – review & editing. LW: Resources, Supervision, Validation, Writing – review & editing. QY: Resources, Supervision, Validation, Writing – review & editing. DW: Resources, Supervision, Validation, Writing – review & editing. BY: Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. WL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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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.

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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.2024.1523909/full#supplementary-material

The supplementary material is the original data table.

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Dose determination of VV116 in COVID-19 patients with severe liver dysfunction: a case report

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VV116 is an oral antiviral drug against SARS-CoV-2, known for its favorable efficacy and safety profile. But its application in patients with severe liver dysfunction has not been evaluated. Here, we report a case in which a patient with aplastic anemia and liver impairment (recovery phase of acute liver failure) was infected with SARS-CoV-2. Based on clinical trials and pharmacokinetic analysis about VV116, we initiated a reduced dose of 300 mg every 12 h on day 1, 200 mg every 12 h on days 2–5 for antiviral therapy. Finally, the patient's viral load rapidly dropped to an undetected level, and no drug-related adverse effects were observed.

KEYWORDS

COVID-19, VV116, liver dysfunction, dosage adjustment, SARS-CoV-2

1 Introduction

Liver dysfunction is prevalent in COVID-19 patients, affecting approximately 50% of infected individuals (1, 2). This prevalence can be attributed to two main factors. On one hand, liver diseases are widespread, affecting over 300 million people in China alone (3); and liver dysfunction is a risk factor of COVID-19 infection and disease progression (4, 5). On the other hand, SARS-CoV-2 infection itself has certain impairment on liver function (6). However, antiviral drugs suitable for COVID-19 patients with liver dysfunction are limited.

Currently, the treatment regimens for COVID-19 mainly include two classes, neutralizing antibodies hindering viral entry by targeting the Spike protein and small-molecule antivirals suppressing the replication of SARS-CoV-2 by targeting the conserved RNA-dependent RNA polymerase (RdRp) or main protease (Mpro). The usage of neutralizing antibody is limited due to the inconvenient administration route and drug resistance to the emerging SARS-CoV-2 subvariants (such as the XBB lineage) (7, 8). The approved or authorized small-molecule antivirals include nirmatrelvir-ritonavir (Paxlovid), remdesivir, molnupiravir, deuremidevir hydrobromide (VV116), etc. Among these small-molecule drugs, Paxlovid ranks first in reducing mortality and hospitalization, and VV116 ranks first in safety outcomes from a network meta-analysis (9). Paxlovid is a co-packaged combination agent consisting of nirmatrelvir and ritonavir, among which nirmatrelvir is

TABLE 1 Laboratory test data of the patient on admission.

Laboratory parameters	Value	Units	Reference ranges
White blood cell count	0.22	10^9/L	3.50–9.50
Absolute neutrophil count	0.01	10^9/L	
Red blood cell count	2.13	10^{12}/L	4.30–5.80
Platelet count	21	10^9/L	125–350
Hemoglobin	64	g/L	130–175
Reticulocyte count	0.4	10^9/L	24.0–84.0
Alanine transaminase	129.0	IU/L	0.0–50.0
Aspartate transaminase	43.0	IU/L	17.0–59.0
Gamma-glutamyl transferase	155.0	IU/L	15.0–73.0
Alkaline phosphatase	90.8	IU/L	38.0–126.0
Total bilirubin	204.9	μmol/L	3.0–22.0

the inhibitor of Mpro and ritonavir is a pharmacologic booster of nirmatrelvir (10). The main concern of Paxlovid in clinic use is the multiple drug-drug interactions, mainly owing to that ritonavir is a strong CYP3A4 inhibitor (11, 12). Additionally, ritonavir has potential hepatotoxicity (13). VV116 is a deuterated remdesivir hydrobromide showing potent anti-SARS-CoV-2 activity by inhibiting RdRp (14, 15). VV116 exhibits no mutagenicity compared with molnupiravir and has fewer drug-drug interactions compared with Paxlovid (16). Moreover, VV116 shows favorable pharmacokinetics properties conferred by its deuteration modification (17).

Here, we present a case of patient with aplastic anemia and severe liver dysfunction who was infected with Omicron XBB.1, treated with VV116, and eventually recovered.

2 Case presentation

A 40-year-old male was diagnosed with acute liver failure on January 17, 2024, followed by timely plasma exchange and systemic anti-inflammatory therapy. One week later, the patients developed pancytopenia and aplastic anemia was diagnosed. On February 3, 2024, he was transferred to the department of hematology of our hospital for further treatment. On admission, his clinical symptoms included skin petechiae and ecchymosis, jaundice, anorexia, nausea, fatigue, cough with expectoration, headache and chest pain. The laboratory test data was shown in Table 1. The initial treatment consisted of hepatoprotective therapy and supportive care like blood transfusion.

On February 4, 2024, the patient developed a fever with temperature of 38.4°C. Routine blood examination showed C-Reactive Protein of 119.77 mg/L, Serum Amyloid A of 148.83 mg/L. On February 5, 2024, the NGS detection of sputum culture indicated the presence of Epstein-Barr virus, herpes simplex virus and SARS-CoV-2 (Omicron XBB.1). Notably, SARS-CoV-2 exhibited a high sequence number of 52839. Chest CT showed ground glass opacities of both lungs, bilateral mild pleural effusions with atelectasis of right lower lobe, and multiple small nodules (Figure 1A). For antiviral therapy against SARS-CoV-2, we consulted previous reports regarding the clinical use of VV116 and determined the schedule as 300 mg every 12 h on day 1 followed by 200 mg every 12 h on days 2–5 for this patient with severe liver dysfunction and aplastic anemia. The patient and his family members all agreed to this regimen and provided written informed consent. Moreover, broad-spectrum antibacterial medications including meropenem and levofloxacin were initiated to prevent secondary infection. On February 14, 2024, 4 days after completing the VV116 course, NGS of sputum confirmed that SARS-CoV-2 was undetected, indicating successful viral clearance. In the process of VV116 treatment from February 6, 2024 to February 10, 2024, the liver function, coagulation function and kidney function were closely monitored. As shown in Figure 2, the TBil, DBil, IBil, ALT, AST, ALP and GGT

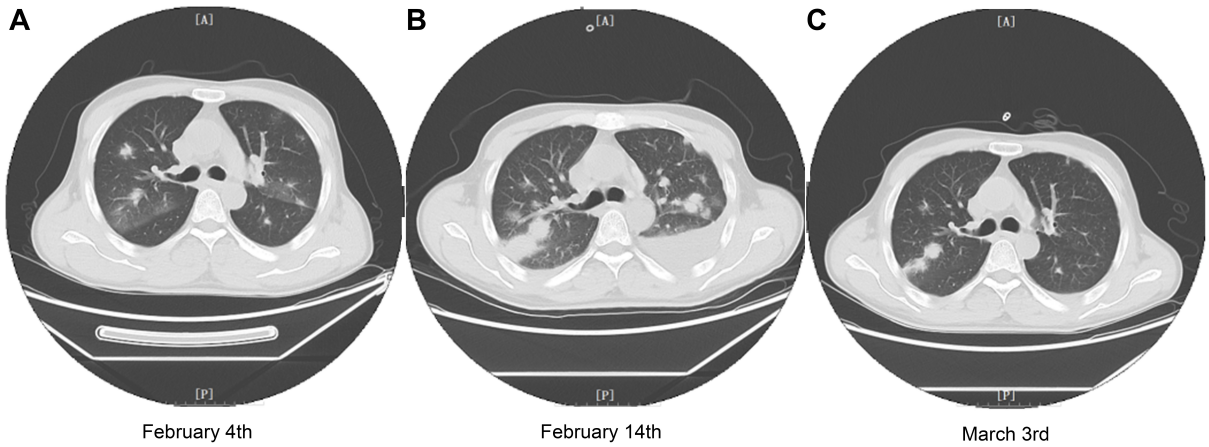

FIGURE 1
Chest CT images of the patient during treatment in our hospital. **(A)** Chest CT image of the patient on February 4th (2 days before VV116 initiation). **(B)** Chest CT image of the patient on February 14th, 4 days after completing the VV116 course. **(C)** Chest CT image of the patient on March 3rd, 3 days before leaving our hospital.

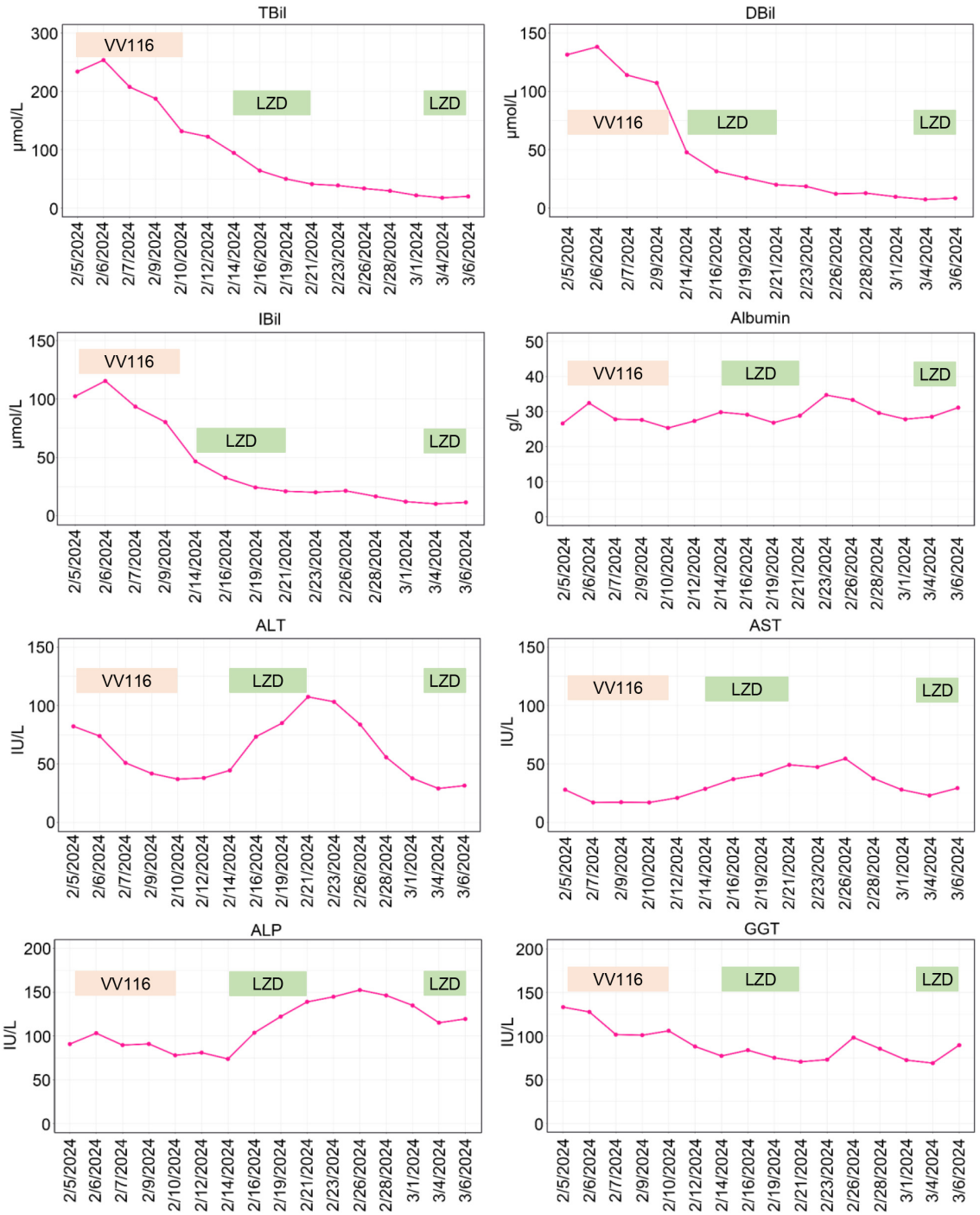

FIGURE 2
Graphical representation of hepatic enzymes and bilirubin during the patient's stay in our hospital. TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; LZD, linezolid.

levels were declined stepwise; and serum albumin levels remained stable throughout the treatment. Meanwhile, coagulation function markers prothrombin time (PT) and international normalized ratio (INR) were steady within the normal range (Figure 3). Furthermore, to comprehensively evaluate the liver function, we calculated the Child-Pugh score and Model for End-Stage Liver Disease (MELD) score according to corresponding formula (18, 19). As shown in Figure 3, the Child-Push score was stable, and the MELD score was falling steadily during VV116 treatment. The kidney function markers (serum creatinine, blood urea nitrogen, uric acid) also showed no obvious changes during the treatment course of VV116 (Supplementary Figure 1).

However, the pulmonary infection was aggravated on February 14 (Figure 1B). Serum galactomannan testing and sputum culture

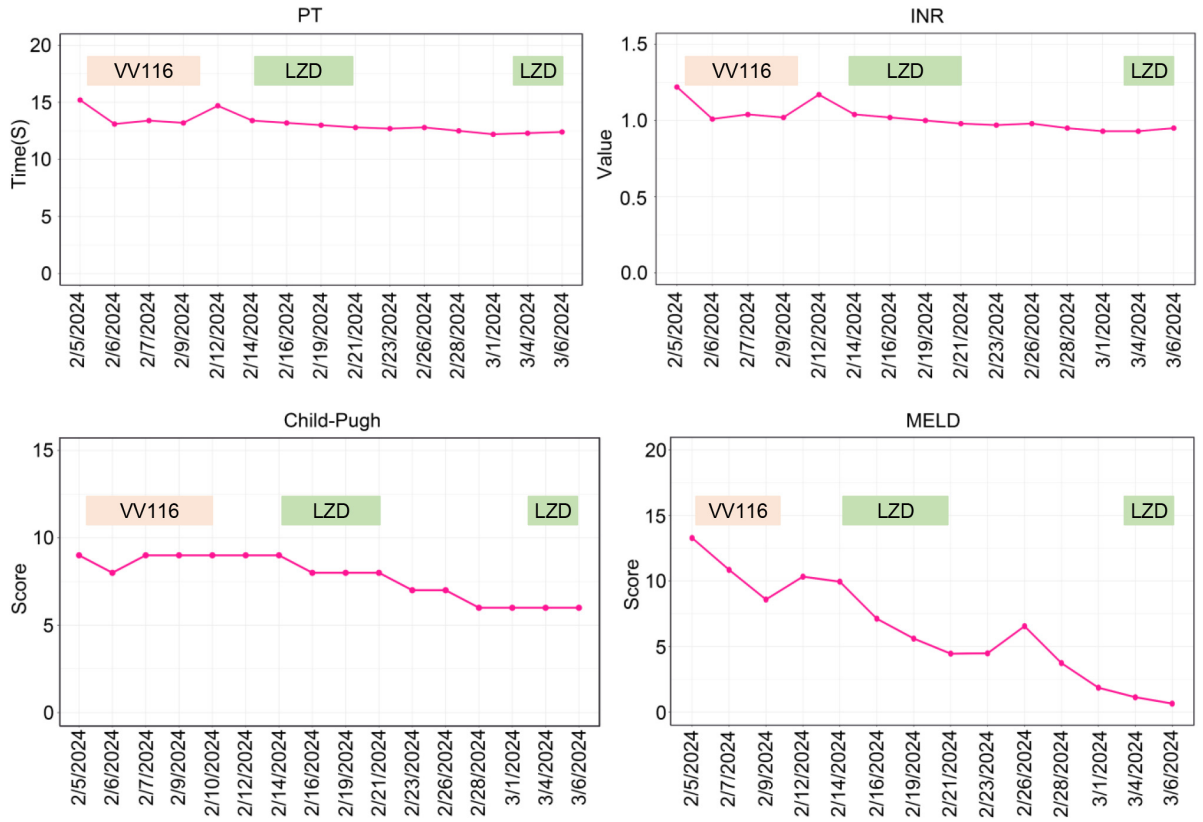

FIGURE 3
Trends of parameters of coagulation function and scores of Child-Pugh and MELD. PT, prothrombin time; INR, international normalized ratio; LZD, linezolid.

indicated the existence of *Candia tropicalis*, *Candida parapsilosis* and *Aspergillus terreus complex*. Caspofungin (50 mg, QD) was administered for antifungal therapy. Besides, linezolid (600 mg, QD) was prescribed to cover suspected gram-positive bacterial infections.

Whilst, the levels of ALT, AST and ALP showed mild increase from February 14 to February 21 (Figure 2). Notably, in this period, except hepatoprotective drugs and antibiotics (meropenem and levofloxacin), caspofungin and linezolid were newly prescribed drugs, which might be the etiological agents responsible for transaminase elevations. To evaluate the hepatotoxicity of linezolid and caspofungin, we consulted the LiverTox Web site,[1] an excellent resource for drug-induced liver injury. Based on LiverTox records, linezolid and caspofungin are both potentially hepatoxic, but linezolid got higher likelihood score of A (Well known cause) compared to caspofungin with a likelihood score of D (Possible cause). Therefore, linezolid was discontinued and replaced by vancomycin (500 mg, Q12H) on February 22, 2024. Then the levels of ALT, AST and ALP were gradually declined (Figure 2). Given the liver function improved significantly on March 3, 2024 and linezolid is superior to vancomycin for the treatment of pneumonia (20), we reintroduced linezolid to replace vancomycin. Notably, the levels of ALT, AST and ALP were back up again on March 6, 2024 as shown in Figure 2, further supporting the earlier elevations of ALT, AST and ALP from February 14 to February 21 might be ascribed to the application of linezolid. In contrast to linezolid treatment, VV116 administration (from February 6, 2024 to February 10, 2024) did not induce any elevation of liver enzymes or bilirubin (Figure 2), demonstrating the good tolerance of VV116 in patients with liver impairment.

On March 3, 2024, NGS detection of sputum demonstrated the sequence number of *Candia tropicalis* and *Aspergillus terreus complex* were significantly declined, and CT scan also confirmed the pulmonary function improved significantly (Figure 1C). By March 6, 2024, fever and cough with expectoration had no happened to the patient for several days. Jaundice nearly disappeared. Considering the improved general conditions and the insufficiency of blood supply in our hospital, the patient was discharged from our hospital and transferred to local hospital for blood transfusion and treatment of aplastic anemia.

3 Discussion

To ascertain the appropriate dosage of VV116 for COVID-19 patients with severe liver dysfunction, we consulted previous reports on the clinical use of VV116. Firstly, phase I clinical trials revealed no serious adverse events happened in healthy subjects receiving VV116 at doses of 200–600 mg Q12H, with only one subject receiving 400 mg VV116 experienced a mild and transient transaminase increase (21). Secondly, an open, prospective cohort

1 https://www.ncbi.nlm.nih.gov/books/NBK547852/

study showed 7 out of 60 COVID-19 patients taking VV116 (300 mg, Q12H for 5 days) had mild liver enzyme elevations, which resolved spontaneously (22). Thirdly, pharmacokinetic analysis indicated a dosage of 200 mg Q12H could achieve effective concentrations against SARS-CoV-2 (21, 23, 24).

VV116 was developed from remdesivir. Remdesivir exhibited no hepatotoxicity in preclinical study. However, in clinical trials, remdesivir might induce transient elevations of aminotransferases (25). In contrast, VV116, which has a wide tissue distribution, exhibits lower liver-targeting capability and enhanced lung-specific delivery (26). Our study indicated VV116 was well-tolerated in COVID-19 patients with liver impairment. Notably, our main concern regarding this conclusion is whether the good tolerance of VV116 can be ascribed to the concomitant use of hepatoprotective drugs. However, as aforementioned, during the treatment course from February 14 to February 21, linezolid induced an elevation of transaminases even under hepatoprotective treatment. Previous studies have also demonstrated linezolid could lead to liver impairment (27). Generally, linezolid-induced liver injury occurs under conditions of prolonged or high-dose administration (27, 28). In this study, a standard dose of linezolid treatment for only a few days induced an elevation of transaminases, indicating our patient was highly sensitive to hepatotoxic drugs even under hepatoprotective therapy. In contrast, during the VV116 treatment for COVID-19, the liver function of the patient continued to improve gradually, supporting the favorable tolerance of VV116 in COVID-19 patients with liver dysfunction.

4 Conclusion

Our study indicated VV116 was well-tolerated in COVID-19 patients with liver impairment. Notably, our main concern regarding this conclusion is whether the blood concentration of VV116 in patients with liver impairment differs from that in the general population. A limitation of this study is the lack of monitoring of blood drug concentrations. Future studies involving similar patients may consider blood drug concentration monitoring or conducting population pharmacokinetic studies.

Data availability statement

The original contributions presented in this study are included in this article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Shanghai Municipal Hospital of Traditional Chinese Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The 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

JY: Writing – original draft, Conceptualization, Data curation, Funding acquisition, Investigation. WJ: Data curation, Formal Analysis, Resources, Writing – original draft. JD: Project administration, Resources, Writing – original draft. ML: Formal Analysis, Investigation, Resources, Writing – original draft. YX: Data curation, Methodology, Software, Writing – original draft. JB: Funding acquisition, Validation, Writing – original draft. TJ: Conceptualization, Formal Analysis, Investigation, Supervision, Writing – review and editing. QH: Conceptualization, Project administration, Resources, Supervision, Writing – review and editing. LZ: Supervision, Validation, Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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The authors declare that no Generative AI was used in the creation of 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/fmed.2025.1541235/full#supplementary-material

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De novo abnormalities identified by fluorescence *in situ* hybridization during follow-up confer poor prognosis in Chinese multiple myeloma

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Background: Although there is evolving consensus to re-evaluate cytogenetic features during follow-up in multiple myeloma (MM), longitudinal studies on cytogenetic evolution in Chinese MM patients are still lacking. Our aim was to highlight the importance of ongoing monitoring of cytogenetic characteristics and shed light on the implications of clonal evolution in Chinese MM patients.

Patients and methods: The clinical data of 230 MM patients were retrospectively analyzed, including 100 patients were continuously monitored for cytogenetic abnormalities by fluorescence *in situ* hybridization (FISH).

Results: 49 out of 100 patients acquired *de novo* FISH abnormalities during follow-up, which were associated with disease progression ($p = 0.003$) and inferior progression free survival (PFS) (median 31 vs. 51 months, $p = 0.032$). Patients with ≥2 *de novo* FISH abnormalities had poorer PFS (median 24 vs. 45 months, $p = 0.003$) when compared to those with l or no *de novo* FISH abnormality. Patients who acquired new abnormalities within 31 months since diagnosis had significantly worse PFS (median: 20 vs. 41 months, $p < 0.001$) and Overall Survival (OS) (median: 61 vs. 100 months, $p = 0.008$) compared to those who acquired new abnormalities after 31 months. When gain/amp *1q21*, *del(17p)*, t(4;14), and t(14;16) were classified as high risk abnormalities (HRA), patients with ≥2 HRA had a shorter PFS (median 28 vs. 49 months, $p = 0.038$) and OS (median 75 vs. 107 months, $p = 0.040$) when compared to those without HRA.

Conclusion: Re-evaluation of cytogenetic characteristics by serial FISH tests is important in MM patients. *De novo* FISH abnormalities during follow-up are adverse prognostic factors, especially when ≥2 new FISH anomalies and acquired new abnormalities within 31 months since diagnosis are presented, and the presence of ≥2 HRA during the disease process are associated with poor survival in Chinese MM patients.

KEYWORDS

multiple myeloma, survival, high-risk, clonal evolution, cytogenetics

Introduction

Multiple myeloma (MM) is the second most commonly diagnosed hematological malignancy, characterized by the proliferation of malignant plasma cells in the bone marrow and excessive production of immunoglobulins (1, 2). In recent years, as new therapies including immunomodulators (IMiDs), proteasome inhibitors (PIs) and monoclonal antibodies (mAbs) have been incorporated into standard treatments, the overall survival (OS) and progression-free survival (PFS) of MM have been significantly improved. However, most cases still remain a chronic and incurable disease due to its typical pattern of remission and relapse (3–6). Heterogeneous cytogenetic abnormalities are the most important characteristics of MM and cytogenetic analysis is essential for prognostic evaluation at diagnosis (7). Many studies had identified that some cytogenetic abnormalities including *del(17p)(p53)*, t(4;14)(p16;q32), t(14;16)(q32;q23), and t(14;20)(q32;q12) were high-risk abnormalities (HRA) in MM patients, and others such as *del(13q)* and t(11;14)(q13;q32) were considered as standard-risk factors, whereas the prognostic value of *1q21* gain/amplication (gain/amp *1q21*) had been controversial (8–13). Of note, most of the previous studies mainly focused on the prognostic impact of the abnormalities identified at diagnosis, only few studies had considered the significance of the new acquired cytogenetic aberrations throughout the course of the disease (14–16). A longitudinal cytogenetic study focusing on cytogenetic evolution of 128 patients from the time of primary diagnosis and at relapse from Merz et al. (17) revealed that the presence of a new acquired HRA during follow-up conferred to poor prognosis as well. The study from Binder et al. (7) showed that the development of additional abnormalities during the 3 years following diagnosis was associated with increased subsequent mortality. While these previous studies had highlighted the importance of ongoing monitoring of MM cytogenetic signatures, they were not sufficient to adequately assess all potentially HRA that occur during the disease process in the case of modern therapies. For example, it is unclear whether HRA emerged at diagnosis or during follow-up has different effects on the outcome of MM patients. In addition, longitudinal studies on cytogenetic evolution in Chinese MM patients are still lacking. Therefore, in the present study, we summarized the clinical data of 230 newly-diagnosed MM (NDMM) patients admitted to our hospital, focusing on the analysis of 100 cases with sequential FISH data, with the aim to emphasize the importance of continuous monitoring of the cytogenetic characteristics and shed light on the implications of cytogenetic clonal evolution in Chinese MM patients.

Methods

Patients and treatments

The patients who were diagnosed with NDMM at our hospital between January 2012 and December 2019 were retrospectively analyzed. One hundred patients who underwent at least twice fluorescence *in situ* hybridization (FISH) evaluations with intervals more than 3 months were included in the longitudinal subgroup. Meanwhile, 130 patients who received only once cytogenetic evaluation with complete clinical data were randomly selected with 15 percents of NDMM in the same period. The group consisted of 146 (63.5%) males and 84 (36.5%) females, with a median age of 61 years (30–83). The ISS stage I, II and III were counted 18.3, 38.2 and 43.5%, respectively. All patients were followed up for survival until March 31, 2022, with a median follow-up time of 41 (28–130) months from diagnosis. The baseline data at diagnosis was extracted from medical records, while follow-up information was recorded after each visit. This study was approved by the ethics committee of Peking University People's Hospital.

