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# Community series in the role of vitamin D as an immunomodulator, volume II

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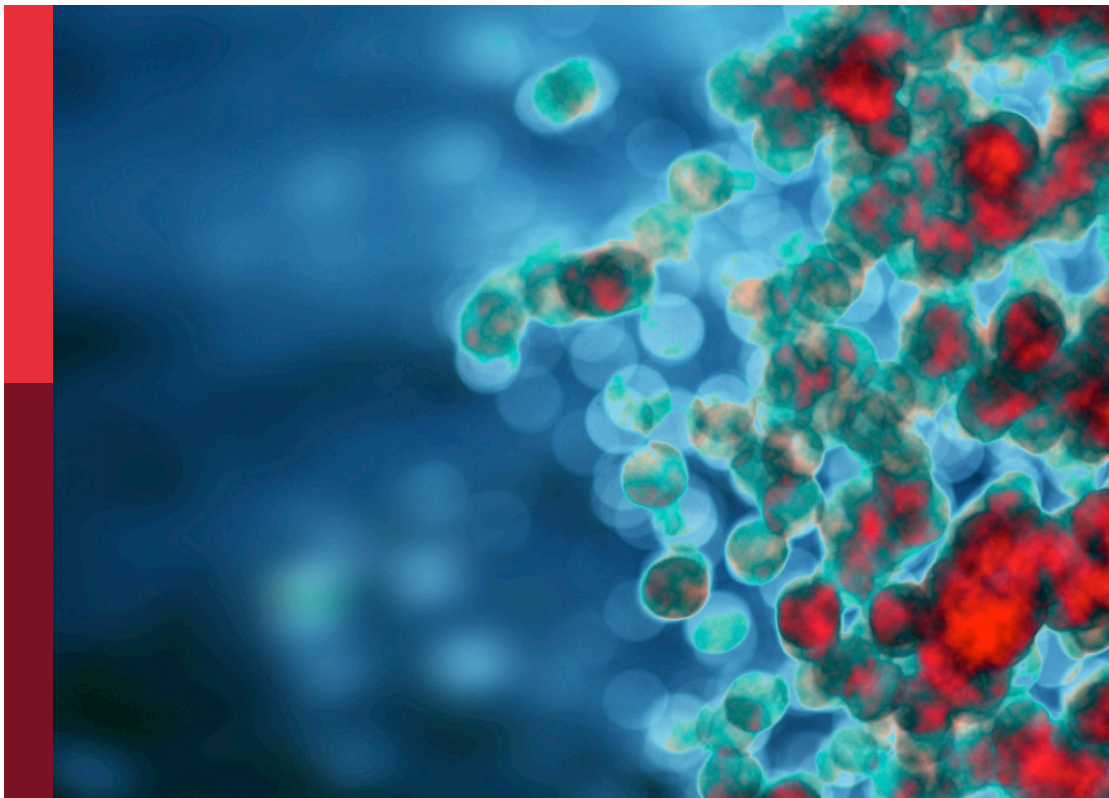

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# Community series in: the role of vitamin D as an immunomodulator, volume II

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# Editorial: Community series in: the role of vitamin D as an immunomodulator, volume II

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Editorial on the Research Topic
Community series in: the role of vitamin D as an immunomodulator, volume II

## 1 Introduction

Vitamin D has emerged as a multifaceted molecule, acting not only as a regulator of metabolic and skeletal homeostasis but also as a modulator of immune responses (1–3). This dual functionality underscores the need to clarify the conceptual distinction between regulation and modulation, particularly within the framework of immunology. The following sections provide an integrated overview of the conceptual principles, molecular foundations, immunomodulatory mechanisms, and systemic relevance of vitamin D as a key homeostatic factor.

### 1.1 Molecular and physiological basis of vitamin D activity

Vitamin D continues to emerge as a pleiotropic regulator of immune and metabolic homeostasis, operating at the crossroads of endocrine, skeletal, and immune systems (4–6). Traditionally recognized for its role in calcium–phosphate balance and bone mineralization, vitamin D—functioning both as a vitamin and a pre-hormone—exerts wide-ranging biological effects through its active metabolite, 1,25-dihydroxyvitamin $D_3$ [$1,25(OH)_2D_3$] (7–10). This metabolite binds to the vitamin D receptor (VDR), a ligand-dependent nuclear transcription factor expressed in numerous immune and non-immune cells, including those in the intestine, pancreas, bone, and skin (11). Upon activation, the $1,25(OH)_2D_3$–VDR complex forms a heterodimer with the retinoid X receptor (RXR-α) and binds to vitamin D response elements (VDREs) in target genes, thereby regulating

transcriptional programs involved in cell proliferation, differentiation, cytokine secretion, and immune tolerance (12–15).

Physiologically, vitamin D is synthesized in the skin from 7-dehydrocholesterol upon exposure to ultraviolet B radiation (290–320 nm) and subsequently hydroxylated in the liver and kidney by CYP2R1 and CYP27B1 to generate its active form (16, 17). The expression of these metabolic enzymes and the VDR within immune and non-immune tissues underscores the body's intrinsic capacity to locally produce and respond to calcitriol, thereby modulating both autocrine and paracrine signaling networks. Beyond its classical skeletal actions, vitamin D plays critical roles in neuromuscular function and in regulating key cellular processes such as growth, differentiation, apoptosis, and glucose metabolism (18–25). Collectively, these multidimensional effects position vitamin D as a central integrator of immune regulation, metabolic balance, and overall systemic health.

### 1.2 Immunomodulation *versus* immune regulation: conceptual distinction

In immunology, the terms *immunomodulation* and *immune regulation* are often used interchangeably, yet they represent distinct biological concepts.

Immune regulation refers to the intrinsic, homeostatic mechanisms by which the immune system maintains balance between activation and tolerance (26–28). These include key processes such as central and peripheral tolerance, the action of regulatory immune cells, cytokine feedback loops, and the suppression of excessive or self-reactive immune responses (29–31). In essence, immune regulation ensures the preservation of immune equilibrium and self-tolerance under physiological conditions. By contrast, immunomodulation encompasses any external or endogenous intervention—pharmacological, nutritional, hormonal, or microbial—that alters immune activity, either by enhancing or suppressing or redirecting specific cellular functions (32–34). Immunomodulators can therefore act as stimulants, suppressants, or balancing agents depending on the immunological context (Figure 1).

### 1.3 The dual and context-dependent immunomodulatory actions of vitamin D

Through interconnected pathways, vitamin D acts as a pivotal modulator of both innate and adaptive immunity. It strengthens antimicrobial defenses by inducing the expression of potent effector peptides, including cathelicidin and β-defensin 2, while simultaneously attenuating excessive inflammation by downregulating proinflammatory mediators (35–37). By fine-tuning cytokine networks and antigen-driven responses, vitamin D maintains a delicate equilibrium between immune activation and tolerance, promoting resolution of inflammation and preserving tissue integrity and homeostasis. Vitamin D thus exemplifies a potent immunomodulatory agent, as it influences both regulatory and effector immune mechanisms (38). It does not merely suppress or activate immune functions, but rather *modulates* their intensity and quality, promoting an adaptive state of functional equilibrium that is essential for immune homeostasis. In this regard, vitamin D serves as a paradigm of *context-dependent immunomodulation* (39)—a molecular sentinel that operates through finely regulated receptor-mediated signaling to maintain immune homeostasis.

## 2 The immunomodulatory power of vitamin D: key insights from volume II

The compelling body of work compiled here, comprising 14 original research and review articles, highlights the growing interest of vitamin D and significantly advances our understanding of its immunomodulatory effects across a broad spectrum of conditions—from allergic and autoimmune diseases to viral infections like COVID-19, pregnancy-related disorders, inflammatory bowel diseases, metabolic syndromes, and cancer.

This editorial synthesizes the key contributions of this volume, emphasizing the convergence of mechanistic insights and clinical applications.

### 2.1 UVB-driven vitamin D metabolism and macrophage reprogramming fine-tuned by physical shielding

The experimental study by Meterfi et al. reveals how environmental factors dynamically shape vitamin D-mediated immune regulation. By exposing peritoneal macrophages to UVB radiation with or without a simulated passive barrier, the authors demonstrate that UVB enhances respiratory burst, MPO expression, and M1-like polarization, while physical shielding fine-tunes these effects by attenuating METosis-related MPO activity and modulating local redox metabolism. This study highlights how physical environmental constraints can calibrate vitamin D metabolism and innate immune effector functions, providing mechanistic insight into how external cues orchestrate immune homeostasis.

### 2.2 Vitamin D in allergic diseases and immunotherapy

Allergic disease receives careful attention: the review by Zhang et al. synthesizes mechanistic and clinical evidence linking vitamin D to atopic dermatitis, allergic rhinitis and asthma. In a complementary piece, Zúñiga and Bazan-Perkins analyze the controversial relationship between vitamin D and atopy, highlighting that vitamin D promotes a pro-tolerogenic Th2 phenotype, suppresses Th1-driven inflammation, and supports immune homeostasis. They further emphasize that vitamin D acts through regulatory

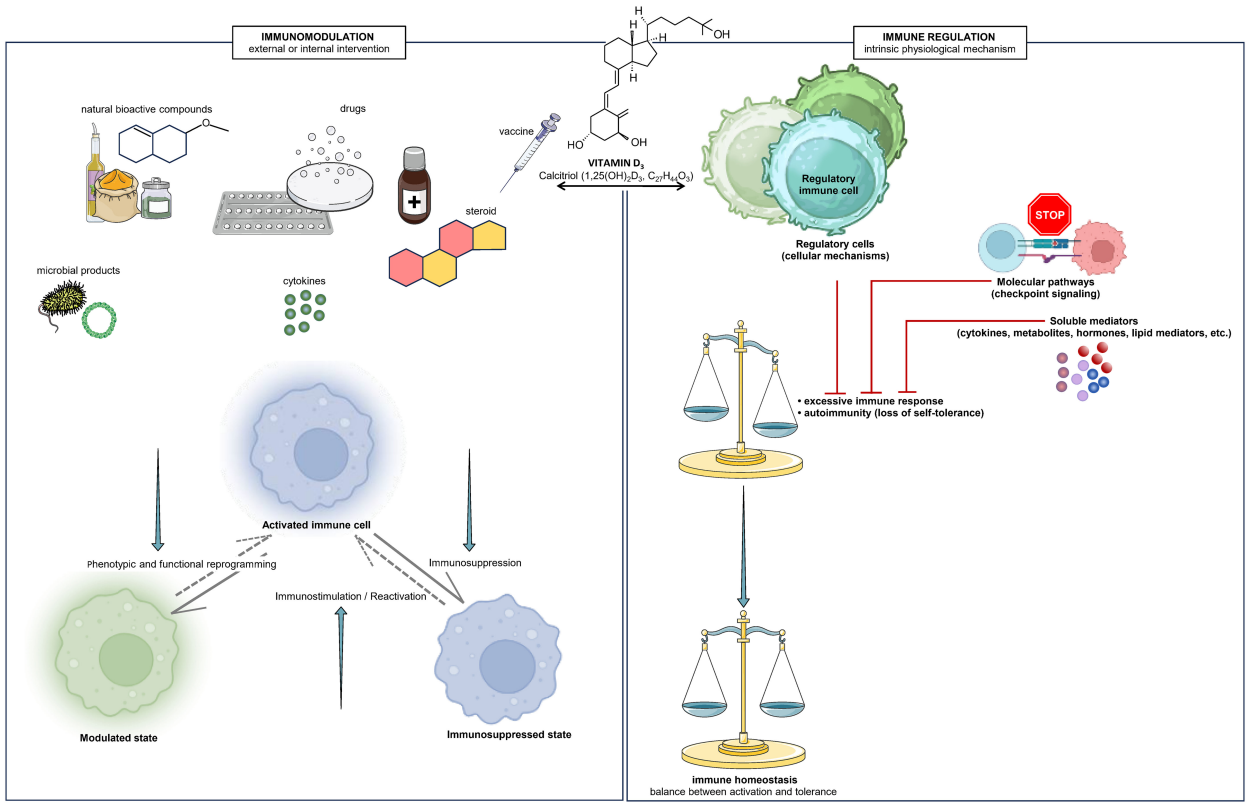


FIGURE 1
Conceptual interplay between immunomodulation and immune regulation: the dual influence of vitamin D. Immunomodulation refers to endogenous or exogenous influences that can bidirectionally modify immune responsiveness, either by stimulating, inhibiting, or redirecting cellular activities, thereby highlighting its adaptive and context-dependent nature. These processes affect immune cells that are already activated or primed, leading to a change in their functional and phenotypic state without complete loss of activation. In contrast, immune regulation represents intrinsic physiological mechanisms that maintain immune homeostasis and prevent both excessive immune activation and loss of self-tolerance. It reflects the immune system's self-governing capacity to restore equilibrium following activation, through cellular mechanisms involving regulatory cells that directly suppress or modulate effector responses, molecular pathways engaging inhibitory checkpoint signaling that sustains immune homeostasis, and soluble mediators that limit inflammation and maintain immune tolerance. The bioactive form of vitamin D, 1,25-dihydroxyvitamin $D_3$ (calcitriol), exemplifies a dual-acting molecule within this conceptual continuum. As a prohormone with immunomodulatory properties, it influences the differentiation, activation, and reprogramming of immune cells, while indirectly supporting immunoregulatory balance through its systemic endocrine and paracrine effects. All conceptual notions presented in this Figure are encompassed within this integrative framework. Schematic elements were partially adapted from Servier Medical Art (https://smart.servier.com), licensed under a Creative Commons Attribution 3.0 Unported License (CC BY 3.0). The molecular structure of the active form of vitamin $D_3$ (calcitriol, $1,25(OH)_2D_3$; $C_{27}H_{44}O_3$) was generated using ACD/ChemSketch, Version 2.0 (Advanced Chemistry Development, Inc., Toronto, Canada).

T-cells to maintain cytokine balance—modulating both pro- and anti-inflammatory pathways—and by limiting B-cell proliferation and differentiation, thereby fostering a state of immune equilibrium.

## 2.3 Pregnancy, hemostasis and vitamin D-associated platelet modulation

In obstetric immuno-hematology, the clinical study by Wang et al. shows that gestational hypertension is associated with lower vitamin D levels and platelet counts (PLT), along with higher mean platelet volume (MPV), platelet distribution width (PDW), and D-dimer levels. Vitamin D was significantly correlated with these parameters, suggesting a potential role in modulating platelet function and coagulation, and highlighting the value of optimizing vitamin D status in managing this condition.

## 2.4 Vitamin D and COVID-19 in risk modulation and clinical outcomes

The role of vitamin D during the COVID-19 pandemic is examined from multiple complementary angles. Missilmani et al. report that while systemic serum 25(OH)D levels alone are not directly associated with COVID-19 status, elevated nasopharyngeal VDR and DEFA1–3 expression correlate with reduced risk of SARS-CoV-2 infection, underscoring the importance of local vitamin D responsiveness. Complementing these findings, Petakh et al., in an umbrella review of systematic reviews, conclude that vitamin D supplementation significantly reduces the risk of Intensive Care Unit (ICU) admissions and mortality in COVID-19, particularly in vitamin D–deficient populations, while underscoring the need for standardized study designs. The cohort study by Konikowska et al. further establishes that sufficient vitamin

D levels (≥30 ng/mL) are associated with improved survival and a reduced risk of death among hospitalized COVID-19 patients, highlighting the prognostic value of 25(OH)D concentrations during hospitalization. In pediatric settings, Perestiuk et al. demonstrate that vitamin D deficiency or insufficiency is associated with more severe acute disease, prolonged hospitalization, and a 2.2-fold higher risk of long COVID—particularly with neurological and musculoskeletal symptoms—underscoring the importance of age-specific preventive approaches.

## 2.5 Vitamin D status across metabolic and inflammatory disease outcomes

Population and clinical cohort studies add epidemiological weight to mechanistic findings. Liu et al. report that lower serum 25(OH)D is associated with a higher incidence of hyperlipidemia in adults, highlighting the link between lifestyle-related vitamin D insufficiency and adverse lipid profiles. In the inflammatory bowel disease field, Zheng et al. report that higher baseline vitamin D levels independently predict clinical remission in infliximab-treated Crohn's disease patients, suggesting that vitamin D status may influence biologic therapy outcomes and patient stratification.

## 2.6 Vitamin D in autoimmunity: immunomodulation and genetic evidence

In their Opinion article, Su et al. revisit the debated field of high-dose vitamin D supplementation as a potential approach to immune recalibration in autoimmune diseases, highlighting its capacity to modulate Th1, Th17, and regulatory T-cells, alongside B-cell activity, in selected conditions, including multiple sclerosis, systemic lupus erythematosus, and Crohn's disease. Adding a genetic perspective, Huang et al. applied a Mendelian randomization framework leveraging genetic variants linked to various circulating micronutrients, including vitamin D, to dissect causal relationships with systemic lupus erythematosus (SLE). Using summary statistics from the IEU OpenGWAS database and supported by multiple sensitivity analyses (including MR-PRESSO, MR-Egger, and leave-one-out), their findings provide genetic evidence for a protective role of selected nutrients—notably vitamin D and calcium—against autoimmune susceptibility, highlighting the value of integrative nutrigenomic approaches.

## 2.7 Mechanistic and nanotechnological advances in vitamin D-based cancer therapy

Two studies in this volume shed light on novel oncological applications of vitamin D. Zhang et al. reveal an immunosuppressive $1,25(OH)_2D_3$–LL-37–tumor-associated macrophage (TAM) axis in hepatocellular carcinoma (HCC), in which LL-37 induction promotes macrophage recruitment and M2 polarization, thereby limiting vitamin D's efficacy. Importantly, the authors show that suramin, an LL-37 inhibitor, disrupts TAM recruitment and M2 polarization, restores M1 polarization, inhibits protein kinase B/mammalian target of rapamycin (Akt/mTOR) and signal transducer and activator of transcription 3 (STAT3) signaling, and enhances $1,25(OH)_2D_3$ therapeutic effects, reducing tumor growth and nodules *in vivo*, highlighting its potential as an adjunct in vitamin D–based immunotherapy for HCC.

To overcome the narrow therapeutic window of vitamin $D_3$ in oncology, Ezcurra-Hualde et al. developed a liposomal cholecalciferol formulation (VD-LP) with high encapsulation efficiency and long-term stability. VD-LP modulated immune-related and metabolic gene expression in THP-1 cells and exerted superior antiproliferative effects across colorectal, breast, and prostate cancer cell lines compared to free vitamin $D_3$. *In vivo*, VD-LP delayed tumor growth and improved survival without inducing hypercalcemia, highlighting its favorable toxicity profile. These findings demonstrate how nanotechnological innovations can safely extend vitamin D's therapeutic applications in oncology field.

# 3 Concluding perspectives: mechanistic integration and emerging frontiers

Together, the studies featured in this volume illuminate how vitamin D acts as a central immunometabolic regulator, bridging molecular mechanisms with translational vision across diverse pathophysiological contexts. From redox-sensitive macrophage reprogramming and modulation of T helper (Th) cells to genetic and nanotechnological innovations, these contributions collectively underscore vitamin D's capacity to fine-tune immune responses, metabolic signaling, and therapeutic efficacy.

The integration of multi-omic approaches, including genomics, transcriptomics, and nutrigenomics, emerges as a powerful avenue for uncovering individualized responses to vitamin D and identifying predictive biomarkers of efficacy across diseases.

Looking ahead, the convergence of precision nutrition, immune engineering, and vitamin D-based interventions define an exciting frontier for research, paving the way toward personalized immunomodulatory strategies that harmonize metabolic, genetic, and environmental dimensions of health.

In conclusion, this Research Topic provides a comprehensive framework for understanding and advancing vitamin D-based interventions across immune, metabolic, and oncological landscapes, charting a path toward more precise and effective clinical and therapeutic applications.

We trust that the insights presented here will catalyze translational breakthroughs and support the rational design of vitamin D-based interventions tailored to individual immune and metabolic profiles.

## Author contributions

MA: Writing – review & editing, Writing – original draft. FM: Writing – review & editing. CT-B: Writing – review & editing.

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## References

1. Sung C-C, Liao M-T, Lu K-C, Wu C-C. Role of vitamin D in insulin resistance. *J BioMed Biotechnol.* (2012) 2012:634195. doi: 10.1155/2012/634195

2. Liang Z, Wang Z, Liu X, He Y. Confronting the global obesity epidemic: investigating the role and underlying mechanisms of vitamin D in metabolic syndrome management. *Front Nutr.* (2024) 11:1416344. doi: 10.3389/fnut.2024.1416344

3. Daryabor G, Gholijani N, Kahmini FR. A review of the critical role of vitamin D axis on the immune system. *Exp Mol Pathol.* (2023) 132–133:104866. doi: 10.1016/j.yexmp.2023.104866

4. Młynarska E, Lisińska W, Hossa K, Krupińska N, Jakubowska P, Rysz J, et al. Vitamin D and chronic disorders: A review of metabolic and cardiovascular diseases. *Pharmaceuticals.* (2025) 18:1467. doi: 10.3390/ph18101467

5. Deepika N, Kumari A, Singh S, Ahmad MF, Chaki D, Poria V, et al. Vitamin D: recent advances, associated factors, and its role in combating non-communicable diseases. *NPJ Sci Food.* (2025) 9:100. doi: 10.1038/s41538-025-00460-5

6. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev.* (2016) 96:365–408. doi: 10.1152/physrev.00014.2015

7. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immuno-modulator. *Immunology.* (2011) 134:123–39. doi: 10.1111/j.1365-2567.2011.03482.x

8. Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. *Endocrinol Metab Clin North Am.* (2017) 46:815–43. doi: 10.1016/j.ecl.2017.07.001

9. Kongsbak M, Levring TB, Geisler C, von Essen MR. The vitamin d receptor and T cell function. *Front Immunol.* (2013) 4:148. doi: 10.3389/fimmu.2013.00148

10. Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). *Endocrinol Metab Clin North Am.* (2010) 39:255–69. doi: 10.1016/j.ecl.2010.02.007

11. Matilainen JM, Husso T, Toropainen S, Seuter S, Turunen MP, Gynther P, et al. Primary effect of 1α,25(OH)$_2$D$_3$ on IL-10 expression in monocytes is short-term down-regulation. *Biochim Biophys Acta.* (2010) 1803:1276–86. doi: 10.1016/j.bbamcr.2010.07.009

12. Lin R. Crosstalk between vitamin D metabolism, VDR signalling, and innate immunity. *BioMed Res Int.* (2016) 2016:1375858. doi: 10.1155/2016/1375858

13. Thompson PD, Jurutka PW, Haussler CA, Whitfield GK, Haussler MR. Heterodimeric DNA binding by the vitamin D receptor and retinoid X receptors is enhanced by 1,25-dihydroxyvitamin D3 and inhibited by 9-cis-retinoic acid. Evidence for allosteric receptor interactions. *J Biol Chem.* (1998) 273:8483–91. doi: 10.1074/jbc.273.14.8483

14. Zhou Q, Qin S, Zhang J, Zhon L, Pen Z, Xing T. 1,25(OH)2D3 induces regulatory T cell differentiation by influencing the VDR/PLC-γ1/TGF-β1/pathway. *Mol Immunol.* (2017) 91:156–64. doi: 10.1016/j.molimm.2017.09.006

15. Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M, et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol.* (2009) 183:5458–67. doi: 10.4049/jimmunol.0803217

16. Patwardhan VG, Khadilkar AV, Chiplonkar SA, Mughal ZM, Khadilkar VV. Varying relationship between 25-hydroxy-vitamin D, high density lipoprotein cholesterol, and serum 7-dehydrocholesterol reductase with sunlight exposure. *J Clin Lipidol.* (2015) 9:652–7. doi: 10.1016/j.jacl.2015.05.007

17. Young AR, Morgan KA, Harrison GI, Lawrence KP, Petersen B, Wulf HC, et al. A revised action spectrum for vitamin D synthesis by suberythemal UV radiation exposure in humans. *Vivo Proc Natl Acad Sci U.S.A.* (2021) 118:e2015867118. doi: 10.1073/pnas.2015867118

18. Fife RS, Sledge GW, Proctor C. Effects of vitamin D3 on proliferation of cancer cells. *Vitro Cancer Lett.* (1997) 120:65–9. doi: 10.1016/s0304-3835(97)00298-x

19. Sergeev IN. Vitamin D-mediated apoptosis in cancer and obesity. *Horm Mol Biol Clin Investig.* (2014) 20:43–9. doi: 10.1515/hmbci-2014-0035

20. Sun L-J, Lu J-X, Li X-Y, Zheng T-S, Zhan X-R. Effects of vitamin D supplementation on glucose and lipid metabolism in patients with type 2 diabetes mellitus and risk factors for insulin resistance. *World J Diabetes.* (2023) 14:1514–23. doi: 10.4239/wjd.v14.i10.1514

21. Zhang Y, Ni P, Miao Y, Chen H, Tang L, Song H, et al. Vitamin D3 improves glucose metabolism and attenuates inflammation in prediabetic human and mice. *J Nutr Biochem.* (2024) 130:109659. doi: 10.1016/j.jnutbio.2024.109659

22. Akimbekov NS, Digel I, Sherelkhan DK, Razzaque MS. Vitamin D and phosphate interactions in health and disease. *Adv Exp Med Biol.* (2022) 1362:37–46. doi: 10.1007/978-3-030-91623-7_5

23. Johnson JM, Peresuh SJ, Singh M, Okewunmi J, Arcand MA, Gil JA. The role of vitamin D in fracture healing: Insights from basic science, clinical studies, and future directions. *J Orthopaedic Rep.* (2025), 100759. doi: 10.1016/j.jorep.2025.100759

24. Wang JJ-D, Quak GS-W, Lee H-B, Foo L-X, Tay P, Mah S-M, et al. The role of vitamin D supplementation in enhancing muscle strength post-surgery: A systemic review. *Nutrients.* (2025) 17:1512. doi: 10.3390/nu17091512

25. Latham CM, Brightwell CR, Keeble AR, Munson BD, Thomas NT, Zagzoog AM, et al. Vitamin D promotes skeletal muscle regeneration and mitochondrial health. *Front Physiol.* (2021) 12:660498. doi: 10.3389/fphys.2021.660498

26. Han L, Wu T, Zhang Q, Qi A, Zhou X. Immune tolerance regulation is critical to immune homeostasis. *J Immunol Res.* (2025) 2025:5006201. doi: 10.1155/jimr/5006201

27. Goldmann O, Nwofor OV, Chen Q, Medina E. Mechanisms underlying immunosuppression by regulatory cells. *Front Immunol*. (2024) 15:1328193. doi: 10.3389/fimmu.2024.1328193

28. Zheng SJ. Immune regulation. In: *Veterinary Molecular Immunology*. Springer Nature Singapore, Singapore (2024). p. 205–17. doi: 10.1007/978-981-99-8929-4_9

29. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell*. (2008) 133:775–87. doi: 10.1016/j.cell.2008.05.009

30. Wang R, Lan C, Benlagha K, Camara NOS, Miller H, Kubo M, et al. The interaction of innate immune and adaptive immune system. *MedComm (2020)*. (2024) 5:e714. doi: 10.1002/mco2.714

31. van de Veen W, Stanic B, Wirz OF, Jansen K, Globinska A, Akdis M. Role of regulatory B cells in immune tolerance to allergens and beyond. *J Allergy Clin Immunol*. (2016) 138:654–65. doi: 10.1016/j.jaci.2016.07.006

32. Costa E, MaChado M, Pintado M, Silva S. Biological macromolecules as immunomodulators. In: *Biological macromolecules*. Amsterdam, Netherlands: Elsevier (2022). p. 273–87. doi: 10.1016/B978-0-323-85759-8.00012-9

33. Ogino S, Nowak JA, Hamada T, Phipps AI, Peters U, Milner DA, et al. Integrative analysis of exogenous, endogenous, tumour and immune factors for precision medicine. *Gut*. (2018) 67:1168–80. doi: 10.1136/gutjnl-2017-315537

34. Strzelec M, Detka J, Mieszczak P, Sobocińska MK, Majka M. Immunomodulation-a general review of the current state-of-the-art and new therapeutic strategies for targeting the immune system. *Front Immunol*. (2023) 14:1127704. doi: 10.3389/fimmu.2023.1127704

35. Bartley J. Vitamin D: emerging roles in infection and immunity. *Expert Rev Anti Infect Ther*. (2010) 8:1359–69. doi: 10.1586/eri.10.102

36. Gombart AF. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol*. (2009) 4:1151–65. doi: 10.2217/fmb.09.87

37. Yin K, Agrawal DK. Vitamin D and inflammatory diseases. *J Inflammation Res*. (2014) 7:69–87. doi: 10.2147/JIR.S63898

38. Aribi M, Mennechet FJD, Touil-Boukoffa C. Editorial: The role of vitamin D as an immunomodulator. *Front Immunol*. (2023) 14:1186635. doi: 10.3389/fimmu.2023.1186635

39. Athanassiou L, Mavragani CP, Koutsilieris M. The immunomodulatory properties of vitamin D. *Mediterr J Rheumatol*. (2022) 33:7–13. doi: 10.31138/mjr.33.1.7

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# Association of serum vitamin D concentration with the final course of hospitalization in patients with COVID-19

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**Background:** Vitamin D deficiency is a substantial public health problem. The present study evaluated the association between vitamin D concentration and hospitalization and mortality risk in patients with coronavirus disease 19 (COVID-19).

**Methods:** This study used the COronavirus in LOwer Silesia (COLOS) dataset collected between February 2020 and June 2021. The medical records of 474 patients with confirmed severe acute respiratory syndrome 2 (SARS-CoV-2) infection, and whose vitamin D concentration was measured, were analyzed.

**Results:** We determined a significant difference in vitamin D concentration between discharged patients and those who died during hospitalization ($p = 0.0096$). We also found an effect of vitamin D concentration on the risk of death in patients hospitalized due to COVID-19. As vitamin D concentration increased, the odds ratio (OR) for death slightly decreased (OR = 0.978; 95% confidence interval [CI] = 0.540-0.669). The vitamin D concentration cutoff point was 15.40 ng/ml. In addition, patients with COVID-19 and serum 25-hydroxyvitamin D (25(OH)D) concentrations < 30 ng/ml had a lower survival rate than those with

serum 25(OH)D ≥ 30 ng/ml (log-rank test p = 0.0018). Moreover, a Cox regression model showed that patients with an estimated glomerular filtration rate (eGFR) ≥ 60 ml/min/1.73 $m^2$ and higher vitamin D concentrations had a 2.8% reduced risk of mortality (hazard ratio HR = 0.972; CI = 0.95-0,99; p = 0.0097).

**Conclusions:** The results indicate an association between 25(OH)D levels in patients with COVID-19 and the final course of hospitalization and risk of death.

KEYWORDS

SARS-CoV-2, vitamin D, COVID-19, mortality, vitamin D deficiency, public health

# 1 Introduction

Vitamin D deficiency is a global problem that affects healthy and sick people (1). Race is a significant risk factor for vitamin D deficiency (2). The overall prevalence rate of vitamin D deficiency in the U.S. population was 41.6%, with the highest rate among African Americans (82.1%), followed by Hispanics (69.2%), and lowest among non-Hispanic Whites (30.9%) (3). The risk group consists mainly of overweight and elderly individuals who require higher doses of vitamin D (4, 5). Vitamin D deficiency is also often detected in patients with liver and kidney disorders (4, 6–8). Health benefits of vitamin D supplementation and sunlight exposure include reduced risk of many chronic diseases, including autoimmunity, cardiovascular disease, infections, and neurocognitive dysfunctions (1). Adequate vitamin D levels also prevent frailty and fractures (9).

Studies show that vitamin D has pleiotropic effects (10, 11). Calcitriol, 1,25-dihydroxycholecalciferol [1,25(OH)2D], is the active form of vitamin D and participates in numerous physiological processes while exhibiting immunomodulatory effects (12). It promotes immune cell proliferation and differentiation, modulates lymphocyte activity, and reduces pro-inflammatory cytokine (tumor necrosis factor alpha [TNF-α] and interleukin-1 [IL-1]) concentration while increasing anti-inflammatory cytokine (IL-4, IL-5, and IL-10) concentration (4). In addition, the action of 1,25(OH)2D is mediated by a highly specific, intracellular vitamin D receptor (VDR) (4, 13). As a nuclear receptor, ligand activation of the VDR leads to protein binding to specific sites in the genome, resulting in modulation of target gene expression (14). The pleiotropic effect of 1,25(OH)2D is believed to be active in the presence of VDR and 25(OH)D-1α-hydroxylase (CYP27B1) in many tissues, which allows the extrarenal synthesis of the active 1,25(OH)2D. The participation of 25(OH)D in the endocrine, autocrine, and paracrine pathways seems to be crucial for reducing the risk of cancer development, autoimmune diseases (e.g., multiple sclerosis, type 1 diabetes, and bronchial asthma), cardiovascular diseases, strokes, type 2 diabetes, and neurocognitive disorders (13, 15). 25(OH)D also reduced the incidence of recurrent infections (2, 16).

Vitamin D deficiency is linked to increased autoimmunity and higher susceptibility to respiratory tract infection (RTI) (17, 18). Nevertheless, the association between antimicrobial, antiviral, and anti-inflammatory effects may reduce the risk of asthma exacerbation, often caused by RTI and characterized by dysregulated pulmonary inflammation (19, 20). In addition, other studies have demonstrated an association between vitamin D deficiency and higher intensive care unit (ICU) mortality rates (21, 22).

The therapeutic role of vitamin D in patients with coronavirus disease 2019 (COVID-19) is currently a subject of discussion (23). Low vitamin D concentration has been linked to an increased predisposition to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (24) and a severe course of COVID-19 (25). Furthermore, it has been hypothesized that vitamin D may prevent and/or alleviate SARS-CoV-2 infection (23). However, the results of such research are not conclusive.

Based on studies conducted in 20 countries in Europe, Illie et al. (26) demonstrated a significant negative correlation between total 25-hydroxyvitamin D [25(OH)D] serum concentration and death in SARS-CoV-2 patients. In contrast, Orchard et al. (27) reported a negligible difference in vitamin D levels among hospitalized and ICU patients with COVID-19. Moreover, a non-significant difference in the clinical course was found between patients with low and normal vitamin D concentrations (27). Similarly, no beneficial effect of vitamin D3 treatment was found in patients with COVID-19 by Marani et al. (28). Administration of a large single dose of vitamin D3 (500,000 IU) did not prevent respiratory function deterioration in patients hospitalized with mild to moderate COVID-19, and there was no significant effect on the length of hospital stay (28).

Our study is one of the first to look at the association of vitamin D with adult mortality in the Polish population. Based on current knowledge, there are only a few published studies from Poland on vitamin concentrations in patients with COVID-19 (29, 30). In the study by Ziuzia-Janiszewska et al. (29), potential predictors of a severe course of COVID-19 in young adults (20-45 years old) were identified, including low vitamin concentration as one of the predictors. In this study, vitamin D concentration was tested in 81 out of 229 patients with SARS-CoV-2. Ziuzia-Janiszewska et al. (29) found that, among others, obesity, comorbidities, higher levels of CRP, IL-6, creatinine, urea, and also lower eGFR value, albumin, calcium, and vitamin D concentration may be associated with poor

COVID-19 outcomes. In another study from Poland, vitamin D concentrations were analyzed in 45 out of 52 pregnant women with confirmed SARS-CoV infection (30). It was shown that approximately 62% of pregnant women with COVID-19 had a decreased concentration of vitamin D (mean value – 27.15 ng/ml) (30).

Our study evaluated the association between vitamin D concentration and the final course of hospitalization and risk of death in patients treated for COVID-19. COVID-19 can affect many organs of the human body, including kidneys (31). The mechanism of kidney involvement in COVID-19 appears to be multifactorial. Thus far, data suggest effects of direct viral infection (viral tropism to the renal system), hypoxia, inflammatory syndrome-mediated injury, hemodynamic instability, vascular injury, and hypercoagulable state (32–35). In our study, an accurate analysis of the relationship between vitamin D concentration and baseline estimated glomerular filtration rate (eGFR) and the probability of a patient's survival outcome up to 90 days after hospital admission was performed. In addition, the association between vitamin D and other predictors of the incidence of death up to 90 days after COVID-19 hospitalization was examined using a Cox regression model. We hypothesized that low 25(OH)D plasma levels can predict poorer survival outcomes. We also aimed to evaluate whether 25(OH)D plasma levels predict mortality in adults with COVID-19 while considering potential confounders.

# 2 Methods

## 2.1 Study design and participants

Our research was part of the COronavirus in LOwer Silesia (COLOS) study of patients with laboratory-confirmed SARS-CoV-2 infection. The medical records of 474 patients with vitamin D concentration measurements were analyzed retrospectively. The patients were hospitalized at the University Hospital in Wroclaw between February 2020 and June 2021.

SARS-CoV-2 infection was confirmed by positive reverse transcriptase-polymerase chain reaction (RT-PCR) for viral ribonucleic acid (RNA) from a nasopharyngeal swab.

The Institutional Review Board and Bioethics Committee of Wroclaw Medical University approved this COLOS study (No: KB-444/2021). The data were collected retrospectively, and written informed consent to participate in the study was not required. The Bioethics Committee approved the publication of anonymized data.

## 2.2 Study procedures

The demographic data and information on medical history, previous medication, symptoms at admission, laboratory tests, and in-hospital clinical courses were derived from electronic hospital medical records. The presence of comorbidities and information about smoking was established from an interview with the patient on admission.

Laboratory assessment measured vitamin D status, IL-6, C-reactive protein (CRP), and renal function tests, including creatinine, were measured and eGFR values were calculated. Different indexes are used for kidney function assessment and the eGFR remains one of the best markers (36). eGFR was calculated based on the Modification of Diet in Renal Disease Study equation (36). A chemiluminescence method (Alinity i 25-OH Vitamin D Reagent Kit, Abbott, USA) evaluated the total serum 25(OH) D concentration.

Surviving patients were followed up by telephone after 3 months. Information was obtained directly from patients, their relatives, or the hospital system. Government General Registry Office data on death were used for the study.

## 2.3 Statistical analysis

Continuous data were expressed as mean and standard deviation (SD) when a normal distribution occurred. In the absence of a normal distribution, medians, and interquartile ranges were reported. The categorical data were presented as frequencies and percentages. Given the sample size in the study, the continuous variable was analyzed using parametric tests. Although parametric tests are designed for normal data distribution in general, it has been shown recently that the ANOVA is robust to the violation of this assumption (37, 38). It matters especially when the sample size is large compared to a threshold sample size (N = 30), which has been proposed as a criterion for reliably using parametric tests (when the assumption of the variance homogeneity is met) (39), with a sample size being considered as "large" when it contains hundreds of data (37, 38). Categorical variables were compared using Pearson's Chi-squared or Fisher's exact tests when the sample size was smaller than five.

ANOVA with Welch correction evaluated the relationship between the vitamin D concentration and the final hospitalization course. As the variance was unequal among the compared group (Levene's test: $F_{3,\ 470\ =}$ 3.18, p = 0.024), Tamhane's T2 *post hoc* test assessed differences between the groups. The final course of hospitalization was described as a categorical variable and divided into four stages: 0 - completed with discharge; 1 - completed transfer from the University Clinical Hospital to another acute hospital for specialized treatment due to new problems/deterioration of the patient's condition; 2 - completed by transfer to another hospital outside of the University Clinical Hospital for rehabilitation or docking of patients who could not be discharged home; 3 - fatal.

The effect of vitamin D concentration on the risk of death during hospitalization was assessed using a receiver operating characteristic (ROC) curve. The area under the curve (AUC) and confidence intervals (CIs) were calculated. The analysis established a Youden index and estimated a cutoff point for vitamin D concentration.

A Cox model evaluated the relationship between vitamin D concentration and eGFR values and the probability of patients' survival up to 90 days from the start of hospitalization. The results of patients' survival probabilities were presented using Breslow

survival curves, as were patients' survival probabilities concerning vitamin D concentration and eGFR value. According to the US National Kidney Foundation, chronic kidney disease (CKD) can be defined as an eGFR < 60 ml/min/1.73 $m^2$ (40). We assumed that the expected normal clinical range of eGFR is ≥ 60 ml/min/1.73 $m^2$. To visualize the effect of vitamin D concentration not disturbed by the effect of eGFR, the latter was assumed to be equal to 76.2 ml/min/1.72 $m^2$ (the arithmetic mean).

As the effects of the vitamin D concentration were statistically significant in the Cox regression, in the next step, Kaplan-Meier analysis with log-rank test compared the survival of patients up to the 90th day after hospital admission, divided into two groups based on the vitamin D concentration determined at the beginning of hospitalization. Patients were grouped into two serum 25(OH)D level categories: < 30 ng/ml and ≥30 ng/ml. Vitamin D sufficiency was defined as a blood level of ≥ 30 ng/ml. Vitamin D levels < 30 ng/ml were considered low.

In the last stage of the analysis, the hazard ratio (HR) and 95% CI of mortality (the incidence of death in patients with COVID-19 up to 90 days from the start of hospitalization) in relation to vitamin D concentration (as a continuous variable) and some other co-variables that are considered potentially important predictors were estimated using Cox proportional hazard regression models. The other variables entered into the model were sex, age at admission, CRP level, and the presence of heart disease. Because eGFR is usually considered an important predictor when assessing COVID-19 severity, a Cox model was used for the two patient groups separately. Patients with a measured vitamin D concentration were divided into two groups according to eGFR at admission: eGFR ≥ 60 ml/min/1.73 $m^2$ or eGFR < 60 ml/min/1.73 $m^2$.

In all the Cox regression models, p was > 0.2 for all the predictors in the test of proportional hazard assumptions. The assumption of a linear form of continuous covariates was assessed by plotting the Martingale residuals against continuous covariates. Goodness-of-fit of the Cox regression model was assessed using the statistics "concordance" (see below).

A p-value < 0.05 was considered statistically significant. Statistical analyses employed Statistica v.13.3 software (TIBCO Software Inc., Krakow, Poland) (descriptive statistics, Levene's test, proportional hazard test, and preparing scatterplots of Martingale residuals), SPSS 28.0 (Welch's ANOVA), and R-packages "survival" (41) and "survminer" (42) (Cox regression, forest plot preparation, Kaplan-Meier curves, and log-rank test "concordance").

# 3 Results

Vitamin D concentration in the blood of 474 patients hospitalized for SARS-CoV-2 infection was evaluated. The mean age of patients was 63 (± 15.3) years, and 276 (58.1%) patients were men. Comorbidities such as diabetes, hypertension, cardiovascular disease, and chronic kidney disease were present in 128 (26.9%), 259 (54.5%), 124 (26.1%), and 48 (10.1%) patients, respectively (Table 1).

TABLE 1 Patients' characteristics and clinical outcomes.

| Variables | |
|---|---|
| Age, mean (SD), years | 63.0 (15.3) |
| Men, No. (%) | 276 (58.1) |
| **Comorbidities occurrence*** | |
| Diabetes, No. (%) | 128 (26.9%) |
| Hypertension, No. (%) | 259 (54.5%) |
| Cardiovascular disease, No. (%) | 124 (26.1%) |
| Chronic kidney disease, No. (%) | 48 (10.1%) |
| Tumor, No. (%) | 29 (6.1%) |
| **Smoking, No. (%)*** | |
| Never | 421 (88.6%) |
| Former | 32 (6.7%) |
| Current | 21 (4.4%) |
| **Hospitalization course** | |
| The number of days in hospital, mean (SD), days | 20.3 (15.5) |
| Transferring the patient to ICU, No. (%) | 103 (21.7%) |
| Tracheostomy, No. (%) | 47 (9.9%) |
| Intubation during hospitalization, No. (%) | 98 (20.6%) |
| Shock during hospitalization, No. (%) | 89 (18.7%) |
| Deterioration of the patient's condition during hospital stay, No. (%) | 172 (36.2%) |
| Initiation of renal replacement therapy, No. (%) | 41 (8.6%) |
| SIRS, No. (%) | 65 (13.7%) |
| Death in hospital, No. (%) | 96 (20.2%) |
| **Laboratory values** | |
| 25(OH)D, ng/ml (Me, IQR) | 20.55 (20.30) |
| Creatinine, mg/dl (Me, IQR) | 0.95 (0.44) |
| CRP, mg/l (Me, IQR) | 61.18 (101.29) |
| IL-6, pg/mL (Me, IQR) | 21.85 (43.15) |

* data from the interview with the patient on admission; SD, standard deviation; No., number; ICU, Intensive Care Unit; SIRS, systematic inflammatory response syndrome; 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein, IL-6, Interleukin-6, Me, median; IQR, interquartile range.

The relationship between vitamin D concentration and the final hospitalization course was statistically significant (ANOVA: $F_{3, 470} = 4.82$, $p = 0.003$) (Table 2). A statistically significant difference in vitamin D concentration compared to discharged patients and those who were transferred urgently to another hospital due to new health problems/deterioration of their condition was determined. Moreover, discharged patients had significantly higher mean vitamin D concentrations than patients who died during hospitalization. In addition, patients who were transferred urgently to another hospital due to new health problems/deterioration of their condition had significantly lower

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TABLE 2 Vitamin D concentration and the course of hospitalization (ANOVA test, p-values for post-hoc Tamhane's T2 test).

| | | n (%) | mean (95% CI) | Me | | | Pairwise post-hoc comparisons | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 0 | 1 | 2 | 3 |
| Hospitalization * | 0 | 302 (63.6%) | 26.0 (23.9-28.1) | 23.1 | Hospitalization * | 0 | – | 0.0002 | 0.5503 | 0.0096 |
| | 1 | 21 (4.4%) | 16.0 (12.2-19.8) | 13.4 | | 1 | 0.0002 | – | 0.0362 | 0.4434 |
| | 2 | 55 (11.6%) | 23.0 (19.7-26.3) | 22.0 | | 2 | 0.5503 | 0.0362 | – | 0.7251 |
| | 3 | 96 (20.2%) | 20.1 (17.0-23.1) | 14.9 | | 3 | 0.0096 | 0.4434 | 0.7251 | – |

Hospitalization*: 0 - completed with discharge; 1 - completed transfer from the University Clinical Hospital to another acute hospital for specialized treatment due to new problems/deterioration of the patient's condition; 2 - completed by transfer to another hospital outside the University Clinical Hospital for rehabilitation or docking of patients who cannot be discharged home; 3 – fatal; CI, confidence interval; Me, median.

mean vitamin D concentrations than patients transferred to another hospital for rehabilitation or docking.

In our study, the effect of vitamin D concentration on death risk due to COVID hospitalization was also analyzed. Figure 1 shows the area under the ROC curve of vitamin D serum level for in-hospital mortality of patients with COVID-19. The AUC of the 25(OH)D ROC curve was 0.605 (CI 95% = 0.540-0.669; p = 0.0106) and shows that as vitamin D concentration increased, the odds ratio (OR) for death slightly decreased (OR = 0.978). Without adjusting for confounders, the sensitivity and specificity of vitamin D concentration as a single predictor for mortality in patients hospitalized for COVID-19 were poor (AUC = 0.605; 95% CI = 0.540-0.669). The cutoff point for vitamin D concentration was 15.40 ng/ml.

The study also assessed the probability of survival up to 90 days after the onset of hospitalization for SARS-CoV-2 infection, taking into account vitamin D concentration and eGFR values during hospital admission. Using Cox regression, we demonstrated a statistically significant effect of vitamin D concentration and baseline eGFR on the probability of survival (vitamin D: HR = 0.98; 95% CI = 0.969-0.995; p = 0.0075; eGFR value: HR = 0.99; 95% CI = 0.981-0.993; p = 0.000009, model concordance - 0.631).

To illustrate the effect of vitamin D concentration on survival probability, Breslow survival curves were prepared for two vitamin D concentrations to include the first and third quartiles. Survival probability at a low vitamin D concentration was markedly lower when compared to the high vitamin D concentration quartile (68.9% vs. 77.2%) at the same eGFR value (see "Statistical analysis" section and Figure 2 for details).

Figure 3 shows Kaplan-Meier curves for the survival probability of patients with COVID-19 up to 90-days after admission to the hospital according to vitamin D status. Patients with COVID-19 and serum 25 (OH)D concentrations < 30 ng/ml presented a lower survival rate than those with serum 25(OH)D ≥ 30 ng/ml (log-rank test p = 0.0018).

The Cox regression model showed that the association between vitamin D concentration and mortality risk in patients with

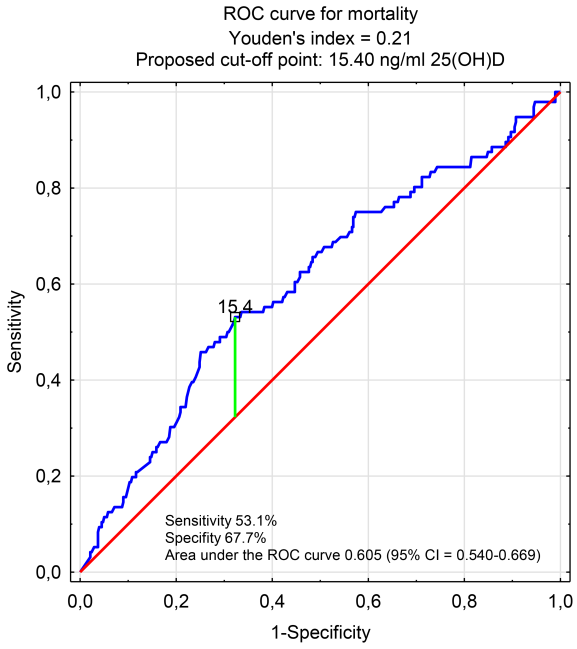


FIGURE 1
Area under the ROC curve of vitamin D serum level for in-hospital mortality of patients with COVID-19.

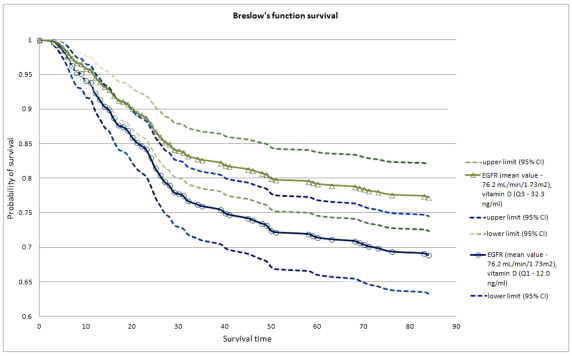


FIGURE 2
Breslow survival curves for patients with COVID-19 up to 90 days after hospitalization in relation to low (1st quartile Q1,) and high (3rd quartile Q3) vitamin D level, at constant eGFR level, equal to 76.2 mL/min/1.73m$^2$ (arithmetic mean).

COVID-19 depended on baseline eGFR (Figures 4, 5). In patients with COVID-19, it relied on baseline eGFR (Figures 4, 5), though in those with eGFR < 60 ml/min/1.73m$^2$, vitamin D concentration was not significantly associated with mortality risk (p = 0.882). In contrast, patients with an eGFR ≥ 60 ml/min/1.73 m$^2$ and higher vitamin D concentrations had a 2.8% reduced mortality risk (HR = 0.972; CI = 0.95-0.99; p = 0.0097) even after adjusting for potential confounders such as sex, heart disease, and CRP value. The mortality risk also increased by 4% with age in COVID-19 patients with eGFR values ≥ 60 ml/min/1.73 m2. Model concordance for the patient groups amounted to 0.63 for patients with eGFR < 60 ml/min/1.73 m$^2$ and 0.71 for patients with an eGFR ≥ 60 ml/min/1.73 m$^2$.

# 4 Discussion

This study presented the results of the association between vitamin D concentrations and the risk of death in patients hospitalized with COVID-19. Campi et al. (43) evaluated the vitamin D concentrations of 103 patients treated for severe COVID-19 (study group) and compared them to 52 patients with mild COVID-19 and 206 patients without confirmed SARS-CoV-2 infection (control group) and found that 25(OH)D concentrations were lower in COVID-19 patients who died in hospital (13.2 ± 6.4 ng/ml) compared to patients who survived (19.3 ± 12.0 ng/ml; p=0.0003). Moreover, they discovered an inverse correlation between 25(OH)D and mortality regardless of age, sex, diabetes, platelet count, and levels of IL-6, CRP, lactate dehydrogenase (LDH), neutrophils, and lymphocytes. The study found that a 1 ng/ml 25(OH)D increase was associated with a 4% reduction in the risk of death from COVID-19 (43).

In the current study, vitamin 25(OH)D values relevant to survival were in analogous convergence. We found that patients with COVID-19 and vitamin D values of 15.40 ng/ml or less at admission had a higher risk of death during hospitalization than patients whose vitamin D concentrations were above 15.40 ng/ml. Hafez et al. (44) reported similar findings. This study showed a statistically significant correlation between 25(OH)D deficiency (<12 ng/ml) and in-hospital mortality (p = 0.04). In an unadjusted logistic regression model, the probability of mortality increased significantly among patients with serum 25(OH)D

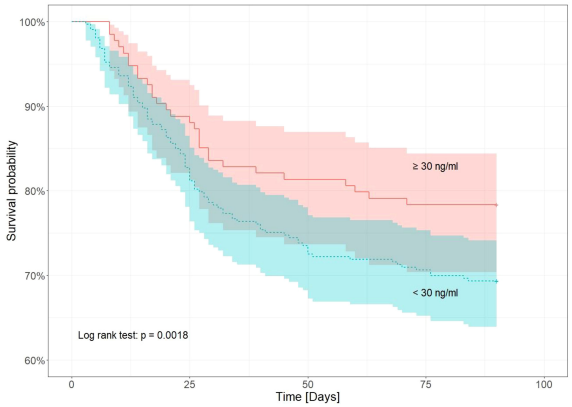


FIGURE 3
Kaplan–Meier survival analysis of COVID-19 patients until the 90th day from admission to hospital in relation to vitamin D concentration (<30 ng/ml and ≥ 30 ng/ml).

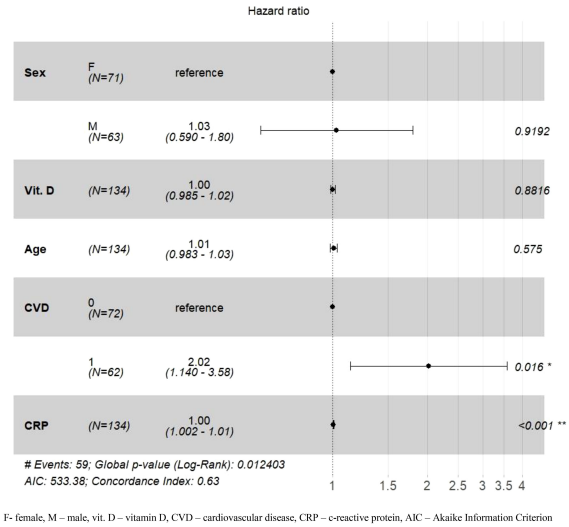


FIGURE 4
Adjusted hazard ratios (HR) for 90-day mortality in the group of patients with eGFR < 60 ml/min/1.73 $m^2$. *p-value <0.05; **p-value <0.01.

concentration <12 ng/ml compared to those with a higher serum concentration (OR = 7.86, 95% CI =1.43–37.49). In addition, Hafez et al. (44) used an adjusted logistic regression model for sex and age (Model 1), with a cutoff point of 25(OH)D < 12 ng/ml and 25(OH)D < 20 ng/ml adjusted for sex, age, race, and comorbidities in multivariate logistic regression in Models 2 and 3, respectively. The authors revealed that a serum 25(OH)D concentration <12 ng/ml significantly increased mortality 12-fold in Model 1 and 62-fold in Model 2.

In our study, the Cox regression model showed that the mortality risk also increased by 4% with age in COVID-19 patients with eGFR values ≥ 60 ml/min/1.73 $m^2$. It is worth mentioning that the value of eGFR decreases with age by approximately 1 ml/min/$m^2$ per year starting from the third decade of life (45). In addition, renal hyperfiltration is associated with a higher risk of cardiovascular disease and all-cause mortality (46, 47). Hafez et al. (44) also showed increased mortality of 13% for each 1-year increase in age. Researchers suggest that immune system functions decline with increasing age, which may be one of the risk factors for death (44).

Another study involving 464 patients with COVID-19 found that 25(OH)D concentrations < 12 ng/ml were significantly associated with a higher risk of severe SARS-CoV-2 infection and mortality (48). Al Safar et al. (48) determined predictors of

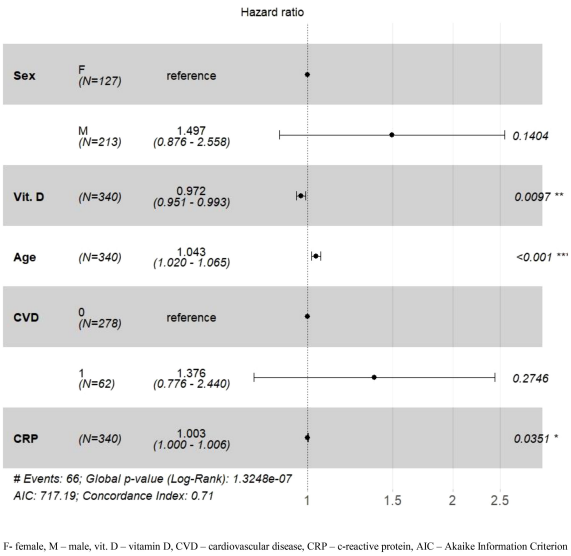


FIGURE 5
Adjusted hazard ratios (HR) for 90-day mortality in the group of patients with eGFR ≥ 60 ml/min/1.73 $m^2$. *p-value <0.05; **p-value <0.01; ***p-value <0.001.

mortality using binary regression analysis with adjusted and unadjusted models. Age was significantly associated with the risk of death regardless of the model used. In addition, a significant predictor of death in the adjusted models was serum vitamin D concentration < 12 ng/mL, which was associated with a 2.55-fold increased risk of death after adjusting for sex and age, and a 2.58-fold increased risk of death after adjusting for sex, age, and comorbidities (48). Comorbidities such as heart disease, diabetes, kidney disease, and metabolic disease only manifested as risk factors for death in unadjusted models (48). In our study, heart diseases were also a significant risk factor for death, but only for patients with an eGFR < 60 ml/min/1.73 $m^2$.

In our study, Cox regression found no association between sex and the risk of death up to 90 days from hospitalization for COVID-19. In the Al Safar et al. (48) study, as in our study, sex was not a significant predictor of death in patients with COVID-19. Evaluating the association between sex and 25(OH)D with risk of death requires further studies in a homogeneous group of patients.

The current study showed a significant effect of eGFR values and vitamin D concentration on survival probability up to 90 days after hospitalization, with a proportionally higher survival probability and vitamin D concentration observed when comparing the same baseline eGFR values (Figure 2). Thus, it may be suggested that higher 25(OH)D concentrations are important for a better prognosis in patients with COVID-19. On the other hand, the Cox regression model showed that, while analyzing additional variables such as age, gender, heart disease, and CRP value, the association between vitamin D concentration and mortality risk in patients with COVID-19 depended on baseline eGFR. Kidneys are the main site for the conversion of 25-hydroxyvitamin D into circulating calcitriol, and are essential for the health benefits of endocrine VDR activation (49). Vitamin D deficiency increases progressively in the course of kidney disease and is associated with accelerated disease progression and death (49). It has also been shown that eGFR values were significantly correlated with COVID-19-related kidney injury, and eGFR values < 60 ml/min/1.73 $m^2$ were independently associated with in-hospital mortality (50).

In our previous study, we assessed renal function, according to eGFR values, in patients admitted to the hospital due to COVID-19 (51). Mortality during hospitalization and after 90 and 180 days was significantly higher in the patients with an eGFR < 60 ml/min/1.73 $m^2$ (Group B) compared to patients with eGFR values ≥ 60 ml/min/1.73 $m^2$ (Group A) ($p < 0.001$) (51). Mortality in Group B patients was associated with comorbidities, immune impairment, and the frequent development of acute kidney injury. This study showed that the baseline eGFR values determine the course of COVID-19 (51). Our current study also showed that vitamin D concentrations are a significant risk factor for mortality and COVID-19, and patients with lower vitamin D concentrations have a worse prognosis.

D'Avolio et al. (24) stated that patients with COVID-19 had significantly decreased 25(OH)D concentration compared to healthy people (11.1 ng/ml vs. 24.6 ng/ml; $p = 0.004$). In a study by Meltzer et al. (52), a multivariate analysis showed that a positive test result for COVID-19 was associated with probable vitamin D deficiency status, compared to probable vitamin D sufficiency status (risk ratio [RR] 1.77; 95% CI = 1.12-2.81; $p = 0.02$). On the other hand, Annweiler et al. (53) found that vitamin D3 supplementation was linked to better three-month survival in elderly patients with COVID-19. Finally, based on systematic reviews and meta-analyses, low vitamin D concentrations were related to a higher risk, severity, and mortality from SARS-CoV-2 infection in most studies (54, 55). Thus, taking into account all the references presented, it seems reasonable to increase blood 25(OH)D concentration as a therapeutic element in patients with COVID-19 (56, 57).

How vitamin D interferes with the outcomes of COVID-19 remains unknown (44). The protective effects of vitamin D in SARS-CoV-2 infection may be mediated through the production of antimicrobial peptides such as cathelicidin and defensin, respiratory barriers, reduced inflammation through tolerogenic effects, and the induction of T-regulatory cells and IL-10, inhibition of IL-12, gamma interferon (IFN-γ), TNF-α, IL-2, and IL-17, along with the modulation of the renin-angiotensin pathway and angiotensin-converting enzyme 2 (ACE-2) downregulation (44, 58–60).

According to current knowledge, it cannot be stated unequivocally for many diseases, disorders, and consequences that vitamin D deficiency is their direct cause (4). The reverse causality hypothesis is gaining traction in light of the results of systematic reviews, meta-analyses, and randomized controlled trials (RCTs) (61, 62). In a review of meta-analyses and RCTs, the authors supported the hypothesis that lower vitamin D concentrations are a consequence of poor health rather than a cause of it (61, 62). In addition, recent research indicates that vitamin D supplementation may prevent frequent upper RTI and asthma exacerbation (62).

The dose of vitamin D has to be appropriately selected depending on the subjects' baseline 25(OH)D concentration. In a normal-weight adult with a 25(OH)D concentration of 20 ng/ml, 100 IU of vitamin D is needed to increase blood 25(OH)D concentrations by approximately 0.6-1 ng/ml (1). This explains why giving an adult, whose blood 25(OH)D level is 18-20 ng/ml, 1,000 IU of vitamin D is not effective in achieving a blood 25(OH)D of more than 30 ng/ml (1). Central European guidelines consider a vitamin D concentration of 30 to 50 ng/ml as optimal for all potential health advantages (63). On the other hand, the Vitamin D recommendations of the Endocrine Society's Practice Guidelines refer to 25(OH)D ranging from 40 to 60 ng/ml (64). The authors believe that there should be greater clinical awareness in recognizing specific populations with COVID-19 that require vitamin D supplementation over and above the recommended dose (56, 57). In patients diagnosed with SARS-CoV-2, the daily dose should be higher than those recommended for the elderly, those who are obese, and those with comorbidities. Most of the study results show that vitamin D supplementation would have clinical benefits for patients with COVID-19 in terms of prevention and treatment, including length of hospital stay, mortality, and prognosis recovery (65).

This study was large, homogenous, and only included those with a PCR-confirmed COVID-19 diagnosis who were treated in one center according to the same rules, which may increase the strength of the obtained results.

In this study, some limitations occurred. As a retrospective study, the causality cannot be inferred. Moreover, vitamin D levels were monitored while patients were hospitalized due to SARS-CoV-2 infection. As such, the findings may have been due to reverse causality, which cannot be excluded. SARS-CoV-2 infection may have led to a decreased 25(OH)D concentration, where an enhancement of 25(OH)D1-alpha-hydroxylase enzyme activity due to the systemic inflammatory response associated with COVID-19 can be considered (28). The study did not collect data on vitamin D supplementation or dietary intake.

## 5 Conclusions

The findings indicate an association between SARS-CoV-2 infected patients' vitamin D concentration and the final course of hospitalization and risk of death. Based on the results of our research, it seems that low vitamin D concentrations should be considered a risk factor for poor prognosis in SARS-CoV-2 infection. From the point of view of public health, it seems important to monitor the concentration of vitamin D, in particular in the elderly and people with heart diseases, and to take preventive measures to achieve the optimal concentrations of vitamin D. Nevertheless, the importance of vitamin D in susceptibility to infection and disease course requires further research.

## 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 Institutional Review Board and Bioethics Committee of Wroclaw Medical University, Poland (No: KB-444/2021). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because The data were collected retrospectively, and written informed consent to participate in the study was not required. The Bioethics Committee approved the publication of anonymized data.

## Author contributions

KK-P and KKo contributed to the conception and design of the study. KK-P, KKo, and KKu contributed to the methodology of the study. KKu performed the statistical analysis. KKo, KK-P, AM-W, KKu, BA, AD, KKa, MPo, MPr, JS, KM, and EJ conducted research, investigation process, and data curation of the study. KKo and KK-P wrote the first draft of the manuscript. KKu and AM-W wrote sections of the manuscript. KM and EJ supervised the study. All authors contributed to the article and approved the submitted version.

## 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



## References

1. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Rev Endocr. Metab Disord* (2017) 18:153–65. doi: 10.1007/s11154-017-9424-1

2. Akbari AR, Khan M, Adeboye W, Hai Lee LH, Chowdhury SI. Ethnicity as a risk factor for vitamin D deficiency and undesirable COVID-19 outcomes. *Rev Med Virol* (2022) 32:e2291. doi: 10.1002/rmv.2291

3. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res* (2011) 31:48–54. doi: 10.1016/j.nutres.2010.12.001

4. Rusińska A, Płudowski P, Walczak M, Borszewska-Kornacka MK, Bossowski A, Chlebna-Sokół D, et al. Vitamin D supplementation guidelines for general population and groups at risk of vitamin D deficiency in Poland-recommendations of the polish society of pediatric endocrinology and diabetes and the expert panel with participation of national specialist consultants and representatives of scientific societies-2018 update. *Front Endocrinol* (2018) 9:246. doi: 10.3389/fendo.2018.00246

5. Koziarska-Rościszewska M, Rysz J, Stępień M. High prevalence of vitamin D deficiency and its association with metabolic disorders in elderly patients. *Fam. Med Prim. Care Rev* (2017) 19:372–6. doi: 10.5114/fmpcr.2017.70809

6. Franca Gois PH, Wolley M, Ranganathan D, Seguro AC. Vitamin D deficiency in chronic kidney disease: Recent evidence and controversies. *Int J Environ Res Public Health* (2018) 15:1773. doi: 10.3390/ijerph15081773

7. González EA, Sachdeva A, Oliver DA, Martin KJ. Vitamin D Insufficiency and Deficiency in Chronic Kidney Disease. A single center observational study. *Am J Nephrol.* (2004) 24:503–10. doi: 10.1159/000081023

8. Zand L, Kumar R. The use of vitamin D metabolites and analogues in the treatment of chronic kidney disease. *Endocrinol Metab Clin North. Am* (2017) 46:983–1007. doi: 10.1016/j.ecl.2017.07.008

9. Shaan ZH, Alvi Y, Ahmad S, Jilani LZ, Faizan M, Fatima ZH. Assessment of Vitamin D deficiency among older adult patients with an orthopaedic fracture and its association with increasing age. *Fam. Med Prim. Care Rev* (2019) 21:372–6. doi: 10.5114/fmpcr.2019.90171

10. Egierska D, Pietruszka P, Burzyńska P, Chruścicka I, Buchta J. Pleiotropic effects of vitamin D3. *J Educ Health Sport.* (2021) 11:143–55. doi: 10.12775/JEHS.2021.11.07.013

11. Restrepo Valencia CA, Aguirre Arango JV. Vitamin D (25(OH)D) in patients with chronic kidney disease stages 2-5. *Colomb Med (Cali).* (2016) 47:160–6.