The 230 patients received different regimens of initial therapy as follows, 162 (70.4%) patients were treated with bortezomib-based regimens, including BD (bortezomib, dexamethasone), BCD (bortezomib, cyclophosphamide, dexamethasone), and BAD (bortezomib, doxorubicin, dexamethasone). 41 (17.8%) patients received immunomodulator-based regimens, including RD (lenalidomide, dexamethasone), TAD (thalidomide, doxorubicin, dexamethasone) and TCD (thalidomide, cyclophosphamide, dexamethasone). 21 (9.1%) patients received bortezomib combined with immunomodulator regimens, including VTD (bortezomib, thalidomide and dexamethasone), VRD (bortezomib, lenalidomide and dexamethasone). 6 (2.6%) patients were treated with conventional VAD (vincristine, adriamycin, dexamethasone) chemotherapy. After induction therapy, 38 (16.5%) patients received first-line autologous stem cell transplantation (ASCT) as consolidation, and the others received lenalidomide, bortezomib or thalidomide plus dexamethasone as maintenance therapy.

ASCT

Patients underwent high-dose cyclophosphamide chemotherapy in combination with granulocyte colony-stimulating factor (G-CSF) for peripheral blood hematopoietic stem cell mobilization. The specific mobilization regimen was as follows: cyclophosphamide was administered intravenously over 2 days. Following chemotherapy, G-CSF was administered at a dose of 5–10 μg/(kg·d) to mobilize stem cells. Peripheral blood stem cell collection typically began on day 4–5 of G-CSF mobilization and continued for 1–2 days, with a maximum duration of 3 days. After collection, stem cells were reinfused electively, following pre-treatment with Mafran 2–3 days prior to reinfusion.

Metaphase karyotype analysis and interphase FISH

A 24 h short-term culture and G-banding technique were routinely used for metaphase karyotyping in all 230 patients. At least 20 metaphase cells were analyzed as possible in each G-banding analysis and the karyotypes were described according to the International Nomenclature System of Human Cytogenetics (ISCN2020). All patients were analyzed for gain/amp *1q21, del(17p), del(13q)* and *IgH* rearrangement by iFISH on enrichment of CD138+ plasma cells which was performed by magnetic-activated cell sorting (MACS) (purchased by Miltenyi Biotec, Germany) using gene locus-specific probes (GLP) including GLP *1q21*, GLP *P53*, GLP *D13S391*, GLP *RB1*, GLP *IgH* at diagnosis. If an *IgH* rearrangement was suspected, dual-color and dual-fusion translocation probes such as *IGH/FGFR3*, *IGH/MAF* and *IGH/CCND1* were used for the detection of t(4;14)(p16;q32), t(14;16)(q32;q23) and t(11;14)(q13;q32) when the samples were available.

Continuous FISH detections were performed in 100 patients during follow-up. In this study, for many patients with relatively stable disease following treatment, FISH assessments were typically conducted at regular intervals of 6 months to 1 year. However, for patients with disease progression, FISH re-evaluations were performed at any time. All probes were purchased from Peking GP Medical Technologies (Peking, China). At least 200 nuclei were counted for each probe with each sample, if the count value was near the threshold, the number of counted nuclei was increased to 500. The cut-off points for positive values (the mean of the normal control plus three standard deviations) were established in bone marrow from 20 healthy donors and 5.0% for gain/amp *1q21*, 8.0% for *D13S319* and *RB1* deletions, 8.0% for p53 deletion, 5.0% for *IgH* rearrangement and 3.0% for translocations.

Definition and statistical analysis

The abnormalities of gain/amp(*1q21*), *del(17p)*, t(4;14), and t(14;16) identified by FISH were classified as HRA and the others were classified as non-HRA in this study. Among the patients with longitudinal FISH analysis, new emerging FISH abnormalities during follow-up were defined as "*de novo*" abnormalities. Cytogenetic clonal evolution was defined as any new acquired abnormality during follow-up. Treatment response was evaluated according to the international uniform response criteria (18). PFS was defined from the date of diagnosis to the date of death, disease progression, or the last follow-up. OS was defined from the date of diagnosis to the date of death or the last contact. The survival curves were generated using the Kaplan–Meier method and the survival comparisons were performed by the log-rank test. Fisher exact test were performed to make the comparison of categorical variables among groups. A p-value <0.05 was considered statistically significant. All p-values were two-sided. All statistical analyses were performed using SPSS version 22.0 (SPSS, Inc).

Results

Karyotyping and FISH results of 230 patients

Among the whole cohort of 230 patients, 219 (95.2%) had successful G-banding cytogenetic analysis at diagnosis including 159 (69.1%) with normal karyotypes and 60 (26.1%) with clonal abnormalities, 11 (4.8%) patients with failure karyotyping results or with less than 5 normal metaphases were not considered. Meanwhile, FISH was performed in all patients and revealed abnormalities in 180 (78.3%) patients, and the incidence of gain/amp *1q21*, *del(13q)*, *del(17p)*, and abnormal *IGH* were 47.8% (110/230), 42.6% (98/230), 6.1% (14/230), 63.5% (146/230), respectively. Among 146 patients with abnormal signal patterns by *IGH* break apart probes in whom *IGH* translocations were suspected, 135 (92.5%) were analyzed for t(11;14), t(4;14), and t(14;16), and the incidence of each translocation was 37.0% (50/135), 17.8% (24/135) and 2.2% (3/135). The cytogenetic characteristics in 230 patients at diagnosis were summarized in Table 1.

TABLE 1 Cytogenetic characteristics in 230 patients at diagnosis.

Cytogenetic characteristics	No. (%)
G-banding	*N* = 230
Normal karyotypes	159 (69.1%)
Unnormal karyotypes	60 (26.1%)
Complex karyotypes	36 (15.7%)
Less than 5 normal metaphases	11 (4.8%)
FISH	*N* = 230
Non-HRA	
del(13q)	108 (47%)
IGH/CCND1	55 (23.9%)
HRA	
1q21	111 (48.3%)
del(17p)	25 (10.9%)
IGH/FGFR3	29 (12.6%)
IGH/MAF	3 (1.3%)

FISH, fluorescence in situ hybridization; HRA, high-risk abnormalities.

Cytogenetic clonal alterations

Continuous FISH detections were performed in 100 patients, and the results showed that 31 patients had unchanged FISH results during follow-up, including 10 with normal and 21 with abnormalities at diagnosis, and cytogenetic alterations were observed in 69 patients, out of whom 49 patients had *de novo* FISH abnormalities and 20 patients lost at least one or more previous existing abnormalities. Among 49 patients with *de novo* FISH abnormalities during follow-up, 26 patients had only 1 *de novo* abnormality while 23 patients had 2 or more new acquired abnormalities. According to the risk stratification, 35 patients acquired de novo HRA and 14 acquired non-HRA, and the new emerging aberrations included gain/amp (*1q21*) (25 cases), *del(13q)* (17 cases), *del(17p)* (11 cases), abnormal *IGH* (32 cases), *IGH::CCND1* (7 cases), *IGH::FGFR3* (6 cases) and *IGH::MAF* (1 case).

Among the 100 patients with continuous FISH detections, 67 patients underwent continuous G-banding analysis, and the results showed no change in 34 (50.8%) patients while 25 (37.3%) patients acquired new abnormalities and 8 (11.9%) lost at least one or more previous abnormalities.

Totally, regarding both G-banding and FISH results, 53% (53/100) of patients had cytogenetic evolution and the detailed clonal evolution types based on the initial and *de novo* FISH abnormalities and their prognostic risk stratification were listed in Table 2.

Prognostic significance of the cytogenetic clonal evolution

Impact of cytogenetic clonal evolution on disease progression in MM patients: a longitudinal cytogenetic analysis

Among 100 patients with longitudinal FISH analysis, disease progression and death events were observed in 67 and 16 patients, respectively. It was observed that 83.7% (41/49) of patients with *de novo* FISH abnormalities suffered from disease progression, which

TABLE 2 Cytogenetic alterations in 100 patients with continuous FISH detections.

Cytogenetic alterations	No. (%)
G-banding	*N* = 67
Unchanged	34 (50.8%)
de novo abnormalities	25 (37.3%)
Loss at least one previous abnormalities	8 (11.9%)
FISH	*N* = 100
Unchanged	31 (31%)
de novo HRA	35 (35%)
de novo non-HRA	14 (14%)
Loss at least one previous abnormalities	20 (20%)
Clonal evolution types by FISH	*N* = 49
Initial HRA + de novo HRA	4 (8.2%)
Initial HRA + de novo non-HRA	5 (10.2%)
Initial non-HRA + de novo HRA	30 (61.2%)
Initial non-HRA + de novo non-HRA	10 (20.4%)

FISH, fluorescence in situ hybridization; HRA, high-risk abnormalities.

was much higher than 56.9% (29/51) of those without *de novo* FISH aberrations ($\chi^2 = 0.003$). There was no significant difference on the frequencies of disease progression between the patients with *de novo* HRA and those with de novo non-HRA (84.8% vs. 62.5%, $\chi^2 = 0.082$), suggesting that de novo FISH abnormalities during follow-up were associated with disease progression regardless of new emerging HRA or non-HRA. Moreover, among 20 patients who experienced abnormalities loss after treatment during follow-up, 16 patients (80%) showed a good response to treatment (10 cases were evaluated as VGPR, and 6 cases were evaluated as CR), while 4 patients (20%) experienced disease progression. These findings suggested that the majority of patients with abnormalities loss demonstrated treatment efficacy.

As shown in Table 2, 100 MM patients underwent continuous cytogenetic analysis. Among them, 49 patients acquired new cytogenetic abnormalities, including 21 were treated with the BD/BCD regimen, and 28 received Non-BD/BCD regimens. Among the 51 patients without new acquired abnormalities, 28 were treated with the BD/BCD regimen, and 23 with Non-BD/BCD regimens. Statistical analysis revealed that regardless of the treatment regimens (whether BD/BCD or Non-BD/BCD), there was no significant difference in the probability of acquiring newly cytogenetic abnormalities ($\chi^2 = 0.231$), suggesting that the treatment regimen had no apparent effect on cytogenetic clonal evolution.

Impact of the number and timing of *de novo* FISH abnormalities during follow-up on survival outcomes

Furthermore, we investigated the effect of the number of de novo FISH abnormalities on the survival, and the results showed that there were no significant difference in PFS (median 31 vs. 49 months, $p = 0.113$) (Figure 1A) and OS (median 90 vs. 101 months, $p = 0.949$) (Figure 1B) between the patients with ≥1 *de novo* FISH abnormality (49 cases) and those without *de novo* FISH abnormality (51 cases), whereas the patients with ≥2 de novo FISH abnormalities (23 cases) had an inferior PFS (median 24 vs. 45 months, $p = 0.003$) when compared to those with only 1 or no *de novo* FISH abnormality (77 cases) and there was no significant difference in OS between two groups (median 78 vs. 107 months, $p = 0.119$) (Figures 1C,D).

Among the 49 patients who developed de novo FISH abnormalities during follow-up, the median time to acquisition of new FISH abnormalities was 31 months (range: 4–71 months). We further analyzed the relationship between the timing of these abnormalities and survival outcomes. Our results indicated that patients who acquired new abnormalities within 31 months since diagnosis had significantly worse PFS (median: 20 vs. 41 months, $p < 0.001$) and OS (median: 61 vs. 100 months, $p = 0.008$) compared to those who acquired new abnormalities after 31 months (Figures 1E,F).

Impact of high risk abnormalities and treatment on survival

To determine whether the initial HRA at diagnosis and *de novo* HRA during follow-up confer to different prognosis, patients with initial HRA and without de novo HRA during follow-up (120 cases) were defined as the initial HRA group, and patients with *de novo* HRA during follow-up and initial normal FISH (23 cases) or initial non-HRA (7 cases) were defined as the de novo HRA group. It was observed that there were no significant difference in PFS (median 38 vs. 27 months, $p = 0.530$) (Figure 2A) and OS (median 72 vs. 85 months, $p = 0.111$) (Figure 2B) between the initial HRA group and the de novo HRA group.

Among 100 patients with serial FISH analysis, considering the FISH results during the disease process, there were 48 cases with 1 HRA, 18 cases with 2 HRA, 1 case with 3 HRA, and 33 cases without HRA. Regarding the prognostic effect of the HRA number on the survival, the results showed that there were no significant difference in PFS (median 37 vs. 49 months, $p = 0.187$) (Figure 3A) and OS (median 91 vs. 107 months, $p = 0.381$) (Figure 3B) between the patients with 1 HRA and those without HRA. However, the patients with≥2 HRA (19 cases) had shorter PFS (median 28 vs. 49 months, $p = 0.038$) and OS (median 75 vs. 107 months, $p = 0.040$) when compared to those without HRA (33 cases) (Figures 3C,D).

Among the 100 MM patients who underwent continuous cytogenetic analysis, 11 patients received ASCT. Of the 49 patients who acquired new cytogenetic abnormalities, 5 underwent ASCT. Survival analysis indicated that there were no significant differences in PFS(median: 36 vs. 31 months, $p = 0.705$) and OS (median: 71 vs. 90 months, $p = 0.471$) between patients who underwent ASCT and those who received chemotherapy alone among the 49 patients.

Discussion

The prognostic significance of baseline cytogenetic aberrations in NDMM is well-documented, which have been shown to have a significantly greater prognostic impact in MM than mutations in specific genes (19) and there is increasing evidence that the evolution of cytogenetic aberrations over time has an adverse effect on the prognosis of MM patients (20–22). The study from Aleksander et al. (23) showed that presence of clonal evolution, particularly the acquisition of new *del(17p)* at relapse negatively affect the outcome of

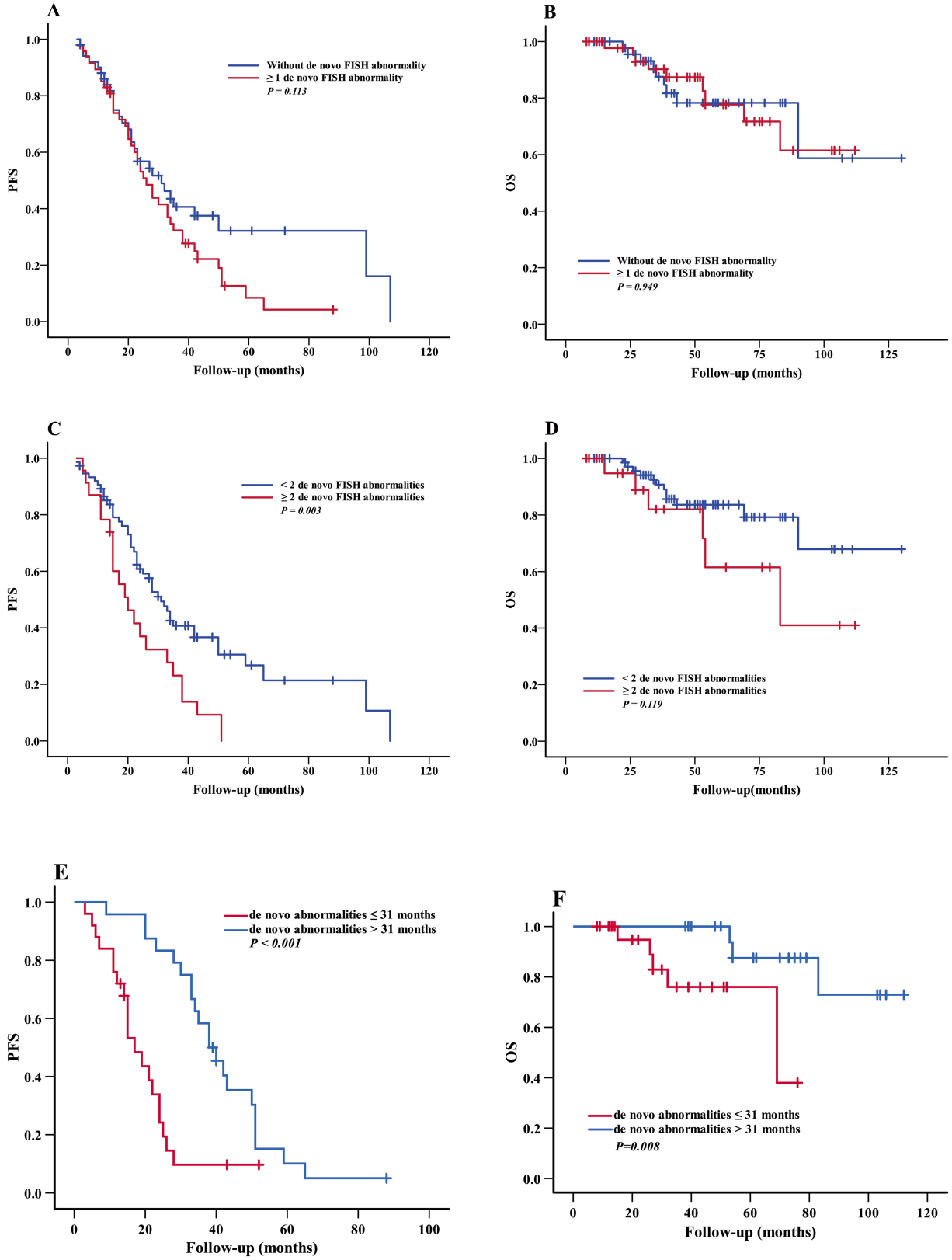

FIGURE 1
Impact of the number of de novo FISH abnormalities on survival. PFS **(A)** and OS **(B)** of patients with without de novo FISH abnormality and ≥ 1 de novo FISH abnormality. PFS **(C)** and OS **(D)** of patients with < 2 de novo FISH abnormalities and ≥ 2 de novo FISH abnormalities. PFS **(E)** and OS **(F)** of patients with de novo FISH abnormalities ≤ 31 months and > 31 months. OS, overall survival; PFS, progression-free survival.

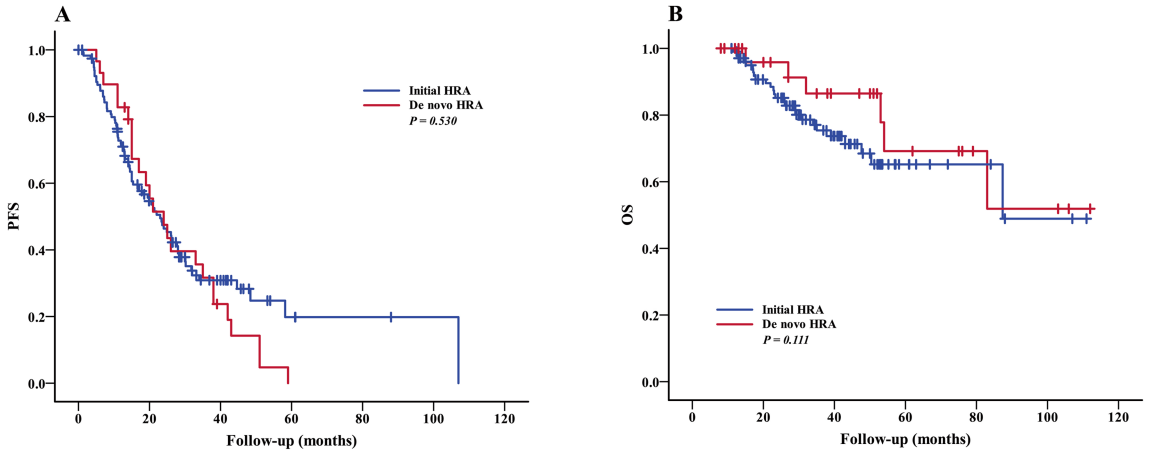

FIGURE 2
Impact of high risk abnormalities on survival. PFS **(A)** and OS **(B)** of patients with initial HRA and de novo HRA. OS, overall survival; PFS, progression-free survival; HRA, high-risk abnormalities.

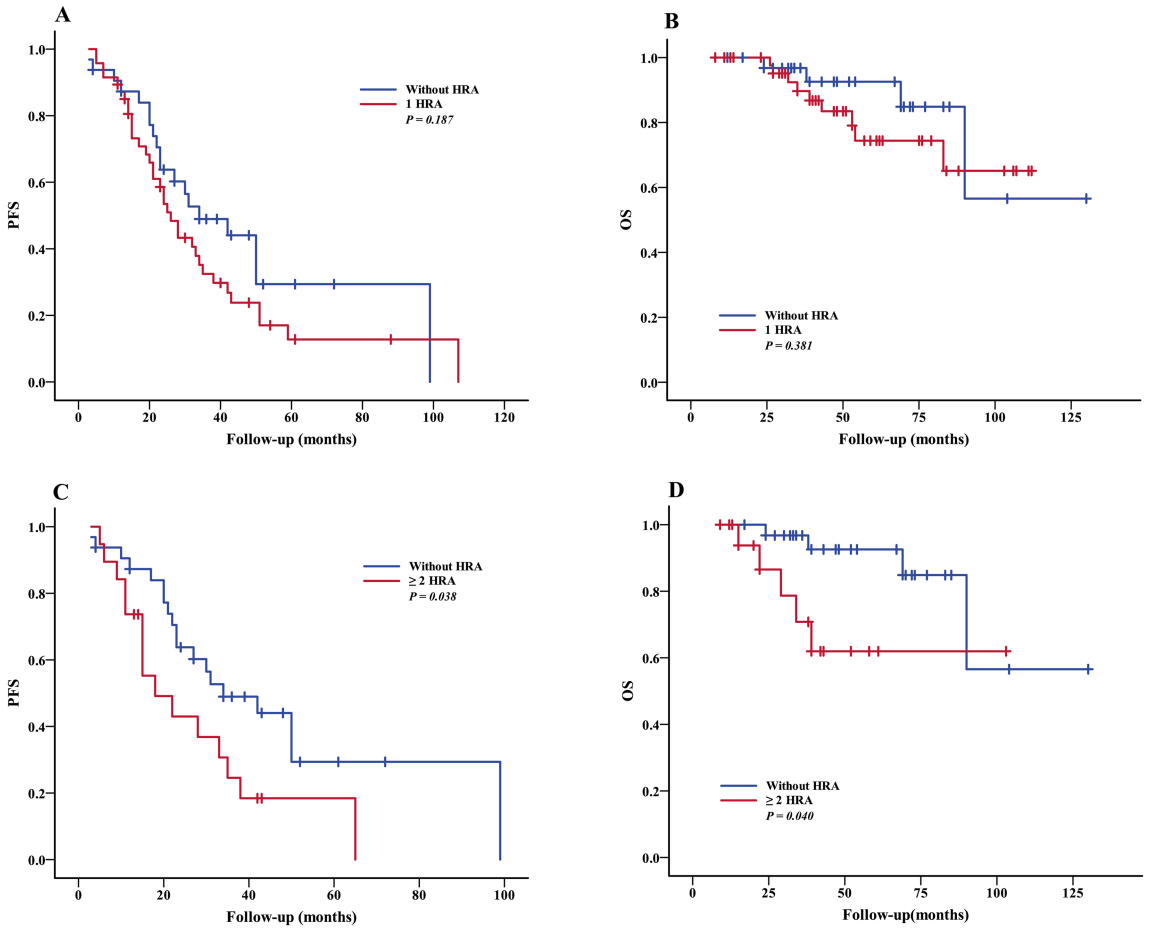

FIGURE 3
Impact of high risk abnormalities on survival. PFS **(A)** and OS **(B)** of patients without HRA and 1 HRA. PFS **(C)** and OS **(D)** of patients without HRA and 2 HRA. OS, overall survival; PFS, progression-free survival; HRA, high-risk abnormalities.

MM, and similar results were observed in the Lakshman et al. (24) study. The study from Binder et al. (7) enrolled 989 MM patients including 304 with at least twice cytogenetic evaluations showed that the presence of t(11;14) at the time of diagnosis was associated with decreased odds of cytogenetic evolution during follow-up, while the presence of at least one trisomy or tetrasomy was associated with

increased odds, and the development of additional abnormalities during the 3 years following diagnosis was associated with increased subsequent mortality. In addition, they also found that the prognostic significance of baseline cytogenetic abnormalities was most pronounced at the time of diagnosis and attenuated over time, the presence of cytogenetic high-risk features at diagnosis were associated with shorter OS but the presence of high-risk features were no longer associated with OS in those who survived 3 years after diagnosis, which highlighted the importance of continuous monitoring of cytogenetic characteristics and suggested that risk factors emerged at different times in the disease process may have different prognostic implications for MM patients. As more and more data suggest that disease progression, dissemination, and relapse in MM is driven by clonal evolution (25–28), an evolving consensus to reevaluate for cytogenetic high-risk features during follow-up has been reached, but the clinical implication of cytogenetic clonal evolution especially the prognostic significance of *de novo* HRA remains to be further clarified.

Our study revealed that 49% of Chinese MM patients acquired de novo FISH abnormalities during follow-up, and de novo FISH aberrations were associated with disease progression regardless of HRA or non-HRA (83.7% vs. 56.9%, $X^2 = 0.003$) and they were also conferred to an inferior median PFS (31 vs. 51 months, $p = 0.032$). In addition, the patients with 2 or more de novo FISH abnormalities had shorter median PFS (median 24 vs. 45 months, $p = 0.003$) when compared to those with one or no de novo FISH abnormality, and patients who acquired new abnormalities within 31 months since diagnosis had significantly worse PFS (median: 20 vs. 41 months, $p < 0.001$) and OS (median: 61 vs. 100 months, $p = 0.008$) compared to those who acquired new abnormalities after 31 months. Since clonal evolution may reflect the genomic instability, which is the hallmark of all neoplastic diseases and is the source of genetic heterogeneity of MM, it is reasonable to speculate that the more new emerging cytogenetic anomalies and the earlier new FISH abnormalities acquired, the greater the tumor instability and the worse the prognosis of MM. Consistent with this conjecture, our study showed that the higher number of *de novo* FISH abnormalities, the worse the survival, suggesting a cumulative adverse effect of the number of de novo FISH aberrations.

Although cytogenetic risk stratification of MM patients is widely used in clinical practice, there are some controversies about the prognostic impact of HRA in MM patients as new treatment strategies are constantly updated. Related study reported that with appropriately treatments, the survival of patients with certain high risk categories can approach that of patients with standard risk disease. In a large trial using bortezomib-based induction, early ASCT, and bortezomib maintenance, the median OS of patients with *del(17p)* was approximately 8 years (8-year survival rate of 52%), which was identical to patients with standard risk MM. In contrast, survival was lower for patients with t(4;14) (8-year survival rate, 33%) and for patients with gain(*1q21*) (8-year survival rate, 36%). These findings underscore the limitations of current risk stratification models in the context of modern therapy and highlight the need to stratify MM based on individual cytogenetic groups rather than arbitrary heterogeneous risk categories (29, 30). Considering the impact of the number of cytogenetic abnormalities on prognosis, Binder et al. (31) found that the greater the number of HRA at the time of diagnosis, the worse the prognosis of MM patients. In our study, almost all patients received modern therapies such as bortezomib, immunomodulator or ASCT as induction or maintenance, and no significant difference in PFS (median 37 vs. 49 months, $p = 0.187$) and OS (median 91 vs. 107 months, $p = 0.381$) were observed between the patients with 1 HRA and those without HRA, but the patients with ≥2 HRA had shorter median PFS (28 vs. 49 months, $p = 0.038$) and OS (75 vs. 107 months, $p = 0.040$) than the patients without HRA, suggesting by modern strategies of therapy, only two or more HRA were definitely adverse prognostic factor in Chinese MM patients, which highlighted the potential for risk stratification to change as treatments were updated.