12. Bivona G, Agnello L, Ciaccio M. Vitamin D and immunomodulation: Is it time to change the reference values? *Ann Clin Lab Sci* (2017) 47:508–10.

13. Marcinkowska M, Mickiewicz A, Fijałkowski M. Plejotropowe działanie witaminy D. *Choroby Serca i Naczyń.* (2019) 16:45–52. doi: 10.5603/ChSiN.2019.0007

14. Lee SM, Pike JW. The vitamin D receptor functions as a transcription regulator in the absence of 1,25-dihydroxyvitamin $D_3$. *J Steroid. Biochem Mol Biol* (2016) 164:265–70. doi: 10.1016/j.jsbmb.2015.08.018

15. Płudowski P, Hollick MF, Grant WB, Konstantynowicz J, Mascarenhas MR, Haq A, et al. Vitamin D supplementation guidelines. *J Steroid. Biochem Mol Biol* (2018) 175:125–35. doi: 10.1016/j.jsbmb.2017.01.021

16. Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR) – mediated actions of 1α25(OH)2vitamin D3: genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab* (2011) 25:543–59. doi: 10.1016/j.beem.2011.05.010

17. Aranow C. Vitamin D and the immune system. *J Investig Med* (2011) 59:881–6. doi: 10.2310/JIM.0b013e31821b8755

18. Bergman P, Lindh AU, Björkhem-Bergman L, Lindh JD. Vitamin D and respiratory tract infections: A systematic review and meta-analysis of randomized controlled trials. *PLoS One* (2013) 8:e65835. doi: 10.1371/journal.pone.0065835

19. Martineau AR, Cates CJ, Urashima M, Jensen M, Griffiths AP, Nurmatov U, et al. Vitamin D for the management of asthma. *Cochrane Database Syst Rev* (2016) 9:CD011511. doi: 10.1002/14651858.CD011511.pub2

20. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ* (2017) 356:i6583. doi: 10.1136/bmj.i6583

21. Quraishi SA, Bittner EA, Blum L, McCarthy CM, Bhan I, Camargo CA Jr. Prospective study of vitamin D status at initiation of care in critically ill surgical patients and risk of 90-day mortality. *Crit Care Med* (2014) 42:1365–71. doi: 10.1097/CCM.0000000000000210

22. Zapatero A, Dot I, Diaz Y, Gracia MP, Pérez-Terán P, Climent C, et al. La hipovitaminosis D grave al ingreso en el paciente crítico se asocia a fracaso renal agudo y mal pronóstico. *Med Intensiva.* (2018) 42:216–24. doi: 10.1016/j.medin.2017.07.004

23. Mariani J, Tajer C, Antonietti L, Inserra F, Ferder L, Manucha W. High-dose vitamin D versus placebo to prevent complications in COVID-19 patients: A structured summary of a study protocol for a randomised controlled trial (CARED-TRIAL). *Trials.* (2021) 22:111. doi: 10.1186/s13063-021-05073-3

24. D'Avolio A, Avataneo V, Manca A, Cusato J, De Nicolò A, Lucchini R, et al. 25-hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-coV-2. *Nutrients.* (2020) 12:1359. doi: 10.3390/nu12051359

25. Panagiotou G, Tee SA, Ihsan Y, Athar W, Marchitelli G, Kelly D, et al. Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalized with COVID-19 are associated with greater disease severity. *Clin Endocrinol* (2020) 93:508–11. doi: 10.1111/cen.14276

26. Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. *Aging Clin Exp Res* (2020) 32:1195–8. doi: 10.1007/s40520-020-01570-8

27. Orchard L, Baldry M, Nasim-Mohi M, Monck C, Saeed K, Grocott MPW, et al. Vitamin-D levels and intensive care unit outcomes of a cohort of critically ill COVID-19 patients. *Clin Chem Lab Med* (2021) 59:1155–63. doi: 10.1515/cclm-2020-1567

28. Mariani J, Antonietti L, Tajer C, Ferder L, Inserra F, Sanchez Cunto M, et al. High-dose vitamin D versus placebo to prevent complications in COVID-19 patients: Multicentre randomized controlled clinical trial. *PLoS One* (2022) 17:e0267918. doi: 10.1371/journal.pone.0267918

29. Ziuzia-Januszewska L, Januszewski M, Sosnowska-Nowak J, Janiszewski M, Dobrzyński P, Jakimiuk AA, et al. COVID-19 severity and mortality in two pandemic waves in Poland and predictors of poor outcomes of SARS-CoV-2 infection in hospitalized young adults. *Viruses.* (2022) 14:1700. doi: 10.3390/v14081700

30. Januszewski M, Ziuzia-Januszewska L, Jakimiuk AA, Oleksik T, Pokulniewicz M, Wierzba W, et al. Predictors of COVID-19 severity among pregnant patients. *Bosn. J Basic Med Sci* (2022) 22:1005–15. doi: 10.17305/bjbms.2022.7181

31. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

32. Cheng Y, Luo R, Wang K, Zhang M, Wang Z, Dong L, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int* (2020) 97:829–38. doi: 10.1016/j.kint.2020.03.005

33. Peiris S, Mesa H, Aysola A, Manivel J, Toledo J, Borges-Sa M, et al. Pathological findings in organs and tissues of patients with COVID-19: A systematic review. *PLoS One* (2021) 16:e0250708. doi: 10.1371/journal.pone.0250708

34. Parmar MS. Acute kidney injury associated with COVID-19-Cumulative evidence and rationale supporting against direct kidney injury (infection). *Nephrology.* (2021) 26:239–47. doi: 10.1111/nep.13814

35. Liakopoulos V, Roumeliotis S, Papachristou S, Papanas N. COVID-19 and the kidney: Time to take a closer look. *Int Urol Nephrol.* (2021) 54:1–5. doi: 10.1007/s11255-021-02976-7

36. Levey AS, Coresh J, Green T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* (2006) 145:247–54. doi: 10.7334/psicothema2016.383

37. Blanca MJ, Alarcón R, Arnau J, Bono R, Bendayan R. Non-normal data: Is ANOVA still a valid option? *Psicothema* (2017) 29:552–7. doi: 10.7334/psicothema2016.383

38. Schmider E, Ziegler M, Danay E, Beyer L, Bühner M. Is it really robust? Reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. *Methodology: Eur J Res Methods Behav Soc Sci* (2010) 6:157–1. doi: 10.1027/1614-2241/a000016

39. Kwak SG, Kim JH. Central limit theorem: the cornerstone of modern statistics. *Korean J Anesthesiol.* (2017) 70:144–56. doi: 10.4097/kjae.2017.70.2.144

40. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification and stratification. *Am J Kidney Dis* (2002) 39 (suppl 1):S1–S266.

41. Therneau T. *A Package for Survival Analysis in R. R package version 3.5-5* (2023). Available at: https://CRAN.R-project.org/package=survival.

42. Kassambara A, Kosinski M, Biecek P. Drawing Survival Curves using 'ggplot2'. *R package survminer version 0.4.9* (2021). Available at: https://CRAN.R-project.org/package=survminer.

43. Campi I, Gennari L, Merlotti D, Mingiano C, Frosali A, Giovanelli L, et al. Vitamin D and COVID-19 severity and related mortality: a prospective study in Italy. *BMC Infect Dis* (2021) 21:566. doi: 10.1186/s12879-021-06281-7

44. Hafez W, Saleh H, Arya A, Alzouhbi M, Fdl Alla O, Lal K, et al. Vitamin D status in relation to the clinical outcome of hospitalized COVID-19 patients. *Front Med* (2022) 9:843737. doi: 10.3389/fmed.2022.843737

45. Waas T, Schulz A, Lotz J, Rossmann H, Pfeiffer N, Beutel ME, et al. Distribution of estimated glomerular filtration rate and determinants of its age dependent loss in a German population-based study. *Sci Rep* (2020) 11:10165. doi: 10.1038/s41598-021-89442-7

46. Kanbay M, Ertuglu LA, Afsar B, Ozdoğan EB, Kucuksumer ZS, Ortiz A, et al. Renal hyperfiltration defined by high estimated glomerular filtration rate: A risk factor for cardiovascular disease and mortality. *Diabetes.* (2019) 21:2368–83. doi: 10.1111/dom.13831

47. Park M, Yoon E, Lim YH, Kim H, Choi J, Yoon HJ. Renal hyperfiltration as a novel marker of all-ccause mortality. *J Am Soc Nephrol.* (2015) 26:1426–33. doi: 10.1681/ASN.2014010115

48. AlSafar. H, Grant WB, Hijazi R, Uddin M, Alkaabi N, Tay G, et al. COVID-19 disease severity and death in relation to vitamin D status among SARS-coV-2-positive UAE residents. *Nutrients.* (2021) 13:1714. doi: 10.3390/nu13051714

49. Dusso AS. Kidney disease and vitamin D levels: 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and VDR activation. *Kidney Inter.* (2011) Suppl. 1:136–41. doi: 10.1038/kisup.2011.30

50. Cei F, Chiarugi L, Brancati S, Montini MS, Dolenti S, Di Stefano D, et al. Early reduction of estimated Glomerular Filtration Rate (eGFR) predicts poor outcome in acutely ill hospitalized COVID-19 patients firstly admitted to medical regular wards (eGFR-COV19 study). *BioMed Pharmacother.* (2022) 153:113454. doi: 10.1016/j.biopha.2022.113454

51. Kilis-Pstrusinska K, Akutko K, Braksator J, Dancewicz A, Grosman-Dziewiszek P, Jamer T, et al. Kidney dysfunction and its progression in patients hospitalized duo to COVID-19: Contribution to the clinical course and outcomes. *J Clin Med* (2021) 10:5522. doi: 10.3390/jcm10235522

52. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of vitamin D status and other clinical characteristics with COVID-19 test results. *JAMA Netw Open* (2020) 3:e2019722. doi: 10.1001/jamanetworkopen.2020.19722

53. Annweiler C, Beaudenon M, Simon R, Guenet M, Otekpo M, Célarier T, et al. GERIA-COVID study group. Vitamin D supplementation prior to or during COVID-19 associated with better 3-month survival in geriatric patients: Extension phase of the GERIA-COVID study. *J Steroid. Biochem Mol Biol* (2021) 213:105958. doi: 10.1016/j.jsbmb.2021.105958

54. Petrelli F, Luciani A, Perego G, Dognini G, Colombelli PL, Ghidini A. Therapeutic and prognostic role of vitamin D for COVID-19 infection: A systematic review and meta-analysis of 43 observational studies. *J Steroid. Biochem Mol Biol* (2021) 211:105883. doi: 10.1016/j.jsbmb.2021.105883

55. Yisak H, Ewunetei A, Kefale B, Mamuye M, Teshome F, Ambaw B, et al. Effects of vitamin D on COVID-19 infection and prognosis: A systematic review. *Risk Manag Healthc Policy.* (2021) 14:31–8. doi: 10.2147/RMHP.S291584

56. Beatrice Paulus I, Aryana IS, Wahyu Purnamasidhi C, Daniella D. Benefits of standard dose and pulse dose vitamin D in the high-risk and confirmed hypovitaminosis D population with COVID-19: An Evidence-Based Case Report (EBCR). *Fam. Med Prim. Care Rev* (2022) 24:151–5. doi: 10.5114/fmpcr.2022.115877

57. Ferder L, Martín Giménez VM, Inserra F, Tajer C, Antonietti L, Mariani J, et al. Vitamin D supplementation as a rational pharmacological approach in the COVID-19 pandemic. *Am J Physiol Lung Cell Mol Physiol* (2020) 319:L941–8. doi: 10.1152/ajplung.00186.2020

58. Xu J, Yang J, Chen J, Luo Q, Zhang Q, Zhang H. Vitamin D alleviates lipopolysaccharide induced acute lung injury via regulation of the renin angiotensin system. *Mol Med Rep* (2017) 16:7432–8. doi: 10.3892/mmr.2017.7546

59. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science.* (2006) 311:1770–3. doi: 10.1126/science.1123933

60. Cantorna MT. Vitamin D and lung infection. *Infect Immun* (2016) 84:3094–6. doi: 10.1128/IAI.00679-16

61. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol* (2014) 2:76–89. doi: 10.1016/S2213-8587(13)70165-7

62. Autier P, Mullie P, Macacu A, Dragomir M, Boniol M, Coppens K, et al. Effect of vitamin D supplementation on non-skeletal disorders: a systematic review of meta-analyses and randomised trials. *Lancet Diabetes Endocrinol* (2017) 5:986–1004. doi: 10.1016/S2213-8587(17)30357-1

63. Płudowski P, Karczmarewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe — recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. *Endokrynol. Pol* (2013) 64:319–27. doi: 10.5603/EP.2013.0012

64. Bae JH, Choe HJ, Holick MF, Lim S. Association of vitamin D status with COVID-19 and its severity: Vitamin D and COVID-19: a narrative review. *Rev Endocr. Metab Disord* (2022) 23:579–99. doi: 10.1007/s11154-021-09705-6

65. Li B, Yang S, Hou N. Could vitamin D supplementation play a role against COVID-19? *Front Immunol* (2022) 13:967215. doi: 10.3389/fimmu.2022.967215

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# Vitamin D and allergic diseases

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In recent years, the relationship between vitamin D and allergic diseases has received widespread attention. As a fat-soluble vitamin, vitamin D plays a crucial role in regulating the immune system and may influence the onset and progression of diseases such as atopic dermatitis, allergic rhinitis, and asthma. To understand the underlying mechanisms, we have summarized the current research on the association between vitamin D and allergic diseases. We also discuss the impact of vitamin D on the immune system and its role in the course of allergic diseases, particularly focusing on how vitamin D supplementation affects the treatment outcomes of these conditions. We aim to provide a theoretical basis and practical guidance for optimizing the management and treatment of allergic diseases by modulating vitamin D levels.

KEYWORDS

vitamin D, allergic rhinitis, asthma, atopic dermatitis, food allergy, allergen immunotherapy

## 1 Introduction

Allergic diseases are a result of the immune system's overreacted response to allergens, with a diverse set of immune cells (such as lymphocytes, mast cells/basophils) and immune molecules like IgE playing a role in the pathogenetic process. Studies suggest that a complex interplay between genetic, environmental, and nutritional factors can lead to the onset of allergic diseases (1). Over the past decades, there has been a dramatic increase in the prevalence of allergic diseases, such as atopic dermatitis (AD), allergic rhinitis (AR), and allergic asthma (AA), posing a significant societal burden (2).

Vitamin D, a fat-soluble vitamin, exists in two forms: D2 (ergocalciferol) and D3 (cholecalciferol) (3). Initially, vitamin D is hydroxylated by the 25-hydroxylase enzyme in the liver to form 25-hydroxyvitamin D (25(OH)D), which is then metabolized in the kidneys by the 1α-hydroxylase enzyme into the biologically active form, 1,25-dihydroxyvitamin D (1,25(OH)2D) (4). Vitamin D primarily acts through the vitamin D receptor (VDR) to regulate calcium and phosphorus balance and maintain bone health (5). Given that mast cells, monocytes, macrophages, T cells, B cells, and dendritic cells (DCs) express nuclear receptors (nVDR) and membrane receptors (mVDR) (6), vitamin D also plays a vital role in modulating immune responses (7, 8).

Recent research has indicated that vitamin D, through its regulatory effect on the immune system, could be involved in the onset and progression of allergic diseases. This

article provides a review of the influence of vitamin D on the immune system, the relationship between vitamin D and allergic diseases, and the impact of vitamin D supplementation on allergic outcomes.

## 2 The impact of vitamin D on the immune system

Vitamin D primarily exerts its immunoregulatory effects through the VDR. The expression of VDR in immune cells such as DCs, macrophages, monocytes, and lymphocytes provides a foundation for its role in immune regulation (9, 10).

### 2.1 Vitamin D and innate immune cells

Innate immunity is the body's frontline defense, swiftly fending off pathogen invasions. Vitamin D showcases distinct impacts on various innate immune cells through different routes.

Vitamin D mainly exerts immunosuppressive effects on human innate lymphoid cells (ILCs), inhibiting the ability of vitamin A-induced ILC2 cells to produce cytokines such as IL-5 and IL-13, and the expression of gut-directed integrin α4β7 induced by vitamin A (11). Vitamin D inhibits the response of ILC3 cells to IL-23 through its receptor, thereby inhibiting the production of cytokines such as IL-22, IL-17F, and granulocyte-macrophage colony-stimulating factor (GM-CSF), while enhancing the expression of genes associated with the IL-1β signaling pathway, converting the production of ILC3 cell factors to the production of innate cytokines, such as IL-6, IL-8, macrophage inflammatory proteins 1α/β (MIFs) (12).

Vitamin D mainly exerts inhibitory effects on eosinophils. 1,25(OH)2D can upregulate the expression of C-X-C motif chemokine receptor 4 (CXCR4) on them, promoting the transfer of eosinophils from allergic inflammation sites to non-inflammatory tissues outside the blood vessels induced by the latter (13, 14), and can inhibit the production of eosinophil mediators, such as major basic protein (MBP), eosinophil peroxidase (EPX), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) (15). In a mouse asthma model, vitamin D reduced the infiltration of eosinophils in the lungs (16).

In mast cells, 1,25(OH)2D can increase the number of VDRs in mast cells, maintain the stability of mast cells, and inhibit the production of inflammatory and vasodilatory mediators mediated by IgE in human mast cells (17, 18). *In vitro* studies have shown that vitamin D upregulates the expression of IL-10 mRNA in mouse mast cells and induces the secretion of IL-10 (19).

Vitamin D enhances the formation of neutrophil extracellular traps (NETs), upregulates the production of IL-4, and downregulates the expression of pro-inflammatory cytokines IL-1β, IL-6, IL-8, and IL-12 in neutrophils (20–22). Some studies have shown that vitamin D induces apoptosis of peripheral blood neutrophils in patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD) through the p38 MAPK signaling pathway (23).

For NK cells, vitamin D can promote their secretion of IFN-α, making them more successful in exerting antibody-dependent cellular cytotoxicity (ADCC) effects (24).

Monocytes/macrophages can recognize components of bacteria, viruses, and fungi through their surface-expressed toll-like receptors. Vitamin D can form a 1,25/VDR/RXR complex with VDR and retinoid X receptor (RXR) on monocytes/macrophages, promoting the expression of toll-like receptors (25), enhancing the chemotaxis and phagocytosis capabilities of monocytes/macrophages, and inducing the production of antimicrobial peptides (26). Additionally, 1,25(OH)2D can promote the development of macrophages, which play a key role in the phagocytosis and clearance of bacteria, as evidenced by the increased expression of complement receptor immunoglobulin (CRIg) mRNA, protein, and cell surface expression. The phagocytic ability of macrophages treated with 1,25(OH)2D is also significantly enhanced (26, 27). In general, macrophages polarize into different phenotypes under various inflammatory conditions (28). Resting macrophages (M0) become polarized into pro-inflammatory M1-like macrophages (M1) when exposed to stimuli such as lipopolysaccharide (LPS), interferon-alpha (IFN-α), IL-12, and IL-23. These M1 macrophages primarily produce pro-inflammatory cytokines such as TNF-α, IL-23, IL-12, and IL-1β, thereby promoting inflammatory responses. Conversely, IL-4 and IL-10 enhance the development of anti-inflammatory M2-like macrophages (M2), which produce anti-inflammatory cytokines IL-10 and TGF-β, promoting wound healing and maintaining tissue homeostasis (29, 30).

Studies have shown that vitamin D, through the VDR pathway, downregulates the expression of IL-12, TNF-α, and IL-1β in M1 macrophages, as well as the expression of co-stimulatory molecules CD80 and CD86 on macrophages, thereby reducing the macrophages' ability to stimulate T cells. Simultaneously, vitamin D upregulates the production of IL-10 and TGF-β in M2 macrophages, promoting the differentiation of macrophages towards the M2 phenotype (31, 32). This polarization alleviates the development of allergic diseases such as AR and AD (33–35).

For dendritic cells (DCs), it has been found that 1,25(OH)2D can inhibit the expression of MHC class II and co-stimulatory molecules CD40, CD80, and CD86 on the surface of DCs, thereby reducing their antigen-presenting and T cell-activating capabilities. It also inhibits the release of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-2 (IL-12) (which influences Th cell differentiation into Th1 cells), and IL-23 (which influences Th cell differentiation into Th17 cells). Additionally, it upregulates IL-10 (an anti-inflammatory cytokine that inhibits Th2-type immune responses) and IL-6, reduces the production of C-C chemokine ligand 17 (CCL17), and inhibits the differentiation, maturation, and chemotactic abilities of DCs (36–41). Moreover, vitamin D promotes the induction of FOXP3 transcription by DCs to enhance the generation of Tregs, thus boosting immune tolerance and reducing allergic reactions (42, 43). Brulefert et al. collected human skin samples to investigate the effects of vitamin D on DCs. They found that vitamin D-induced CD14+ skin DCs significantly increased the production of IL-4 and IL-13, promoting T helper cell

2 (Th2) responses even in the absence of TSLP (44). This seems contradictory, suggesting that the mechanisms by which vitamin D affects DCs need further investigation.

Through the above various ways, vitamin D regulates the function of innate immune cells, playing a crucial role in the body's first line of defense (Figure 1).

## 2.2 Vitamin D and adaptive immune cells

The influence of vitamin D on T cells varies across different subsets, primarily showcasing inhibitory effects on T helper cell 1 (Th1) and T helper cell 17 (Th17) subsets and stimulatory effects on Th2 and regulatory T cell (Treg) subsets. Sloka et al. used an experimental autoimmune encephalomyelitis (EAE) model and *in vitro* cultures of human and mouse cells to demonstrate that 1,25 (OH)2D upregulates GATA-3 through a STAT6-dependent mechanism, promoting Th2 cell polarization and inhibits the differentiation of Th1 and Th17 cells and the production of inflammatory cytokines (45). Zhang et al. constructed vitamin D receptor-deficient (VDR-/-) and wild-type (WT) mouse models. *In vitro* experiments showed that 1,25(OH)2D significantly inhibited Th1 cell differentiation and the production of related cytokines (such as IL-2, IFN-γ, and TNF-β) activated by Bacillus Calmette-Guérin (BCG). *In vivo* experiments further demonstrated that in vitamin D-deficient mice vaccinated with BCG, 1,25(OH)2D reduced inflammatory infiltration in the spleen, decreased the expression of inflammatory cytokines, and promoted the development of Th2 cells. These results indicate that 1,25(OH)2D alleviates inflammatory responses by inhibiting Th1 cell differentiation and cytokine production through the JAK/STAT pathway (46), while fostering the expression of Th2 cell factors (IL-4, IL-5, IL-9, IL-13) (36, 43). Vitamin D also lowers the levels of IL-12 and IL-23, the Th1/Th17 polarizing cytokines produced by DCs, inhibits the differentiation of naive $CD4^{+}$T cells into Th17 and Th1 cells, and significantly bolsters the development of $FoxP3^{+}CD127^{low}CD25^{+}$ regulatory T cells (Tregs) and IL-10-producing T cells. The induction of $ICOS^{+}$Tregs (mainly IL-10 producers), $CD69^{+}FoxP3^{+}$ and $TIGIT^{+}FoxP3^{+}$Tregs is also significantly increased (47). Moreover, 1,25(OH)2D curbs the expression of IL-17A, IL-22, TNF-α, IFN-γ and chemokine receptor CCR6 in Th17 cells, thereby stopping Th17 cells from migrating to inflamed tissues (48–50). It can also induce the differentiation of Tregs by promoting the expression of IL-10 and FoxP3, thereby curbing pro-inflammatory immune responses (51, 52).

VDR also exists in human B lymphocytes. Studies indicate that 1,25(OH)2D can curb the generation of plasma cells and memory B cells (53), downregulate CD40, NF-κB signaling, lessen the activation of human peripheral B cells and induce their apoptosis, and curb the production of IgE (54–56). Simultaneously, 1,25(OH)2D enhances the expression of IL-10 in activated B cells by

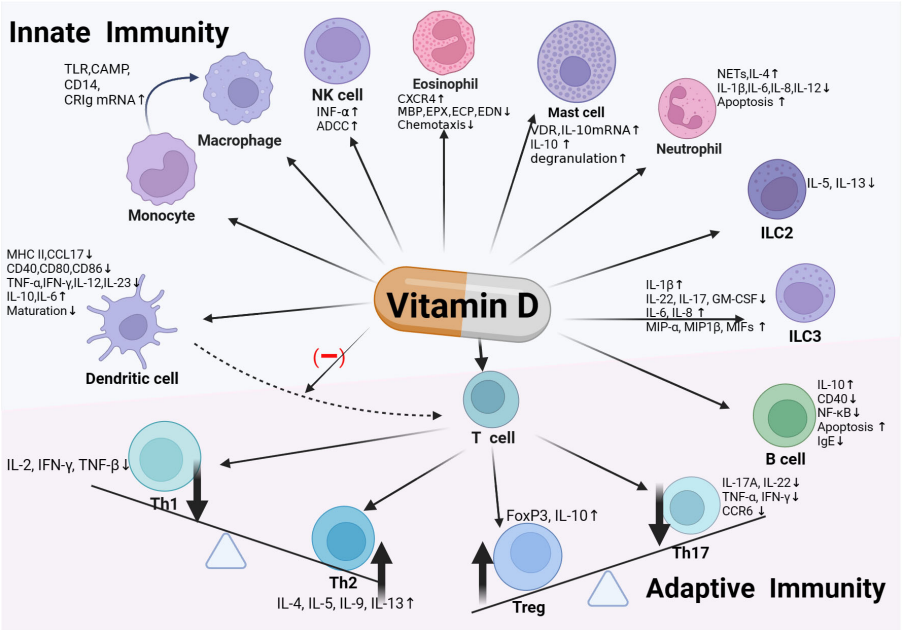


FIGURE 1
Vitamin D and immune system. Vitamin D exerts immunoregulatory effects by binding to the Vitamin D Receptor (VDR) expressed on various immune cells, including monocytes/macrophages, dendritic cells, innate lymphoid cells (ILC), as well as T and B cells within the adaptive immune system. In innate immunity, vitamin D enhances monocyte/macrophage chemotaxis and phagocytosis, and induces antimicrobial peptide production. It modulates dendritic cell maturation, activation, and chemotactic and immunostimulatory capabilities, along with affecting the functions of ILCs and eosinophils. In the realm of adaptive immunity, vitamin D promotes the development of Th2 and regulatory T cells (Treg), while inhibiting the differentiation and activation of Th1 and Th17 cells. It also modulates B cell activity and IgE production. Through these pathways, vitamin D contributes to maintaining immune homeostasis and preventing excessive inflammatory responses, thereby playing a vital role in preserving human health.

recruiting VDR to the promoter of IL-10, thereby participating in the inhibition of T cell activation (57).

In summary, through various pathways to regulate the activity of various T cell subgroups and B cells, maintain immune balance and suppress inflammatory responses, vitamin D is of great significance for maintaining the stability of the immune system and preventing excessive immune responses.

# 3 Vitamin D and allergic diseases

## 3.1 Vitamin D and atopic dermatitis

Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin allergic disease, characterized by the disorder of skin barrier function leading to dry skin, itching, eczematous skin lesions, and IgE-mediated sensitization to food and environmental allergens (58). In an ovalbumin (OVA)-induced AD mouse model (59), vitamin D significantly improved the skin condition of mice, decreased IgE and IL-5 levels, but increased IL-4 and IL-13 levels, reduced filaggrin levels, and decreased epidermal thickness. Histological studies further confirmed that vitamin D has significant effects in alleviating inflammation and improving the pathological state of the skin. Most studies support the negative correlation between vitamin D levels and AD. A case-control study by El Taieb et al. (60) found that the average vitamin D level in children with AD was much lower than the normal value. A nationwide cross-sectional survey conducted by Heimbeck et al. (61) in Germany found that low serum vitamin D levels were negatively correlated with eczema in German children and adolescents. Ahmed Mohamed et al. (62) also observed a dose-response relationship between vitamin D deficiency and the prevalence of AD in a comparison of 100 AD patients and 1001 normal controls in the dermatology outpatient department in Cairo, Egypt. Moreover, most studies have observed a negative correlation between the severity of AD and serum 25(OH)D levels; the more severe the vitamin D deficiency, the higher the scoring atopic dermatitis (SCORAD) score (63–67). A recent case-control study (64) involving 96 AD patients and 90 healthy controls found that compared with atopy and eosinophilia, the reduction of serum vitamin D levels seems to have a more significant impact on the severity of AD. For each unit increase in serum vitamin D levels, the SCORAD index decreases by 0.449 units, while an increase of 1 unit in eosinophil count will cause the SCORAD index to increase by 0.009 units. However, several other cohort studies believe that there is no association between the risk of AD in offspring at 3–5 years and the level of vitamin D during pregnancy, at birth, and early life (68–70). Overall, the majority of existing studies suggest that vitamin D is associated with the risk and severity of atopic dermatitis.

## 3.2 Vitamin D and allergic rhinitis

Allergic rhinitis (AR) is a common allergic disease mediated by immunoglobulin E (IgE), caused by inhaled allergens, and clinically manifested as sneezing, nasal congestion, nasal itching, and rhinorrhea. In an ovalbumin-induced AR mouse model, 1,25 (OH)2D reduced serum levels of ovalbumin-specific IgE and spleen IL-17 levels, as well as IL-5 and IL-13 levels in nasal lavage fluid (71). Additionally, studies on human serum have shown that the level of 1,25(OH)2D is related to the Th1/Th2 balance in AR patients, and vitamin D deficiency shifts the Th1/Th2 balance to Th2 (72). Most studies believe that the serum vitamin D level of AR patients is lower than that of healthy people or the control group (73–78). Jung et al. (79) conducted a large-scale national survey of 8,012 Korean adults over 18 years old, indicating that the lower the 25(OH)D level, the higher the prevalence of AR. A recent secondary study (80) of the Vitamin D Antenatal Asthma Reduction Trial (VDAART) birth cohort showed that compared with patients with vitamin D deficiency in early and late pregnancy, the occurrence of AR and sensitization to airborne allergens at 3 and 6 years old in the offspring of mothers with sufficient prenatal vitamin D in late pregnancy was reduced (OR= 0.47; 95% CI, 0.26–0.84). Bunyavanich et al. (81) studied 1,248 mother-child pairs in the US prenatal cohort and found that every 100 IU/d of dietary vitamin D intake in the first three months and the last three months of pregnancy reduced the chance of school-age children suffering from AR by 21% and 20% respectively. Saad et al. (73) found in a cohort study of 120 Egyptian children with AR and 100 healthy children that the average 25(OH)D level of patients with moderate/severe AR was significantly lower than that of patients with mild AR, and the average 25(OH)D level of the AR group was negatively correlated with the total nasal symptom score and total IgE level. However, it has been observed that the association between Vitamin D and AR is affected by race, age, gender, etc. (82–84). For example, Mai et al. (84) reported that lower levels of vitamin D in the Norwegian adult population are related to an increased risk of AR in men and a reduced risk of AR in women. The authors speculated that this might be related to female sex hormones enhancing Th1 responses and reducing Th2 responses. Wegienka et al. (83) found that higher prenatal and cord blood 25 (OH)D levels were generally associated with fewer allergic outcomes, such as eczema and sensitization to airborne allergens. This association was more significant in white children and less evident in black children. Additionally, they observed that 25(OH) D levels were negatively associated with sensitization to airborne allergens only in black children.

Some research has refuted the link between vitamin D and AR. A cross-sectional study conducted by Wu et al. (85), which included 32 patients with persistent AR and 25 controls, found no significant difference in serum 25(OH)D levels between the two groups. A large cross-sectional study (86) in Korea involving 15,212 adults aged 19 or above indicated, through multivariate linear regression analysis, that adults with vitamin D deficiency did not have an increased likelihood of asthma, AR, or IgE sensitization. A cohort study (87) collected the cord blood 25(OH)D levels of 239 newborns. Using a symptom questionnaire based on the International Study of Asthma and Allergies in Childhood (ISAAC) and following up these children until they were 5 years old, it was found that there was no correlation between cord serum 25(OH)D levels and asthma and AR at age 5. The most recent Mendelian randomization study (88) also did not find evidence of a causal relationship between serum vitamin D levels and AR risk in individuals of European descent.

Therefore, more research is needed to confirm the relationship between vitamin D and the development of AR.

## 3.3 Vitamin D and asthma

Asthma is a common chronic respiratory disease, characterized by chronic inflammation of the airways and high airway reactivity, manifested as coughing, wheezing, chest tightness, and difficulty breathing. The most common phenotype is allergic asthma. Vasiliou et al. (89) investigated the immune responses and inflammatory markers in neonatal allergic airway disease using a vitamin D-deficient mouse model. Their findings indicated that vitamin D deficiency resulted in an elevated proportion of Th2 cells, a decrease in IL-10-secreting regulatory T cells, and exacerbated eosinophilic inflammation and airway remodeling following exposure to house dust mites, thereby fostering the development of allergic diseases (90). Vitamin D supplementation significantly mitigated these pathological changes. Hamzaoui et al. collected peripheral blood samples from young children with asthma and found that vitamin D significantly inhibited the differentiation of Th17 cells and the production of IL-17 while increasing the levels of the anti-inflammatory cytokine IL-10 (91). A cross-sectional study in the Cyprus region (92) included 69 active asthmatics and 671 never wheezing/never asthmatic teenagers aged 16–17. It was found that the average vitamin D level of asthmatic children was lower, and in the AA group, the vitamin D level was negatively correlated with the severity of asthma. Previously, Bener et al. (93) compared the vitamin D levels of 483 asthmatic children with healthy children in Qatar, and also proposed that vitamin D deficiency is a major predictor of childhood asthma. A cross-sectional study (94) in the UK of 435,040 adults found that compared with vitamin D deficiency, the risk of asthma in individuals with low and sufficient vitamin D concentrations was reduced by 6.4% and 9.8% respectively, and their lung function would also improve. Similarly, many studies have reported that 25(OH)D deficiency is related to increased risk of asthma in newborns, adolescents, adults, and decreased lung function (95–100), and is affected by many factors such as gender, race, ethnicity, smoking, whether to use ICS, sleep mode and genetic susceptibility (98–101). As Chang et al. (98) discovered in a large-scale prospective cohort study based on the UK Biobank, the protective effect of vitamin D against asthma was strongest under healthy sleep patterns. In individuals with moderate genetic risk, higher levels of vitamin D were associated with a significantly reduced risk of asthma. The protective effect of vitamin D was most notable in males, individuals under 60 years old, overweight individuals, and current or former smokers. Another Norwegian cohort study reported that the association between vitamin D levels and lung function varied by gender and allergy status, with this association being particularly significant among male asthma patients (99).

Studies have shown that vitamin D has a protective effect on airway smooth muscle cell contraction and remodeling in asthma. Vitamin D inhibits the growth of airway smooth muscle cells by reducing the expression of cyclin D1 and inducing the phosphorylation of retinoblastoma protein and checkpoint kinase 1 (102). It also inhibits vascular endothelial growth factor (VEGF)-induced ADAM Metallopeptidase Domain 33 (ADAM33) expression and proliferation, reducing airway remodeling (103). Furthermore, Plesa et al. demonstrated that vitamin D can inhibit the proliferation and migration of bronchial fibroblasts by suppressing ERK1/2 and Akt signaling pathways and upregulating genes involved in cell cycle arrest, such as p21 and p27. It also reduces the expression of genes involved in extracellular matrix remodeling, such as type I collagen and matrix metallopeptidase 2 (MMP2) (104). Vitamin D inhibits NF-κB activation, reducing the expression of pro-inflammatory cytokines like IL-6 and IL-8 (105), and decreases the expression of type I collagen and protein arginine methyltransferase 1 (PRMT1) activity, exhibiting anti-inflammatory and antifibrotic effects (106). These mechanisms indicate that vitamin D may play a pivotal role in regulating airway remodeling in asthma, thereby reinforcing its association with the condition and its potential as an adjunctive therapy for asthma management (107, 108).

Recent studies underscore a close interrelationship between vitamin D, gut microbiota, and asthma. Vitamin D deficiency may compromise barrier integrity and alter microbiome composition, with gut dysbiosis potentially impairing both local and pulmonary immune functions, thus heightening asthma susceptibility. Respiratory infections can disrupt the gut microbiome, decreasing bacteria that produce short-chain fatty acids (SCFAs), which in turn impacts the function and fate of immune cells, further exacerbating asthma symptoms (109–111).

Contradictorily, as mentioned earlier, *in vitro* experiments have proven that vitamin D can promote Th2 cell shift (45, 112), which seems to contradict the protective effect of vitamin D on allergies. A cohort study (113) based on a large population of adults only reported that vitamin D deficiency is related to acute asthma attacks, but there is no significant connection with doctor-diagnosed asthma. Cheng et al. (86) investigated the data of 15,212 people aged 19 and above in South Korea, and also found that adults with vitamin D deficiency did not increase the likelihood of asthma or IgE sensitization. Overall, the majority of studies support the association between vitamin D and the risk and severity of asthma.

## 3.4 Vitamin D and food allergies

Food allergies (FAs) are pathological reactions triggered by the immune system mistakenly identifying one or more protein antigens in food as harmful substances. Symptoms can accumulate in multiple systems such as the skin, digestion, respiration, circulation, and even lead to anaphylactic shock. They can be classified as IgE-mediated, IgE-dependent and IgE non-dependent pathways co-mediated (mixed), and non-IgE-mediated (114). Studies have shown that light, latitude, and season of birth are related to FAs (115). For example, a survey by Vassallo (116) and others showed that the proportion of children under 5 years old born in autumn or winter with FA is 50% higher than those born in spring or summer. In the United States and Australia, the overall risk of allergies, FAs, and FA markers in the population farthest from the equator is higher than those closest to the equator (117, 118). Seasons and latitude affect the exposure of the human body to sunlight and solar radiation (with fewer megajoules of sunlight per square meter

in the world's southernmost and northernmost parts and shorter daylight hours in autumn and winter). The synthesis of vitamin D is also related to light with 80%-90% of the serum 25(OH)D levels deriving from sun exposure. Its level changes periodically with the seasons, because the time for sun exposure to synthesize vitamin D is longer in winter than in summer (119). There is also direct evidence indicating that insufficient sunlight exposure before the age of 24 months may elevate the risk of developing FAs, asthma, AR, and AD in school-aged children (120). This connects vitamin D with FAs, AR, AD and other allergic diseases. A cross-sectional study (121) by Silva and others found that infants with cow's milk protein allergy had lower average vitamin D levels compared with the healthy control group. A large study (122) reported a cross-sectional association between vitamin D deficiency (VDI; 25(OH)D <50 nmol/L) in one-year-old infants of Australian-born parents and positive provocation test IgE-mediated food allergies, with evidence suggesting a dose-response relationship, where infants deficient in vitamin D had a 3-fold increased risk of egg allergy, an 11-fold increased risk of peanut allergy, and a 10-fold increased risk in infants with two or more FAs. In addition, in infants who already have food sensitization, those who are deficient in vitamin D have a 6-fold risk of developing FAs compared to those who develop food tolerance. A recent systematic review suggests that maternal vitamin D deficiency and infant vitamin D deficiency appear to increase the risk of FAs, especially in the second year after the baby's birth (123). In contrast, Weisse et al. (124) observed in a cohort study that the higher the maternal and cord blood 25(OH)D levels, the higher the risk of FAs in children in the first two years, and they believe that this association can be explained by the observed decrease in the number of Treg cells at birth. Similarly, a case-cohort study by Molloy (125) and others also believes that vitamin deficiency in the first 6 months of infancy is not significantly associated with FAs at one year old. In summary, most of the literature supports a significant association between vitamin D and food allergies, but the specific mechanism needs to be further studied.

Indeed, the relationship between vitamin D and allergies may depend on several factors, including an individual's vitamin D levels, the type of allergic disease, gender, ethnicity, and other potential immune regulatory mechanisms. Therefore, further research is necessary to clarify the exact role of vitamin D in allergic diseases, and how to effectively use vitamin D in the clinic to regulate immune responses and improve the treatment of allergic diseases.

# 4 The clinical efficacy of vitamin D supplementation in allergic diseases

## 4.1 The impact of vitamin D supplementation on the outcomes of allergic diseases

With the established association between vitamin D deficiency and allergic diseases, numerous studies have been dedicated to investigating the clinical benefits of vitamin D supplementation in various populations, and the results have been relatively promising (Table 1). A significant, large-scale randomized controlled trial (RCT) study is the VDAART trial (126). The VDAART trial was a randomized, double-blind, placebo-controlled study conducted across three centers in the United States. It included 881 non-smoking pregnant women aged between 18–39 years, who were at 10–18 weeks of gestation and had a high risk of their offspring developing asthma. These women were randomly divided to receive either the intervention group (4400 IU of vitamin D daily) or placebo (a multivitamin containing 400 IU of vitamin D daily) until childbirth. The study examined the maternal 25(OH)D levels in the late stages of pregnancy and the conditions of asthma and recurrent wheezing in the offspring. While the intention-to-treat analysis and stratified analysis based on the 25(OH)D levels of the mothers during pregnancy indicated that maternal vitamin D supplementation did not impact the occurrence of asthma and recurrent wheezing in the offspring at ages 3 and 6 (127, 128), further analysis of early and late prenatal vitamin D status, baseline vitamin D levels of the mothers at the beginning of the study, and the timing of supplementation initiation led researchers to conclude that adequate prenatal vitamin D throughout pregnancy provides a protective effect against the development of asthma/recurrent wheezing in children before the age of 3 (129). The study also found that earlier intervention during pregnancy can significantly reduce the risk of asthma or recurrent wheezing in offspring, with each week of earlier intervention reducing the odds of the offspring developing asthma and recurrent wheezing by 15%. When compared with daily supplementation of 400 IU of vitamin D, initiating daily intake of 4400 IU of vitamin D between the 9th and 12th weeks can decrease the odds of asthma or recurrent wheezing by a maximum of 55% (130). Concurrently, a secondary analysis of VDAART by Chen et al. (80) also highlighted that prenatal vitamin D supplementation has a protective effect on the incidence of AR and sensitization to airborne allergens at ages 3 and 6.

A randomized, triple-blind, parallel, placebo-controlled study (131) conducted in Spain included 112 patients with an average age of 55 years suffering from asthma and with serum 25(OH)D levels below 30ng/mL. The study period was 6 months. The intervention group received 16,000 IU of oral cholecalciferol supplements weekly, while the control group added a placebo to the routine asthma treatment. The results showed that compared with the placebo, weekly oral supplementation of 25(OH)D can significantly improve Asthma Control Test (ACT) scores within 6 months. It can also improve the quality of life of patients, reduce the use of oral corticosteroids and the number of asthma attacks, and reduce the risk of hospital treatment for asthma.

In a prospective double-blind study conducted by Nabavizadeh et al. (132), 80 patients with chronic spontaneous urticaria were included. These patients were given low-dose vitamin D (4200IU/week, Group 1) and high-dose vitamin D (28,000 IU/week, Group 2) supplements for 12 weeks, in addition to their baseline treatment plan. The results indicated that both groups experienced a significant decrease in the total scores of urticaria severity, medication scores, and quality of life scores. Moreover, the high vitamin D group exhibited a more significant reduction in the total score of urticaria severity at the 6th week, and a more noticeable decrease in the quality-of-life score at the 6th and 12th weeks, compared to the low vitamin D group. Another study by Mohamed

TABLE 1 Studies on the impact of vitamin D supplementation on allergy outcomes.

| Reference | Design | Country | Sample size and age | Subject characteristics | Treatment | Primary outcome | Conclusion |
|---|---|---|---|---|---|---|---|
| Litonjua et al. (2014,2016,2020) (126–128) | Multicenter, double blind, randomized, placebo-controlled trial | Boston, Massachusetts; St Louis, Missouri; San Diego, USA | n=806, vitamin D group: 27.5 (5.5) y; Placebo group: 27.32 (5.5) y | Pregnant women with either history of asthma or allergies in themselves or the biological father | Vitamin D group: vitamin D3 4400 IU/day; Placebo group: vitamin D3 400 IU/day, duration the woman's pregnancy, about 22 to 30 weeks | Offspring asthma or recurrent wheeze | • Prenatal VD supplementation alone<br>• Has no effects on offspring asthma and recurrent wheeze development up to age 6 |
| Lu et al. (2021) (129); Shadid et al. (2023) (130) | Multicenter, double blind, randomized, placebo-controlled trial, secondary analysis | Boston, Massachusetts; St Louis, Missouri; San Diego, USA | n=806, vitamin D group: 27.5 (5.5) y; Placebo group: 27.32 (5.5) y | Pregnant women with either history of asthma or allergies in themselves or the biological father | Vitamin D group: vitamin D3 4400 IU/day; Placebo group: vitamin D3 400 IU/day, duration the woman's pregnancy, about 22 to 30 weeks | Offspring asthma or recurrent wheeze | •VD sufficiency throughout pregnancy<br>• Reducing the risk of asthma and recurrent wheeze in offspring<br>•The earlier the intervention, the better the effect |
| Chen et al. (2021) (80) | Multicenter, double blind, randomized, placebo-controlled trial, secondary analysis | Boston, Massachusetts; St Louis, Missouri; San Diego, USA | n=806, vitamin D group: 27.5 (5.5) y; Placebo group: 27.32 (5.5) y | Pregnant women with either history of asthma or allergies in themselves or the biological father | Vitamin D group: vitamin D3 4400 IU/day; Placebo group: vitamin D3 400 IU/day, duration the woman's pregnancy, about 22 to 30 weeks | Offspring aeroallergen sensitization and allergic Rhinitis | •VD sufficiency throughout pregnancy<br>•Attenuating the risk of offspring allergic rhinitis with sensitization by age 6 years. |
| Andújar-Espinosa et al. (2021) (131) | Prospective, randomized, triple-blind, placebo-controlled, parallel-group study | Murcia, Spain | n=112, Calcifediol group: 54.57 (15.83) y; Placebo group: 56.61(15.00) y | Adult asthmatic patients with serum 25(OH)D3 <30 ng/mL | 25(OH)D group: 25 (OH)D 16000 IU/week; Placebo group: placebo + usual asthma treatment, 6months | Asthma control degree: ACT; Life quality: Mini-AQLQ, Asthma attacks, Oral corticosteroid cycles, Emergency visits, Unscheduled consultations with the primary care physician and hospitalizations for asthma. | •Weekly oral calcifediol compared with placebo<br>•Improving asthma control among asthmaic adults with VD deficiency at 6 months. |
| Nabavizadeh et al. (2023) (132) | Prospective, randomized, double-blinded clinical trial | Shiraz, Iran | n=69, vitamin D group: 27.5 (5.5) y; Placebo group: 27.32 (5.5) y | Patients with chronic spontaneous urticaria | Low vitamin D3 group: 4200 IU/week; High vitamin D3 group: 28,000 IU/week, 12 weeks | Quality of life (CU-Q2oL questionnaire), urticaria severity (USS questionnaire) and medication scores | •High dose of vitamin D<br>• Reducing CU symptoms severity and the required doses of allergy medications. |
| Mohamed et al. (2022) (133) | Prospective, randomized, controlled and single blinded clinical trial | Cairo, Egypt | n=77, Study group: 35.2 (4.37) y; Placebo group: 34.6(9.8) y | Adults >18 y with urticaria episodes at least 2 days per week for 6 weeks or longer | Study group: 0.25μg alfacalcidol + Hydroxyzine 25 mg/day; Placebo group: 0.25μg placebo + Hydroxyzine 25 mg/day, 12 weeks | UAS7 total score, serum IL-6, hsCRP, TNF-α | •VD supplementation for 12 weeks<br>•Improveing UAS7 total score and the level of the inflammatory markers<br>•Having a beneficial effect on CSU patients |

*(Continued)*

TABLE 1 Continued

| Reference | Design | Country | Sample size and age | Subject characteristics | Treatment | Primary outcome | Conclusion |
|---|---|---|---|---|---|---|---|
| El-Heis et al. (2022) (134) | Multicenter, double-blind, randomized placebo-controlled trial | Southampton, Oxford and Sheffield, UK | n=703, Cholecalciferol group: 31.0 (4.9) y; Placebo group: 31.1 (5.0) y | Pregnant women aged over 18 years, gestational age < 17 week, and serum 25 (OH)D between 25 and 100 nmol/L and calcium < 2.75 mmol/L | Cholecalciferol group: cholecalciferol 1000 IU daily; Placebo group: matched placebo; from 14 weeks' gestation until delivery | Offspring atopic eczema at ages 12, 24 and 48 months | •Maternal cholecalciferol supplementation •Reducing the risk of atopic eczema in offspring during their first year of life. |
| Aldaghi et al. (2022) (135) | Single-center, double-blind, randomized, parallel-group clinical trial | Sabzevar, Iran | n=81, Synbiotic group: 4.09 (2.78) y; vitamin D3 group: 4.44 (2.84) y; Control group: 6.07(4.50) y | Infants under 12 months of age, without other chronic diseases, SCORAD score>14 | Synbiotic group: synbiotic 5 drops/day +routine treatment; vitamin D3 group: vitamin D3 1000IU/day + routine treatment; Control group: routine treatments, 2months | SCORAD score | •VD supplements administration along with routine treatments •Reducing the severity of AD in infants. |
| Mansour et al. (2020) (136) | Double-blind, randomized, parallel, placebo-controlled clinical trial | Cairo, Egypt | n=86, vitamin D group: 12 (4.75) y; Placebo group: 11(5.5) y | Patients aged from 5 to 16 years old, with a diagnosis of severe AD | Vitamin D3 group: vitamin D3 1600 IU/day + 1% hydrocortisone cream twice daily; Placebo group: placebo + 1% hydrocortisone cream twice daily, 12 weeks | EASI score | •VD supplements in children with severe AD •Providing clinical improvement |
| Cabalín et al. (2023) (137) | Open-label pilot trial | Santiago, Chile | n=86, 6.8 (3.8) y | Children aged 2–18 years with AD, SCORAD ≥ 25 | Oral doses of liquid VD3 8000 IU/week for 2–5.9 years; 12,000 IU/week for 6–11.9 years; 16,000 IU/week for 12–18 years, 6weeks | Stratum corneum RNA expression of the VDR, CAMP, and TSLP genes, and LL-37 protein | •VD supplementation in children with AD. •Improving AD severity, VDR and Cathelicidin expression in lesional skin |
| Guo(2023) (138) | Single-center, Assessor/statistician-blinded, randomized, parallel study | Ganzhou, China | n=128, Experimental group: 32.8 (10.2) y; Control group: 32.1(11.1) y | Patients aged between 16 and 60 years, with moderate-to-severe AR, did not receive any AR-related treatment within two weeks of diagnosis, had good drug compliance | Experimental group: vitamin D 1600 IU/day + 200 μg mometasone nasal spray twice/day; Control group: 200 μg mometasone nasal spray twice/day, 4weeks | TNSS, RQLQ, T lymphocyte subsets (CD3+, CD4+ and CD8+), IL-10, TNF-α, and IFN-γ | •VD supplementation in AR patients •Improving AR symptoms and quality-of-life •Decreasing TNF-α levels and increased IFN-γ and IL-10 levels. |
| Liu et al. (2022) (139) | Single-center, randomized, controlled trial | Hohhot, China | n= 90, vitamin D group: 27.2 (8.8) y; DCD group: 27.3 (7.1) y; Control group: 31.2 (10.6) y | Patients with mild seasonal pollen AR | Vitamin D group: oral desloratadine citrate disodium (DCD, 8.8 mg/day) + vitamin D3 nasal drops (1.5 × 106 IU, once/week; DCD group: DCD, 8.8 mg/day; Control group: no medication | Peripheral blood eosinophils, IL-4 levels, and nasal symptoms | •VD3 as an adjuvant therapy •Alleviating the nasal symptoms and decrease serum IL-4 and blood eosinophil count in patients with AR. |

VD, vitamin D; ACT, asthma control test; mini-AQLQ, the mini asthma quality of life questionnaire; CU-Q2oL, chronic urticaria quality of life questionnaire; USS, urticaria severity score; CU, chronic urticaria; CSU, Chronic spontaneous urticaria; UAS7, urticaria activity score over 7 days; AD, atopic dermatitis; SCORAD, scoring atopic dermatitis; EASI, eczema area and severity index; VDR, vitamin D receptor; CAMP, cathelicidin antimicrobial peptide; TSLP, thymic stromal lymphopoietin; AR, allergic rhinitis; TNSS, total nasal symptom score; RQLQ, rhinoconjunctivitis quality of life questionnaire.

et al. (133), which focused on adults aged 18 and above in Egypt, corroborated these findings. They also observed that, in comparison with the placebo group and baseline results, the study group had significantly lower average serum IL-6, hypersensitive C-reactive protein (hs-CRP), and TNF-α levels.

For AD, El-Heis et al. (134) observed that supplementing mothers with 1000 IU of vitamin D daily from 14 weeks of pregnancy to delivery could reduce the incidence of AD in the first year after birth. Most RCT studies have confirmed that the addition of vitamin D to the basic treatment of AD significantly reduces the severity of the disease in children, including reducing SCORAD scores and eczema area and severity index (EASI) scores (135, 136). A study in the United States further found that oral vitamin D supplementation may be related to the increase in the expression of VDR and Cathelicidin in lesion skin (137).

In the context of AR, an RCT study carried out by Guo et al. (138) discovered that supplementing vitamin D can enhance the therapeutic effect of mometasone nasal spray on moderate to severe AR. This resulted in a more significant decrease in patients' TNSS total score, T lymphocyte subsets (CD3+, CD4+), CD4+/CD8+ ratio, TNF-α, and rhinoconjunctivitis quality of life questionnaire (RQLQ) total score. The levels of CD8+, IFN-γ, IL-10, and serum vitamin D were found to be more significantly increased compared to the control group and the initial test. Liu et al. (139) also noticed that patients who received vitamin D as an adjunct therapy had higher serum 25(OH)D levels, lower AR symptom scores, IL-4 levels, and peripheral blood eosinophils, and a higher effective rate of AR treatment, compared to those treated with desloratadine citrate dihydrate (DCD) alone. Hence, supplementing vitamin D in routine treatment can serve as an effective adjuvant treatment for AR patients by suppressing inflammation (Table 1).

The above studies have many limitations. First, the study population may be single-center, short-term, and small-scale. Second, the selection of the severity of the study subjects may be overly broad. Third, most studies may not consider the intake of vitamin D in the diet and the data of the patient's sun exposure time. Fourth, many self-filled questionnaires may have recall bias. Therefore, subsequent studies should consider the impact of differences in age, gender, severity, race, etc. on the clinical efficacy of vitamin D supplementation, and further large-scale, long-term follow-up, multi-center clinical trials and randomized controlled trials are needed. Also, it's necessary to determine the optimal dosage and duration of vitamin D supplementation and to deeply understand the impact of vitamin D on the treatment effect of allergic diseases.

## 4.2 Vitamin D and allergen immunotherapy

Allergen immunotherapy (AIT) is a therapeutic approach for allergic diseases that modulates the patient's immune system by progressively increasing the dose of allergens, thus reducing the allergic response to specific allergens. This method is commonly used for treating conditions such as pollen allergy, house dust mite (HDM) allergy, certain FAs, and bee venom allergy. AIT can be administered via subcutaneous injections, sublingual drops, or sublingual tablets. It can decrease allergen-specific Th2, stimulate regulatory T cells and B cells, and produce IgG and IgA blocking antibodies, thereby inducing tolerance to allergens in patients, reducing symptoms, and enhancing the quality of life. Given its long treatment cycle and high demand for patient compliance, new strategies are being explored currently, such as novel adjuvants, recombinant allergens, and immunomodulators, to provide safer, more effective, and convenient treatment plans and more lasting long-term tolerance (140). In this context, vitamin D has been identified as a potential enhancer, improving the effectiveness of AIT.

Numerous animal studies have demonstrated the enhancing effect of vitamin D on AIT. In a murine model of grass pollen-induced allergic asthma, vitamin D supplementation reduced the Th2 cell factor responses and innate cell factor responses to allergens in lung tissue, increased IL-10 in lung tissue, and reduced airway hyperresponsiveness (AHR). Researchers observed that, compared to subcutaneous immunotherapy (SCIT) or sublingual immunotherapy (SLIT) alone, the combination of 1,25 (OH)2D with SCIT or SLIT resulted in a more significant reduction in eosinophil counts and IL-5 and IL-13 levels in bronchoalveolar lavage fluid, as well as marked improvement in lung function. The authors concluded that vitamin D enhances the efficacy of grass pollen SLIT and SCIT in mice (141, 142).

Li (143) and colleagues conducted a regression analysis on 153 AR patients who received SLIT, revealing that a deficiency in serum Vitamin D could impact the effectiveness of SLIT in children with AR. Majak (144) and others carried out a retrospective secondary analysis of the combined data from a prospective, randomized, placebo-controlled trial involving 36 children with asthma undergoing AIT. They discovered that patients with higher serum 25(OH)D levels experienced more significant reductions in asthma symptom scores and AIT-induced corticosteroid reduction effects over the 12-month AIT period. These patients also exhibited higher peripheral blood TGF-β production and greater expression of Foxp3 positive cells, suggesting that vitamin D might serve as an effective adjuvant for AIT. A randomized, double-blind, placebo-controlled trial (145) in Poland, which included 50 children aged 5–12 who were allergic to grass pollen and had AR (with 8 also having asthma), used a daily 5-grass pollen sublingual 300 IR tablet and supplemented with either 1000 IU of vitamin D or a placebo for 5 months. The study found that the SLIT plus vitamin D group was more effective in alleviating nasal symptoms, asthma symptoms, and symptom-medication combined scores compared to the placebo group. In a study on children with asthma who were allergic to HDMs, the SCIT plus vitamin D group had a lower total asthma symptom score at the 6th month and the highest average fluorescence intensity of Foxp3 at the 12th month, compared to using SCIT alone (146).

A study conducted in Bangkok, Thailand (147), demonstrated that, compared to a placebo, adult patients allergic to HDMs who received subcutaneous AIT and supplemented with vitamin D experienced significantly reduced symptom-drug scores and increased treatment response rates. This improvement in allergic

symptoms is thought to be achieved by vitamin D significantly reducing the quantity of dysfunctional regulatory T cells (CRTH2 +Treg). This lends further support to the potential value of vitamin D in AIT. These findings also offer new treatment strategies for AIT and pave the way for new possibilities in the treatment of allergic diseases.

## 5 Summary

Based on existing research, the role of vitamin D in allergic diseases cannot be ignored. Vitamin D can affect the occurrence and development of allergic diseases through its immune regulatory function. Although existing research shows that vitamin D deficiency is related to an increased risk of allergic diseases, its correlation is not consistent among different populations, and the effect of vitamin D supplementation on improving the outcomes of these diseases still needs further research. In terms of mechanism, there are many contradictions in vitamin D's regulation of Th1/Th2 balance, Th17/Treg, ILC2 cells, etc. Due to the complexity of the immune system, the occurrence and development of allergies by vitamin D cannot be explained by a single regulatory method, and further research is needed to discuss which regulation predominates. In terms of clinical efficacy, future research should explore the optimal supplement dose and duration of vitamin D more deeply, considering patients' lifestyles, dietary habits, and basic health conditions, and carry out more rigorous and detailed research design. This includes cross-racial and regional studies, as well as analyses of different age and gender groups, to ensure the wide applicability and accuracy of the research results, and how to use vitamin D more effectively to regulate immune responses and improve the treatment effects of patients with allergic diseases. At the same time, we need to pay attention to the potential of vitamin D as an adjuvant combined with AIT, to develop safer, more effective and convenient treatment methods. In summary, vitamin D plays an important role in the prevention and treatment of allergic diseases, but its specific mechanisms and application strategies still need to be clarified by further research in the future.

## Author contributions

PZ: Writing – original draft, Writing – review & editing. QX: Writing – review & editing. RZ: Conceptualization, 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.

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## Publisher's note



## References

1. Haahtela T. A biodiversity hypothesis. *Allergy*. (2019) 74:1445–56. doi: 10.1111/all.13763

2. Nwaru BI, Virtanen SM. Allergenic food introduction and childhood risk of allergic or autoimmune disease. *JAMA*. (2017) 317:86. doi: 10.1001/jama.2016.18329

3. Aranow C. Vitamin D and the immune system. *J Investig Med*. (2011) 59:881–6. doi: 10.2310/JIM.0b013e31821b8755

4. Hewison M. Vitamin D and innate and adaptive immunity. *Vitam Horm*. (2011) 86:23–62. doi: 10.1016/B978-0-12-386960-9.00002-2

5. Bora SA, Kennett MJ, Smith PB, Patterson AD, Cantorna MT. Regulation of vitamin D metabolism following disruption of the microbiota using broad spectrum antibiotics. *J Nutr Biochem*. (2018) 56:65–73. doi: 10.1016/j.jnutbio.2018.01.011

6. Agmon-Levin N, Theodor E, Segal RM, Shoenfeld Y. Vitamin D in systemic and organ-specific autoimmune diseases. *Clin Rev Allergy Immunol*. (2013) 45:256–66. doi: 10.1007/s12016-012-8342-y

7. Malaguarnera L. Vitamin D and microbiota: Two sides of the same coin in the immunomodulatory aspects. *Int Immunopharmacol*. (2020) 79:106112. doi: 10.1016/j.intimp.2019.106112

8. Murdaca G, Tonacci A, Negrini S, Greco M, Borro M, Puppo F, et al. Emerging role of vitamin D in autoimmune diseases: An update on evidence and therapeutic implications. *Autoimmun Rev*. (2019) 18:102350. doi: 10.1016/j.autrev.2019.102350

9. Bizzaro G, Antico A, Fortunato A, Bizzaro N. Vitamin D and autoimmune diseases: is vitamin D receptor (VDR) polymorphism the culprit? *Isr Med Assoc J*. (2017) 19:438–43.