In the era of new drugs, the role of ASCT has been questioned. However, ASCT remains the standard treatment recommended by international guidelines, including those of the American Society of Clinical Oncology and the European Society for Medical Oncology (32). Our study did not demonstrate that ASCT could improve the prognosis of high-risk patients (those who acquired new cytogenetic abnormalities during follow-up). We speculate that the limited number of patients undergoing ASCT in our cohort may have introduced statistical bias. In future studies, we plan to collect more cases to further explore this issue.

The ability to draw firm conclusions from our data is limited by the retrospective nature and a relatively small number of enrolled patients, but our results reaffirm the importance of continuous monitoring of the cytogenetic characteristics of MM during follow-up. *De novo* FISH abnormalities during follow-up are adverse prognostic factors in MM patients, especially when ≥2 new FISH anomalies are presented, and the presence of ≥2 HRA during the disease process are associated with poor survival in Chinese MM patients, which remains to be further confirmed in larger scale of studies.

Conclusion

Re-evaluation of cytogenetic characteristics by serial FISH tests is important in MM patients. De novo FISH abnormalities during follow-up are adverse prognostic factors, especially when ≥2 new FISH anomalies and acquired new abnormalities within 31 months since diagnosis are presented, and the presence of ≥2 HRA during the disease process are associated with poor survival in Chinese MM patients.

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

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

SC: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. LG: Investigation, Writing – original draft. LF: Investigation, Writing – original draft. ZW: Investigation, Writing – original draft. YeL: Investigation, Writing – original draft. QL: Investigation, Writing – original draft. WS: Investigation, Writing – original draft. SK: Investigation, Writing – original draft. YaL: Investigation, Writing – review & editing. JL: Investigation, Writing – review & editing. YC: Investigation, Writing – review & editing. XH: Investigation, Writing – review & editing. YuL: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing.

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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.

Generative AI statement

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Publisher's note

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Successfully salvaging a HIV-positive patient with mixed CIDP and meningoencephalitis: a case report

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We describe an acquired immunodeficiency syndrome (AIDS) patient who first exhibited chronic inflammatory demyelinating polyneuropathy (CIDP) and subsequently developed meningoencephalitis due to ganciclovir (GCV)-resistant cytomegalovirus (CMV) infection. The patient first presented with peripheral nervous system (PNS) symptoms, followed by central nervous system (CNS) symptoms. Based on auxiliary examinations, including cerebrospinal fluid (CSF) tests, electromyography (EMG), brain magnetic resonance imaging (MRI) and GCV drug resistance tests, the patient was diagnosed with CIDP and meningoencephalitis due to CSF GCV-resistant CMV. After the combined application of intravenous immunoglobulin treatment, corticosteroid treatment, antiretroviral therapy (ART), and adjusted anti-CMV treatment, the patient achieved persistent relief. This case underscores the importance of considering CMV as a common etiology of neurological disorders in AIDS patients. It also highlights the necessity of prompt drug resistance testing when anti-CMV therapy yields suboptimal responses.

KEYWORDS

cytomegalovirus, meningoencephalitis, chronic inflammatory demyelinating polyradiculoneuropathy, ganciclovir resistance, acquired immunodeficiency syndrome

Introduction

Cytomegalovirus (CMV) is a common pathogenic agent in central nervous system (CNS) opportunistic infections, and CMV meningoencephalitis is a major cause of mortality in acquired immunodeficiency syndrome (AIDS) patients (1, 2). CMV is also associated with peripheral nervous system (PNS) dysfunction in AIDS patients (3). As the first-line antiviral agent, ganciclovir (GCV) resistance primarily arises from mutations in the UL97 kinase gene (phosphotransferase) and/or UL54 DNA polymerase gene. Although primary resistance (pre-therapy mutations) is rare, secondary resistance due to prolonged antiviral exposure is increasingly reported in immunocompromised hosts (4). While drug-resistant CMV infections in the HIV-positive population are uncommon, the prevalence of GCV resistance among the HIV-positive population is the highest, followed by that of the organ transplant population (5). Resistance testing, including genotypic assays for UL97 and UL54 mutations, is critical for guiding therapy in refractory cases. Here, we report an AIDS patient with a concurrent neurological complications caused by GCV-resistant CMV.

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Case description

A 49-year-old male presented with bilateral lower limb weakness on March 1, 2022 that had existed for the prior 2 months. The patient was conscious and had no obvious sensory disorders, however, the muscle strength of both lower limbs decreased. Muscle atrophy of both lower limbs was noted, and the reflex of both knee tendons was weakened. The results of laboratory examinations were as follows: HIV-Ab (+), serum HIV RNA concentration of 1.92×10^6 copies/mL, and $CD4^+$ T-cell count of 6/μL. Lumbar puncture was performed, and cerebrospinal fluid (CSF) analysis revealed the following results: WBC count, 276×10^9/L (0–8); protein concentration, 1.4 g/L (0.12–0.6); normal glucose and chloride concentration. The concentration of CSF CMV-DNA was 3×10^6 copies/mL, while the CSF HIV-1 viral load was 2.89×10^5 copies/mL (Table 1). Initial brain magnetic resonance imaging (MRI) revealed no significant lesions. An electromyogram (EMG) showed neurogenic damage. The motor nerve conduction velocities of the left and right median nerves, the right common peroneal nerve, and the left and right tibial nerves decreased as the distal latency increased. The patient met the electrodiagnostic criteria for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) recommended by the European Academy of Neurology/Peripheral Nerve Society (6). The patient was diagnosed with AIDS, CMV-related peripheral neuropathy and meningitis and was treated with GCV, intravenous immunoglobulin, methylprednisolone. Antiretroviral therapy (ART) (bictegravir sodium, emtricitabine, and tenofovir alafenamide fumarate tablets) was initiated 15 days after anti-CMV therapy. After treatment, the muscle strength of the patient gradually improved, and his CSF cell count decreased, but his CSF CMV DNA test remained positive after 2 months of anti-CMV treatment (Table 1). The coadministration of GCV and foscarnet was used as an adjusted anti-CMV regimen, while methylprednisolone was then tapered.

Three months later, the patient suffered from headache, ataxia, muscle weakness, and temporal disorientation. The Mini-Mental State Examination (MMSE) score was 18, and the CMV DNA and HIV RNA in CSF remained positive (Table 1). No mutation in the UL97 gene of CMV in the serum was detected by Sequence-Specific PCR followed by Sanger sequencing. Regrettably, due to the limitations of detection technology, we could not detect UL54 gene mutations in

TABLE 1 Laboratory examination and treatment of the patient.

Date of examination and treatment	Reference range	Onset	2 months	3 months	8 months	1 year
Event		Decreased lower limb muscle strength	Muscle strength improved	Consciousness disorders, muscle weakness	UL97 gene mutation	Mental and muscular recovery, CSF HIV escape
CSF cell count ($\times 10^6$/L)	0–8	276	10	50	16	31
CSF protein (mg/L)	120–600	1,415	1,142	1,990	1,010	1,441
Lumbar puncture pressure(mmH_2O)	80–180	135	180	100	120	236
CSF HIV-RNA (copies/mL)	<20	2.89×10^5	253	1,651	<20	1,210
CSF CMV-DNA (copies/mL)	$<1.0 \times 10^3$	3.04×10^6	8.36×10^4	2.30×10^3	3.30×10^4	$<1.0 \times 10^3$
CSF mNGS	0	CMV 2402series				CMV 279series
Plasma CD4+ T cell count (cells/uL)	410–1,590	6	18	22	11	148
Plasma CD8+ T cell count(cells/uL)	190–1,140	471	356	419	317	1,703
Plasma HIV-RNA (copies/mL)	<20	1.92×10^6	90.1	75	<20	141
Serum CMV-DNA (copies/mL)	$<1.0 \times 10^3$	1.22×10^5	4.26×10^3	8.60×10^4	$<1.0 \times 10^3$	$<1.0 \times 10^3$
ART regimen		B/F/TAF	B/F/TAF	F/TAF + DTG	F/TAF + DTG	F/TAF + DTG
Corticosteroid therapy		Methylprednisolone, 40 mg twice per day	Methylprednisolone, 12 mg once per day	Methylprednisolone, 20 mg twice per day	Hydrocortisone, 30 mg each morning and 20 mg each afternoon	Hydrocortisone, 15 mg each morning and 5 mg each afternoon
Anti-CMV treatment		Ganciclovir	Ganciclovir and foscarnet	Ganciclovir and foscarnet	Foscarnet	Foscarnet

CSF, cerebrospinal fluid; CMV, Cytomegalovirus; mNGS, metagenomics next-generation sequencing; ART, antiretroviral therapy; B/F/TAF, bictegravir sodium, emtricitabine and tenofovir alafenamide fumarate tablets; F/TAF, emtricitabine and tenofovir alafenamide fumarate tablets; DTG, dolutegravir.

blood samples, nor could we detect UL97 and UL54 gene mutations in CSF. Diffusion weighted imaging (DWI) of the brain MR images were consistent with the diagnosis of CMV meningoencephalitis (Figure 1A). The coadministration of GCV and foscarnet continued, while bictegravir sodium was replaced by dolutegravir to increase the penetration of ART into the CNS. The patient became negative for CSF HIV RNA 2 weeks later. The patient's neurological symptoms gradually improved in 2 months.

Eight months later, the patient's MMSE score increased from 18 to 27, and his muscle strength also recovered. However, his CSF remained positive for CMV DNA. The A594V mutation in the UL97 gene of CMV in the CSF was detected by Sequence-Specific PCR followed by Sanger sequencing, but no drug resistance mutations were detected in the UL54 gene. GCV treatment was stopped, and foscarnet treatment was continued (Table 1). Moreover, hormonal testing revealed adrenal cortical dysfunction (data not shown).

After 1 year of treatment, the patient exhibited normal consciousness and muscle strength, CMV DNA was undetectable in CSF by PCR (<1,000 copies/mL), his $CD4^+$ T-cell count was 148/μL (Table 1), and the patient's brain MRI and electromyography also showed significant improvement (Figure 1A). The patient subsequently received reduced corticosteroids for CIDP. Due to adrenal cortex dysfunction, the patient received 20 mg/d hydrocortisone for maintenance treatment. Anti-CMV treatment was discontinued after 15 months of onset. At the three-year follow-up, the patient reached remission, with normal consciousness and muscle strength. The timeline for treatment adjustment and follow-up of this patient is clearly outlined in Figure 1B.

Discussion

This patient had simultaneous involvement of the PNS and CNS, which is associated with CSF GCV-resistant CMV infection. The patient had no prior GCV exposure, and CMV resistance occurred during anti-CMV therapy, which suggested secondary CMV resistance rather than a primary drug-resistant CMV infection. Initial response to GCV was favorable, with reduced CMV DNA in serum and CSF, decreased CSF cell count, and improved peripheral neuropathy. However, by the third month, CMV DNA persisted in serum and CSF, and serum levels increased. UL97 resistance testing in blood was negative, prompting continued GCV and foscarnet

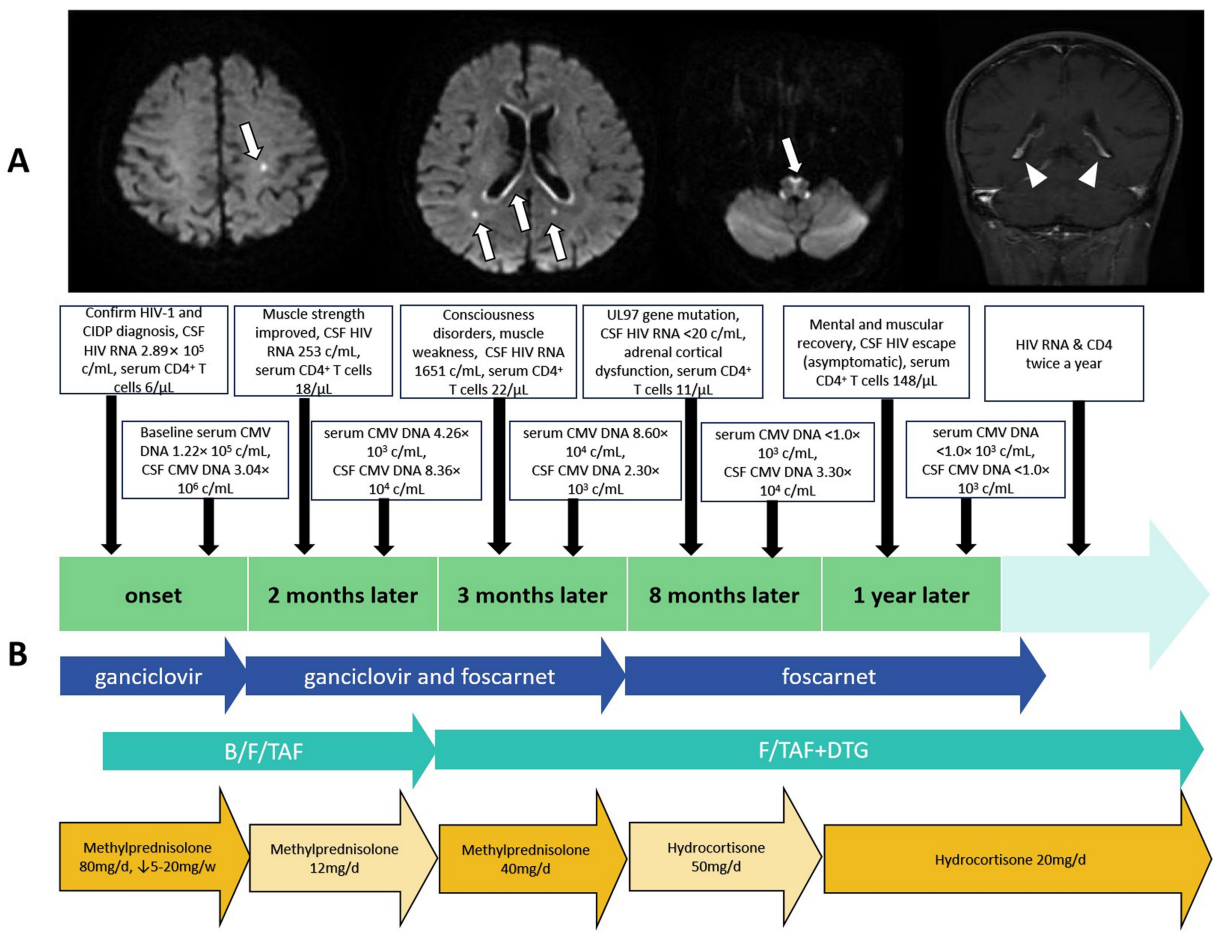

FIGURE 1
(A) Brain magnetic resonance imaging (MRI) of the patient. Diffusion-weighted imaging sequence revealed high signals in the left half of the oval center, near the posterior horn of the bilateral ventricles, bilateral ventricular walls, and anterior edge of the medulla oblongata (white arrows). The coronal image showed abnormal enhancement of the bilateral ventricles (Triangles). **(B)** The timeline for treatment adjustment and follow-up of this patient.

therapy. By the eighth month, serum CMV DNA was undetectable, but CSF levels remained elevated, and the A594V mutation in the UL97 gene was detected in CSF CMV. This suggests resistance developed due to prolonged CMV infection and GCV therapy. Despite rapid HIV suppression with ART, inadequate immune restoration also contributed to poor anti-CMV response.

HIV and CMV infections, especially CMV infections, might play a key role in CIDP (7, 8). CIDP most likely results from an immunopathogenic mechanism, reflecting altered immune regulation caused by HIV and CMV infections. CIDP is triggered by an immune stimulus such as an infection (9). The relationship between CIDP and CMV infection in this case is complex. While CIDP is typically considered an immune-mediated disorder, the presence of CMV in the CSF suggests that CMV may have directly contributed to peripheral nerve dysfunction. This dual pathology complicates the decision to initiate corticosteroids, as their use in the setting of active CMV infection carries risks of exacerbating viral replication. However, in this case, the clinical improvement following corticosteroid administration supports the hypothesis that CIDP was primarily immune-mediated, with CMV acting as a cofactor. CSF pleocytosis could be a feature of HIV-positive CIDP patients (10, 11). For our patient, concomitant CMV infection might be another reason for CSF pleocytosis.

In AIDS patients with cryptococcal meningitis, ART initiation generally is deferred for 4 to 6 weeks after antifungal agents are started to avoid immune reconstitution inflammatory syndrome (IRIS). However, for CMV-related conditions, ART is usually started within 1–2 weeks of anti-CMV therapy. In this patient, ART was initiated 15 days after anti-CMV treatment, and no IRIS occurred. The subsequent encephalitis was consistent with CMV ventriculoencephalitis rather than IRIS, and corticosteroid use likely reduced IRIS risk.

For CIDP treatment, intravenous immunoglobulin, plasma exchange, and corticosteroid were recommended. AIDS patients with CIDP are generally younger, more steroid-responsive, and have a monophasic progressive course compared to HIV-negative patients (12). In treating CMV infection in AIDS patients, although the optimal duration of initial therapies has not been established, the combination of GCV and foscarnet is the preferred regimen. Except for GCV and foscarnet, other anti-CMV drugs were unavailable or difficult to obtain in China. The intravenous infusion of CMV-cytotoxic T lymphocytes (CTLs) and anti-CMV specific immunoglobulins were only reported in transplant patients (13). Continuous CMV disease progression after multiple weeks of GCV therapy can be an indication of drug resistance. Although the correlation between CMV genetic resistance and phenotypic resistance is undetermined and optimal testing methods are nonuniform, the detection of CMV genetic resistance still facilitates the improvement of anti-CMV therapies (14).

Conclusion

In conclusion, patients with refractory CMV-associated nervous system disease progression should be tested for CMV drug resistance using CSF samples. In addition to GCV and foscarnet, other new anti-CMV drugs such as Maribavir will require being validated in HIV-positive population. Optimized therapies should include adjusting anti-CMV drugs, prolonging the treatment period and improving the immune status. Long-term maintenance therapy is also needed.

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

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

WW: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. JY: Supervision, Writing – review & editing. XL: Conceptualization, Writing – review & editing, Data curation, Formal analysis, Methodology. YW: Conceptualization, Writing – review & editing, Supervision, Validation, Visualization.

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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.

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Chinese expert consensus on the application of intravenous immunoglobulin in hematological diseases

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Intravenous immunoglobulin (IVIG), first developed for the treatment of patients with antibody deficiencies, is now widely used in clinical practice, especially in hematological and immune system diseases, and its application in hematological oncology chemotherapy, cellular immunotherapy and hematopoietic stem cell

transplantation (HSCT) is becoming more and more common. The Chinese Collaborative Group for Infection Immunology and Microecology Research Translation Collaborative Group organized relevant experts to discuss and propose the "Chinese expert consensus on the application of intravenous immunoglobulin in hematological diseases," which was formulated based on the progress of research on the application of IVIG in blood diseases, and provides a basis for the standardization of the use of IVIG in hematologic disorders.

KEYWORDS

IVIG, clinical application, hematologic disorders, chimeric antigen receptor T-Cell, hematopoietic stem cell transplant (HSCT), expert consensus

1 Introduction

Intravenous immunoglobulin (IVIG) is a blood product obtained from the plasma of healthy donors, consisting primarily of polyclonal immunoglobulin G (IgG). It has anti-inflammatory and immunomodulatory effects, and was first introduced in the early 1980s for the treatment of primary humoral immunodeficiency (1). At present, IVIG has been widely used in clinic, such as the treatment of immune system diseases (including systemic lupus erythematosus, Kawasaki disease, primary immunodeficiency, etc.), hematologic disorders (including immune thrombocytopenia, autoimmune hemolytic anemias, hemolytic disease of the newborn, etc.) and neurological disease (including Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, myasthenia gravis, polymyositis, multiple sclerosis and autoimmune encephalitis) (2–5). Its application in chemotherapy, targeted therapy, cellular immunotherapy and HSCT for malignant hematological tumors is also becoming more prevalent (6–8). In order to further improve the standardized application of IVIG in hematological diseases, the Chinese Collaborative Group for Infection Immunology and Microecology Research Translation Collaborative Group has organized a multi-disciplinary team (MDT) steering committee composed of experts from hematology (including HSCT), infectious diseases, critical care medicine, pharmacy and other specialties to discuss the issue, and to synthesize the current status of related research at home and abroad to formulate expert recommendations for the standardized application of IVIG in hematological diseases. The expert recommendation on the application of IVIG in hematological diseases was formulated based on the progress of domestic and international research on the application of IVIG in hematological diseases, which provides a basis for the standardization of the use of IVIG in hematological diseases. This expert recommendation uses the GRADE/DECISION Evidence to Decision Making Framework to determine the direction and strength of the recommendation, with the GRADE methodology assessing the quality of evidence rated as high (A), moderate (B), low (C), or very low (D). Based on the GRADE evidence the MDT panel rated the strength of the recommendation as strong and weak recommendation (for or against interest intervention), or not recommended if the overall quality of the evidence in the key endpoints was very low, and ultimately the full MDT experts voted and reached consensus. Strength of evidence recommendations and level of evidence criteria in treatment guidelines (Table 1).

2 Overview of IVIG

2.1 Mechanism of IVIG

IVIG is used as a therapeutic substitution in primary and secondary immunodeficiencies as well as an immunomodulatory agent. IVIG has many immunomodulatory effects, however the exact mechanism for of this is not completely clear. At present, the mechanisms of IVIG include: (1) the Fc fragment of IgG specifically binds to some immune effector cells, closes the monocyte macrophage Fc receptor, and inhibits antibody-dependent cell-mediated cytotoxicity. (2) IVIG plays an immunosubstitutive role by binding to antigens in the $F(ab)_2$ fraction. A variety of specific antibodies in IVIG can either directly seal off the site of action of the organism's antigens, resulting in a decrease in the titer of pathogenic antibodies, or bind to the organism's antigens to form an antigen–antibody complex that is phagocytosed by the phagocytes. (3) IVIG has a broad spectrum of anti-normal human protein and anti-idiotypic antibody, which can accelerate the clearance and neutralization of circulating autoantibodies by seizing the action site of autoantibodies. (4) Anti-inflammatory effect: IVIG can regulate the secretion of various cytokines and inhibit the production of pro-inflammatory cytokines, thus achieving anti-inflammatory effect. In addition, IVIG may also exert potential anti-inflammatory effects through complement inhibition, blockade of Fas ligand-mediated apoptosis, and other mechanisms, and these mechanisms are not mutually exclusive but synergistic. (5) Eliminating pathogenic microorganisms: IVIG contains a variety of anti-bacterial toxin antibodies, neutralizing superantigens contained in bacterial toxins, and can eliminate pathogenic microorganisms such as viruses and bacterial toxins that persist in the body (9–12). (6) FcγRIII regulates dendritic cell properties: IVIG enhances the production of IL-1 and inhibits the production of IL-12 by inhibiting the differentiation and maturation of DCs (13) (Figure 1).

2.2 Adverse reactions

The risk of infectious complications from IVIG is extremely low. The requirements for donor screening and infectious disease testing for imported plasma are very stringent. In addition, at least one step in the IVIG manufacturing process must remove the enveloped virus, while at least two complementary viral inactivation methods must be used to prevent infectious pathogens that may be present during the screening

TABLE 1 Strength of evidence recommendations and level of evidence criteria in treatment guidelines.

Level of evidence standards	
Grade A	Meta-analysis or systematic review of multiple randomized controlled trials; Multiple randomized controlled trials (RCT) or one RCT with sufficient sample size
Grade B	At least one high-quality RCT
Grade C	Controlled trials that are not randomized but are well-designed; or well-designed cohort or case–control studies
Grade D	Case series without concurrent controls or expert consensus
Strength of evidence recommendations	
Class I	If a randomized controlled trial cannot be done, strong recommendation was defined when > 85% of panelists, agreed with a statement
Class II	If a randomized controlled trial cannot be done, weak recommendation was defined when 75–85% of panelists, agreed with a statement
Class III	If a randomized controlled trial cannot be done, no recommendation was defined when < 75% of panelists, agreed with a statement

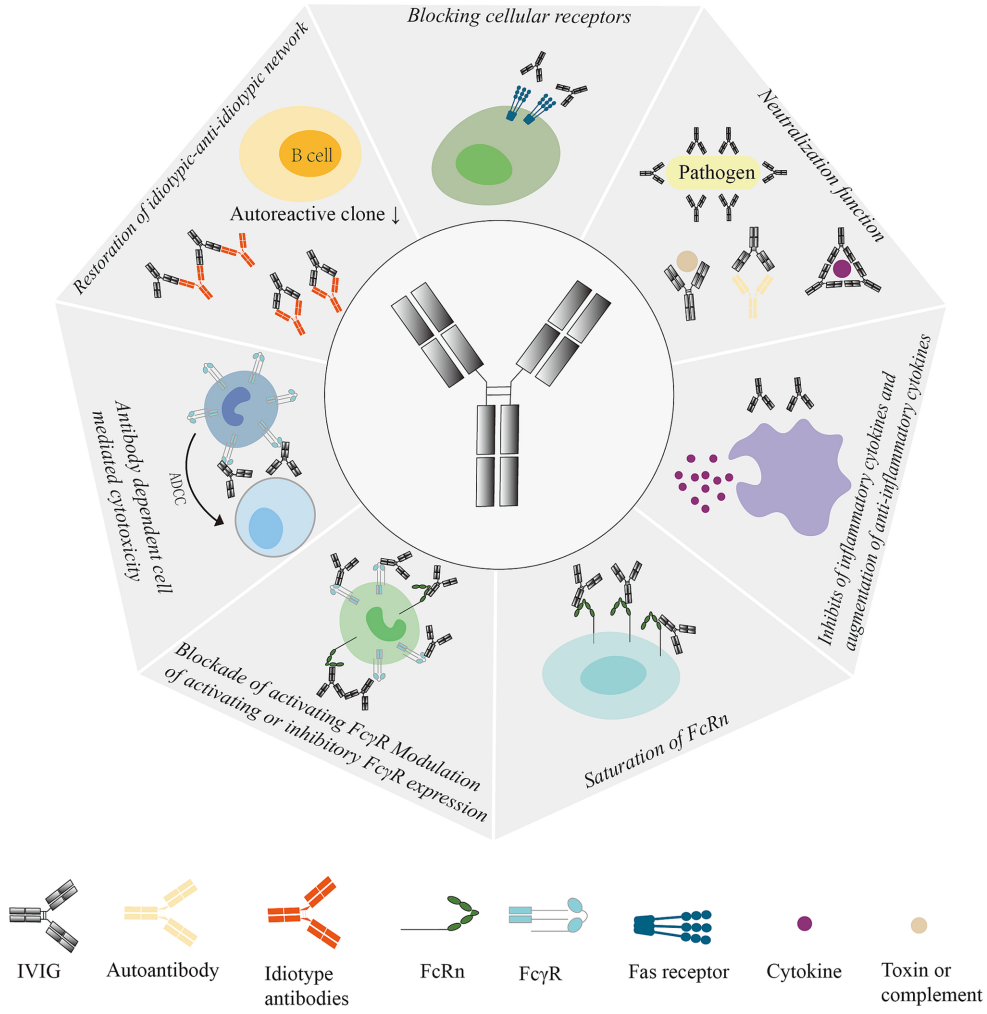

FIGURE 1
Mechanism of action of IVIG: Regulates Fc receptor expression and function, interferes with complement activation and regulates cytokine secretion, delivers anit-idiotype antibodies, neutralizes bacterial toxins, and regulates dendritic cell, T-cell, and B-cell activation, differentiation, and their effector functions. ADCC, antibody-dependent cell-mediated cytotoxicity; IVIG, Intravenous Immunoglobulin; Fas, Factor-related Apoptosis; FcRn, neonatal Fc receptor; FcγR, IgG Fc receptor.

process. Recently, significant advances have been made in the way IVIG is produced, reducing the likelihood of the presence of infectious pathogens while ensuring safety and that side effects are minimized (14). However, IVIG, a blood product isolated from the combined plasma of thousands of healthy blood donors, still carries the risk of infectious agent transmission (15). There are few reports on the prospective data of IVIG-related adverse reactions. The overall incidence of IVIG infusion-related reactions is between 3 and 15%, and these reactions are usually phlogistic

in nature and self-limited (16). IVIG related reactions are headache, nausea, vomiting, fever, rash, etc. Mild to moderate adverse reactions can usually be alleviated or avoided by slowing down the infusion speed of IVIG or stopping the infusion. Patients with multiple reactions are given antipyretics and antihistamines in advance. Contraindications to IVIG include individuals who are allergic to human immunoglobulin and individuals with selective IgA deficiency with anti-IgA antibodies. Serious adverse reactions are severe allergic reactions, acute renal failure, thromboembolic events, aseptic meningitis occurrences, neutropenia, autoimmune hemolytic anemia and rare events of arthritis. In view of the lack of data on the severity and incidence of their potential adverse events, this Expert Advice concludes that clinicians should limit the prescription of IVIG and use it only when there is sufficient evidence (17), and since cost is a limiting factor as well as the huge cost of IVIG.