10. Hewison M, Burke F, Evans KN, Lammas DA, Sansom DM, Liu P, et al. Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. *J Steroid Biochem Mol Biol*. (2007) 103:316–21. doi: 10.1016/j.jsbmb.2006.12.078

11. Ruiter B, Patil SU, Shreffler WG. Vitamins A and D have antagonistic effects on expression of effector cytokines and gut-homing integrin in human innate lymphoid cells. *Clin Exp Allergy*. (2015) 45:1214–25. doi: 10.1111/cea.12568

12. Konya V, Czarnewski P, Forkel M, Rao A, Kokkinou E, Villablanca EJ, et al. Vitamin D downregulates the IL-23 receptor pathway in human mucosal group 3 innate lymphoid cells. *J Allergy Clin Immunol*. (2018) 141:279–92. doi: 10.1016/j.jaci.2017.01.045

13. Hiraguchi Y, Tanida H, Sugimoto M, Hosoki K, Nagao M, Tokuda R, et al. 1,25-Dihydroxyvitamin D3 upregulates functional C-x-C chemokine receptor type 4 expression in human eosinophils. *Int Arch Allergy Immunol.* (2012) 158 Suppl 1:51–7. doi: 10.1159/000337767

14. Nagase H, Miyamasu M, Yamaguchi M, Fujisawa T, Ohta K, Yamamoto K, et al. Expression of CXCR4 in eosinophils: functional analyses and cytokine-mediated regulation. *J Immunol.* (2000) 164:5935–43. doi: 10.4049/jimmunol.164.11.5935

15. Lu H, Xie R-D, Lin R, Zhang C, Xiao X-J, Li L-J, et al. Vitamin D-deficiency induces eosinophil spontaneous activation. *Cell Immunol.* (2017) 322:56–63. doi: 10.1016/j.cellimm.2017.10.003

16. Gorman S, Judge MA, Burchell JT, Turner DJ, Hart PH. 1,25-dihydroxyvitamin D3 enhances the ability of transferred CD4+ CD25+ cells to modulate T helper type 2-driven asthmatic responses. *Immunology.* (2010) 130:181–92. doi: 10.1111/j.1365-2567.2009.03222.x

17. Liu Z-Q, Li X-X, Qiu S-Q, Yu Y, Li M-G, Yang L-T, et al. Vitamin D contributes to mast cell stabilization. *Allergy.* (2017) 72:1184–92. doi: 10.1111/all.13110

18. Yip K-H, Kolesnikoff N, Yu C, Hauschild N, Taing H, Biggs L, et al. Mechanisms of vitamin $D_3$ metabolite repression of IgE-dependent mast cell activation. *J Allergy Clin Immunol.* (2014) 133:1356–1364, 1364.e1–14. doi: 10.1016/j.jaci.2013.11.030

19. Biggs L, Yu C, Fedoric B, Lopez AF, Galli SJ, Grimbaldeston MA. Evidence that vitamin D(3) promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice. *J Exp Med.* (2010) 207:455–63. doi: 10.1084/jem.20091725

20. Chen L, Eapen MS, Zosky GR. Vitamin D both facilitates and attenuates the cellular response to lipopolysaccharide. *Sci Rep.* (2017) 7:45172. doi: 10.1038/srep45172

21. Bishop E L, Ismailova A, Dimeloe S, Hewison M, White JH. Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory. *JBMR Plus.* (2021) 5: e10405. doi: 10.1002/jbm4.10405

22. Subramanian K, Bergman P, Henriques-Normark B. Vitamin D promotes pneumococcal killing and modulates inflammatory responses in primary human neutrophils. *J Innate Immun.* (2017) 9:375–86. doi: 10.1159/000455969

23. Yang H, Long F, Zhang Y, Yu R, Zhang P, Li W, et al. 1α,25-Dihydroxyvitamin D3 Induces Neutrophil Apoptosis through the p38 MAPK Signaling Pathway in Chronic Obstructive Pulmonary Disease Patients. *PloS One.* (2015) 10:e0120515. doi: 10.1371/journal.pone.0120515

24. Christofyllakis K, Neumann F, Bewarder M, Thurner L, Kaddu-Mulindwa D, Kos IA, et al. Vitamin D enhances immune effector pathways of NK cells thus providing a mechanistic explanation for the increased effectiveness of therapeutic monoclonal antibodies. *Nutrients.* (2023) 15:3498. doi: 10.3390/nu15163498

25. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science.* (2006) 311:1770–3. doi: 10.1126/science.1123933

26. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB journal.* (2005) 19:1067–77. doi: 10.1096/fj.04-3284com

27. Small AG, Harvey S, Kaur J, Putty T, Quach A, Munawara U, et al. Vitamin D upregulates the macrophage complement receptor immunoglobulin in innate immunity to microbial pathogens. *Commun Biol.* (2021) 4:401. doi: 10.1038/s42003-021-01943-3

28. Luo M, Zhao F, Cheng H, Su M, Wang Y. Macrophage polarization: an important role in inflammatory diseases. *Front Immunol.* (2024) 15:1352946. doi: 10.3389/fimmu.2024.1352946

29. Atri C, Guerfali FZ, Laouini D. Role of human macrophage polarization in inflammation during infectious diseases. *Int J Mol Sci.* (2018) 19:1801. doi: 10.3390/ijms19061801

30. Abe C, Bhaswant M, Miyazawa T, Miyazawa T. The potential use of exosomes in anti-cancer effect induced by polarized macrophages. *Pharmaceutics.* (2023) 15:1024. doi: 10.3390/pharmaceutics15031024

31. Zhang X, Zhou M, Guo Y, Song Z, Liu B. 1,25-dihydroxyvitamin $D_3$ Promotes high glucose-induced M1 macrophage switching to M2 via the VDR-PPARγ Signaling pathway. *BioMed Res Int.* (2015) 2015:157834. doi: 10.1155/2015/157834

32. Gunasekar P, Swier VJ, Fleegel JP, Boosani CS, Radwan MM, Agrawal DK. Vitamin D and macrophage polarization in epicardial adipose tissue of atherosclerotic swine. *PloS One.* (2018) 13:e0199411. doi: 10.1371/journal.pone.0199411

33. Xia T, Fu S, Yang R, Yang K, Lei W, Yang Y, et al. Advances in the study of macrophage polarization in inflammatory immune skin diseases. *J Inflammation (Lond).* (2023) 20:33. doi: 10.1186/s12950-023-00360-z

34. Zhu X, Sun Y, Yu Q, Wang X, Wang Y, Zhao Y. Exosomal lncRNA GAS5 promotes M1 macrophage polarization in allergic rhinitis via restraining mTORC1/ULK1/ATG13-mediated autophagy and subsequently activating NF-κB signaling. *Int Immunopharmacol.* (2023) 121:110450. doi: 10.1016/j.intimp.2023.110450

35. Wen S, Li F, Tang Y, Dong L, He Y, Deng Y, et al. MIR222HG attenuates macrophage M2 polarization and allergic inflammation in allergic rhinitis by targeting the miR146a-5p/TRAF6/NF-κB axis. *Front Immunol.* (2023) 14:1168920. doi: 10.3389/fimmu.2023.1168920

36. Ao T, Kikuta J, Ishii M. The effects of vitamin D on immune system and inflammatory diseases. *Biomolecules.* (2021) 11:1624. doi: 10.3390/biom11111624

37. Barragan M, Good M, Kolls JK. Regulation of dendritic cell function by vitamin D. *Nutrients.* (2015) 7:8127–51. doi: 10.3390/nu7095383

38. Bartels LE, Hvas CL, Agnholt J, Dahlerup JF, Agger R. Human dendritic cell antigen presentation and chemotaxis are inhibited by intrinsic 25-hydroxy vitamin D activation. *Int Immunopharmacol.* (2010) 10:922–8. doi: 10.1016/j.intimp.2010.05.003

39. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol.* (2000) 164:2405–11. doi: 10.4049/jimmunol.164.5.2405

40. Canning MO, Grotenhuis K, de Wit H, Ruwhof C, Drexhage HA. 1-alpha,25-Dihydroxyvitamin D3 (1,25(OH)(2)D(3)) hampers the maturation of fully active immature dendritic cells from monocytes. *Eur J Endocrinol.* (2001) 145:351–7. doi: 10.1530/eje.0.1450351

41. Penna G, Amuchastegui S, Giarratana N, Daniel KC, Vulcano M, Sozzani S, et al. 1,25-Dihydroxyvitamin D3 selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells. *J Immunol.* (2007) 178:145–53. doi: 10.4049/jimmunol.178.1.145

42. Gallo D, Baci D, Kustrimovic N, Lanzo N, Patera B, Tanda ML, et al. How does vitamin D affect immune cells crosstalk in autoimmune diseases? *Int J Mol Sci.* (2023) 24:4689. doi: 10.3390/ijms24054689

43. Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and disease. *Nutrients.* (2020) 12:2097. doi: 10.3390/nu12072097

44. Brulefert A, Hoste A, Muller Q, Fauny J-D, Mueller CG, Flacher V. Vitamin D3-elicited CD14+ human skin dendritic cells promote thymic stromal lymphopoietin-independent type 2 T-helper responses. *Allergy.* (2021) 76:2044–56. doi: 10.1111/all.14718

45. Sloka S, Silva C, Wang J, Yong VW. Predominance of Th2 polarization by vitamin D through a STAT6-dependent mechanism. *J Neuroinflamm.* (2011) 8:56. doi: 10.1186/1742-2094-8-56

46. Zhang Z, Chen F, Li J, Luo F, Hou T, Xu J, et al. 1,25(OH)2D3 suppresses proinflammatory responses by inhibiting Th1 cell differentiation and cytokine production through the JAK/STAT pathway. *Am J Transl Res.* (2018) 10:2737–46.

47. Hafkamp FMJ, Taanman-Kueter EWM, van Capel TMM, Kormelink TG, de Jong EC. Vitamin D3 priming of dendritic cells shifts human neutrophil-dependent th17 cell development to regulatory T cells. *Front Immunol.* (2022) 13:872665. doi: 10.3389/fimmu.2022.872665

48. Anderson J, Do LAH, Toh ZQ, Hoe E, Reitsma A, Mulholland K, et al. Vitamin D induces differential effects on inflammatory responses during bacterial and/or viral stimulation of human peripheral blood mononuclear cells. *Front Immunol.* (2020) 11:602. doi: 10.3389/fimmu.2020.00602

49. Dankers W, Davelaar N, van Hamburg JP, van de Peppel J, Colin EM, Lubberts E. Human memory Th17 cell populations change into anti-inflammatory cells with regulatory capacity upon exposure to active vitamin D. *Front Immunol.* (2019) 10:1504. doi: 10.3389/fimmu.2019.01504

50. Colin EM, Asmawidjaja PS, van Hamburg JP, Mus AMC, van Driel M, Hazes JMW, et al. 1,25-dihydroxyvitamin D3 modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. *Arthritis Rheum.* (2010) 62:132–42. doi: 10.1002/art.25043

51. Urry Z, Chambers ES, Xystrakis E, Dimeloe S, Richards DF, Gabryšová L, et al. The role of 1α,25-dihydroxyvitamin D3 and cytokines in the promotion of distinct Foxp3+ and IL-10+ CD4+ T cells. *Eur J Immunol.* (2012) 42:2697–708. doi: 10.1002/eji.201242370

52. Cantorna MT, Snyder L, Lin Y-D, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. *Nutrients.* (2015) 7:3011–21. doi: 10.3390/nu7043011

53. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol.* (2007) 179:1634–47. doi: 10.4049/jimmunol.179.3.1634

54. Geldmeyer-Hilt K, Heine G, Hartmann B, Baumgrass R, Radbruch A, Worm M. 1,25-dihydroxyvitamin D3 impairs NF-κB activation in human naïve B cells. *Biochem Biophys Res Commun.* (2011) 407:699–702. doi: 10.1016/j.bbrc.2011.03.078

55. Wu C-Y, Yang H-Y, Luo S-F, Huang J-L, Lai J-H. Vitamin D supplementation in patients with juvenile idiopathic arthritis. *Nutrients.* (2022) 14:1538. doi: 10.3390/nu14081538

56. Martens P-J, Gysemans C, Verstuyf A, Mathieu AC. Vitamin D's effect on immune function. *Nutrients.* (2020) 12:1248. doi: 10.3390/nu12051248

57. Heine G, Niesner U, Chang H-D, Steinmeyer A, Zügel U, Zuberbier T, et al. 1,25-dihydroxyvitamin D(3) promotes IL-10 production in human B cells. *Eur J Immunol.* (2008) 38:2210–8. doi: 10.1002/eji.200838216

58. Bieber T. Atopic dermatitis. *N Engl J Med.* (2008) 358:1483–94. doi: 10.1056/NEJMra074081

59. Alosaimi NS, Sattar Ahmad M a. A, Alkreathy HM, Ali AS, Khan LM. Pharmacological basis of the putative therapeutic effect of Topical Vitamin D3 on the experimental model of atopic dermatitis in mice. *Eur Rev Med Pharmacol Sci.* (2022) 26:6827–36. doi: 10.26355/eurrev_202209_29785

60. El Taieb MA, Fayed HM, Aly SS, Ibrahim AK. Assessment of serum 25-hydroxyvitamin d levels in children with atopic dermatitis: correlation with SCORAD index. *Dermatitis.* (2013) 24:296–301. doi: 10.1097/DER.0000000000000010

61. Heimbeck I, Wjst M, Apfelbacher CJ. Low vitamin D serum level is inversely associated with eczema in children and adolescents in Germany. *Allergy.* (2013) 68:906–10. doi: 10.1111/all.12167

62. Ahmed Mohamed A, Salah Ahmed EM, Farag YMK, Bedair NI, Nassar NA, Ghanem AIM. Dose-response association between vitamin D deficiency and atopic dermatitis in children, and effect modification by gender: a case-control study. *J Dermatolog Treat*. (2021) 32:174–9. doi: 10.1080/09546634.2019.1643447

63. Ng JC, Yew YW. Effect of vitamin D serum levels and supplementation on atopic dermatitis: A systematic review and meta-analysis. *Am J Clin Dermatol*. (2022) 23:267–75. doi: 10.1007/s40257-022-00677-0

64. Çiçek F, Köle MT. Evaluation of the impact of serum vitamin D levels on the scoring atopic dermatitis index in pediatric atopic dermatitis. *Children (Basel)*. (2023) 10:1522. doi: 10.3390/children10091522

65. Baek JH, Shin YH, Chung IH, Kim HJ, Yoo E-G, Yoon JW, et al. The link between serum vitamin D level, sensitization to food allergens, and the severity of atopic dermatitis in infancy. *J Pediatr*. (2014) 165:849–854.e1. doi: 10.1016/j.jpeds.2014.06.058

66. Barlianto W, Wulandari D, Sari TL, Firdayanti VH, Avandi MI. Vitamin D, cytokine profiles, and disease severity in infants with atopic dermatitis: a single centre, cross-sectional study. *Postepy Dermatol Alergol*. (2022) 39:793–9. doi: 10.5114/ada.2022.118923

67. Peroni DG, Piacentini GL, Cametti E, Chinellato I, Boner AL. Correlation between serum 25-hydroxyvitamin D levels and severity of atopic dermatitis in children. *Br J Dermatol*. (2011) 164:1078–82. doi: 10.1111/j.1365-2133.2010.10147.x

68. Yang L, Sato M, Saito-Abe M, Nishizato M, Mezawa H, Yamamoto-Hanada K, et al. Serum 25-hydroxyvitamin D concentrations and atopic dermatitis in early childhood: findings from the Japan environment and children's study. *Nutrients*. (2021) 13:2761. doi: 10.3390/nu13082761

69. Berents TL, Lødrup Carlsen KC, Mowinckel P, Sandvik L, Skjerven HO, Rolfsjord LB, et al. Vitamin D levels and atopic eczema in infancy and early childhood in Norway: a cohort study. *Br J Dermatol*. (2016) 175:95–101. doi: 10.1111/bjd.14537

70. Hennessy Á, Hourihane JO, Malvisi L, Irvine AD, Kenny LC, Murray DM, et al. Antenatal vitamin D exposure and childhood eczema, food allergy, asthma and allergic rhinitis at 2 and 5 years of age in the atopic disease-specific Cork BASELINE Birth Cohort Study. *Allergy*. (2018) 73:2182–91. doi: 10.1111/all.13590

71. Chen B, Qu S, Li M, Ye L, Zhang S, Qin T, et al. Effects of 1,25-dihydroxyvitamin D3 in an ovalbumin-induced allergic rhinitis model. *Int Immunopharmacol*. (2017) 47:182–9. doi: 10.1016/j.intimp.2017.04.005

72. Keleş E, Özkara Ş, İlhan N, Güngör H, Karlıdağ T, Yalçın Ş. The relationship between th1/th2 balance and 1α, 25-dihydroxyvitamin D3 in patients with allergic rhinitis. *Turk Arch Otorhinolaryngol*. (2015) 53:139–43. doi: 10.5152/tao.2015.1187

73. Saad K, Abdelmoghny A, Aboul-Khair MD, Abdel-Raheem YF, Gad EF, Hammour AE-S, et al. Vitamin D status in Egyptian children with allergic rhinitis. *Ear Nose Throat J*. (2020) 99:508–12. doi: 10.1177/0145561319850814

74. Kajal S, Shrestha P, Gupta Y, Deepak R, Verma H. Vitamin D deficiency and interleukin levels in allergic rhinitis: A case-control study. *Indian J Otolaryngol Head Neck Surg*. (2022) 74:1720–4. doi: 10.1007/s12070-021-02897-y

75. Çoban K, Öz I, Topçu Dİ, Aydın E. The impact of serum 25-hydroxyvitamin D3 levels on allergic rhinitis. *Ear Nose Throat J*. (2021) 100:NP236–41. doi: 10.1177/0145561319874310

76. Restimulia L, Pawarti DR, Ekorini HM. The relationship between serum vitamin D levels with allergic rhinitis incidence and total nasal symptom score in allergic rhinitis patients. *Open Access Maced J Med Sci*. (2018) 6:1405–9. doi: 10.3889/oamjms.2018.247

77. Ma Y, Liu Y, Li X, Qiu J, Fang P. Low serum 25-hydroxyvitamin D levels are associated with perennial allergic rhinitis but not disease severity. *J Clin Lab Anal*. (2020) 34:e23516. doi: 10.1002/jcla.23516

78. Dogru M, Suleyman A. Serum 25-hydroxyvitamin D3 levels in children with allergic or nonallergic rhinitis. *Int J Pediatr Otorhinolaryngol*. (2016) 80:39–42. doi: 10.1016/j.ijporl.2015.11.013

79. Jung J-W, Kim J-Y, Cho S-H, Choi B-W, Min K-U, Kang H-R. Allergic rhinitis and serum 25-hydroxyvitamin D level in Korean adults. *Ann Allergy Asthma Immunol*. (2013) 111:352–7. doi: 10.1016/j.anai.2013.08.018

80. Chen Y-CS, Mirzakhani H, Lu M, Zeiger RS, O'Connor GT, Sandel MT, et al. The association of prenatal vitamin D sufficiency with aeroallergen sensitization and allergic rhinitis in early childhood. *J Allergy Clin Immunol Pract*. (2021) 9:3788–3796.e3. doi: 10.1016/j.jaip.2021.06.009

81. Bunyavanich S, Rifas-Shiman SL, Platts-Mills TA, Workman L, Sordillo JE, Camargo CA, et al. Prenatal, perinatal, and childhood vitamin D exposure and their association with childhood allergic rhinitis and allergic sensitization. *J Allergy Clin Immunol*. (2016) 137:1063–1070.e2. doi: 10.1016/j.jaci.2015.11.031

82. Tian H-Q, Chen X-Y, Lu Y, Lu W-M, Wang M-L, Zhao H-L, et al. Association of VDR and CYP2R1 polymorphisms with mite-sensitized persistent allergic rhinitis in a Chinese population. *PloS One*. (2015) 10:e0133162. doi: 10.1371/journal.pone.0133162

83. Wegienka G, Havstad S, Zoratti EM, Kim H, Ownby DR, Johnson CC. Association between vitamin D levels and allergy-related outcomes vary by race and other factors. *J Allergy Clin Immunol*. (2015) 136:1309–1314.e1–4. doi: 10.1016/j.jaci.2015.04.017

84. Mai X-M, Chen Y, Camargo CA, Langhammer A. Serum 25-hydroxyvitamin D levels and self-reported allergic rhinitis in Norwegian adults - The HUNT Study. *Allergy*. (2014) 69:488–93. doi: 10.1111/all.12365

85. Wu H-Y, Chen J-X, Tian H-Q, Zhang X-L, Bian H-Y, Cheng L. Serum 25-hydroxyvitamin D inversely associated with blood eosinophils in patients with persistent allergic rhinitis. *Asia Pac Allergy*. (2017) 7:213–20. doi: 10.5415/apallergy.2017.7.4.213

86. Cheng HM, Kim S, Park G-H, Chang SE, Bang S, Won CH, et al. Low vitamin D levels are associated with atopic dermatitis, but not allergic rhinitis, asthma, or IgE sensitization, in the adult Korean population. *J Allergy Clin Immunol*. (2014) 133:1048–55. doi: 10.1016/j.jaci.2013.10.055

87. Baïz N, Dargent-Molina P, Wark JD, Souberbielle J-C, Annesi-Maesano IEDEN Mother-Child Cohort Study Group. Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. *J Allergy Clin Immunol*. (2014) 133:147–53. doi: 10.1016/j.jaci.2013.05.017

88. Feng Q, Bønnelykke K, Ek WE, Chawes BL, Yuan S, Cheung CL, et al. Null association between serum 25-hydroxyvitamin D levels with allergic rhinitis, allergic sensitization and non-allergic rhinitis: A Mendelian randomization study. *Clin Exp Allergy*. (2021) 51:78–86. doi: 10.1111/cea.13739

89. Vasiliou JE, Lui S, Walker SA, Chohan V, Xystrakis E, Bush A, et al. Vitamin D deficiency induces Th2 skewing and eosinophilia in neonatal allergic airways disease. *Allergy*. (2014) 69:1380–9. doi: 10.1111/all.12465

90. Nahm D. Regulatory T cell-targeted immunomodulatory therapy for long-term clinical improvement of atopic dermatitis: hypotheses and perspectives. *Life (Basel)*. (2023) 13:1674. doi: 10.3390/life13081674

91. Hamzaoui A, Berraïes A, Hamdi B, Kaabachi W, Ammar J, Hamzaoui K. Vitamin D reduces the differentiation and expansion of Th17 cells in young asthmatic children. *Immunobiology*. (2014) 219:873–9. doi: 10.1016/j.imbio.2014.07.009

92. Kolokotroni O, Papadopoulou A, Middleton N, Kouta C, Raftopoulos V, Nicolaidou P, et al. Vitamin D levels and status amongst asthmatic and non-asthmatic adolescents in Cyprus: a comparative cross-sectional study. *BMC Public Health*. (2015) 15:48. doi: 10.1186/s12889-015-1385-2

93. Bener A, Ehlayel MS, Tulic MK, Hamid Q. Vitamin D deficiency as a strong predictor of asthma in children. *Int Arch Allergy Immunol*. (2012) 157:168–75. doi: 10.1159/000323941

94. Zhu Y, Jing D, Liang H, Li D, Chang Q, Shen M, et al. Vitamin D status and asthma, lung function, and hospitalization among British adults. *Front Nutr*. (2022) 9:954768. doi: 10.3389/fnut.2022.954768

95. Thorsteinsdottir F, Cardoso I, Keller A, Stougaard M, Frederiksen P, Cohen AS, et al. Neonatal vitamin D status and risk of asthma in childhood: results from the D-tect study. *Nutrients*. (2020) 12:842. doi: 10.3390/nu12030842

96. Hollams EM, Teo SM, Kusel M, Holt BJ, Holt KE, Inouye M, et al. Vitamin D over the first decade and susceptibility to childhood allergy and asthma. *J Allergy Clin Immunol*. (2017) 139:472–481.e9. doi: 10.1016/j.jaci.2016.07.032

97. Niruban SJ, Alagiakrishnan K, Beach J, Senthilselvan A. Association between vitamin D and respiratory outcomes in Canadian adolescents and adults. *J Asthma*. (2015) 52:653–61. doi: 10.3109/02770903.2015.1004339

98. Chang Q, Zhu Y, Zhou G, Liang H, Li D, Cheng J, et al. Vitamin D status, sleep patterns, genetic susceptibility, and the risk of incident adult-onset asthma: a large prospective cohort study. *Front Nutr*. (2023) 10:1222499. doi: 10.3389/fnut.2023.1222499

99. Larose TL, Langhammer A, Chen Y, Camargo CA, Romundstad P, Mai X-M. Serum 25-hydroxyvitamin D levels and lung function in adults with asthma: the HUNT Study. *Eur Respir J*. (2015) 45:1019–26. doi: 10.1183/09031936.00069714

100. Brumpton BM, Langhammer A, Henriksen AH, Camargo CA, Chen Y, Romundstad PR, et al. Vitamin D and lung function decline in adults with asthma: the HUNT study. *Am J Epidemiol*. (2016) 183:739–46. doi: 10.1093/aje/kwv243

101. Han Y-Y, Forno E, Celedón JC. Vitamin D insufficiency and asthma in a US nationwide study. *J Allergy Clin Immunol Pract*. (2017) 5:790–796.e1. doi: 10.1016/j.jaip.2016.10.013

102. Damera G, Fogle HW, Lim P, Goncharova EA, Zhao H, Banerjee A, et al. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *Br J Pharmacol*. (2009) 158:1429–41. doi: 10.1111/j.1476-5381.2009.00428.x

103. Kim S-H, Pei Q-M, Jiang P, Yang M, Qian X-J, Liu J-B. Effect of active vitamin D3 on VEGF-induced ADAM33 expression and proliferation in human airway smooth muscle cells: implications for asthma treatment. *Respir Res*. (2017) 18:7. doi: 10.1186/s12931-016-0490-9

104. Plesa M, Gaudet M, Mogas A, Olivenstein R, Al Heialy S, Hamid Q. Action of 1,25(OH)2D3 on human asthmatic bronchial fibroblasts: implications for airway remodeling in asthma. *J Asthma Allergy*. (2020) 13:249–64. doi: 10.2147/JAA.S261271

105. Song Y, Hong J, Liu D, Lin Q, Lai G. 1,25-dihydroxyvitamin D3 inhibits nuclear factor kappa B activation by stabilizing inhibitor IκBα via mRNA stability and reduced phosphorylation in passively sensitized human airway smooth muscle cells. *Scand J Immunol*. (2013) 77:109–16. doi: 10.1111/sji.12006

106. Jin A, Tang X, Zhai W, Li Y, Sun Q, Liu L, et al. TSLP-induced collagen type-I synthesis through STAT3 and PRMT1 is sensitive to calcitriol in human lung fibroblasts. *Biochim Biophys Acta Mol Cell Res*. (2021) 1868:119083. doi: 10.1016/j.bbamcr.2021.119083

107. Salameh L, Mahmood W, Hamoudi R, Almazrouei K, Lochanan M, Seyhoglu S, et al. The role of vitamin D supplementation on airway remodeling in asthma: A systematic review. *Nutrients*. (2023) 15:2477. doi: 10.3390/nu15112477

108. Gupta A, Sjoukes A, Richards D, Banya W, Hawrylowicz C, Bush A, et al. Relationship between serum vitamin D, disease severity, and airway remodeling in children with asthma. *Am J Respir Crit Care Med*. (2011) 184:1342. doi: 10.1164/rccm.201107-1239OC

109. Murdaca G, Tagliafico L, Page E, Paladin F, Gangemi S. Gender differences in the interplay between vitamin D and microbiota in allergic and autoimmune diseases. *Biomedicines*. (2024) 12:1023. doi: 10.3390/biomedicines12051023

110. Zhao X, Hu M, Zhou H, Yang Y, Shen S, You Y, et al. The role of gut microbiome in the complex relationship between respiratory tract infection and asthma. *Front Microbiol*. (2023) 14:1219942. doi: 10.3389/fmicb.2023.1219942

111. Murdaca G, Gerosa A, Paladin F, Petrocchi L, Banchero S, Gangemi S. Vitamin D and microbiota: is there a link with allergies? *Int J Mol Sci*. (2021) 22:4288. doi: 10.3390/ijms22084288

112. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A. 1α,25-dihydroxyvitamin D3 has a direct effect on naive CD4+ T cells to enhance the development of Th2 cells1. *J Immunol*. (2001) 167:4974–80. doi: 10.4049/jimmunol.167.9.4974

113. Confino-Cohen R, Brufman I, Goldberg A, Feldman BS. Vitamin D, asthma prevalence and asthma exacerbations: a large adult population-based study. *Allergy*. (2014) 69:1673–80. doi: 10.1111/all.2014.69.issue-12

114. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol*. (2016) 16:751–65. doi: 10.1038/nri.2016.111

115. Matsui T, Tanaka K, Yamashita H, Saneyasu K-I, Tanaka H, Takasato Y, et al. Food allergy is linked to season of birth, sun exposure, and vitamin D deficiency. *Allergol Int*. (2019) 68:172–7. doi: 10.1016/j.alit.2018.12.003

116. Vassallo MF, Banerji A, Rudders SA, Clark S, Mullins RJ, Camargo CA. Season of birth and food allergy in children. *Ann Allergy Asthma Immunol*. (2010) 104:307–13. doi: 10.1016/j.anai.2010.01.019

117. Mullins RJ, Clark S, Camargo CA. Regional variation in epinephrine autoinjector prescriptions in Australia: more evidence for the vitamin D-anaphylaxis hypothesis. *Ann Allergy Asthma Immunol*. (2009) 103:488–95. doi: 10.1016/S1081-1206(10)60265-7

118. Camargo CA, Clark S, Kaplan MS, Lieberman P, Wood RA. Regional differences in EpiPen prescriptions in the United States: the potential role of vitamin D. *J Allergy Clin Immunol*. (2007) 120:131–6. doi: 10.1016/j.jaci.2007.03.049

119. Antonucci R, Locci C, Clemente MG, Chicconi E, Antonucci L. Vitamin D deficiency in childhood: old lessons and current challenges. *J Pediatr Endocrinol Metab*. (2018) 31:247–60. doi: 10.1515/jpem-2017-0391

120. Hwang JM, Oh SH, Shin MY. The relationships among birth season, sunlight exposure during infancy, and allergic disease. *Korean J Pediatr*. (2016) 59:218–25. doi: 10.3345/kjp.2016.59.5.218

121. Silva CM, da Silva SA, Antunes MM de C, da Silva GAP, Sarinho ESC, Brandt KG. Do infants with cow's milk protein allergy have inadequate levels of vitamin D? *J Pediatr (Rio J)*. (2017) 93:632–8. doi: 10.1016/j.jped.2017.01.006

122. Allen KJ, Koplin JJ, Ponsonby A-L, Gurrin LC, Wake M, Vuillermin P, et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol*. (2013) 131:1109–1116, 1116.e1–6. doi: 10.1016/j.jaci.2013.01.017

123. Psaroulaki E, Katsaras GN, Samartzi P, Chatziravdeli V, Psaroulaki D, Oikonomou E, et al. Association of food allergy in children with vitamin D insufficiency: a systematic review and meta-analysis. *Eur J Pediatr*. (2023) 182:1533–54. doi: 10.1007/s00431-023-04843-2

124. Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M, et al. Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. *Allergy*. (2013) 68:220–8. doi: 10.1111/all.12081

125. Molloy J, Koplin JJ, Allen KJ, Tang MLK, Collier F, Carlin JB, et al. Vitamin D insufficiency in the first 6 months of infancy and challenge-proven IgE-mediated food allergy at 1 year of age: a case-cohort study. *Allergy*. (2017) 72:1222–31. doi: 10.1111/all.13122

126. Litonjua AA, Lange NE, Carey VJ, Brown S, Laranjo N, Harshfield BJ, et al. The Vitamin D Antenatal Asthma Reduction Trial (VDAART): rationale, design, and methods of a randomized, controlled trial of vitamin D supplementation in pregnancy for the primary prevention of asthma and allergies in children. *Contemp Clin Trials*. (2014) 38:37–50. doi: 10.1016/j.cct.2014.02.006

127. Litonjua AA, Carey VJ, Laranjo N, Harshfield BJ, McElrath TF, O'Connor GT, et al. Effect of prenatal supplementation with vitamin D on asthma or recurrent wheezing in offspring by age 3 years: the VDAART randomized clinical trial. *JAMA*. (2016) 315:362–70. doi: 10.1001/jama.2015.18589

128. Litonjua AA, Carey VJ, Laranjo N, Stubbs BJ, Mirzakhani H, O'Connor GT, et al. Six-year follow-up of a trial of antenatal vitamin D for asthma reduction. *N Engl J Med*. (2020) 382:525–33. doi: 10.1056/NEJMoa1906137

129. Lu M, Litonjua AA, O'Connor GT, Zeiger RS, Bacharier L, Schatz M, et al. Effect of early and late prenatal vitamin D and maternal asthma status on offspring asthma or recurrent wheeze. *J Allergy Clin Immunol*. (2021) 147:1234–1241.e3. doi: 10.1016/j.jaci.2020.06.041

130. Shadid IL, Brustad N, Lu M, Chawes BL, Bisgaard H, Zeiger RS, et al. The impact of baseline 25-hydroxyvitamin D level and gestational age on prenatal vitamin D supplementation to prevent offspring asthma or recurrent wheezing. *Am J Clin Nutr*. (2023) 117:1342–52. doi: 10.1016/j.ajcnut.2023.04.019

131. Andújar-Espinosa R, Salinero-González L, Illán-Gómez F, Castilla-Martínez M, Hu-Yang C, Ruiz-López FJ. Effect of vitamin D supplementation on asthma control in patients with vitamin D deficiency: the ACVID randomised clinical trial. *Thorax*. (2021) 76:126–33. doi: 10.1136/thoraxjnl-2019-213936

132. Nabavizadeh SH, Alyasin S, Esmaeilzadeh H, Mosavat F, Ebrahimi N. The effect of vitamin D add-on therapy on the improvement of quality of life and clinical symptoms of patients with chronic spontaneous urticaria. *Asian Pac J Allergy Immunol*. (2023) 41:150–7. doi: 10.12932/AP-021219-0705

133. Mohamed AA, Hussein MS, Salah EM, Eldemery A, Darwish MM, Ghaith DM, et al. Efficacy and safety of active vitamin D supplementation in chronic spontaneous urticaria patients. *J Dermatolog Treat*. (2022) 33:427–32. doi: 10.1080/09546634.2020.1762838

134. El-Heis S, D'Angelo S, Curtis EM, Healy E, Moon RJ, Crozier SR, et al. Maternal antenatal vitamin D supplementation and offspring risk of atopic eczema in the first 4 years of life: evidence from a randomized controlled trial. *Br J Dermatol*. (2022) 187:659–66. doi: 10.1111/bjd.21721

135. Aldaghi M, Tehrani H, Karrabi M, Abadi FS, Sahebkar M. The effect of multistrain synbiotic and vitamin D3 supplements on the severity of atopic dermatitis among infants under 1 year of age: a double-blind, randomized clinical trial study. *J Dermatolog Treat*. (2022) 33:812–7. doi: 10.1080/09546634.2020.1782319

136. Mansour NO, Mohamed AA, Hussein M, Eldemiry E, Daifalla A, Hassanin S, et al. The impact of vitamin D supplementation as an adjuvant therapy on clinical outcomes in patients with severe atopic dermatitis: A randomized controlled trial. *Pharmacol Res Perspect*. (2020) 8:e00679. doi: 10.1002/prp2.679

137. Cabalín C, Pérez-Mateluna G, Iturriaga C, Camargo CA, Borzutzky A. Oral vitamin D modulates the epidermal expression of the vitamin D receptor and cathelicidin in children with atopic dermatitis. *Arch Dermatol Res*. (2023) 315:761–70. doi: 10.1007/s00403-022-02416-1

138. Guo M. Vitamin D supplementation improves the therapeutic effect of mometasone on allergic rhinitis. *Acta Biochim Pol*. (2023) 70:583–9. doi: 10.18388/abp.2020_6637

139. Liu X, Liu X, Ren Y, Yang H, Sun X, Huang H. Clinical efficacy of vitamin D3 adjuvant therapy in allergic rhinitis: A randomized controlled trial. *Iran J Immunol*. (2020) 17:283–91. doi: 10.22034/iji.2020.84336.1652

140. Durham SR, Shamji MH. Allergen immunotherapy: past, present and future. *Nat Rev Immunol*. (2023) 23:317–28. doi: 10.1038/s41577-022-00786-1

141. Hesse L, van Ieperen N, Petersen AH, Elberink JNGO, van Oosterhout AJM, Nawijn MC. High dose vitamin D3 empowers effects of subcutaneous immunotherapy in a grass pollen-driven mouse model of asthma. *Sci Rep*. (2020) 10:20876. doi: 10.1038/s41598-020-77947-6

142. Hesse L, Petersen AH, Oude Elberink JNG, van Oosterhout AJM, Nawijn MC. 1,25(OH)2VitD3 supplementation enhances suppression of grass pollen-induced allergic asthma by subcutaneous and sublingual immunotherapy in a mouse model. *Sci Rep*. (2020) 10:8960. doi: 10.1038/s41598-020-65946-6

143. Li L, Cui X, Zhang X, Zheng L, Sun X, Yang C, et al. Serum vitamin D3 deficiency can affect the efficacy of sublingual immunotherapy in children with allergic rhinitis: a retrospective cohort study. *J Thorac Dis*. (2023) 15:649–57. doi: 10.21037/jtd-22-1883

144. Majak P, Jerzyńska J, Smejda K, Stelmach I, Timler D, Stelmach W. Correlation of vitamin D with Foxp3 induction and steroid-sparing effect of immunotherapy in asthmatic children. *Ann Allergy Asthma Immunol*. (2012) 109:329–35. doi: 10.1016/j.anai.2012.08.002

145. Jerzynska J, Stelmach W, Rychlik B, Lechańska J, Podlecka D, Stelmach I. The clinical effect of vitamin D supplementation combined with grass-specific sublingual immunotherapy in children with allergic rhinitis. *Allergy Asthma Proc*. (2016) 37:105–14. doi: 10.2500/aap.2016.37.3921

146. Baris S, Kiykim A, Ozen A, Tulunay A, Karakoc-Aydiner E, Barlan IB. Vitamin D as an adjunct to subcutaneous allergen immunotherapy in asthmatic children sensitized to house dust mite. *Allergy*. (2014) 69:246–53. doi: 10.1111/all.12278

147. Chiewchalermsri C, Sangkanjanavanich S, Pradubpongsa P, Mitthamsiri W, Jaisupa N, Jindarat S, et al. Randomized, double-blind, placebo-controlled trial of vitamin D supplementation in the build-up phase of house dust mite-specific immunotherapy. *Allergy Asthma Immunol Res*. (2023) 15:336–47. doi: 10.4168/aair.2023.15.3.336

# Glossary

| | |
|---|---|
| AD | Atopic Dermatitis |
| AR | Allergic Rhinitis |
| AA | Allergic Asthma |
| VDR | Vitamin D Receptor |
| DCs | Dendritic Cells |
| ILCs | Innate Lymphoid Cells |
| GM-CSF | Granulocyte-Macrophage Colony-Stimulating Factor |
| CXCR4 | C-X-C Motif Chemokine Receptor 4 |
| MBP | Major Basic Protein |
| EPX | Eosinophil Peroxidase |
| ECP | Eosinophil Cationic Protein |
| EDN | Eosinophil-Derived Neurotoxin |
| NETs | Neutrophil Extracellular Traps |
| AECOPD | Acute Exacerbation of Chronic Obstructive Pulmonary Disease |
| ADCC | Antibody-Dependent Cellular Cytotoxicity |
| RXR | Retinoid X Receptor |
| CRIg | Complement Receptor Immunoglobulin |
| LPS | Lipopolysaccharide |
| INF-α | Interferon-Alpha |
| TGF-β | Transforming Growth Factor Beta |
| MHC | Major Histocompatibility Complex |
| TNF-α | Tumor Necrosis Factor Alpha |
| IFN-γ | Interferon-Gamma |
| IL | Interleukin |
| CCL17 | C-C Chemokine Ligand 17 |
| FOXP3 | Forkhead Box P3 |
| Th1 | T Helper Cell 1 |
| Th2 | T Helper Cell 2 |
| Th17 | T Helper Cell 17 |
| Tregs | Regulatory T Cells |
| SCORAD | Scoring Atopic Dermatitis |
| JAK/STAT | Janus Kinase/Signal Transducer and Activator of Transcription |
| ICOS | Inducible T-Cell Costimulator |
| CCR6 | C-C Chemokine Receptor Type 6 |
| NF-κB | Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells |
| IgE | Immunoglobulin E |
| SCFAs | Short-Chain Fatty Acids |
| AHR | Airway Hyperresponsiveness |
| ERK1/2 | Extracellular Signal-Regulated Kinase 1/2 |
| PRMT1 | Protein Arginine Methyltransferase 1 |
| VEGF | vascular endothelial growth factor |
| MMP2 | matrix metallopeptidase 2 |
| ACT | Asthma Control Test |
| hs-CRP | High-Sensitivity C-Reactive Protein |
| EASI | Eczema Area and Severity Index |
| RQLQ | Rhinoconjunctivitis Quality of Life Questionnaire |
| DCD | Desloratadine Citrate Dihydrate |
| AIT | Allergen Immunotherapy |
| SCIT | Subcutaneous Immunotherapy |
| SLIT | Sublingual Immunotherapy |
| HDM | House Dust Mite |

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# Assessing causal association of circulating micronutrients and systemic lupus erythematosus susceptibility: a Mendelian randomization study

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**Background:** Previous studies showed the conflicting associations between circulating micronutrient levels and systemic lupus erythematosus (SLE). Therefore, we aimed to clarify the causal association between circulating micronutrient levels and the risk of SLE by two-sample Mendelian randomization (MR) analysis.

**Methods:** 56 single nucleotide polymorphisms (SNPs) significantly associated with 14 circulating micronutrients (vitamin A, B6, B9, B12, C, D and E, phosphorus, calcium, magnesium, copper, iron, zinc, and selenium) in published genome-wide association studies (GWAS) were used as instrumental variables (IVs). And summary statistics related to SLE were obtained from the IEU OpenGWAS database. We used the MR Steiger test to estimate the possible causal direction between circulating micronutrients and SLE. In the MR analysis, inverse variance weighting (IVW) method and the Wald ratio was as the main methods., Moreover, the MR-Pleiotropy residuals and outliers method (MR-PRESSO), Cochrane's Q-test, MR-Egger intercept method and leave-one-out analyses were applied as sensitivity analyses. Additionally, we conducted a retrospective analysis involving the 20,045 participants from the Third National Health and Nutritional Examination Survey (NHANES III). Weight variables were provided in the NHANES data files. Univariate and multivariate logistic regression analyses were performed to determine the associations between circulating micronutrients and SLE.

**Results:** The MR estimates obtained from the IVW method revealed potential negative correlations between circulating calcium (OR: 0.06, 95% CI: 0.01–0.49, $P$ = 0.009), iron levels (OR: 0.63, 95% CI: 0.43–0.92, $P$ = 0.016) and the risk of SLE. The results remained robust, even under various pairs of sensitivity analyses. Our retrospective analysis demonstrated that the levels of vitamin D, serum total calcium, and serum iron were significantly lower in SLE patients ($N$ = 40) when compared to the control group ($N$ = 20,005). Multivariate logistic regression

analysis further established that increased levels of vitamin D and serum total calcium served as protective factors against SLE.

**Conclusion:** Our results provided genetic evidence supporting the potential protective role of increasing circulating calcium in the risk of SLE. Maintaining adequate levels of calcium may help reduce the risk of SLE.

KEYWORDS

**circulating micronutrients, minerals, vitamins, systemic lupus erythematosus, Mendelian randomization**

# 1 Introduction

Systemic lupus erythematosus (SLE) is a chronic, debilitating, multi-system autoimmune disease characterized by wide-ranging clinical manifestations (1), with high morbidity and mortality (2). The global prevalence and fatality rates of SLE have been documented as 13-7713.5/100,000 and 0.01–2.71/100,000, respectively (3). Recently, dietary interventions in preventing autoimmune diseases have garnered increasing interest among researchers. Circulating micronutrients primarily obtained through dietary intake can notably influence physiological functions in the conditions of both overabundance and deficiency. Despite extensive research, the circulating micronutrients associated with SLE remain only partially understood.

Micronutrients are typically nutrients that cannot be synthesized by the body and generally consist of water-soluble vitamins, fat-soluble vitamins, trace elements, and trace minerals. In the past two decades, many studies have indicated that circulating micronutrients play a significant role in developing immunoinflammatory diseases, but the findings are still confusing (4–10). In numerous studies of patients with SLE, vitamin D deficiency was more common compared to those without SLE (11–13). However, two prolonged follow-up studies showed that vitamin D supplementation during adolescence had no preventive effect on the development of SLE in adulthood and adult women (9, 14). As an essential trace element, iron has been reported to be involved in a diversity of biological processes. Nevertheless, there are still limited findings on the role of iron in the pathogenesis of SLE. It was observed from two recent studies that sufficient iron status was inversely associated with the risk of developing SLE (15, 16). In contrast, a case report conducted by Oh VM illustrated that iron supplementation resulted in the manifestation of SLE-like symptoms in a woman of childbearing age suffering from iron deficiency anemia (17). Given that most of these studies are observational studies and prone to confounding factors and reverse causation. Therefore, a more detailed elucidation of the potential causal relationship and causal direction between circulating micronutrients and SLE is urgently necessary.

Mendelian randomization (MR) leverages genetic variations associated with exposure as unconfounded instrumental variables (IVs) to evaluate the causal relationship between exposure and outcome (17). This method limits both bias and reverse causality, which commonly occurs in observational epidemiological studies (18). In theory, MR operates on a similar principle to naturally occurring randomized controlled trials (RCTs) and serves as a pivotal approach to strengthening causal inference in situations where conducting RCTs is impractical or unethical (19). Given the multiple advantages of MR in inferring the causal relationship between exposure and outcome, our study utilized a two-sample MR analysis to investigate potential causal relationships between genetically predicted 14 circulating micronutrients (including vitamins and minerals) and the risk of SLE.

# 2 Materials and methods

## 2.1 Study design

This study adhered to the guidelines stipulated by the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian randomization (STROBE-MR) (20). The STROBE-MR checklist for the reporting of MR studies was showed in Supplementary Table 1. We utilized the two-sample MR method to investigate the potential causal relationships between circulating micronutrients and the risk of SLE. Given that our study harnessed data extracted from pre-existing published literature, it circumvented the need for further ethical approval or informed consent. The architecture of our study was based on the three core assumptions underpinning MR (Figure 1).

## 2.2 Selection of genetic instrumental variables

A systematic search of PubMed was conducted to identify observational studies published on circulating micronutrients in relation to SLE. This resulted in an initial list of such micronutrients, comprising vitamin A, vitamin B6, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E, sodium, phosphorus, calcium, magnesium, copper, iron, zinc, and selenium (4). Although several MR studies have evaluated the role of vitamin B9 (21), vitamin B12 (21), vitamin D (22), and iron status (16) in SLE, the Genome-Wide Association Study (GWAS) data for both exposures and outcomes used in our research exhibit slight variations. As a result, we have undertaken a replication of these analyses. Following this, we explored the GWAS catalog and PubMed for published GWAS centered on

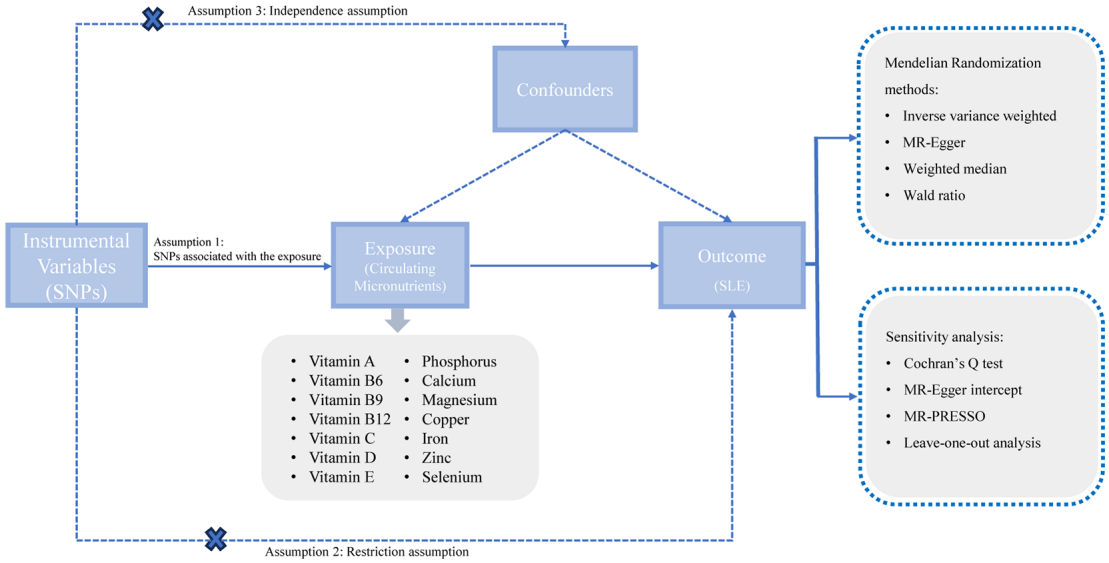


FIGURE 1
A schematic representation of our study design. SNPs, single nucleotide polymorphisms; SLE, systemic lupus erythematosus; MR, Mendelian randomization.

circulating micronutrients in European populations (the search was last updated in September 2023). Owing to the lack of relevant studies on sodium, it was excluded from our analysis. Ultimately, our research encompassed GWAS of 14 different circulating micronutrients: vitamin A (23), vitamin B6 (24), vitamin B9 (25), vitamin B12 (25), vitamin C (26), vitamin D (27), vitamin E (28), phosphorus (29), calcium (30), magnesium (31), copper (32), iron (33), zinc (32), and selenium (32). In this research, Single Nucleotide Polymorphisms (SNPs) linked to these 14 circulating micronutrients were designated as instrumental variables (IVs) adhering to the following standards: (1) The SNP demonstrates significant association with circulating micronutrient ($P < 5e\text{-}08$) and lacks linkage disequilibrium ($r^2 < 0.001$, KB = 10,000) (34); (2) The SNP with a minor allele frequency (MAF) of $\geq$ 5% (35); (3) The SNP showing no evidence of reverse causality, as determined by the Steiger filtering test (36); (4) In cases where the SNP is not found in the results dataset, a closely associated SNP ($r^2 > 0.8$) is chosen as a proxy in the 1000 Genomes database. If proxy SNP was unavailable, it was excluded from the analysis (37). (5) The chosen SNP is confirmed to be unassociated with confounding factors through inspection via the PhenoScanner database[1] ($P < 5e\text{-}08$,$r^2 = 0.8$) (38). Furthermore, we calculated the $R^2$ to denote the variance explained by the SNP and the F-statistic to signify potential weak IV bias in MR analysis. The $R^2$ was calculated as follows (39): $R^2 = 2 \times \text{Beta}^2 \times (1\text{-EAF}) \times \text{EAF/SD}^2$, and the F-statistic was calculated as (40): $F = (\text{Beta})^2/(\text{SE})^2$, where Beta is the per allele effect size of the association between each SNP and phenotype, EAF is the effect allele frequency, SE is the standard error, SD is the standard deviation. The IV is deemed strong when the F-statistic $\geq$ 10 (40). Ultimately, we identified 56 SNPs correlated with 14 circulating micronutrients, serving as IVs. The summary statistics of these SNPs utilized for MR analysis are presented in Table 1 and Supplementary Table 2.

## 2.3 SLE data source

The GWAS summary data (GCST90018917) for SLE were sourced from a recent large-scale GWAS in the IEU OpenGWAS database. This dataset comprises 647 cases of European ancestry (from Finland and the UK) and 482,264 control subjects of European ancestry. Then, cases of non-European ancestry (from Japan) have been excluded.

## 2.4 Statistical analysis

Following the harmonization of SNPs in both exposure and outcome using identical alleles, a two-sample MR analysis was conducted. When the MR estimate contained only one single SNP, the Wald ratio method was adopted as the primary analysis method (41); when the number of SNPs was $\geq$ 2, we employed the inverse variance weighted (IVW) method as the primary analysis method (42). When the number of SNPs was $\geq$ 3, the MR-Egger and Weighted median methods were applied for supplementary approaches to test the robustness of the primary analysis (43, 44). Furthermore, an MR analysis was conducted separately for each SNP associated with exposures. In addition, to ensure that the MR effects were oriented in the correct direction (from exposure to SLE), we conducted the MR Steiger test to confirm that each instrumental variable (IV) explained more variance in the exposure than in the outcome (36).

The degree of heterogeneity amongst the IVs was evaluated using Cochrane's Q test (45). When $P < 0.05$, it signifies the presence of heterogeneity. In cases of observed heterogeneity, the

1 http://www.phenoscanner.medschl.cam.ac.uk/(1-3)

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TABLE 1 Circulating micronutrient-associated SNPs used as instrumental variables in the Mendelian randomization analyses.

| Exposure | SNPs | EA | OA | EAF | Beta | SE | *P*-value | MR Steiger test (*P*-value) |
|---|---|---|---|---|---|---|---|---|
| **Vitamins** | | | | | | | | |
| Vitamin A | rs10882272 | C | T | 0.35 | −0.03 | 0.004 | 7.80E-12 | 9.65E-14 |
| | rs1667255 | C | A | 0.31 | 0.03 | 0.004 | 6.35E-14 | 4.04E-13 |
| Vitamin B6 | rs4654748 | C | T | 0.50 | −1.45 | 0.280 | 8.30E-18 | 3.17E-07 |
| vitamin B9 | rs1801133 | G | A | 0.67 | 0.11 | 0.008 | 6.65E-53 | 8.74E-43 |
| | rs652197 | C | T | 0.18 | 0.07 | 0.010 | 5.73E-13 | 2.96E-10 |
| | rs76630415 | G | T | 0.21 | −0.04 | 0.007 | 2.40E-08 | 1.04E-06 |
| Vitamin B12 | rs1131603 | C | T | 0.06 | 0.19 | 0.017 | 4.30E-28 | 1.55E-25 |
| | rs1141321 | C | T | 0.63 | 0.06 | 0.007 | 1.40E-16 | 2.17E-16 |
| | rs12272669 | A | G | 0.00 | 0.51 | 0.086 | 3.00E-09 | 2.48E-08 |
| | rs1801222 | G | A | 0.59 | 0.11 | 0.007 | 1.10E-52 | 2.37E-48 |
| | rs2270655 | G | C | 0.94 | 0.07 | 0.016 | 3.50E-05 | 1.30E-04 |
| | rs2336573 | T | C | 0.03 | 0.32 | 0.021 | 1.10E-51 | 1.69E-47 |
| | rs34324219 | C | A | 0.88 | 0.21 | 0.012 | 8.80E-71 | 6.86E-58 |
| | rs3742801 | T | C | 0.29 | 0.05 | 0.008 | 5.30E-08 | 1.73E-07 |
| | rs41281112 | C | T | 0.95 | 0.17 | 0.016 | 9.60E-27 | 5.76E-23 |
| | rs602662 | A | G | 0.60 | 0.16 | 0.008 | 4.10E-96 | 1.40E-79 |
| Vitamin C | rs10051765 | C | T | 0.34 | 0.04 | 0.007 | 3.64E-09 | 2.95E-07 |
| | rs10136000 | A | G | 0.28 | 0.04 | 0.007 | 1.33E-08 | 5.87E-08 |
| | rs117885456 | A | G | 0.09 | 0.08 | 0.012 | 1.70E-11 | 1.12E-09 |
| | rs13028225 | T | C | 0.86 | 0.10 | 0.009 | 2.38E-30 | 6.66E-27 |
| | rs174547 | C | T | 0.33 | 0.04 | 0.007 | 3.84E-08 | 1.06E-05 |
| | rs2559850 | A | G | 0.60 | 0.06 | 0.006 | 6.30E-20 | 6.69E-20 |
| | rs56738967 | C | G | 0.32 | 0.04 | 0.007 | 7.62E-10 | 8.98E-06 |
| | rs6693447 | T | G | 0.55 | 0.04 | 0.006 | 6.25E-10 | 1.56E-09 |
| | rs9895661 | T | C | 0.82 | 0.06 | 0.008 | 1.05E-14 | 5.65E-13 |
| Vitamin D | rs10741657 | A | G | 0.40 | 0.03 | 0.002 | 2.05E-46 | 4.88E-44 |
| | rs10745742 | T | C | 0.40 | 0.02 | 0.002 | 1.88E-14 | 4.17E-15 |
| | rs12785878 | T | G | 0.75 | 0.04 | 0.002 | 3.80E-62 | 6.05E-61 |
| | rs17216707 | T | C | 0.79 | 0.03 | 0.003 | 8.14E-23 | 3.73E-15 |
| | rs3755967 | T | C | 0.28 | −0.09 | 0.002 | 1.00E-200 | 0.00E+00 |
| | rs8018720 | C | G | 0.82 | −0.02 | 0.003 | 4.72E-09 | 3.86E-07 |
| Vitamin E | rs964184 | G | C | 0.21 | 0.04 | 0.010 | 7.80E-12 | 7.53E-05 |
| **Minerals** | | | | | | | | |
| Phosphorus | rs2970818 | T | A | 0.09 | 0.05 | 0.008 | 4.38E-09 | 8.73E-09 |
| | rs9469578 | C | T | 0.92 | 0.06 | 0.009 | 1.11E-11 | 2.34E-10 |
| | rs947583 | T | C | 0.29 | 0.04 | 0.005 | 3.45E-12 | 1.32E-10 |
| Calcium | rs10491003 | T | C | 0.09 | 0.03 | 0.005 | 1.60E-06 | 2.05E-06 |
| | rs1550532 | C | G | 0.31 | 0.02 | 0.003 | 4.60E-08 | 1.22E-08 |
| | rs1570669 | G | A | 0.66 | 0.02 | 0.003 | 4.00E-08 | 1.07E-07 |
| | rs7336933 | G | A | 0.15 | 0.02 | 0.004 | 1.60E-07 | 9.71E-08 |
| | rs7481584 | G | A | 0.30 | 0.02 | 0.003 | 9.20E-10 | 1.39E-10 |
| | rs780094 | T | C | 0.42 | 0.02 | 0.003 | 3.70E-11 | 9.17E-10 |

*(Continued)*

TABLE 1 (Continued)

| Exposure | SNPs | EA | OA | EAF | Beta | SE | *P*-value | MR Steiger test (*P*-value) |
|---|---|---|---|---|---|---|---|---|
| Magnesium | rs11144134 | C | T | 0.08 | 0.01 | 0.001 | 8.20E-15 | 5.76E-26 |
| | rs13146355 | A | G | 0.44 | 0.01 | 0.001 | 6.30E-13 | 2.53E-06 |
| | rs3925584 | T | C | 0.55 | 0.01 | 0.001 | 5.20E-16 | 1.23E-08 |
| | rs4072037 | T | C | 0.54 | 0.01 | 0.001 | 2.00E-36 | 8.49E-22 |
| | rs448378 | A | G | 0.53 | 0.00 | 0.001 | 1.25E-08 | 1.12E-04 |
| | [a]rs7965584 | A | G | 0.71 | 0.01 | 0.001 | 1.10E-16 | 4.56E-11 |
| Copper | rs1175550 | G | A | 0.23 | 0.20 | 0.032 | 5.03E-10 | 1.23E-09 |
| | rs2769264 | G | T | 0.19 | 0.31 | 0.034 | 2.63E-20 | 1.14E-19 |
| Iron | rs1800562 | A | G | 0.07 | 0.33 | 0.016 | 2.72E-97 | 1.89E-69 |
| | rs7385804 | T | G | 0.67 | −0.07 | 0.007 | 6.65E-20 | 7.52E-79 |
| | rs8177240 | T | G | 0.67 | −0.07 | 0.007 | 6.65E-20 | 8.91E-18 |
| | rs855791 | G | A | 0.55 | 0.18 | 0.007 | 1.32E-139 | 3.21E-18 |
| Zinc | rs1532423 | A | G | 0.43 | 0.18 | 0.026 | 9.00E-12 | 1.39E-11 |
| | rs2120019 | T | C | 0.81 | 0.29 | 0.033 | 1.50E-18 | 1.35E-17 |
| Selenium | rs921943 | T | C | 0.29 | 0.25 | 0.023 | 9.40E-28 | 1.05E-25 |

SNPs, single nucleotide polymorphisms; OA, other allele; EA, effect allele; SE, standard error. [a]rs7965584 was not available in the outcome dataset, and rs11105470 was found to replace it in the 1000 Genomes database.

random effects IVW method is deployed to ascertain the causal relationship between exposure and outcome, thereby mitigating bias from heterogeneous IVs. The MR-Egger intercept detected horizontal pleiotropy in the IVs, with $P < 0.05$ indicating its presence (44). The MR-Pleiotropy Residual Sum and Outlier method (MR-PRESSO) was employed to identify outlying SNPs and rerun the analysis after outlier removal (46). Finally, the leave-one-out analysis was implemented to ascertain the MR analysis's robustness and determine whether a specific SNP drove any association (47).

In this study, $P < 0.05$ was considered statistically significant. All analyses were performed using R software, with the "TwoSampleMR" and "MR-PRESSO" packages facilitating the two-sample MR analysis.

## 2.5 External validation in the NHANES III cohort

We utilized the NHANES III (1988–1994) data as the external validation dataset for this study. The NHANES III participants were restricted to adults aged 17 years and older. After excluding 5 participants with unknown SLE status, a total of 20,045 participants with the completed household interview and physical examination were included in the analysis. The participant's SLE status was determined by the item in the questionnaire: "Doctor ever told you had: lupus?" The other variables including age, gender, and race were also derived from the household interview data, while BMI was calculated using the formula: BMI = weight (kg)/[height (m)]$^2$. The serum levels of the 6 circulating micronutrients (vitamin A, vitamin C, vitamin D, serum calcium, iron, and selenium) were obtained from the laboratory examination data.

Considering the complex survey design, the weight variables were provided in the NHANES data files and *t*-tests, chi-square tests, and rank-sum tests were utilized to compare demographic disparities between the SLE group ($N = 40$) and the control group ($N = 20{,}005$). In the univariate regression analysis, we first constructed a preliminary rude model using only age, gender, race, and BMI. Then 6 circulating micronutrients were individually analyzed based on this rude model. Finally, variables with a significance level of $P < 0.10$ in above univariate regression analysis were included in the next multivariate regression analysis, which aimed to identify the most significant factors associated with SLE. And both the multivariate and univariate regression analyses were adjusted for the same covariates.

# 3 Results

## 3.1 The causal relationship of 14 circulating micronutrients on SLE in the European populations

The MR estimates obtained from the IVW method revealed potential negative correlations between circulating calcium (OR: 0.06, 95% CI: 0.01–0.49, $P = 0.009$), iron levels (OR: 0.63, 95% CI: 0.43–0.92, $P = 0.016$) and the risk of SLE (Figure 2). Concurrently, the weighted median method also derived similar results regarding the causal relationship between circulating iron level and the risk of SLE (OR: 0.60, 95% CI: 0.39–0.92, $P = 0.020$). The directional consistency of the causal relationship between circulating calcium level and the risk of SLE was maintained in the weighted median analysis (OR: 0.08, 95% CI: 0.00–1.18, $P = 0.066$) and IVW analysis, albeit without statistical significance (Supplementary Table 3). However, we did not observe significant correlations between

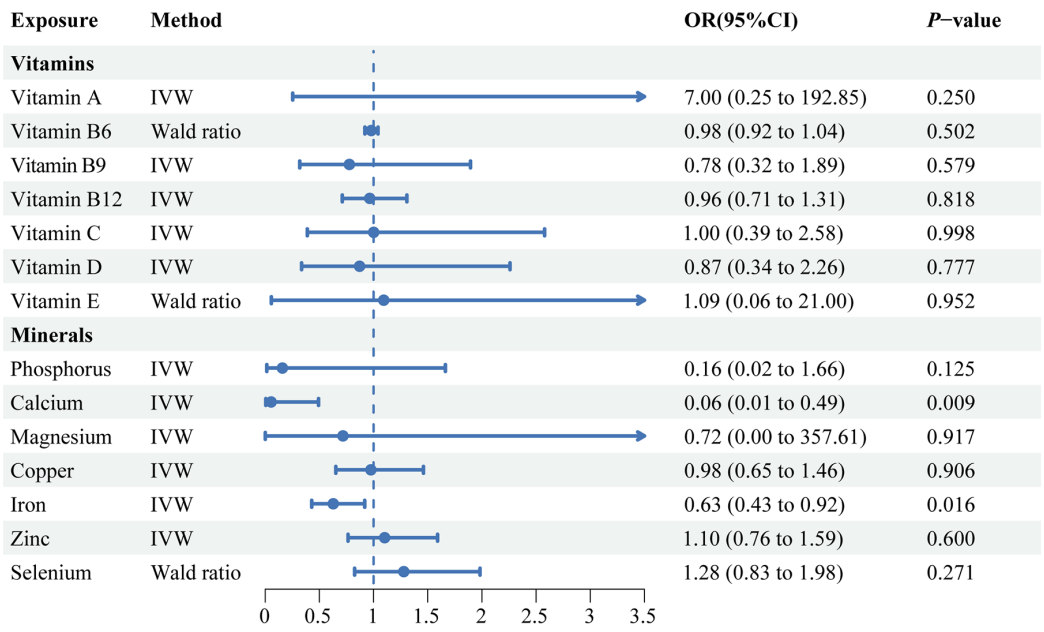


FIGURE 2
A forest plot showing the associations between genetically determined circulating micronutrients and systemic lupus erythematosus, based on Mendelian randomization analysis. IVW, inverse variance weighted; OR, odds ratio; CI, confidence interval.

vitamin A, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E, phosphorus, magnesium, copper, zinc, selenium and the risk of SLE, as detailed in Supplementary Table 3.

## 3.2 Sensitive analysis

As indicated in Supplementary Table 4, both Cochrane's Q test and the MR-Egger intercept suggest no heterogeneity and horizontal pleiotropy present in our MR analyses ($P > 0.05$). In the MR-PRESSO analyses, the rs1697421 ($P = 0.01$), rs17265703 ($P = 0.005$) and rs1801725 ($P = 0.005$) were identified as outliers. Then, no outlier SNPs were detected after removing and re-testing ($P > 0.05$) (Supplementary Table 4). When conducting the MR analyses using individual SNPs for either circulating calcium or iron levels, the results aligned with those obtained through the MR-PRESSO method (Supplementary Figure 1). The scatter plots, funnel plots, and leave-one-out plots all showed that the MR analysis results for the relationship between circulating calcium and iron levels with the risk of SLE remained robust, even under various pairs of sensitivity analyses (Supplementary Figures 2–4). Furthermore, as shown in Supplementary Table 5, the F-statistics for all 56 SNPs exceed 10, indicating no weak instrumental bias in our MR analyses.

## 3.3 Validation analysis in the NHANES III cohort

To further validate the findings in our MR analysis, we compared and analyzed the levels of circulating micronutrients in the serum of patients with SLE and those without SLE which sourced from a large cohort, known as the NHANES III cohort.

A total of 20,045 participants were ultimately included in this study. The demographic characteristics of the NHANES III participants by SLE status are presented in Supplementary Table 6. After applying appropriate weighting for the analysis, we observed that the mean age of the SLE group [52.06 (13.72)] was significantly higher than that of the control group [57.80 (13.92)] ($P < 0.01$). However, there were no significant differences observed in other demographic characteristics, including BMI, gender, and race. In the comparative analysis of 6 circulating micronutrients between the two groups, the SLE group exhibited significantly lower levels of vitamin D ($P < 0.01$), serum total calcium ($P = 0.01$), and serum iron levels ($P = 0.04$) (Supplementary Table 7). Consistent with the results of univariate Logistic regression analyses, multivariate Logistic regression analyses also found that vitamin D (OR: 0.98, 95% CI: 0.97–1.00, $P = 0.01$) and serum total calcium (OR: 0.03, 95% CI: 0.00–0.58, $P = 0.02$) had a protective effect against SLE (Supplementary Table 8).

# 4 Discussion

The precise etiology of SLE remains unclear. Recently, the potential of dietary interventions in preventing autoimmune diseases has garnered increasing interest among researchers. While there have been prior causal analyses involving single exposure, such as vitamin D, vitamin B, and iron status with SLE, to the best of our knowledge, this is the first comprehensive study to explore the causal associations between multiple circulating micronutrients and SLE. Our MR analyses showed the causal association between genetically predicted reductions in circulating calcium, iron and susceptibility to SLE in European populations. However, in the external validation analysis using the NHANES III cohort, only circulating calcium emerged as a protective factor for SLE.

In the present study, for the first time, we support a causal association between circulating calcium and SLE, and circulating calcium can serve as a potential protective factor against SLE. An earlier observational case-control study supported our results by finding a correlation between serum total calcium levels and activity of SLE (48). The researchers also observed that the serum calcium levels in SLE patients were significantly lower than those of healthy individuals (48). A retrospective analysis likewise discovered a significant reduction in the serum calcium levels of SLE patients when compared to those of healthy controls (49). Furthermore, a significant proportion of patients with SLE, as identified by numerous cross-sectional studies, exhibit insufficient levels of calcium intake, seldom reaching the recommended dietary allowance (15, 50). Calcium is an essential trace metal required for biological growth, and calcium signaling regulates many immune tolerance and inflammation pathways. Studies have found that the disruption of B-cell tolerance is a core key to the onset of SLE, and calcium signaling plays an important role in the development and fate of B cells (two key aspects of immune tolerance) through specific activation of transcription programs (51). Additionally, calcium signaling transmission can regulate the activation of the cGAS-STING axis, thus participating in innate immunity and autoimmune regulation through Type I interferon (52). Calcium exists in the blood in three forms (the ionic form, the form bound primarily to albumin, and the form bound to anions), with $Ca^{2+}$ being the physiologically active form of calcium. Nonetheless, contemporary clinical laboratory routines continue to measure overall serum calcium levels to represent the calcium status of the body. Thus, serum calcium may serve as a potential biomarker for the onset and progression of SLE.