3 Application of IVIG in hematologic disorders

3.1 Immune thrombocytopenia

The earliest discovery of the efficacy of high-dose IVIG was in the treatment of primary immune thrombocytopenia (ITP) (18). ITP is an autoimmune disorder characterized by a decrease in platelet count due to destruction of platelets by the immune system. ITP treatment guidelines mention that the first line treatment of choice is glucocorticoids and IVIG (19). ITP was the first autoimmune disease to be treated with IVIG, which is as effective as corticosteroid treatment (20, 21). In cases of active bleeding or when corticosteroids are contraindicated in patients, IVIG can induce a rapid increase in platelet count and quickly stop bleeding. The effect begins 1–3 days and the peak lasts for 2–7 days (22). Different centers use different IVIG doses and regimens. A meta-analysis showed that there were no significant differences in clinical outcomes and progression to chronic ITP with low-dose IVIG (<2 g/kg) versus high-dose IVIG (>2 g/kg), and that low-dose IVIG had fewer adverse effects and was less costly (23). The American Society of Hematology guideline panel recommends a single dose of IVIG 0.8 to 1 g/kg or a short course of glucocorticoids as first-line treatment for ITP, but both can be used in combination in order to rapidly elevate platelet levels (24). Patients with ITP treated with IVIG must have weekly complete blood counts (CBC) to assess efficacy and duration of therapy. In addition, patients should be informed that headache due to aseptic meningitis may occur after administration (25).

Expert recommendation 1: For adult patients with acute ITP requiring treatment, the recommended first-line treatment is a single dose of IVIG 0.8–1.0 g/kg or 0.4 g/kg/d for 3 to 5 days, with repeated administration if necessary. For emergency treatment of patients with hemorrhage, glucocorticoids in combination with IVIG 1 g/kg/d for 2 days are recommended (Class I).

3.2 Hemophagocytic syndrome

Hemophagocytic syndrome (HPS), also known as hemophagocytic lymphohistiocytosis (HLH), is a syndrome of excessive inflammatory response caused by abnormal activation and proliferation of the lymphocyte, monocyte and macrophage systems, and secretion of large amounts of inflammatory cytokines, causing a series of inflammatory reactions. Depending on the etiology, HLH is categorized as primary and secondary. Primary HLH results in defective cytotoxic function of natural killer cells and T lymphocytes. Secondary HLH can be triggered by infection, malignancy, autoimmunity, etc. It is considered to be caused by temporary acquired immunodeficiency leading to defective NK cells, and the clinical manifestations are characterized by persistent fever, hepatosplenomegaly, pancytopenia, and hemophagocytosis found in bone marrow, liver, spleen, and lymph node tissues. The HLH-1994 regimen is suitable for all types of HLH First-line induction therapy, including etoposide and dexamethasone (26–28). The application of IVIG can inhibit the activity of macrophages and reduce the damage of tissues and cells through various anti-inflammatory mechanisms. At present, IVIG can be considered as an auxiliary treatment for HLH, especially in the early stage of infection-related HLH. Some patients show a good response to IVIG alone, and the treatment with IVIG can avoid the adverse reactions of other treatment medications. IVIG should be supplemented with treatment of infection and criteria for hematopoietic stem cell transplantation in accordance with treatment guidelines (29–31).

Expert recommendation 2: IVIG 0.4 g/kg/week can be added as co-infection supportive therapy or neutropenia co-infection in patients with HLH (Class I).

3.3 Thrombotic thrombocytopenic purpura

Thrombotic Thrombocytopenic Purpura (TTP) is a severe diffuse thrombotic microangiopathy characterized by microangiopathic hemolytic anemia, consumptive reduction of platelets, and organ damage (e.g., kidneys, central nervous system, etc.) due to microthrombosis. The pathogenesis of TTP mainly involves factors such as lack of activity of von Willebrand factor (vWF) lyase (ADAMTS13), abnormal release of vascular endothelial cell vWF, abnormal activation of complement, and abnormal activation of platelets, leading to microvascular thrombosis, microvascular hemolysis, and subsequent organ ischemia, hypoxia, and dysfunction, resulting in clinical symptoms of TTP pentad syndrome, namely thrombocytopenic purpura, microvascular hemolysis, central nervous system symptoms, fever, and kidney damage. Most TTP patients have a sudden onset and dangerous condition, with a mortality rate of up to 90% if left untreated. High-dose immunoglobulin may be effective in some patients who fail plasma exchange by inhibiting platelet aggregation and splenic destruction of platelets and red blood cells (32).

Expert recommendation 3: High-dose IVIG is recommended for patients with recurrent or refractory TTP, at a dose of 1.0 g/kg/d for 2 days or 0.4 g/kg/d for 5 days. If necessary, it can be given repeatedly, but the therapeutic effect may not be as good as plasma exchange (Class II).

3.4 Acquired hemophilia A

Acquired Hemophilia A (AHA) is an acquired hemorrhagic disease characterized by a decrease in FVIII activity (FVIII: C) due to the production of specific autoantibodies that inhibit FVIII in the body. AHA is often associated with severe life-threatening bleeding, and subcutaneous hematomas are a characteristic manifestation of AHA, in addition to muscle, joint, gastrointestinal, and vaginal bleeding. Effective hemostasis is achieved with correct diagnosis and prompt treatment. The principles of treatment include initial

hemostatic therapy (bypass, and activated human or activated porcine FVII are the current standard of care) and etiologic therapy (human or porcine FVIII), which depends on the severity of the hemorrhage and the characteristics of the antibodies (33, 34).

Expert recommendation 4: In patients with AHA who do not respond to immunosuppressive regimens, the administration of IVIG 1.0 g/kg/d for 2 days or 0.4 g/kg/d for 5 days is recommendedIVIG has poor efficacy in the AHA, and therefore its use for the purpose of blocking autoantibodies to FVIII is not recommended (Class II).

3.5 Autoimmune hemolytic anemia

Autoimmune hemolytic anemia (AIHA) is a type of hemolytic anemia caused by disorders in the regulation of the body's immune function, leading to the production of autoantibodies attached to the surface of red blood cells accelerating the destruction of red blood cells through the antigen–antibody reaction, typically mediated by complement activation. The production of autoantibodies involves many links of the immune system, such as the cross reaction between endogenous red blood cells and exogenous/environmental antigens, acquired factors such as infection and malignant tumors, structural change of auto-antigens and the disorder of antigen presentation, and the dysfunction of B cells and T cells. AIHA is usually categorized based on a positive direct antiglobulin test (DAT) and the optimal temperature required for the antibody to act on the erythrocyte membrane (hot, cold, and mixed forms), with the IgG-mediated warm-antibody phenotype being the most common. First line treatment for AIHA consists of glucocorticosteroids or glucocorticosteroids in combination with rituximab. For patients who fail glucocorticoid therapy, relapse, intolerant and dependent, second-line therapy is administered and the preferred regimen is rituximab. Third-line treatment includes splenectomy and immunosuppressive agents such as cyclosporine A, sirolimus, and azathioprine. AIHA may occur secondary to diseases that may trigger autoantibody production, such as chronic lymphocytic leukemia and systemic autoimmune diseases. In addition, the incidence of AIHA after HSCT is increasing (35). In common variable immunodeficiency, disease-associated warm antibody-type secondary AIHA, maybe present in some patients with this condition. In addition to the risk of infection caused by the underlying immunodeficiency itself, such patients are further prone to severe and life threatening infections especially after glucocotricoids, immunosuppressants or rituximab, and the administration of intravenous immunoglobulin is recommended to elevate the level of immunoglobulin during the course of treatment and reduce the risk of infection (36).

Expert recommendation 5: High-dose IVIG can be used for life-threatening hemolysis or hemolysis for which other treatments are ineffective, and it is recommended that high-dose corticosteroids be given in combination with IVIG 1.0 g/kg/d for 2 days or 0.4 g/kg/d for 5 days as salvage therapy only in cases of severe or rapid hemolysis (Class I).

3.6 Hypogammaglobulinemia

Hypogammaglobulinemia (HG) is an immune system disorder, defined as serum IgG < 7 g/L, which results in lower antibody serum levels and an increased risk of infection due to the failure of the immune system to produce enough immunoglobulin (37). HG may be caused by various underlying primary/congenital immune system defects or secondary immune deficiency states (such as hematological malignancies, protein loss diseases, etc.). Primary humoral immune deficiencies are most commonly X-linked agammaglobulinemia (XLA) and common variable immune deficiencies. The most common clinical features of HG are recurrent bacterial infections, malabsorption syndrome, steatorrhea, protein-losing enteropathy, etc. Primary immunodeficiencies may be associated with a variety of autoimmune diseases, such as AIHA, ITP, dermatomyositis, etc., and malignant tumors (38, 39).

Expert recommendation 6: When complications related to primary immunodeficiency, such as infection, occur in HG patients, prophylactic treatment IVIG infusion, IVIG 0.4 g/kg/dose, is used, recommended every 3 weeks typically for the remainder of the patient's life.

4 Application of IVIG in the treatment of hematological tumors

4.1 Chimeric antigen receptor T-cell immunotherapy

Chimeric antigen receptor T-Cells (CAR-T) are genetically engineered to integrate gene fragments of single-chain variable regions and co-stimulatory molecules targeting tumor antigens into the T-cell genome and express them on T-cells, which specifically recognizes tumor antigens and initiates the downstream signaling pathway to proliferate, activate, and exert a CAR-T cell targeted tumor-killing effect, and the common targets of CAR-T cell therapy for refractory/relapsed acute B-lymphoblastic leukemia are CD19 and CD22 (40). CAR-T cell products have been used to achieve good efficacy in the treatment of relapsed refractory B-cell tumors, with CD19 as the main target, and the indications mainly include diffuse large B-cell lymphoma, condylomatous cell lymphoma, and follicular lymphoma (41). CAR-T cell in the treatment of relapsed and refractory multiple myeloma is targeted at B cell maturation antigen (BCMA), and many other CAR-T targets such as CS1 (CS1 is a cell surface glycoprotein of the signaling lymphocyte activation molecule (SLAM) receptor family), G protein-coupled receptor class C group 5 member D (GPRC5D), CD38 and CD138 have also entered clinical trials (42). Pretreatment chemotherapy regimens prior to infusing CAR-T cells back into the body can cause lymphocyte exhaustion (43), CAR-T cell therapy also destroys normal B cells, and the majority of patients receiving CAR-T therapy have varying degrees of hypogammaglobulinemia or B-cell deficiencies (44, 45).This along with other factors may lead to increased risk of infections after CAR-T therapy. After receiving BCMA CAR-T cell therapy, 76% of patients with multiple myeloma developed hypogammaglobulinemia, increasing the risk of infection (46). The incidence of infection within 28 days after reinfusion of CAR-T in patients with acute lymphoblastic leukemia was 40% (47), while in another clinical trial of BCMA CAR-T cell therapy for multiple myeloma, the incidence of infections ranged from 42 to 69% (48).The consensus among Chinese experts is that prophylactic intravenous immunoglobulin (IVIG) is a routine adjuvant therapy for patients receiving CAR-T cell therapy. National Comprehensive Cancer Network (NCCN) guidelines recommend that patients receiving CAR-T cell therapy should be regularly supplemented

with immunoglobulin infusions. The European Society for Hematology and Bone Marrow Transplantation and the American Society for Hematology and Bone Marrow Transplantation recommend that IVIG replacement therapy after CAR-T therapy should follow the X-linked absence of gammaglobulinemia principle (48).

Expert recommendation 7: After CAR-T cell therapy, the number of B lymphocytes and immunoglobulin level should be checked regularly, and IVIG infused at least once a month, at a dose of 0.4 g/kg/dose, until immunoglobulins and B cells returned to normal range or 6 months after CAR-T cell therapy (Class I).

Expert recommendation 8: Patients with serum IgG < 4 g/L and severe or recurrent infections after CAR-T cell therapy should continue to receive IVIG infusion at least once a month at a dose of 0.4 g/kg/dose until the risk factors are eliminated. If the serum IgG level is 4–6 g/L and there is still severe or recurrent infections after treatment, IVIG infusion should be given at least once a month at a dose of 0.4 g/kg/dose. If the serum IgG is greater than 6 g/L and there are recurrent infections, it is recommended to further evaluate the levels of the types of immunoglobulins (IgG, IgA, IgM and IgE) and the number of B cells (Class I).

4.2 Chemotherapy for acute leukemia

Intense chemotherapy is the main method for patients with acute leukemia (AL) to alleviate their illness and prolong their survival. However, AL supplemented is affected by various factors such as severe granulocyte deficiency after chemotherapy and increased risk of infection associated with the use of immunosuppressants and glucocoticoids causing significant economic burden and increased morbidity and mortality. Therefore, it is extremely important to prevent and actively control the occurrence of infection after AL chemotherapy. Although B cells are affected and immunoglobulin levels maybe decreased in AL patients receiving chemotherapy, IVIG infusion has not been widely and systematically studied for the prevention or treatment of related infections in AL patients. Most infections are due to the disease itself and/or chemotherapy-related neutropenia, and for patients who are in remission and have completed treatment, the immune deficiency may last for 6–12 months after the treatment is completed (49). When neutropenia occurs after AL chemotherapy, the inflammatory response mediated by neutrophils is not significant. Severe neutropenia most commonly occurs during hematopoietic stem cell transplantation, AL initial induction chemotherapy, and high-dose chemotherapy consolidation stage. It is necessary to identify neutropenic fever early and start empirical systemic antimicrobial therapy in a timely manner to avoid sepsis and death. It is important to administer IVIG simultaneously with anti-infective therapy (50).

Expert recommendation 9: Individualized assessment of the benefits and risks of IVIG infusion in patients with AL is recommended, and after weighing the risks and costs associated with treatment, at least one IVIG infusion per month is recommended at IVIG 0.4 g/kg/dose, with a target serum IgG of 4 to 6 g/L, and in the presence of breakthrough infections, a serum IgG > 6 to 8 g/L. Serum IgG levels, specific antibody titers and infection pattern are used to guide the IVIG treatment (Class II).

AL-intense chemotherapy can also cause severe myelosuppression, and repeated transfusion therapy is required after chemotherapy. Repeated transfusions can lead to a number of adverse transfusion reactions and platelet ineffective transfusion can occur in some patients with repeated platelet transfusion, which is mainly attributed to the development of specific antibodies against human leukocyte antigens. AL can also cause a decrease in ABO blood type antigens in patients, affecting clinical blood matching, increasing the risk of hemolytic reactions due to blood type incompatibility, and even leading to patient death in severe cases. The application of IVIG can block the Fc receptors of monocytes and macrophages, reducing the destruction of tissue cells mediated by allogenic antibodies and thereby, reducing adverse reactions to blood transfusion, and improving safety.

Expert recommendation 10: For AL patients with ineffective repeated platelet transfusions and obvious adverse reactions to blood transfusion, IVIG before transfusion can be recommended at a dose of 0.2–0.4 g/kg/dose (Class II).

4.3 Treatment of multiple myeloma

Infection remains a major cause of morbidity and mortality in patients with multiple myeloma (MM) due to the cumulative effect of disease, treatment and host-related factors. Disease-related plasma cell abnormalities, the effects of antitumor therapy, older age of onset and disease-related complications (e.g., renal failure) all contribute to an increased susceptibility to infections in patients with myeloma, and therefore the prevention of infections during the treatment of MM is of utmost importance. Prevention of infection during the process is crucial, and optimal prevention strategies include antimicrobial prophylaxis, infection control measures, and IVIG infusion in some patients (51). The NCCN guidelines mention that the risk of infection in MM patients is associated with treatments such as autologous HSCT, bispecific antibodies, CAR-T cell therapy, cytotoxic chemotherapy drugs, proteasome inhibitors, anti-CD38 monoclonal antibodies, glucocorticoids, etc. 75% of patients receiving bispecific antibody therapy in the MajesTEC-1 study developed hypogammaglobulinemia (52). An earlier study retrospectively analyzed 3,000 MM patients showed 10% died within 60 days, 45% of whom died of infections (53). It has also been shown that myeloma patients receiving IVIG prophylaxis had fewer cases of serious infections than patients who did not receive IVIG, and that prophylactic use of IVIG reduced the risk of serious infections by 90% in MM patients treated with bispecific antibodies to BCMA (54, 55). Therefore, 0.4 to 0.6 g/kg IVIG infusion every month for 6–12 months is beneficial to MM patients, and the dosage and time should be adjusted according to the patient's condition to ensure full prevention of infection (56). IVIG has a major limitation: it accelerates immunoglobulin metabolism in people with high levels of myeloma protein. There are two points to note when administering IVIG: (1) Immune modulators such as thalidomide, lenalidomide, and pomalidomide increase the risk of thrombosis during treatment, and IVIG infusion can further increase the risk of thrombosis (57). (2) Some MM patients may experience renal dysfunction, and IVIG infusion may further increase the risk of renal injury. Currently, most IVIG products remove sucrose (previously used as a stabilizer), which can reduce the risk of IVIG renal injury (58).

Expert recommendation 11: For MM patients with serum IgG ≤4 g/L and severe or recurrent infections, at least one IVIG infusion

of 0.4 g/kg/dose per month is recommended, and for MM patients after CAR-T cell therapy, at least one IVIG infusion of 0.4 g/kg/dose per month is recommended until 1 year after the end of CAR-T therapy (Class II).

4.4 Treatment of non-Hodgkin lymphoma

Non-Hodgkin's lymphoma (NHL) is the most common malignant tumor of lymphatic system, among which B cell-derived lymphoma accounts for more than 85% of the cases (59). Chemotherapy can effectively treat lymphoma, but chemotherapy not only leads to bone marrow suppression, but also has an immunosuppressive effect. CD20 monoclonal antibody is a major breakthrough in the treatment of B-cell lymphoma. However, CD20 monoclonal antibody in combination with chemotherapy leads to a further decrease in the immune function of the patient, predisposing to a variety of infections, such as herpes zoster, lung infections and others. CD20 monoclonal antibody kills abnormal and normal B lymphocytes at the same time, which reduces the number of B lymphocytes in peripheral blood and antibody production, eventually leading to humoral immune deficiency and increasing the risk of infection in NHL patients (60). The use of targeted drugs (such as Bruton's tyrosine kinase inhibitors), immune modulators (such as lenalidomide), immune checkpoint inhibitors, and other drugs can also increase the incidence of infection (61, 62). The guidelines of the British Hematology Standards Committee suggest that NHL patients should receive regular IVIG infusions during treatment to reduce the occurrence of infections (63), while the NCCN guidelines recommend that NHL patients receiving CD20 monoclonal antibody and CAR-T cell therapy should receive regular IVIG infusions.

Expert recommendation 12: NHL patients need regular infusion of IVIG to enhance immunity and prevent infection during treatment, especially for NHL patients with IgG $\leq$ 4 g/L, recurrent or severe infection, using IVIG infusion every 3–4 weeks, IVIG 0.4 g/kg/dose (Class II).

5 Application of hematopoietic stem cell transplantation

HSCT, especially allogenetic stem cell transplantation (allo-HSCT), is still the only cure for many benign and malignant hematological diseases (64, 65). Meanwhile, post-transplant complications such as infection, graft-versus-host disease (GVHD), thrombotic microangiopathy, and sinusoidal obstruction syndrome (SOS), also know as Veno-occlusive Disease (VOD) are closely related to transplantation-related mortality, and these complications have hindered transplantation development (66, 67). Human immunoglobulin has synergistic anti-infection and immune regulation functions, and is often used to control infection and complications such as GVHD after transplantation. After HSCT, IVIG can effectively prevent cytomegalovirus (CMV) infection, GVHD, bacterial infection, etc. The FDA of the United States approved IVIG as a preventive drug for patients with HSCT over 20 years old to reduce the incidence of infection complications such as pneumonia (11).

5.1 Application in post-transplant CMV infection

CMV infection is one of the main causes of infection and poor prognosis in patients undergoing allogeneic HSCT. CMV can lead to CMV disease, acute and chronic GVHD, opportunistic infection, bone marrow suppression and other serious adverse events, affecting the prognosis of patients undergoing HSCT. The incidence of CMV disease ranges from 10 to 40%, with CMV pneumonia being the most predominant type, with a mortality rate as high as 70%. Due to the wide application of prevention and preemptive treatment, the incidence of CMV disease has been reduced to less than 10%, with a case fatality rate of about 20 to 60% (68). The reactivation of CMV within 100 days after transplantation is accompanied by the increase of transplant-related mortality. The preemptive Treatment with antiviral drugs and the use of CMV-negative or leukocyte-depleted blood products have greatly reduced the incidence of CMV infection after transplantation (69). Early studies showed that preventive application of IVIG reduced CMV infection rate and mortality of interstitial pneumonia without CMV infection (70). IVIG combined with ganciclovir significantly changed the prognosis of patients with CMV pneumonia after transplantation. However, Schmidt et al. gave prophylactic IVIG to patients at high risk of CMV disease after allograft transplantation and showed no significant difference in the rate of CMV infection, or in the cumulative incidence of GVHD, when compared to a control group that was not given prophylactic IVIG (70). Another study evaluated the weekly preventive use of IVIG 5 g from −7 days to +98 days after transplantation. The results showed that the cumulative incidence of CMV infection was similar between the subjects treated with IVIG and the control group at +100 days after transplantation. These data showed that IVIG had no obvious advantage for CMV reactivation in transplant patients who could receive antiviral drugs. However, whether the high-risk population of CMV reactivation can benefit from these interventions has not been confirmed by research (71).

Expert recommendation 13: It is recommended that IVIG can be used prophylactically in patients with CMV high-risk activators in allo-HSCT, with 1 to 2 weekly infusions of IVIG, IVIG 5 g to 10 g/dose, up to 100 days post-transplantation (Class II).

5.2 Application in GVHD after transplantation

At least 50% or more of transplant related deaths are directly or indirectly related to GVHD, and effective prevention and treatment methods for GVHD need to be sought. GVHD is often closely related to delayed immune function reconstruction in transplant patients, and promoting the formation of immune reconstruction is the fundamental way to solve complications after allo HSCT. Induced immune tolerance means that after transplantation the recipient is resistant to rejection and the graft maintains stable function for a long time (72). The research hotspots in recent years mentioned that the role of intestinal flora in the body's anti-tumor immune response including chemotherapy and allo-HSCT has received increasing attention.

TABLE 2 15 recommendations were proposed for the application of IVIG in the treatment of hematological diseases, hematological tumors, and hematopoietic stem cell transplantation.

Disease	Uses and dosage	Strength of evidence recommendation and expert agreement
Application of IVIG in hematologic disorders		
ITP	For adult patients with acute ITP requiring treatment, the recommended first-line treatment is a single dose of IVIG 0.8–1.0 g/kg or 0.4 g/kg/d for 3 to 5 days, with repeated administration if necessary.	Class I; 100%
	For emergency treatment of patients with hemorrhage, glucocorticoids in combination with IVIG 1 g/kg/d for 2 days is recommended.	
HPS	IVIG 0.4 g/kg/week can be added as co-infection supportive therapy or neutropenia co-infection in patients with HLH.	Class I; 87.9%
TTP	High-dose IVIG is recommended for patients with recurrent or refractory TTP, at a dose of 1.0 g/kg/d for 2 days or 0.4 g/kg/d for 5 days. If necessary, it can be given repeatedly, but the therapeutic effect may not be as good as plasma exchange.	Class II; 78.8%
AHA	In patients with AHA who do not respond to immunosuppressive regimens, the administration of IVIG 1.0 g/kg/d for 2 days or 0.4 g/kg/d for 5 days is recommended IVIG has poor efficacy in the AHA, and therefore its use for the purpose of blocking autoantibodies to FVIII is not recommended.	Class II;78.8%
AIHA	High-dose IVIG can be used for life-threatening hemolysis or hemolysis for which other treatments are ineffective, and it is recommended that high-dose corticosteroids be given in combination with IVIG 1.0 g/kg/d for 2 days or 0.4 g/kg/d for 5 days as salvage therapy only in cases of severe or rapid hemolysis.	Class I; 93.9%
HG	When complications related to primary immunodeficiency, such as infection, occur in HG patients, prophylactic treatment IVIG infusion, IVIG 0.4 g/kg/dose, is used, recommended every 3 weeks typically for the remainder of the patient's life.	Class II; 75.8%
Application of IVIG in the treatment of hematological tumors		
Chimeric antigen receptor T cell immunotherapy	After CAR-T cell therapy, the number of B lymphocytes and immunoglobulins level should be checked regularly, and IVIG infused at least once a month, at a dose of 0.4 g/kg/dose, until the immunoglobulins and B cells returned to normal range or 6 months after CAR-T cell therapy.	Class I; 90.9%
	Patients with serum IgG < 4 g/L and severe or recurrent infections after CAR-T cell therapy should continue to receive IVIG infusion at least once a month at a dose of 0.4 g/kg/dose until the risk factors are eliminated.	Class I; 90.9%
	If the serum IgG level is 4–6 g/L and there is still severe or recurrent infections after treatment, IVIG infusion should be given at least once a month at a dose of 0.4 g/kg/dose.	
	If the serum IgG is greater than 6 g/L and there are recurrent infections, it is recommended to further evaluate the levels of other types of immunoglobulins (IgG, IgA, IgM and IgE) and the number of B cells as well as specific antibody titers.	
Chemotherapy for acute leukemia	Individualized assessment of the benefits and risks of IVIG infusion in patients with AL is recommended, and after weighing the risks and costs associated with treatment, at least one IVIG infusion per month is recommended at IVIG 0.4 g/kg/dose, with a target serum IgG of 4 to 6 g/L, and in the presence of breakthrough infections, a serum IgG > 6 to 8 g/L. Serum IgG levels, specific antibody titers and infection pattern are used to guide the IVIG treatment.	Class II; 75.8%
	For AL patients with ineffective repeated platelet transfusion and obvious adverse reactions to blood transfusion, IVIG before transfusion can be recommended, at a dose of 0.2–0.4 g/kg/dose.	Class II; 78.8%
Treatment of multiple myeloma	For MM patients with serum IgG ≤4 g/L and severe or recurrent infections, at least one IVIG infusion of 0.4 g/kg/dose per month is recommended, and for MM patients after CAR-T cell therapy, at least one IVIG infusion of 0.4 g/kg/dose per month is recommended until 1 year after the end of CAR-T therapy.	Class II; 81.8%
Treatment of non-Hodgkin lymphoma	NHL patients need regular infusion of IVIG to enhance immunity and prevent infection during treatment, especially for NHL patients with IgG ≤ 4 g/L or recurrent infection or severe infection. The dose of IVIG is 0.4 g/kg/dose every 3–4 weeks.	Class II; 75.8%
Application of hematopoietic stem cell transplantation		
Application in post-transplant CMV infection	It is recommended that IVIG can be used prophylactically in patients with high risk for CMV activation in allo-HSCT, with 1 to 2 weekly infusions of IVIG, 5 g to 10 g/dose, up to 100 days post-transplantation.	Class II; 84.8%

(Continued)

TABLE 2 (Continued)

Disease	Uses and dosage	Strength of evidence recommendation and expert agreement
Application in GVHD after transplantation	IVIG 0.5 g/kg per week from pre-transplantation −7 days to post-transplantation day 90 and 0.5 g/kg per month from post-transplantation day 90 to 1 year post-transplantation is recommended for those at high risk for GVHD when mismatched with allo-HSCT.	Class II; 84.8%
Application in post-transplant bacterial infections	IVIG 0.5 g/kg per week is recommended for patients with serum IgG <4 g/L from 7 days pretransplant to 90 days posttransplant, and 0.5 g/kg per month from 90 days posttransplant to 1 year posttransplant, so as to prevent serious bacterial infection. Serum IgG concentration should be monitored every 2 weeks, and individualized treatment should be carried out according to serum IgG level and infection.	Class II; 75.8%

The gut microbiota plays an important role in the pathological and physiological processes of GVHD (73). Changes in gut microbiota may determine the severity of GVHD. After transplantation, the composition of gut microbiota is altered, leading to dysbiosis. Gut microbiota can easily penetrate damaged intestinal mucosa, causing abnormal immune responses, activating T lymphocytes, promoting the release of inflammatory mediators, and causing damage to the gastrointestinal mucosal barrier, thereby damaging target organs such as the gastrointestinal tract (74–76). Based on the immunomodulatory effect of IVIG, a large randomized controlled study was conducted on allo-HSCT patients, comparing the weekly administration of IVIG 0.5 g/kg from −7 days to +90 days after transplantation, followed by monthly administration of IVIG 0.5 g/kg from 90 days to 360 days. Multivariate analysis showed that compared with IVIG recipients, the control group had an increased risk of acute GVHD > grade 2 (RR 1.63, $p < 0.0056$) (77). In another study, more than 600 subjects received 0.1 g/kg, 0.25 g/kg or 0.5 g/kg IVIG at random, once a week until the 90th day after transplantation, and then once a month until 1 year after transplantation. The study showed that the incidence of acute GVHD was the lowest in the HLA-matched unrelated donor group and the subjects who received the highest dose of IVIG (78).

Expert recommendation 14: IVIG 0.5 g/kg per week from pre-transplantation −7 days to post-transplantation day 90 and 0.5 g/kg per month from post-transplantation day 90 to 1 year post-transplantation is recommended for those at high risk for GVHD when mismatched with allo-HSCT (Class II).

5.3 Application in post-transplant bacterial infections

HSCT patients undergo myeloablative chemotherapy. After ultra-intensive pretreatment and radiotherapy, the patients are in the ablative period of bone marrow, and the process of immune function reconstruction is longer, with longer period of severe neutrophil deficiency. In addition, the incidence of various infections such as bacteria, fungi and viruses is higher after long-term use of immunosuppressants such as corticosteroids. Regarding whether IVIG is needed to prevent bacterial infection during transplantation, the relevant guidelines of the American Society of Blood and Bone Marrow Transplantation unanimously recommend that IVIG should not be routinely used to prevent bacterial infection after transplantation (79). However, when the patient is complicated with severe hypogammaglobulinemia (serum IgG < 4 g/L), it is suggested to receive preventive IVIG infusion from the beginning of pretreatment chemotherapy before transplantation to 100 days after transplantation to maintain serum IgG > 4 g/L. There is a lack of sufficient randomized controlled studies to support this recommendation, and the only objective data are based on IVIG pharmacokinetic studies showing a half-life of approximately 6 days in transplanted patients and 22 days in normal subjects, and the explanation for this discrepancy may be the increased proteolytic metabolism and reduced protein conversion and synthesis due to GVHD (80).

Expert recommendation 15: IVIG 0.5 g/kg per week is recommended for patients with serum IgG <4 g/L from 7 days pretransplant to 90 days posttransplant, and 0.5 g/kg per month from 90 days posttransplant to 1 year posttransplant, so as to prevent serious bacterial infection. Serum IgG concentration should be monitored every 2 weeks, and individualized treatment should be carried out according to serum IgG level and infection (Class II).

6 Conclusion

This consensus is based on the existing evidence both domestically and internationally, as well as the careful discussions organized by the Chinese Infection Immunology and Microecology Research Translation Collaborative Group with relevant experts. Based on the evidence grading method, 15 suggestions were proposed for the application of IVIG in the treatment of hematological diseases, hematological tumors, and hematopoietic stem cell transplantation (Table 2). It is hoped to serve as a reference for clinicians and help standardize the dosage of IVIG used to treat hematological diseases.

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Validation, Writing – review & editing. ZhX: Data curation, Supervision, Validation, Writing – review & editing. LL: Data curation, Supervision, Validation, Writing – review & editing. DW: Data curation, Supervision, Validation, Writing – review & editing. NZ: Data curation, Supervision, Validation, Writing – review & editing. HuZ: Data curation, Supervision, Validation, Writing – review & editing. ZZ: Data curation, Supervision, Validation, Writing – review & editing. WD: Data curation, Supervision, Validation, Writing – review & editing. XJX: Data curation, Supervision, Validation, Writing – review & editing. HaZ: Data curation, Supervision, Validation, Writing – review & editing. LD: Data curation, Supervision, Validation, Writing – review & editing. JM: Data curation, Supervision, Validation, Writing – review & editing. ZS: Data curation, Supervision, Validation, Writing – review & editing. LS: Writing – review & editing. YC: Data curation, Supervision, Validation, Writing – review & editing. YaL: Data curation, Supervision, Validation, Writing – review & editing. RQ: Data curation, Supervision, Validation, Writing – review & editing. GL: Data curation, Supervision, Validation, Writing – review & editing. PC: Data curation, Supervision, Validation, Writing – review & editing. HoZ: Data curation, Supervision, Validation, Writing – review & editing. JL: Supervision, Validation, Writing – review & editing, Data curation. YuL: Data curation, Supervision, Validation, Writing – review & editing. JJL: Data curation, Supervision, Validation, Writing – review & editing. ZiX: Data curation, Supervision, Validation, Writing – review & editing. SS: Data curation, Supervision, Validation, Writing – review & editing. XCX: Data curation, Supervision, Validation, Writing – review & editing. QMW: Data curation, Supervision, Validation, Writing – review & editing. QW: Data curation, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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COVID-19 and blood group-related antigens: can natural anti-carbohydrate antibodies provide innate protection from symptomatic SARS-CoV-2 infection?

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Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily targets respiratory mucosa, causing coronavirus disease 2019 (COVID-19). Susceptibility and severity of COVID-19 may be influenced by predisposing factors including blood groups. In this study, we investigated whether natural anti-carbohydrate antibodies provide innate protection against SARS-CoV-2 and influence disease severity.

Methodology: We used samples (plasma and saliva) from a longitudinal cohort study in Bangladesh that enrolled 100 COVID-19 symptomatic and asymptomatic patients. We also enrolled 21 and 38 healthy controls during the pandemic period and pre-pandemic period, respectively. We phenotype ABO blood grouping from blood and determined Lewis and secretor status (H antigen) from the saliva samples. We quantified natural anti-carbohydrate antibodies (anti-A, anti-B, anti-Tn-Mono and anti-αGal IgG, IgA, and IgM) from plasma collected at enrollment. We also explored the trend of natural anti-carbohydrate antibodies until 3 months of convalescence period among the COVID-19 patients (day 14 and day 90 from enrollment). Antibody quantification and ABH/Lewis phenotyping were performed using enzyme-linked immunosorbent assay (ELISA).

Results: We included 99 COVID-19 patients and 59 healthy controls assessing the differences of natural antibody titer during enrollment, while 95 patients were analyzed exploring Lewis and secretor status with natural antibody titer and disease status. We did not find significant difference in the distribution for neither ABO blood groups nor non-secretors and Lewis-negative individuals among asymptomatic or symptomatic patients and healthy controls. Nonetheless, we observed lower anti-A antibody titers among symptomatic patients compared to healthy controls. We also identified slight differences in antibody titers linked to age and gender. Anti-A and anti-B antibodies among asymptomatic patients had a higher trend up to 3 months from infection compared to symptomatic patients.

Conclusion: Higher natural anti-A and anti-B antibody titers may offer protection against symptomatic COVID-19 infections. Gender and blood group differences indicate potential innate immune factors influencing disease severity, but larger studies are needed to confirm these findings.

KEYWORDS

Lewis status, secretor status, natural anti-carbohydrate antibodies, COVID-19, Bangladesh, SARS-CoV-2, HBGA, blood group

1 Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets the respiratory mucosa, and it can infect and replicate in the gastric and intestinal epithelium, causing coronavirus disease 2019 (COVID-19). COVID-19 is a complex and multifactorial disease, where inherited predispositions with comorbidities and risk factors are likely to influence the severity of the disease (1, 2). Most studies have found that blood group O individuals were protected to some extent against SARS-CoV-2 infection while A and/or B blood group individuals were more susceptible to infection (3–6). Some studies also reported some protection of the O individuals against severe disease. Whereas meta-analyses provided evidence that there may be a link between ABO and susceptibility to infection, the link between ABO and severity of the disease appears poorly reliable (7–9). The histo-blood group antigen (HBGA) family, including the ABO blood groups, is expressed on many cell types, particularly epithelial cells of the gastrointestinal tract, upper respiratory tract, and lower genito-urinary tract. Coronaviruses are enveloped viruses whose main envelope protein, the Spike S protein is heavily glycosylated. The sugar coat provides the so-called glycan shield that protects the virus from the adaptive immune response. However, since coronaviruses replicate in cells of the upper respiratory tract, the S protein of virion can express HBGAs, depending on the patient's genetic polymorphism for these antigens (10, 11).

Individuals who possess the active α1, 2-fucosyltransferase enzyme (FUT2) are known as secretor (Se), while mutations on *FUT2* gene lead to lack or decreased α1,2-fucosyltransferase activity. Therefore, absence of the α1, 2 fucosylated antigens in mucosal tissues and secretions (e.g., saliva) results in individual phenotype as non-secretor (se) which represents approximately 20% of Caucasian and African populations (12–15). Similarly, the *FUT3* gene codes an α 1, 3 or 1, 4 fucosyltransferase which can generate Lewis antigens by modifying precursor oligosaccharides (type 1)/H-type 1 or precursor oligosaccharides (type 2)/H-type 2 antigens to form Le^a /Le^b and Le^x/Le^y antigens, respectively, thus making an individual Lewis-positive (Le+). Lewis negative (le-) individuals lack Le^a and Le^b regardless secretor status (16). The European population have approximately 4–11% Lewis negative (Le a- b-) and non-secretors (se), whereas approximately 29 and 11% of individuals contribute to African and Asian populations, respectively (15, 17).

Earlier study of SARS-CoV outbreak in Hong Kong during the 2003 outbreak showed that blood group O individuals have low risk of being infected by SARS-CoV-1 compared to non-O blood group individuals (18). The anti-A antibodies were observed to have the ability to inhibit the interaction between SARS-CoV spike protein, produced in cells expressing the A antigen, and its cellular receptor ACE2 (19). It is thus conceivable that this association can be attributed to protection exerted by anti-blood group antibodies and not the blood group antigens (19, 20). The expression of ABH antigens in epithelial cells where SARS-CoV replicates is also controlled by polymorphisms of the *FUT2* gene. Thus, individuals with two *FUT2* null alleles, the so-called non-secretors, are unable to synthesize H antigen and hence A or B antigens in these cells (19).

Since SARS-CoV-2 replicates primarily in epithelial cells of the upper respiratory tract epithelial cells that express these carbohydrate antigens and also use ACE2 as a receptor, our working hypothesis was to verify that the presence of natural anti-carbohydrate antibodies, including anti-blood group A and B antibodies, could confer a certain level of innate protection against infection by SARS-CoV-2 and can explain the association between ABO phenotype and the severity of infection by SARS-Cov2.

2 Methods

2.1 Study participants

This is a prospective cohort study as mentioned previously (1). In brief, we enrolled 100 COVID-19 patients between November 2020 and February 2021 in Dhaka, Bangladesh. All patients aged 18 years and above were confirmed SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) positive for the first time prior to or during enrollment. We used WHO guideline of COVID-19 (clinical symptoms and oxygen saturation) for determining severity of the patients which were collected from the hospital records on admission or the patient's condition during enrollment (21) and categorized them into asymptomatic, mild, moderate, and severe (25 patients per severity group). Patients who gave confirmed history of previous SARS-CoV-2 infection with RT-PCR positive results were excluded. Thirty-one age- and sex-matched healthy controls (pandemic controls) were enrolled at the same time period who were judged healthy by medical personnel, had no history of COVID-19, were RT-PCR negative for SARS-CoV-2 during enrollment, and had no clinical signs and symptoms of COVID-19 in the 2 weeks prior to enrollment. Blood specimens from COVID-19 patients for measuring antibody titer were collected prospectively on day 1(enrollment), day 14, and day 90. Saliva specimens were collected on day 1(enrollment), day 7, day 14, and day 28. However, in this analysis, we used saliva specimen only from any one of the day points for Lewis phenotyping and secretory/non-secretory characteristics. As for healthy controls, all samples were collected only during enrollment. ABO blood

grouping was done from blood specimen during enrollment by agglutination method. Additional available stored plasma and saliva samples from 38 participants of pre-pandemic period were included in this analysis as a representative of the local population in terms of the distribution of HBGA phenotypes (Lewis and secretor status).

2.2 Quantification of natural antibodies in plasma

The circulating anti-carbohydrate antibodies were assessed with the enzyme-linked immunosorbent assay (ELISA) from stored plasma specimen of patients and healthy controls. ELISA plates (F96 Maxisorp, Nunc, Thermo Fisher Scientific, Roskilde, Denmark) were coated with synthetic sugars (from Glyco NZ, Auckland New Zealand): A trisaccharide or A-Tri (GalNAcα1,3-(Fucα1,2)-Galß-PAA), B trisaccharide or B-Tri (Galα1,3-(Fucα1,2)-Galß-PAA), Tn monosaccharide or Tn-Mono (GalNAcα-PAA), or α-Gal trisaccharide (Galα1,3-Galβ1,4-GlcNAcβ-PAA) at 10 μg/mL in 0.1 M carbonate buffer pH 9.0 at 4°C overnight. The plates were washed six times in 1X phosphate buffer saline with 0.05% Tween 20 (PBS-0.05%(T), unbound sites were blocked with PBS 5% bovine serum albumin (BSA) for 2 h at 37°C. After six additional washes with PBS-T, plasma samples from COVID-19 patients or controls were added to the plate at a 1:100 dilution in PBS-1% BSA (duplicate wells for coated wells and single non-coated well) for 4 h at 4°C. Optimal dilutions had been chosen based on preliminary analyses performed using plasma samples from healthy blood donors and COVID-19 patients mentioned earlier (20). The plates were then washed six times with PBS-T, and biotinylated Goat anti-human Fcγ (Jackson ImmunoResearch Laboratories Inc., Ely, United Kingdom), biotinylated Goat anti-human IgA (Novus), and biotinylated-Goat anti-human IgM (Novus) were added at a 1/10000, 1/5000, and 1/5000 dilutions, respectively, in PBS-1% BSA for 1 h at 37°C. After another six washes with PBS-T, secondary conjugate Avidin-HRP (vector laboratories) was added to the plates at 1:3000 dilution in PBS-1% BSA and incubated for 1 h at 37°C. Finally, after five last washes with PBS-T and one with plain PBS, revelation was performed with 50 μL/well of 3,3′,5,5′-tetramethylbenzidine (Sigma Aldrich, St Louis, MO), and the reaction was stopped with 50 μL/well of 1 M phosphorous acid after 3 min. Optical densities were read twice at 450 nm with an EON BioTek spectrophotometer. The final value was obtained after subtracting the background value (non-coated wells) from the average value of the coated wells.

2.3 Secretor and Lewis phenotyping by ELISA on saliva

ABH and Lewis phenotyping was done by enzyme-linked immunosorbent assay (ELISA) on saliva. Saliva specimens were boiled for 10 min and briefly centrifuged, and the supernatants were diluted in carbonate–bicarbonate buffer solution at 1:1000 along with control positive saliva. ELISA plates (NUNC 96F Maxisorp; Thermo Fisher Scientific, Roskilde, Denmark) were coated with the diluted mucins, sealed with parafilm, and incubated at 4°C overnight. On the following day, plates were first washed with PBS-0.05%(T) for three times and then saturated in PBS 5% milk to block the non-specific sites and incubated for 1 h at 37°C in humid atmosphere. Afterward, the plates were washed again like before and 100 μL per well of primary antibodies anti-Lewis a (7Le, Thermo Fisher Scientific) and anti-Lewis b (2-25Le, Thermo Fisher Scientific) diluted at 1:500 in 5% PBS milk were introduced accordingly. After three washes with PBS-0.05%(T), plates were coated with secondary reagents anti-mouse-HRP (Uptima up446330, Interchim, Montluçon, France) at 1:1000 in 5% PBS milk and incubated for 1 h at 37°C. To determine the secretor status, 100 μL/well of biotinylated UEA-I lectin anti-H type 2 (Vector Laboratories, Newark, CA) were added to the corresponding wells. The lectin UEA-I was diluted at 1:500 saturated in PBS 5% milk. The plates were incubated for 1 h at 37°C like before and then coated with secondary reagents streptavidin-HRP (Vector Laboratories) at 1:3000 dilution in 5% PBS milk after three washes like before.

Finally, after three washes with PBS-0.05%(T) and two with PBS, reaction was initiated with 50 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) per well (BD OptEIA, BD Biosciences), incubated for 5–7 min at room temperature, and afterward stopped by loading 50 μL of 1 M phosphoric acid. Optical density (OD) of each plate was read at 450 nm by EON BioTek, and values equivalent to twice the background were considered positive. Individuals with a positive response to Ulex were considered to be secretors, and individuals with a positive response to anti Lewis a and/or or anti Lewis b were considered to be Lewis positive.

2.4 Statistical analysis

The mild, moderate, and severe COVID-19 patients from the original cohort were grouped into symptomatic COVID-19 patients. Demographic information, blood group (A, B, AB, and O), and clinical characteristics of the participants were stratified by health status (healthy control, asymptomatic, and symptomatic). Antibody titer was measured as geometric mean (GM) with 95% confidence interval (CI). Continuous variables were described as mean with 95% confidence interval (CI) or median with inter-quartile range (IQR) and frequency with percentage for categorical data. To identify the significant difference, *t*-test was used to compare the mean among the different groups, while for median and percentage, non-parametric Kruskal–Wallis rank-sum test or chi-square test was used, respectively. Antibody titers were measured as geometric mean (GM) with 95% CI. To assess the difference in antibody response between the groups, we conducted linear regression analysis on log-transformed antibody titers adjusted for age. We included age as a covariate to adjust for its potential confounding effect and an interaction term between age and symptom status to evaluate whether age modifies the relationship between symptom status and antibody titers. The significance of the coefficients of the models was tested using *t*-test that represents the difference in the log-transformed means between the groups adjusted for age. Since patients were enrolled at different time points from disease onset, we analyzed the trend of antibody titers over the convalescent period by categorizing the intervals from the date of disease onset to study follow-up dates, up to study day 90. Box plots and line plots with scattered points were created to see the distribution of clinical laboratory values in different groups. All analyses were done using GraphPad Prism version 6.0 and R statistical software version 4.2.2

("ggplot2" and "ggpubr" packages for the scatter and boxplot diagram and "dplyr" package for data).

3 Results

3.1 Demographic

A total of 99 COVID-19 patients (asymptomatic, $n = 25$; mild, $n = 25$; moderate, $n = 25$; and severe, $n = 24$) and 59 healthy controls were included in the analysis who had plasma during enrollment (Figure 1). From the pandemic healthy control participants, 10 participants were excluded from the final analysis due to SARS-CoV-2 RBD-specific antibody response.

The overall median age of the COVID-19 patients in this cohort was 46 years (IQR: 35, 57.5). Asymptomatic infection was observed among the younger patients [median age 35 years (IQR: 31, 44)], while older patients suffered from symptomatic SARS-CoV-2 infection [median age 50 years (IQR: 38, 62)] and the median age of healthy control (both pandemic and pre-pandemic) was 33 (IQR: 28.5, 41) (Table 1). Male patients suffered mostly from symptomatic infection (69%), while majority of the female patients (64%) had asymptomatic infection. Distribution of individuals by blood group was similar between the patient and healthy control populations.

In this cohort, we phenotyped Lewis and secretor status from 95 COVID-19 patients who had sufficient saliva samples stored. Among the healthy controls, 30.5% were non-secretors, whereas 25% asymptomatic patients and 40.8% symptomatic patients were non-secretors. Lewis negative individuals (Lewis a- b-) among the control groups were 11.9%, while asymptomatic and symptomatic patients consisted of 20.8 and 16.9% individuals (Table 2). However, no significant difference was found between the control group and the patient group for the secretor and Lewis characteristics.

3.2 Age stratified comparison of natural antibody titer

Given the age differences among healthy controls, asymptomatic, and symptomatic COVID-19 patients, we stratified participants into two groups: <45 and ≥45 years. Anti-B IgG and IgA titers were significantly higher in asymptomatic patients <45 years compared to both symptomatic patients and healthy controls in the same age group (Supplementary Figure 1B). Younger healthy controls (<45 years) had higher anti-A IgM titers than older controls (Supplementary Figure 1I). Asymptomatic patients <45 years also showed significantly elevated anti-αGal IgG, IgA, and IgM titers compared to healthy controls (Supplementary Figures 1C,G,K). No significant differences were observed in Tn-Mono antibody titers across groups (Supplementary Figures 1D,H,L).

The regression analysis on antibody titer and adjusted age (Supplementary Table 1) indicates that age and symptomatic status were not significantly associated with IgA antibody levels or with IgG antibody levels against anti-A, anti-Tn, and anti-α Gal. However, for anti-B IgG, symptomatic status was significantly associated with lower titers ($p = 0.002$), while age alone was not a significant predictor. In addition, among anti-Tn-Mono IgM titer, age showed a modest but significant negative association ($p = 0.030$). The interaction term between age and symptomatic status was significant for both anti-B IgG ($p = 0.018$) and anti-Tn-Mono IgM ($p = 0.047$), suggesting that the effect of age on these antibody levels may differ depending on symptom status.

3.3 Natural antibody titers among different blood groups, COVID-19 patients, and healthy controls

Since anti-A antibody is present among blood groups O and B, we compared the age-adjusted IgG, IgA, and IgM titers between

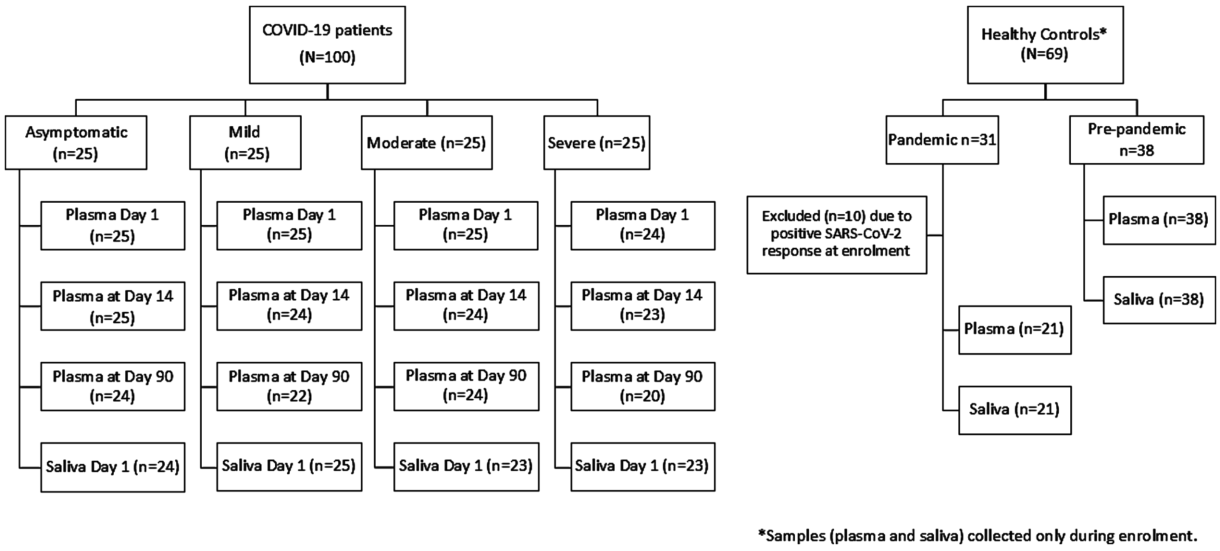

FIGURE 1
Participant flow diagram for blood and saliva sample collection.