In the MR analysis, an elevation in serum iron levels is associated with a decreased risk of SLE, the findings congruent with those derived from recent MR investigations (16). However, our validation analysis in the NHANES III cohort revealed that, after adjusting for demographic characteristics, there was no significant association between serum iron and SLE, as indicated by the univariate analysis using Logistic regression. In fact, the association between serum iron and SLE is not clear, with inconsistent conclusions reported. A recent substantial cohort study conducted in China revealed that the risk of developing SLE is notably higher in patients with iron deficiency anemia (53). Another case-control study conducted in Bangladesh also revealed similar results (54). However, in two additional small-scale case-control studies, no substantial difference was observed in the serum iron levels between patients with SLE and their control counterparts (55, 56). There are also indeed conflicting research findings regarding the association between serum iron and the mechanisms of inflammation induction. Prior studies have established that iron serves as a crucial micronutrient required for the proliferation of B cells and the production of antigen-antibodies (57). Iron homeostasis is critical in the incidence and progression of autoimmune inflammatory diseases (58). Research has demonstrated a substantial correlation between iron homeostasis and immune inflammation. Iron deficiency could potentially influence the expression of cytokines such as IL-6, IL-1, TNF-α, and IFN-γ, contributing to tissue damage (59). However, Wang et al. discovered that an overabundance of iron could stimulate the generation of pro-inflammatory cytokines via poly(rC)-binding protein 1 (Pcbp1), consequently leading to the direct induction of autoimmune diseases (60). Therefore, further investigation through large-scale experimental epidemiological studies is needed to explore the association between serum iron and SLE.

The strengths of this study are as follows: First, we built the causal relationship between multiple circulating micronutrients and the risk of SLE in European populations and validate in the NHANES III cohort. This comprehensive analysis can provide a more global understanding of them. Second, the exposure and outcome of our study come from different regions of the same lineage, the overlap of samples is relatively light, and the bias of population stratification is small. Finally, we excluded SNPs that may have a reverse causality and overcame the limitations of observational studies (confounding factors, recall bias).

There are also some limitations in this study. First, although multiple MR methods were used to prevent confounding caused by pleiotropy, residual bias cannot be eliminated. We cannot be sure that the SNPs chosen concerning circulating micronutrients will not affect SLE-related outcomes through other causal pathways. Second, there are significant gender differences in SLE, but we cannot stratify the outcome data due to the lack of individual-level data in the summary statistics.

In conclusion, by a two-sample MR analysis and an external validation analysis, our results provided genetic evidence supporting the potential protective role of circulating calcium levels in the risk of SLE. Our findings will provide a crucial scientific basis for dietary intervention in the development and progression of SLE.

## 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

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

## Author contributions

SH: Data curation, Formal analysis, Methodology, Writing – original draft. XW: Data curation, Formal analysis, Methodology, Writing – original draft. FQ: Data curation, Formal analysis, Methodology, Writing – original draft. ZY: Resources, Visualization, Writing – review & editing. CM: Visualization, Writing – review & editing. YK: Validation, Writing – review & editing. CH: Conceptualization, Project administration, Writing – review & editing. JJ: Conceptualization, Project administration, Resources, Writing – review & editing. LY: Conceptualization, Project administration, Resources, 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/fnut.2024.1359697/full#supplementary-material

## References

1. Wincup C, Sawford N, Rahman A. Pathological mechanisms of abnormal iron metabolism and mitochondrial dysfunction in systemic lupus erythematosus. *Expert Rev Clin Immunol.* (2021) 17:957–67.

2. Lazar S, Kahlenberg J. Systemic lupus erythematosus: New diagnostic and therapeutic approaches. *Annu Rev Med.* (2023) 74:339–52.

3. Barber M, Drenkard C, Falasinnu T, Hoi A, Mak A, Kow N, et al. Global epidemiology of systemic lupus erythematosus. *Nat Rev Rheumatol.* (2021) 17:515–32.

4. Islam M, Khandker S, Kotyla P, Hassan R. Immunomodulatory effects of diet and nutrients in systemic lupus erythematosus (SLE): A systematic review. *Front Immunol.* (2020) 11:1147. doi: 10.3389/fimmu.2020.01477

5. Minami Y, Hirabayashi Y, Nagata C, Ishii T, Harigae H, Sasaki T. Intakes of vitamin B6 and dietary fiber and clinical course of systemic lupus erythematosus: A prospective study of Japanese female patients. *J Epidemiol.* (2011) 21:246–54. doi: 10.2188/jea.je20100157

6. Minami Y, Sasaki T, Arai Y, Kurisu Y, Hisamichi S. Diet and systemic lupus erythematosus: A 4 year prospective study of Japanese patients. *J Rheumatol.* (2003) 30:747–54.

7. Ritterhouse L, Crowe S, Niewold T, Kamen D, Macwana S, Roberts V, et al. Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. *Ann Rheum Dis.* (2011) 70:1569–74. doi: 10.1136/ard.2010.148494

8. Comstock G, Burke A, Hoffman S, Helzlsouer K, Bendich A, Masi A, et al. Serum concentrations of α tocopherol, β carotene, and retinol preceding the diagnosis of rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis.* (1997) 56:323–5.

9. Costenbader K, Feskanich D, Holmes M, Karlson E, Benito-Garcia E. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. *Ann Rheum Dis.* (2008) 67:530–5.

10. Athanassiou L, Kostoglou-Athanassiou I, Koutsilieris M, Shoenfeld Y. Vitamin D and autoimmune rheumatic diseases. *Biomolecules.* (2023) 13:709.

11. Wu P, Rhew E, Dyer A, Dunlop D, Langman C, Price H, et al. 25-Hydroxyvitamin D and cardiovascular risk factors in women with systemic lupus erythematosus. *Arthritis Rheum.* (2009) 61:1387–95.

12. Sabio J, Vargas-Hitos J, Martinez-Bordonado J, Navarrete-Navarrete N, Díaz-Chamorro A, Olvera-Porcel C, et al. Association between low 25-hydroxyvitamin D, insulin resistance and arterial stiffness in nondiabetic women with systemic lupus erythematosus. *Lupus.* (2015) 24:155–63. doi: 10.1177/0961203314551811

13. Sakthiswary R, Raymond A. The clinical significance of vitamin d in systemic lupus erythematosus: A systematic review. *PLoS One.* (2013) 8:e55275.

14. Hiraki L, Munger K, Costenbader K, Karlson E. Dietary intake of vitamin D during adolescence and risk of adult-onset systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Care Res.* (2012) 64:1829–36. doi: 10.1002/acr.21776

15. Meza-Meza M, Vizmanos-Lamotte B, Muñoz-Valle J, Parra-Rojas I, Garaulet M, Campos-López B, et al. Relationship of excess weight with clinical activity and dietary intake deficiencies in systemic lupus erythematosus patients. *Nutrients.* (2019) 11:2683.

16. Ye D, Zhu Z, Huang H, Sun X, Liu B, Xu X, et al. Genetically predicted serum iron status is associated with altered risk of systemic lupus erythematosus among european populations. *J Nutr.* (2021) 151:1473–8. doi: 10.1093/jn/nxab015

17. Kember R, Levin M, Cousminer D, Tsao N, Judy R, Schur G, et al. Genetically determined birthweight associates with atrial fibrillation. *Circulation.* (2020) 13:e002553.

18. Afshar M, Luk K, Do R, Dufresne L, Owens D, Harris T, et al. Association of triglyceride-related genetic variants with mitral annular calcification. *J Am Coll Cardiol.* (2017) 69:2941–8. doi: 10.1016/j.jacc.2017.04.051

19. Larsson S, Burgess S, Michaëlsson K. Genetic association between adiposity and gout: A Mendelian randomization study. *Rheumatology (Oxford).* (2018) 57:2145–8. doi: 10.1093/rheumatology/key229

20. Skrivankova V, Richmond R, Woolf B, Yarmolinsky J, Davies N, Swanson S, et al. Strengthening the reporting of observational studies in epidemiology using mendelian randomization: The STROBE-MR statement. *JAMA.* (2021) 326:1614–21. doi: 10.1001/jama.2021.18236

21. Yang H, Song J, Li A, Lv L, Sun X, Mao Y, et al. Genetically predicted levels of folate, vitamin B12, and risk of autoimmune diseases: A Mendelian randomization study. *Front Immunol.* (2023) 14:1139799. doi: 10.3389/fimmu.2023.1139799

22. Zhao S, Mason A, Gjekmarkaj E, Yanaoka H, Burgess S. Associations between vitamin D and autoimmune diseases: Mendelian randomization analysis. *Semin Arthritis Rheum.* (2023) 62:152238.

23. Mondul A, Yu K, Wheeler W, Zhang H, Weinstein S, Major J, et al. Genome-wide association study of circulating retinol levels. *Hum Mol Genet.* (2011) 20:4724–31.

24. Tanaka T, Scheet P, Giusti B, Bandinelli S, Piras M, Usala G, et al. Genome-wide association study of vitamin B6, Vitamin B12, folate, and homocysteine blood concentrations. *Am J Hum Genet.* (2009) 84:477–82. doi: 10.1016/j.ajhg.2009.02.011

25. Grarup N, Sulem P, Sandholt C, Thorleifsson G, Ahluwalia T, Steinthorsdottir V, et al. Genetic architecture of vitamin B12 and folate levels uncovered applying deeply

sequenced large datasets. *PLoS Genet.* (2013) 9:e1003530. doi: 10.1371/journal.pgen.1003530

26. Zheng J, Luan J, Sofianopoulou E, Imamura F, Stewart I, Day F, et al. Plasma vitamin C and type 2 diabetes: Genome-wide association study and Mendelian randomization analysis in European populations. *Diabetes Care.* (2020) 44:98–106. doi: 10.2337/dc20-1328

27. Jiang X, O'Reilly P, Aschard H, Hsu Y, Richards J, Dupuis J, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nat Commun.* (2018) 9:260. doi: 10.1038/s41467-017-02662-2

28. Major J, Yu K, Wheeler W, Zhang H, Cornelis M, Wright M, et al. Genome-wide association study identifies common variants associated with circulating vitamin E levels. *Hum Mol Genet.* (2011) 20:3876–83.

29. Kestenbaum B, Glazer N, Köttgen A, Felix J, Hwang S, Liu Y, et al. Common genetic variants associate with serum phosphorus concentration. *J Am Soc Nephrol.* (2010) 21:1223–32. doi: 10.1681/ASN.2009111104

30. O'Seaghdha C, Wu H, Yang Q, Kapur K, Guessous I, Zuber A, et al. Meta-analysis of genome-wide association studies identifies six new loci for serum calcium concentrations. *PLoS Genet.* (2013) 9:e1003796. doi: 10.1371/journal.pgen.1003796

31. Meyer T, Verwoert G, Hwang S, Glazer N, Smith A, Rooij F, et al. Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six loci influencing serum magnesium levels. *PLoS Genet.* (2010) 6:e1001045. doi: 10.1371/journal.pgen.1001045

32. Evans D, Zhu G, Dy V, Heath A, Madden P, Kemp J, et al. Genome-wide association study identifies loci affecting blood copper, selenium and zinc. *Hum Mol Genet.* (2013) 22:3998–4006. doi: 10.1093/hmg/ddt239

33. Benyamin B, Esko T, Ried J, Radhakrishnan A, Vermeulen S, Traglia M, et al. Novel loci affecting iron homeostasis and their effects in individuals at risk for hemochromatosis. *Nat Commun.* (2014) 5:4926.

34. Durbin R, Altshuler D, Durbin R, Abecasis G, Bentley D, Chakravarti A, et al. A map of human genome variation from population-scale sequencing. *Nature.* (2010) 467:1061–73.

35. Daniel N, Bouras E, Tsilidis K, Hughes D. Genetically predicted circulating concentrations of micronutrients and COVID-19 susceptibility and severity: A Mendelian randomization study. *Front Nutr.* (2022) 9:842315. doi: 10.3389/fnut.2022.842315

36. Hemani G, Tilling K, Smith G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet.* (2017) 13:e1007081.

37. Richard M, Huan T, Ligthart S, Gondalia R, Jhun M, Brody J, et al. DNA methylation analysis identifies loci for blood pressure regulation. *Am J Hum Genet.* (2017) 101:888–902.

38. Kamat M, Blackshaw J, Young R, Surendran P, Burgess S, Danesh J, et al. PhenoScanner V2: An expanded tool for searching human genotype–phenotype associations. *Bioinformatics.* (2019) 35:4851–3. doi: 10.1093/bioinformatics/btz469

39. Liu B, Ye D, Yang H, Song J, Sun X, Mao Y, et al. Two-sample Mendelian randomization analysis investigates causal associations between gut microbial genera and inflammatory bowel disease, and specificity causal associations in ulcerative colitis or Crohn's disease. *Front Immunol.* (2022) 13:921546. doi: 10.3389/fimmu.2022.921546

40. Huang W, Xiao J, Ji J, Chen L. Association of lipid-lowering drugs with COVID-19 outcomes from a Mendelian randomization study. *Elife.* (2021) 10:e73873. doi: 10.7554/eLife.73873

41. Saunders C, Cornish A, Kinnersley B, Law P, Claus E, Il'yasova D, et al. Lack of association between modifiable exposures and glioma risk: A Mendelian randomization analysis. *Neuro Oncol.* (2020) 22:207–15. doi: 10.1093/neuonc/noz209

42. Xu F, Fu Y, Sun T, Jiang Z, Miao Z, Shuai M, et al. The interplay between host genetics and the gut microbiome reveals common and distinct microbiome features for complex human diseases. *Microbiome.* (2020) 8:145. doi: 10.1186/s40168-020-00923-9

43. Bowden J, Davey Smith G, Haycock P, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14.

44. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: Effect estimation and bias detection through Egger regression. *Int J Epidemiol.* (2015) 44:512–25.

45. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. *Stat Med.* (2017) 36:1783–802.

46. Verbanck M, Chen C, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8.

47. Hong H, Chen L, Zhong Y, Yang Z, Li W, Song C, et al. Associations of homocysteine, folate, and vitamin B12 with osteoarthritis: A Mendelian randomization study. *Nutrients.* (2023) 15:1636.

48. Sha Y, Rui Z, Dong Y, Wei X, Zhou Y, Ye X, et al. Total serum calcium level is negatively correlated with systemic lupus erythematosus activity. *Dose Resp.* (2020) 18:1559325820926764.

49. Du X, Zhao D, Wang Y, Sun Z, Yu Q, Jiang H, et al. Low serum calcium concentration in patients with systemic lupus erythematosus accompanied by the enhanced peripheral cellular immunity. *Front Immunol.* (2022) 13:901854. doi: 10.3389/fimmu.2022.901854

50. Pocovi-Gerardino G, Correa-Rodríguez M, Callejas-Rubio J, Ríos-Fernández R, Ortego-Centeno N, Rueda-Medina B. Dietary intake and nutritional status in patients with systemic lupus erythematosus. *Endocrinol Diabetes Nutr.* (2018) 65:533–9.

51. Hemon P, Renaudineau Y, Debant M, Le Goux N, Mukherjee S, Brooks W, et al. Calcium signaling: From normal B cell development to tolerance breakdown and autoimmunity. *Clin Rev Allerg Immunol.* (2017) 53:141–65.

52. Mathavarajah S, Salsman J, Dellaire G. An emerging role for calcium signalling in innate and autoimmunity via the cGAS-STING axis. *Cytokine Growth Factor Rev.* (2019) 50:43–51. doi: 10.1016/j.cytogfr.2019.04.003

53. Chang R, Chu K, Lin M, Chu Y, Hung Y, Wei J. Newly diagnosed iron deficiency anemia and subsequent autoimmune disease: A matched cohort study in Taiwan. *Curr Med Res Opin.* (2020) 36:985–92. doi: 10.1080/03007995.2020.1748585

54. Jahan I, Das D, Hussain MS, Hossain MM, Chowdhury MAZ, Fardous Z, et al. Alterations of serum trace elements and other biochemical parameters are correlated with the pathogenesis of systemic lupus erythematosus: A preliminary study on Bangladeshi population. *J Trace Elem Med Biol.* (2021) 68:126861. doi: 10.1016/j.jtemb.2021.126861

55. Tóth C, Baranyai E, Csípő I, Tarr T, Zeher M, Posta J, et al. Elemental analysis of whole and protein separated blood serum of patients with systemic lupus erythematosus and Sjögren's syndrome. *Biol Trace Elem Res.* (2017) 179:14–22. doi: 10.1007/s12011-017-0945-y

56. Yilmaz A, Sari R, Gundogdu M, Kose N, Dag E. Trace elements and some extracellular antioxidant proteins levels in serum of patients with systemic lupus erythematosus. *Clin Rheumatol.* (2005) 24:331–5. doi: 10.1007/s10067-004-1028-y

57. Jiang Y, Li C, Wu Q, An P, Huang L, Wang J, et al. Iron-dependent histone 3 lysine 9 demethylation controls B cell proliferation and humoral immune responses. *Nat Commun.* (2019) 10:2935. doi: 10.1038/s41467-019-11002-5

58. Baker J, Ghio A. Iron homoeostasis in rheumatic disease. *Rheumatology.* (2009) 48:1339–44.

59. Velo-García A, Castro S, Isenberg D. The diagnosis and management of the haematologic manifestations of lupus. *J Autoimmun.* (2016) 74:139–60. doi: 10.1016/j.jaut.2016.07.001

60. Wang Z, Yin W, Zhu L, Li J, Yao Y, Chen F, et al. Iron drives T helper cell pathogenicity by promoting RNA-binding protein PCBP1-mediated proinflammatory cytokine production. *Immunity.* (2018) 49:80–92.e7. doi: 10.1016/j.immuni.2018.05.008

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# A cohort study of serum 25-hydroxyvitamin D levels and the risk of hyperlipidaemia in adults

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**Objective:** This study aims to investigate the potential association between serum 25(OH)D levels and the risk of hyperlipidemia in adults through a prospective cohort study conducted in Zhejiang Province.

**Methods:** Baseline surveys and follow-up studies were conducted to collect and analyze follow-up data over a three-year period. Vitamin D deficiency was defined as 25(OH)D < 20 ng/mL, insufficiency as 20–29 ng/mL, and sufficiency as 25(OH)D ≥ 30 ng/mL. Hyperlipidemia or dyslipidemia was defined as the presence of hypercholesterolemia, hypertriglyceridemia, or both. The relationship between demographic characteristics and the incidence of hyperlipidemia among the study participants was explored.

**Results:** A total of 1,210 participants were included in this study, with 43.80% being male. The mean age of the participants was 51.84 ± 14.37 years, and the average serum 25(OH)D level was 25.89 (21.50, 29.82) ng/mL. A significant difference in the proportion of vitamin D deficiency was observed between males and females (22.06% vs. 10.94%, $p < 0.001$). Vitamin D deficiency and insufficiency were prevalent among the middle-aged and elderly population (78.24%). Significant differences were found between the two groups in multiple sociodemographic variables, behavioral factors, and metabolic risk factors ($p < 0.05$). The incidence of hyperlipidemia among vitamin D-deficient individuals was 1.612 times higher than that among vitamin D-sufficient individuals (95% confidence interval [CI]: 1.228–2.116; $p < 0.001$). After fully adjusting for confounding factors, the multivariate-adjusted hazard ratio (HR) was 1.572 (95% CI: 1.187–2.08; $p = 0.002$), indicating a difference in the incidence of hyperlipidemia across different serum vitamin D levels.

**Conclusion:** This cohort study reveals a significant association between serum 25(OH)D levels and the incidence of hyperlipidemia. Additionally, lifestyle factors associated with vitamin D deficiency are also correlated with the incidence of hyperlipidemia. These findings provide further evidence for improving blood lipid profiles through adjustments in vitamin D intake or related lifestyle modifications.

KEYWORDS

**vitamin D, hyperlipidaemia, cohort study, 25(OH)D, adults**

# 1 Introduction

Hyperlipidemia, as a chronic metabolic disorder characterized by abnormal lipid metabolism, exhibits a complex pathogenesis involving interactions between genetic and environmental factors (1, 2). Adverse dietary patterns, particularly excessive intake of high-fat and high-calorie foods and nutritional imbalances, coupled with lifestyle changes such as lack of regular exercise and irregular sleep patterns, along with specific medical interventions like drug side effects, collectively contribute to the development of hyperlipidemia (3, 4). This condition has emerged as a global public health challenge, not only severely impacting individuals' quality of life but also significantly increasing the risk of severe complications such as cardiovascular disease, diabetes, and metabolic syndrome, thereby imposing a substantial burden on national healthcare systems (5). In recent years, with shifts in lifestyle, the incidence of hyperlipidemia has risen markedly (6). Data indicates that the prevalence of hypercholesterolemia among adults in the Americas and Europe is as high as 47.7 and 53.7%, respectively (7), while the prevalence of dyslipidemia among adults aged 65 and older in the United States has reached 60.3% (8). A recent meta-analysis from Malaysia also revealed high prevalence rates for various subtypes of dyslipidemia, emphasizing the global ubiquity of hyperlipidemia and the urgency for in-depth research (9).

Vitamin D (calciferol), a fat-soluble steroid hormone, primarily exists in the forms of ergocalciferol (Vitamin $D_2$), derived from plant-based foods, and cholecalciferol (Vitamin $D_3$), sourced from animal-based foods and endogenously synthesized in the epidermis under UVB light (10). In circulation, vitamin D binds primarily to vitamin D-binding protein (VDBP) and is transported to the liver for the initial hydroxylation to produce the main circulating metabolite, 25(OH)D (11). 7-dehydrocholesterol is converted into the vitamin D3 precursor by microsomal cytochrome P450 (12). Subsequently, 25(OH)D is transported to the kidneys or other extrarenal tissues (VAN ETTEN and MATHIEU (13)) for further hydroxylation into the biologically active form, 1,25-dihydroxyvitamin D [1,25(OH)2D] (14). Vitamin D is not only crucial for bone health (15), but also participates in regulating immune responses, cell proliferation and differentiation, and cardiovascular physiology among various biological processes (14). Recently, the association between vitamin D deficiency and an increased risk of metabolic, immune, and various chronic diseases has garnered considerable attention (16, 17).

When exploring the complex relationship between vitamin D levels and lipid metabolism, existing studies provide mixed evidence. Some studies have found that lower concentrations of 25(OH)D correlate with higher total and abdominal fat levels (18), and vitamin D deficiency is associated with elevated triglyceride (TG) concentrations in obese adults (19). A potential correlation between vitamin D deficiency and obesity may arise because adipose tissue can sequester vitamin D or because obese individuals may be less inclined to expose themselves to sunlight, thereby reducing skin synthesis of vitamin D. Numerous studies have pointed out the association between vitamin D deficiency and overweight, which further exacerbates the risk of lipid metabolism disorders (20, 21). Additionally, a potential correlation between vitamin D deficiency and increased total cholesterol (TC) levels and decreased low-density lipoprotein cholesterol (LDL-C) levels has been observed (22, 23). However, other studies have reported different trends, such as Ponda et al. (24) finding that patients with optimal 25(OH)D concentrations had lower LDL-C and TG concentrations but higher high-density lipoprotein cholesterol (HDL-C) concentrations. These discrepancies may stem from differences in study design, sample selection, and adjustment for confounding factors (25, 26).

This study aims to conduct a well-designed cohort study to delve into the potential association between serum 25(OH)D levels and the risk of hyperlipidemia in adults. By systematically analyzing the impact of different vitamin D concentrations on lipid metabolism patterns in hyperlipidemia patients, we hope to provide scientific support for the prevention and treatment of hyperlipidemia (27, 28). Although the specific mechanisms by which vitamin D regulates blood lipids are not fully understood, its positive effects are widely recognized (29, 30). Given the wide availability, cost-effectiveness, and safety of vitamin D, its potential as a blood lipid-regulating supplement is noteworthy (31). However, more in-depth research is needed regarding optimal dosage and intervention timing. This study employs a prospective cohort design to assess the relationship between serum vitamin D concentrations and the incidence of hyperlipidemia in the general population, aiming to validate and deepen the knowledge in this field, open new perspectives for the prevention and treatment of hyperlipidemia, and provide a scientific basis for future clinical practice.

# 2 Materials and methods

## 2.1 Study population

A multi-stage sampling method was employed for sample recruitment. Two communities/administrative villages were selected from 16 streets/towns in the Lishui area of southeastern Zhejiang Province using random integer sampling. Then, the corresponding number of participants was randomly selected from each community. The specific sampling approach was as follows. In the first step, cluster sampling was employed, with all communities/administrative villages within each street/township being assigned numerical labels. Two communities/administrative villages were selected through a random drawing process. In the second step, systematic sampling was utilized to select survey participants from the chosen communities/administrative villages. This involved sorting households by house number and using a random number generator to select the first household. Subsequent sample households were determined at intervals based on the total number of households in the community and the desired sample size. All household members over 18 years of age in the sampled households were selected as survey subjects. The sample of the study included 3,828 adult permanent residents.

From this larger sample, subjects were selected to participate in the current study. Subjects for the current study were selected based on the following inclusion criteria: (1) no hyperlipidaemia: serum TC < 6.2 mmol/L (240 mg/dL), serum TG < 2.3 mmol/L (200 mg/dL), serum LDL-C < 4.1 mmol/L (160 mg/dL) and serum HDL-C ≥ 1.0 mmol/L (40 mg/dL) (32), (2) no malignant diseases, chronic liver disease or chronic kidney disease; (3) no hyperlipidaemia drug treatment; (4) no vitamin D supplementation. At the end of the follow-up period, a total of 1,210 subjects were included in the statistical analysis. This study was designed as a cohort study, including baseline measurements and follow-up measurements over a

three-year period. The data included survey data, anthropometry data and laboratory testing. All subjects provided written informed consent and the study was performed in strict accordance with the Helsinki Declaration. This topic has been approved by the Ethics Committee of Soochow University (No. ECSU-20160001).

## 2.2 Instruments and equipment

After fasting for 12 h, 5 mL of peripheral venous blood was collected from each subject. The blood was centrifuged at 3,000 r/min for 10 min, and the upper serum was taken to detect related indicators. Fasting blood glucose levels were measured by the hexokinase method using a fully automatic biochemical analyzer (COBAS c702, Roche Diagnostics, Mannheim, Germany). The levels of TG, TC, HDL-C and LDL-C were measured by the enzymatic method using an automatic biochemical analyzer (COBAS e601, Roche Diagnostics Co., Ltd., Mannheim, Germany). Serum 25(OH)D concentrations were determined by chemiluminescence immunoassays using an automatic chemiluminescence immunoassay analyzer (ADVIA Centaur XP, Siemens Medical Diagnostics, New York, United States). The intra-and inter-assay coefficients of variation for this method were <5%. These analyses were completed by Hangzhou Dean Medical Laboratory Centre Co., Ltd.

## 2.3 Baseline data collection

Standard questionnaires were used to collect demographic data (age, gender, area of residence, etc.), socioeconomic status (education level), smoking habits (current smoking, smoking cessation, or never smoking), drinking status (current drinking, smoking cessation, or never drinking), physical activity, family income, marital status, cognitive attitude toward chronic diseases, family history of hypertension and family history of diabetes. The education level is divided into four groups: no school, primary school, junior high school, college and above. This study used the World Health Organization's physical activity recommendations (at least 150 min of moderate activity per week or the same amount of exercise) as a reference value for regular exercise. Family history of hypertension was defined as any first-degree relative (mother, father, sister, or brother) diagnosed with hypertension, and family history of diabetes was defined as any first-degree relative (mother, father, sister, or brother) diagnosed with diabetes.

## 2.4 Measurement of physical parameters

Physical examinations were performed in the early morning on an empty stomach and included measurements of height, weight, waist circumference and blood pressure. Height was measured by a height meter with a length of 2.0 m and an accuracy of 0.1 cm. Body weight is measured by a weight meter with a maximum weight of 150 kg and an accuracy of 0.1 kg. Blood pressure was measured using a standard mercury sphygmomanometer. Blood pressure was measured in millimeters of mercury, with an accuracy of up to 1 mm. After the subjects rested for 15 min, their blood pressure was measured three times in a relaxed seated position. There was a 5-min break between each measurement. The average of the three measurements was used for analysis. Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg and/or taking antihypertensive drugs and/or diagnosed with hypertension by a medical unit at or above the county level (33). Body mass index (BMI) was calculated as weight divided by the square of height (BMI = kg/m$^2$). BMI $\geq$ 28 kg/m$^2$ was defined as obese, 24 kg/m$^2$ $\leq$ BMI < 28 kg/m$^2$ as overweight and 18.5 kg/m$^2$ $\leq$ BMI < 24 kg/m$^2$ as normal (34).

## 2.5 Follow-ups and study outcome

All subjects were followed up by strictly trained community physicians. Data on relevant hyperlipidaemia events during the follow-up period were collected. The occurrence of hyperlipidaemia was the primary endpoint of this study. New-onset hyperlipidaemia was defined as a self-reported history of physician diagnosis during follow-up and/or receiving medication for hyperlipidaemia and/or serum TC $\geq$ 6.2 mmol/L (240 mg/dL) and/or serum TG $\geq$ 2.3 mmol/L (200 mg/dL) and/or serum LDL-C $\geq$ 4.1 mmol/L (160 mg/dL) and/or serum HDL-C < 1.0 mmol/L (40 mg/dL) (35).

## 2.6 Index division standards

(1) According to the Chinese Guidelines for the Prevention of Dyslipidaemia in Adults (2016 Edition) (32), serum TC $\geq$ 6.2 mmol/L (240 mg/dL) was defined as hypercholesterolemia, serum TG $\geq$ 2.3 mmol/L (200 mg/dL) was defined as hypertriglyceridemia, serum LDL-C $\geq$ 4.1 mmol/L (160 mg/dL) was defined as LDL-hypercholesterolemia, and serum HDL-C < 1.0 mmol/L (40 mg/dL) was defined as HDL-hypocholesterolemia. Hypercholesterolemia, hypertriglyceridemia, LDL-hypercholesterolemia or HDL-hypocholesterolemia were defined as hyperlipidaemia or dyslipidaemia.

(2) According to the diagnostic criteria of hypertension of the National Health Commission: (1) In the absence of antihypertensive drugs, the clinic blood pressure was measured three times on different days, SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg. Simple systolic hypertension was defined as SBP $\geq$ 140 mmHg and DBP < 90 mmHg; (2) The patient has a history of hypertension and is currently using antihypertensive drugs. Although the blood pressure is lower than 140/90 mmHg, it should still be diagnosed as hypertension (33).

(3) According to the Endocrine Society's Clinical Practice Guidelines, serum 25(OH)D deficiency was defined as 25(OH)D < 20 ng/mL, insufficiency as (20–29 ng/mL), and sufficiency was defined as 25(OH)D $\geq$ 30 ng/mL.

## 2.7 Quality control

To ensure standardization of the project and obtain high-quality survey data, before the implementation of the survey, the community doctors, public health doctors, nursing staff and questionnaire survey personnel were uniformly trained. After the implementation of the project, on-site quality control was carried out, and the quality of the

survey data, physical measurements, blood sample collection and laboratory testing were dynamically monitored in real time. When a quality problem was found, timely feedback and correction were performed to prevent the diffusion of the problem. All data are input by a unified program, and double data entry is used to check data logic and abnormal data.

## 2.8 Statistical analysis

EpiData 3.1 software was used for double data entry, logical error correction and consistency testing. Normally distributed data were expressed as the mean ± standard deviation ($\overline{x} \pm s$), non-normally distributed data were expressed as the median and interquartile range [M(QR)] and categorical variables were expressed as frequencies (%). Analysis of variance (ANOVA) and the Chi-square test were used to analyze the differences in quantitative variables between 25(OH)D level groups. Between-group differences in qualitative variables were tested by the Chi-square test. A Cox proportional hazard model was used to evaluate HR and 95% CI for the relationship between baseline serum 25(OH)D levels and hyperlipidaemia. In model 1, only serum 25(OH)D was included as an independent predictor. Age, gender and BMI levels were also included in model 2. Model 3 was based on model 2 with further adjustment for the following variables: residence area (rural or urban), education level, SBP, DBP, smoking, alcohol consumption, physical activity and family history of hypertension. The median serum 25(OH)D value for each group was input into the model as a continuous variable for trend testing. Then, the association between serum 25(OH)D levels and the risk of hyperlipidaemia was examined in subgroups stratified by age, sex, BMI, area of residence, education, SBP, DBP, smoking, alcohol consumption, physical activity and family history of hypertension. The subjects were divided into two groups according to a 25(OH)D cut-point of 30 ng/mL. All data were analyzed using SPSS version 26.0, and statistical significance was defined as a two-tailed $p < 0.05$.

# 3 Results

## 3.1 Demographic characteristics and incidence of hyperlipidaemia

The study sample comprised 1,210 individuals, among which, 43.80% were male (530 subjects) and 56.19% were female (680 subjects). The average age of the subjects was 51.84 ± 14.37 years. As shown in Table 1, there were 368 new cases of hyperlipidaemia (167 males and 201 females) during the three-year follow-up period, and the cumulative incidence of hyperlipidaemia was 30.41%. Specifically, the cumulative incidence of hyperlipidaemia was 31.51% in males and 29.56% in females. There was no statistically significant difference in the prevalence of hyperlipidaemia between male and female subjects ($p > 0.05$). In the relationship between age and the incidence of hyperlipidemia shown in Table 1, with the increase of age, the incidence of hyperlipidemia in male and female participants showed a significant upward trend, and when the age reached a certain stage, this upward trend did not continue, but there was a slight decline in the reversal. The incidence of hyperlipidaemia increased the fastest among men in the 30–39 years age group.

## 3.2 Comparison of the baseline characteristics of subjects classified by vitamin D status

Among the 1,210 participants, the overall mean serum 25(OH)D level was 25.87 ± 6.56 ng/mL, and the respective ratios of vitamin D deficiency (<20 ng/mL) and insufficiency (20–29 ng/mL) were 17.19 and 58.76%. According to Table 2, the rates of vitamin D deficiency and insufficiency in male subjects were 10.94% (58 people) and 52.45% (278 people), respectively, and the rates of vitamin D deficiency and insufficiency in female subjects were 22.06% (150 people) and 63.68% (433 people), respectively. Vitamin D deficiency was more common in women than in men (22.06% vs. 10.96%). With increases in age, the vitamin D deficiency rate showed an upward trend; only 21.43% of the elderly subjects aged over 80 years had sufficient vitamin D. The rates of vitamin D deficiency and insufficiency among rural residents were 12.93% (67 people) and 57.92% (300 people), respectively. The rates of vitamin D deficiency and insufficiency among urban residents were 20.36% (141 people) and 59.39% (411 people), respectively. The rates of vitamin D deficiency and insufficiency in subjects with BMI < 24 kg/m2 were 16.80% (128 people) and 26.90% (205 people), respectively. The rate of vitamin D deficiency was higher in women and participants living in urban areas ($p < 0.01$). Aside from baseline age, education level, SBP, DBP, dyslipidaemia and family history of hypertension, the differences between other sociodemographic, behavioral and metabolic risk factors and vitamin D levels were statistically significant (all $p < 0.05$, Table 3).

## 3.3 The relationship between serum 25(OH) D levels and hyperlipidaemia

As shown in Table 4, when the clinical classification of vitamin D status was used, the subjects with sufficient vitamin D (≥30 ng/mL) were used as a reference. The incidence of hyperlipidemia in vitamin D deficiency (20–29 ng/mL) was statistically different ($p < 0.05$), and the incidence of hyperlipidemia was significantly higher than that of vitamin D sufficient subjects. When using the clinical classification of vitamin D status, compared with the survey of vitamin D sufficiency (≥30 ng/mL), the incidence of hyperlipidemia in patients with vitamin D insufficiency (<30 ng/mL) was significantly increased ($p < 0.05$). According to the quartile of baseline 25(OH)D level, the study population was divided into four groups (Q1, Q2, Q3, and Q4). Taking the respondents in the Q4 group as a reference, the incidence of hyperlipidemia in the Q2 and Q3 groups was higher than that in the Q4 group ($p < 0.05$).

After adjusting the potential confounding factors of age, gender and BMI, when the vitamin D status was classified into three clinical categories, the incidence of hyperlipidemia in vitamin D deficiency subjects was still significantly higher than that in vitamin D sufficient subjects ($p < 0.05$). When the clinical classification of vitamin D status was used, the investigation of vitamin D adequacy was used as a reference. There was a statistically significant difference in the incidence of hyperlipidemia between vitamin D insufficiency and sufficiency ($p < 0.05$), and the incidence of hyperlipidemia in vitamin D insufficiency was higher. According to the baseline 25(OH)D level quartiles, the study population was

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TABLE 1 Incidence of hyperlipidemia in different sex and age groups.

| Age group (years) | Baseline number | Hyperlipidemia | Follow-up years | Cumulative incidence (%) | Incidence density (per 1,000 person-years) |
|---|---|---|---|---|---|
| Whole crowd | | | | | |
| 18- | 80 | 14 | 26.88 | 17.50 | 520.91 |
| 30- | 180 | 51 | 80.86 | 28.33 | 630.73 |
| 40- | 277 | 84 | 134.64 | 30.32 | 623.89 |
| 50- | 314 | 111 | 201.07 | 35.35 | 552.04 |
| 60- | 236 | 72 | 122.53 | 30.51 | 587.61 |
| 70- | 95 | 32 | 52.82 | 33.68 | 605.78 |
| 80- | 28 | 4 | 8.65 | 14.29 | 462.36 |
| Total | 1,210 | 368 | 3153.45 | 30.41 | 116.70 |
| Male | | | | | |
| 18- | 27 | 5 | 75.63 | 18.52 | 66.11 |
| 30- | 67 | 34 | 148.11 | 50.75 | 229.55 |
| 40- | 110 | 42 | 280.25 | 38.18 | 149.87 |
| 50- | 136 | 39 | 363.45 | 28.68 | 107.31 |
| 60- | 118 | 29 | 321.64 | 24.58 | 90.16 |
| 70- | 56 | 18 | 149.16 | 32.14 | 120.67 |
| 80- | 16 | 0 | 48.00 | 0.00 | 0.00 |
| Total | 530 | 167 | 1386.24 | 31.51 | 120.47 |
| Female | | | | | |
| 18- | 53 | 9 | 149.25 | 16.98 | 60.30 |
| 30- | 113 | 17 | 327.04 | 15.04 | 51.98 |
| 40- | 167 | 42 | 455.50 | 25.15 | 92.21 |
| 50- | 178 | 72 | 456.85 | 40.45 | 157.60 |
| 60- | 118 | 43 | 304.66 | 36.44 | 141.14 |
| 70- | 39 | 14 | 98.37 | 35.90 | 142.32 |
| 80- | 12 | 4 | 32.65 | 33.33 | 122.51 |
| Total | 680 | 201 | 1824.32 | 29.56 | 110.18 |

We recorded the baseline survey time and follow-up time of each participant. Participants were tracked at different times during the three-year period. After completing the calculation of the number of follow-up years at the individual level, summary statistics were performed to calculate the total follow-up years and average follow-up years of the entire cohort.

divided into four groups. The subjects in the Q4 group were used as a reference. There was a statistically significant difference in the incidence of hyperlipidemia between the subjects in the Q2 group and the Q4 group ($p < 0.05$).

After adjusting the potential confounding factors such as residence, education level, SBP, DBP, smoking status, drinking status, physical activity and family history of hypertension, when the vitamin D status was classified into three clinical categories, the subjects with sufficient vitamin D were used as a reference, and the incidence of hyperlipidemia in vitamin D deficiency was still high ($p < 0.05$). When using the clinical classification of vitamin D status, the incidence of hyperlipidemia in vitamin D deficiency and sufficient subjects was higher than that in vitamin D sufficient subjects ($p < 0.05$). According to the quartile of baseline 25(OH)D level, the study population was divided into four groups. Taking the respondents in the Q4 group as a reference, the incidence of hyperlipidemia in the Q4 group was lower than that in the Q2 group ($p < 0.05$).

As shown in Table 5, when analyzing the relationship between baseline serum 25-hydroxyvitamin D (25(OH)D) levels and the incidence of hyperlipidemia among individuals with normal weight, after excluding those who were overweight or obese, it was found that there was no statistically significant association between vitamin D and the risk of hyperlipidemia, regardless of the vitamin D clinical classification used. However, in the subset of overweight and obese individuals (Table 6), under the clinical binary classification of vitamin D status, those with vitamin D deficiency (<30 ng/mL) exhibited a significantly higher incidence of hyperlipidemia compared to those with sufficient vitamin D (≥30 ng/mL) ($p < 0.05$). Similarly, under the clinical ternary classification of vitamin D status, with those having sufficient vitamin D (≥30 ng/mL) as the reference group, individuals with vitamin D insufficiency (20–29 ng/mL) demonstrated a statistically significant difference in hyperlipidemia incidence ($p < 0.05$). When the study population was stratified into four groups based on quartiles of baseline 25(OH)D levels (Q1, Q2, Q3, and Q4),

TABLE 2 Incidence of hyperlipidemia in different sex and age groups.

| Characteristic | Vitamin D deficiency | Vitamin D insufficiency | Vitamin D sufficiency | Total |
|---|---|---|---|---|
| Male, *n*(%) | | | | |
| 18- | 2(7.41) | 16(59.26) | 9(33.33) | 27 |
| 30- | 8(11.94) | 32(47.76) | 27(40.3) | 67 |
| 40- | 12(10.91) | 60(54.55) | 38(34.55) | 110 |
| 50- | 18(13.24) | 64(47.06) | 54(39.71) | 136 |
| 60- | 9(7.63) | 69(58.47) | 40(33.9) | 118 |
| 70- | 6(10.71) | 30(53.57) | 20(35.71) | 56 |
| 80- | 3(18.75) | 7(43.75) | 6(37.5) | 16 |
| Total | 58(10.94) | 278(52.45) | 194(36.6) | 530 |
| Female, *n*(%) | | | | |
| 18- | 10(18.87) | 36(67.92) | 7(13.21) | 27 |
| 30- | 29(25.66) | 67(59.29) | 17(15.04) | 67 |
| 40- | 31(18.56) | 112(67.07) | 24(14.37) | 110 |
| 50- | 31(17.42) | 120(67.42) | 27(15.17) | 136 |
| 60- | 25(21.19) | 75(63.56) | 18(15.2) | 118 |
| 70- | 16(41.03) | 19(48.72) | 4(10.26) | 56 |
| 80- | 8(66.67) | 4(33.33) | 0(0) | 16 |
| Total | 150(22.06) | 433(63.68) | 97(14.26) | 530 |

with Q4 serving as the reference group, the incidence of hyperlipidemia was higher in Q1, Q2, and Q3 compared to Q4 ($p < 0.05$).

After fully adjusting for potential confounding factors such as age, gender, and BMI, under the clinical ternary classification of vitamin D status, with those having sufficient vitamin D as the reference group, individuals with vitamin D insufficiency (20–29 ng/mL) still exhibited a significantly higher incidence of hyperlipidemia ($p < 0.05$). When using the clinical binary classification of vitamin D status, with those having sufficient vitamin D as the reference, there was a statistically significant difference in hyperlipidemia incidence between vitamin D-deficient and sufficient individuals ($p < 0.05$), with a higher incidence among the deficient group. Additionally, when stratifying the study population into four groups based on quartiles of baseline 25(OH)D levels and using Q4 as the reference, there was a statistically significant difference in hyperlipidemia incidence between Q2 and Q4 ($p < 0.05$). After fully adjusting for potential confounding factors such as residence, education level, systolic blood pressure, diastolic blood pressure, smoking status, alcohol consumption, physical activity, and family history of hypertension, the results remained consistent with the previous findings.

## 4 Discussion

A substantial body of literature has established a strong association between vitamin D and various disease states, including, but not limited to, type 2 diabetes, dyslipidemia, cardiovascular diseases, autoimmune disorders, certain cancers, metabolic syndrome, schizophrenia, and depression (CHEN (3, 36)). Clinical trial data, in particular, reveal a close relationship between 25(OH)D levels and lipid metabolism, indicating that elevated lipid levels can lead to a decrease in vitamin D levels, while vitamin D deficiency further exacerbates dyslipidemia and weight gain (37). Vitamin D deficiency has become a global public health issue, affecting individuals across all age groups, ethnicities, and socioeconomic statuses (38). Certain groups, such as individuals with obesity, the elderly, children, pregnant women, and postmenopausal women, exhibit especially high rates of vitamin D deficiency (39). Hyperlipidemia, as an increasingly prevalent metabolic disorder, not only poses a threat to individual health but also places a considerable burden on global healthcare systems (40, 41). For instance, by 2017, over 100 million people in the United States had been diagnosed with hypercholesterolemia, with 31 million adults experiencing elevated LDL-C levels (42). Therefore, investigating effective treatment strategies for hyperlipidemia, particularly regarding the potential role of vitamin D, is essential for managing lipid levels and improving patient outcomes.

Research has demonstrated that vitamin D plays a crucial role in reducing lipid levels and mitigating mortality risk (43), especially in patients with hypertension and type 2 diabetes (44). Theoretically, vitamin D not only exerts a direct influence on lipid levels but may also indirectly impact lipid metabolism by modulating serum parathyroid hormone and/or calcium balance (45). Specifically, sufficient vitamin D increases intracellular calcium levels in adipocytes, promoting the activity of fatty acid synthase, inhibiting lipolysis, and enhancing the storage of lipids within adipocytes (46). Additionally, vitamin D can inhibit macrophage migration and phagocytic activity, reducing the deposition of LDL-C (47). Conversely, as vitamin D levels decline, calcium absorption in the gastrointestinal tract diminishes (48), impairing the lipid storage function of adipocytes, which leads to the release of lipids into the bloodstream, causing elevated concentrations of TC, TG, and LDL-C, as well as a decrease in HDL-C levels (49). Studies from Australia (50) and animal models (51) further support that adequate vitamin D supplementation effectively inhibits hepatic steatosis and fibrosis,

TABLE 3 Baseline characteristics of the study population ($n$ = 1,210).

| Characteristic | Total | Serum 25(OH)D concentrations (ng/ml) | | | $F/\chi^2$ | $P$ |
|---|---|---|---|---|---|---|
| | ($n$ = 1,210) | <20($n$ = 208) | 20–29($n$ = 711) | ≥30($n$ = 291) | | |
| 25(OH)D | 25.87 ± 6.56 | 16.16 ± 2.96 | 25.32 ± 2.78 | 34.15 ± 4.16 | | |
| Age (years) | 51.84 ± 14.37 | 52.92 ± 15.74 | 51.39 ± 14.07 | 52.17 ± 14.04 | 1.01 | 0.365 |
| Sex (men), $n$(%) | 530(43.80) | 58(10.94)* | 278(52.45) | 194(36.60) | 89.598 | <0.001 |
| (Women), $n$(%) | 680(56.20) | 150(22.06) | 433(63.68) | 97(14.26) | | |
| District (rural), $n$(%) | 518(42.81) | 67(12.93)* | 300(57.92) | 151(29.15) | 19.453 | <0.001 |
| (Urban), $n$(%) | 692(57.19) | 141(20.38) | 411(59.39) | 140(20.23) | | |
| Education, $n$(%) | | | | | 7.148 | 0.307 |
| No school | 210(17.36) | 36(17.14) | 129(61.43) | 45(21.43) | | |
| Primary school | 328(27.11) | 56(17.07) | 182(55.49) | 90(27.44) | | |
| Middle school | 611(50.50) | 105(17.18) | 358(58.59) | 148(24.22) | | |
| Junior college or higher | 61(5.04) | 11(18.03) | 42(68.85) | 8(13.11) | | |
| BMI (kg/m$^2$) | 23.28 ± 3.24 | 23.34 ± 3.34 | 23.44 ± 3.26* | 22.86 ± 3.09 | 3.364 | 0.035 |
| <24 | 762(62.98) | 128(61.54) | 429(60.34) | 205(70.45) | 9.414 | 0.052 |
| 24–27.9 | 346(28.60) | 62(29.81) | 219(30.80) | 65(22.34) | | |
| ≥28 | 102(8.43) | 18(8.65) | 63(8.86) | 21(7.22) | | |
| TC | 4.95 ± 0.72 | 5.13 ± 0.72 | 4.95 ± 0.72* | 4.84 ± 0.69 | 9.978 | <0.001 |
| TG | 1.25 ± 0.45 | 1.32 ± 0.46 | 1.26 ± 0.45* | 1.17 ± 0.46 | 6.674 | 0.001 |
| LDL-C | 2.84 ± 0.60 | 2.94 ± 0.63 | 2.85 ± 0.60* | 2.75 ± 0.59 | 5.726 | 0.003 |
| HDL-C | 1.58 ± 0.34 | 1.66 ± 0.48 | 1.57 ± 0.31* | 1.57 ± 0.31 | 6.078 | 0.002 |
| Systolic pressure | 125.01 ± 18.77 | 126.70 ± 20.32 | 124.14 ± 18.45 | 125.93 ± 19.04 | 1.954 | 0.142 |
| Diastolic pressure | 78.72 ± 10.21 | 78.62 ± 11.13 | 78.90 ± 10.11 | 78.36 ± 9.81 | 0.298 | 0.743 |
| Family history of hypertension, $n$(%) | 258(21.32) | 46(17.83) | 46(17.83) | 46(17.83) | 0.989 | 0.610 |
| Dyslipidemia, $n$(%) | 53(4.38) | 12(5.77) | 30(4.22) | 11(3.78) | 7.150 | 0.128 |
| Current smoker, $n$(%) | 267(22.08) | 39(18.84) | 146(20.53)* | 82(28.18) | 8.539 | 0.014 |
| Current drinker, $n$(%) | 370(30.63) | 41(19.81) | 217(30.52)* | 112(38.62) | 20.131 | <0.001 |
| Sufficient physical activity, $n$(%) | 292(24.17) | 56(27.18) | 185(26.02)* | 51(17.53) | 9.357 | 0.009 |

*There was a significant difference compared with the 25(OH) D sufficient group [serum 25(OH)D ≥ 30 ng/mL]; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

promotes hepatic calcium absorption, and thereby helps regulate lipid profiles.

However, it is worth noting that when we limited the analysis to individuals with normal weight, the results showed a very different pattern (73). We discovered that restricting the analysis to overweight and obese individuals can unveil a significant statistical correlation between vitamin D status and hyperlipidemia risk. In the clinical dichotomous classification of vitamin D status, individuals with vitamin D insufficiency (<30 ng/mL) exhibited a significantly higher incidence of hyperlipidemia compared to those with sufficient vitamin D (≥30 ng/mL) ($p < 0.05$). When further refining the classification into trichotomous categories, individuals with vitamin D levels in the insufficient range (20–29 ng/mL), using those with sufficient vitamin D as a reference, meanwhile demonstrated a statistical difference in hyperlipidemia incidence ($p < 0.05$). These observations strongly indicate that, in overweight and obese adults, the nutritional status of vitamin D may exert a notable impact on lipid metabolism, thereby increasing the risk of hyperlipidemia. To further explore this relationship, we grouped the study population based on quartiles of baseline 25(OH)D levels. The results showed that, using the highest quartile group (Q4) of 25(OH)D levels as a reference, individuals in the lower quartiles (Q1, Q2, and Q3) had significantly higher incidences of hyperlipidemia ($p < 0.05$). This trend analysis not only reinforces the negative correlation between vitamin D and hyperlipidemia risk but also suggests a potential dose–response effect of increasing vitamin D levels on reducing hyperlipidemia risk. Notably, this effect is particularly evident in overweight and obese populations, providing robust evidence to support vitamin D supplementation strategies tailored for these groups.

However, it is worth noting that when we expanded the analysis to individuals with normal body weight, the research findings presented a starkly different pattern. Regardless of the vitamin D clinical classification standard employed (i.e., dichotomous, trichotomous, or more detailed quartile classification), no statistically significant correlation was observed between baseline serum 25(OH) D levels and the risk of hyperlipidemia. This finding may suggest that, in adults with normal body weight, the nutritional status of vitamin D may not be a primary influencing factor in the development of

TABLE 4 Relationship between baseline serum 25(OH)D levels and the incidence of hyperlipidemia.

| 25(OH)D (ng/ml) | Events (%) | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|---|
| | | HR(95% CI) | *P* | HR(95% CI) | *P* | HR(95% CI) | *P* |
| Clinical triad | | | | | | | |
| <20 | 208(60) | 1.306(0.921–1.853) | 0.134 | 1.309(0.915–1.873) | 0.141 | 1.315(0.914–1.89) | 0.140 |
| 20–29 | 711(242) | 1.612(1.228–2.116) | 0.001 | 1.581(1.197–2.087) | 0.001 | 1.572(1.187–2.08) | 0.002 |
| ≥30 | 291(66) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 0.984(0.965–1.003) | 0.100 | 0.985(0.965–1.004) | 0.123 | 0.984(0.965–1.004) | 0.123 |
| Dichotomy | | | | | | | |
| <30 | 919(302) | 1.540(1.180–2.010) | 0.001 | 1.523(1.159–2.001) | 0.003 | 1.519(1.130–2.001) | 0.003 |
| ≥30 | 291(66) | 1.00 | – | 1.00 | – | 1.00 | – |
| P for trend† | | 0.953(0.925–0.982) | 0.001 | 0.954(0.925–0.984) | 0.003 | 0.954(0.925–0.984) | 0.003 |
| Quartile | | | | | | | |
| Q1(<21.50) | 301(90) | 1.280(0.940–1.743) | 0.117 | 1.259(0.916–1.731) | 0.156 | 1.265(0.916–1.746) | 0.153 |
| Q2(21.50–25.88) | 303(109) | 1.580(1.175–2.125) | 0.002 | 1.565(1.156–2.119) | 0.004 | 1.535(1.130–2.084) | 0.006 |
| Q3(25.89–29.82) | 303(96) | 1.392(1.027–1.888) | 0.033 | 1.351(0.993–1.837) | 0.055 | 1.350(0.989–1.843) | 0.059 |
| Q4(≥29.83) | 303(73) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 0.984(0.965–1.003) | 0.107 | 0.985(0.966–1.005) | 0.150 | 0.985(0.965–1.006) | 0.153 |
| Continuous‡ | 1,210(368) | 0.987(0.972–1.003) | 0.104 | 0.988(0.972–1.004) | 0.139 | 0.987(0.971–1.004) | 0.136 |

Model 1: no adjustment; model 2: adjust gender, age and BMI; model 3: adjust residence, education level, systolic blood pressure, diastolic blood pressure, smoking status, drinking status, physical activity and family history of hypertension.
HR, hazard ratio; CI, confidence interval.
†Test trends based on median variables in each group (medians for Q1, Q2, Q3, and Q4 were 18.55, 24.17, 27.92, and 32.88 ng/mL, respectively).
‡HR is the standard deviation (6.43 ng/mL) proportionally scaled to serum 25(OH)D levels.

TABLE 5 Relationship between baseline serum 25(OH)D levels and the incidence of hyperlipidemia (excluding overweight and obese people from normal-weight individuals).

| 25(OH)D | Events (%) | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|---|
| (ng/ml) | | HR(95% CI) | *P* | HR(95% CI) | *P* | HR(95% CI) | *P* |
| Clinical triad | | | | | | | |
| <20 | 81(32) | 0.982(0.612–1.576) | 0.939 | 0.956(0.497–1.838) | 0.892 | 0.999(0.549–1.817) | 0.997 |
| 20–29 | 287(127) | 1.125(0.780–1.623) | 0.528 | 0.930(0.552–1.565) | 0.784 | 1.024(0.625–1.677) | 0.925 |
| ≥30 | 87(37) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 0.989(0.965–1.015) | 0.410 | 0.990(0.965–1.016) | 0.451 | 0.990(0.965–1.017) | 0.472 |
| Dichotomy | | | | | | | |
| <30 | 368(159) | 1.093(0.764–1.563) | 0.626 | 0.935(0.561–1.558) | 0.796 | 1.017(0.633–1.635) | 0.944 |
| ≥30 | 87(37) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 0.988(0.954–1.023) | 0.508 | 0.988(0.953–1.024) | 0.514 | 0.989(0.954–1.026) | 0.550 |
| Quartile | | | | | | | |
| Q1(<21.15) | 114(45) | 0.923(0.617–1.38) | 0.697 | 0.898(0.513–1.570) | 0.705 | 0.952(0.568–1.596) | 0.851 |
| Q2(21.16–25.38) | 113(52) | 1.086(0.737–1.602) | 0.676 | 0.828(0.477–1.439) | 0.504 | 0.955(0.565–1.612) | 0.862 |
| Q3(25.39–28.80) | 113(49) | 1.068(0.720–1.584) | 0.742 | 1.074(0.618–1.865) | 0.801 | 1.021(0.603–1.730) | 0.937 |
| Q4(≥28.81) | 115(50) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 0.993(0.969–1.018) | 0.579 | 0.994(0.969–1.019) | 0.625 | 0.994(0.969–1.020) | 0.669 |
| Continuous‡ | 455(196) | 0.993(0.973–1.013) | 0.490 | 0.993(0.973–1.014) | 0.508 | 0.994(0.973–1.015) | 0.585 |

Model 1: no adjustment; model 2: adjust gender, age and BMI; model 3: adjust residence, education level, systolic blood pressure, diastolic blood pressure, smoking status, drinking status, physical activity and family history of hypertension.
HR, hazard ratio; CI, confidence interval.
†Test trends based on median variables in each group (medians for Q1, Q2, Q3, and Q4 were 18.62, 23.86, 26.94, and 32.15 ng/mL, respectively).
‡HR is the standard deviation (6.00 ng/mL) proportionally scaled to serum 25(OH)D levels.

TABLE 6 Relationship between baseline serum 25(OH)D levels and the incidence of hyperlipidemia (overweight and obese people).

| 25(OH)D (ng/ml) | Events (%) | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|---|
| | | HR(95% CI) | *P* | HR(95% CI) | *P* | HR(95% CI) | *P* |
| **Clinical triad** | | | | | | | |
| <20 | 127(28) | 1.571(0.935–2.641) | 0.088 | 1.637(0.957–2.802) | 0.072 | 1.643(0.955–2.826) | 0.073 |
| 20–29 | 424(115) | 1.987(1.322–2.986) | 0.001 | 2.052(1.351–3.118) | 0.001 | 2.098(1.374–3.201) | 0.001 |
| ≥30 | 204(29) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 1.005(0.991–1.021) | 0.475 | 1.005(0.989–1.020) | 0.551 | 1.004(0.988–1.020) | 0.660 |
| **Dichotomy** | | | | | | | |
| <30 | 551(143) | 1.889(1.267–2.816) | 0.002 | 1.967(1.302–2.972) | 0.001 | 2.013(1.323–3.061) | 0.001 |
| ≥30 | 204(29) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 1.012(0.992–1.032) | 0.253 | 1.011(0.990–1.032) | 0.309 | 1.010(0.989–1.032) | 0.348 |
| **Quartile** | | | | | | | |
| Q1(<21.73) | 186(44) | 1.821(1.115–2.975) | 0.017 | 1.884(1.131–3.140) | 0.015 | 1.894(1.128–3.181) | 0.016 |
| Q2(21.73–26.47) | 191(51) | 2.114(1.310–3.411) | 0.002 | 2.201(1.351–3.585) | 0.002 | 2.239(1.369–3.664) | 0.001 |
| Q3(26.48–30.45) | 188(52) | 2.287(1.419–3,684) | 0.001 | 2.315(1.429–3.749) | 0.001 | 2.360(1.448–3.847) | 0.001 |
| Q4(≥30.46) | 190(25) | 1.00 | – | 1.00 | – | 1.00 | – |
| *P* for trend† | | 1.008(0.993–1.023) | 0.313 | 1.007(0.992 ~ 1.023) | 0.374 | 1.006-(0.9911.022) | 0.441 |
| Continuous‡ | 755(172) | 1.005(0.993–1.017) | 0.394 | 1.004(0.9921.017) | 0.484 | 1.005(0.993–1.017) | 0.394 |

Model 1: no adjustment; model 2: adjust gender, age and BMI; model 3: adjust residence, education level, systolic blood pressure, diastolic blood pressure, smoking status, drinking status, physical activity and family history of hypertension.
HR, hazard ratio; CI, confidence interval.
†Test trends based on median variables in each group (medians for Q1, Q2, Q3, and Q4 were 18.53, 24.42, 28.38, and 33.34 ng/mL, respectively).
‡HR is the standard deviation (6.86 ng/mL) proportionally scaled to serum 25 (OH)D levels.

hyperlipidemia, or its effect may be obscured by other more dominant metabolic factors. This result challenges the view that vitamin D is generally beneficial to lipid metabolism in some previous studies, and further emphasizes that there may be differences in the potential mechanism of vitamin D on lipid metabolism under overweight (74, 75).

The results of this study further confirm a positive association between vitamin D deficiency and the incidence of hyperlipidemia. When baseline 25(OH)D levels were considered as the sole predictor, vitamin D deficiency was significantly associated with an increased incidence of dyslipidemia. Even after extensive adjustment for confounding variables, a low vitamin D status remained significantly associated with dyslipidemia. Notably, the cumulative incidence of hyperlipidemia did not show a statistically significant difference between women and men, suggesting that the mechanisms underlying hyperlipidemia may transcend gender and are likely driven by similar fundamental physiological and genetic factors. However, in terms of vitamin D deficiency prevalence, a gender disparity was observed, with women exhibiting a higher frequency of vitamin D deficiency compared to men (22.06% vs. 10.94%), consistent with findings from a cross-sectional study in the Netherlands (52). This gender-specific variation may stem from differences in lifestyle, physiological characteristics, time spent outdoors, sun protection practices, and occupational activities between men and women (53). For instance, traditional or societal expectations may lead women to adopt sun protection measures or spend more time indoors, thereby reducing natural sunlight exposure—a primary pathway for endogenous vitamin D synthesis. Additionally, women experience unique life stages and hormonal changes, including menarche, potential pregnancy, breastfeeding, and contraceptive use, through to menopause, where the production of hormones such as estrogen and progesterone may predispose them to vitamin D deficiency (54). Furthermore, occupational differences could contribute to unequal sunlight exposure opportunities between genders. These factors collectively influence the efficiency of vitamin D synthesis in the skin, either directly or indirectly. Additionally, dietary habits—another crucial source of vitamin D—may also vary by gender. Women, in their pursuit of healthier eating, may inadvertently restrict foods rich in vitamin D, especially within specific dietary cultures or societal norms. In contrast, men may be less affected by these factors, or their dietary patterns may naturally include more vitamin D-rich foods. These hypotheses warrant further research, with detailed dietary surveys and lifestyle assessments needed to substantiate these findings.

With advancing age, the incidence of hyperlipidemia and vitamin D deficiency increases in both men and women, aligning with findings from previous literature (55). This age-related rise may be attributable to reduced outdoor activity, diminished gastrointestinal function, and imbalances in dietary intake and nutrition, which can lead to decreased vitamin D absorption and greater intracellular deposition of LDL-C in older adults (56). Interestingly, the incidence of hyperlipidemia tends to slightly decline among the elderly at certain advanced ages. This may be due to the premature attrition of high-risk individuals from the cohort because of the onset or progression of other health conditions, such as cardiovascular disease or diabetes, which reduces their representativeness in the study's data on hyperlipidemia incidence (57). Such individuals may experience limited ability or willingness to participate due to health complications, thereby decreasing their representation in the cohort. Additionally, it is essential to consider the effects of aging on physiological function and metabolic processes (58,

59). With age, physiological functions, including lipid metabolism, undergo gradual decline, potentially impacting the incidence of hyperlipidemia either directly or indirectly (60). To further investigate this phenomenon, we plan to conduct a refined age-stratified analysis in future studies to elucidate similarities and differences in the mechanisms of hyperlipidemia onset across different age groups. Furthermore, we observed that individuals with lower educational attainment, lower BMI, or a family history of hyperlipidemia are more susceptible to both vitamin D deficiency and dyslipidemia. This trend may be associated with limited health literacy, imbalanced diets, inadequate nutrient intake, and genetic predispositions (61). First, individuals with lower educational levels may lack health knowledge and practices, such as regular monitoring of vitamin D levels and taking appropriate supplements, which could increase their risk for vitamin D deficiency and consequently dyslipidemia. Second, those with lower BMI, particularly underweight individuals, may experience vitamin D deficiency and other nutritional deficiencies due to imbalanced diets or inadequate nutrient intake. This state of nutrient deficiency could further impact lipid metabolism, elevating the risk of dyslipidemia. Finally, individuals with a family history of hyperlipidemia may inherit gene variants that affect vitamin D or lipid metabolism, rendering them more vulnerable to both vitamin D deficiency and dyslipidemia (62). We intend to explore these potential factors further to develop a more comprehensive predictive model for hyperlipidemia risk.

In this study, a threshold of 30 ng/mL was set for vitamin D levels, and significant differences in the prevalence of dyslipidemia and individual lipid profile measures were observed between the two groups. Even after adjusting for various potential confounders, vitamin D deficiency remained significantly associated with an elevated risk of dyslipidemic events. This finding aligns with prior meta-analyses (63) and individual studies (64–66), which collectively indicate a close relationship between serum vitamin D levels and lipid markers such as TC, TG, LDL-C, and HDL-C. An increase in serum 25(OH)D levels has been shown to aid in reducing blood lipid levels and improving dyslipidemia (67). Although adjustments were made for multiple traditional risk factors, residual confounders such as dietary habits, genetic background, and medication use may still influence the relationship between vitamin D levels and hyperlipidemia (68, 69). Insufficient intake of vitamin D-rich foods, such as fish, dairy products, and fortified foods, may contribute to vitamin D deficiency. Concurrently, diets high in fat, sugar, and salt could exacerbate lipid metabolism disorders, increasing the risk of dyslipidemia. Additionally, genetic background plays a key role in both vitamin D metabolism and lipid metabolism. Genetic variants may influence the activity of vitamin D receptors or related enzymes, affecting vitamin D bioavailability and lipid processing. Furthermore, medication use is a critical factor impacting both vitamin D levels and lipid abnormalities; certain drugs, such as antiepileptics, antibiotics, and lipid-lowering agents, may alter vitamin D metabolism or elevate the risk of dyslipidemia. Given individual variability and the complexity of these interactions, unidentified or insufficiently understood confounders may still impact the association between vitamin D levels and hyperlipidemia. Future studies should more deeply explore these potential factors and utilize more precise study designs and sensitive detection methods to uncover the subtler relationships between vitamin D and lipid abnormalities.

Given the extensive physiological effects of vitamin D (70), particularly its pivotal role in metabolic regulation, we emphasize the importance of ensuring adequate vitamin D levels. The long half-life of vitamin D, which allows it to persist in the human body for 3 weeks and maintain a relatively stable blood concentration, provides a rationale for its use as a mild and effective control measure (56). Consequently, it is reasonable to encourage patients to ensure sufficient vitamin D intake during glycemic and lipid-lowering therapy (27). However, there is currently a lack of support from large-scale, long-term studies regarding the optimal dosage and frequency of vitamin D supplementation, necessitating cautious consideration of individual differences and potential risks when formulating supplementation strategies (71). Our study has made certain progress in exploring the relationship between serum 25(OH)D levels and the risk of hyperlipidemia in adults. Its strengths lie in the rigorous design, employing a cohort study approach, which enables the observation of the impact of 25(OH)D levels over time on hyperlipidemia risk. Furthermore, the study considered various potential confounding factors, such as age, gender, and BMI, thereby enhancing the accuracy and reliability of the findings. Nonetheless, our study also has some limitations. Firstly, during data collection, factors that may influence vitamin D levels, such as habitual diet, sun exposure time, and sunscreen use, were not considered. These factors could potentially interfere with the study results and affect the accuracy of the conclusions. Secondly, the study sample was regionally limited, and the follow-up period was relatively short, which may impact the reliability of the correlation test between 25(OH)D and hyperlipidemia. This limitation may be particularly evident in subgroup analyses, challenging the robustness of the conclusions. Additionally, the insufficient sample size and follow-up duration may restrict the generality and applicability of the study results. Future research is needed to conduct a series of targeted studies to clarify the recommended dosage and frequency of vitamin D supplements, maximizing their benefits while minimizing potential risks. It is essential to identify target populations, such as elderly individuals and specific high-risk groups (e.g., obese and diabetic patients), who are likely to benefit most from these interventions, in order to provide more precise vitamin D management guidelines to support personalized treatment in clinical practice. At the same time, we also need to consider factors such as habitual diet, sunshine time, and sunscreen use into the study to more comprehensively and accurately assess the effect of vitamin D on the risk of hyperlipidemia. This study also provides some implications for enhancing the intake of vitamin D-rich foods such as milk, salted fish and cod liver oil (72). By continuously exploring and optimizing vitamin D management strategies, we are expected to make a greater contribution to improving metabolic health and preventing chronic diseases. Future studies need to further increase the sample size and prolong the follow-up time, so as to improve the accuracy and reliability of the study and provide more strong evidence support for clinical practice.

## 5 Conclusion

This study is a three-year prospective cohort analysis conducted in Zhejiang, which delves into the relationship between serum 25(OH)D levels and the risk of hyperlipidemia in adults. The results indicate a significant correlation between vitamin D status and the incidence of hyperlipidemia, and this association remains robust even after extensive adjustment for confounding factors, further confirming the crucial role of vitamin D in lipid metabolism. Women are more prone to vitamin D deficiency compared to men, and this gender disparity may be associated with factors such as lifestyle, physiological characteristics, and occupational activities. Additionally, older age, lower educational attainment, lower BMI, and a family history are predictive of both vitamin D deficiency and dyslipidemia. Compared to existing research, this study not only validates the close correlation between vitamin D and lipid levels but also uncovers the distribution characteristics of vitamin

D deficiency across different populations and its independent impact on the risk of hyperlipidemia. Future research should further explore the optimal dosage and frequency of vitamin D supplementation and how to optimize vitamin D management strategies for specific high-risk populations to improve metabolic health, providing novel insights into the prevention and treatment of hyperlipidemia. The findings of this study offer compelling evidence for the potential role of vitamin D in the prevention and control of hyperlipidemia, emphasizing the importance of maintaining adequate vitamin D levels.

## Data availability statement

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

## Ethics statement

The studies involving humans were approved by the Ethics Committee of Soochow University (ESCU-20160001). 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), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

## Author contributions

Z-yL: Writing – original draft, Writing – review & editing. SL: Writing – review & editing. XY: Writing – review & editing. C-yW: Writing – review & editing. Y-hS: Writing – review & editing. Y-mB: Writing – review & editing. J-XW: Writing – review & editing. YL: Writing – review & editing. T-lS: Writing – review & editing. WM: Writing – review & editing. C-yC: Writing – review & editing. Z-YH: 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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## References

1. Li N. Platelets as an inter-player between hyperlipidaemia and atherosclerosis. *J Intern Med*. (2024) 296:39–52. doi: 10.1111/joim.13794

2. Nohria A, Shah JT, Desai D, Alhanshali L, Ingrassia J, Femia A, et al. Alopecia areata and cardiovascular comorbidities: a cross-sectional analysis of the all of us research program. *JAAD Int*. (2024) 16:46–8. doi: 10.1016/j.jdin.2024.03.024

3. Faluk M, Wardhere A, Mohamoud A, Nor M, Bampastsias D, Oikonomou E, et al. Evolution of LDL-C lowering medications and their cardiovascular benefits: past, present, and future. *Curr Probl Cardiol*. (2024) 49:102637–7. doi: 10.1016/j.cpcardiol.2024.102637

4. Yang M, Song Z, Wang S, He Y, Wang W, Gong H, et al. Protective effect of bamboo root dietary fibre on hyperlipidaemia mice induced by high-fat diet. *CYTA J Food*. (2023) 21:674–81. doi: 10.1080/19476337.2023.2274371

5. Takaoka M, Zhao X, Lim HY, Magnussen CG, Ang O, Suffee N, et al. Early intermittent hyperlipidaemia alters tissue macrophages to fuel atherosclerosis. *Nature*. (2024) 634:457–65. doi: 10.1038/s41586-024-07993-x

6. Huang X, Hui H, Zhu W, Chen N, Wei Y, Wang Z, et al. Effect of the interaction between alcohol and meat consumption on the hyperlipidaemia risk among elderly individuals: evidence from Shanghai, China. *Front Nutr*. (2022) 9:982626–6. doi: 10.3389/fnut.2022.982626

7. Vardell E. Global Health Observatory data repository. *Med Ref Serv Q*. (2020) 39:67–74. doi: 10.1080/02763869.2019.1693231

8. McDermott CM, Feola R, Plude J. Detection of cyanobacterial toxins (microcystins) in waters of northeastern Wisconsin by a new immunoassay technique. *Toxicon*. (1995) 33:1433–42. doi: 10.1016/0041-0101(95)00095-4

9. Mohamed-Yassin M-S, Rosman N, Kamaruddin KN, Miptah HN, Baharudin N, Ramli AS, et al. A systematic review and meta-analysis of the prevalence of dyslipidaemia among adults in Malaysia. *Sci Rep*. (2023) 13:11036–6. doi: 10.1038/s41598-023-38275-7

10. Meza-Meza MR, Ruiz-Ballesteros AI, de la Cruz-Mosso U. Functional effects of vitamin D: from nutrient to immunomodulator. *Crit Rev Food Sci Nutr*. (2022) 62:3042–62. doi: 10.1080/10408398.2020.1862753

11. Carlberg C. Nutrigenomics of vitamin D. *Nutrients*. (2019) 11:676. doi: 10.3390/nu11030676

12. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev*. (2008) 29:726–76. doi: 10.1210/er.2008-0004

13. Van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol*. (2005) 97:93–101. doi: 10.1016/j.jsbmb.2005.06.002

14. Martinelli RP, Rayego-Mateos S, Alique M, Márquez-Expósito L, Tejedor-Santamaria L, Ortiz A, et al. Vitamin D, cellular senescence and chronic kidney diseases: what is missing in the equation? *Nutrients*. (2023) 15:1349. doi: 10.3390/nu15061349

15. Kim D-H, Meza CA, Clarke H, Kim J-S, Hickner RC. Vitamin D and endothelial function. *Nutrients*. (2020) 12:575. doi: 10.3390/nu12020575

16. Borges MC, Martini LA, Rogero MM. Current perspectives on vitamin D, immune system, and chronic diseases. *Nutrition*. (2011) 27:399–404. doi: 10.1016/j.nut.2010.07.022

17. Soto-Dávila M, Valderrama K, Inkpen SM, Hall JR, Rise ML, Santander J. Effects of vitamin D 2 (Ergocalciferol) and D 3 (cholecalciferol) on Atlantic Salmon (*Salmo salar*) primary macrophage immune response to *Aeromonas salmonicida* subsp *salmonicida* infection. *Front Immunol*. (2020) 10:3011. doi: 10.3389/fimmu.2019.03011

18. Rajakumar K, de las Heras J, Chen TC, Lee SJ, Holick MF, Arslanian SA. Vitamin D status, adiposity, and lipids in black American and Caucasian children. *J Clin Endocrinol Metab*. (2011) 96:1560–7. doi: 10.1210/jc.2010-2388

19. Huang X, Yang Y, Jiang Y, Zhou Z, Zhang J. Association between vitamin D deficiency and lipid profiles in overweight and obese adults: a systematic review and meta-analysis. *BMC Public Health*. (2023) 23:1653–3. doi: 10.1186/s12889-023-16447-4

20. Alzohily B, AlMenhali A, Gariballa S, Munawar N, Yasin J, Shah I. Unraveling the complex interplay between obesity and vitamin D metabolism. *Sci Rep*. (2024) 14:7583–3. doi: 10.1038/s41598-024-58154-z

21. Song X, Qin S, Chen S, Zhang C, Lin L, Song Z. Bibliometric analysis of vitamin D and obesity research over the period 2000 to 2023. *Front Pharmacol*. (2024) 15:1445061. doi: 10.3389/fphar.2024.1445061

22. Di Filippo L, De Lorenzo R, Giustina A, Rovere-Querini P, Conte C. Vitamin D in osteosarcopenic obesity. *Nutrients*. (2022) 14:1816. doi: 10.3390/nu14091816

23. Song S, Yuan Y, Wu X, Zhang D, Qi Q, Wang H, et al. Additive effects of obesity and vitamin D insufficiency on all-cause and cause-specific mortality. *Front Nutr*. (2022) 9:999489–9. doi: 10.3389/fnut.2022.999489

24. Ponda MP, Dowd K, Finkielstein D, Holt PR, Breslow JL. The short-term effects of vitamin D repletion on cholesterol: a randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol*. (2012) 32:2510–5. doi: 10.1161/ATVBAHA.112.254110

25. Lu L, Yu Z, Lin X. Plasma 25-Hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. *Diabetes Care*. (2010) 33:e14. doi: 10.2337/dc09-1713

26. Miñambres I, Sánchez-Quesada JL, Sánchez-Hernández J, Rodríguez J, de Leiva A, Pérez A. Vitamin D concentrations in familial combined hyperlipidemia: effects of lipid lowering treatment. *Diabetol Metab Syndr*. (2014) 6:7. doi: 10.1186/1758-5996-6-7

27. Al-Qusous MN, Al Madanat WKJ, Mohamed Hussein R. Association of Vitamins D, B6, and B12 deficiencies with hyperlipidemia among Jordanian adults. *Rep Biochem Mol Biol*. (2023) 12:415–24. doi: 10.61186/rbmb.12.3.415

28. Yang Y, Yan S, Yao N, Guo Y, Wang H, Sun M, et al. Effects of vitamin D supplementation on the regulation of blood lipid levels in prediabetic subjects: a meta-analysis. *Front Nutr*. (2023) 10:983515–5. doi: 10.3389/fnut.2023.983515

29. Harreiter J, Mendoza LC, Simmons D, Desoye G, Devlieger R, Galjaard S, et al. Vitamin D3 supplementation in overweight/obese pregnant women: no effects on the maternal or fetal lipid profile and body fat distribution-a secondary analysis of the multicentric, randomized, controlled vitamin D and lifestyle for gestational diabetes prevention trial (DALI). *Nutrients*. (2022) 14:3781. doi: 10.3390/nu14183781

30. Li J-M, Yang H-Y, Wu S-H, Dharmage SC, Jalaludin B, Knibbs LD, et al. The associations of particulate matter short-term exposure and serum lipids are modified by vitamin D status: a panel study of young healthy adults. *Environ Pollut*. (2023) 317:120686. doi: 10.1016/j.envpol.2022.120686

31. Alsufiani HM, AlGhamdi SA, AlShaibi HF, Khoja SO, Saif SF, Carlberg C. A single vitamin D3 bolus supplementation improves vitamin D status and reduces proinflammatory cytokines in healthy females. *Nutrients*. (2022) 14:3963. doi: 10.3390/nu14193963

32. Zhu JG. Guidelines for prevention and treatment of dyslipidemia in Chinese adults (2016 revised edition). *Chin Circulat J*. (2016) 31:937–53.

33. Phanmaneelux N, Buranakitjaroen P, Kunanon S, Roubsanthisuk W, Chotruangnapa C. The ASSOCIATION between hypertension subtypes defined by home blood pressure in hypertensive patients and incidence of hypertension mediated organ damage. *J Hypertens*. (2024) 42:e203. doi: 10.1097/01.hjh.0001021500.24571.d8

34. de Waal E, Pienaar AE. Influences of persistent overweight on perceptual-motor proficiency of primary school children: the north-west CHILD longitudinal study. *BMC Pediatr*. (2021) 21:245–10. doi: 10.1186/s12887-021-02708-x

35. Stach K, Richter H, Fraass U, Stein A. Quantifying the "distance to LDC-goal" in patients at very high cardiovascular risk with hyperlipidaemia in Germany: a retrospective claims database analysis. *Ther Adv Cardiovasc Dis*. (2024) 18:17539447241277402. doi: 10.1177/17539447241277402

36. Chen Zengli JY, Yutong L, Jie L, Shiqin L, Mingting L. Dyslipidemia prevalence in Chinese older adults: a Meta-analysis. *Zhongguo Quanke Yixue*. (2022) 25:115–21. doi: 10.12114/j.issn.1007-9572.2021.00.328

37. van den Heuvel EG, Lips P, Schoonmade LJ, Lanham-New SA, van Schoor NM. Comparison of the effect of daily vitamin D2 and vitamin D3 supplementation on serum 25-Hydroxyvitamin D concentration (Total 25(OH)D, 25(OH)D2, and 25(OH)D3) and importance of body mass index: a systematic review and Meta-analysis. *Adv Nutr*. (2024) 15:100133. doi: 10.1016/j.advnut.2023.09.016

38. Liu C, Chen L. Letter to the editor, "effects of vitamin D deficiency on blood lipids and bone metabolism: a large cross-sectional study.". *J Orthop Surg Res*. (2023) 18:307–7. doi: 10.1186/s13018-023-03773-x

39. Herrmann M, Keppel M, Zelzer S, Pilz S, Scharnagl H, Kleber M, et al. The role of functional vitamin D deficiency and low vitamin D reservoirs in relation to cardiovascular health and mortality. *Clin Chim Acta*. (2024) 558:118230. doi: 10.1016/j.cca.2024.118230

40. Ren C, Zhang S, Hong B, Guan L, Huang W, Feng J, et al. Germinated brown rice relieves hyperlipidemia by alleviating gut microbiota dysbiosis. *J Integr Agric*. (2023) 22:945–57. doi: 10.1016/j.jia.2023.02.015

41. Xie Z, Li E, Gao G, Du Y, Wang M, Wang H, et al. Zexie tang targeting FKBP38/mTOR/SREBPs pathway improves hyperlipidemia. *J Ethnopharmacol*. (2022) 290:115101–1. doi: 10.1016/j.jep.2022.115101

42. Karr S. Epidemiology and management of hyperlipidemia. *Am J Manag Care*. (2017) 23:S139–48.

43. Wan Z, Guo J, Pan A, Chen C, Liu L, Liu G. Association of Serum 25-Hydroxyvitamin D concentrations with all-cause and cause-specific mortality among individuals with diabetes. *Diabetes Care*. (2021) 44:350–7. doi: 10.2337/dc20-1485

44. Li X. The FGF metabolic axis. *Front Med*. (2019) 13:511–30. doi: 10.1007/s11684-019-0711-y

45. Harahap IA, Landrier J-F, Suliburska J. Interrelationship between vitamin D and calcium in obesity and its comorbid conditions. *Nutrients*. (2022) 14:3187. doi: 10.3390/nu14153187

46. Ruiz-Ojeda FJ, Anguita-Ruiz A, Leis R, Aguilera CM. Genetic factors and molecular mechanisms of vitamin D and obesity relationship. *Ann Nutr Metab*. (2018) 73:89–99. doi: 10.1159/000490669

47. Riek AE, Oh J, Bernal-Mizrachi C. Vitamin D regulates macrophage cholesterol metabolism in diabetes. *J Steroid Biochem Mol Biol*. (2010) 121:430–3. doi: 10.1016/j.jsbmb.2010.03.018

48. Li C, Fu J, Ye Y, Li J, He Y, Fang T. The impact of vitamin D on the etiopathogenesis and the progression of type 1 and type 2 diabetes in children and adults. *Front Endocrinol*. (2024) 15:1360525–5. doi: 10.3389/fendo.2024.1360525

49. Pittas AG, Jorde R, Kawahara T, Dawson-Hughes B. Vitamin D supplementation for prevention of type 2 diabetes mellitus: to D or not to D? *J Clin Endocrinol Metab*. (2020) 105:3721–33. doi: 10.1210/clinem/dgaa594

50. Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Shaw JE, Zimmet PZ, et al. Low serum 25-Hydroxyvitamin D is associated with increased risk of the development of the metabolic syndrome at five years: results from a national, population-based prospective study (The Australian diabetes, obesity and lifestyle study: AusDiab). *J Clin Endocrinol Metab*. (2012) 97:1953–61. doi: 10.1210/jc.2011-3187

51. Muscella A, Stefàno E, Lunetti P, Capobianco L, Marsigliante S. The regulation of fat metabolism during aerobic exercise. *Biomolecules*. (2020) 10:1699. doi: 10.3390/biom10121699

52. Janssen HCJP, Emmelot-Vonk MH, Verhaar HJJ, van der Schouw YT. Determinants of vitamin D status in healthy men and women aged 40–80 years. *Maturitas*. (2013) 74:79–83. doi: 10.1016/j.maturitas.2012.10.008

53. Zaydieva YZ, Balan VE. Vitamin D and women's reproductive health (literature review). *Medicinskij Sovet*. (2018):164–72. doi: 10.21518/2079-701X-2018-12-164-172

54. Ciarambino T, Crispino P, Minervini G, Giordano M. Vitamin D: can gender medicine have a role? *Biomedicines*. (2023) 11:1762. doi: 10.3390/biomedicines11061762

55. Lupton JR, Faridi KF, Martin SS, Sharma S, Kulkarni K, Jones SR, et al. Deficient serum 25-hydroxyvitamin D is associated with an atherogenic lipid profile: The very large database of lipids (VLDL-3) study. *J Clin Lipidol*. (2016) 10:72–81.e1. doi: 10.1016/j.jacl.2015.09.006

56. Jorde R, Figenschau Y, Hutchinson M, Emaus N, Grimnes G. High serum 25-hydroxyvitamin D concentrations are associated with a favorable serum lipid profile. *Eur J Clin Nutr*. (2010) 64:1457–64. doi: 10.1038/ejcn.2010.176

57. Giustina A, Bouillon R, Dawson-Hughes B, Ebeling PR, Lazaretti-Castro M, Lips P, et al. Vitamin D in the older population: a consensus statement. *Endocrine*. (2023) 79:31–44. doi: 10.1007/s12020-022-03208-3

58. Qorbani M, Zarei M, Moradi Y, Appannah G, Djalainia S, Pourrostami K, et al. Effect of vitamin D supplementation on cardiac-metabolic risk factors in elderly: a systematic review and meta-analysis of clinical trials. *Diabetol Metab Syndr*. (2022) 14:88–8. doi: 10.1186/s13098-022-00859-0

59. Tanaka K, Ao M, Tamaru J, Kuwabara A. Vitamin D insufficiency and disease risk in the elderly. *J Clin Biochem Nutr*. (2024) 74:9–16. doi: 10.3164/jcbn.23-59

60. Wyskida M, Wieczorowska-Tobis K, Chudek J. Prevalence and factors promoting the occurrence of vitamin D deficiency in the elderly. *Postepy Hig Med Dosw*. (2017) 71:0–204. doi: 10.5604/01.3001.0010.3804

61. Harvey NC, Ward KA, Agnusdei D, Binkley N, Biver E, Campusano C, et al. Optimisation of vitamin D status in global populations. *Osteoporos Int*. (2024) 35:1313–22. doi: 10.1007/s00198-024-07127-z

62. Mendes MM, Gomes APO, Araújo MM, Coelho ASG, Carvalho KMB, Botelho PB. Prevalence of vitamin D deficiency in South America: a systematic review and meta-analysis. *Nutr Rev*. (2023) 81:1290–309. doi: 10.1093/nutrit/nuad010

63. Dibaba DT. Effect of vitamin D supplementation on serum lipid profiles: a systematic review and meta-analysis. *Nutr Rev*. (2019) 77:890–902. doi: 10.1093/nutrit/nuz037

64. Chen X, Zhou M, Yan H, Chen J, Wang Y, Mo X. Association of serum total 25-hydroxy-vitamin D concentration and risk of all-cause, cardiovascular and malignancies-specific mortality in patients with hyperlipidemia in the United States. *Front Nutr*. (2022) 9:971720–0. doi: 10.3389/fnut.2022.971720

65. Liu W, Wu Z, Zhu D, Chen G, Yan G, Zhang S, et al. Vitamin D and lipid profiles in postmenopausal women: a meta-analysis and systematic review of randomized controlled trials. *Front Mol Biosci*. (2021) 8:799934–4. doi: 10.3389/fmolb.2021.799934

66. Williams DM, Fraser A, Lawlor DA. Associations of vitamin D, parathyroid hormone and calcium with cardiovascular risk factors in US adolescents. *Heart*. (2011) 97:315–20. doi: 10.1136/hrt.2010.203224

67. Zittermann A, Trummer C, Theiler-Schwetz V, Lerchbaum E, März W, Pilz S. Vitamin D and cardiovascular disease: an updated narrative review. *Int J Mol Sci*. (2021) 22:2896. doi: 10.3390/ijms22062896

68. Lee SS, Ling KH, Tusimin M, Subramaniam R, Rahim KF, Loh SP. Interplay between maternal and neonatal vitamin D deficiency and vitamin-D-related gene polymorphism with neonatal birth anthropometry. *Nutrients*. (2022) 14:564. doi: 10.3390/nu14030564

69. Lin S, Li J, Zhang Y, Song X, Chen G, Pei L. Maternal passive smoking, vitamin D deficiency and risk of spontaneous abortion. *Nutrients*. (2022) 14:3674. doi: 10.3390/nu14183674

70. Khoshnaw M, Dizaye K. Beneficial effects of vitamin D in the management of untreated hyperlipidemia in diabetic patients in Erbil, Iraq. *Farmacija*. (2022) 69:847–53. doi: 10.3897/pharmacia.69.e90908

71. Jaun F, Boesing M, Luethi-Corridori G, Abig K, Bloch N, Giezendanner S, et al. Effect of single high dose vitamin D substitution in hospitalized COVID-19 patients with vitamin D deficiency on length of hospital stay. *Biomedicines*. (2023) 11:1277. doi: 10.3390/biomedicines11051277

72. Pludowski P. Supplementing vitamin D in different patient groups to reduce deficiency. *Nutrients*. (2023) 15:3725. doi: 10.3390/nu15173725

73. Patriota P, Rezzi S, Guessous I, Marques-Vidal P. Vitamin D and Weight Change: A Mendelian Randomization, Prospective Study. *International journal of Molecular Sciences*. (2022) 23:11100. doi: 10.3390/ijms231911100

74. Rajakumar K, Moore CG, Khalid AT, Vallejo AN, Virji MA, Holick MF, et al. Effect of vitamin D3 supplementation on vascular and metabolic health of vitamin D–deficient overweight and obese children: a randomized clinical trial. *The American Journal of Clinical Nutrition*. (2020) 111:757–768. doi: 10.1093/ajcn/nqz340

75. Mead MJ, McWhorter CA, Rodgers MD, Ebeling MD, Shary JR, Gregoski MJ, et al. Does maternal vitamin D status influence placental weight or vascular and inflammatory pathology? Secondary analysis from the Kellogg Pregnancy Study. *The Journal of steroid biochemistry and molecular biology*. (2023) 233:106358–106358. doi: 10.1016/j.jsbmb.2023.106358

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# Liposomal encapsulation of cholecalciferol mitigates *in vivo* toxicity and delays tumor growth

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**Introduction:** Vitamin $D_3$ (cholecalciferol) has demonstrated potential anticancer properties, but its clinical application is limited by associated toxicity at effective doses. This study investigated the use of liposomal encapsulation to increase the therapeutic efficacy of vitamin $D_3$ while mitigating its toxicity.

**Methods:** Liposomal vitamin $D_3$ (VD-LP) was prepared via the film-hydration method and characterized for particle size, polydispersity index, encapsulation efficiency, and long-term stability. *In vitro* gene expression modulation was evaluated in monocytic THP-1 cells, and antiproliferative effects were assessed in HT29 (colorectal), BT474 (breast), and TRAMP-C1 (prostate) cancer cell lines. *In vivo* antitumor efficacy and toxicity were tested in a mouse model with subcutaneously implanted MC38 tumors. Tumor growth, survival rates, and serum calcium and phosphate levels were analyzed.

**Results:** VD-LP demonstrated high encapsulation efficiency and stability over 90 days, with a consistent particle size of approximately 83 nm. VD-LP modulated immune-related and metabolic gene expression in THP-1 cells, including upregulation of antimicrobial peptides and vitamin D receptor genes. VD-LP showed superior antiproliferative effects compared to free vitamin $D_3$ in all tested cancer cell lines. *In vivo*, VD-LP delayed tumor growth and improved survival without causing hypercalcemia, highlighting its favorable toxicity profile.

**Discussion:** Liposomal encapsulation of vitamin $D_3$ significantly improves its anticancer efficacy while mitigating toxicity, making it a promising strategy for future cancer therapies. VD-LP shows potential for enhanced therapeutic applications with reduced adverse effects, warranting further clinical exploration.

KEYWORDS

**liposomal encapsulation, vitamin $D_3$, anticancer efficacy, gene expression, tumor growth**

# 1 Introduction

Vitamin D, particularly its active form, vitamin $D_3$ (cholecalciferol), is traditionally known for its role in calcium homeostasis and bone health (1). However, emerging evidence over the past few decades has revealed a much broader spectrum of biological functions, including modulation of the immune system, regulation of cell proliferation and differentiation, and a potential role in the prevention and treatment of various malignancies (2). Vitamin $D_3$ exerts its effects primarily through the vitamin D receptor (VDR), a nuclear receptor that regulates the expression of numerous genes involved in cellular growth, immune responses, and metabolic processes (3).

Several epidemiological studies have suggested an inverse relationship between vitamin D levels and the incidence of various cancers, including colorectal, breast, and prostate cancers (2). *In vitro* and *in vivo* studies have further supported these observations, demonstrating that vitamin $D_3$ can induce cell cycle arrest, promote apoptosis, inhibit angiogenesis, and modulate the tumor microenvironment. These anticancer effects are believed to be mediated through the activation of the VDR and subsequent transcriptional regulation of target genes involved in these processes (4).

Despite the promising anticancer properties of vitamin $D_3$, its clinical application has been significantly hindered by its narrow therapeutic window (5). At the therapeutic doses required to exert anticancer effects, vitamin $D_3$ can induce hypercalcemia, hypercalciuria, and other toxic effects, limiting its safe administration (6). Hypercalcemia resulting from vitamin $D_3$ toxicity can manifest as nephrocalcinosis, renal failure, cardiac arrhythmias, and soft tissue calcification, posing significant risks to patients (7). The primary mechanism of this toxicity involves the dysregulation of calcium absorption and mobilization due to the overactivation of the VDR (8). Consequently, vitamin $D_3$'s therapeutic potential is often overshadowed by its narrow therapeutic index, which limits its safe and effective use, especially in high doses required for treating conditions such as cancer. This challenge has prompted the exploration of various strategies aimed at enhancing its efficacy while minimizing toxicity.

One widely explored approach involves the development of vitamin $D_3$ analogues with modified structures to retain biological activity while reducing the risk of hypercalcemia. These analogues, such as calcipotriol and paricalcitol, have shown promise in specific applications, particularly in dermatological and renal contexts. However, their broader use remains limited due to variable efficacy and potential off-target effects (9–13).

Another strategy involves combining vitamin $D_3$ with agents that either enhance its antitumor activity or mitigate toxicity. For instance, pairing vitamin $D_3$ with chemotherapeutic drugs or immune modulators has demonstrated synergistic effects in preclinical models. Despite these advances, such combinations require careful dose calibration to prevent adverse interactions and maintain safety (14–17).

Liposomal encapsulation of vitamin $D_3$ represents a complementary and innovative approach that directly addresses the dual challenges of bioavailability and toxicity. Unlike free vitamin $D_3$ or its analogues, liposomal formulations provide enhanced stability, targeted delivery, and sustained release, which collectively reduce systemic toxicity while improving therapeutic efficacy. While not the only solution, liposomal encapsulation offers unique advantages that make it a particularly promising strategy for clinical applications, especially in oncology. Liposomal encapsulation has emerged as a promising approach to improve the therapeutic index of various drugs, including those with poor solubility, poor stability, or significant toxicity. Liposomes are spherical vesicles composed of phospholipid bilayers that can encapsulate hydrophobic or hydrophilic drugs within their core or membrane. This encapsulation can protect the drug from degradation, increase its bioavailability, and provide controlled release, thereby reducing the frequency and dose of administration required (18).

The use of liposomal formulations has been particularly advantageous in cancer therapy, as they can facilitate targeted delivery to tumor tissues while minimizing systemic exposure and toxicity. Liposomes can preferentially accumulate in tumor tissues through the enhanced permeability and retention (EPR) effect, a phenomenon resulting from the leaky vasculature and poor lymphatic drainage typically associated with tumors. This targeted delivery not only enhances the therapeutic efficacy of the drug but also reduces the adverse effects on healthy tissues (19).

Given the challenges associated with the systemic administration of vitamin $D_3$, liposomal encapsulation represents a potential strategy to enhance its anticancer effects while minimizing toxicity. Previous studies have demonstrated that liposomal vitamin $D_3$ (VD-LP) can increase bioavailability, leading to more pronounced biological effects at lower doses (20). Furthermore, liposomal encapsulation may protect vitamin $D_3$ from rapid degradation in the bloodstream, thereby extending its half-life and improving its therapeutic efficacy (21).

In this context, our study aimed to explore the potential of liposomal encapsulation to overcome the limitations associated with vitamin $D_3$ therapy in cancer. We hypothesized that compared with VD, VD-LP would demonstrate enhanced stability, reduced toxicity, and improved anticancer efficacy. To test this hypothesis, we conducted a series of *in vitro* and *in vivo* experiments to evaluate the physicochemical properties, biological activity, and therapeutic potential of VD-LP. Our findings suggest that liposomal encapsulation not only enhances the delivery and efficacy of vitamin $D_3$ but also significantly reduces the risk of hypercalcemia and other toxic effects, thus offering a safer alternative for clinical use.

# 2 Materials and methods

## 2.1 Vitamin $D_3$ liposome preparation

Vitamin $D_3$ (cholecalciferol) was purchased from Sigma (Spain); phosphatidylcholine-hydrogenated (HSPC), cholesterol (CH), and DSPE-PEG2K were purchased from Avanti Polar Lipids (USA). Other reagents were of analytical grade.

VD-LP was prepared via the film-hydration method. The methodology was based on a previous protocol (22, 23).

Briefly, lipids and VD (HSPC: CH : VD: DSPE-PEG2K 60:35:4.5:5, molar ratio) were dissolved in a solution of chloroform:methanol [9:1 (v/v)]. The mixture was dried by rotary evaporation at 40°C (Büchi, Switzerland) to form a film, which was hydrated with HEPES buffer (pH 6.7) (Gibco, Waltham, Massachusetts, USA). Finally, for homogenization of the particle size, the liposomal solution was extruded through several polycarbonate membranes (from 200 nm in size to 80 nm in size). This protocol was also followed to formulate empty liposomes (HSPC: CH : DSPE-PEG2K, 65:35:5 molar ratio). The purification of the VD-LP was carried out by size exclusion chromatography using a PD10 column (loaded with Sephadex-25) (GE Healthcare, Madrid, Spain).

## 2.2 Characterization of vitamin $D_3$ liposomes

The particle size, polydispersity index (PDI) and zeta potential were analyzed via laser diffractometry (DLS) using a Zetasizer Nano Series system (Malvern Instruments, UK). The lipid concentration was measured via a phosphate assay, and the encapsulation efficiency of VD was measured via a Nanodrop at 265 nm. The long-term stability of the VD-LP preserved at 4°C was assayed for 90 days.

For the morphological characterization of the formulations, transmission electronic microscopy (FE-SEM Zeiss Sigma 300 VP) was used to analyze the samples. Briefly, the samples suspended in ddH2O were laid on copper grids with a film of formvar (EMS, FF200-cu) for 2.5 min at room temperature. These samples were washed twice with $ddH_2O$, and negative staining with 1% uranyl acetate for 15 s was performed.

## 2.3 Cell lines and culture

Tumor cell lines were grown with appropriate culture media. Adherent murine MC38 (University of Washington, Seattle, USA) and human HT29 (ATCC; HTB-38) colorectal cancer cells were cultured with RPMI 1640 (Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco), 100 IU penicillin and 100 µg/mL streptomycin (1% P/S; Gibco). The human BT474 breast cancer cell line (Hospital del Mar, Barcelona, Spain) was seeded with DMEM F12, 10% (v/v) FBS and 1% P/S. The murine TRAMP-C1 prostate cancer cell line (ATCC; CRL-2730) was cultured with DMEM F12, 10% (v/v) FBS, 1% P/S, 0.005 mg/mL bovine insulin (Sigma) and 10 $nmol{\cdot}L^{-1}$ dehydroisoandrosterone 90% (ACROS Organics, Thermo Fisher Scientific, Spain). The human monocytic leukemia cell line THP-1 (ATCC, TIB-202) was grown in suspension with RPMI 1640, 10% (v/v) FBS, 1% P/S and 0.9 µL/mL 2-mercaptoethanol. All the samples were incubated in a 5% $CO_2$ humidified atmosphere at 37°C.

## 2.4 Evaluation of the modulation of gene expression

In a 96-well plate, $3{\times}10^5$ cells/well/150 µL were seeded, and the cells were subsequently stimulated with 10 µL of VD-LP containing 0.25 µM vitamin $D_3$, 10 µL of empty-liposomes (Empty-LP) or 0.25 µM VD mixed in a volume of 50 µL/well in triplicate. Vitamin $D_3$ was prepared as a concentrated stock solution by dissolving it in absolute ethanol. The stock solution was diluted in the cell culture medium to achieve the final working concentration. The final ethanol concentration in the culture medium was less than 0.1%, a level that does not induce toxicity in the cells. This preparation ensured the solubility of $VD_3$ and its bioavailability for comparison with the liposomal-encapsulated vitamin $D_3$ formulation. For 24 h, the plates were incubated in a 5% $CO_2$ atmosphere at 37°C.

RNA extraction was carried out with a Maxwell® RSC simple RNA Tissue Kit and Instrument (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. The RNA was quantified with a NanoDrop spectrophotometer (Thermo Scientific Wilmington, Delaware, USA).

Reverse transcription was performed from 300 ng of RNA with reverse transcriptase (Promega). Amplification from generated cDNA was performed with iQ SYBR Green Supermix (Bio-Rad, Hercules, California, USA). Specific forward (Fw) and reverse (Rv) primer sequences for each gene were purchased from Invitrogen (Thermo Fisher). Housekeeping gene RPLP0 Fw 5′-aacatctccccttctcctt-3′ Rv5′-gaaggccttgacctttcag-3′; cathelicidin antimicrobial peptide (hCAMP) Fw 5′-tgggcctggtgatgcct-3′ Rv 5′-cgaaggacagcttccttgtagc-3′. Vitamin D receptor (hVDR) Fw 5'-gtggacatcggcatgatgaag-3' Rv 5'-ggtcg aggtcttatggtggg-3'. ATP-binding cassette subfamily D member 2 (ABCD2) Fw 5'-aatggaccagatcgagtgctg-3' Rv 5'-tgggatagagggttttcagagc-3'. Fructose-1,6-biphosphatase 1 (FBP1) Fw 5'-cgcgcacctctatggcatt-3' Rv 5'-ttcttctgacacgagaacacac-3'. Neuronal growth regulator 1 (NEGR1) Fw 5'-gcttgttgctcgaaccagtg-3' Rv 5'-ccccttttctgaccatcatgtt-3'. The resulting amount of each transcript was expressed via the formula $2^{\Delta Ct}$.

## 2.5 RNA sequencing

RNA sequencing (RNAseq) analysis was performed to analyze MC38 tumors treated *in vivo* and THP-1 cells treated *in vitro*. MC38 tumors were isolated 48 h after the last dose. A total of $3.5 \times 10^5$ THP-1 cells were stimulated with 10 µL of VD-LP containing 0.25 µM vitamin $D_3$, 10 µL of Empty-LP or 0.25 µM VD in triplicate for 24 hours. In both cases, RNA was isolated with a Maxwell® RSC simple RNA Tissue Kit and Instrument (Promega) and quantified with a NanoDrop spectrophotometer (ThermoScientific). Starting from the isolated RNA of the induction assay. The 20 ng/µL samples were sequenced by the Genomic Unit of the Center for Applied Medical Research (CIMA, University of Navarra). All the RNA samples were high-quality, with RIN values greater than 7. Library preparation was performed via the Illumina Stranded mRNA Prep Ligation Kit (Illumina) following the manufacturer's protocol. All sequencing libraries were constructed from 100 ng of total RNA according to the manufacturer's instructions. The protocol selects and purifies poly(A)-containing RNA molecules via magnetic beads coated with poly(T) oligos. Poly(A)-RNAs are fragmented and reverse transcribed into the first cDNA strand via random primers. The second cDNA strand is synthesized in the presence

of dUTP to ensure strand specificity. The resulting cDNA fragments were purified with AMPure XP beads (Beckman Coulter), adenylated at 3′ ends and then ligated with uniquely indexed sequencing adapters. Ligated fragments are purified and PCR amplified to obtain the final libraries. The quality and quantity of the libraries were verified via a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and 4200 TapeStation with High Sensitivity D1000 ScreenTape (Agilent Technologies). Libraries were then sequenced via a NextSeq2000 sequencer (Illumina). Forty million pair-end reads (100 bp; Rd1:51; Rd2:51) were sequenced for each sample and demultiplexed via Cutadapt. RNA-seq was carried out at the Genomics Unit of CIMA.

## 2.6 *In vitro* proliferation assay

Analyses were carried out on a Real-Time Cell Analyzer xCELLigence. The HT29, BT474 and TRAMP-C1 cell lines were seeded in a 16-well E-Plate 16 PET (Aligent, Adelaida, South Australia, AUS) at a concentration of $3.5 \times 10^4$ cells/well/100 µL. For 4–6 hours, readings were collected from the plate until exponential cell growth occurred. The treatments were subsequently added in duplicate: 0.25 µM VD, 10 µL of VD-LP containing 0.25 µM vitamin $D_3$ and 10 µL of Empty-LP. Readings were collected for an additional 72–96 hours.

## 2.7 *In vivo* antitumor efficacy

C57BL/6J mice (female, 5 weeks old) were purchased from Harlan Laboratories (Barcelona, Spain) and maintained under a 12 h light/dark cycle with free access to food and water. After trypsinization of the MC38 cells and counting of the cells (98% viability) and previous shaving, each mouse received a subcutaneous injection in the flank of the right hind leg of $5 \times 10^5$ cells resuspended in 100 µL of PBS and randomly assigned to treatment cohorts: control/HEPES, VD, VD-LP and Empty-LP. A dose of 30 µg of vitamin $D_3$ was given to each mouse in the VD and VD-LP groups, and the equivalent quantity of lipids in Empty-LP and VD-LP was determined. All the treatments were administered intravenously through the lateral tail vein in a volume of 200 µL every 2 days for a total of 3 doses/mouse. The tumors were measured 2 days a week. Twenty-four hours after the last administration, serum samples were drawn to analyze the calcium ($Ca^{2+}$) and phosphate ($PO_4^{3-}$) levels via a chemical analyzer (Cobas c311, Roche).

## 2.8 Statistical analysis

The software used for statistical analysis was GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, California, USA). The data were analyzed via one-way ANOVA followed by ordinary ANOVA, the Kruskal-Wallis test, Sidak's multiple comparisons test or the log-rank Mantel-Cox test. Significant differences *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

# 3 Results

## 3.1 Characterization and stability of the VD-LP formulation

The physicochemical properties of the VD-LP formulation were evaluated over a 90-day period at 4°C to assess its stability. The particle size, polydispersity index (PDI), and encapsulation efficiency (EE) of VD-LP remained stable throughout the study. The mean particle size of the VD-LP was consistently less than 100 nm (mean ± SD: 82.69 ± 0.984 nm), with a PDI of 0.041 ± 0.012, indicating a narrow size distribution. The encapsulation efficiency was high, with a mean value of 94.85% ± 4.82% (Figure 1A, Table 1). Transmission electron microscopy (TEM) confirmed the spherical morphology of the liposomes and their uniform size distribution (Figure 1B). These results indicate that the VD-LP formulation is stable and uniform throughout the storage period.

## 3.2 Modulation of gene expression by VD-LP in THP-1 cells

To evaluate the biological activity of VD-LP, we performed gene expression analysis on human monocytic THP-1 cells following treatment with VD-LP, VD, or Empty-LP (Figure 2A). RNA-seq revealed significant differences in gene expression profiles among the treatment groups. Cells treated with free VD presented the fewest changes in gene expression, likely reflecting the limited ability of free VD to penetrate the cytoplasm and activate the vitamin D receptor (Figures 2B, C). In contrast, both VD-LP and Empty-LP had more pronounced effects on gene expression (Figures 2B, C). Notably, Empty-LP induced the greatest number of gene expression changes (Figure 2B), likely due to its promotion of inflammatory pathways, with TNF signaling being the most prominently upregulated pathway (Figure 2D). For VD-LP, the upregulated pathways were related primarily to metabolic processes. These findings indicate that incorporating vitamin D into liposomes helps prevent the activation of inflammatory responses while facilitating intracellular vitamin $D_3$ activity (Figure 2D). To validate these findings, real-time PCR analysis was conducted, confirming that VD-LP treatment led to the upregulation of key genes such as cathelicidin antimicrobial peptide (hCAMP), vitamin D receptor (hVDR), ATP-binding cassette subfamily D member 2 (ABCD2), neuronal growth regulator 1 (NEGR1) and fructose-1,6-bisphosphatase 1 (FBP1). Specifically, fold-change increases in expression were: hCAMP 5-fold, hVDR 1.5-fold, ABCD2 2.3-fold, NEGR1 1.6-fold, and FBP1 3.7-fold (Figure 2E). Overall, VD-LP treatment effectively modulated the expression of genes involved in the immune response and metabolism in THP-1 cells.

## 3.3 Antiproliferative effects of VD-LP in cancer cell lines

The antiproliferative effects of VD-LP were assessed in three cancer cell lines: HT29 (human colorectal cancer), BT474 (human

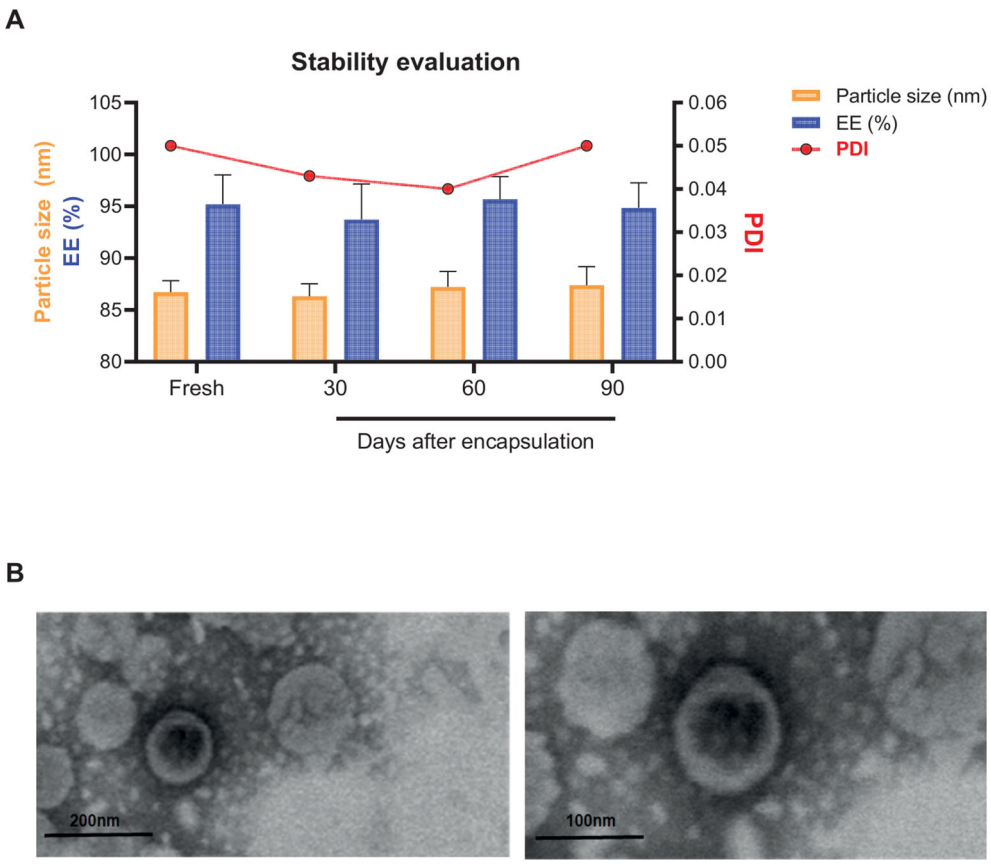


FIGURE 1
Characterization and stability of the VD-LP formulation. **(A)** Stability evaluation of VD-LP over 90 days at 4°C. The particle size (nm), polydispersity index (PDI), and encapsulation efficiency (EE%) were monitored and remained stable throughout the study period. The data represent the means ± SDs of three independent batches. **(B)** Transmission electron microscopy (TEM) images of the VD-LP showing spherical liposomes with sizes of less than 100 nm. The images confirmed the uniform morphology of the liposomes.

breast cancer), and TRAMP-C1 (mouse prostate cancer). Using real-time cell analysis via the xCELLigence system, we found that compared with no treatment, VD-LP treatment significantly inhibited the proliferation of all three cell lines. At 40 hours, VD-LP treatment resulted in a significant reduction in cell proliferation: 86.4% in HT29 cells, 56.1% in BT474 cells, and 84.5% in TRAMP-C1 cells. These results demonstrate a pronounced sensitivity of HT29 and TRAMP-C1 cells to VD-LP, with a more moderate effect observed in BT474 cells (Figure 3).

TABLE 1 Physicochemical characterization of empty and VD liposomal formulations.

| | Empty-LP | VD-LP |
|---|---|---|
| **Particle size (nm)** | 96.72 ± 0.949 | 82.69 ± 0.984 |
| **PDI** | 0.046 ± 0.009 | 0.041 ± 0.012 |
| **Zeta potential (mV)** | -6.24 ± 1.05 | -24.6 ± 2.54 |
| **EE (%)** | – | 94.85 ± 4.82 |

PDI, polydispersity index; EE, encapsulation efficiency.
The data represent the means ± SDs of three independent batches.

## 3.4 *In vivo* antitumor efficacy and toxicity of VD-LP

To evaluate the antitumor efficacy of VD-LP *in vivo*, we employed a mouse model in which MC38 colon carcinoma cells were subcutaneously implanted. The mice were treated with VD-LP, VD, Empty-LP, or HEPES buffer as a control (Figure 4A). The administration of VD induced acute toxicity, with only one animal surviving long enough to assess tumor growth (Figure 4B). In the remaining experimental groups, no early signs of acute toxicity were observed, allowing for the evaluation of tumor progression. Compared with control mice (median survival time: 26 days) and Empty-LP-treated mice (median survival time: 26 days), which exhibited similar survival rates, those treated with VD-LP showed significantly reduced tumor growth. Moreover, the survival of the VD-LP-treated group was significantly prolonged, with a median survival time of 35.5 days ($p < 0.01$ when compared to the control group) (Figures 4B, C).

To evaluate the potential toxicity of VD-LP, we analyzed the serum calcium and phosphate levels. Interestingly, VD-LP treatment did not cause significant changes in these parameters, suggesting a favorable toxicity profile (Figure 4D). These results

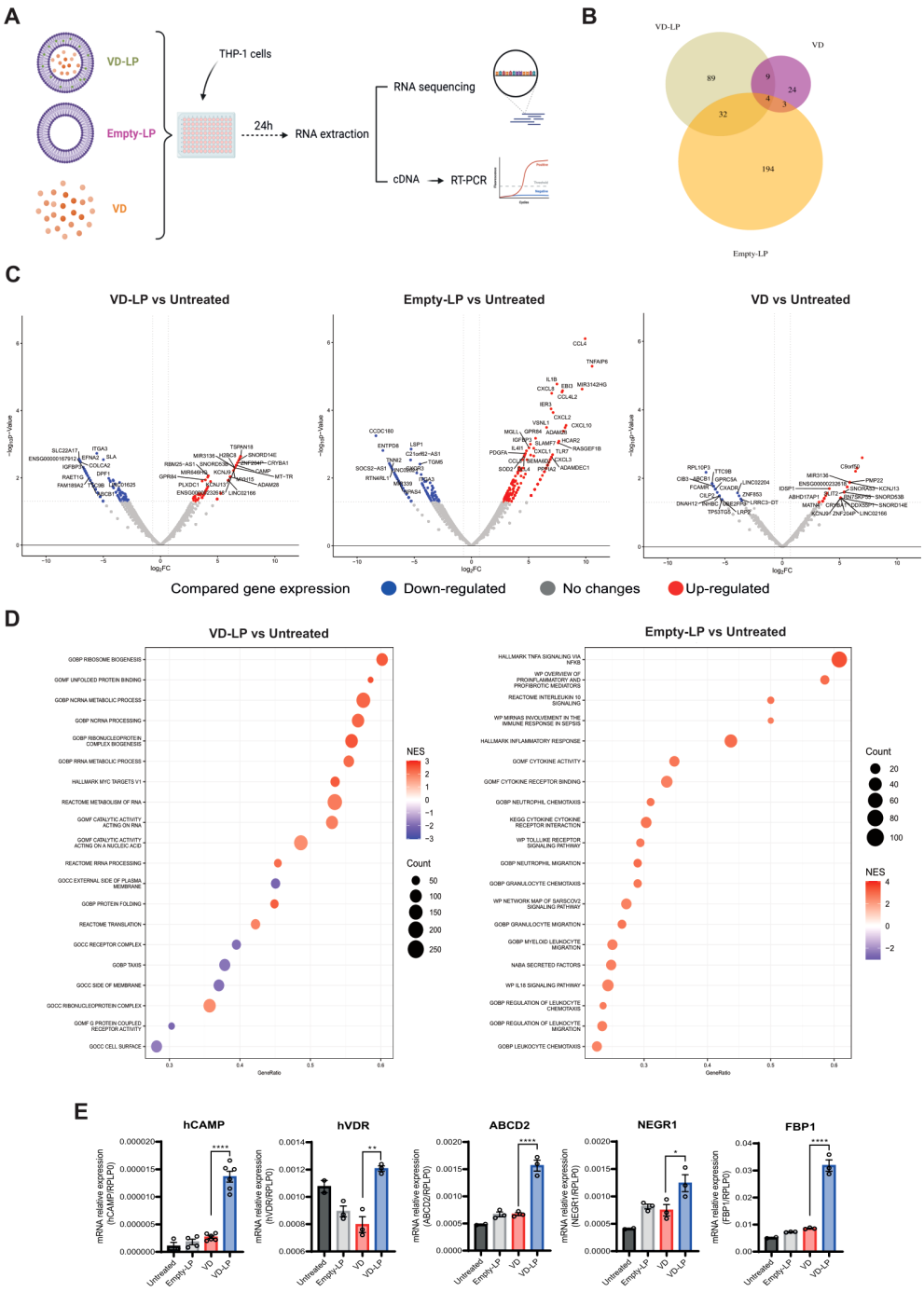


FIGURE 2
Modulation of gene expression by VD-LP in THP-1 cells. **(A)** A total of 300000 THP-1 cells were seeded in a 96-well plate and stimulated for 24 h with VD-LP, VD or Empty-LP. RNA was extracted for RNA-seq, and cDNA was generated for PCR. **(B)** Venn diagram representing the number of modulated genes in each experimental group. **(C)** Volcano plots representing down- and upregulated genes. **(D)** GSEA of differentially regulated pathways. **(E)** Real-time PCR analysis of cathelicidin antimicrobial peptide (hCAMP), vitamin D receptor (hVDR), ATP-binding cassette subfamily D member 2 (ABCD2), fructose-1,6-biphosphatase 1 (FBP1), and neuronal growth regulator 1 (NEGR1). One-way ANOVA with Sidak's multiple comparisons test was used. *p ≤ 0.05, **p ≤ 0.01, ****p ≤0.0001.

underscore the enhanced safety and therapeutic efficacy of the liposomal formulation.

Gene expression analysis was conducted on tumor tissues from VD-LP-treated and control mice to investigate the molecular mechanisms underlying the observed antitumor effects. RNA-seq data revealed significant upregulation and downregulation of genes involved in cell proliferation, apoptosis, and immune response pathways following VD-LP treatment. Volcano plot analysis revealed clear differences in gene expression profiles between the VD-LP-treated group and the control group (Figure 4E). Furthermore, gene set enrichment analysis (GSEA) revealed significant downregulation of multiple pathways related to the

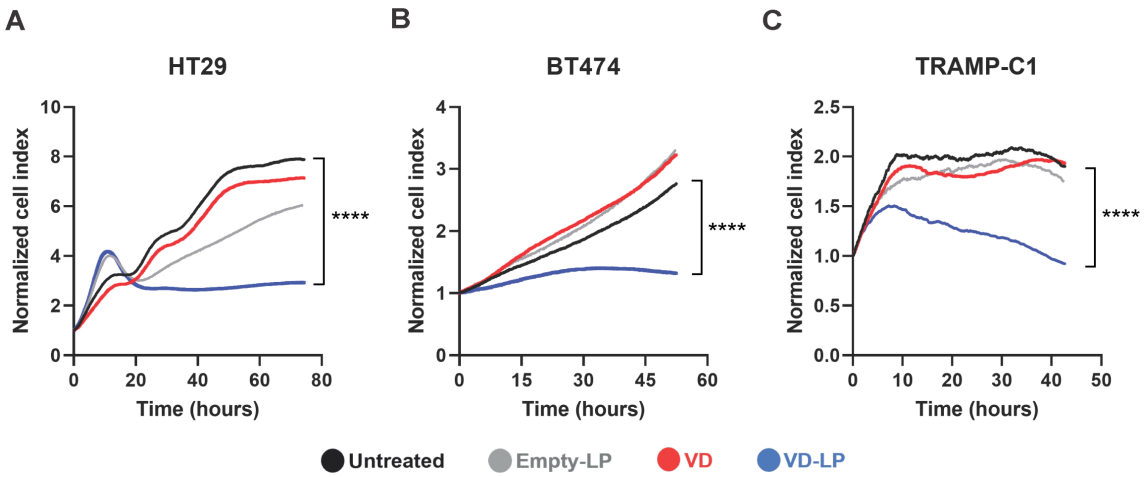


FIGURE 3
Antiproliferative effects of VD-LP in cancer cell lines. **(A–C)** Evaluation of the antiproliferative effects of VD-LP on the HT29 (human colorectal cancer), BT474 (human breast cancer), and TRAMP-C1 (prostate cancer) cell lines. The cells were treated with VD-LP, free VD, or Empty-LP or left untreated, and cell proliferation was monitored via xCELLigence real-time cell analysis. One-way ANOVA with the Kruskal-Wallis test; ****$p \leq 0.0001$.

immune response and inflammation (Figure 4F). These findings suggest that VD-LP exerts its antitumor effects by modulating key inflammatory pathways within the tumor microenvironment.

## 4 Discussion

This study highlights the potential of liposomal encapsulation to enhance the therapeutic efficacy and safety profile of vitamin $D_3$ (cholecalciferol) for cancer treatment. Our findings demonstrate that VD-LP exhibits superior stability, enhanced biological activity, and significant anticancer effects both *in vitro* and *in vivo*, while minimizing the toxicity typically associated with free vitamin $D_3$. These results extend the current knowledge on the advantages of liposomal drug delivery systems, particularly in improving the pharmacokinetics and therapeutic outcomes of hydrophobic drugs such as vitamin $D_3$ (18, 24, 25).

The physicochemical stability of VD-LP, as evidenced by the consistent particle size, polydispersity index (PDI), and encapsulation efficiency (EE) over 90 days at 4°C, suggests that this formulation is highly stable under storage conditions. Our data indicate that VD-LP maintained a mean particle size of ~83 nm with a narrow size distribution (PDI < 0.05) and high EE (~95%). These findings align with previous studies indicating that liposomal encapsulation can improve the absorption of hydrophobic drugs such as vitamin $D_3$, which are otherwise prone to degradation in oily formulations (20). The uniform size and spherical morphology of VD-LP, as confirmed by transmission electron microscopy (TEM), further support its potential for controlled drug delivery, with small, well-defined particles facilitating better biodistribution and tumor-targeting properties (8). In terms of biological activity, gene expression analysis of THP-1 cells revealed that VD-LP significantly modulated the expression of genes involved in the immune response and metabolism. Notably, compared with free vitamin $D_3$, VD-LP treatment led to the upregulation of the cathelicidin antimicrobial peptide (hCAMP) and vitamin D receptor (hVDR) genes, suggesting enhanced immune modulation. This aligns with earlier findings that vitamin $D_3$ induces antimicrobial peptide expression, although the liposomal formulation appeared to further enhance this effect, likely due to improved cellular uptake and sustained release. The limited activity observed in the free vitamin $D_3$ treatment group may be explained by reduced intracellular delivery in the presence of serum. Our previous research has demonstrated that scavenger receptor class B type I is required for 25-hydroxycholecalciferol cellular uptake and signaling. In serum-rich conditions, the lipoproteins may restrict its cellular entry and subsequent activity (26). The liposomal formulation appears to bypass these limitations, facilitating more efficient intracellular delivery and enhancing stability, which likely accounts for the observed differences in gene expression profiles between VD-LP and free vitamin $D_3$ treatments.

The modulation of immune-related genes and pathways, such as hCAMP and VDR, by VD-LP treatment may have implications for its potential role in antitumor responses. These genes are known to influence the immune microenvironment, particularly by enhancing antimicrobial peptide expression and promoting the activation of the vitamin D receptor, which plays a role in immune regulation. Enhanced expression of these genes could contribute to a more robust immune activation, potentially facilitating tumor immune surveillance and control (27, 28).

The observed upregulation of metabolic genes, such as ABCD2 and FBP1, following VD-LP treatment, suggests that the liposomal formulation may influence key pathways in cancer metabolism. ABCD2 is part of the peroxisomal transporter family and has been implicated in the regulation of lipid metabolism, which is critical for energy homeostasis and cellular proliferation in cancer. Enhanced ABCD2 expression may reflect a shift in the metabolic state of tumor cells toward pathways less favorable for tumor progression,

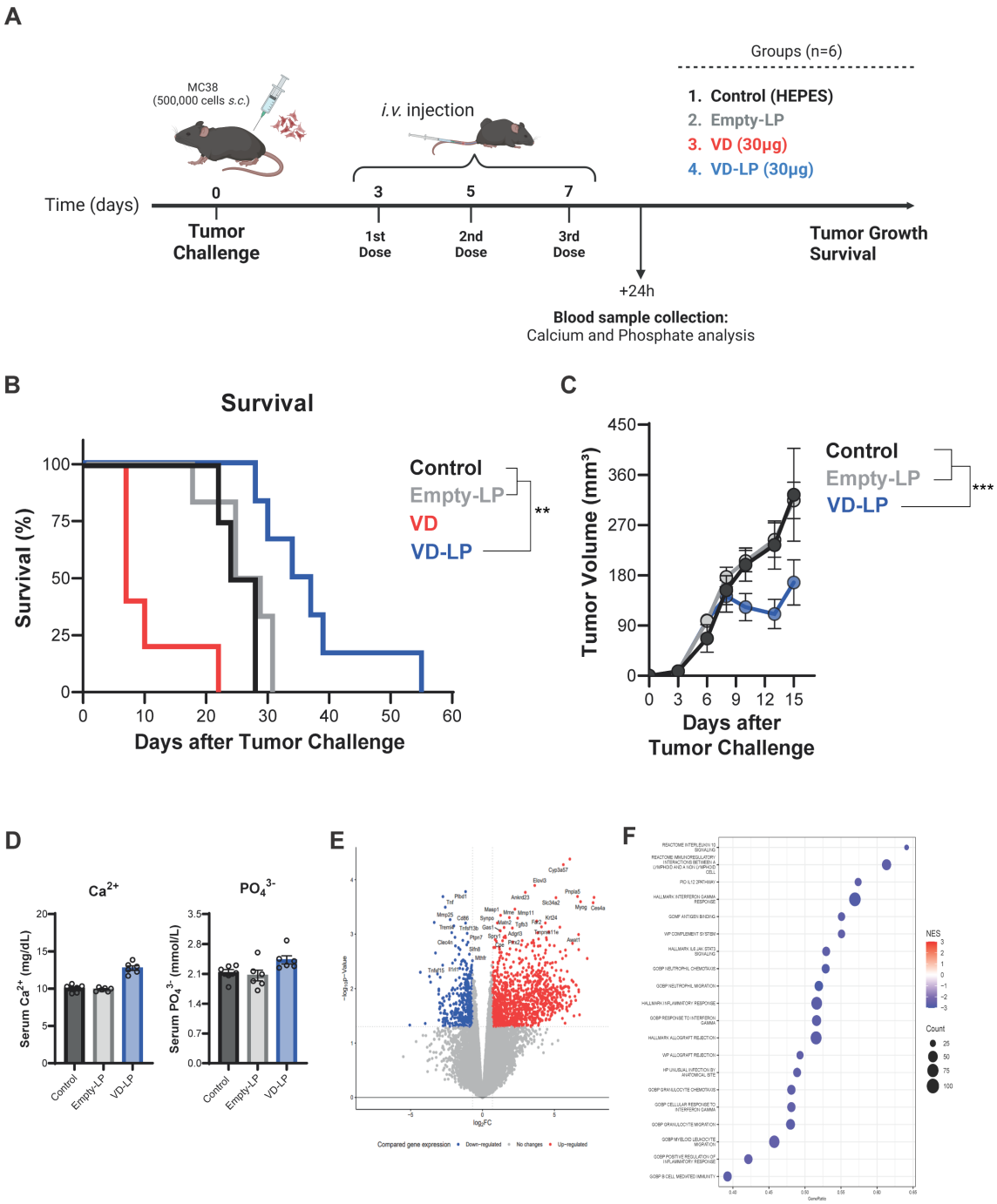


FIGURE 4
*In Vivo* Antitumor Efficacy and Toxicity of VD-LP in a Mouse Model **(A)** Graphical representation of the *in vivo* assay. C57BL/6J mice were subcutaneously inoculated with $5 \times 10^5$ MC38 cells and treated with VD-LP, free VD, Empty-LP, or HEPES buffer (control). **(B)** Kaplan-Meier survival curve for mice treated with VD-LP, free VD, Empty-LP, or HEPES. VD-LP treatment resulted in improved survival rates compared with those of the other groups. Log-rank (Mantel-Cox) test. **(C)** Tumor volume over time in mice treated with VD-LP, free VD, Empty-LP, or HEPES. VD-LP significantly reduced tumor growth compared with that in the control groups. One-way ANOVA with Sidak's multiple comparisons test was used. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. **(D)** Measurement of toxicity: serum calcium ($Ca^{2+}$) and phosphate ($PO_4^{3-}$) levels. **(E)** Volcano plot of differentially expressed genes in tumor tissues from VD-LP-treated versus control mice, highlighting upregulated and downregulated genes. **(F)** GSEA plot showing significant pathways that were activated or inhibited in tumor tissues following VD-LP treatment.

potentially through altered fatty acid oxidation or lipid biosynthesis (29, 30). Similarly, FBP1, a gluconeogenesis-related enzyme, has been associated with tumor suppression in various cancers. Its upregulation may disrupt the glycolytic phenotype typically exhibited by cancer cells, known as the Warburg effect, thereby impairing their metabolic adaptability and proliferation (31–33). These findings point to a broader antitumor potential of VD-LP, not only through immune modulation but also by altering cancer

cell metabolism (34). Future studies could investigate whether these changes in metabolic gene expression directly contribute to tumor growth inhibition or interact synergistically with immune pathways to enhance therapeutic efficacy. This line of research could provide novel insights into the metabolic vulnerabilities of tumors and inform the development of combination therapies targeting both immune and metabolic axes.

Compared with those of untreated controls, the antiproliferative effects of VD-LP on multiple cancer cell lines—HT29, BT474, and TRAMP-C1—were particularly striking, with significant reductions in cell proliferation. These findings build on the work of Krishnan et al., who demonstrated the antiproliferative effects of vitamin $D_3$ on various cancer cell lines through cell cycle arrest and apoptosis (35). However, VD-LP was more effective than free vitamin $D_3$, suggesting that liposomal encapsulation enhances drug efficacy, likely by improving cellular delivery and therefore increasing the intracellular availability of vitamin $D_3$. This finding is consistent with other studies demonstrating the enhanced efficacy of liposomal formulations, such as liposomal paclitaxel and liposomal doxorubicin, compared with their free forms (36).

In an *in vivo* MC38 colon carcinoma mouse model, compared with free vitamin $D_3$ and control treatments, VD-LP treatment significantly inhibited tumor growth and improved survival rates. These findings underscore the potential of VD-LP as a more potent antitumor agent, with enhanced bioavailability and efficacy due to the liposomal delivery system. The reduced toxicity of VD-LP, as evidenced by the absence of hypercalcemia and weight loss, further supports its therapeutic advantage over free vitamin $D_3$, which is often associated with toxicity at high doses. This favorable toxicity profile suggests that VD-LP allows for increased therapeutic dosing without the adverse effects typically linked to high-dose vitamin $D_3$ administration (37).