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TABLE 1 Demographic and distribution of histo-blood group antigens among the COVID-19 patients and controls.

Parameters	All patients *n* = 99	Asymptomatic *n* = 25	Symptomatic[a] *n* = 74	Healthy controls and pre-pandemic[b] *n* = 59	*p*-value[c]
Sex					
Female	39 (39.4%)	16 (64.0%)	23 (31.1%)	27 (45.8%)	**0.011**
Male	60 (60.6%)	9 (36.0%)	51 (68.9%)	32 (54.2%)	
Age Median (IQR)	46 (35, 57.5)	35 (31, 44)	50 (38, 62)	33 (28.5, 41)	**<0.001**[d]
Blood group					
A Blood group	25 (25.3%)	8 (32.0%)	17 (23.0%)	17 (28.8%)	0.791
AB Blood group	9 (9.1%)	2 (8.0%)	7 (9.5%)	3 (5.1%)	
B Blood group	35 (35.3%)	7 (28.0%)	28 (37.8%)	17 (28.8%)	
O Blood group	30 (30.3%)	8 (32.0%)	22 (29.7%)	22 (37.3%)	
Duration between disease onset and enrollment, Median days (IQR)	10 (8, 12)	10 (2, 16)	10 (8, 12)	–	0.680[d]

Data presented as *n* (%).
[a]Symptomatic patient: mild: *n* = 25, moderate: *n* = 25, severe: *n* = 24.
[b]Pandemic healthy controls: *n* = 21; pre-pandemic healthy controls: *n* = 38.
[c]Pearson's chi-squared test for association.
[d]Non-parametric Kruskal–Wallis rank-sum test.
Bold values indicate significant difference between asymptomatic, symptomatic and healthy controls.

TABLE 2 Distribution of Lewis and secretor status by asymptomatic, symptomatic, and healthy control group.

Label	Asymptomatic, *n* = 24	Symptomatic, *n* = 71	Control, *n* = 59*	*p*-value[$]
Lewis status				
Lewis positive *n* = 130	19 (79.17%)	59 (83.10%)	52 (88.14%)	0.544
Lewis negative *n* = 24	5 (20.83%)	12 (16.90%)	7 (11.86%)	
Secretor status				
Secretor, *n* = 101	18 (75%)	42 (59.15%)	41 (69.49%)	0.267
Non-secretor, *n* = 53	6 (25%)	29 (40.85%)	18 (30.51%)	

[$]Chi-square test.
*Pandemic healthy control, *n* = 21; pre-pandemic healthy control, *n* = 38.

healthy controls, asymptomatic, and symptomatic COVID-19 patients from O and B blood group individuals only (Figure 2). Anti-A IgG and IgA antibody titers (Figures 2A,B) were significantly lower in both symptomatic and combined (asymptomatic + symptomatic) COVID-19 patient groups compared to healthy controls, while no significant difference was observed between asymptomatic and symptomatic patients. In contrast, anti-A IgM titers did not differ significantly among any of the groups (Figure 2C).

Similarly, we compared age-adjusted anti-B IgG, IgA, and IgM antibody titers among O and A blood group individuals, across healthy controls, asymptomatic, and symptomatic COVID-19 patients (Figure 3). For anti-B IgG (Figure 3A), asymptomatic individuals had significantly higher titers compared to healthy controls ($p < 0.05$) and significantly higher than symptomatic patients ($p < 0.01$), while no significant difference was observed between symptomatic patients and healthy controls. In contrast, no significant differences were observed in anti-B IgA (Figure 3B) or IgM (Figure 3C) titers among any of the groups.

We further explored the anti-Tn and anti-α Gal age-adjusted titers. Since the Tn-Mono antigen (αGalNAc) and the αGal antigen (Galα1,3-Galß1,4-GlcNAc) are structurally close, respectively, to the A and B antigens, we compared the anti-Tn titers between "non-A" (B + O) versus "A" blood groups (A + AB) and the anti-αGal titers between "non-B" (A + O) versus "B" (B + AB) blood groups, respectively (Supplementary Figure 2). We compared the antibody titer of the respective blood groups within the COVID-19 patients, within the healthy control and between the patients and healthy control groups, respectively. The anti-Tn IgA and IgM antibody titers were significantly higher among the "non-A" blood group than the "A" blood groups (A + AB) within COVID-19 patients (Supplementary Figures 2C,E). Similarly, the anti-α gal

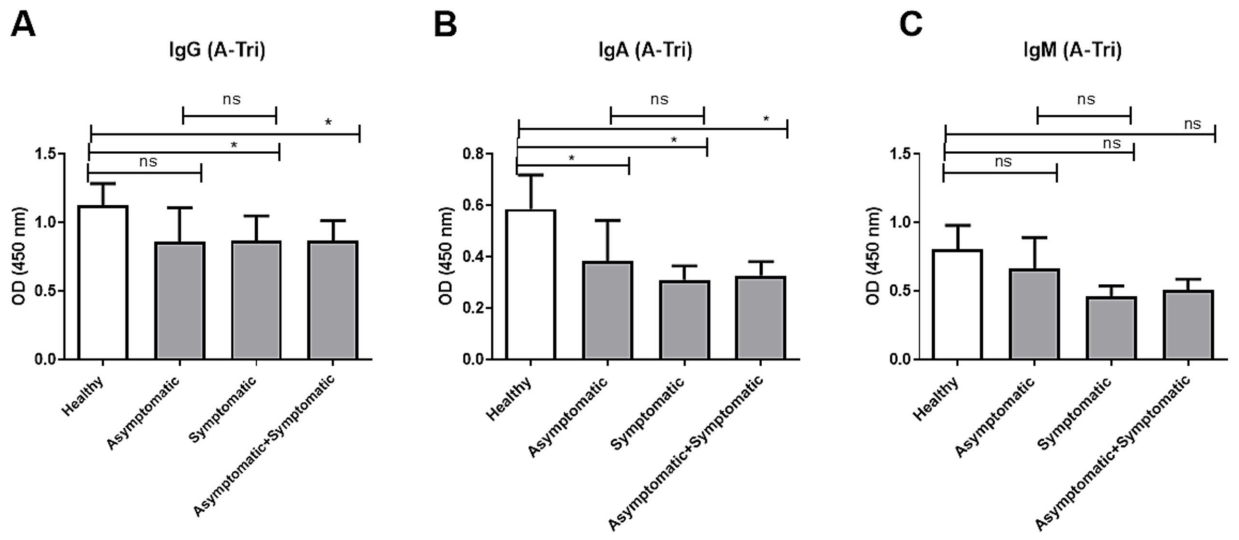

FIGURE 2
Anti-A antibody titer in O and B blood group patients and controls. Comparison of anti-A antibody (A-Tri) titer between healthy control (n = 39), asymptomatic patients (n = 15), symptomatic patients (n = 50), and combined (asymptomatic+ symptomatic) patients (n = 65) of individuals with O and B blood groups. **(A)** Anti-A IgG antibody, **(B)** anti-A IgA antibody, and **(C)** anti-A IgM antibody. *** and ** denote $p < 0.005$.

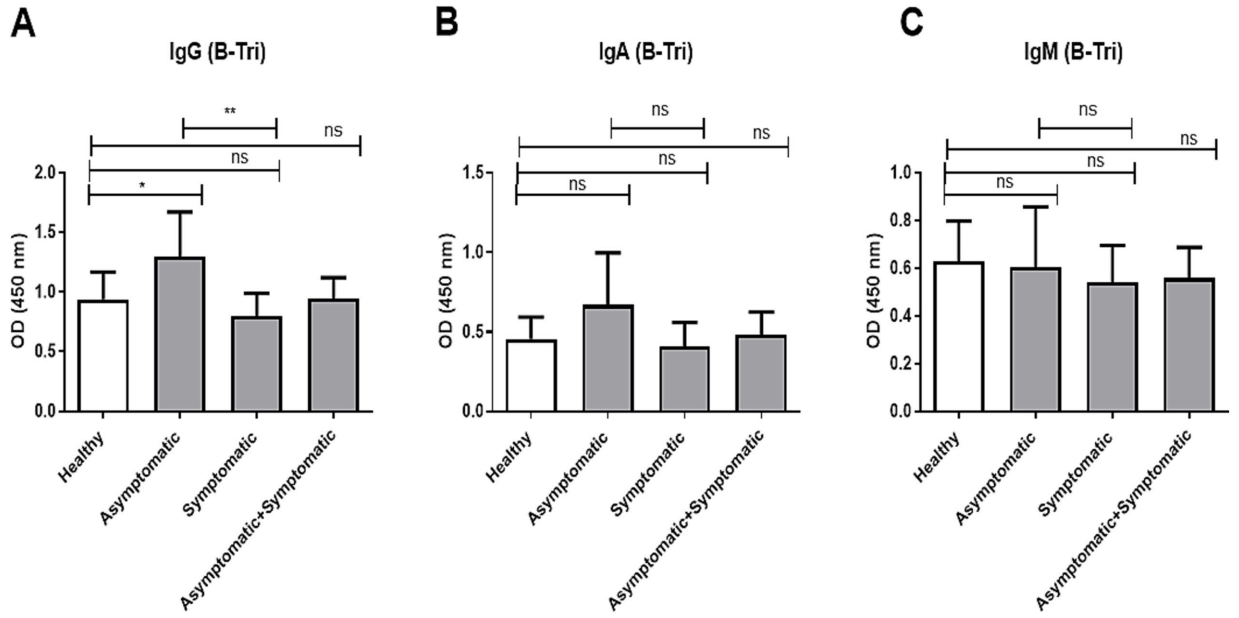

FIGURE 3
Anti-B antibody titer in O and A blood group patients and controls. Comparison of anti-B antibody (B-Tri) titer between healthy control (n = 39), asymptomatic patients (n = 16), symptomatic patients (n = 39), and combined (asymptomatic+ symptomatic) patients (n = 56) of individuals with O and A blood groups. **(A)** Anti-A IgG antibody, **(B)** anti-A IgA antibody, and **(C)** anti-A IgM antibody. *** and ** denote $p < 0.005$.

antibody titers were significantly higher among the "non-B" blood groups than the "B" blood groups (Supplementary Figures 1B,D,F). Interestingly, the anti-α-Gal antibody titers were found to be significantly higher in COVID-19 non-B blood group patients compared to healthy controls.

Comparing the differences of natural antibody titers among the COVID-19 patients and controls stratified by secretor status, we observed secretor healthy controls had significantly higher anti-A IgG ($p = 0.006$) antibody titer than secretor patients (Table 3). However, no difference was observed in anti-A IgA ($p = 0.081$) and IgM ($p = 0.663$) antibody titer between the secretor patients and controls. No significant differences in anti-B IgG, anti-Tn-Mono, or anti-α Gal antibody titers were observed between healthy controls and patients, regardless of secretor status (Table 3).

TABLE 3 Natural antibody titer according to secretor status by COVID-19 patients and healthy controls.

Factor	Labels	IgA	*p*-value[$]	IgG	*p*-value[$]	IgM	*p*-value[$]
A-Tri (B + O)							
Secretors	COVID-19 Patients ($n = 39$)	0.26 (0.21, 0.33)	0.081	0.62 (0.48, 0.81)	**0.006**	0.41 (0.32, 0.54)	0.663
	Control ($n = 27$)	0.44 (0.33, 0.58)		0.97 (0.80, 1.19)		0.62 (0.45, 0.85)	
Non-secretors	COVID-19 Patients ($n = 24$)	0.26 (0.19, 0.35)	0.144	0.73 (0.51, 1.04)	0.722	0.40 (0.33, 0.49)	0.838
	Control ($n = 12$)	0.49 (0.32, 0.76)		0.92 (0.60, 1.42)		0.50 (0.27, 0.94)	
B-Tri (A + O)							
Secretors	COVID-19 Patients ($n = 32$)	0.34 (0.26, 0.45)	0.639	0.66 (0.51, 0.85)	0.840	0.47 (0.35, 0.63)	0.596
	Control ($n = 28$)	0.33 (0.26, 0.42)		0.68 (0.50, 0.91)		0.45 (0.32, 0.63)	
Non-secretors	COVID-19 Patients ($n = 23$)	0.36 (0.27, 0.48)	0.238	0.88 (0.67, 1.16)	0.319	0.32 (0.23, 0.44)	0.692
	Control ($n = 11$)	0.36 (0.21, 0.62)		0.74 (0.41, 1.31)		0.43 (0.27, 0.68)	
Tn-Mono (A + AB)							
Secretors	COVID-19 Patients ($n = 23$)	0.38 (0.31, 0.46)	0.982	0.64 (0.53, 0.78)	0.623	0.69 (0.53, 0.89)	0.348
	Control ($n = 14$)	0.37 (0.30, 0.46)		0.73 (0.53, 1.00)		0.87 (0.71, 1.08)	
Non-secretors	COVID-19 Patients ($n = 11$)	0.31 (0.24, 0.39)	0.472	0.55 (0.43, 0.70)	0.664	0.63 (0.44, 0.91)	0.448
	Control ($n = 6$)	0.40 (0.26, 0.62)		0.66 (0.35, 1.27)		0.88 (0.64, 1.20)	
α-Gal (B + AB)							
Secretors	COVID-19 Patients ($n = 30$)	0.53 (0.45, 0.62)	0.712	1.27 (1.14, 1.41)	0.159	0.87 (0.74, 1.02)	0.948
	Control ($n = 13$)	0.53 (0.45, 0.63)		1.01 (0.87, 1.17)		0.89 (0.72, 1.12)	
Non-secretors	COVID-19 Patients ($n = 12$)	0.45 (0.35, 0.59)	0.838	1.03 (0.76, 1.39)	0.664	0.67 (0.51, 0.90)	0.689
	Control ($n = 7$)	0.49 (0.37, 0.64)		1.13 (0.90, 1.41)		0.79 (0.65, 0.95)	

[$]t-test for difference adjusted by age. $p < 0.05$ indicates significant difference between the groups.
Data presented as geometric mean (95% CI).
Bold values indicated a significant difference between COVID-19 patients and controls.

3.4 Gender-stratified difference of natural antibody titers among COVID-19 patients and healthy controls

We further explored the age-adjusted differences in natural antibody titer between male and female COVID-19 patients and controls (Figure 4). Anti-A IgG titers (Figure 4A) were significantly higher in female healthy controls and female asymptomatic patients compared to their male counterparts.

Anti-B IgG titer (Figure 4B) was significantly higher in asymptomatic females than symptomatic males.

Anti-αGal IgG titer (Figure 4C) was elevated in both asymptomatic and symptomatic females than healthy female controls.

For IgA, anti-A IgA titer (Figure 4E) was higher in female healthy controls (O and B blood groups) than in healthy males and symptomatic females. Anti-αGal IgA titers (Figure 4G) were elevated in asymptomatic females than both healthy and symptomatic females. Anti-Tn IgA titers (Figure 4H) were higher in females (pooled) than males. In the IgM isotype, anti-A IgM titer (Figure 4I) was higher in female healthy controls and asymptomatic patients than in male counterparts, and lower in symptomatic females compared to healthy females. Anti-B IgM titer (Figure 4J) was significantly higher in asymptomatic and symptomatic females than in corresponding males. Anti-αGal and anti-Tn IgM titers were significantly higher in asymptomatic females compared to symptomatic and healthy females, and in both asymptomatic and symptomatic females than their male counterparts (Figures 4K,L).

3.5 Trend of natural antibody titer over convalescence period among COVID-19 patients

We further explored the trend of natural antibody titer during the convalescence period from the disease onset or exposure (asymptomatic cases) stratified by symptomatic status and blood group. Anti-A antibody (IgG and IgA) among blood groups B and O individuals remained steady over time, except for anti-A IgM, which showed a significant decrease among symptomatic patients

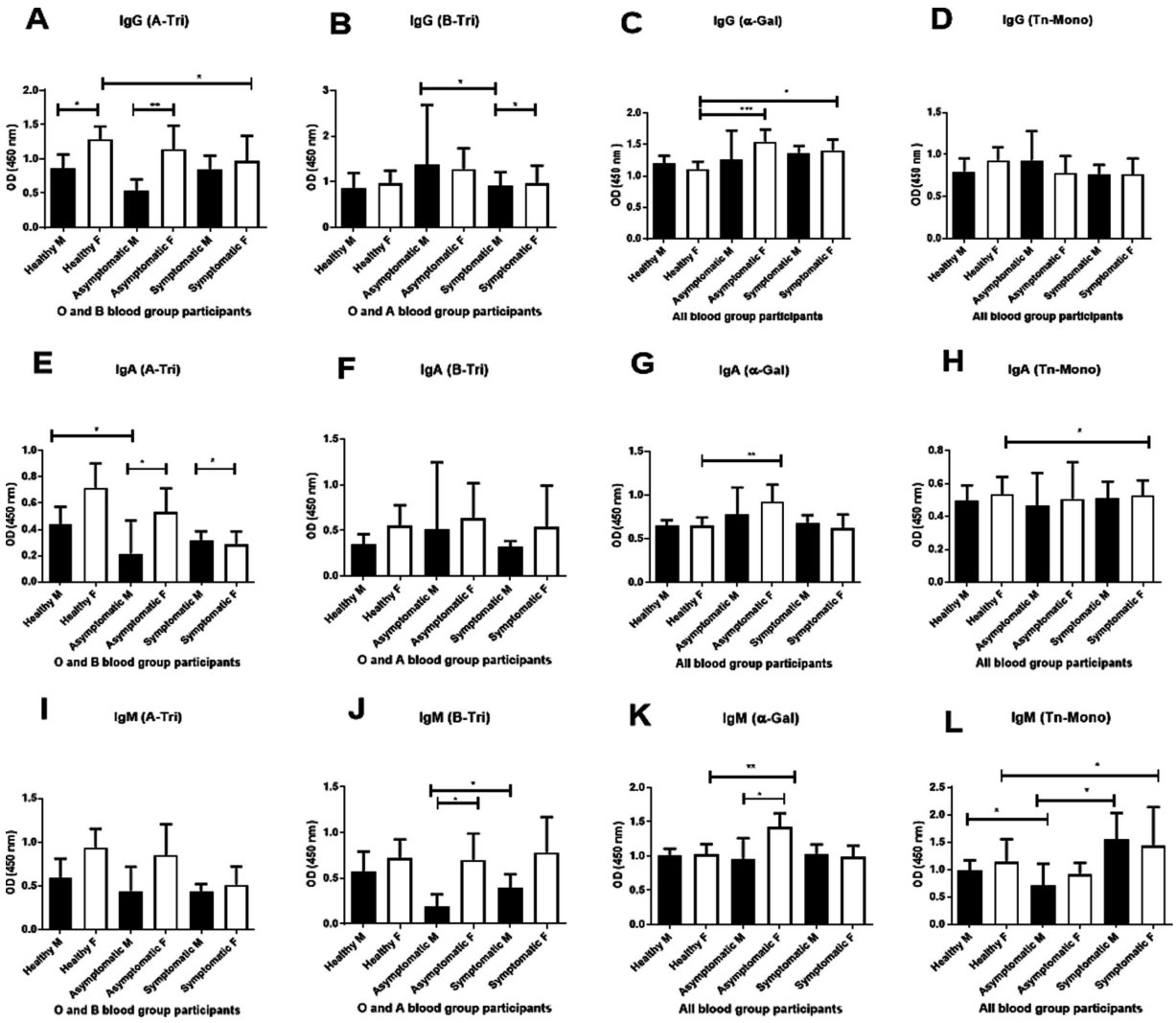

FIGURE 4
Gender-based analysis of natural antibody. Comparison of natural anti-A (A-Tri), anti-B (B-Tri), anti α-Gal, and anti-Tn-Mono (Tn-Mono) IgG **(A–D)**, IgA **(E–H)** and IgM **(I–L)** antibodies between healthy controls, asymptomatic, and symptomatic patients stratified by gender. For anti-A antibody: healthy male (M) n = 18, female (F) n = 21; asymptomatic male (M) n = 6, female (F) n = 8; symptomatic male (M) n = 33, female (F) n = 15. Anti-B IgG antibody (healthy male = 21, female = 18), asymptomatic male (M) n = 3, female (F) n = 13; symptomatic male (M) n = 24, female (F) n = 15; Alpha-Gal or Tn-Mono (healthy male = 32, female = 27); asymptomatic male (M) n = 8, female (F) n = 16; symptomatic male (M) n = 49, female (F) n = 23. Asterisks *** and ** denote statistical significance, $p < 0.005$.

between Days 8 to 12 and Days 21 to 25 (Figure 5I). However, for anti-B IgG antibody among blood group A and O individuals, there were significant differences between symptomatic and asymptomatic patients at Days 8 to 12 ($p = 0.0038$) and Days 21 to 25 ($p = 0.004$) (Figure 5B) from disease onset. The Tn-Mono antibody titer for IgG among the symptomatic patients with all blood groups significantly increased after 3 months ($p = 0.01$ between Days 8 to 12 and Days 93 to 101; $p = 0.003$ between Days 21 to 25 and Days 93 to 101) (Figure 5C). Additionally, anti-Tn-Mono IgM antibody titers were higher in symptomatic patients compared to asymptomatic patients, and this differences persisted throughout the convalescence period (Figure 5K). Although no increase was observed in alpha-gal antibody titer over the 3 months, however, a significant difference in IgA and IgM was observed between the symptomatic and asymptomatic patients (Figures 5H,L).

4 Discussion and conclusion

This is the very first study in South Asia looking into the interference of host natural antibody in relation to blood group status with symptomatic of SARS-CoV-2 infection. The current study was part of an exploratory analysis on the relation of natural anti-carbohydrate antibodies, Lewis phenotype, and secretor status with COVID-19 disease severity. The demographic characteristics in our cohort were described previously in our first study (1) having younger patients of median age 35 years old suffering from asymptomatic SARS-CoV-2 infection while elder patients of median age 50 years old suffering mostly from symptomatic infection. As discussed earlier, male patients suffer more from severe disease than female patients and majority of the asymptomatic infection was presented by female patients (1). Thus, from this analysis, we have further investigated the

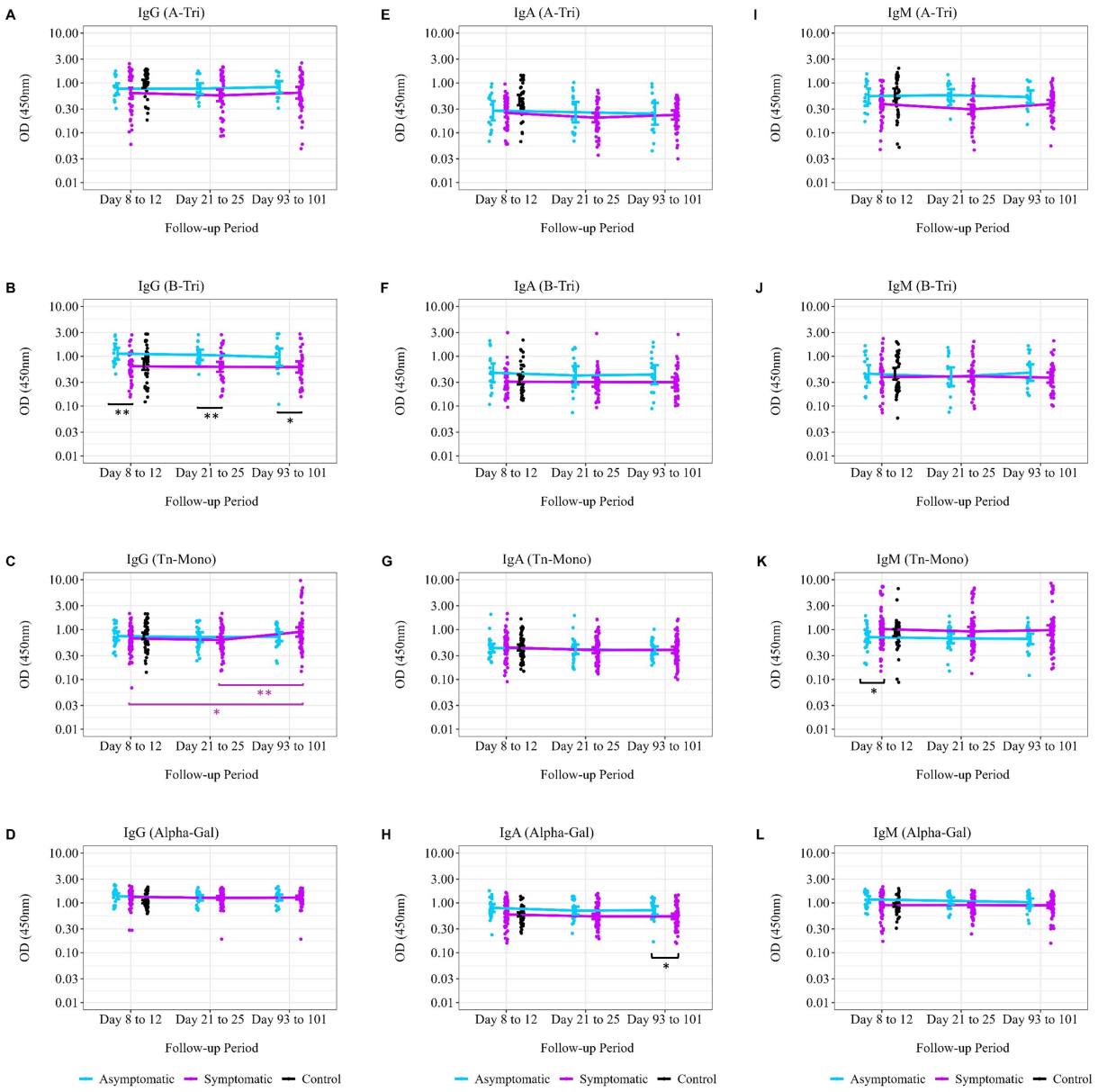

FIGURE 5
Trend of natural antibodies over 3 months post-SARS-CoV-2 infection. Comparing the trend of natural antibody dynamics over the convalescence period from date of disease onset of exposure. The follow-up period, displayed on the *x*-axis, was calculated from the date of disease onset to each study follow-up time point. **(A)** Anti-A IgG antibody titer (asymptomatic, *n* = 15; symptomatic, *n* = 50; control, *n* = 39), **(B)** anti-B IgG antibody titer (asymptomatic, *n* = 16; symptomatic, *n* = 39; control, *n* = 39), **(C)** anti-Tn-Mono IgG antibody titer (asymptomatic, *n* = 25; symptomatic, *n* = 74; control, *n* = 59), and **(D)** anti-αGal IgG antibody titer (asymptomatic, *n* = 25; symptomatic, *n* = 74; control, *n* = 59). **(E)** Anti-A IgA antibody titer (asymptomatic, *n* = 15; symptomatic, *n* = 50; control, *n* = 39), **(F)** anti-B IgA antibody titer (asymptomatic, *n* = 16; symptomatic, *n* = 39; control, *n* = 39), **(G)** anti-Tn-Mono IgA antibody titer (asymptomatic, *n* = 25; symptomatic, *n* = 74; control, *n* = 59), and **(H)** anti-αGal IgA antibody titer (asymptomatic, *n* = 25; symptomatic, *n* = 74; control, *n* = 59). **(I)** Anti-A IgM antibody titer (asymptomatic, *n* = 15; symptomatic, *n* = 50; control, *n* = 39), **(J)** anti-B IgM antibody titer (asymptomatic, *n* = 16; symptomatic, *n* = 39; control, *n* = 39), **(K)** anti-Tn-Mono IgM antibody titer (asymptomatic, *n* = 25; symptomatic, *n* = 74; control, *n* = 59), and **(L)** anti-αGal IgM antibody titer (asymptomatic, *n* = 25; symptomatic, *n* = 74; control, *n* = 59). The purple asterisks denote geometric mean of antibody titer comparisons between two time points among symptomatic patients, while black asterisks denote difference in geometric mean antibody titer between the asymptomatic and symptomatic patients at a single time point. * denotes $p = 0.05–0.01$; ** denotes $p = 0.01–0.001$; and *** denotes $p < 0.001$.

host factors which may support the reason behind this trend of SARS-CoV-2 infection.

In our cohort, we found 38% of the COVID-19 patients with B blood group suffered from symptomatic SARS-CoV-2 infection while O blood group patients represented 33% of asymptomatic infections and 37% of healthy controls. The association between blood group type and susceptibility to symptomatic COVID-19 has been a topic of research. Studies have suggested that individuals with O blood type may have a lower risk of developing severe symptoms or being infected with SARS-CoV-2 (2, 22). However, in our cohort, we did not find any difference in the proportion of A, B, AB, and O blood group distribution among the asymptomatic, symptomatic,

and healthy controls which was debatable with the results obtained in other studies where blood group A was predominant among the COVID-19 patients (4, 23). It has been discussed elsewhere that O blood group individuals naturally possess both anti-A and anti-B antibodies which may provide protection against SARS-CoV-2 by preventing the binding of SARS-CoV-2 to its receptor thereby inhibiting the virus entry into the targeted human cells (24, 25). As discussed above, the presence of the HBGAs in the epithelial cells has previously been implicated in the genetic susceptibility to several infectious diseases, including viral diseases (24). Previously, our group have shown that anti-A could block the binding of SARS-CoV spike protein produced in a A antigen positive cell to ACE2 (19), while another group have shown that SARS-CoV-2 spike can bind to the A antigen (like other viral proteins such as some norovirus strain VP1 or some rotavirus strain VP8 capsid proteins) (26). These two distinct mechanisms could explain that different blood groups have different susceptibility to SARS-CoV-2 infection. The H-antigen and A/B antigens in respiratory cells may enhance SARS-CoV-2 binding to host receptors, influencing infection susceptibility (2). In this cohort, we have observed approximately 30.5% individuals with inactive *FUT2* enzymes making them non-secretors and 11.86% Lewis negative individuals among the control groups (Table 2) which is similar to other studies in Bangladesh and Asian population (27–29). However, this distribution is higher than most Caucasian population and Chinese population (2, 30–33).

Natural antibodies are part of the innate immune system, which is the first line of defense against infections in the human body (34). Previous studies, including observations from the SARS-CoV 2003 outbreak and other viral infections, suggest that anti-A and anti-B antibodies can inhibit viral entry by blocking interactions between viral proteins and host blood group antigens (19). In our cohort, anti-A and anti-B antibody titers did not differ significantly between healthy controls, asymptomatic, and symptomatic patients when stratified by age (<45 vs. ≥45 years), suggesting that age is not a confounding factor in the observed association between lower natural antibody titers and symptomatic COVID-19 (Supplementary Figure 1). A minor difference in anti-αGal IgG titers observed among healthy controls likely reflects sampling variability due to small group size and should be interpreted cautiously. Larger studies are needed to further confirm that age does not influence natural antibody levels in the context of SARS-CoV-2 infection.

Our data showed that anti-A IgG and IgA titers were significantly lower in symptomatic and combined COVID-19 patient groups compared to healthy controls (Figure 2), while anti-B IgG was significantly lower in symptomatic patients compared to asymptomatic patients but not compared to controls (Figure 3). This finding is consistent with the observation from the study conducted in Belgium that hospitalized patients who had lower anti-A and/ or anti-B IgM antibody titer compared to healthy control were at risk of developing SARS-CoV-2 infection (35). The elevated anti-A and anti-B IgG levels observed in asymptomatic individuals may reflect a protective antibody profile, while IgA and IgM levels remained unchanged between patient groups (24, 35). This is further supported by epidemiological data, including our review of 35 studies, which found that blood group A (who lack anti-A antibodies) was associated with higher infection risk in over 50% of studies, compared to only 20% for blood group B (who lack anti-B) (24). These findings reinforce the hypothesis that anti-A antibodies may provide stronger protection than anti-B, possibly due to the presence of A-like antigens on the viral envelope.

The increased susceptibility of blood group A supports the idea that anti-A antibodies may interfere with viral binding to ACE2, reducing viral entry and disease severity. Collectively, these results highlight the potential role of natural anti-carbohydrate antibodies in modulating COVID-19 susceptibility and clinical outcome. Histo-blood group antigens (HBGAs) are expressed on various epithelial surfaces, including those in the respiratory and digestive tracts, where SARS-CoV-2 is known to replicate and be transmitted. The viral spike protein is heavily glycosylated, and the specific glycan structures it carries depend on the host cell type (24, 35). However, glycans detected on recombinant spike protein differ from those found on viral particles produced by infected individuals' epithelial cells, making recombinant spike unreliable for inhibition studies involving anti-A or anti-B antibodies (2). Given this discrepancy, we focused on studying and comparing natural antibody titers (anti-A and anti-B) between COVID-19 patients and healthy controls, particularly regarding secretor status.

The secretor status (*FUT2* gene) and Lewis blood group system (*FUT3* gene) influence mucosal antigen expression, impacting SARS-CoV-2 susceptibility and immune response (36). Since secretor individuals express ABO and Lewis antigens on mucosal surfaces, their viral particles may acquire host-derived glycans, potentially altering immune recognition and neutralization. In contrast, non-secretors lack these antigens, which may limit viral binding and reduce disease severity, as observed in gastroenteritis cases such as rotavirus infection making non-secretors less susceptible to rotavirus (37). It is anticipated that infectious viral particles generated by respiratory epithelial cells in individuals of the "secretor" phenotype would bear the H, A, and B antigens (24). Therefore, we compared the anti-A and anti-B antibody titer between the COVID-19 patients and controls stratified by secretor status.

Our findings revealed that secretor healthy controls (B and O groups) had higher anti-A antibody titers compared to COVID-19 patients, suggesting a protective role of these antibodies (Table 3). In addition, Lewis-negative individuals (Le a- b-) have been associated with lower hospitalization rates, further supporting a potential protective effect (2).The significant reduction in IgA and IgM levels in secretor COVID-19 patients may indicate immune modulation tied to prolonged antigen exposure, leading to immune tolerance or antigen–antibody complex formation, which can dampen mucosal immunity (36). Since IgA is important for respiratory defense, lower levels in secretors may impair mucosal protection, increasing viral shedding and disease severity (24). These findings emphasize that host glycan expression plays a key role in shaping SARS-CoV-2 susceptibility, immune response, and disease outcomes although a larger study is required to determine the magnitude of the effect of secretor status and Lewis phenotypes on SARS-CoV-2 infections (2).

We observed a consistent trend of higher anti-A (IgG, IgM, and IgA) and anti-B IgM antibody titers in female healthy controls and asymptomatic patients compared to their male counterparts (Figure 4). Although not statistically significant for all groups, there is a trend of higher ABO antibody titer among the female individuals than male (Figure 4). Similar phenomenon of higher ABO antibody titer among female healthy blood donors than male healthy donors has been observed in a Japanese population (38). This finding further supports

the hypothesis that natural antibodies may protect against severity of COVID-19, and hence female suffers milder or asymptomatic infection compared to male individuals (Figure 4) (1, 34, 35).

Comparing the anti-A, anti-B, anti-Tn, and anti-αGal IgG antibodies up to 3 months from disease onset, we found that the antibody titer remained steady over the time (Figure 5). Asymptomatic patients maintained higher anti-A and anti-B IgG antibody titers over time than symptomatic patients although not significant except for anti-B IgG antibody titer at days 8–12 and days 21–25 follow-up period from disease onset. This gives the confidence that natural antibody titers provide innate immunity against symptomatic disease.

The strength of this analysis is that this is the first study looking into the natural anti-A and anti-B antibody titers from both asymptomatic and symptomatic COVID-19 patients compared to healthy control from both pandemic and pre-pandemic period. The results observed from this analysis may further provide evidence supporting the role of natural antibodies in protecting against symptomatic infection. However, due to the small sample size of the healthy controls from the pandemic period, we could not carry out strong statistical tests to confirm the risk of infection according to the ABH and Lewis phenotypes.

In conclusion, from our observation on natural antibody titers in this cohort, higher natural anti-A and possibly anti-B antibody titers may provide protection against symptomatic infection. These findings could help guide further studies into whether certain blood group and secretor combinations are linked to differences in SARS-CoV-2 susceptibility or severity and potentially support more personalized approaches to understanding immune responses in viral infections.

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 was approved by the Institutional Review Board (IRB) of the International Centre for Diarrhoeal Disease Research (icddr,b). Written informed consent to participate in the study was obtained from all participants according to the 'Declaration of Helsinki' regulation and guidelines.

Author contributions

TA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. AB: Conceptualization, Resources, Validation, Writing – original draft, Writing – review & editing. MA: Formal analysis, Validation, Writing – original draft, Writing – review & editing. GB: Validation, Writing – original draft, Writing – review & editing. NP: Validation, Writing – original draft, Writing – review & editing. MF: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. AA: Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. FQ: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. FC: Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. TB: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. JP: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. NR-C: Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

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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.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2025.1554785/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Comparison of natural antibody titer by age. **(A,E,I)** anti-A antibodies (B and O blood groups) with healthy control <45 years old (*n*=32), healthy control ≥45 years old (*n*=6), asymptomatic patients <45 years old (*n*=13) and ≥45 years old (*n*=2), symptomatic patients <45 years old (*n*=14) and ≥45 years old (*n*=36). **(B,F,J)** Anti-B antibodies (IgG, IgA and IgM) with healthy control <45 years old (*n*=32), healthy control ≥45 years old (*n*=7), asymptomatic patients <45 years old (*n*=11), and ≥45 years old (*n*=5), symptomatic patients <45 years old (*n*=14) and ≥45 years old (*n*=26). **(C,G,K)** Anti-αGal antibodies (IgG, IgA and IgM) with healthy control <45 years old (*n*=49), healthy control ≥45 years old (*n*=9), asymptomatic patients <45 years old (*n*=19), and ≥45 years old (*n*=6), symptomatic patients <45 years old (*n*=25) and ≥45 years old (*n*=49). **(D,H,L)** Anti-Tn mono antibodies (IgG, IgA and IgM) with healthy control <45 years old (*n*=49), healthy control ≥45 years old (*n*=9), asymptomatic patients <45 years old (*n*=19), and ≥45 years old (*n*=6), symptomatic patients <45 years old (*n*=25) and ≥45 years old (*n*=49). *** and ** denotes $p < 0.005$

SUPPLEMENTARY FIGURE 2
Anti-Tn-Mono and Alpha-Gal antibodies among COVID-19 patients and controls. Comparing the natural antibody titers between healthy controls (*n*=59) and COVID-19 patients (*n*=99). **(A)** anti-Tn-Mono IgG antibody titer, **(B)** anti-αGal IgG antibody titer, **(C)** anti-Tn-Mono IgA antibody titer, **(D)** anti-αGal IgA antibody titer **(E)** anti-Tn-Mono IgM antibody titer and **(F)** anti-αGal IgM antibody titer. We stratified antibody titer for anti-Tn-Mono by "non-A" [B+O (*n*=104, patients (65) vs control (39)] and "A" blood group [A+AB (*n*=54, patients (34) vs control (20)] blood groups. We stratified antibody titer for anti-αGal by "non-B" [A+O (*n*=94, patients (55) vs control (39)] and "B" [B+AB (*n*=64, patients (44) vs control (20)] blood groups. Significant difference between patients and controls are presented with black asterisks. Comparisons are also made between blood groups within the healthy controls and patients and only the significant difference is presented with purple asterisks. * p = 0.05–0.01, ** denotes p = 0.01–0.001 and *** denotes $p < 0.001$.

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Case Report: Persistent COVID-19 in a fully vaccinated Japanese man being treated with rituximab and epcoritamab for diffuse large B-cell lymphoma

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The management of persistent coronavirus disease 2019 (COVID-19) in patients with hematological malignancies who are immunocompromised because of underlying disease or iatrogenic immunosuppression remains clinically challenging. Herein, we report an 84-year-old man with stage 3 diffuse large B-cell lymphoma treated with rituximab and epcoritamab who subsequently developed persistent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, despite having received seven doses of COVID-19 mRNA vaccine and remdesivir. The patient was treated with a combination of remdesivir, sotrovimab, and nirmatrelvir/ritonavir, and recovered clinically. SARS-CoV-2 polymerase chain reaction and antigen tests eventually turned negative, and he was discharged after 28 days of hospitalization. This case highlights the challenges associated with managing persistent SARS-CoV-2 infection in immunocompromised patients with hematological malignancies. Combined treatment with antivirals and monoclonal antibodies may be an effective strategy.

KEYWORDS

persistent COVID-19, diffuse large B-cell lymphoma, rituximab, epcoritamab, SARS-CoV-2

1 Introduction

Immunocompromised individuals, particularly those with hematological malignancies are susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Despite the effectiveness of vaccines and antiviral treatment, the risk of coronavirus disease 2019 (COVID-19)-related morbidity and mortality persists, even in the era of the Omicron variant (1). Patients with humoral immunodeficiency, such as those with B-cell malignancies and those receiving anti-CD20 therapy, are particularly susceptible to developing persistent COVID-19. Persistent SARS-CoV-2 infection can lead to decreased viral clearance, prolonged viral shedding, and an increased risk of viral mutations (2–4). The management of persistent COVID-19 in patients who are immunocompromised because of underlying disease or iatrogenic immunosuppression remains clinically challenging. Herein, we report the case of a patient with advanced

diffuse large B-cell lymphoma (DLBCL) who developed persistent SARS-CoV-2 infection following treatment with rituximab and epcoritamab.

2 Case report

An 84-year-old Japanese man with stage 3 DLBCL received rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone therapy, followed by polatuzumab vedotin, bendamustine, and rituximab therapy. Owing to tumor progression, the patient was treated with epcoritamab in June 2024. Although it had been approved for prophylactic use against SARS-CoV-2 infection in Japan, tixagevimab/cilgavimab was not available at the time. The patient had received seven doses of an mRNA-based COVID-19 vaccine according to the schedule recommended in Japan, with the last dose administered in December 2023.

In July 2024, after the first cycle of epcoritamab, the patient developed a high fever and mild cough. The SARS-CoV-2 rapid antigen test (QuickNavi-COVID19 Ag; Denka Co., Ltd., Tokyo, Japan) was positive, and chest computed tomography (CT) revealed ground-glass opacities in the right lower lobe, left lingual lobe, and left lower lobe of his lungs (Figure 1). The patient was diagnosed with COVID-19 pneumonia and treated with remdesivir for 8 days (200 mg on the first day and 100 mg on the subsequent 7 days), tocilizumab (400 mg on the first day), and cefepime (2 g twice daily for 6 days), before being discharged from hospital on day 9. Nine days after the last administration of epcoritamab in the second cycle (i.e., 40 days after the previous COVID-19 onset), the patient presented with recurrent high fever, cough, and anorexia. Physical examination revealed no evidence of heart failure, including no peripheral edema, weight gain, or wheezing. SARS-CoV-2 antigen and polymerase chain reaction (PCR) tests (Xpert Xpress SARS-CoV-2 Cepheid; Beckman Coulter, Inc., Brea, CA, USA) were positive (cycle threshold [Ct]: 22.8). Chest CT revealed ground-glass opacities in both upper lobes and the right middle lobe of his lungs, although the previously identified abnormal shadows had almost disappeared (Figure 1). The patient was diagnosed with persistent SARS-CoV-2 infection. He had no history of foreign travel, sexually transmitted infections, or inhalational exposures. He was taking fluconazole (100 mg/day) for prophylaxis against fungal infections.

The patient's vital signs on admission revealed fever (body temperature, 38.0°C), tachycardia (125 beats/min), and hypoxia (SpO_2: 92% breathing ambient air), with a normal blood pressure (127/76 mmHg). His blood test results showed elevated inflammatory marker levels and hypogammaglobulinemia (Table 1). D-dimer levels exhibited a slight elevation, remaining essentially unchanged from the baseline prior to admission. He was treated with intravenous remdesivir for 10 days (200 mg on the first day, followed by 100 mg for 9 days), dexamethasone (6.6 mg/day for 5 days), ceftriaxone (2 g/day for 5 days), and azithromycin (500 mg/day for 3 days) (Figure 2), and oxygen was administered through a nasal cannula. Anticoagulant thromboprophylaxis was not implemented. Tests for autoantibodies associated with connective tissue diseases, serum anti-neutrophil cytoplasmic antibodies, *Mycoplasma* antibody, *Aspergillus* antigen, and cytomegalovirus immunoglobulin M (IgM), and an interferon-γ release assay to screen for tuberculosis were all negative. As the patient had a moderately elevated serum (1→3)-β-D-glucan level, sulfamethoxazole/trimethoprim (3,600/720 mg per day) was administered to treat possible *Pneumocystis jirovecii* pneumonia; however, a sputum PCR test for *P. jirovecii* DNA was negative.

Although the patient's high fever and hypoxia resolved, a follow-up SARS-CoV-2 PCR test remained positive (Ct: 24.8) (Figure 2), and his symptoms of cough, fatigue, and anorexia persisted. On day 9 of hospitalization, he received sotrovimab (500 mg) monoclonal antibody treatment. On day 14, another SARS-CoV-2 PCR test was again positive (Ct: 24.0). Nirmatrelvir/ritonavir was administered on day 15. On day 21, the SARS-CoV-2 antigen and PCR test results were negative (Ct: 34.8). By day 24, the CT abnormalities had almost disappeared (Figure 1), and the patient was discharged on day 28 of hospitalization. One month after discharge, a follow-up PCR test for SARS-CoV-2 remained negative (Ct > 45.0) and there was no evidence of recurrent abnormalities on the CT scan. The CT severity of lung abnormalities was evaluated by three clinicians using a semi-quantitative scoring method as described by Pan et al. (5). The mean severity scores were 10.7, 11.0, 7.3, and 4.0 in the initial CT, second CT (admission in this report), pre-discharge follow-up, and 1-month post-discharge follow-up, respectively. The scores for each lung lobe are shown in Supplementary Table 1.

3 Discussion

Anti-CD20 therapy is frequently used to treat B-cell hematological malignancies. However, anti-CD20 agents can lead to prolonged immunosuppression that makes patients more susceptible to infection, including SARS-CoV-2 infection (2, 3). Rituximab, a commonly used immunosuppressive drug targeting B-cells, is associated with reduced response to SARS-CoV-2 vaccination (6). Patients with hematological malignancies who receive anti-CD20 therapy may have a reduced immune response to SARS-CoV-2 infection for as long as 12 months, which increases their risk of persistent COVID-19 (7). Epcoritamab is a novel bispecific antibody that targets both CD20 and CD3 and activates T-cells to target and eliminate CD20-expressing cells (8). The effect of epcoritamab-induced CD20 depletion in SARS-CoV-2 infection remains unclear, and there are only a few reports on the clinical course of persistent SARS-CoV-2 infection following rituximab and epcoritamab treatment. Faxén and Edvinsson (9) described a case of persistent COVID-19 treated using a combination therapy of remdesivir, tixagevimab/cilgavimab, and nirmatrelvir/ritonavir. Longo et al. (10) reported three cases of persistent COVID-19 and highlighted the effectiveness of combination therapy with antiviral agents and monoclonal antibodies. In our case, despite receiving rituximab and epcoritamab, which may have exerted an additive immunosuppressive effect, the patient recovered after sequential administration of remdesivir, sotrovimab, and nirmatrelvir/ritonavir.

Abbreviations: COVID-19, coronavirus disease 2019; Ct, cycle threshold; CT, computed tomography; DLBCL, diffuse large B-cell lymphoma; IgM, immunoglobulin M; IVIg, intravenous immunoglobulin; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SpO_2, peripheral oxygen saturation.

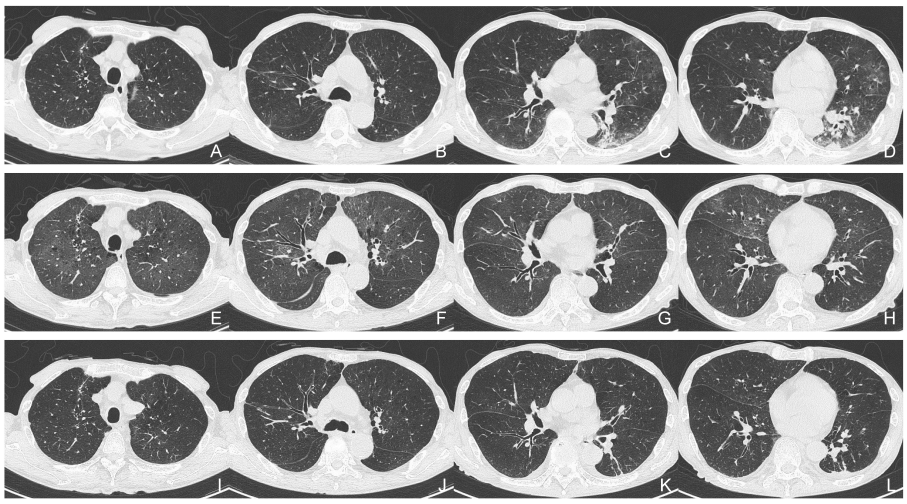

FIGURE 1
Serial chest computed tomography (CT). **(A–D)** Initial CT scan obtained during the patient's first episode of COVID-19. **(E–H)** Second CT scan obtained on the patient's readmission in August 2024. **(I–L)** Follow-up CT scan obtained before the patient's discharge from hospital.

Identifying patients who are most likely to experience persistent COVID-19 and establishing appropriate diagnostic criteria and management strategies for patients at high risk remain major clinical challenges. As humoral immunity plays a crucial role in the clearance of SARS-CoV-2, patients with B-cell depletion caused by conditions such as hematological malignancy, anti-CD20 therapy, hematopoietic stem cell/solid organ transplant, and common variable immunodeficiency, are more susceptible to persistent SARS-CoV-2 infection (1, 2). Although local protocols for the management of prolonged COVID-19 in patients with severe immunosuppression have been introduced (11), the optimal diagnostic evaluation and therapeutic strategies for patients with different degrees of immunodeficiency remain unclear. Moreover, although the US National Institutes of Health COVID-19 treatment guidelines acknowledge the occurrence of prolonged viral shedding in immunocompromised individuals (12), standardized clinical terminology for this phenomenon has not yet been established. Given our patient's clinical course, prolonged viral shedding, symptoms, and CT findings, we diagnosed him with persistent COVID-19 based on the proposed criteria for persistent COVID-19 (11, 13).

Consensus regarding the optimal treatment strategy for persistent SARS-CoV-2 infection in patients who are immunocompromised, such as those undergoing B-cell depletion therapy, is lacking. Remdesivir suppresses viral replication by blocking the RNA-dependent RNA polymerase, which is essential for SARS-CoV-2 replication (14). Similarly, nirmatrelvir inhibits 3CL protease, an enzyme necessary for SARS-CoV-2 viral replication, and ritonavir inhibits its degradation. Dexamethasone, in combination with remdesivir is effective for treating COVID-19 (15). However, the potential for adverse outcomes due to steroid-induced suppression of interferon production and prolonged viral shedding has concerns (16). Therefore, cautious use of dexamethasone in combination with remdesivir with attention to dosage and duration is warranted in patients with hematological malignancies. The use of single antiviral agents for persistent SARS-CoV-2 infection is associated with a risk of drug resistance and promotes viral evolution (4). Prolonged use of nirmatrelvir/ritonavir treatment has been reported to be effective for treating persistent SARS-CoV-2 infection (17). However, clinically significant resistance to remdesivir and nirmatrelvir/ritonavir has been reported, particularly in patients with hematological malignancies and persistent SARS-CoV-2 infection (14). Recent reviews and case series have highlighted the effectiveness of therapies involving a combination of antiviral agents and monoclonal antibodies in patients who are immunocompromised with persistent COVID-19 (9, 10, 18–20). Both sequential and simultaneous combination therapies have been reported to be safe and effective (10). The Japanese guidelines for COVID-19 do not mention simultaneous combination therapy with antiviral agents (21). In this case, we used sequential combination therapy, which resulted in a favorable outcome. Intravenous immunoglobulin (IVIg) boosts and modulates the immune system and helps to fight infection in patients with immunodeficiency. Although the effectiveness of IVIg against persistent COVID-19 has not been confirmed, Maruki et al. (22) reported successful use of IVIg and antiviral agents to treat persistent COVID-19 in an immunocompromised patient who was receiving CD20-depleting therapy for follicular lymphoma. Convalescent plasma may offer therapeutic benefits for persistent COVID-19 (18), but its availability in general medical practice is limited. Optimizing personalized patient care and the choice and prioritization of drug combinations is challenging owing to the heterogeneity of immunosuppression according to the type and severity of the underlying disease. Further research in this area is warranted.

Previous studies have demonstrated the effectiveness of neutralizing antibodies for treating persistent COVID-19 (9, 10, 18–20). Although sotrovimab has been reported to be less effective at neutralizing SARS-CoV-2 variants BA.2.12.1, BA.4, and BA.5 *in vitro* (23), in real-world settings, it continued to be effective at preventing severe disease and complications during the period when the BA.2 and BA.5 variants were predominant (24). In patients with hematological malignancy, sotrovimab

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TABLE 1 Laboratory test results of the patient on admission.