Gene expression analysis of tumor tissues provided additional insights into the molecular mechanisms underlying the antitumor effects of VD-LP. Significant modulation of genes involved in cell proliferation, apoptosis, and immune response pathways was observed following VD-LP treatment. Notably, GSEA revealed downregulation of immune and inflammatory pathways, suggesting that VD-LP may also exert its antitumor effects by modulating the tumor microenvironment. These findings support previous studies demonstrating the immunomodulatory potential of vitamin $D_3$ and its role in enhancing the immune response against tumors (38). The ability of VD-LP to influence both tumor-intrinsic and immune-related pathways reinforces its potential as a powerful anticancer agent.

To advance the translational potential of the VD-LP formulation, future efforts should focus on defining a clinically viable target product profile. Intravenous administration appears most suitable for systemic delivery, ensuring efficient distribution and bioavailability while minimizing first-pass metabolism. Alternatively, subcutaneous administration could be explored for its convenience in outpatient settings or for use in extended-release formulations. Regarding formulation, a lyophilized product format would offer advantages in terms of long-term stability and ease of storage and transportation. This format could be reconstituted into an injectable solution prior to administration. The final dosage and volume of administration will require optimization to ensure safety and efficacy, particularly in preventing hypercalcemia while maintaining therapeutic benefits. The scalability of the VD-LP formulation is promising, as established liposomal manufacturing technologies, such as high-pressure homogenization or ethanol injection methods, are already compliant with pharmaceutical production standards. These features support the potential for large-scale production and clinical application. Targeting VD-LP as an adjunctive therapy in cancer or other diseases leveraging its immunomodulatory and antitumor properties could position it as a valuable addition to existing therapeutic regimens. Future studies should aim to evaluate its pharmacokinetics, pharmacodynamics, and compatibility with current treatment modalities to refine its clinical potential and ensure its successful translation to patient care. VD-LP offers distinct advantages over other strategies for mitigating vitamin $D_3$ toxicity. Unlike analogues, which require chemical modification and may lack the full biological activity of native vitamin D3, liposomal encapsulation preserves its natural structure while enhancing bioavailability and reducing systemic toxicity (10). Compared to combination therapies, which involve additional agents and require precise dose calibration, VD-LP provides a simpler and more direct approach, ensuring sustained release and targeted delivery (17). These features make liposomal encapsulation a scalable and versatile solution, positioning it as a promising strategy for reducing toxicity while maintaining the therapeutic potential of vitamin $D_3$, particularly in oncology.

The carrier material DSPE-PEG2000, widely used for its biocompatibility and low immunogenicity, is not entirely free from immunogenic potential. Reports of anti-PEG antibody development and hypersensitivity reactions highlight the need for safety considerations. While our study focused on acute toxicity and efficacy, no immediate adverse effects related to DSPE-PEG2000 were observed. However, long-term immunogenicity assessments are crucial, particularly for repeated dosing. Future strategies, such as optimizing PEG density or exploring alternative materials, could further enhance the formulation's safety and clinical viability.

While our study demonstrates the potential of VD-LP in reducing toxicity and enhancing antitumor efficacy, several limitations should be acknowledged. First, the long-term safety and immunogenicity of the liposomal formulation, particularly with repeated dosing, remain to be thoroughly evaluated. Second, although VD-LP was compared to free vitamin D3 in this study, direct comparisons with other advanced drug delivery systems were not included and could provide additional context for its relative advantages. Third, the mechanistic pathways underlying the improved efficacy and reduced toxicity of VD-LP require further exploration to fully understand its therapeutic potential. Finally, the efficacy of VD-LP as a standalone treatment is limited. Vitamin $D_3$, including its liposomal formulation, may be best suited for use in combination therapies rather than as a monotherapy in cancer treatment. Integration with other modalities, such as chemotherapy, immunotherapy, or targeted therapies, could potentially enhance its antitumor effects by leveraging complementary mechanisms of action. For instance, combining VD-LP with immune checkpoint inhibitors might amplify immune activation, while pairing it with cytotoxic agents could exploit its modulatory effects on tumor

metabolism. Addressing these limitations in future studies will strengthen the evidence base and facilitate the translation of VD-LP into clinical applications.

In conclusion, this study demonstrated that liposomal encapsulation of vitamin $D_3$ enhances its stability, bioavailability, and therapeutic efficacy while reducing its associated toxicity. VD-LP exhibits potent anticancer activity across multiple cancer cell lines and in an *in vivo* tumor model, making it a promising candidate for future clinical applications. The ability of VD-LP to modulate key molecular pathways involved in tumor growth and the immune response further supports its potential as a novel therapeutic approach for cancer treatment. Future research should focus on optimizing the formulation and exploring its effects in clinical settings to fully realize its therapeutic potential.

## Data availability statement

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

## Ethics statement

The animal study was approved by the Ethics Committee of the University of Navarra (Ref. 108-23). The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

ME-H: Investigation, Writing – original draft, Writing – review & editing. SZ: Investigation, Writing – original draft, Writing – review & editing. ÁB: Investigation, Writing – original draft, Writing – review & editing. LA: Investigation, Writing – original draft, Writing – review & editing. AR: Investigation, Writing – original draft, Writing – review & editing. RG-F: Investigation, Writing – original draft, Writing – review & editing. CG: Investigation, Writing – original draft, Writing – review & editing. NA: Investigation, Writing – original draft, Writing – review & editing. VB: Investigation, Writing – original draft, Writing – review & editing. DR-G: Formal analysis, Writing – original draft, Writing – review & editing. AS-A: Investigation, Writing – original draft, Writing – review & editing. AS: Investigation, Writing – original draft, Writing – review & editing. FA: Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. MG: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. PB: Conceptualization, Formal analysis, Funding acquisition, 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.

PB declared that he was an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Generative AI statement

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## Publisher's note



## References

1. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: A systematic review and meta-analysis. *Lancet*. (2014) 383:146–55. doi: 10.1016/S0140-6736(13)61647-5

2. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer*. (2014) 14:342–57. doi: 10.1038/nrc3691

3. Carlberg C, Munoz A. An update on vitamin D signaling and cancer. *Semin Cancer Biol*. (2022) 79:217–30. doi: 10.1016/j.semcancer.2020.05.018

4. Munoz A, Grant WB. Vitamin D and cancer: an historical overview of the epidemiology and mechanisms. *Nutrients*. (2022) 14:1448. doi: 10.3390/nu14071448

5. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med*. (2019) 380:33–44. doi: 10.1056/NEJMoa1809944

6. Marcinowska-Suchowierska E, Kupisz-Urbanska M, Lukaszkiewicz J, Pludowski P, Jones G. Vitamin D toxicity-a clinical perspective. *Front Endocrinol (Lausanne)*. (2018) 9:550. doi: 10.3389/fendo.2018.00550

7. Bell DA, Crooke MJ, Hay N, Glendenning P. Prolonged vitamin D intoxication: presentation, pathogenesis and progress. *Intern Med J*. (2013) 43:1148–50. doi: 10.1111/imj.12269

8. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. *Adv Drug Delivery Rev*. (2013) 65:36–48. doi: 10.1016/j.addr.2012.09.037

9. Ferronato MJ, Salomon DG, Fermento ME, Gandini NA, Lopez Romero A, Rivadulla ML, et al. Vitamin D analogue: potent antiproliferative effects on cancer cell lines and lack of hypercalcemic activity. *Arch Pharm (Weinheim)*. (2015) 348:315–29. doi: 10.1002/ardp.201400448

10. Zhang CF, Liu ZP. Recent developments of 2-substituted analogs of 1,25(Oh)(2) D(3). *Curr Med Chem*. (2013) 20:2080–95. doi: 10.2174/0929867311320160003

11. Wierzbicka JM, Binek A, Ahrends T, Nowacka JD, Szydlowska A, Turczyk L, et al. Differential antitumor effects of vitamin D analogues on colorectal carcinoma in culture. *Int J Oncol*. (2015) 47:1084–96. doi: 10.3892/ijo.2015.3088

12. Seoane S, Gogoi P, Zarate-Ruiz A, Peluso-Iltis C, Peters S, Guiberteau T, et al. Design, synthesis, biological activity, and structural analysis of novel des-C-ring and aromatic-D-ring analogues of 1alpha,25-dihydroxyvitamin D(3). *J Med Chem*. (2022) 65:13112–24. doi: 10.1021/acs.jmedchem.2c00900

13. Drueke TB. Which vitamin D derivative to prescribe for renal patients. *Curr Opin Nephrol Hypertens*. (2005) 14:343–9. doi: 10.1097/01.mnh.0000172720.34229.39

14. Attia YM, Hammam OA, Ammar RA, Mansour MT, Elmazar MM. Crosstalk between aldehyde dehydrogenase-1 and chemoresistance in breast cancer: insights into the role of vitamin D3. *Life Sci*. (2020) 253:117733. doi: 10.1016/j.lfs.2020.117733

15. Brosseau C, Colston K, Dalgleish AG, Galustian C. The immunomodulatory drug lenalidomide restores a vitamin D sensitive phenotype to the vitamin D resistant breast cancer cell line mda-mb-231 through inhibition of bcl-2: potential for breast cancer therapeutics. *Apoptosis*. (2012) 17:164–73. doi: 10.1007/s10495-011-0670-5

16. Galus L, Michalak M, Lorenz M, Stoinska-Swiniarek R, Tusien Malecka D, Galus A, et al. Vitamin D supplementation increases objective response rate and prolongs progression-free time in patients with advanced melanoma undergoing anti-pd-1 therapy. *Cancer*. (2023) 129:2047–55. doi: 10.1002/cncr.34718

17. Young MR, Lathers DM. Combination docetaxel plus vitamin D(3) as an immune therapy in animals bearing squamous cell carcinomas. *Otolaryngol Head Neck Surg*. (2005) 133:611–8. doi: 10.1016/j.otohns.2005.05.658

18. Filipczak N, Pan J, Yalamarty SSK, Torchilin VP. Recent advancements in liposome technology. *Adv Drug Delivery Rev*. (2020) 156:4–22. doi: 10.1016/j.addr.2020.06.022

19. Olusanya TOB, Haj Ahmad RR, Ibegbu DM, Smith JR, Elkordy AA. Liposomal drug delivery systems and anticancer drugs. *Molecules*. (2018) 23:907. doi: 10.3390/molecules23040907

20. Dalek P, Drabik D, Wolczanska H, Forys A, Jagas M, Jedruchniewicz N, et al. Bioavailability by design - vitamin D(3) liposomal delivery vehicles. *Nanomedicine*. (2022) 43:102552. doi: 10.1016/j.nano.2022.102552

21. Zurek G, Przybylo M, Witkiewicz W, Langner M. Novel approach for the approximation of vitamin D(3) pharmacokinetics from *in vivo* absorption studies. *Pharmaceutics*. (2023) 15:783. doi: 10.3390/pharmaceutics15030783

22. Zalba S, Navarro I, Troconiz IF, Tros de Ilarduya C, Garrido MJ. Application of different methods to formulate peg-liposomes of oxaliplatin: evaluation *in vitro* and *in vivo*. *Eur J Pharm Biopharm*. (2012) 81:273–80. doi: 10.1016/j.ejpb.2012.02.007

23. Merino M, Contreras A, Casares N, Troconiz IF, Ten Hagen TL, Berraondo P, et al. A new immune-nanoplatform for promoting adaptive antitumor immune response. *Nanomedicine*. (2019) 17:13–25. doi: 10.1016/j.nano.2018.12.016

24. Daraee H, Etemadi A, Kouhi M, Alimirzalu S, Akbarzadeh A. Application of liposomes in medicine and drug delivery. *Artif Cells Nanomed Biotechnol*. (2016) 44:381–91. doi: 10.3109/21691401.2014.953633

25. Yetisgin AA, Cetinel S, Zuvin M, Kosar A, Kutlu O. Therapeutic nanoparticles and their targeted delivery applications. *Molecules*. (2020) 25:2193. doi: 10.3390/molecules25092193

26. Tenesaca S, Vasquez M, Fernandez-Sendin M, Di Trani CA, Ardaiz N, Gomar C, et al. Scavenger receptor class B type I is required for 25-hydroxycholecalciferol cellular uptake and signaling in myeloid cells. *Mol Nutr Food Res*. (2020) 64:e1901213. doi: 10.1002/mnfr.201901213

27. Luo J, Chen H, Ma F, Xiao C, Sun B, Liu Y, et al. Vitamin D metabolism pathway polymorphisms are associated with efficacy and safety in patients under anti-pd-1 inhibitor therapy. *Front Immunol*. (2022) 13:937476. doi: 10.3389/fimmu.2022.937476

28. Chen X, Zou X, Qi G, Tang Y, Guo Y, Si J, et al. Roles and mechanisms of human cathelicidin ll-37 in cancer. *Cell Physiol Biochem*. (2018) 47:1060–73. doi: 10.1159/000490183

29. Kozawa S, Honda A, Kajiwara N, Takemoto Y, Nagase T, Nikami H, et al. Induction of peroxisomal lipid metabolism in mice fed a high-fat diet. *Mol Med Rep*. (2011) 4:1157–62. doi: 10.3892/mmr.2011.560

30. Cheng H, Wang M, Su J, Li Y, Long J, Chu J, et al. Lipid metabolism and cancer. *Life (Basel)*. (2022) 12:784. doi: 10.3390/life12060784

31. Li H, Qi Z, Niu Y, Yang Y, Li M, Pang Y, et al. Fbp1 regulates proliferation, metastasis, and chemoresistance by participating in C-myc/stat3 signaling axis in ovarian cancer. *Oncogene*. (2021) 40:5938–49. doi: 10.1038/s41388-021-01957-5

32. Ju D, Liang Y, Hou G, Zheng W, Zhang G, Dun X, et al. Fbp1/mir-24-1/enhancer axis activation blocks renal cell carcinoma progression via warburg effect. *Front Oncol*. (2022) 12:928373. doi: 10.3389/fonc.2022.928373

33. Song C, Zhang J, Liu X, Li M, Wang D, Kang Z, et al. Pten loss promotes warburg effect and prostate cancer cell growth by inducing fbp1 degradation. *Front Oncol*. (2022) 12:911466. doi: 10.3389/fonc.2022.911466

34. Vander Heiden MG, DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. *Cell*. (2017) 168:657–69. doi: 10.1016/j.cell.2016.12.039

35. Krishnan AV, Swami S, Feldman D. Vitamin D and breast cancer: inhibition of estrogen synthesis and signaling. *J Steroid Biochem Mol Biol*. (2010) 121:343–8. doi: 10.1016/j.jsbmb.2010.02.009

36. Chang HI, Yeh MK. Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. *Int J Nanomedicine*. (2012) 7:49–60. doi: 10.2147/IJN.S26766

37. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *Am J Clin Nutr*. (2007) 85:6–18. doi: 10.1093/ajcn/85.1.6

38. Fleet JC, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: A review of molecular mechanisms. *Biochem J*. (2012) 441:61–76. doi: 10.1042/BJ20110744

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# Vitamin D status in children with COVID-19: does it affect the development of long COVID and its symptoms?

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**Introduction:** Long COVID is characterized by diverse symptoms persisting after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Given the immunomodulatory and neuroprotective properties of vitamin D, understanding its role in long COVID symptoms is of growing interest. This study aimed to determine vitamin D status in children with COVID-19 and assess its impact on the clinical course of disease and long COVID development.
**Methods:** A prospective cohort study included hospitalized children with confirmed COVID-19, aged 1 month to 18 years, diagnosed between September 2022 and March 2024. Serum 25-hydroxyvitamin D (25(OH)D) concentrations were measured upon hospital admission, and follow-up was done to identify long COVID symptoms.
**Results:** In total, 162 hospitalized patients with COVID-19 were examined. Vitamin D deficiency was determined in 8.0%, insufficiency in 25.3%, and optimal levels in 66.7% of children with COVID-19. Vitamin D deficiency/insufficiency was observed in 73% of children over 6 years and 21.6% of children under 6 years of age. Comorbid conditions were 1.4 times more frequent in children with vitamin D insufficiency, with undernutrition and obesity playing the most significant roles ($p = 0.0023$, $p = 0.0245$, respectively). Serum 25(OH)D concentration depends on COVID-19 severity ($p = 0.0405$) and children with vitamin D deficiency/insufficiency had a longer hospital stay (4 vs. 3 days, $p = 0.0197$). The vitamin D status affected the median levels of neutrophils, lymphocytes, their ratio, prothrombin time, fibrinogen levels, and the frequency of increased immunoglobulins M and E levels. Among 134 children who agreed to follow up, 56 (41.8%) experienced long COVID symptoms, while 78 (58.2%) recovered fully. Long COVID was frequently observed in children with vitamin D deficiency/insufficiency ($p = 0.0331$). The odds of developing long COVID were 2.2 times higher ($p = 0.0346$) in children with vitamin D deficiency/insufficiency compared to those with optimal levels. Children with vitamin D deficiency/insufficiency more often exhibited neurological (80% vs. 41.9%, $p = 0.0040$) and musculoskeletal symptoms (16% vs. 0%, $p = 0.0208$).
**Conclusion:** The 25(OH)D concentrations in children with COVID-19 depended on their age. Comorbid conditions affect the vitamin D status in children with COVID-19. Vitamin D influenced the COVID-19 severity and duration of hospitalization. There was an increased risk of developing long COVID in children with vitamin D deficiency/insufficiency, and its impact on the development of neurological symptoms associated with long COVID was established.

KEYWORDS

COVID-19, SARS-CoV-2 infection, long COVID, vitamin D, 25(OH)D, children vitamin D, long COVID

# Introduction

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), primarily affects the respiratory system, leading to conditions like interstitial pneumonia and acute respiratory distress syndrome (1). Although COVID-19 can cause severe complications in adults, especially those with comorbidities, most children experience mild or asymptomatic cases, with very few requiring hospitalization and a low mortality rate globally (2, 3).

As the incidence of SARS-CoV-2 infection increases, concerns are rising regarding persistent symptoms following acute infection, widely known as "long COVID" (4). Long COVID (sometimes referred to as "post-acute sequelae of COVID-19" or "post-COVID-19 syndrome") is a multisystem condition characterized by a variety of symptoms, including cardiorespiratory, neurological, psychosomatic, sensory, cognitive, and psychological symptoms that arise after infection with coronavirus type 2 (4, 5). As of September 1, 2024, the World Health Organization (WHO) reports that more than 776 million cases of COVID-19 have been documented worldwide, and 10%–30% of non-hospitalized individuals and 50%–70% of hospitalized patients may experience long COVID (6, 7).

Long COVID raises growing health concerns as its persistence can affect multiple organ systems with potentially negative impacts on quality of life (8, 9). Only a few prospective studies collect systematic data in larger cohorts of children with multidisciplinary clinical assessment (4, 5, 10, 11).

Post-COVID syndrome in adults is more often associated with prolonged tissue damage following persistent inflammation caused by the virus, immune dysregulation, autoimmune processes, endothelial damage, and microthrombosis (12, 13). In children and adolescents, similar pathogenic mechanisms are plausible, but the significance of vitamin and micronutrient deficiencies is also being discussed (14). In developing long COVID symptoms in children, age, certain comorbid conditions, and hospitalization in intensive care units were considered important factors (14–16).

Despite the importance of control measures during the COVID-19 pandemic (17–19), prolonged stay at home negatively impacted the health and development of children (20). Children who remain at home for extended periods are more prone to physical inactivity, unhealthy diets, and limited sunlight exposure, which may put them at greater risk of vitamin D deficiency and insufficiency (20–22).

Vitamin D plays a key role in maintaining calcium homeostasis and bone health, as well as having immunoregulatory effects as a potent regulator of innate and adaptive immune responses, influencing the expression of antimicrobial peptides and the inflammatory cascade (23–25). It has been shown to influence gene expression, modulating immune response, inflammation, oxidative stress, and the gut microbiota (26). Optimal serum vitamin D levels are fundamental for promoting health in both pediatric and adult populations (27).

Several studies have shown a connection between symptoms, severity, mortality, and outcomes of COVID-19 and vitamin D concentration in patients, regardless of age (28, 29). However, the majority of studies are related to the adult population. Szerszeń et al. (30) noted a correlation between patient mortality, the need for oxygen therapy, and vitamin D levels in older patients. Some publications have shown the impact of vitamin D status on the development of long COVID, including multisystem inflammatory syndrome (31). However, the studies on the significance of vitamin D in the development and course of COVID-19 in children, and its role in the emergence of long COVID in the pediatric population remain limited and their results are contradictory. The aim of our study was to determine vitamin D status in children with COVID-19 and assess its impact on the clinical course of disease and long COVID development.

# Materials and methods

## Study design

A prospective cohort study was conducted from September 2022 to March 2024 in the pediatric infectious diseases department of a tertiary-level hospital in Ternopil, Ukraine.

## Participants

The study included hospitalized patients aged 1 month to 18 years diagnosed with COVID-19. All cases of SARS-CoV-2 infection were confirmed using polymerase chain reaction (PCR), rapid tests, or serological methods (detection of class M antibodies).

The inclusion criteria for the study were age up to 18 years, confirmed cases of SARS-CoV-2 infection, informed consent from parents or patients, and the ability to determine the concentration of 25(OH)D in serum during hospitalization. Exclusion criteria included parental refusal for examination and unconfirmed cases of COVID-19.

## Data collection and laboratory assessments

A thorough collection of baseline and clinical data was conducted upon patient admission. The baseline characteristics included age and sex, while the clinical signs encompassed comorbidities, the severity of COVID-19, and the duration of hospitalization. The severity of COVID-19 was determined according to the WHO definition (32).

A comprehensive laboratory examination at admission included a complete blood count, determination of biochemical blood analysis indicators, coagulogram, and serum immunoglobulins (Ig) A, M, G, and E. Serum immunoglobulins were determined using the Monobind enzyme-linked immunosorbent assay (ELISA) kit, AccuBind ELISA Kits, USA. The evaluation of immunoglobulin levels was conducted according to age-specific norms.

To assess vitamin D status, its quantitative measurement was conducted in serum. For this purpose, the concentration of 25-hydroxyvitamin D (25(OH)D) in serum was determined using the ELISA, FCCu Bind ELISA Microwells, USA.

According to the recommendations of the European Vitamin D Association (EVIDAS), a concentration of 25(OH)D between 30 and 100 ng/ml (75–250 nmol/L) was considered optimal; 20–30 ng/ml (50–75 nmol/L) was classified as vitamin D insufficiency; and concentrations below 20 ng/ml (<50 nmol/L) indicated vitamin D deficiency (33).

## Long COVID definition and monitoring

After discharge from the hospital, patients were monitored for the presence of long-term symptoms of COVID-19. For this purpose, we conducted surveys at intervals of 1–3, 3–6, 6–9, and 9–12 months after the acute phase of infection, using the questionnaire developed by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC)/IP4C Global Pediatric COVID-19 Follow-Up Form. Patients or their parents, in cases where the children were under 8 years old, answered the questions. The presence of "long COVID" was determined according to WHO criteria, defined as the continuation or development of new symptoms at least 3 months after the initial SARS-CoV-2 infection, with a duration of at least 2 months with no other explanations (6).

Patients who did not have symptoms during the follow-up period after the onset of acute COVID-19 symptoms for at least 8 weeks were defined as fully recovered. The selection of patients for the study is shown in Figure 1.

## Ethical considerations

Throughout our study, we adhered to all recommendations of the 1975 Declaration of Helsinki (as revised in 2000). The study was approved by the I. Horbachevsky Ternopil National Medical University Ethics Committee (Minutes № 70 from August 1, 2022). Upon admission, all parents or children who reached the age of 16 signed an informed, voluntary consent for the study, as well as for the use of diagnostic and treatment results in scientific works.

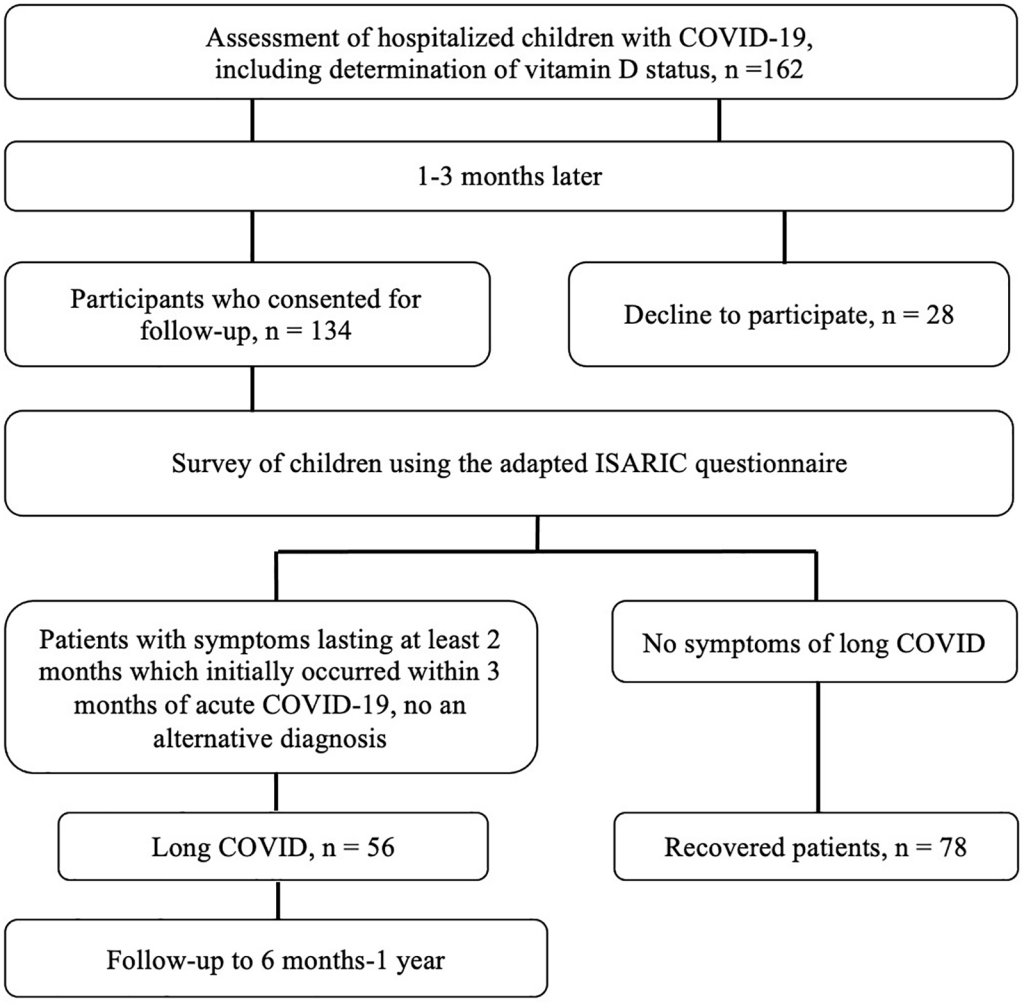


FIGURE 1
Patients flow diagram.

## Statistical analysis

Statistical analysis of the results was performed using the STATISTICA 12 software. All data were described as the mean ± standard deviation (SD) for normally distributed data or median and interquartile range (IQR) for skewed distributions and categorical variables expressed as frequency (percentage). Differences in variables with a normal distribution between two independent samples were compared using the Student's t-test, while the results with a non-normal distribution were analyzed using the Mann–Whitney *U*-test, and categorical variables were compared using the Chi-square test. A *p*-value of less than 0.05 was defined as statistically significant and highlighted in the tables in bold font.

Odds ratio (OR) and 95% confidence intervals (CI) were determined to explore the influence of vitamin D deficiency on the development of long COVID. For this purpose, we used only statistically significant features.

# Results

## Characteristics of children with COVID-19

In total, 162 hospitalized patients with COVID-19 were examined. Clinical and laboratory characteristics of children with COVID-19 are presented in Table 1. The average age of hospitalized patients with COVID-19 was 3.62 ± 4.55 years, ranging from 1 month to 18 years. Boys predominated over girls in the overall cohort of patients (56.8%).

Comorbidities were present in 75 (46.3%) patients, with 31 children (19.1%) having two or more conditions. Nutritional disorders were the most common comorbidities (25.9%), followed by allergic conditions (22.2%). Disorders of the nervous, cardiovascular, gastrointestinal systems, and kidneys were less frequently observed (Table 1).

Children with mild COVID-19 predominated in the cohort (90.1%). Moderate cases were observed in 4.3%, severe cases in 4.3%, and critical cases in 1.2% of patients. Eight patients (4.9%) developed COVID-19-related pneumonia. Seven patients (4.3%) required treatment in the intensive care unit, and four children (2.5%) required oxygen therapy during their treatment. Oxygen therapy was administered using a nasal cannula or a face mask, with the duration of oxygen supply depending on the effectiveness of the therapy and the severity of respiratory disorders. The average duration of oxygen therapy was 3 days. Mechanical ventilation was provided briefly for two children who developed acute respiratory failure. No deaths were reported among the children in this cohort. The average length of hospitalization was 4.6 ± 3.0 days, ranging from 1 to 20 days.

Leukocytosis was observed in 9.9% of children, lymphopenia in 53.5%, and neutrophilia in 8.3% of patients. A neutrophil-to-lymphocyte ratio greater than 4 was noted in 12.7% of patients. In most children with COVID-19 (85.7%), platelet counts were within the normal range. An elevated C-reactive protein (CRP) level was found in 54.5% of patients.

A reduced fibrinogen level was identified in 42.5% of patients, while an elevated fibrinogen level was noted in 5.8%. D-dimer levels were elevated in 50.9% of patients upon admission. Ferritin levels ranged from 4.03 to 440 ng/ml, with elevated levels observed in only two cases (4.2%).

An increase in IgA was observed in 42.9% of children, IgM in 66.1%, IgG in 14.3%, and IgE in 29.6% of children. Additionally, decreased levels of IgM were detected in 3.6% of children and IgG in 12.5% of children.

## Vitamin D status in patients with the acute phase of SARS-CoV-2 infection

Vitamin D deficiency was identified in 13 (8.0%), insufficiency in 41 (25.3%), and optimal levels in 108 (66.7%) children with COVID-19. Vitamin D status depended on the age of the children (Figure 2). In children under 6 years old, optimal vitamin D levels were more frequently observed (78.4% vs. 21.6%, $p < 0.0001$), while in children over 6 years, only 27% had optimal levels, and 73% exhibited deficiency and insufficiency. An inverse correlation was observed between the concentration of 25(OH)D and the age of the children ($r = -0.4989$, $p < 0.05$) (Figure 3).

## Comparison of clinical characteristics of COVID-19 based on vitamin D status

In the first group, with 25(OH)D concentrations below 30.0 ng/ml, there were 54 (33.3%) children, while the remaining 108 (66.7%) were classified in the second group with optimal vitamin D levels (30.0–100.0 ng/ml). The comparison of clinical characteristics of COVID-19 patients based on vitamin D status is presented in Table 1.

Overall, comorbid conditions were 1.4 times more prevalent in children with vitamin D insufficiency, and this difference was statistically significant ($p = 0.0449$). Specifically, nutritional disorders, particularly obesity, were significantly more common in children with low vitamin D levels ($p = 0.0023$ and $p = 0.0245$, respectively).

We did not find a correlation between the severity of COVID-19 and vitamin D status. However, the mean level of serum 25(OH)D concentration in children with mild course was significantly higher than in children with moderate and severe/critical course (49.19 ng/ml vs. 34.40 ng/ml, $p = 0.0405$) (Figure 4). Children with vitamin D deficiency and insufficiency had longer hospital stays (4 vs. 3 days, $p = 0.0197$).

The leukocyte level, as well as the percentage of children with leukocytosis, did not depend on vitamin D status in COVID-19 patients. The median neutrophil level was higher in the group of patients with low vitamin D levels ($p = 0.0021$), while the median lymphocyte level was higher in patients with optimal vitamin D levels ($p = 0.0044$). When examining the ratio of neutrophils to lymphocytes, it was found that the ratio was lower in the group with normal vitamin D levels, $p < 0.0001$. However, the percentage of children with neutrophilia, lymphopenia, and an elevated neutrophil-to-lymphocyte ratio did not differ between the two groups, which may indicate a greater age dependence, as these indicators change with age, and considering that most children under 6 years had

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TABLE 1 Clinical characteristics of the patients with COVID-19 and their dependence on vitamin D status.

| Characteristics | Total, *n* = 162 | Vitamin D deficiency/insufficiency, *n* = 54 | Optimal vitamin D level, *n* = 108 | *P* |
|---|---|---|---|---|
| | Median (interquartile range, IQR) or *n* (%) | | | |
| Age of children, years | 1.3 (0.7;5.33) | 5.91 (2.08;11.0) | 0.82 (0.53;1.5) | **<0.0001** |
| Gender | | | | |
| Female | 70 (43.2) | 28 (51.9) | 42 (38.9) | 0.1164 |
| Male | 92 (56.8) | 26 (48.1) | 66 (61.1) | 0.1164 |
| Comorbid conditions: | 75 (46.3) | 31 (57.4) | 44 (40.7) | **0.0449** |
| Allergic diseases | 36 (22.2) | 9 (16.7) | 27 (25.0) | 0.2291 |
| Nutritional disorders | 42 (25.9) | 22 (40.7) | 20 (18.5) | **0.0023** |
| Overweight | 15 (9.3) | 8 (14.8) | 7 (6.5) | 0.0845 |
| Obesity | 13 (8.0) | 8 (14.8) | 5 (4.6) | **0.0245** |
| Undernutrition | 14 (8.6) | 6 (11.1) | 8 (7.4) | 0.4290 |
| Cardiovascular pathologies | 10 (6.2) | 2 (3.7) | 8 (7.4) | 0.3558 |
| Nervous system diseases | 16 (9.9) | 5 (9.3) | 11 (10.2) | 0.8523 |
| Digestive system diseases | 7 (4.3) | 4 (7.4) | 3 (2.8) | 0.1719 |
| Urinary system diseases | 5 (3.1) | 2 (3.7) | 3 (2.8) | 0.7480 |
| COVID-19 severity | | | | |
| Mild | 146 (90.1) | 47 (87.0) | 99 (91,7) | 0.4056 |
| Moderate | 7 (4.3) | 4 (7.4) | 3 (2.8) | 0.2232 |
| Severe/critical | 9 (5.6) | 3 (5.6) | 6 (5.6) | 1.0000 |
| Duration of hospitalization, days | 4.0 (3.0; 5.0) | 4.0 (3.0; 6.0) | 3.0 (3.0; 5.0) | **0.0197** |
| Leukocytes, $10^9$/L | 5.94 (4.44; 8.8) | 5.93 (4.37; 8.44) | 5.94 (4.53; 8.89) | 0.7979 |
| Leukocytosis | 16/161 (9.9) | 7/54 (13.0) | 9/107 (8.4) | 0.4074 |
| Neutrophils, $10^9$/L | 2.37 (1.33; 3.69) | 3.1 (2.13; 4.06) | 1.88 (1.06; 3.25) | **0.0021** |
| Neutrophilia | 13/157 (8.3) | 6/51 (11.8) | 7/106 (6.6) | 0.2718 |
| Lymphocytes, $10^9$/L | 2.12 (1.21; 3.99) | 1.65 (0.97; 2.56) | 2.38 (1.42; 4.41) | **0.0044** |
| Lymphopenia | 84/157 (53.5) | 25/51 (49.0) | 59/106 (55.7) | 0.4346 |
| Neutrophil-to-lymphocyte ratio | 1.16 (0.44; 2.64) | 2.19 (1.09; 3.5) | 0.83 (0.34; 2.08) | **<0.0001** |
| More than 4 | 20/157 (12.7) | 10/51 (19.6) | 10/106 (9.4) | 0.0734 |
| Thrombocytes, $10^9$/L | 244.0 (204.0; 310.0) | 218.5 (187.0; 263.0) | 264.5 (208.0;323.0) | **0.0071** |
| Thrombocytopenia, % | 11/161 (6.8) | 3/54 (5.6) | 8/107 (7.5) | 0.7522 |
| CRP, mg/L | 5.48 (1.41; 15.9) | 5.86 (3.4; 20.8) | 5,25 (1.4; 14.5) | 0.1259 |
| Elevated CRP, % | 79/145 (54.5) | 30/49 (61.2) | 49/96 (51.0) | 0.2914 |
| Prothrombin time (PT), sec | 14.7 (13.4; 15.9) | 15.5 (13.8; 16.4) | 14.5 (13.3; 15.6) | **0.0058** |
| Prolonged PT (more than 15 s) | 3/147 (42.9) | 31/49 (63.3) | 32/98 (32.7) | **0.0007** |
| Activated partial thromboplastin time (aPTT), sec | 38.3 (34.0; 44.0) | 37.1 (32.2; 41.7) | 39.6 (34.8; 45.3) | 0.1074 |
| Prolonged aPTT (more than 35 s) | 98/147 (66.7) | 30/49 (61.2) | 68/98 (69.4) | 0.3563 |
| Fibrinogen, g/L | 2.17 (1.67; 2.98) | 2.66 (1.97; 3.42) | 2.0 (1.52; 2.86) | **0.0037** |
| More than 4 g/L | 8/139 (5.8) | 4/45 (8.9) | 4/94 (4.3) | 0.2734 |
| D-dimer, ng/ml | 270.0 (100; 750) | 239.0 (90; 390) | 360.5 (150; 840) | 0.1269 |
| More than 250 ng/ml | 30/59 (50.9) | 10/25 (40.0) | 20/34 (58.8) | 0.1923 |
| Alanine aminotransferase (ALT), U/L | 21.0 (15.0; 30.0) | 15.0 (12.0; 19.0) | 24.0 (18.0; 33.0) | **<0.0001** |
| Elevated ALT level, % | 11/155 (7.1) | 3/51 (5.9) | 8/104 (7.7) | 1.000 |
| Ferritin | 49.1 (27.8; 88.9) | 47.29 (25.2; 86.6) | 49.15 (33.81; 118,96) | 0.4088 |
| Hyperferritinemia, % | 2/48 (4.2) | 0/20 (0) | 2/28 (7.1) | 0.5035 |
| Immunoglobulin A | | | | |
| Optimal level | 32/56 (57.1) | 13/29 (44.8) | 19/27 (70.4) | 0.0644 |
| Increase level | 24/56 (42.9) | 16/29 (55.2) | 8/27 (29.6) | 0.0644 |
| Immunoglobulin M | | | | |
| Optimal level | 17/56 (30.4) | 6/29 (20.7) | 11/27 (40.7) | 0.1475 |
| Decrease level | 2/56 (3.6) | 0 | 2/27 (7.4) | 0.2279 |
| Increase level | 37/56 (66.1) | 23/29 (79.3) | 14/27 (51.9) | **0.0476** |
| Immunoglobulin G | | | | |
| Optimal level | 41/56 (73.2) | 19/29 (65.6) | 22/27 (81.5) | 0.2329 |
| Decrease level | 7/56 (12.5) | 5/29 (17.2) | 2/27 (7.4) | 0.4239 |
| Increase level | 8/56 (14.3) | 5/29 (17.2) | 3/27 (11.1) | 0.7066 |

(*Continued*)

TABLE 1 Continued

| Characteristics | Total, $n = 162$ | Vitamin D deficiency/insufficiency, $n = 54$ | Optimal vitamin D level, $n = 108$ | $P$ |
|---|---|---|---|---|
| | Median (interquartile range, IQR) or $n$ (%) | | | |
| Immunoglobulin E | | | | |
| Optimal level | 38/54 (70.4) | 16/29 (55.2) | 22/25 (88.0) | **0.0154** |
| Increase level | 16/54 (29.6) | 13/29 (44.8) | 3/25 (12.0) | **0.0154** |

Statistically significant values are highlighted in bold.

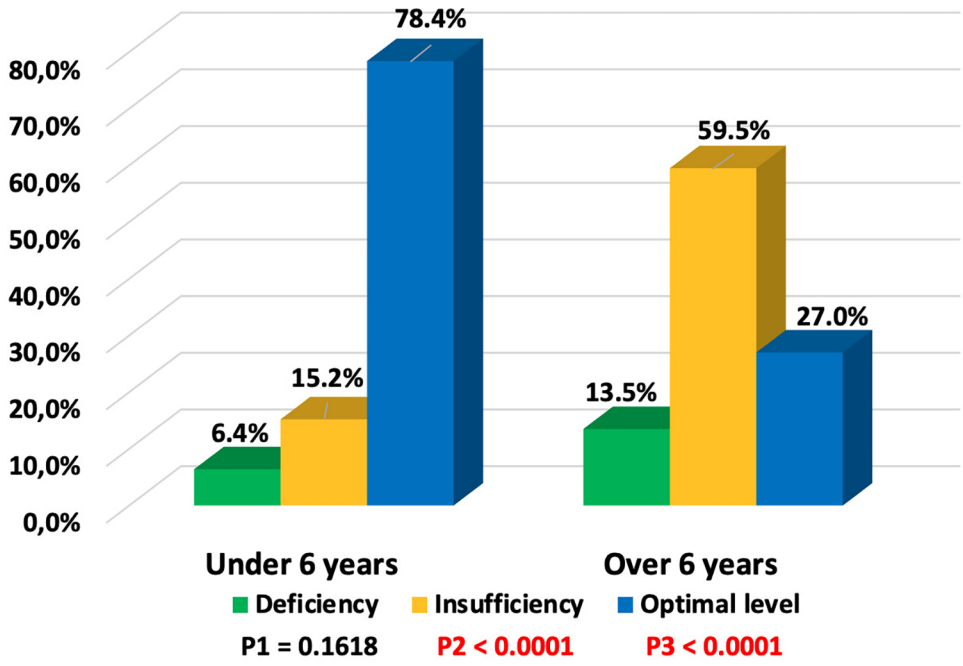


FIGURE 2
Dependence of vitamin D status on age in children with COVID-19 (P1—value between groups under 6 years and over 6 years with vitamin D deficiency, P2—value between groups under 6 years and over 6 years with vitamin D insufficiency, P3—value between groups under 6 years and over 6 years with optimal vitamin D levels).

optimal vitamin D levels, while those over 6 predominantly had vitamin D insufficiency. However, the number of children with a neutrophil-to-lymphocyte ratio greater than 4 was twice as high among those with vitamin D deficiency/insufficiency, $p = 0.0734$. In patients with optimal vitamin D levels, the median platelets count was significantly higher ($p = 0.0071$), but there was no significant difference in the frequency of thrombocytopenia ($p = 0.6587$).

The CRP level, although somewhat higher in the group of patients with low vitamin D levels, did not differ significantly (Table 1).

Only 11 out of 146 (7.5%) hospitalized children with COVID-19 had all coagulation parameters assessed in this study within the normal range. The medians of PT and fibrinogen were higher in the cohort of patients with low vitamin D levels ($p = 0.0058$ and $p = 0.0037$, respectively). The proportion of children with prolonged PT and elevated fibrinogen levels was twice as high in patients with vitamin D deficiency/insufficiency and COVID-19, although the difference was statistically significant only for PT. The median values of aPTT and D-dimer were not dependent on vitamin D status.

The median ALT level was higher in children with optimal vitamin D levels; however, the proportion of children with elevated ALT and ferritin levels did not differ between the two groups of children with COVID-19 (Table 1).

When comparing immunoglobulin levels in children with deficient or insufficient vitamin D levels to those with optimal vitamin D levels, it was found that children with vitamin D deficiency/insufficiency more frequently exhibited elevated levels of IgA (55.2% vs. 29.6%), IgM (79.3% vs. 51.9%), IgG (17.2% vs. 11.1%), and IgE (44.8% vs. 12.0%). The differences were statistically significant for IgM ($p = 0.0476$) and IgE ($p = 0.0154$), with a trend toward increased IgA levels ($p = 0.0644$).

## Clinical characteristics of children with long COVID

Of the 162 patients included in the study, 134 consented to further observation. Among them, symptoms of long COVID

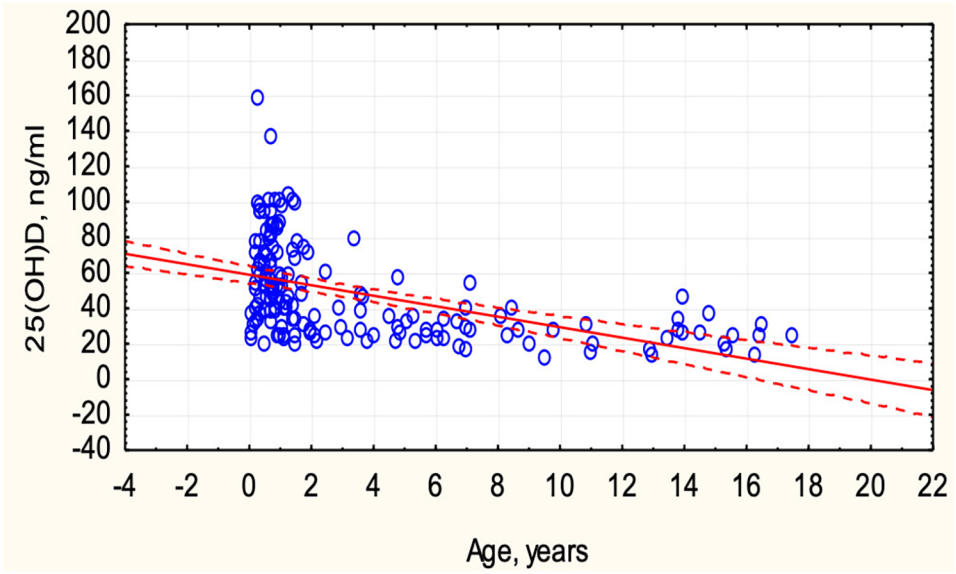


FIGURE 3
Correlation between serum 25(OH)D concentration and children's age.

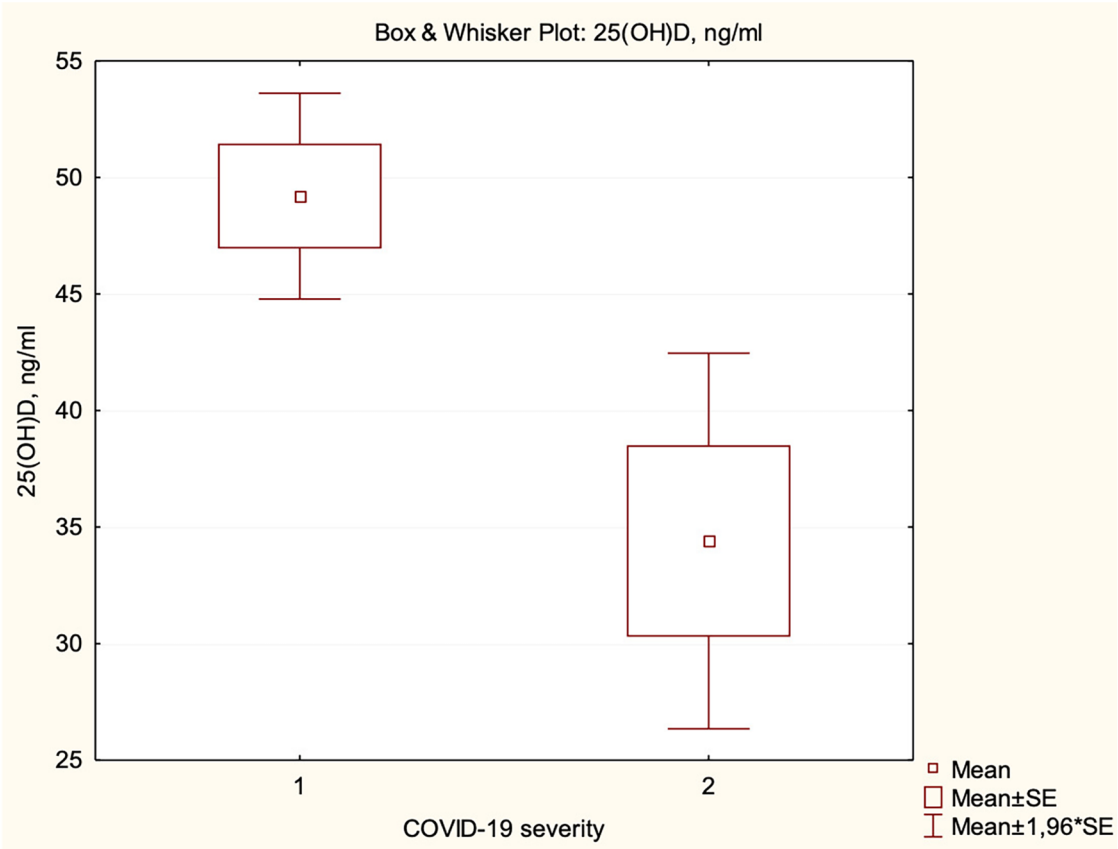


FIGURE 4
Dependence of serum 25(OH)D concentration on the severity of COVID-19 (1—mild, 2—moderate and severe/critical).

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were identified in 56 (41.8%) children, while 78 (58.2%) recovered fully. The observation period ranged from 6 months to 1 year, with an average of 10.4 months. Among the symptoms in children with long COVID, general manifestations predominated, observed in 37 (66.1%) children. These included fatigue, general weakness, decreased appetite, reduced physical activity, and difficulties starting tasks. Neurological symptoms included insomnia or excessive sleepiness, headache, increased irritability, emotional lability, decreased memory and attention, and inability to concentrate on tasks. This group of symptoms was present in 33 (58.9%) children. Gastroenterological symptoms, such as hepatopathy, abdominal pain, nausea, and constipation, were observed in 9 (16.1%) patients. Cardiological manifestations, including tachycardia or conduction disturbances, were less common, occurring in 2 (3.6%) children, while musculoskeletal symptoms (myalgias and arthralgias) were noted in 4 patients (7.1%). Additionally, 20 (35.7%) children experienced frequent acute respiratory infections, which occurred significantly more often than before the SARS-CoV-2 infection.

## Long COVID symptoms depend on vitamin D status

The vitamin D status in patients with long COVID and the symptoms of long COVID based on vitamin D status are shown in Table 2. Vitamin D deficiency was observed in 7 (12.5%) children, insufficiency in 18 (32.1%), and optimal levels in 31 (55.4%) children who subsequently developed long COVID symptoms, while among recovered children, optimal vitamin D levels were found in 57 (73.1%). Long COVID was more frequently observed in children with vitamin D deficiency/insufficiency) (Figure 5), and this difference was statistically significant ($p = 0.0331$). The odds of developing long COVID were 2.2 times higher (OR = 2.1889, 95% CI: 1.0585–4.5267; $p = 0.0346$) in children with vitamin D deficiency/insufficiency compared to those with optimal vitamin D levels. It should be noted that vitamin D status in children with symptoms of both long COVID and those who recovered depended on the age of the children. Deficiency/insufficiency of vitamin D was observed in 15/18 (83.3%) children over 6 years old with long COVID symptoms and in 11/14 (78.5%) children who recovered ($p = 0.7321$). The median concentration of 25(OH)D in patients with long COVID symptoms was lower (34.52 ng/ml; IQR: 23.69; 67.31 ng/ml) compared to those who recovered (44.52 ng/ml; IQR: 28.65; 70.03 ng/ml), but the difference was not statistically significant, $p = 0.1451$.

TABLE 2 Symptoms of long COVID in hospitalized children depends on vitamin D status.

| Characteristics | Vitamin D deficiency/ insufficiency | Optimal vitamin D level | *P* |
|---|---|---|---|
| | *n* (%) | *n* (%) | |
| Long COVID | 25/46 (54.3) | 31/88 (35.2) | **0.0331** |
| General | 16/25 (64.0) | 21/31 (67.7) | 0.7687 |
| Neurological | 20/25 (80.0) | 13/31 (41.9) | **0.0040** |
| Gastroenterological | 1/25 (4.0) | 8/31 (25.8) | **0.0272** |
| Cardiological | 1/25 (4.0) | 1/31 (3.2) | 0.8767 |
| Musculoskeletal | 4/25 (16.0) | 0/31 | **0.0208** |
| Frequent acute respiratory infections | 6/25 (24.0) | 8/31 (25.8) | 0.8767 |

Statistically significant values are highlighted in bold.

General and cardiological symptoms were observed equally frequently in both groups, regardless of vitamin D status. Neurological manifestations were almost twice as common in children with vitamin D deficiency/insufficiency compared to those with optimal levels ($p = 0.0040$). Low vitamin D levels also affected musculoskeletal manifestations (16% vs. 0%, $p = 0.0208$). The odds of developing neurological symptoms of long COVID were 5.5 times higher (OR = 5.5385, 95% CI: 1.6480–18.6133; $p = 0.0056$) in children with vitamin D deficiency/insufficiency compared to those with optimal vitamin D levels. For musculoskeletal symptoms, OR = 13.1860, 95% CI: 0.6746–257.7413; $p = 0.0890$. In contrast, gastroenterological symptoms were more frequently observed in patients with normal vitamin D levels ($p = 0.0272$). The increased incidence of respiratory diseases was nearly the same in both groups of children.

# Discussion

Our study revealed a decrease in vitamin D levels in one-third of children with COVID-19. However, findings from other studies regarding the prevalence of hypovitaminosis D among children with acute SARS-CoV-2 infection remain contradictory. Bayramoğlu et al. (29) reported hypovitaminosis D in 35.4% of hospitalized children with mild and moderate disease. This aligns with our findings, as most children in our cohort had mild COVID-19. In contrast, Bayrak et al. (34) found a higher prevalence of vitamin D deficiency or insufficiency (67.1%) among hospitalized children with COVID-19. Furthermore, the average 25(OH)D concentration in their study was significantly lower compared to that of our cohort.

Vitamin D status is influenced not only by the disease itself but also by various external factors. In our cohort, vitamin D status was notably age-dependent, with vitamin D deficiency or insufficiency observed in 73% of children over six years of age. Other studies have also highlighted an age-related dependence of vitamin D levels in hospitalized COVID-19 patients (35), with adolescents being more susceptible to developing hypovitaminosis D. This increased susceptibility among adolescents may stem from their tendency toward sedentary lifestyles, particularly during extended periods of staying at home, with greater access to computers, televisions, and smartphones (36, 37).

The COVID-19 pandemic has contributed to prolonged home confinement for both children and adults, significantly reducing sun exposure time (38, 39). Epidemiological studies have indicated that the pandemic has altered vitamin D levels, particularly among preschool- and school-aged children (40).

Our study revealed differences in mean 25(OH)D concentrations based on COVID-19 severity and the length of

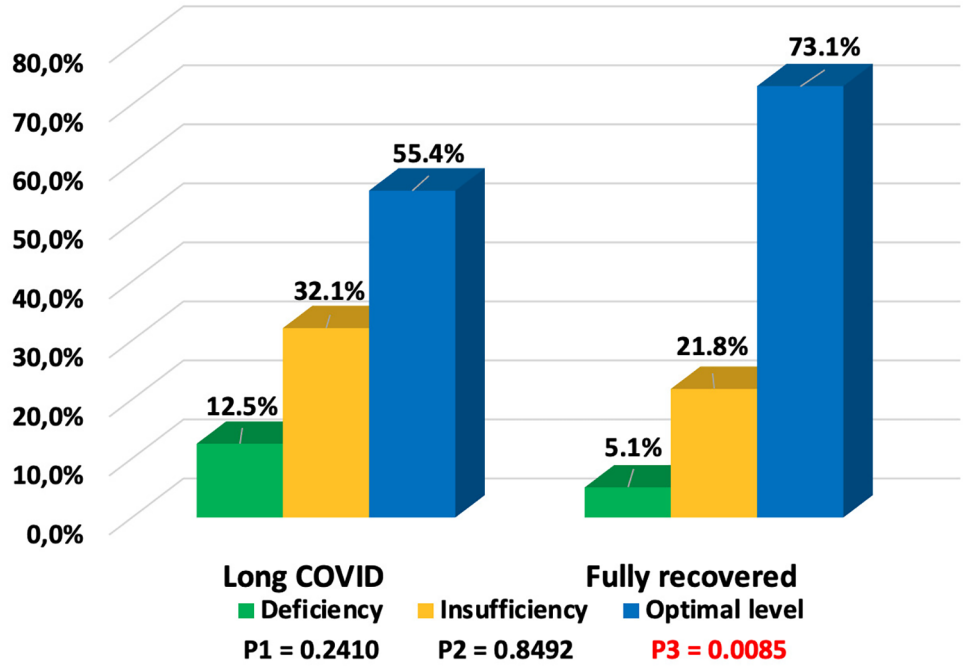


FIGURE 5
Vitamin D status in children with long COVID and fully recovered (P1—value between groups of children with long COVID and fully recovered with vitamin D deficiency, P2—value between groups of children with long COVID and fully recovered with vitamin D insufficiency, P3—value between groups of children with long COVID and fully recovered with optimal vitamin D levels).

hospital stay among children with different vitamin D status. These findings align with other studies that report a direct impact of vitamin D levels on symptoms, severity, and outcomes of COVID-19 (28–30, 41).

Notably, among our patients, pneumonia was diagnosed in only 8 children (4.9%), and 6 (75.0%) of whom had hypovitaminosis D. Nicolae et al. (42) confirmed that hypovitaminosis D could contribute to the development of pneumonia due to its significant influence on immunological processes. Regression analysis in another study also identified low vitamin D levels as a risk factor for developing respiratory distress (41). Furthermore, Kosmeri et al. (43) demonstrated an association between vitamin D insufficiency and the severity of COVID-19 in adults. Their study highlighted 25(OH)D concentration as an independent risk factor for COVID-19 infection and the likelihood of requiring hospitalization.

Other studies have demonstrated the impact of vitamin D status on COVID-19 mortality, especially in adult patients (30, 44, 45). A population-based study revealed a negative correlation between 25(OH) vitamin D average levels and COVID-19 mortality in 19 European countries (44). Seal et al. demonstrated that 25(OH)D concentrations were associated with COVID-19-related hospitalization and mortality in a cohort of veterans (45). A meta-analysis also declared an association between COVID-19 severity and mortality and low serum vitamin D levels (46).

The association between vitamin D deficiency and disease severity or increased mortality is likely related to impaired immune responses. Vitamin D plays a critical role in supporting the immune system. It modulates innate and adaptive immune responses, enhancing the body's ability to fight infections and reducing excessive inflammatory responses (24, 27, 36). Through its effects on immune cells such as macrophages, dendritic cells, and T cells, vitamin D promotes the production of antimicrobial peptides like cathelicidins and defensins, which are vital for neutralizing pathogens (25). Furthermore, vitamin D stabilizes endothelial function and regulates vascular permeability, reducing the risk of cytokine storm and associated complications often observed in severe infections like COVID-19 (29).

An analysis of laboratory characteristics revealed that the median levels of neutrophils and the neutrophil-to-lymphocyte ratio were significantly higher in children with low vitamin D levels ($p = 0.0021$; $p < 0.0001$, respectively), whereas median levels of lymphocytes and platelets were significantly lower in this group of patients ($p = 0.0044$; $p = 0.0071$, respectively). Alpcan et al. (41) reported a positive correlation between vitamin D levels and leukocyte, lymphocyte, and platelet counts. Similarly, another study found that lower vitamin D levels were associated with increased clinical severity and more pronounced inflammatory markers (29). In our study, CRP was elevated in 55.6% of children with COVID-19 and vitamin D deficiency/insufficiency; however, no statistically significant difference was observed compared to patients with optimal vitamin D levels. Ferritin levels were elevated in only two patients, and no dependence on vitamin D status was identified. By contrast, another study demonstrated significantly higher levels of CRP

and ferritin in adult patients with low vitamin D levels (47). Moreover, elevations in CRP, fibrinogen, and lymphopenia were more frequently observed in cases of vitamin D deficiency rather than insufficiency (29). In our cohort, the proportion of children with vitamin D deficiency was relatively small, comprising only 8.0% of the population, while the majority exhibited vitamin D insufficiency. This distribution may explain the lack of statistically significant differences in certain inflammatory markers.

We also observed changes in coagulation markers, specifically increased PT and fibrinogen levels ($p = 0.0058$; $p = 0.0037$, respectively). Our previous research demonstrated age-related characteristics of coagulation markers in children with COVID-19 (48). Other studies have also reported a relationship between vitamin D levels and thrombotic complications in COVID-19 patients (47, 49–51). Cooper et al. (50) noted that vitamin D activation reduces the risk of respiratory infections and decreases coagulation and thrombosis.

Increased IgA levels were nearly twice as common, IgM levels were 1.5 times more frequent, IgE levels were 3.7 times more frequent, and changes in IgG levels were 1.8 times more frequent in children with vitamin D deficiency/insufficiency compared to those with optimal levels. Our findings align with results from other researchers, who demonstrated that the immunomodulatory effects of vitamin D are associated with the inhibition of B-cell proliferation, blockage of their differentiation, and a significant decrease in immunoglobulin secretion (52).

On one hand, the more frequent elevation of immunoglobulin levels in cases of vitamin D deficiency/insufficiency may be linked to a more severe course of illness, as was also shown in our study. Specifically, Peraire et al. (53) investigated the relationship between circulating immunoglobulins (IgA, IgG, IgM) and COVID-19 pneumonia. They established that IgM, IgA, and IgG concentrations were significantly higher in patients with COVID-19 pneumonia (mild, severe, and critical forms) compared to those in the ambulatory group ($P \leq 0.001$).

On the other hand, increased immunoglobulin production in children with reduced vitamin D levels may potentially contribute to the development of autoimmunity and/or symptoms of long COVID (8, 13, 26). Notably, several studies have demonstrated a rise in autoimmune diseases following the COVID-19 pandemic (54).

In our study, symptoms of long COVID were observed in 41.8% of hospitalized children. The prevalence of patients with long COVID symptoms is highly variable, ranging from 3.4% in symptomatic patients (55) to 81.4% in hospitalized patients (56). Numerous factors can influence the prevalence of long COVID, including age, study cohort, follow-up duration, comorbidities, COVID-19 vaccination status, etc. Rao et al. reported that the frequency of at least one systemic, syndromic, or drug-induced sign of post-acute complications of SARS-CoV-2 infection was 41.9% among children with a positive test for the virus (57), which aligns with the results of our study. The authors highlighted a higher prevalence of long COVID symptoms among children under 5 years of age and those with comorbidities. The high prevalence of comorbid conditions in our cohort (46.3%) and the predominance of children under 6 years of age (77.2%) could explain the high frequency of long COVID symptoms. It should also be noted that we studied the presence of symptoms specifically in hospitalized patients, which may have contributed to the high frequency of long COVID in our findings.

Our study showed that children with vitamin D deficiency or insufficiency had more than twice the risk of developing long COVID compared to those with optimal vitamin D levels (OR = 2.1889, $p = 0.0346$). These findings align with results from other studies. For instance, lower 25(OH) vitamin D concentrations were reported six months after the acute phase of the disease in adults with long COVID compared to those who fully recovered from coronavirus infection (20.1 vs. 23.2 ng/ml, $p = 0.0300$) (58). Similarly, Chen et al. demonstrated that vitamin D deficiency was associated with delayed recovery in adult patients with long COVID (59). A study by Guerrero-Romero et al. highlighted a threefold increase in the risk of developing long COVID in adult patients with insufficient vitamin D and magnesium levels (60). However, several investigations involving adult populations did not find a significant association between low serum vitamin D levels and long COVID (59, 61, 62). Pizzini et al. conducted a prospective, multicenter study on the long-term sequelae of COVID-19 and their association with 25(OH)D concentrations (63). While vitamin D deficiency was commonly observed among COVID-19 patients, it was not linked to long-term disease outcomes. Similarly, other researchers found reduced concentrations of vitamins D, A, and E in adult patients with COVID-19 but reported no significant impact on the development of long COVID symptoms (64).

In children, research has primarily focused on vitamin D status concerning multisystem inflammatory syndrome (MIS-C) associated with COVID-19. Studies have shown that children with MIS-C had significantly lower vitamin D levels than those in the non-MIS-C group (65), and vitamin D deficiency was found in 72% of children with MIS-C (31).

The impact of vitamin D levels on long COVID symptoms in children has also been analyzed. We observed an almost twofold increase in the frequency of neurological symptoms in patients with vitamin D deficiency ($p = 0.0040$) and a higher incidence of musculoskeletal symptoms ($p = 0.0208$). However, the odds ratio indicated only an increased risk of neurological symptoms. Another study found lower 25(OH)D levels in patients with neurocognitive symptoms six months after recovering from COVID-19 (60). A multivariable regression analysis conducted by Townsend et al. showed no relationship between persistent fatigue and reduced exercise tolerance following COVID-19 (66).

Given vitamin D's ability to modulate both immune and nerve cells, its role in neuroimmune modulation, anti-inflammatory action, neuroprotection, and endothelial function suggests that it could play a positive role in preventing and managing neuropsychiatric, neuroinflammatory, and other processes in long COVID (67, 68). However, further research is needed to determine optimal dosages and the duration of treatment.

## Strengths and limitations of the study

This study provides valuable insight into the role of vitamin D in pediatric COVID-19 patients, an area where data remains limited. Most studies have focused on adults, making this work a significant contribution to understanding long COVID in children. The study includes a prospective cohort design with thorough follow-up, allowing for detailed observations of long COVID symptoms in relation to vitamin D status.

However, the study has several limitations. The sample size, particularly for children with long COVID, is relatively small, which may affect the generalizability of the results. The study population was drawn from a tertiary-level pediatric hospital, meaning the patients may differ from those in other pediatric hospitals. Additionally, no control group of healthy children did not contract COVID-19, which could have provided additional comparative data on vitamin D status in non-infected populations. The study was conducted during a specific time frame, and seasonal variations in sunlight exposure, which could affect vitamin D levels, were not fully accounted for. Another limitation is the method used to measure vitamin D levels—ELISA—which is less sensitive and specific compared to chemiluminescence and mass spectrometry, the internationally recommended methods.

# Conclusion

The 25(OH)D concentrations in children with COVID-19 depended on their age. Vitamin D deficiency/insufficiency was observed in 73% of children over 6 years of age and 21.6% of children under 6 years of age with COVID-19. The presence of comorbid conditions, particularly undernutrition and obesity, affected the vitamin D status in children with COVID-19. Vitamin D influenced the COVID-19 severity and duration of hospitalization. There was an increased risk of developing long COVID in children with vitamin D deficiency/insufficiency, and its impact on the development of neurological symptoms associated with long COVID was established. Further research is needed to determine independent predictors of long COVID development.