Parameter	Value	Reference range
Total protein (g/dL)	**5.3**	6.6–8.1
Albumin (g/dL)	**3.2**	4.1–5.1
AST (IU/L)	20	13–30
ALT (IU/L)	10	10–42
ALP (IU/L)	93	38–113
LDH (IU/L)	**449**	124–222
BUN (mg/dL)	15.0	8.0–20.0
Creatinine (mg/dL)	0.80	0.65–1.07
Sodium (mEq/L)	**130**	138–145
Chloride (mEq/L)	**97**	101–108
Potassium (mEq/L)	3.8	3.6–4.8
Calcium (mmol/L)	**8.7**	8.8–10.1
CRP (mg/dL)	**5.87**	0.00–0.14
PCT (ng/mL)	**0.20**	< 0.05
IgG (mg/dL)	**483**	861–1,747
IgA (mg/dL)	**56**	93–393
IgM (mg/dL)	**10**	33–183
sIL2-R (U/mL)	**2,256**	145–519
Blood glucose (mg/dL)	**137**	73–109
HbA1c (%)	5.5	4.9–6.0
BNP (pg/mL)	**33.3**	< 18.5
Mycoplasma Ab	Negative	
(1→3)-β-D-glucan (pg/mL)	**123.6**	< 11.0
Aspergillus Ag	< 0.2	< 0.2
Sputum PCR for *P. jirovecii* DNA	Negative	
Cytomegalovirus IgG (AU/mL)	**104**	< 6.0
Cytomegalovirus IgM (index)	0.13	0.00–0.84
T-SPOT.TB	Negative	
HIV Ag/Ab	Negative	
KL-6 (U/mL)	**860**	< 500
SP-D (ng/mL)	**254**	< 110
White blood cell count (cells/μL)	5,400	3,300–8,600
Neutrophils (%)	73	42–74
Lymphocytes (%)	**17**	20–60
Monocytes (%)	9	0–12
Red blood cell count ($\times 10^4$/μL)	**359**	435–555
Hb (g/dL)	**10.5**	13.7–16.8
Hct (%)	**31.2**	40.7–50.1
MCV (fL)	86.9	83.6–98.2
MCHC (g/dL)	33.7	31.7–35.3
Platelet ($\times 10^3$/μL)	174	158–348
PT-INR	1.01	
aPTT (s)	30.3	24.0–34.0
Fibrinogen (mg/dL)	**471**	150–400
FDP (μg/mL)	3.0	< 5.0
D-dimer (μg/mL)	**1.4**	< 1.0

Bold indicates values outside the normal range. Ab, antibody; Ag, antigen; ALP, alkaline phosphatase; ALT, alanine transaminase; aPTT, activated partial thromboplastin time; AST, aspartate transaminase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CRP, C-reactive protein; FDP, fibrin/fibrinogen degradation products; Hb, hemoglobin; HbA1c, glycated hemoglobin; Hct, hematocrit; HIV, human immunodeficiency virus; Ig, immunoglobulin; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; *P. jirovecii*, *Pneumocystis jirovecii*; PCT, procalcitonin; PT-INR, prothrombin time international normalized ratio; sIL2-R, soluble interleukin-2 receptor; SP-D, surfactant protein D.

substantially boosts neutralizing antibody titers, even in those with inadequate humoral immune responses to COVID-19 vaccination (25). These studies suggest that sotrovimab may be a valuable therapeutic option against SARS-CoV-2 Omicron subvariants. In our patient, sotrovimab was administered sequentially owing to the unavailability of casirivimab/imdevimab.

Persistent COVID-19 can present with or without abnormal pulmonary findings, and the severity of respiratory decompensation varies widely (9, 10, 19). Frequently, multiple ground-glass opacities are observed on CT scans, and migration of airspace opacities, as demonstrated in this case, may also be observed (26) (Supplementary Table 1). In cases of ground-glass opacities that appear after the onset of COVID-19, the differential diagnosis includes persistent SARS-CoV-2 infection, as well as infections induced by a broad spectrum of pathogens, non-infectious diseases, pulmonary edema, and exacerbation complicating pre-existing interstitial lung disease. The wide range of conditions to consider in the differential diagnosis contributes to the diagnostic challenge. Challenges to diagnosing persistent COVID-19 include the positioning of imaging patterns and predicting cases of severe or refractory disease that require close monitoring. Even cases with a relatively mild but persistent clinical course of COVID-19 pneumonia can be fatal (10, 27), highlighting the need for further research in this field.

This study has some limitations. First, genetic sequencing of the SARS-CoV-2 virus was not performed in this case. According to Japanese nationwide surveillance data, the Omicron variant JN.1 and its subvariants accounted for approximately 95% of SARS-CoV-2 strains circulating in Japan in July 2024 (28), consistent with global trends (29). Second, no lower respiratory tract specimens (e.g., bronchoalveolar lavage fluid) were collected from the patient; therefore, the possible involvement of other respiratory pathogens cannot be ruled out in this case. However, except for elevated (1→3)-β-D-glucan levels, no other laboratory findings suggested the presence of other infections. Decisions regarding further testing should be personalized based on each patient's unique immune status and the invasiveness of the proposed diagnostic procedures.

This case highlights key aspects of persistent COVID-19 in immunocompromised patients. Although patient backgrounds and treatment strategies vary, most cases are mild, with favorable outcomes from antiviral monotherapy or combination therapy.

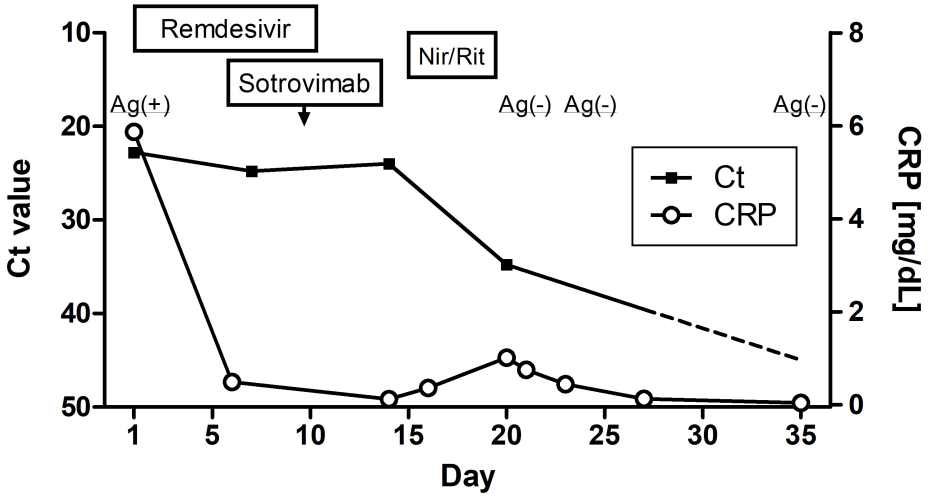

FIGURE 2
Clinical course of this case showing the changes in SARS-CoV-2 nucleic acid and C-reactive protein levels. Nir/Rit, nirmatrelvir/ritonavir.

Notably, viral load appears to rapidly decrease following clinical improvement (10). Few reports are available of persistent infection following rituximab and epcoritamab treatment (9, 10, 30, 31). Among them, Bay et al. (30) suggested that intermittent remdesivir monotherapy may induce resistance through ORF1b:C455F mutation. As with our patient, most patients improve with prolonged (sequential or simultaneous) nirmatrelvir/ritonavir-based therapy. Although Breeden et al. (31) reported pneumonia associated with persistent SARS-CoV-2 infection, to our knowledge, our report is the first report migratory ground-glass opacities observed on serial high-resolution CT scans. Persistent COVID-19 in immunocompromised patients is diverse and clinically challenging to manage. More clinical research is needed for optimal practice.

In conclusion, in immunocompromised patients, particularly those with B-cell hematological disorders who are undergoing chemotherapy, are at risk of persistent SARS-CoV-2 infection. Although no standardized guidelines are available for treatment of persistent SARS-CoV-2 infection, combination therapy should be considered in immunocompromised patients to enhance the effectiveness of treatment and reduce the risk of prolonged viral shedding and the emergence of new SARS-CoV-2 mutations.

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 humans were approved by the Ethics committee in Kashiwa Kosei General Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The 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

MS: Investigation, Resources, Supervision, Writing – original draft, Writing – review and editing. IF: Investigation, Resources, Writing – original draft, Writing – review and editing. TM: Investigation, Resources, Supervision, Writing – original draft, Writing – review and editing.

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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.

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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.2025.1554100/full#supplementary-material

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High sensitivity of HIV antibody screening tests may lead to longer time to diagnosis: a Case Report

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Background: The fourth-generation human immunodeficiency virus (HIV) serology assay, which simultaneously detects the HIV-1 p24 antigen and HIV-1 antibodies, is available either in a combined format or as dual tests that differentiate between the p24 antigen and antibodies. Divergent detection methodologies require distinct confirmatory testing algorithms, which significantly impact the time to HIV infection.

Case presentation: In this report, we present three cases where the HIV-1 p24 antigen tested reactive, while the HIV-1 antibody remained non-reactive in a dual testing scenario—despite both the combined test and the colloidal gold immunochromatographic assay (GICA) for HIV-1 antibodies yielding reactive results. Upon further analysis of subsequent laboratory procedures, we observed that due to the application of various complementary tests, the assay with high antibody sensitivity such as the GICA paradoxically resulted in a prolonged time to diagnosis, extending the diagnostic window for patients from 5 days to 11 days.

Conclusion: Our findings underscore the importance of prioritizing HIV-1 RNA testing in cases of discordant results between combined antigen/antibody testing, dual testing, and stand-alone antibody testing, particularly for patients who have not received pre-exposure or post-exposure prophylaxis.

KEYWORDS

HIV serological assay, P24 antigen, antibody HIV, diagnosis period, algorithm, fourth generation assay

1 Introduction

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are major public health problems worldwide. An HIV screening test is of great importance to identify all HIV-infected people and facilitate their linkage to care (1). The screening and detection of 90% of all HIV-infected people was declared a major goal by the UNAIDS (2). The fourth-generation antigen/antibody (Ag/Ab) immunoassay has become the most commonly used screening test due to its high sensitivity (3). Elecsys® HIV combi PT and Elecsys® HIV Duo are both fourth-generation reagents of electrochemiluminescence immunoassay (ECLIA). A critical distinction between the two assays lies in result interpretation: Elecsys® HIV combi PT yields a single composite result for the simultaneous detection of the p24 antigen and antibodies, whereas the Elecsys® HIV Duo provides discrete

differentiation of antigen and antibody reactivity, thereby distinguishing whether a positive result is attributable to the presence of the HIV-1 p24 antigen or specific antibodies against HIV-1/2 (4). For samples reactive to HIV antibodies, the colloidal gold immunochromatographic assay (GICA) is routinely used as a rapid diagnostic test and employed as the retesting method. Concurrent positive results from both the GICA and the initial screening test generally indicate the need for a Western blot (WB) HIV-1 antibody confirmatory test, and a negative GICA result typically warrants supplemental HIV-1 RNA testing (nucleic acid amplification technologies, NAATs) to rule out acute HIV infection. The National Guideline for HIV/AIDS Detection (2020), issued by the Chinese Center for Disease Control and Prevention (5) (hereinafter referred to as the "Guideline"), stipulates distinct supplementary diagnostic algorithms (Figure 1) for these two types of test assays.

Here, we present three cases demonstrating reactivity in both Elecsys® HIV Duo and Elecsys® HIV combi PT independently. In one case, the diagnostic algorithm prompted GICA testing, which yielded reactive results; however, the time to definitive HIV diagnosis for this patient was nearly twice as long as that for the other two cases.

2 Case presentation

2.1 Case 1

A 43-year-old man was admitted to the emergency department with a fever lasting for 8 days, with the highest recorded temperature reaching 39°C. The inguinal lymph nodes had become enlarged 2 weeks earlier. An adequate, non-leaky qualified serum sample was sent for an HIV screening test. Since Elecsys® HIV combi PT (Roche Diagnostics, Germany, REF 05390095) was reactive (22.01 COI) and the GICA (Lizhu, China, REF20143401976) was positive, the patient was advised to undergo an HIV-1 antibody confirmatory test. Six days later, we obtained another sample from him for WB testing (MP Diagnostics, Singapore, REF 20163401575). Prior to testing, Elecsys® HIV Duo (Roche Diagnostics, REF 07229542190) was used as a conventional additional test according to our workflow.

Notably, Elecsys® HIV Duo demonstrated discordant results compared to the GICA, and HIV antibodies were non-reactive (0.37 COI), whereas the p24 antigen exhibited reactivity (9.94 COI). The WB testing showed gp160 and p24 bands, which were classified as indeterminate. Given this discrepancy, confirmatory HIV-1 RNA testing was performed using the Roche cobas® system (REF 05212294190). Following a 4-day interval, subsequent testing revealed a high HIV-1 viral load of 2.86×10^6 copies/mL, confirming the definitive diagnosis of HIV infection. In this patient, we found low CD4 + T-lymphocyte counts (BD, America, REF 34049) with 55 cells/μL. The diagnostic interval from the initial HIV screening test to the confirmed diagnosis was 11 days (Figure 2).

2.2 Case 2

A 46-year-old male was admitted to the otorhinolaryngology department with upper respiratory tract symptoms and

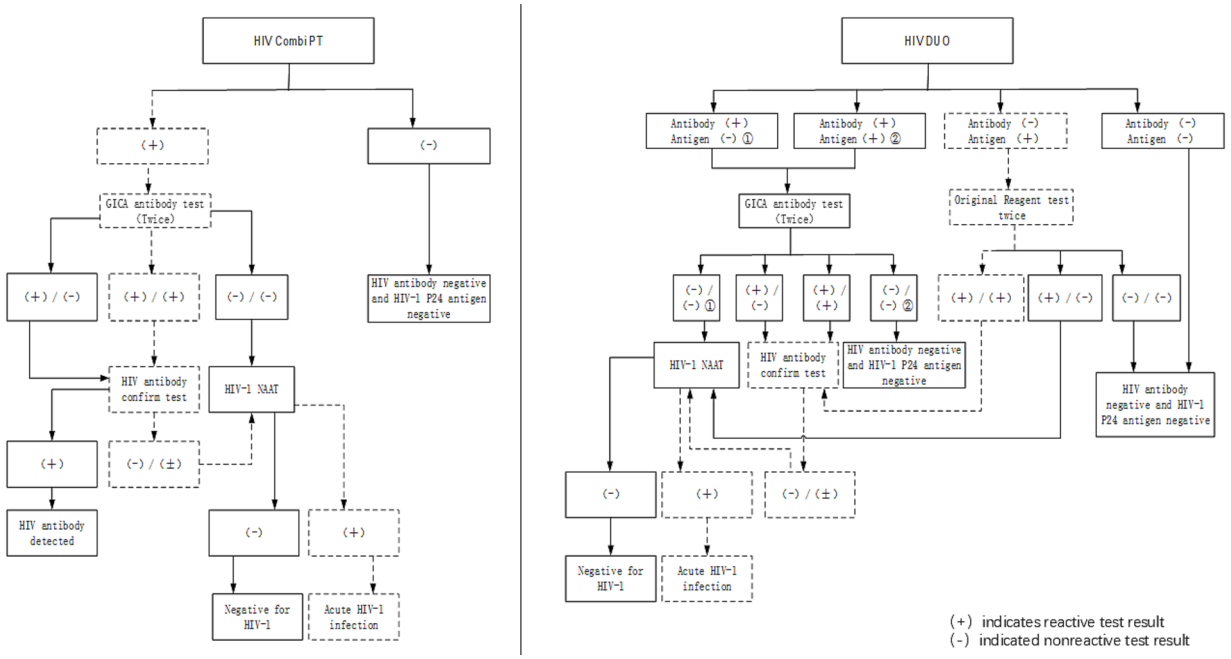

FIGURE 1
Two processing flows for HIV screening positive results. Elecsys® HIV combi PT/ Elecsys® HIV Duo and the GICA antibody test are used as screening procedures for HIV infection. HIV infection can be confirmed in patients with positive NAATs and positive confirmatory tests. The procedure of the Elecsys® HIV combi PT- or Elecsys® HIV Duo-positive supplementary test is shown in the figure. The dotted lines represent the processing flow for our three patients. Case 1 corresponds to the left one, and Cases 2 and 3 correspond to the right one. The HIV-1 antibody confirmatory test uses the Western blot technique to detect antibodies and is regarded as the most important supplementary test for HIV diagnosis in China because it can distinguish the type of HIV proteins targeted by antibodies in patients.

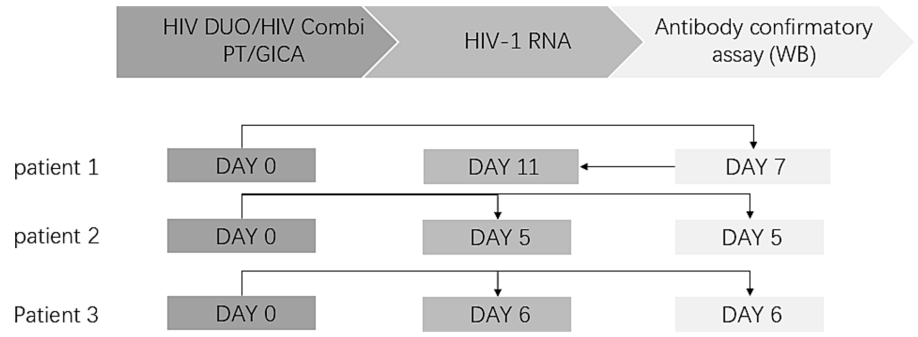

FIGURE 2
Diagnosis timeline of HIV patients. The laboratory diagnostic test procedure for HIV is divided into three sections: the HIV screening test, HIV RNA testing, and the HIV-1 antibody confirmatory test. The arrow indicates the sequence of the tests. In Cases 2 and 3, both HIV-1 RNA testing and Western blot (WB) antibody confirmation were submitted concurrently.

lymphadenopathy. An HIV screening test was immediately conducted. Elecsys® HIV Duo demonstrated reactive p24 antigen (5.52 COI) with concurrent non-reactive HIV antibodies (0.35 COI). To prevent potential false-negative results (as demonstrated in Case 1), antibody retesting was conducted using the GICA, which yielded positive results. To verify this contradictory result, Elecsys® HIV combi PT was added as a third test, and the result was reactive (21.34 COI), prompting us to perform an HIV-1 antibody confirmatory test for this patient. However, in this case, we recommended the concurrent submission of two specimens: one for HIV-1 RNA testing and the other for the HIV-1 antibody confirmatory test. Four days later, the HIV-1 RNA test returned positive with a viral load of more than 1.00×10^7 copies/mL, while the HIV-1 antibody confirmatory test was negative, showing no band. The patient was eventually diagnosed with HIV infection based on a positive HIV-1 RNA result. The diagnostic interval from the initial HIV screening test to the confirmed diagnosis was 5 days (Figure 2), CD4 + T-lymphocyte counts were also low, at a level of 78 cells/μL.

2.3 Case 3

A 46-year-old man with Guillain–Barre syndrome was admitted to the neurology department. Elecsys® HIV Duo showed reactive p24 antigen (8.95 COI) and non-reactive HIV antibodies (0.20COI). Similar to Case 2, retesting with the GICA and Elecsys® HIV combi PT showed positive results. To ensure a comprehensive diagnostic evaluation, we reiterated the recommendation for concurrent HIV-1 RNA testing and confirmatory HIV-1 antibody testing. As a result, the HIV-1 RNA testing was positive, with a viral load above 1.00×10^7 copies/mL, while the WB testing was indeterminate, showing only the gp160 band.CD4 + T-lymphocyte counts were 712 cells/μL. This patient was also diagnosed with HIV infection. The diagnostic interval from the initial HIV screening test to the confirmed diagnosis was 6 days (Figure 2).

The studies involving human participants were reviewed and approved by the Ethics Committee of West China Hospital of Sichuan University. Informed consent was obtained from all patients.

3 Discussion

Serological tests for antigens and antibodies have been the most common method of HIV screening for a long time. Serological testing has evolved through four generations: the first generation used viral lysates for IgG antibody detection, the second generation employed recombinant antigens for IgG antibody detection, the third generation detected immunoglobulin M (IgM) and IgG antibodies, and the fourth generation detected IgM and IgG antibodies alongside the p24 antigen. As a fourth-generation reagent, Elecsys® HIV Duo shows good sensitivity and specificity. A multicenter evaluation of the reagent involving 13,328 blood donor samples showed a specificity of 99.87% (6). Elecsys® HIV Duo is considered to have slightly higher specificity (99.93% vs. 99.84%) and equivalent sensitivity compared to Elecsys® HIV combi PT (7). In a previous study involving 1,505 patients, the BioPlex 4th PLUS assay was assessed and found to have 100% sensitivity and 99.5% specificity (8).

The detection window of fourth-generation reagents is shorter, enabling serological detection within 5–7 days following a positive HIV-1 RNA test, as they can detect the HIV-1 p24 antigen. Approximately 1 week after the appearance of p24, immunoglobulin M (IgM) antibodies are detectable through third-generation immunoassays—several weeks earlier than first- or second-generation immunoassays that only detect immunoglobulin G (IgG) antibodies (9–12). Compared to Elecsys® HIV combi PT, Elecsys® HIV Duo can shorten the window period from 18 days to 14 days after HIV infection (13) because of its slightly different coating of antigens/antibodies, which reduces the proportion of cross-reactions. In addition, Elecsys® HIV Duo can avoid the secondary window period to a certain extent, while the window period for Elecsys® HIV combi PT is more difficult to minimize.

Given reports of HIV misdiagnoses associated with fourth-generation reagents, to avoid false-positive antibody results from Elecsys® HIV Duo and Elecsys® HIV combi PT (3, 14), third-generation reagents such as the GICA with lower detection limits are used as supplementary methods in screening tests. Although only IgG antibodies are detected, due to the high affinity of gp41 and gp120, the GICA reagent has a lower limit for antibody detection than fourth-generation reagents. It usually yields a positive result at the same time as, or earlier

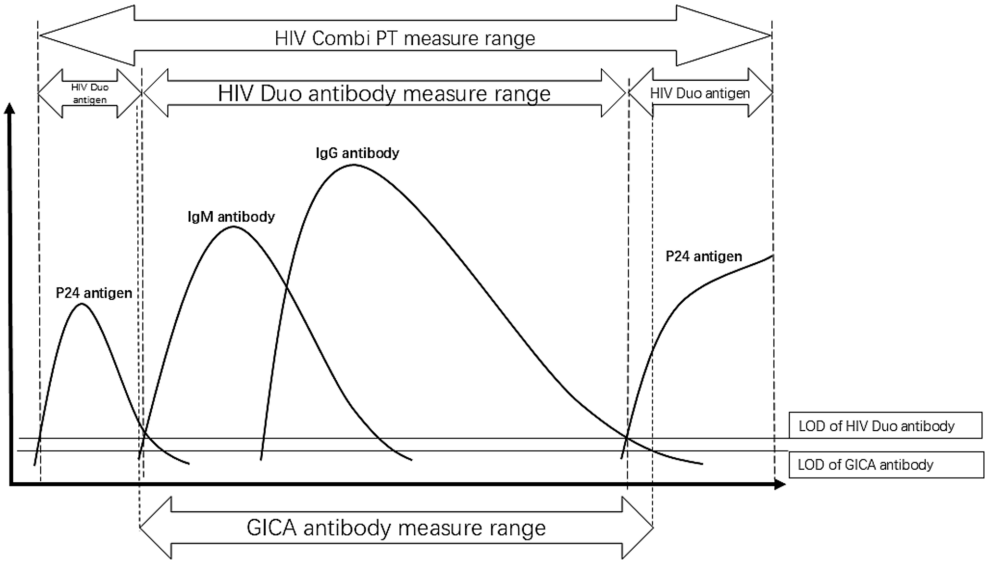

FIGURE 3
Pattern diagram of serological conversion of HIV infection. The dotted lines represent the detection range of different methods, while the solid lines indicate the detection limits of antigens or antibodies. The limit of detection (LOD) for the GICA antibody test is lower than that of the ROCHE HIV DUO antibody test. When the HIV Duo antibody test is negative, the GICA antibody test may be positive, while the HIV Duo antigen test will be positive, as observed in our cases. In the serological conversion process of HIV infection, this situation occurs during two phases—early infection and late infection. At these stages, different tests provide varying guidance. The HIV Duo antigen test takes precedence for patients whose antibodies are not detected, directing them to HIV RNA testing.

than, WB testing (15, 16) (Figure 3). Samples that test positive by ECLIA, the GICA, and WB testing will be used as direct evidence for an antibody-based HIV diagnosis. In contrast, only ECLIA-positive samples will prompt HIV-1 RNA testing (Figure 1). Due to its high specificity and ability to evaluate the stage of infection based on the separation of HIV-1 viral proteins by molecular weight, the WB technique has long been used as a confirmatory assay. However, the HIV-1 Western blot test still relies on first-generation principles, using whole viral lysate as the source of antigens and an enzyme-conjugated anti-IgG to bind to individual HIV proteins. The long detection window period makes it easy to prolong the diagnosis time. HIV-1 RNA is considered the earliest detectable marker of HIV infection and can be detected 7–10 days after infection, even when antigen and antibody tests are still negative (11, 17). It can be detected using PCR or NAATs with high sensitivity. HIV-1 RNA positivity is generally considered direct evidence of HIV infection. However, the risk of false-negative results remains due to viral replication inhibition by antiretroviral therapy and pre-exposure prophylaxis/post-exposure prophylaxis (18–20).

Across all three cases in our series, we observed that, as an antibody supplemental test, the GICA results were inconsistent with the antibody testing results from Elecsys® HIV Duo. Among patients with reactive GICA results, it took much longer for HIV infection to be diagnosed in patients who continued with confirmatory WB testing only (Case 1). We believe that the high sensitivity of the GICA led to reactive results, which subsequently directed patients to WB testing, a method with longer window periods. It is worth noting that in Case 2, the likelihood of missing the diagnosis would have been high if we had not performed additional HIV-1 RNA testing and only performed HIV antibody confirmatory testing based on the supplemental testing procedures of Elecsys® HIV combi PT. Our study demonstrated that incorporating higher-sensitivity antibody detection assays into the initial screening protocol failed to significantly improve diagnostic timeliness. Crucially, HIV-1 RNA testing remained indispensable regardless of the reactivity status in subsequent antibody screening. Furthermore, to preclude the influence of pre-exposure or post-exposure prophylaxis on HIV-1 RNA testing, WB testing is essential and should be conducted concurrently.

In conclusion, our patients presented with unusual cases of discrepant HIV antibody screening results, which led to different recommendations and affected the time to diagnosis and the assessment of infection status. Higher sensitivity antibody screening results lead to HIV-1 antibody confirmatory testing, which, in turn, leads to longer diagnostic times. Therefore, even when the screening procedure confirms HIV antibody reactivity, HIV-1 RNA testing needs to be performed concurrently.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: the dataset contains patient information. Requests to access these datasets should be directed to Yuanfang Wang, 180402617@qq.com.

Ethics statement

The studies involving humans were approved by Ethics Committee of West China Hospital of Sichuan University (No. 920). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired

from a by- product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. 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

YW: Data curation, Writing – original draft. LL: Data curation, Writing – original draft. JD: Formal analysis, Writing – original draft. XL: Formal analysis, Writing – original draft. DL: Project administration, Writing – review & editing. YX: Visualization, Writing – review & editing.

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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.

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