# 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 I. Horbachevsky Ternopil National Medical University Ethics Committee. 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

VP: Conceptualization, Data curation, Formal Analysis, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. TK: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – review & editing, Writing – original draft. OD: Data curation, Formal Analysis, Writing – review & editing, Writing – original draft. LV: Conceptualization, Data curation, Resources, Writing – review & editing, Writing – original draft. OB: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, 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



## References

1. Gemelli Against COVID-19 Post-Acute Care Study Group. Post-COVID-19 global health strategies: the need for an interdisciplinary approach. *Aging Clin Exp Res*. (2020) 32(8):1613–20. doi: 10.1007/s40520-020-01616-x

2. Swann OV, Holden KA, Turtle L, Pollock L, Fairfield CJ, Drake TM, et al. Clinical characteristics of children and young people admitted to hospital with COVID-19 in United Kingdom: prospective multicentre observational cohort study. *Br Med J*. (2020) 370:m3249. doi: 10.1136/bmj.m3249

3. Boyarchuk O, Predyk L, Yuryk I. COVID-19 in patients with juvenile idiopathic arthritis: frequency and severity. *Reumatologia*. (2021) 59(3):197–9. doi: 10.5114/reum.2021.107590

4. Borch L, Holm M, Knudsen M, Ellermann-Eriksen S, Hagstroem S. Long COVID symptoms and duration in SARS-CoV-2 positive children - a nationwide cohort study. *Eur J Pediatr*. (2022) 181(4):1597–607. doi: 10.1007/s00431-021-04345-z

5. Zimmermann P, Pittet LF, Curtis N. How common is long COVID in children and adolescents? *Pediatr Infect Dis J*. (2021) 40(12):e482–7. doi: 10.1097/INF.0000000000003328

6. World Health Organization. *COVID-19 Dashboard*. World Health Organization. (2024). Available online at: https://data.who.int/dashboards/covid19/cases (Accessed July 22, 2023).

7. World Health Organization. *Post COVID-19 Condition (Long COVID)*. World Health Organization (2023). Available online at: https://www.who.int/europe/news-room/fact-sheets/item/postcovid-19-condition#:~:text=It%20is%20defined%20as%20the,months%20with%20no%20other%20explanation (accessed July 11, 2023).

8. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, et al. Post-acute COVID-19 syndrome. *Nat Med*. (2021) 27:601–15. doi: 10.1038/s41591-021-01283-z

9. Venkatesan P. NICE guideline on long COVID. *Lancet Respir Med*. (2021) 9(2):129. doi: 10.1016/S2213-2600(21)00031-X

10. Ashkenazi-Hoffnung L, Shmueli E, Ehrlich S, Ziv A, Bar-On O, Birk E, et al. Long COVID in children: observations from a designated pediatric clinic. *Pediatr Infect Dis J*. (2021) 40(12):e509–11. doi: 10.1097/INF.0000000000003285

11. Mizrahi B, Sudry T, Flaks-Manov N, Yehezkelli Y, Kalkstein N, Akiva P, et al. Long COVID outcomes at one year after mild SARS-CoV-2 infection: nationwide cohort study. *Br Med J*. (2023) 380:e072529. doi: 10.1136/bmj-2022-072529

12. Volianska LA, Burbela EI, Kosovska TM, Perestiuk VO, Boyarchuk OR. Long COVID in children: frequency and diagnostic challenges. *Ukr J Perinatol Pediatr*. (2023) 3(95):101–6. doi: 10.15574/PP.2023.95.101

13. Boyarchuk O, Volianska L. Autoimmunity and long COVID in children. *Reumatologia*. (2023) 61(6):492–501. doi: 10.5114/reum/176464

14. Piazza M, Di Cicco M, Pecoraro L, Ghezzi M, Peroni D, Comberiati P. Long COVID-19 in children: from the pathogenesis to the biologically plausible roots of the syndrome. *Biomolecules*. (2022) 12(4):556. doi: 10.3390/biom12040556

15. Fainardi V, Meoli A, Chiopris G, Motta M, Skenderaj K, Grandinetti R, et al. Long COVID in children and adolescents. *World J Pediatr*. (2021) 17(2):495. doi: 10.1007/s12519-021-00457-6

16. Osmanov IM, Spiridonova E, Bobkova P, Gamirova A, Shikhaleva A, Andreeva M, et al. Risk factors for post-COVID-19 condition in previously hospitalised children using the ISARIC global follow-up protocol: a prospective cohort study. *Eur Respir J*. (2022) 59(2):2101341. doi: 10.1183/13993003.01341-2021

17. Fong MW, Gao H, Wong JY, Xiao J, Shiu EYC, Ryu S, et al. Nonpharmaceutical measures for pandemic influenza in nonhealthcare settings—social distancing measures. *Emerg Infect Dis*. (2020) 26(5):976–84. doi: 10.3201/eid2605.190995

18. Wong SCY, Kwong RT, Wu TC, Chan JWM, Chu MY, Lee SY, et al. Risk of nosocomial transmission of coronavirus disease 2019: an experience in a general ward setting in Hong Kong. *J Hosp Infect*. (2020) 105(2):119–27. doi: 10.1016/j.jhin.2020.03.036

19. Cowling BJ, Ali ST, Ng TWY, Tsang TK, Li JCM, Fong MW, et al. Impact assessment of non-pharmaceutical interventions against coronavirus disease 2019 and influenza in Hong Kong: an observational study. *Lancet Public Health*. (2020) 5(5):e279–88. doi: 10.1016/S2468-2667(20)30090-6

20. Wang G, Zhang Y, Zhao J, Zhang J, Jiang F. Mitigate the effects of home confinement on children during the COVID-19 outbreak. *Lancet*. (2020) 395(10228):945–7. doi: 10.1016/S0140-6736(20)30547-X

21. Nowson CA, McGrath JJ, Ebeling PR, Haikerwal A, Daly RM, Sanders KM, et al. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust*. (2012) 196(11):686–7. doi: 10.5694/mja11.10301

22. Yu L, Ke HJ, Che D, Luo SL, Guo Y, Wu JL. Effect of pandemic-related confinement on vitamin D status among children aged 0–6 years in Guangzhou, China: a cross-sectional study. *Risk Manag Healthc Policy*. (2020) 13:2669–75. doi: 10.2147/RMHP.S282495

23. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr*. (2008) 88(2):491S–9. doi: 10.1093/ajcn/88.2.491S

24. Lips P. Vitamin D physiology. *Prog Biophys Mol Biol*. (2006) 92(1):4–8. doi: 10.1016/j.pbiomolbio.2006.02.016

25. Gombart AF. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol*. (2009) 4:1151–65. doi: 10.2217/fmb.09.87

26. Aribi M, Mennechet FJD, Touil-Boukoffa C. Editorial: the role of vitamin D as an immunomodulator. *Front Immunol*. (2023) 14:1186635. doi: 10.3389/fimmu.2023.1186635

27. Marino R, Misra M. Extra-skeletal effects of vitamin D. *Nutrients*. (2019) 11(7):1460. doi: 10.3390/nu11071460

28. Iacopetta D, Ceramella J, Catalano A, Saturnino C, Pellegrino M, Mariconda A, et al. COVID-19 at a glance: an up-to-date overview on variants, drug design and therapies. *Viruses*. (2022) 14(3):573. doi: 10.3390/v14030573

29. Bayramoğlu E, Akkoç G, Ağbaş A, Akgün Ö, Yurdakul K, Selçuk Duru HN, et al. The association between vitamin D levels and the clinical severity and inflammation markers in pediatric COVID-19 patients: single-center experience from a pandemic hospital. *Eur J Pediatr*. (2021) 180(8):2699–705. doi: 10.1007/s00431-021-04030-1

30. Szerszeń MD, Kucharczyk A, Bojarska-Senderowicz K, Pohorecka M, Śliwczyński A, Engel J, et al. Effect of vitamin D concentration on course of COVID-19. *Med Sci Monit*. (2022) 28:e937741. doi: 10.12659/MSM.937741

31. Darren A, Osman M, Masilamani K, Habib Ali S, Kanthimathinathan HK, Chikermane A, et al. Vitamin D status of children with paediatric inflammatory multisystem syndrome temporally associated with severe acute respiratory syndrome coronavirus 2 (PIMS-TS). *Br J Nutr*. (2022) 127(6):896–903. doi: 10.1017/S0007114521001562

32. World Health Organization. *Clinical Management of COVID-19: Interim Guidance, 27 May 2020*. World Health Organization (2020). Available online at: https://iris.who.int/handle/10665/332196 (Accessed May 27, 2020).

33. Pludowski P. COVID-19 and other pleiotropic actions of vitamin D: proceedings from the fifth international conference "vitamin D—minimum, maximum, optimum" under the auspices of the European vitamin D association (EVIDAS). *Nutrients*. (2023) 15(11):2530. doi: 10.3390/nu15112530

34. Bayrak H, Öztürk D, Bolat A, Ünay B. Association between vitamin D levels and COVID-19 infection in children: a case-control study. *Turk Arch Pediatr*. (2023) 58(3):250–5. doi: 10.5152/TurkArchPediatr.2023.22217

35. Karimian P, Tahami MS, Sayyahfar S, Aghajani Delavar M. Association of vitamin D and severity of COVID-19 in children. *Eur J Transl Myol*. (2022) 32(2):10453. doi: 10.4081/ejtm.2022.10453

36. Kinash MI, Boyarchuk OR. Fat-soluble vitamins and immunodeficiency: mechanisms of influence and opportunities for use. *Vopr Pitan*. (2020) 89(3):22–32. doi: 10.24411/0042-8833-2020-10026

37. Vierucci F, Del Pistoia M, Fanos M, Gori M, Carlone G, Erba P, et al. Vitamin D status and predictors of hypovitaminosis D in Italian children and adolescents: a cross-sectional study. *Eur J Pediatr*. (2013) 172(12):1607–17. doi: 10.1007/s00431-013-2119-z

38. Volianska L, Burbela E, Kosovska T, Perestiuk V, Boyarchuk O. The role of vitamin D in the course of SARS-CoV-2 infection and long COVID in children (literature review). *Child's Health*. (2023) 18(3):225–30. doi: 10.22141/2224-0551.18.3.2023.1590

39. Boyarchuk OR, Monastyrska OI, Suvalko SI, Perestiuk VO, Hariyan TV. Impact of COVID-19, pandemic, and full-scale war on the health of schoolchildren: survey results. *Ukr J Perinatol Pediatr*. (2024) 2(98):77–85. doi: 10.15574/PP.2024.98.77

40. Mosca C, Colucci A, Savoia F, Calì C, Del Bene M, Ranucci G, et al. Vitamin D levels in the pre- and post-COVID-19 pandemic periods and related confinement at pediatric age. *Nutrients*. (2023) 15(9):2089. doi: 10.3390/nu15092089

41. Alpcan A, Tursun S, Kandur Y. Vitamin D levels in children with COVID-19: a report from Turkey. *Epidemiol Infect*. (2021) 149:e180. doi: 10.1017/S0950268821001825

42. Nicolae M, Mihai CM, Chisnoiu T, Balasa AL, Frecus CE, Mihai L, et al. Immunomodulatory effects of vitamin D in respiratory tract infections and COVID-19 in children. *Nutrients*. (2023) 15(15):3430. doi: 10.3390/nu15153430

43. Kosmeri C, Balomenou F, Rallis D, Baltogianni M, Giapros V. The role of serum vitamin 25(OH)D concentration in the COVID-19 pandemic in children. *Br J Nutr*. (2023) 130(3):417–22. doi: 10.1017/S0007114522003476

44. Ahmad AS, Juber NF, Al-Naseri H, Heumann C, Ali R, Oliver T. Association between average vitamin D levels and COVID-19 mortality in 19 European countries-A population-based study. *Nutrients*. (2023) 15(22):4818. doi: 10.3390/nu15224818

45. Seal KH, Bertenthal D, Carey E, Grunfeld C, Bikle DD, Lu CM. Association of vitamin D Status and COVID-19-related hospitalization and mortality. *J Gen Intern Med*. (2022) 37(4):853–61. doi: 10.1007/s11606-021-07170-0

46. Akbar MR, Wibowo A, Pranata R, Setiabudiawan B. Low serum 25-hydroxyvitamin D (vitamin D) level is associated with susceptibility to COVID-19, severity, and mortality: a systematic review and meta-analysis. *Front Nutr*. (2021) 8:660420. doi: 10.3389/fnut.2021.660420

47. Susianti H, Wahono CS, Rahman PA, Pratama MZ, Wulanda IA, Hartanti KD, et al. Low levels of vitamin D were associated with coagulopathy among hospitalized coronavirus disease-19 (COVID-19) patients: a single-centered study in Indonesia. *J Med Biochem*. (2021) 40(4):341–50. doi: 10.5937/jomb0-30228

48. Boyarchuk O, Perestiuk V, Kosovska T, Volianska L. Coagulation profile in hospitalized children with COVID-19: pediatric age dependency and its impact on long COVID development. *Front Immunol*. (2024) 15:1363410. doi: 10.3389/fimmu.2024.1363410

49. Salamanna F, Maglio M, Sartori M, Landini MP, Fini M. Vitamin D and platelets: a menacing duo in COVID-19 and potential relation to bone remodeling. *Int J Mol Sci*. (2021) 22(18):10010. doi: 10.3390/ijms221810010

50. Cooper ID, Crofts CAP, DiNicolantonio JJ, Malhotra A, Elliott B, Kyriakidou Y, et al. Relationships between hyperinsulinaemia, magnesium, vitamin D, thrombosis and COVID-19: rationale for clinical management. *Open Heart*. (2020) 7(2):e001356. doi: 10.1136/openhrt-2020-001356

51. Getachew B, Landis HE, Manaye KF, Tizabi Y. COVID-19-associated coagulopathy: role of vitamins D and K. *Curr Pharm Biotechnol*. (2023) 24(3):401–10. doi: 10.2174/1389201023666220527110455

52. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol*. (2007) 179(3):1634–47. doi: 10.4049/jimmunol.179.3.1634

53. Peraire J, García-Pardo G, Chafino S, Sánchez A, Botero-Gallego M, Olona M, et al. Immunoglobulins in COVID-19 pneumonia: from the acute phase to the recovery phase. *Eur J Med Res*. (2024) 29(1):223. doi: 10.1186/s40001-024-01824-5

54. Votto M, Castagnoli R, Marseglia GL, Licari A, Brambilla I. COVID-19 and autoimmune diseases: is there a connection? *Curr Opin Allergy Clin Immunol*. (2023) 23(2):185–92. doi: 10.1097/ACI.0000000000000888

55. Perlis RH, Green J, Santillana M, Lazer D, Ognyanova K, Simonson M, et al. Persistence of symptoms up to 10 months following acute COVID-19 illness. *medRxiv [preprint]*. (2021) 03:2021.03.07.21253072. doi: 10.1101/2021.03.07.21253072

56. Fernández-de-Las-Peñas C, Palacios-Ceña D, Gómez-Mayordomo V, Rodríuez-Jiménez J, Palacios-Ceña M, Velasco-Arribas M, et al. Long-term post-COVID symptoms and associated risk factors in previously hospitalized patients: a multicenter study. *J Infect*. (2021) 83(2):237–79. doi: 10.1016/j.jinf.2021.04.036

57. Rao S, Lee GM, Razzaghi H, Lorman V, Mejias A, Pajor NM, et al. Clinical features and burden of postacute sequelae of SARS-CoV-2 infection in children and adolescents. *JAMA Pediatr*. (2022) 176(10):1000–9. doi: 10.1001/jamapediatrics.2022.2800

58. di Filippo L, Frara S, Nannipieri F, Cotellessa A, Locatelli M, Rovere Querini P, et al. Low vitamin D levels are associated with long COVID syndrome in COVID-19 survivors. *J Clin Endocrinol Metab*. (2023) 108(10):e1106–16. doi: 10.1210/clinem/dgad207

59. Mohamed Hussein AAR, Galal I, Amin MT, Moshnib AA, Makhlouf NA, Makhlouf HA, et al. Prevalence of vitamin D deficiency among patients attending post COVID-19 follow-up clinic: a cross-sectional study. *Eur Rev Med Pharmacol Sci*. (2022) 26(8):3038–45. doi: 10.26355/eurrev_202204_28635

60. Chen KY, Lin CK, Chen NH. Effects of vitamin D and zinc deficiency in acute and long COVID syndrome. *J Trace Elem Med Biol*. (2023) 80:127278. doi: 10.1016/j.jtemb.2023.127278

61. Guerrero-Romero F, Gamboa-Gómez CI, Rodríguez-Morán M, Orrante M, Rosales-Galindo E, Cisneros-Ramírez I, et al. Hypomagnesemia and 25-hydroxyvitamin D deficiency in patients with long COVID. *Magnes Res*. (2023) 36(3):30–6. doi: 10.1684/mrh.2023.0519

62. Hikmet RG, Wejse C, Agergaard J. Effect of vitamin D in long COVID patients. *Int J Environ Res Public Health*. (2023) 20(22):7058. doi: 10.3390/ijerph20227058

63. Wu JY, Liu MY, Hsu WH, Tsai YW, Liu TH, Huang PY, et al. Association between vitamin D deficiency and post-acute outcomes of SARS-CoV-2 infection. *Eur J Nutr*. (2024) 63(2):613–22. doi: 10.1007/s00394-023-03298-3

64. Pizzini A, Aichner M, Sahanic S, Böhm A, Egger A, Hoermann G, et al. Impact of vitamin D deficiency on COVID-19—a prospective analysis from the CovILD registry. *Nutrients*. (2020) 12:2775. doi: 10.3390/nu12092775

65. Zengin N, Bal A, Goren TA, Bayturan SS, Alkan F, Akcali S. Serum vitamin D levels in relation to development of multisystem inflammatory syndrome in pediatric COVID-19. *J Pediatr Infect Dis*. (2022) 17(06):308–16. doi: 10.1055/s-0042-1756713

66. Townsend L, Dyer AH, McCluskey P, O'Brien K, Dowds J, Laird E, et al. Investigating the relationship between vitamin D and persistent symptoms following SARS-CoV-2 infection. *Nutrients*. (2021) 13(7):2430. doi: 10.3390/nu13072430

67. Chen TB, Chang CM, Yang CC, Tsai IJ, Wei CY, Yang HW, et al. Neuroimmunological effect of vitamin D on neuropsychiatric long COVID syndrome: a review. *Nutrients*. (2023) 15(17):3802. doi: 10.3390/nu15173802

68. Mundula T, Russo E, Curini L, Giudici F, Piccioni A, Franceschi F, et al. Chronic systemic low-grade inflammation and modern lifestyle: the dark role of gut microbiota on related diseases with a focus on COVID-19 pandemic. *Curr Med Chem*. (2022) 29(33):5370–96. doi: 10.2174/0929867329666220430131018

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# The relationship between vitamin D levels, D-dimer and platelet parameter levels in patients with gestational hypertension

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**Introduction:** Hypertension during pregnancy is a common pregnancy complication that has an important impact on maternal and fetal health. In recent years, studies have shown that vitamin D, D dimers and platelet parameters may play a key role in the occurrence and development of gestational hypertension.

**Objective:** This study aimed to explore the relationship between vitamin D levels, D dimers and platelet parameters in patients with gestational hypertension.

**Material and methods:** This study retrospectively analyzed the clinical data of 90 patients with gestational hypertension and 90 normal pregnant women who were treated in our hospital from September 2022 to September 2023. We compared the blood routine indicators between the two groups, including platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), etc., as well as D dimer and vitamin D (Vit D) levels.

**Results:** The results showed that the vitamin D level and PLT in the gestational hypertension group were significantly lower than those in the normal pregnant group, while MPV and PDW were significantly increased. In addition, vitamin D levels were significantly correlated with D dimer, MPV and PDW. Further statistical analysis showed that vitamin D, D dimer and platelet parameters were important predictors of gestational hypertension.

**Conclusion:** This study found that patients with gestational hypertension have vitamin D deficiency and abnormal platelet function. Vitamin D may affect the

development of the disease by regulating platelet activity and coagulation status, which may be closely related to its pathological mechanism. This suggests that improving vitamin D status may have potential value in the management of gestational hypertension.

KEYWORDS

**gestational hypertension, vitamin D, D dimer, platelet parameters, correlation**

# 1 Introduction

Hypertensive Disorders of Pregnancy (HDP) is a common disease unique to pregnant women, with an incidence rate between 5% and 10%. HDP is the second leading cause of maternal death after postpartum hemorrhage, accounting for 10% to 16% of total pregnancy-related deaths (1). It is not only an important cause of maternal death, but also a significant factor in neonatal mortality (2, 3). The clinical manifestations of HDP mainly include proteinuria, edema, and hypertension. In severe cases, coma, convulsions, and even death of mother and child may occur (4). At present, the pathogenesis of HDP is not completely clear, but it may be related to immune regulatory function, genetic factors, and abnormal invasion of trophoblast cells (5). Studies have shown that the main pathological changes of HDP include systemic small vessel spasm, reduced organ perfusion, and excessive hypercoagulability (6, 7). The causes of the disease are complex and involve many aspects, such as ischemia and hypoxia caused by shallow implantation of the placenta, immune disorders, very low-density lipoprotein toxicity, genetic imprinting, and large expression of D-dimer. These factors may cause damage to vascular endothelial cells, leading to systemic microvascular spasm and changes in vascular permeability, ultimately causing leakage of body fluids and proteins, redistributing body water and increasing interstitial fluid (8–10). The influencing factors of HDP are diverse and complex, and there are potential risks to both maternal and fetal health. Therefore, it is of great significance to further study the pathogenesis of HDP and its management measures.

Studies in recent years have shown that 25-hydroxyvitamin D3 (25-(OH)D3) is closely related to gestational hypertension (HDP). 25-(OH)D3 has the functions of inhibiting the proliferation of vascular smooth muscle cells, maintaining blood pressure balance and producing anti-inflammatory effects, which makes it play an important role in the prevention of cardiovascular diseases (11). With the development of society and changes in lifestyle, such as increased indoor work and excessive sun protection, the content of vitamin D (VitD) produced by sunlight in the human body has decreased, and the impact of seasons on vitamin D has become smaller and smaller. In China, the proportion of VitD deficiency or insufficiency is about 71%, and among pregnant women, the proportion of severe VitD deficiency is as high as 80% (12), especially pregnant women with a gestational age of more than 30 weeks (13). At present, a serum 25-hydroxyvitamin D concentration below 25nmol/L is usually defined as severe vitamin D deficiency. It is generally believed that the serum 25-hydroxyvitamin D concentration maintained at 50-125nmol/L is a relatively safe range. Within this range, the body's various physiological functions can be better maintained, which is beneficial to the health of pregnant women and fetuses (14).

VitD deficiency is closely related to the incidence of HDP. VitD has the function of maintaining the stability of vascular endothelial cells, protecting and improving the function of vascular endothelial cells. When VitD deficiency occurs, the content of placental growth factor decreases, the stability and protection of vascular endothelial cells decrease, resulting in the decrease of vascular endothelial anterograde, the increase of hardness, and the increase of both systolic and diastolic blood pressure (15). In addition, VitD can inhibit the renin-angiotensin system, and when VitD is deficient, this inhibition decreases, resulting in the increase of both systolic and diastolic blood pressure in pregnant women (16, 17). Insufficient VitD may also lead to decreased absorption of calcium ions, which can directly affect angiotensin II, and its reduced content can promote angiotensin II to contract vascular smooth muscle function, and eventually lead to preeclampsia like changes in pregnant women (18). *In vivo*, 25-(OH)D3 is the active form of VitD, which can regulate the transcriptional activity of immune cells and up-regulate or inhibit the expression of macrophages, dendritic cells and other cytokines after binding with VitD receptors. When VitD is deficient, the expression of anti-inflammatory factors in pregnant women is reduced, which increases the risk of preeclampsia (19).

In addition, patients with hypertension during pregnancy are often accompanied by obvious hypercoagulable state and platelet activation. Abnormal coagulation function may aggravate the progression of the disease (20). As the disease progresses, vascular endothelial damage intensifies, which not only promotes platelet adhesion and aggregation, but is also accompanied by a decline in microvascular system function, leading to increased platelet destruction. Therefore, the platelet count (PLT) may decrease, while the platelet volume distribution width (PDW) may increase (21). These changes reflect the abnormal characteristics of

**Abbreviations:** HDP, Hypertensive Disorders of Pregnancy; PLT, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; Vit D, vitamin D.

coagulation function in patients with hypertension during pregnancy. Therefore, understanding and monitoring the levels of VitD, D-dimer and platelet parameters in pregnant women may help prevent and manage HDP early, thereby improving maternal and infant outcomes (22).

Through in-depth understanding and evaluation of the pathogenesis of HDP, it can provide an important theoretical basis for the early diagnosis and effective treatment of the disease. However, despite the progress made in existing research, the complexity and diversity of HDP still need further research and exploration.

# 2 Materials and methods

The subjects of this study were pregnant women with singleton pregnancy who were diagnosed with gestational hypertension in the obstetrics department of a municipal tertiary-level A-class specialist hospital in China from September 2022 to September 2023, with an age range of 25 to 39 years. The diagnostic criteria for hypertensive disorders complicating pregnancy refer to the ninth edition of the textbook of obstetrics and gynecology and the guidelines for the diagnosis and treatment of hypertensive disorders complicating pregnancy (2020). Hypertension complicating pregnancy: hypertension after 20 weeks of pregnancy, systolic blood pressure ≥140 mmHg and (or) diastolic blood pressure ≥90 mmHg, returning to normal within 12 weeks after delivery; urine protein (-); diagnosis can only be made after delivery. A total of 180 pregnant women were included to ensure sufficient statistical power. Exclusion criteria included: genetic metabolic diseases, tumors, vitamin D supplementation in the previous 3 months, and patients taking anticoagulants or platelet inhibitors in the past six months. The control group consisted of pregnant women with singleton pregnancy who had normal prenatal examinations in the obstetrics outpatient clinic of the same hospital, and the exclusion criteria were the same as those of the study group to ensure consistent health conditions. This study was approved by the hospital ethics committee (approval number: KY2022052003.0), and all participants signed informed consent.

The demographic characteristics of each pregnant woman, including BMI and the proportion of primiparas, were recorded through the hospital LIS system. Venous blood was drawn to test CBC, D-dimer and vitamin D levels. CBC was performed using the 6800PLUS fully automatic blood cell analysis system produced by Shenzhen Mindray Company, and blood samples using ethylenediaminetetraacetic acid (EDTA) were collected. The samples were analyzed within 1 hour after collection to prevent platelet swelling and result bias.

Plasma D-dimer was detected using latex turbidimetry, using the Sysmex EX810 fully automatic coagulation analyzer and supporting reagents. Vitamin D (25(OH)D) was determined using chemiluminescence immunoassay, using the Mindray 2000i fully automatic biochemical analyzer and supporting kits produced by Shenzhen Mindray Bio-Medical Electronics Co., Ltd. All measurements were strictly performed in accordance with the manufacturer's instructions, and corresponding quality control was performed.

## 2.1 Statistical analysis

Statistical analysis was performed using SPSS 25 software. The Kolmogorov-Smirnov normality test was used for the normal distribution of continuous variables. Continuous variables were expressed as mean ± standard deviation (SD) or median (percentile 25, 75) and compared using unpaired Student's t test or nonparametric Mann-Whitney test. Categorical variables were expressed as counts and percentages and compared using the $\chi 2$ test or Fisher's exact test. Binary logistic regression was used to analyze the influencing factors of gestational hypertension, and ROC curves were used to analyze the diagnostic efficacy of these indicators for gestational hypertension, with $P < 0.05$ as statistically significant.

# 3 Results

## 3.1 Demographic characteristics and clinical symptoms

A total of 180 pregnant women were included in this study, divided into 90 patients with gestational hypertension (research group) and 90 healthy pregnant women undergoing prenatal care (control group). We compared demographic characteristics such as age, days of gestation, and proportion of primigravida between the two groups. The results showed that there was no significant difference in these basic characteristics between the two groups (see Table 1), which laid the foundation for the comparison of subsequent experimental data, allowing the data of different groups to be effectively compared.

TABLE 1 General information comparison of research subjects.

| | Age (years) | Pregnancy days | BMI | Primiparous women [cases (%)] |
|---|---|---|---|---|
| Research group | 30.63 ± 4.45 | 160.24 ± 38.76 | 58.9 ± 5.2 | 52 (57.78) |
| control group | 29.77 ± 4.51 | 163.23 ± 77.75 | 60.2 ± 4.3 | 49 (54.44) |
| t/X2 | 1.73 | -0.574 | -0.825 | 0.207 |
| P | 0.086 | 0.567 | 0.411 | 0.648 |

## 3.2 Laboratory results

We compared the biochemical indicators of the study group and the control group and found that the 25-hydroxyvitamin D level of the study group was significantly lower than that of the control group [17.46 (12.35, 22.98) vs 27.77 (26.36, 29.24), P < 0.001]. This result shows that vitamin D deficiency is common in patients with gestational hypertension, which may be related to the pathogenesis of gestational hypertension. In addition, the D-dimer ([1.49(1.05,2.46) vs 1.2(0.91,1.7), P=0.002]), PDW ([16.3(16,16.6) vs 15.85(15.18,16.23), P <0.001]) and MPV ([9.6(9.1,10.65) vs 8.95 (7.9,10), P<0.001]) were significantly higher than the control group. These results suggest that patients with gestational hypertension not only suffer from vitamin D deficiency, but are also accompanied by platelet function abnormalities and changes in coagulation status, reflecting that the blood system of patients with gestational hypertension may be in a hypercoagulable state. The results are shown in Table 2. The comparison between the two groups is shown in Figures 1– 4.

## 3.3 Correlation analysis

Correlation analysis further revealed the relationship between 25-hydroxyvitamin D and other blood indicators. Our results show that there is a significant negative correlation between 25-hydroxyvitamin D and D-dimer (r=-0.365, P<0.001), and a negative correlation with MPV (r=-0.496, P<0.001). There is also a negative correlation with PDW (r=-0.491, P<0.001). These results suggest that reduced vitamin D levels may lead to enhanced platelet activation and coagulation capacity, thereby promoting the development of gestational hypertension. Through these analyses, we can speculate that vitamin D deficiency may be one of the important driving factors for the onset of gestational hypertension. The results are shown in Table 3 and Figures 5–10.

TABLE 2 Comparison of VitD, D-dimer, MPV, and PDW between the study group and the control group.

| | Cases no. | 25 hydroxyvitamin D ng/mL | D-dimer mg/L | Platelet distribution width % | Mean platelet volume fL |
|---|---|---|---|---|---|
| Research group | 90 | 17.46 (12.35,22.98) | 1.49 (1.05,2.46) | 16.3 (16,16.6) | 9.6 (9.1,10.65) |
| control group | 90 | 27.77 (26.36,29.24) | 1.2 (0.91,1.7) | 15.85 (15.18,16.23) | 8.95 (7.9,10) |
| Z | | -8.498 | -3.074 | -5.733 | -3.771 |
| P | | <0.001 | 0.002 | <0.001 | <0.001 |

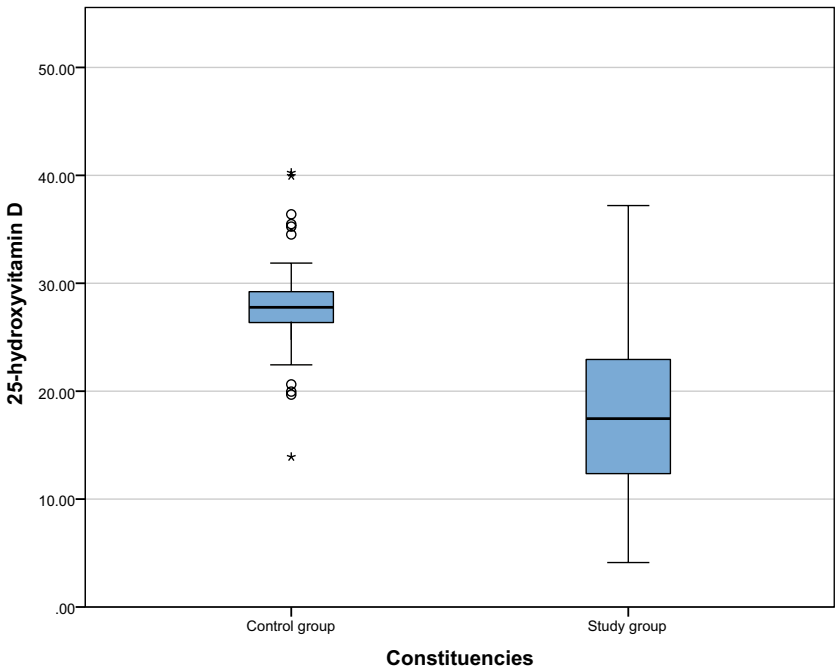


FIGURE 1
Comparison of 25 hydroxyvitamin D levels between the study group and the control group.

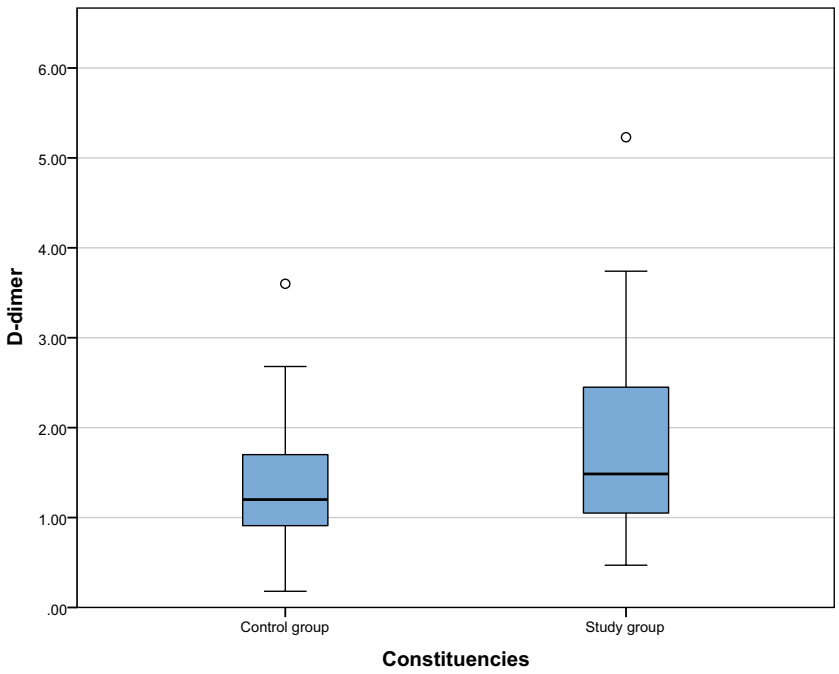


FIGURE 2
Comparison of PDW between the study group and the control group.

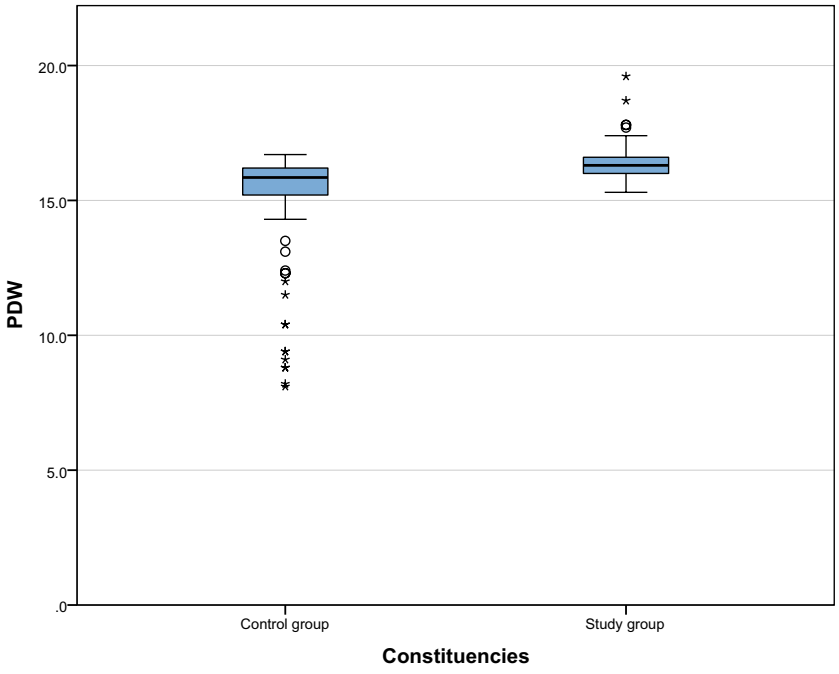


FIGURE 3
Comparison of PDW between the study group and the control group.

## 3.4 Logistic regression analysis

To explore the independent effects of 25-hydroxyvitamin D, D-dimer, PDW and MPV on the occurrence of gestational hypertension, we performed multivariate logistic regression analysis. The results showed that low levels of 25-hydroxyvitamin D (OR=0.43, 95% CI: 0.26-0.72, P=0.002), high D-dimer (OR=2.67, 95% CI: 1.56-4.56, P<0.001), and increased PDW (OR=3.21, 95%

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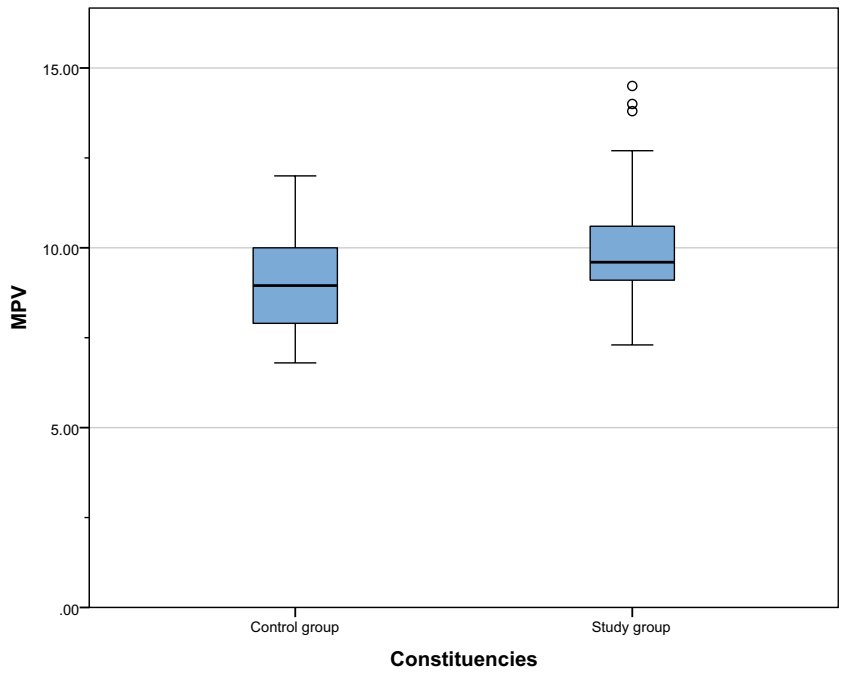


FIGURE 4
Comparison of MPV between the study group and the control group.

TABLE 3 Correlation analysis of 25 hydroxyvitamin D with D-dimer, MPV, and PDW.

| | | D-dimer | platelet distribution width | mean platelet volume |
|---|---|---|---|---|
| 25-hydroxyvitamin D | correlation coefficient | -0.365** | -0.496** | -0.491** |
| | P | <0.01 | <0.01 | <0.01 |

** indicates that the correlation is significant at the 0.01 level.

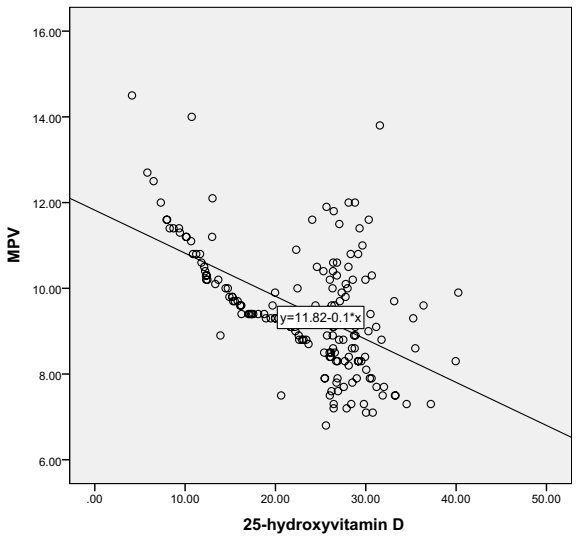


FIGURE 5
Scatter Plot of the Correlation between 25-Hydroxyvitamin D and MPV.

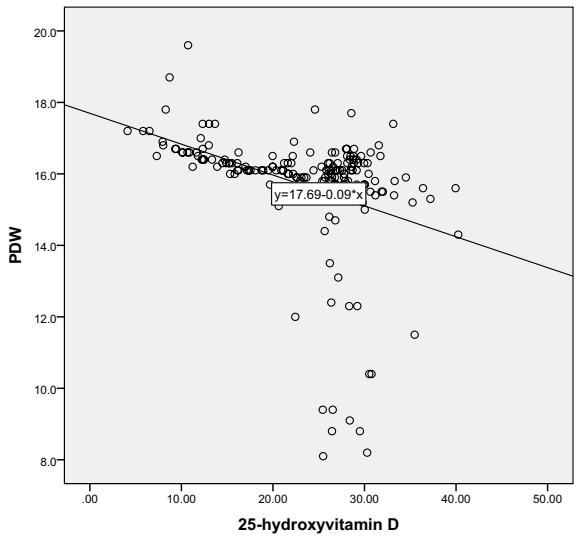


FIGURE 6
Scatter Plot of the Correlation between 25-Hydroxyvitamin D and PDW.

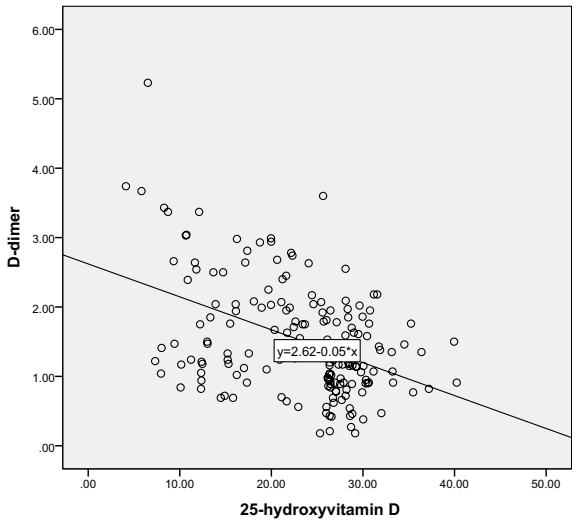


FIGURE 7
Scatter plot of the correlation between 25-hydroxyvitamin D and D-dimer.

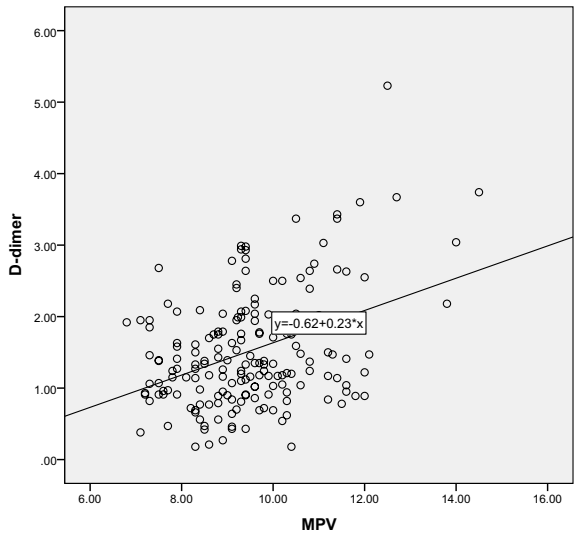


FIGURE 9
Scatter Plot of the Correlation between D-dimer and MPV.

CI: 1.86-5.55, P<0.001) and MPV (OR=2.94, 95% CI: 1.68-5.14, P<0.001) were all independent risk factors for gestational hypertension, see Table 4. These findings reinforce our previous hypothesis that these biomarkers play an important role in the pathogenesis of gestational hypertension.

## 3.5 ROC curve analysis

Based on our previous results, in order to evaluate the effectiveness of these biomarkers in gestational hypertension screening, we performed ROC curve analysis. The results showed that the AUC of 25-hydroxyvitamin D was 0.82, the AUC of PDW was 0.75, and the AUC of the combined index reached 0.89, indicating that it has good diagnostic ability. In terms of sensitivity and specificity, the performance of the combined index is particularly outstanding, which means that the combination of these indicators in clinical practice can more effectively identify patients with gestational hypertension. The results are shown in Table 5, Figures 11, 12.

# 4 Discussion

Preeclampsia is a serious pregnancy complication with complex etiology. Studies have shown that when the vitamin D level of pregnant women is <50nmol/L, the concentration of placental

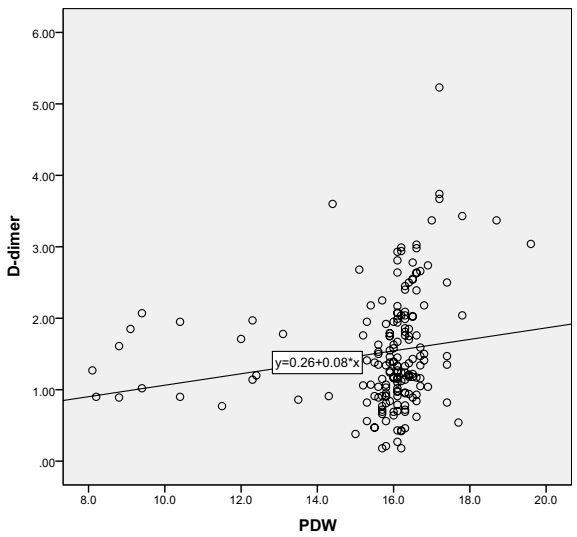


FIGURE 8
Scatter Plot of the Correlation between D-dimer and PDW.

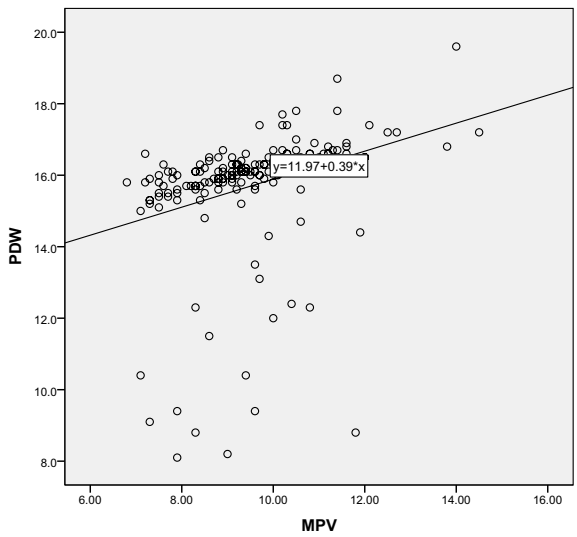


FIGURE 10
Scatter Plot of the Correlation between MPV and PDW.

TABLE 4 Binary logistic regression analysis of the influencing factors of 25 hydroxyvitamin D, D-dimer, MPV, and PDW on the occurrence of preeclampsia.

| | B | Standard error | Wald | Significance | Exp(B) | 95% confidence interval of EXP (B) | |
|---|---|---|---|---|---|---|---|
| | | | | | | lower limit | upper limit |
| 25 hydroxyvitamin D | -0.296 | 0.046 | 41.963 | <0.001 | 0.744 | 0.68 | 0.813 |
| D-dimer | 0.796 | 0.221 | 12.985 | <0.001 | 2.217 | 1.438 | 3.419 |
| Platelet distribution width | 1.605 | 0.368 | 18.99 | <0.001 | 4.977 | 2.418 | 10.243 |
| Mean platelet volume | 0.468 | 0.125 | 13.944 | <0.001 | 1.597 | 1.249 | 2.042 |

TABLE 5 ROC curve analysis of the diagnostic value of 25 hydroxyvitamin D, D-dimer, MPV, and PDW for preeclampsia.

| Test result variable | region | Cutoff value | Sensitivity | Specificity | Maximum Yoden Index | P | 95% asymptotic confidence interval | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | lower limit | upper limit |
| 25 hydroxyvitamin D | 0.867 | 24.95 | 0.822 | 0.944 | 0.766 | <0.001 | 0.805 | 0.928 |
| D-dimer | 0.633 | 1.98 | 0.367 | 0.889 | 0.256 | 0.002 | 0.551 | 0.714 |
| Platelet distribution width | 0.747 | 15.95 | 0.8 | 0.556 | 0.356 | <0.001 | 0.677 | 0.817 |
| Mean platelet volume | 0.663 | 9.15 | 0.744 | 0.556 | 0.3 | <0.001 | 0.583 | 0.742 |
| Merge indicators | 0.89 | – | 0.822 | 0.944 | 0.766 | <0.001 | – | – |

growth factor decreases, which is not conducive to fetal development; vitamin D can also regulate the activity of immune cells and inhibit the expression of cytokines such as macrophages and monocytes. Pregnant women with gestational hypertension have higher levels of inflammatory factors, and vitamin D can promote the production of anti-inflammatory factors and play a certain anti-inflammatory role (23–25). Vitamin D plays an important role in the etiology and pathophysiology of hypertensive diseases during pregnancy. Our research results support this view, pointing out that there is a clear association between 25-hydroxyvitamin D deficiency and increased coagulation activity and changes in platelet function. Vitamin D deficiency may lead to endothelial cell dysfunction, thereby increasing platelet activation and coagulation tendency, forming a vicious cycle of

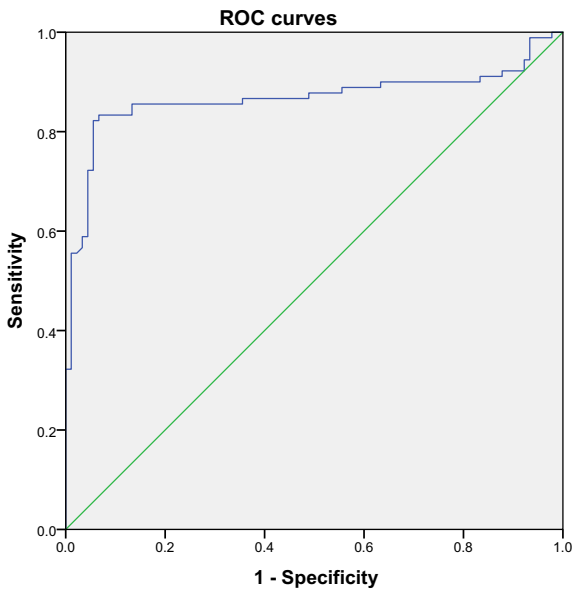


FIGURE 11
ROC curve analysis of specificity and sensitivity of 25 hydroxyvitamin D.

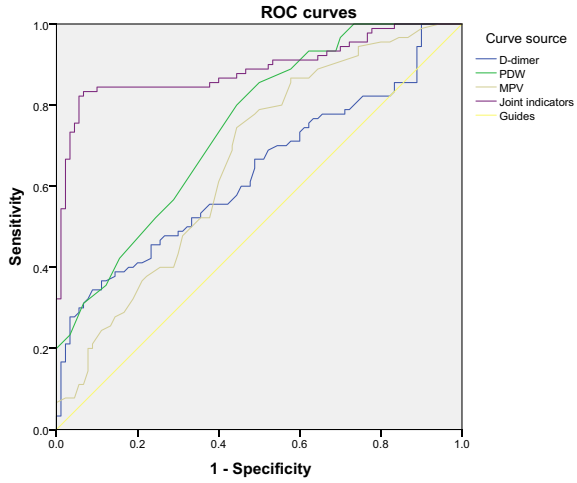


FIGURE 12
ROC curve analysis of specificity and sensitivity of D-dimer, MPV, and PDW.

the development of gestational hypertension. Many studies have shown that vitamin D supplementation during pregnancy can reduce the incidence of hypertensive diseases during pregnancy in people with vitamin D deficiency and is related to the severity of the disease (26, 27), but there is controversy as to whether vitamin D supplementation during pregnancy can improve pregnancy complications and adverse fetal outcomes (28–30).

As a marker of fibrinolysis, elevated levels of D-dimer are usually associated with an increased risk of thrombosis. In our study, the negative correlation between 25-hydroxyvitamin D and D-dimer suggests that low vitamin D levels may lead to overactivation of the coagulation system, thereby increasing the incidence of thrombosis in patients with gestational hypertension. This result is consistent with existing literature (31, 32) and further emphasizes the importance of monitoring D-dimer levels in pregnant women in order to identify potential risks of gestational hypertension early. In addition, HDP patients are often in a chronic diffuse vascular coagulation or prothrombotic state, and dynamic observation of patients' platelet parameters and coagulation indicators can help reduce the incidence of thrombotic complications (33–35).

Studies have found that vitamin D can inhibit platelet aggregation through endothelial cells. Compared with platelets incubated with untreated endothelial cells, platelets in direct contact with umbilical vein endothelial cells incubated with 1α, 25-dihydroxyvitamin D3 showed lower activation (36). Lower vitamin D levels in the blood are associated with higher platelet reactivity. Lower vit D levels lead to lower VDR expression, thereby reducing PLT activation (37). Our results complement this view. The reduction in 25-hydroxyvitamin D levels is negatively correlated with both MPV and PDW, reflecting the role of vitamin D in regulating platelet function and inhibiting inflammation. Vitamin D deficiency may aggravate the pathophysiological changes of pregnancy-induced hypertension by promoting platelet activation and inflammatory response.

In view of our findings, joint detection of 25-hydroxyvitamin D and other blood indicators can significantly improve the diagnostic rate of gestational hypertension, indicating broad prospects for clinical application. It is recommended that clinicians consider these biomarkers as early screening tools. In this study, the 25-hydroxyvitamin D level in the pregnancy-induced hypertension group was 17.46 (12.35, 22.98) ng/ml. According to the "Crowd VD Deficiency Screening Method (WS/T 677- 2020)" released by China in 2020, serum (or plasma) 25 (OH) D ≥ 20 ng/mL is considered normal VD, 12~<20ng/mL is considered VD insufficiency, and <12ng/mL is considered VD deficiency. It can be seen that the 25-hydroxyvitamin D level in the pregnancy-induced hypertension group is generally insufficient, and some have even reached the level of deficiency, far below the lower limit of the normal level of 20ng/ml, which is a more serious vitamin D deficiency. It is generally believed that the serum 25-hydroxyvitamin D concentration is maintained at 20~50ng/ml as a relatively safe range. Within this range, the body's various physiological functions can be well maintained, which is beneficial to the health of pregnant women and fetuses. However, the vitamin D levels in the pregnancy-induced hypertension group in this study deviated significantly from this safe range, indicating that vitamin D deficiency is not only common but also severe in patients with pregnancy-induced hypertension. Check pregnant women's vitamin D levels regularly during pregnancy and supplement when necessary. By improving vitamin D status, it may be possible to reduce the risk of pregnancy-induced hypertension. In addition, our data provide a theoretical basis for vitamin D as a potential target for the prevention and treatment of gestational hypertension, and future clinical trials will help verify the effectiveness of vitamin D supplementation for the prevention and management of gestational hypertension.

The limitations of this study include a relatively small sample size and a cross-sectional design that limits causal inference. To more comprehensively evaluate the relationship between vitamin D and gestational hypertension, large-scale prospective studies should be conducted in the future to validate our findings and explore their potential mechanisms. In addition, the optimal dose of vitamin D supplementation and its efficacy in different populations need to be further explored.

## 5 Conclusion

In summary, the significant correlation between 25-hydroxyvitamin D and gestational hypertension-related blood indicators reveals the important role of vitamin D in hemodynamic regulation during pregnancy. Our study provides a theoretical basis for vitamin D as a new strategy for the prevention and treatment of pregnancy-induced hypertension. Future research should further explore its mechanism and clinical application value.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## Ethics statement

The studies involving humans were approved by The Ethics Committee of Luoyang Maternal and Child Health Hospital (No.KY2022052003.0). 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

PW: Writing – original draft, Conceptualization, Methodology. JY: Writing – review & editing. YL: Writing – review & editing. ZZ:

Supervision, Writing – review & editing. RZ: Formal Analysis, Project administration, Writing – review & editing. SL: Formal Analysis, Project administration, Writing – review & editing. MS: Software, Writing – review & editing. XH: Funding acquisition, Software, 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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## References

1. Qu Y, Wang W, Wang Q. A review of the relationship between blood pressure control level and pregnancy outcomes in hypertensive disorders complicating pregnancy. *Chin J Maternal Child Health Care*. (2018) 33:2632–5. doi: 10.7620/zgfybj.j.issn.1001-4411.2018.11.77

2. Gyselaers W. Hemodynamic pathways of gestational hypertension and preeclampsia. *Am J Obstetrics Gynecology*. (2022) 226:S988–S1005. doi: 10.1016/j.ajog.2021.11.022

3. Lama S, Gurung P, Malla AP. Pregnancy induced hypertensive disorders among patients admitted to the department of obstetric and gynecology in a tertiary care centre: A descriptive cross-sectional study. *JNMA J Nepal Med Assoc*. (2023) 61:195–9. doi: 10.31729/jnma.8060

4. Li W, Xia Z, Wu S, Zhang G. Analysis of pregnancy outcomes in 300 cases of hypertensive disorder complicating pregnancy. *Chin J Pract Gynecology Obstetrics*. (2021) 37:588–91. doi: 10.19538/j.fk2021050120

5. Yang Z. Analysis of hypertensive disorders in pregnancy in real clinical practice. *Chin J Pract Gynecology Obstetrics*. (2019) 35:408–16. doi: 10.19538/j.fk2019040111

6. Yao Q, Yuan X, Zhao Y. Clinical study on Mailuoning injection combined with magnesium sulfate in the treatment of pregnancy-induced hypertension syndrome. *Modern Drugs Clinics*. (2021) 36:538–42. doi: 10.7501/j.issn.1674-5515.2021.03.025

7. Jiang Y. Analysis of urine protein and blood routine levels in patients with gestational hypertension and their impact on pregnancy outcomes. *Shaanxi Med J*. (2019) 48:128–30. doi: 10.3969/j.issn.1000-7377.2019.01.040

8. Wenli H, Wu J. Effects of low-dose aspirin on vascular endothelial function and coagulation function in patients with mild gestational hypertension. *Thromb Hemostasis*. (2021) 27:614–5. doi: 10.3969/j.issn.1009-6213.2021.04.031

9. Metoki H, Iwama N, Hamada H, Satoh M, Murakami T, Ishikuro M, et al. Hypertensive disorders of pregnancy: definition, management, and out-of-office blood pressure measurement. *Hypertens Res*. (2022) 45:1298–309. doi: 10.1038/s41440-022-00965-6

10. Magee LA, Smith GN, Bloch C, Côté AM, Jain V, Nerenberg K, et al. Guideline No. 426: hypertensive disorders of pregnancy: diagnosis, prediction, prevention, and management. *J Obstetrics Gynaecology Canada*. (2022) 44:547–71. doi: 10.1016/j.jogc.2022.03.002

11. Ren B, Gao J. Clinical significance of serum 25-hydroxyvitamin D detection in patients with gestational hypertension. *Chin J Drugs Clin Practice*. (2021) 21:806–8. doi: 10.11655/zgywylc2021.05.037

12. Zhou J, Wang S. Progress in research on vitamin D supplementation for pregnant and postpartum women. *Chin J Clin Obstetrics Gynecology*. (2018) 19:277–9. doi: 10.13390/j.issn.1672-1861.2018.03.036

13. Wang H, Zhang F, Li B, Fu M, Shan X, Ma Y. Three-stage pattern of rapid increase, plateau, and subsequent decline in vitamin D concentration during pregnancy among Chinese women: a large-scale survey. *Front Nutritio*. (2023) 10:1238389. doi: 10.3389/fnut.2023.1238389

14. Ma Y, Bai L, Chen Y. Study on the preventive effect of high-dose vitamin D during pregnancy on the prevention and treatment of gestational diabetes and pregnancy outcomes in obese pregnant women with vitamin D deficiency. *Chin J Med Front (Electronic Edition)*. (2022) 14:55–8. doi: 10.12037/YXQY.2022.07-11

15. Beveridge LA, Khan F, Struthers AD, Armitage J, Barchetta I, Bressendorff I, et al. Effect of vitamin D supplementation on markers of vascular function: a systematic review and individual participant meta-analysis. *J Am Heart Assoc*. (2018) 7:e008273. doi: 10.1161/JAHA.117.008273

16. Lan K, Dong Q, Liu S, Zhang X, Ni J, Zhang G. Relationship between vitamin D level and renin, angiotensin II levels and proteinuria in patients with diabetic nephropathy. *Chin J Integrated Traditional Western Med Nephrology*. (2021) 22:152–4. doi: 10.3969/j.issn.1009-587X.2021.02.017

17. Cui C, Xu P, Li G, Qiao Y, Han W, Geng C, et al. Vitamin D receptor activation regulates microglia polarization and oxidative stress in spontaneously hypertensive rats and angiotensin II-exposed microglial cells: role of renin-angiotensin system. *Redox Biol*. (2019) 26:101295. doi: 10.1016/j.redox.2019.101295

18. Wang L, Liu M. The predictive value of serum 25-hydroxyvitamin D and calcium ion levels for the occurrence and development of preeclampsia. *Chin J Drugs Clin Practice*. (2022) 22:350–2. doi: 10.11655/zgywylc2022.04.014

19. Zhang Yu. Research progress of 1,25-dihydroxyvitamin D3 in autoimmune diseases. *Chinese J Urban Rural Enterprise Health*. (2024) 39:23–5. doi: 10.16286/j.1003-5052.2024.10.007

20. Wang Y, Wei M, Liu H, Wu Y, Luo C, Shan Y, et al. Correlation between serum aPS/PT titer and platelet parameters and coagulation indicators in patients with pre-eclampsia. *J Med Res*. (2022) 51:110–4. doi: 10.11969/j.issn.1673-548X.2022.02.026

21. Wang Q, Liu W, Zhang J, Han Z, Yi W. Analysis of coagulation function changes in patients with gestational hypertension. *Chin J Maternal Child Health Care*. (2023) 38:3625–8. doi: 10.19829/j.zgfybj.issn.1001-4411.2023.19.002

22. Yang Z, Zhang W. Interpretation of the Guidelines for the Diagnosis and Treatment of Hypertensive Disorders Complicating Pregnancy (2020). *Chin J Obstetrics Gynecol*. (2020) 55:425–32. doi: 10.3760/cma.j.cn112141-20200302-00159

23. Adela R, Borkar RM, Mishra N, Bhandi MM, Vishwakarma G, Varma BA, et al. Lower serum vitamin D metabolite levels in relation to circulating cytokines/chemokines and metabolic hormones in pregnant women with hypertensive disorders. *Front Immunol*. (2017) 8:273. doi: 10.3389/fimmu.2017.00273

24. O'Callaghan KM, Kiely M. Systematic review of vitamin D and hypertensive disorders of pregnancy. *Nutrients*. (2018) 10:294. doi: 10.3390/nu10030294

25. Yang J, Wang D. Progress in the study of the correlation between vitamin D deficiency during pregnancy and adverse pregnancy outcomes. *Chin J Maternal Child Health Care*. (2019) 34:235–8. doi: 10.7620/zgfybj.j.issn.1001-4411.2019.01.76

26. Jinhong H, Ren L, Fu S, Zhang C. Correlation between hypertensive disorder complicating pregnancy and serum fat-soluble vitamins and serum lipid levels. *J Shanxi Med University*. (2018) 49:1100–3. doi: 10.13753/j.issn.1007-6611.2018.09.019

27. Huang L, Zhao J, Du J, Zhang J, Dong X, Li Y, et al. Correlation between serum vitamin A, D and vitamin D receptor levels in placental tissue and the pathogenesis of pre-eclampsia. *J Clin Exp Med*. (2020) 19:1188–91. doi: 10.3969/j.issn.1671-4695.2020.11.020

28. You Z, Mei H, Zhang Y, Song D, Zhang Y, Liu C. The effect of vitamin D deficiency during pregnancy on adverse birth outcomes in neonates: a systematic review and meta-analysis. *Front Pediatr*. (2024) 12:1399615. doi: 10.3389/fped.2024.1399615

29. Zheng X, Lai K, Liu C, Chen Y, Zhang X, Wu W, et al. Association between maternal lipid profiles and vitamin D status in second trimester and risk of LGA or SGA: a retrospective study. *Front Endocrinol*. (2024) 15:1297373. doi: 10.3389/fendo.2024.1297373

30. Bi WG, Nuyt AM, Weiler H, Leduc L, Santamaria C, Wei SQ. Association between vitamin D supplementation during pregnancy and offspring growth, morbidity, and mortality: A systematic review and meta-analysis. *JAMA Pediatr*. (2018) 172:635–45. doi: 10.1001/jamapediatrics.2018.0302

31. Dong G, Wang X, Qi H. The effect of magnesium sulfate combined with labetalol on inflammatory stress response and vascular endothelial function in patients with pregnancy-induced hypertension syndrome. *J Pract Hosp Clin Med*. (2021) 18:100–3. doi: 10.3969/j.issn.1672-6170.2021.03.031

32. Lewandowska M, Więckowska B, Sajdak S, Lubiński J. PrePregnancy obesity vs. other risk factors in probability models of preeclampsia and gestational hypertension. *Nutrients*. (2020) 12:2681. doi: 10.3390/nu12092681

33. Zhang X, Li Q. Application effect of preventive nursing intervention based on quantitative evaluation strategy in severe pregnancy-induced hypertension syndrome. *Thromb Hemostasis*. (2021) 27:1044–6. doi: 10.3969/j.issn.1000-7377.2021.04.007

34. Yang F, Gao P. Expression and clinical significance of platelet parameters and D-dimer in patients with early renal injury due to pregnancy-induced hypertension syndrome. *Shaanxi Med J*. (2021) 50:413–6. doi: 10.3969/j.issn.1004-8189.2022.09.038

35. Zhang X, Zhang S, Hu Y. Changes in coagulation function indicators in pregnant women with gestational hypertension syndrome and their clinical significance. *Chin J Family Plann*. (2022) 30:2116–2119, 2123. doi: 10.3969/j.issn.1009-6213.2021.06.064

36. Stach K, Kälsch AI, Nguyen XD, Elmas E, Kralev S, Lang S, et al. 1α,25-dihydroxyvitamin D3 attenuates platelet activation and the expression of VCAM-1 and MT1-MMP in human endothelial cells. *Cardiology*. (2011) 118:107–15. doi: 10.1159/000327547

37. Verdoia M, Pergolini P, Rolla R, C1 S, Nardin M, Schaffer A, et al. Vitamin D levels and high-residual platelet reactivity in patients receiving dual antiplatelet therapy with clopidogrel or ticagrelor. *Platelets*. (2016) 27:576–82. doi: 10.3109/09537104.2016.1149159

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# The impact of vitamin D on atopic disorders: assessing evidence for a causal relationship

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Since the beginning of COVID-19 pandemic, there has been a noticeable increase in the consumption of vitamin D. Evidence accentuates the generation of a pro-tolerogenic T helper 2 cell state with vitamin D, suppressing T helper 1 inflammatory response. T helper 2 cell polarization is characteristic of atopy. However, although the literature on vitamin D and atopy has yielded controversial results, multiple studies have described an inverse relationship between vitamin D levels and the severity of atopy, as well as an improvement of the pathology with vitamin D supplementation. A different approach is offered in the analysis of the immunological mechanisms by which vitamin D acts in the human body, supporting its use as a promoter of homeostasis. In this sense, vitamin D promotes a balanced state through the action of regulatory T cells, controlling cytokines, both pro- and anti-inflammatory, and by reducing B cell prolif eration and differentiation, thus preventing the possible development of atopy.

KEYWORDS

**atopy, vitamin D, T helper 2 cell, dendritic cells, regulatory T cells**

## 1 Introduction

Coronavirus disease (COVID-19), caused by the SARS-CoV-2 virus, is an infectious disease that has posed significant threats to global public health and resulted in millions of deaths worldwide (1). Research indicates that a cytokine storm in COVID-19 is linked to higher mortality rates and clinical worsening, with severe cases being attributed to elevated levels of interleukin (IL)-1, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-6 (2).

Vitamin D deficiency is a widespread global health issue, with an estimated 50% of the world's population experiencing insufficient levels (3). This has led to a rise in the use of vitamin D supplements. While the risk of toxicity from excessive intake is rare and often underestimated, severe complications such as hypercalcemia, delayed teething, late walking, bone demineralization, and pain have been reported as potential effects of vitamin D toxicity (4–6). Since the beginning of the COVID-19 pandemic, studies have suggested a link between vitamin D deficiency and a higher incidence of infection (7, 8) as well as increased severity of COVID-19. As a result, the sale of vitamin supplements, including vitamin D, has surged during the pandemic due to its potential prophylactic or therapeutic benefits (9).

## 2 Vitamin D's biological role

Prohormone and vitamin D can be acquired through endogenous production triggered by ultraviolet B radiation, dietary sources, or supplements. Vitamin D plays a diverse regulatory role in the human body (Figure 1), supported by evidence of its receptor being present in various tissues (10). It serves as a key regulator of mineral homeostasis, influencing the parathyroid glands, bones, and intestines. Additionally, vitamin D has a wide range of other biological functions, including significant effects on the immune system (11).

## 3 Vitamin D and immune system modulation

For this reason, vitamin D can influence both innate and adaptive immunity, promoting the induction of monocyte and macrophage signaling, particularly through antimicrobial peptides like cathelicidins and β-defensin 2. Additionally, vitamin D inhibits the activity of B and T cells, reduces levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, and suppresses antigen-presenting cells like dendritic cells (11, 12). Research indicates that vitamin D shifts the immune response from an inflammatory T-helper (Th) 1-cell response to a pro-tolerogenic Th2 response, halting cytotoxic T lymphocyte infiltration and increasing CD4+ CD25+ regulatory T cells (Tregs) (10). Th2 cells function by releasing mediators and influencing the activity of other cells in the immune system. Th2 cells are a subset of T-helper cells that play a vital role in immune responses, particularly in allergic reactions and the defense against parasitic infections. One of their key functions is the production of IL-5, IL-13 and IL-4, cytokines that are essential for the differentiation and activation of various immune cells. IL-4 not only promotes the differentiation of naive T cells into Th2 cells but also stimulates B cells to produce immunoglobulin E (IgE), which is crucial in allergic responses. Additionally, IL-4 helps in the activation of other immune cells, such as macrophages and eosinophils, further amplifying the immune response. The production of IL-4 by Th2 cells is tightly regulated and is a hallmark of Th2-mediated immunity, making it a critical cytokine in both normal immune function and in the development of allergic diseases (13). This has raised concerns about the potential development of atopy in individuals who consume excessive amounts of vitamin D.

Atopy refers to a predisposition toward an exaggerated immune response to allergens and antigens, characterized by overproduction of IgE (14), state that can be obtained by two types of hypersensitivity, type I and type IVb, of the many described. Type I hypersensitivity, allergens trigger an immune response through IgE and Th2 cells. The sensitization phase involves the activation of antigen-presenting cells, leading to the production of allergen-specific IgE by B cells. Type IVb hypersensitivity, driven by Th2 cells and eosinophils, contributes to chronic inflammation. In this response, IL-4, IL-5, and IL-13 mediate IgE synthesis, eosinophil recruitment, and tissue remodeling. Group 2 innate lymphoid cells (ILC2) amplify the response and contribute to chronicity by promoting eosinophil and basophil recruitment. The interplay between Type I and Type IVb hypersensitivity, the named T2 high phenotype, involves overlapping mechanisms such as eosinophil activation, barrier dysfunction and IgE production, which drive persistent tissue damage and chronic allergic inflammation (15).

Scrutinizing with the aforementioned, vitamin D suppresses the maturation, differentiation, and survival of dendritic cells. It also reduces the expression of major histocompatibility complex type II and costimulatory receptors CD40, CD80, and CD86 (10), which are typically elevated in atopic conditions (16), thereby impairing T cell interaction and activation. Furthermore, vitamin D decreases the proliferation and differentiation of B cells, including their tendency to produce IgE. Additionally, this vitamin promotes the development of regulatory T-lymphocytes (11), which can enhance immune tolerance by increasing the production of IL-10 and transforming growth factor β (16).

### 3.1 Vitamin D and atopic diseases

Debates have emerged about the potential effects of vitamin D and its relationship to the severity of atopic diseases. However, some researches support the notion that insufficient vitamin D levels are associated with heightened atopic dermatitis (AD) severity, and correcting this deficiency through supplementation may lessen symptom severity (17–20).

Vitamin D supplementation demonstrates clinically meaningful benefits for AD, particularly through its immunomodulatory effects on T2 inflammation. Borzutzky et al.'s (18) pivotal randomized controlled trial revealed that high-dose weekly vitamin D (50,000 IU) not only reduced AD severity scores but also significantly lowered IL-13 levels and showed trends toward decreased IgE. These findings provide direct evidence of vitamin D's ability to downregulate key T2 immunity biomarkers, offering a mechanistic explanation for its therapeutic effects. The immunomodulatory potential is further supported by Ng and Yew's (19) meta-analysis, which found the strongest treatment effects in patients with baseline vitamin D deficiency, a population known to exhibit exaggerated T2 immune responses. The studies collectively suggest that vitamin D supplementation may help restore immune balance in AD by suppressing Th2-mediated inflammation, particularly in pediatric and deficient populations where this pathway is most active.

The clinical benefits of vitamin D extend beyond biomarker modulation to measurable improvements in disease severity and patient outcomes. Both Ng and Yew's meta-analysis (19) and Borzutzky's (18) trial reported statistically significant reductions in standardized AD severity scores with supplementation, with effect sizes comparable to some conventional therapies. Notably, the benefits appear dose-dependent, with Ng's study showing superior outcomes at ≥4,000 IU/day and Borzutzky's regimen demonstrating rapid efficacy at pharmacologic weekly doses (18, 19). While Nielsen et al. (20) caution against universal application due to heterogeneous responses, their subgroup analyses still support vitamin D's utility in deficient patients. From a clinical

**Abbreviations:** COVID-19, coronavirus disease; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; Th, T-helper cell; Tregs, T regulatory cells; IgE, immunoglobulin E; ILC2, group 2 innate lymphoid cells; AD, atopic dermatitis.

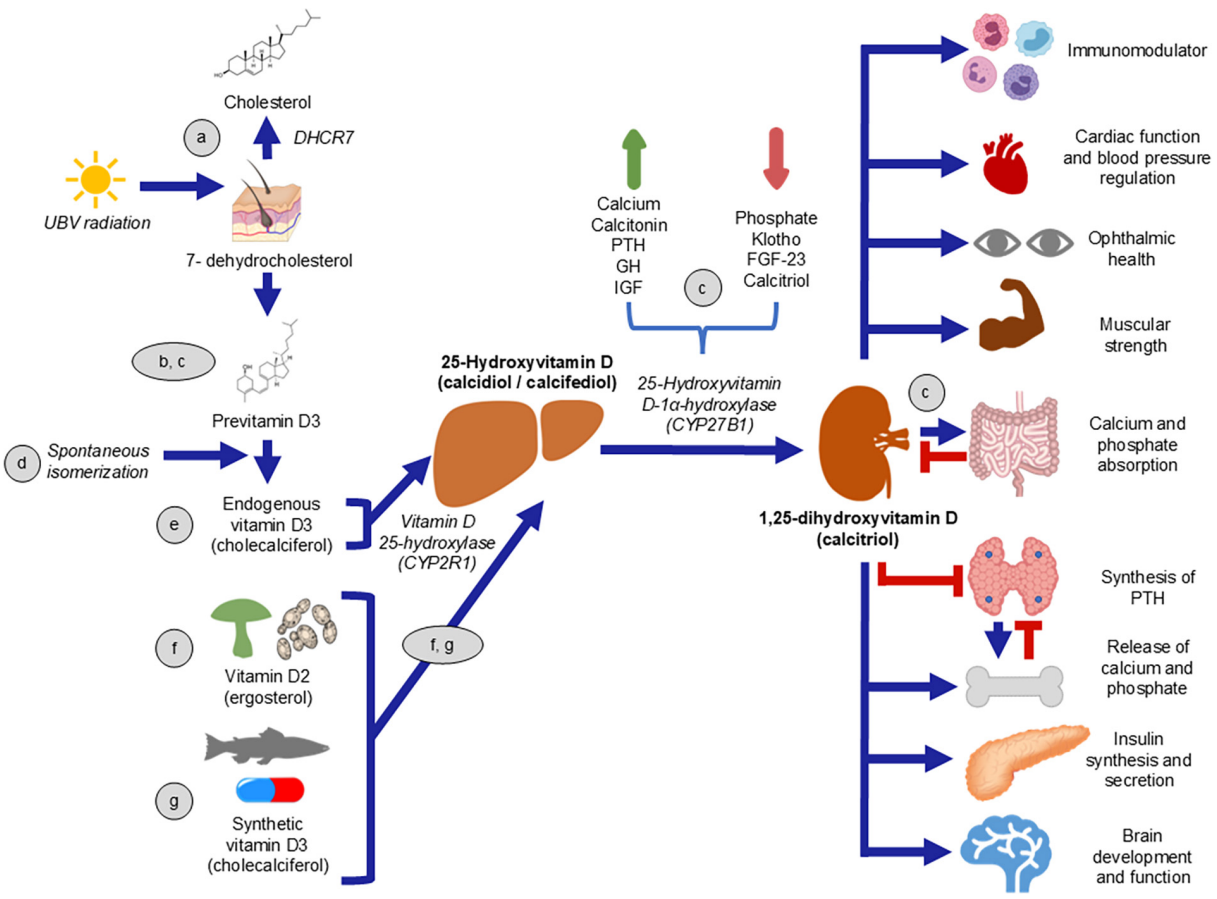


FIGURE 1
Vitamin D (VD) synthesis and its effects. VD is obtained through the skin production or by alimentary consumption. a) 7-dehydrocholesterol, which can be transformed to cholesterol (24); b,c) converts to previtamin D3 (25, 26) through ultraviolet B radiation. d) Via spontaneous isomeration (24), precholecalciferol changes to cholecalciferol. e) VD can be obtained from food, both of animal (cholecalciferol) and plant origin (ergocalciferol) (27). f) Cholecalciferol is metabolized in the liver to 25-hydroxyvitamin D (28). g) The active form of VD, 1,25-dihydroxyvitamin D, is obtained in the kidneys by the enzyme 25-Hydroxyvitamin D-1α-hydroxylase (which, in turn, is regulated by stimuli). c) Calcitriol has diverse effects on different tissues (29).

perspective, these immunomodulatory and therapeutic effects position vitamin D as a safe, low-cost adjunct therapy that may reduce reliance on immunosuppressants in select AD populations, while addressing the common comorbidity of vitamin D deficiency prevalent in AD patients.

The prevention or absence of development of allergic or atopic diseases in individuals who consume vitamin D may be also attributed to the role of Tregs. There are various reasons why vitamin D can explain its benefits in atopic diseases, which are heterogeneous in nature. Nevertheless, it is important to mention that the mechanism that can explain the benefit of vitamin D supplementation is related to this vitamin's immunomodulatory effects that may indirectly suppress IL-4 production by inhibiting Th2 cell proliferation and promoting Tregs function, by which they can possibly decrease levels of ILC2 (21). As previously mentioned, ILC2 cells are known to drive T2 immune responses, which are central to the pathogenesis of atopic diseases. The supplementation of vitamin D appeared to modulate the immune system by diminishing the number and activity of ILC2 cells, thus reducing inflammation and improving the clinical symptoms of AD (18). The latter mentioned, Tregs, can inhibit the proliferation and activation of effector Th cells, such as Th2 and Th17, while also restricting the functions and migration of Th1, Th2, Th9, and Th17 cells, including the production of IFN-γ (16, 18). As a result, Th2 cytokines are suppressed (22) through the possible action of Tregs during atopic conditions, affecting mast cells, IgE, basophils, and eosinophils (22) (Figure 2). Tregs exert their suppressive effects through inhibitory cytokines and signaling molecules like CTLA-4. This receptor plays an immunoregulatory role by preventing the interaction between the CD28 costimulatory molecule on T cells and its ligands, CD80 and CD86. However, recent studies indicate that vitamin D supplementation during pregnancy or infancy does not significantly impact the primary prevention of allergic diseases (23).

## 4 Discussion

Vitamin D plays a critical role in supporting immune homeostasis, acting as a modulator of both the innate and adaptive immune systems. By enhancing the possible activity of

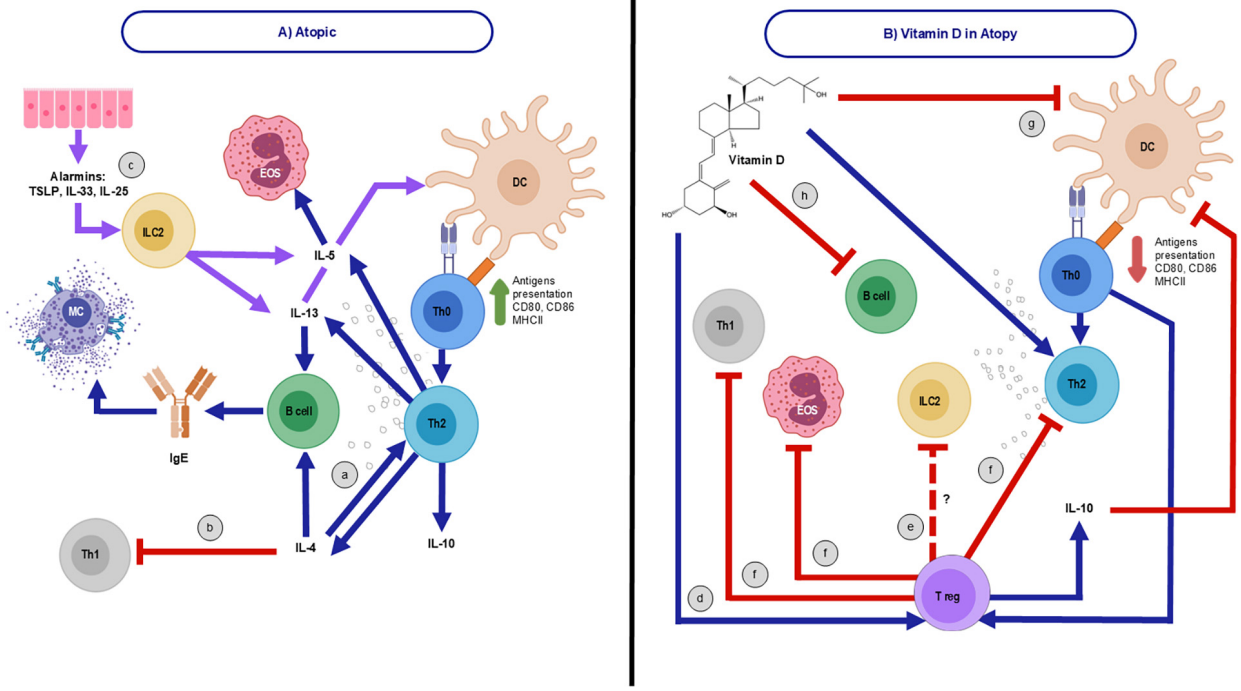


FIGURE 2
Immunological behavior of atopy as a disease and with vitamin D consumption. **(A)** Immune system in atopy. T helper (Th)-2 cells, an atopy feature, a) release cytokines such as interleukin (IL)-4, that inhibits Th1 cells generation (b), IL-5, IL-10, and IL-13. Likewise, there is a notable elevation of antigens presentation, CD80/CD86 expression, major histocompatibility complex II (MHCII) action, immunoglobulin E (IgE) production, mast cell (MC) release, and eosinophils (EOS) activation. c) Also, group 2 innate lymphoid cells (ILC2) are triggered by cytokines released from epithelial cells, known as alarmins, including IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). Once activated, they secrete significant amounts of type 2 (T2) cytokines, such as IL-5 and IL-13, which further enhance the T2-cell response (15). **(B)** Possible Vitamin D's (VD) regulatory mechanisms in atopy. d) VD may regulate atopy's hyperresponsiveness through T regulatory cells (Tregs) differentiation (10), e) possibly inhibiting ILC2 (21) and f) suppressing EOS and impeding Th1 and Th2 functions, which regulates cytokines of the latter. g) Additionally, VD suppresses dendritic cells (DC), h) and B cell differentiation (30), reducing antibody production, including IgE, and antigen presentation.

Tregs, vitamin D helps to suppress the overproduction of pro-inflammatory cytokines and shift immune responses toward a more balanced state. This not only aids in reducing inflammation but also promotes the production of antimicrobial peptides, providing an enhanced defense against infections.

However, while vitamin D offers several immune benefits, concerns have been raised regarding its excessive intake, particularly in relation to the development of allergic or atopic conditions. Despite vitamin D's role in shifting immune responses from Th1 to Th2 pathways, which theoretically could contribute to atopy, no concrete evidence has supported this hypothesis. On the contrary, vitamin D seems to promote immune tolerance and reduce the likelihood of allergic reactions by regulating and enhancing Treg activity and cytokine production.

Vitamin D remains a crucial factor in immune regulation, offering potential therapeutic and prophylactic benefits, particularly in the context of infectious diseases. However, its effects on allergic disease prevention and atopy development are still inconclusive, caution should be taken regarding excessive supplementation. As research continues to evolve, public health strategies should focus on maintaining adequate vitamin D levels, ensuring a balance between deficiency prevention and avoiding the risks associated with hypervitaminosis D. Ultimately, vitamin D supplementation should be tailored to individual needs, considering factors such as baseline levels, health status, and environmental exposure to sunlight.

In conclusion, vitamin D supports immune homeostasis by enhancing the activity of Tregs. As a result, the potential development of atopic diseases, which might arise from an assumed rise in Th2 lymphocytes, is likely prevented through the consumption of this vitamin. It is important to mention that the direct mechanism by which ILC2 activity may be decreased by consuming vitamin D is unknown, therefore this panorama should be studied in the future for a better understanding of the possible benefit of vitamin supplementation and atopy.

## 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 author.

## Author contributions

VZ: Visualization, Writing – original draft, Writing – review and editing. BB-P: Conceptualization, Writing – review and editing.

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## References

1. World Health Organization [WHO]. *Coronavirus Disease (COVID-19) Pandemic*. Geneva: World Health Organization (2025).

2. Kim J, Lee J, Yang J, Lee K, Effenberger M, Szpirt W, et al. Immunopathogenesis and treatment of cytokine storm in COVID-19. *Theranostics.* (2021) 11:316–29. doi: 10.7150/thno.49713

3. Holick M. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord.* (2017) 18:153–65. doi: 10.1007/s11154-017-9424-1

4. Wani M, Wani I, Banday K, Ashraf M. The other side of vitamin D therapy: A case series of acute kidney injury due to malpractice-related vitamin D intoxication. *Clin Nephrol.* (2016) 86:236–41. doi: 10.5414/CN108904

5. Feghali K, Papamarkakis K, Clark J, Malhotra N, Stoddart L, Osakwe I. Vitamin D toxicity managed with peritoneal dialysis. *Case Rep Endocrinol.* (2021) 2021:9912068. doi: 10.1155/2021/9912068

6. Muneer S, Siddiqui I, Majid H, Zehra N, Jafri L, Khan A. Practices of vitamin D supplementation leading to vitamin D toxicity: Experience from a low-middle income Country. *Ann Med Surg.* (2022) 73:103227. doi: 10.1016/j.amsu.2021.103227

7. Szarpak L, Rafique Z, Gasecka A, Chirico F, Gawel W, Hernik J, et al. A systematic review and meta-analysis of effect of vitamin D levels on the incidence of COVID-19. *Cardiol J.* (2021) 28:647–54. doi: 10.5603/CJ.a2021.0072

8. Martineau A. Vitamin D in the prevention or treatment of COVID-19. *Proc Nutr Soc.* (2023) 82:200–7. doi: 10.1017/S0029665122002798

9. Hadizadeh F. Supplementation with vitamin D in the COVID-19 pandemic? *Nutr Rev.* (2021) 79:200–8. doi: 10.1093/nutrit/nuaa081

10. Wang Y, Zhu J, DeLuca H. Where is the vitamin D receptor? *Arch Biochem Biophys.* (2012) 523:123–33. doi: 10.1016/j.abb.2012.04.001

11. Martens P, Gysemans C, Verstuyf A, Mathieu A. Vitamin D's effect on immune function. *Nutrients.* (2020) 12:1248. doi: 10.3390/nu12051248

12. Ashique S, Gupta K, Gupta G, Mishra N, Singh S, Wadhwa S, et al. Vitamin D-A prominent immunomodulator to prevent COVID-19 infection. *Int J Rheum Dis.* (2023) 26:13–30. doi: 10.1111/1756-185X.14477

13. Kokubo K, Onodera A, Kiuchi M, Tsuji K, Hirahara K, Nakayama T. Conventional and pathogenic Th2 cells in inflammation, tissue repair, and fibrosis. *Front Immunol.* (2022) 13:945063. doi: 10.3389/fimmu.2022.945063

14. Justiz Vaillant A, Modi P, Jan A. *StatPearls*. Island, FL: StatPearls Publishing (2025).

15. Jutel M, Agache I, Zemelka-Wiacek M, Akdis M, Chivato T, Del Giacco S, et al. Nomenclature of allergic diseases and hypersensitivity reactions: Adapted to modern needs: An EAACI position paper. *Allergy.* (2023) 78:2851–74. doi: 10.1111/all.15889

16. Boonpiyathad T, Sözener Z, Akdis M, Akdis C. The role of Treg cell subsets in allergic disease. *Asian Pac J Allergy Immunol.* (2020) 38:139–49. doi: 10.12932/AP-030220-0754

17. Borzutzky A, Camargo C. Role of vitamin D in the pathogenesis and treatment of atopic dermatitis. *Expert Rev Clin Immunol.* (2013) 9:751–60. doi: 10.1586/1744666X.2013.816493

18. Borzutzky A, Iturriaga C, Pérez-Mateluna G, Cristi F, Cifuentes L, Silva-Valenzuela S, et al. Effect of weekly vitamin D supplementation on the severity of atopic dermatitis and type 2 immunity biomarkers in children: A randomized controlled trial. *J Eur Acad Dermatol Venereol.* (2024) 38:1760–8. doi: 10.1111/jdv.19959

19. Ng J, Yew Y. Effect of vitamin D serum levels and supplementation on atopic dermatitis: A systematic review and meta-analysis. *Am J Clin Dermatol.* (2022) 23:267–75. doi: 10.1007/s40257-022-00677-0

20. Nielsen A, Høj S, Thomsen S, Meteran H. Vitamin D supplementation for treating atopic dermatitis in children and adults: A systematic review and meta-analysis. *Nutrients.* (2024) 16:4128. doi: 10.3390/nu16234128

21. López D, Griffin A, Aguilar L, Deering-Rice C, Myers E, Warren K, et al. Prenatal inflammation remodels lung immunity and function by programming ILC2 hyperactivation. *Cell Rep.* (2024) 43:114365. doi: 10.1016/j.celrep.2024.114365

22. Luo C, Sun Y, Zeng Z, Liu Y, Peng S. Vitamin D supplementation in pregnant women or infants for preventing allergic diseases: A systematic review and meta-analysis of randomized controlled trials. *Chin Med J.* (2022) 135:276–84. doi: 10.1097/CM9.0000000000001951

23. Martín-Orozco E, Norte-Muñoz M, Martínez-García J. Regulatory T cells in allergy and asthma. *Front Pediatr.* (2017) 5:117. doi: 10.3389/fped.2017.00117

24. Prabhu A, Luu W, Sharpe L, Brown A. Cholesterol-mediated degradation of 7-dehydrocholesterol reductase switches the balance from cholesterol to vitamin D synthesis. *J Biol Chem.* (2016) 291:8363–73. doi: 10.1074/jbc.M115.699546

25. Wacker M, Holick M. Sunlight and Vitamin D: A global perspective for health. *Dermatoendocrinol.* (2013) 5:51–108. doi: 10.4161/derm.24494

26. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: Modulator of the immune system. *Curr Opin Pharmacol.* (2010) 10:482–96. doi: 10.1016/j.coph.2010.04.001

27. Tian X, Holick M. Catalyzed thermal isomerization between previtamin D3 and vitamin D3 via beta-cyclodextrin complexation. *J Biol Chem.* (1995) 270:8706–11. doi: 10.1074/jbc.270.15.8706

28. Martin J. Analysis of selected elements in tobacco by wavelength dispersive X-ray fluorescence spectrometry. *J Assoc Off Anal Chem.* (1988) 71:1038–41. doi: 10.1093/jaoac/71.5.1038

29. Sassi F, Tamone C, D'Amelio P. Vitamin D: Nutrient, hormone, and immunomodulator. *Nutrients.* (2018) 10:1656. doi: 10.3390/nu10111656

30. Charoenngam N, Holick M. Immunologic effects of vitamin D on human health and disease. *Nutrients.* (2020) 12:2097. doi: 10.3390/nu12072097

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# Suramin blocked hCAP18/LL-37-induced macrophage recruitment and M2 polarization to enhance the therapeutic efficacy of 1,25(OH)$_2$D$_3$ against hepatocellular carcinoma *in vitro* and *in vivo* mouse model

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**Background:** 1,25(OH)$_2$D$_3$ supplementation alone does not provide sufficient benefit to hepatocellular carcinoma (HCC) patients in clinical trials. Tumor-associated macrophages (TAMs)-mediated immunosuppression is regarded as a major hurdle for the effectiveness of several treatments. Previous studies revealed that hCAP18/LL-37 was an important factor which directly suppresses the anticancer activity of 1,25(OH)$_2$D$_3$ on HCC cells. However, whether TAMs contribute to the limited clinical efficacy of 1,25(OH)$_2$D$_3$ through hCAP18/LL-37 remains unclear.

**Methods:** Co-culture systems of HCC cells (PLC/PRF-5, Huh7) with THP-1-derived macrophages and co-xenograft mouse models were established. Anticancer activity was evaluated *in vitro* and *in vivo* mouse models using standard assays. Mechanistic investigations utilized qRT-PCR, Western blot, flow cytometry, ELISA, and immunohistochemistry. Therapeutic efficacy of 1,25(OH)$_2$D$_3$/suramin combination was assessed in co-xenograft and N-Nitrosodiethylamine (DEN)/Carbon tetrachloride ($CCl_4$)-induced HCC models.

**Results:** 1,25(OH)$_2$D$_3$ (200–500 nM) promoted macrophage recruitment, M2 polarization, Akt/mTOR signal and STAT3 signal activation in HCC/macrophage co-culture systems. This effect was mediated by 1,25(OH)$_2$D$_3$-induced hCAP18/LL-37 overexpression, which facilitated TAM infiltration and M2 reprogramming. Suramin, a potent LL-37 inhibitor, abrogated these immunosuppressive effects by blocking LL-37 internalization, restoring M1 polarization and suppressing Akt/mTOR and STAT3 pathways. Notably, 1,25(OH)$_2$D$_3$/suramin combination therapy synergistically inhibited HCC proliferation, colony formation, and invasion *in vitro*. In xenograft models and DEN/$CCl_4$-induced HCC models, suramin enhanced 1,25(OH)$_2$D$_3$'s efficacy by promoting M1 polarization, increasing intratumoral M1/M2 ratios, reducing tumor growth, and diminishing macroscopic nodules.

**Conclusion:** The 1,25(OH)$_2$D$_3$-LL-37-TAM axis drives immunosuppression in HCC by modulating macrophage phenotypes. While suramin potently disrupts this axis, blocking LL-37-mediated TAMs recruitment and M2 polarization, while

promoting antitumor M1 phenotype responses. These findings highlight suramin as a promising adjunct to $1,25(OH)_2D_3$-based immunotherapy for HCC.

KEYWORDS

**$1,25(OH)_2D_3$, hCAP18/LL-37, suramin, hepatocellular carcinoma, macrophage**

# 1 Introduction

Hepatocellular carcinoma (HCC) is among the five most common cancers and the third leading cause of cancer-related deaths worldwide. Scientists estimate that 1.4 million people could be diagnosed with liver cancer and 1.3 million people will die from the disease in 2040 (1). Currently, tyrosine kinase inhibitors, including two first-line therapies (sorafenib and lenvatinib) and three second-line therapies (regorafenib, cabozantinib and ramucirumab), immune checkpoint inhibitors (ICIs) and combination regimens with ICIs are approved by the FDA (Food and drug administration) for HCC systemic treatment (2, 3). Despite great breakthroughs in systemic treatments, the majority of HCC patients still obtain limited benefits (4). Therefore, there is an urgent need to develop more therapeutic strategies and overcome the mechanism of tumor microenvironment (TME) mediated drug resistance.

Tumor-associated macrophages (TAMs) are the most abundant innate immune population in the TME of HCC, mediated immunosuppression and is regarded as a major hurdle for the effectiveness of several treatments (5). In addition to liver-resident macrophages (Kupffer cells), most TAMs originate from circulating monocytes that are recruited by chemotactic signals (cytokines and chemokines) to the tumour sites, and subsequently are polarized into different types of TAMs in the TME. TAMs predominantly exhibit immunosuppressive M2-like phenotypes characterized by CD163/Arg-1 expression and IL-10 secretion, which promote angiogenesis, immunosuppression, and metastasis. Conversely, M1-like TAMs expressing iNOS/TNF-$\alpha$ exert antitumor effects. The plasticity of TAMs enables their reprogramming between these phenotypes, making them pivotal regulators of treatment efficacy (6).

Vitamin D3 (1,25-dihydroxyvitamin D3; $1,25(OH)_2D_3$) has been widely studied for its potential role in cancer treatment.[1] Although epidemiological studies report vitamin D deficiency in 90% of HCC patients (7), and experimental studies also show $1,25(OH)_2D_3$ a direct anticancer role against HCC cells by inhibiting cancer cell proliferation, promoting apoptosis and reducing angiogenesis (8), clinical trials demonstrate limited efficacy of monotherapy (9, 10). Recent studies have highlighted the immunomodulatory role of $1,25(OH)_2D_3$ in anti-tumor immunity and immunotherapy response (11). In microbial infections and some inflammatory conditions, $1,25(OH)_2D_3$ promotes macrophage polarization toward an anti-inflammatory M2 phenotype, thereby attenuating excessive immune activation (12–14). Intriguingly, recent evidence also suggests that $1,25(OH)_2D_3$ may similarly enhance M2 polarization, potentially facilitating metastasis in 4 T1 mammary carcinoma models (15), raising concerns regarding its immunosuppressive potential within the HCC TME.

Cathelicidin hCAP18/LL-37 is one of the most relevant vitamin D receptor (VDR) regulated target genes in human immune cells (16). This 16 kDa secreted protein undergoes proteolytic processing from its 19 kDa precursor (pre-hCAP18) to yield the bioactive 4.5 kDa LL-37 peptide (17). Substantial evidence demonstrates that macrophage-derived hCAP18/LL-37 mediates both the antimicrobial and immunomodulatory effects of $1,25(OH)_2D_3$ during infection and inflammatory conditions (18, 19). Our recent work identified hCAP18/LL-37 as a tumor-promoting factor that stimulates HCC proliferation and growth, thereby attenuating the anticancer efficacy of $1,25(OH)_2D_3$ in mouse models (20). However, whether hCAP18/LL-37 further enhances inhibition of $1,25(OH)_2D_3$'s anticancer activity through TAMs remains unknown.

Here, we investigate the role of the $1,25(OH)_2D_3$-LL-37-TAM axis in HCC using *in vitro* co-culture systems and in mouse co-xenograft models. We demonstrate that LL-37 enhances macrophage recruitment and M2 polarization, key mediators of $1,25(OH)_2D_3$-induced immunosuppression. Additionally, an old drug (suramin) synergized with $1,25(OH)_2D_3$ to suppress tumor growth, by reprogramming immunosuppressive M1-TAMs into antitumor effectors. These findings provide a mechanistic rationale for improving $1,25(OH)_2D_3$-based therapy via TAM-targeted combination strategies in HCC.

# 2 Materials and methods

## 2.1 Regents

We purchased Dulbecco's Modified Eagle's Medium (DMEM), RPMI 1640 Medium, IFN-$\gamma$ and IL-4 for cell culture from Gibco (Thermo Fisher Scientific, USA). Lipopolysaccharide (LPS), phorbol 12-myristate 13-acetate (PMA), phenyl methane sulfonyl fluoride (PMSF), phosphatase inhibitor, 0.1% crystal violet and BeyoClick™ EdU-555 kit were sourced from Beyotime (Nanjing, China). The hCAP18/LL-37 (AS014), VDR (sc-13133), p-Akt (Ser473) (sc-514032), Arg-1 (sc-271430), CD68 (sc-17832), CD163 (sc-20066), p-4EBP1 (Ser65) (sc-293124), MMP9 (sc-393859), iNOS (sc-7271), CD163 (sc-20066) antibodies were provided from Santa (Texas, USA). The p-mTOR (Ser2448) (CY6571) and mTOR (P42345) antibodies were purchased from Abways (Shanghai, China). The Akt (9272) and PCNA (13110) antibodies were purchased from Cell Signaling Technology (Massachusetts, USA). The p-STAT3 (AP0070), STAT3 (A1192), $\beta$-actin (AC026) antibodies, horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (AS014) and anti-mouse secondary antibody (AS003) were purchased from ABclonal (Wuhan, China). The LaminB1 (12987-1-AP) antibody was purchased from Proteintech (Wuhan, China). Brilliant Violet 605™ anti mouse CD45 (103155), PE-cy7-F4/80 (123114), Alexa Fluor® 488 anti-mouse/human CD11b (101219), APC anti-mouse CD206 (141708), and Brilliant Violet 421™ anti-mouse CD86 (105032) antibodies were sourced from Biolegend (Cal, USA). Additionally, TRIzol reagent, HiScriptIII RT SuperMix

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(+gDNA wiper), AceQ qPCR SYBR Green Master Mix and BCA protein assay kit were purchased from Vazyme (Nanjing, China). Suramin was purchased from MedMol (Shanghai, China). $1,25(OH)_2D_3$ was purchased from MCE (New Jersey, USA). DAB kit was purchased from Solarbio (Beijing, China). LipoPlus transfection reagent was purhcased from Synthgene (Nanjing, China). Cytokines IL-10, TNF-$\alpha$ and human cathelicidin LL-37 ELISA kits were purchased from DUMA Technology (Shanghai, China). Calcitriol and N-Nitrosodiethylamine (DEN) were sourced from Medchemexpress (New Jersey, USA). Carbon tetrachloride ($CCl_4$) was purhcased from Macklin (Shanghai, China). DNase I was purchased from Sigma (Darmstadt, Germany).

## 2.2 Cell lines, cell culture, and transfection

THP-1, PLC/PRF-5, Huh7 cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science. THP-1 cells were cultured in RPMI 1640 medium. Huh7, PLC/PRF-5 cells were maintained in DMEM. LL-37 peptide was synthesized by Synpeptide Inc. (Nanjing, China). pRNAT-U6.1-GFP and pcDNA3.0-Flag expression vector were kept in our laboratory. All primers were synthesized by General Biol (Anhui, China) and listed in the Supplementary Table S1. LL-37 coding sequences (GenBank accession no. 820) were amplified by qRT-PCR from mRNA of PLC/PRF-5 cells using Oligo $dT_{23}$ that incorporated *Bam*HI and *XbaI* restriction sites, and then inserted into pcDNA3.0 via *BamHI*/*XbaI* restriction sites to generate the pcDNA3.0$^{LL\text{-}37}$. The sequence (5'-GTCCAGAGAAT CAAGGATT-3′) specifically targets the encoding LL-37 of *CAMP* gene (si-LL-37) and negative control siRNA (scrambled siRNA, si-control) were synthesized by General Biol, respectively. si-LL-37 was specifically transfected into HCC cells (PLC/PRF-5 or Huh7) using LipoPlus transfection reagent. Briefly, HCC cells were seeded in 6-well plates ($2 \times 10^5$cells/well) and transfected with si-LL-37 (or scrambled siRNA control) for 48 h, then co-cultured with macrophages to conduct the functional assays. LL-37-overexpressed HCC cells were constructed by transfection with pcDNA3.0$^{LL\text{-}37}$ vectors using LipoPlus transfection reagent. Conditioned medium (CM) was obtained by collecting supernatants after filtration through 0.22 μm pore size membranes. CM was mixed with fresh RPMI 1640 or DMEM medium at a ratio of 1:1 (CM/fresh medium).

## 2.3 M1 and M2 macrophage polarization induction

M1-type and M2-type macrophages were induced from THP-1 as described previously (21). Briefly, THP-1 cells were differentiated into an intermediate stage M0 by stimulation with 100 ng/mL PMA for 24 h. For M1 macrophage polarization, 100 ng/mL LPS and 20 ng/mL IFN-$\gamma$ were added to M0 macrophages for 48 h. For macrophage M2 polarization, 20 ng/mL IL-4 was added to M0 macrophages for 48 h.

## 2.4 Transwell recruitment assay and invasion assay chemotaxis

A co-culture system of macrophages and HCC cells (PLC/PRF-5, Huh7) was established using 24-well transwell plates with transwell chambers with 8-μm poresize (Biofil, Guangzhou, China). HCC cells or macrophages were seeded in the bottom chamber, while macrophages or HCC cells were cultured in the upper chamber. For transwell recruitment assay, $1 \times 10^5$ M0 macrophages were resuspended in 200 μL of 1% FBS RPMI 1640 medium and plated in the upper chamber of a 24-well plate. $1 \times 10^5$ HCC cells were placed the lower chamber. For invasion assay, the transwell chambers were coated with 100 μL of 1:8 diluted Matrigel and incubated at 37°C for 4 h. The HCC cells ($0.5–1 \times 10^5$ cells) were cultured in the upper chamber and macrophages ($0.5–1 \times 10^5$ cells) were cultured in the bottom chamber. After different treatments for 48 h, the cells in the upper chamber were fixed with 4% paraformaldehyde (PFA) for 15 min and then stained with 0.1% crystal violet for 20 min. The non-migrated cells in the upper chamber were removed with a cotton swab, and migratory cells were observed with a microscopy. Four random visual fields in each well were visualized under a microscope for cell counting. After elution with 33% glacial acetic acid, cells were detected at 570 nm. Each independent experiment was repeated four times.

## 2.5 Western blot assay

Protein lysates from isolated tissues or cultured cells were extracted using RIPA buffer containing PMSF and phosphatase inhibitors. The protein concentration was determined by BCA protein assay kits. Protein (20 μg) was separated by SDS-PAGE, and then transfered to a PVDF membrane (Millipore, Darmstadt, Germany). After blocking, the membranes were incubated with primary antibodies specific to hCAP18/LL-37, VDR, p-Akt (Ser473), Arg-1, CD68, CD163, p-4EBP1 (Ser65), MMP9, iNOS, p-mTOR (Ser2448), mTOR Akt, $\beta$-actin, p-STAT3 (Tyr705), STAT3 or LaminB1 overnight at 4°C. Subsequently, the PVDF membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody or anti-mouse secondary antibody for 1 h at room temperature. Membranes were incubated with ECL substrate and the targeted proteins were visualized with a chemiluminescence imaging system (Tanon, China).

## 2.6 Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cells using TRIzol reagent and cDNA was synthesized using HiScriptIII RT SuperMix with Oligo $(dT)_{23}$/random hexamer primers. qPCR was performed on Thermal Cycler 96-well Real-Time PCR Detection System (Thermo scientific, MA, USA) with AceQ qPCR SYBR Green Master Mix. Gene expression was normalized to $\beta$-actin and calculated using the $2^{-\Delta\Delta Ct}$ method. All primers were synthesized by General Biol (Anhui, China) and listed in the Supplementary Table S2.

## 2.7 EdU incorporation assay

Cell proliferation was assessed using the BeyoClick™ EdU-555 Kit following the manufacturer's protocol. Briefly, after different treatments, cells were incubated with 10 μM EdU for 2 h, fixed with 4% PFA for 15 min and permeabilized with 0.2% Triton X-100 for

15 min. Then Cells were incubated with Click Addictive Buffer for 30 min at room temperature (protected from light). After washing with PBS, cells were stained with 4′,6-diamidino-2-phenylindole (DAPI) at a concentration of 10 μg/mL for 15 min to visualize nuclei. More than five random fields per well were quantified as a percentage of EdU-positive cells.

## 2.8 Colony formation assay

Cells were seeded in 12-well plates and cultured in either standard DEME (control) or conditioned medium (CM:DMEM = 1:1). After 10 days of culture, cells were fixed with 4% PFA for 15 min and then stained with 0.1% crystal violet for 20 min. Colonies were photographed and counted. Each independent experiment was repeated four times.

## 2.9 Confocal laser scanning microscopy

PLC/PRF-5 and macrophages were incubated with FITC-labeled LL-37 (1 μM), suramin (5 μM) alone or combination (1 μM LL-37 + 5 μM suramin) for 30 min in the dark. After washing, the cells were incubated with DAPI (5 μM) for 15 min in the dark, followed by washing with PBS. Cells were observed using a Nikon Ti-E-A1R confocal microscope (Tokyo, Japan).

## 2.10 Subcutaneous xenograft tumor model

Male Balb/c nude mice (4–6 weeks old, 18.0 ± 2.0 g; GemPharmatech, China) were housed under SPF conditions. All procedures were approved by Nanjing Normal University's Ethics Committee (IACUC-20220218). Two models were established: PLC/PRF-5 cells ($6 \times 10^6$) were injected subcutaneously ($n = 10$) to establish HCC xenograft model, and PLC/PRF-5 ($6 \times 10^6$) plus M0 macrophages ($1.5 \times 10^6$) were injected subcutaneously to establish HCC/macrophage co-xenograft model ($n = 40$). At tumor volumes of 50–100 $mm^3$, co-xenograft mice were randomized into four treatment groups ($n = 8$/group): (1) PBS group, (2) $1,25(OH)_2D_3$ group (a dose of 0.5 μg/kg/per day), (3) suramin group (10 mg/kg twice a week), and (4) combination therapy $1,25(OH)_2D_3$/suramin group. Tumor volume was calculated using the formula $V = ab^2/2$, where "a" and "b" are tumor dimensions at the longest and widest points, respectively. The mice were sacrificed by isoflurane/cervical dislocation. Tumors were excised, weighed, and the inhibition rate calculated as: $(TW_{control} - TW_{Treatment}) / TW_{control} \times 100\%$.

## 2.11 Immunofluorescence and immunohistochemistry microscopy observation

For IF assay, cells platedon glass slides were fixed (4% PFA), permeated (1% Triton X-100), and blocked (5% BSA, 37°C, 1 h). Then the slides were incubated with indicated promary antibodies at 4°C overnight, followed by incubating with fluorescence-conjugated secondary antibodies at room temperature for 1 h. Slides were stained with DAPI for 15 min to visualize nuclear DNA. After washing, cells were imaged with a Ti-E-A1R confocal laser microscope. For IHC assay, paraffin-embedded tissue sections were prepared as previously described (20). After repairing antigen with boiled Ethylene Diamine Tetraacetic Acid (EDTA) solution and quenching endogenous peroxidase with 3% $H_2O_2$, the sections were incubated by primary antibodies CD163, hCAP18/LL-37, Arg-1, iNOS, PCNA at 4°C overnight. After washing, the sections were incubated with HRP-conjugated goat anti-rabbit IgG or HRP-conjugated goat anti-mouse IgG secondary antibody for 1 h at room temperature. Finally, the sections were visualized with DAB kit, counterstained with hematoxylin, dehydrated and examined by an Olympus IX51 fluorescence microscope. Approximately 4–6 fields were randomly selected for each sample. Data were collected from 4 to 6 independent experiments.

## 2.12 Enzyme-linked immunosorbent assay (ELISA)

Cytokines (IL-10 and TNF-$\alpha$) levels in the culture supernatant was quantified using the commercial ELISA kits. Mouse blood was collected and centrifuged at 1500 rpm for 10 min to collect serum. Serum level of hCAP18/LL-37 was determined by human cathelicidin ELISA kit. All assays followed manufacturers' protocols.

## 2.13 DEN/$CCl_4$- induced HCC mouse model

Male C57BL/6 mice (3-weeks-old) were purchased from GemPharmatech Co. Ltd. and maintained under SPF conditions. Animal procedures and experimental methods were approved by the Ethics Committee of Nanjing Normal University (IACUC-2024210). To establish a HCC model, mice received intraperitoneal injections of DEN at 50 mg/kg biweekly for 4 weeks, followed by $CCl_4$ dissolved in olive oil (1:4) administered intraperitoneally biweekly for an additional 16 weeks. After the successful construction of the model, the mice were randomly divided into 4 groups ($n = 8$ per group): (1) PBS group, (2) $1,25(OH)_2D_3$ group (a dose of 0.5 μg/kg per day), (3) suramin group (10 mg/kg twice a week), and (4) combination therapy ($1,25(OH)_2D_3$/suramin) group. All treatments were initiated post-model confirmation and continued for 3 weeks. The mice were sacrificed by isoflurane/cervical dislocation, livers were excised, weighed. and macroscopic nodules (≥1 mm) counted for flow cytometry analysis.

## 2.14 Flow cytometry analysis

Tissues were enzymatically dissociated into single-cell suspensions using Hank's Balanced Salt Solution (HBSS) containing 1 mg/mL collagenase and 50 μg/mL DNase I at 37°C for 30 min. After erythrocyte lysis and 70-μm filtration, cells were resuspended in PBS containing 3% FBS for Fc receptor blocking (10 min). The following primary antibodies were incubated for 30 min: Brilliant Violet 605™ anti mouse CD45, PE-cy7-F4/80, Alexa Fluor® 488 anti-mouse/human CD11b, APC anti-mouse CD206, or Brilliant Violet 421™ anti-mouse CD86 antibodies.

Additionally, cells from xenograft tumor were stained with mouse anti-human CD163 antibody at 4°C overnight. After washing with PBS, cells were incubated with Alexa Fluor® 488-conjugated mouse IgG secondary antibody at 4°C for 1 h. Flow cytometry was performed using a Cytek® NL-CLC full spectrum flow cytometry (CYTEK, CA, USA). Data were analyzed using FlowJo 10.8.1 software (TreeStar, Inc.).

### 2.15 Statistical analysis

Experiments were independently repeated 4–6 times with biological replicates. Data are expressed as means ± SEM. Two-tailed Student's t-test for pairwise comparisons and one-way ANOVA followed by Tukey's test for multiple group comparisons were used to determine the significance of differences. All statistical analyses were performed using GraphPad Prism 6 (La Jolla, CA). $p$-value < 0.05 was considered statistically significant.

## 3 Results

### 3.1 1,25(OH)$_2$D$_3$ enhances macrophage recruitment/M2 polarization associated with LL-37 level

THP-1-derived M1-type and M2-type macrophages were identified by the classical M1-type markers (iNOS) and M2-type markers (CD163, Arg-1), respectively (Figures 1A–C). Subsequently, we established a HCC/macrophage co-culture system to investigate the effect of 1,25(OH)$_2$D$_3$ on macrophage recruitment and polarization. THP-1-derived M0 macrophages were cultured in upper inserts, while HCC cells (PLC/PRF-5/Huh7) were seeded in lower chambers. Transwell recruitment assays revealed that 1,25(OH)$_2$D$_3$ treatment (200–500 nM) significantly enhanced macrophage recruitment to HCC cells ($p < 0.001$) (Figure 1D). Notably, siRNA-mediated LL-37 knockdown (si-LL-37) completely abolished this chemotactic response ($p < 0.001$), confirming LL-37 as the critical mediator. Furthermore, 1,25(OH)$_2$D$_3$ treatment significantly upregulated canonical M2 polarization markers (Arg-1 and CD163, $p < 0.001$) in co-cultured macrophages (Figure 1E), suggesting an increase of M2 polarization induced by 1,25(OH)$_2$D$_3$. However, this pro-tumorigenic polarization was entirely negated by si-LL-37 pretreatment, confirming the essential role of LL-37 in mediating 1,25(OH)$_2$D$_3$-dependent macrophage reprogramming. Functional analyses demonstrated that, combined treatment with si-LL-37 significantly enhanced 1,25(OH)$_2$D$_3$-induced the suppression of HCC cell proliferation, colony formation, and invasion through Matrigel in co-culture models ($p < 0.001$) (Figures 1F–H). These results demonstrate that 1,25(OH)$_2$D$_3$ enhances macrophage recruitment and M2 polarization, as well as limits the anticancer activity of 1,25(OH)$_2$D$_3$ in HCC/macrophage co-culture model, while LL-37 as the critical mediator during these processes.

### 3.2 LL-37 stimulates macrophages recruitment and M2 polarization *in vitro*

To elucidate the mechanisms by which si-LL-37 inhibited 1,25(OH)$_2$D$_3$-mediated macrophage recruitment and M2 polarization, we first investigated the impact of 1,25(OH)$_2$D$_3$ treatment on hCAP18/LL-37 levels in HCC/macrophage co-culture systems. Here, we confirmed that 1,25(OH)$_2$D$_3$ (200 nM, 24 h) significantly upregulated secreted hCAP18/LL-37 protein levels in both THP-1 and M0 macrophages ($p < 0.05$) (Figure 2A). In HCC/macrophage co-cultures, 1,25(OH)$_2$D$_3$ treatment resulted in ~ 4 fold induction of secreted LL-37 protein (Figure 2B) and 3 ~ 4 fold increase in *CAMP* mRNA ($p < 0.001$) (Figure 2C). Mechanistic investigations revealed that 1,25(OH)$_2$D$_3$ induced nuclear translocation of VDR in both PLC/PRF-5 and macrophages (Figure S1A). ChIP-PCR assay and dual luciferase reporter gene assay demonstrated direct VDR binding to the vitamin D response element (VDRE) in the *CAMP* promoter, driving transcriptional activation in both cell types (Supplementary Figures S1B,C). These findings establish VDR-mediated transcriptional regulation of hCAP18/LL-37 in HCC cells and macrophages.

Subsequently, we investigated the effect of LL-37 on macrophage recruitment and polarization. M0 macrophages were cultured in upper inserts, while HCC cells (PLC/PRF-5/Huh7 wild-type or their LL-37-overexpressing/knockdown derivatives) were seeded in lower chambers. Chemotaxis assay revealed that LL-37-overexpressing HCC cells (PLC/PRF-5$^{\text{LL-37/high}}$ and Huh7$^{\text{LL-37/high}}$) significantly enhanced macrophage recruitment compared to control ($p < 0.001$) (Figure 2D), whereas LL-37-knockdowned HCC cells (PLC/PRF-5$^{\text{LL-37/low}}$ and Huh7$^{\text{LL-37/low}}$) showed markedly reduced recruitment capacity ($p < 0.05$). The effect of LL-37 on M2 polarization was also detected by M2-type markers (Arg-1 and CD163) levels using exogenous addition of LL-37. Similar to the IL-4 induction, LL-37 significantly increased the levels of M2 markers in macrophages ($p < 0.01$) (Figure 2E). Flow cytometry further demonstrated that CD163 (Alexa Fluor® 488 conjugate) fluorescence was increased approximately double higher after LL-37 treatment ($p < 0.001$) (Figure 2F). Together, these results suggest that LL-37 stimulates the recruitment and M2 polarization of THP-1 derived macrophages *in vitro*.

### 3.3 Akt/mTOR and STAT3 signals mediate LL-37-stimulated M2 macrophage polarization

To elucidate the signal mechanisms driving LL-37-mediated M2 polarization, we investigated the regulatory role of the Akt/mTOR and STAT3 signaling pathways. Exogenous LL-37 treatment (100 ng/mL, 48 h) induced significant upregulation of M2 markers (CD163/Arg-1) and MMP9, accompanied by marked phosphorylation of Akt (p-Akt), mTOR (p-mTOR), and 4E-BP1 (p-4E-BP1) in macrophages ($p < 0.001$) (Figure 3A). Using Akt inhibitor MK2206 or mTOR inhibitor Rapamycin (Rapa) effectively reversed these LL-37-mediated enhancements (Figures 3A,B). Meanwhile, p-STAT3 level was increased after LL-37 treatment, while blockade of p-STAT3 by S3I201 obviously decreased the LL-37-induced increase of p-STAT3 level (Figure 3C). Functional validation through ELISA revealed that LL-37 stimulation significantly increased IL-10 secretion (M2-specific cytokine; $p < 0.001$), while MK2206, Rapa or S3I201 completely abolished LL-37-induced IL-10 upregulation ($p < 0.001$) (Figures 3D,E). Notably, LL-37 treatment does not significantly alter TNF-$\alpha$ (M1-specific cytokine) production in macrophages. Together, these findings demonstrate that both Akt/mTOR and STAT3 pathways are involved in LL-37-stimulated macrophage M2 polarization.

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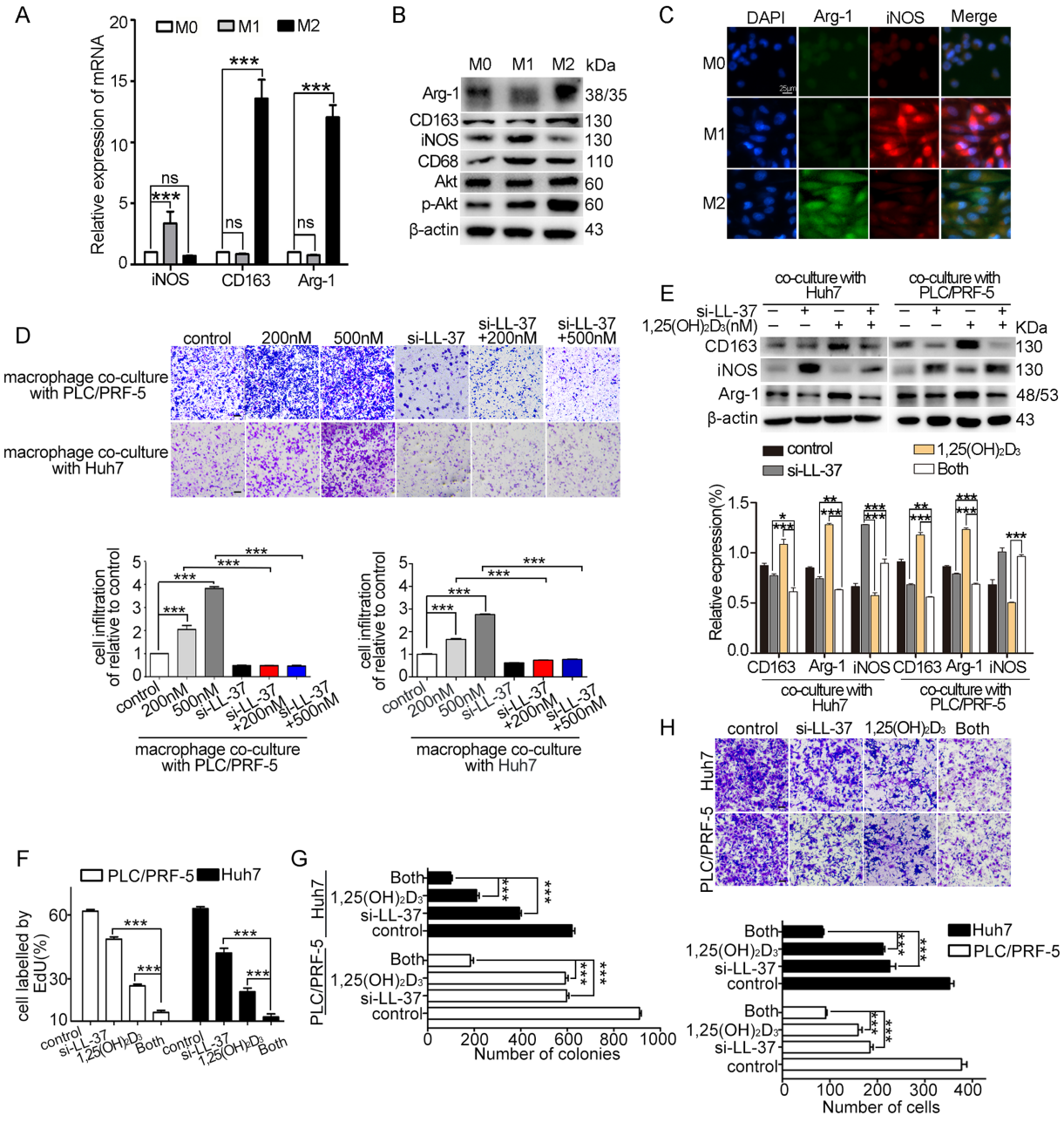


FIGURE 1
Effects of $1,25(OH)_2D_3$ and si-LL-37 on macrophage recruitment and polarization in HCC/macrophage co-culture systems. **(A)** THP-1 derived M1-type and M2-type macrophages were identified using the M1 markers (iNOS) and M2 markers (CD163, Arg-1) by qRT-PCR, respectively. **(B)** Western blot analysis of M1 markers and M2 markers in macrophages. **(C)** Immunofluorescence staining of iNOS and Arg-1 in M0, M1-type and M2-type macrophages. Scale bars, 20 μm. **(D)** PLC/PRF-5 and Huh7 cells were transfected with si-LL-37 for 8 h to obtain LL-37-knockdowned cells (PLC/PRF-$5^{LL\text{-}37/low}$ or $Huh7^{LL\text{-}37/low}$). These modified HCC cells were cultured in the bottom chamber. Then transwell recruitment assay was conducted to assess the macrophage migration to HCC cells after $1,25(OH)_2D_3$ treatment when co-cultured with HCC cells or modified HCC cells (si-LL-37 transfection). Scale bars, 50 μm. **(E)** Western blot assay was conducted to detect the expression of M1-type and M2-type markers in macrophages with or without $1,25(OH)_2D_3$. EdU assay **(F)**, colony formation assay **(G)** and invasion assay **(H)** were conducted to detect proliferation, clonogenesis and invasion of PLC/PRF-5 and Huh7 cells in HCC/M0 co-culture model after $1,25(OH)_2D_3$ mono-treatment or combination with si-LL-37. Scale bars, 50 μm. Data are represented as the mean ± SEM of 4–6 different experiments. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

## 3.4 Suramin reverses the effect of $1,25(OH)_2D_3$ on macrophages

Suramin, an old antiparasitic agent with established LL-37-binding capacity (22), was evaluated for its capacity to reverse $1,25(OH)_2D_3$-induced macrophage recruitment and M2 polarization in HCC/macrophage co-culture model. Results showed that suramin treatment (5 μM) completely inhibited the membrane binding and internalization of LL-37 in both PLC/PRF-5 cells and macrophages (Figure 4A). Functional analyses revealed that suramin treatment

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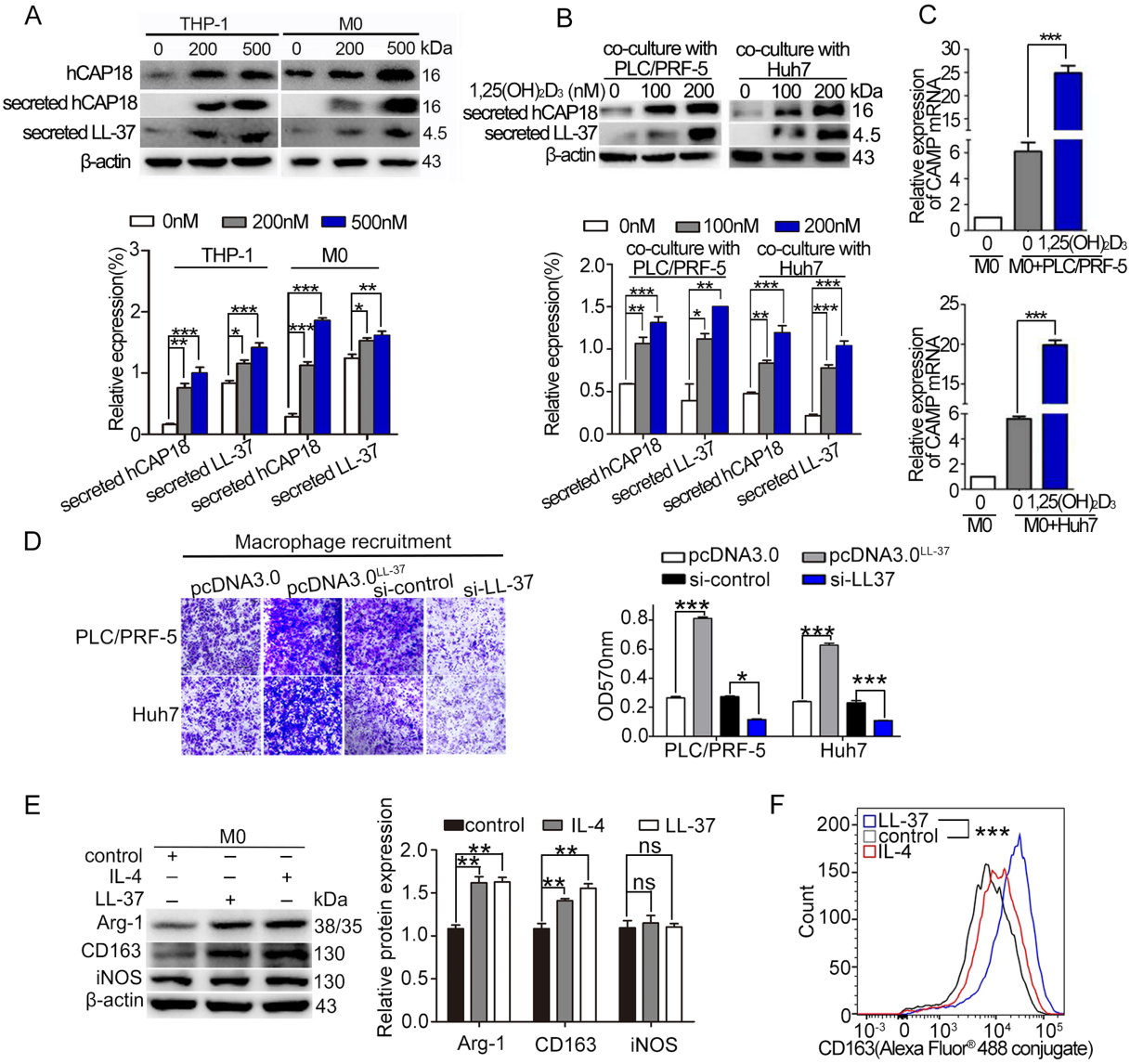


FIGURE 2
$1,25(OH)_2D_3$ modulates hCAP18/LL-37 expression and macrophage recruitment/polarization. **(A)** Western blot analysis of the levels of intracellular hCAP18 (cell lysates) and secreted hCAP18/LL-37 (media) hCAP18/LL-37 in THP-1-derived macrophages treated with $1,25(OH)_2D_3$ for 48 h. **(B)** Secreted hCAP18/LL-37 protein levels were detected in HCC/macrophage co-cultures after $1,25(OH)_2D_3$ treated for 48 h. **(C)** *CAMP* mRNA level in co-cultured macrophages were quantified by qRT-PCR after $1,25(OH)_2D_3$ (200 nM) treated for 48 h. **(D)** Transwell recruitment assays were performed in transwell chambers. PLC/PRF-5 and Huh7 cells were transfected with $pcDNA^{LL-37}$ or si-LL-37 for 8 h to obtain LL-37-overexpressed cells ($PLC/PRF\text{-}5^{LL\text{-}37/high}$, $Huh7^{LL\text{-}37/high}$) or LL-37-knockdowned cells ($PLC/PRF\text{-}5^{LL\text{-}37/low}$ or $Huh7^{LL\text{-}37/low}$). These modified HCC cells were cultured in the bottom chamber. After 48 h, macrophages (in the upper chamber) were stained with crystal violet and detected at 570 nm. Scale bars, 50 μm. **(E)** Western blot analysis of CD163, Arg-1 and iNOS in M0 macrophages treated with LL-37 (2 μM) or IL-4 (20 ng/mL) for 48 h. **(F)** Flow cytometric quantification of CD163 level in macrophages following LL-37 treatment. Data are mean±SEM ($n$ = 4–6). ns, no significance, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

abolished $1,25(OH)_2D_3$-induced macrophage recruitment ($p < 0.001$) (Figure 4B). Suramin decreased $1,25(OH)_2D_3$-upregulated M2 markers (Arg-1, CD163) while increased M1 marker iNOS level when co-cultured with HCC cells (PLC/PRF-5 or Huh7) ($p < 0.001$) (Figures 4C,D). During the process, suramin potently suppressed $1,25(OH)_2D_3$-activated Akt/mTOR signaling, as judged by the phosphorylation inhibition of Akt, mTOR, and 4EBP1 ($p < 0.001$) (Figure 4C). Flow cytometric validation confirmed suramin's capacity to reverse $1,25(OH)_2D_3$-mediated M2 polarization, showing 150% reduction in $CD163^+$ macrophage populations compared to $1,25(OH)_2D_3$-treated control ($p < 0.001$) (Figure 4E). Secreted cytokines analysis further demonstrated that suramin significantly decreased the $1,25(OH)_2D_3$-upregulated IL-10 (M2 type) release, while increased $1,25(OH)_2D_3$-downregulated TNF-$\alpha$ (M1 type) release from macrophages ($p < 0.001$) (Figure 4F). Together, these data demonstrate that suramin effectively reverses $1,25(OH)_2D_3$-induced macrophage recruitment and M2 polarization in HCC/macrophage co-culture model *in vitro*.

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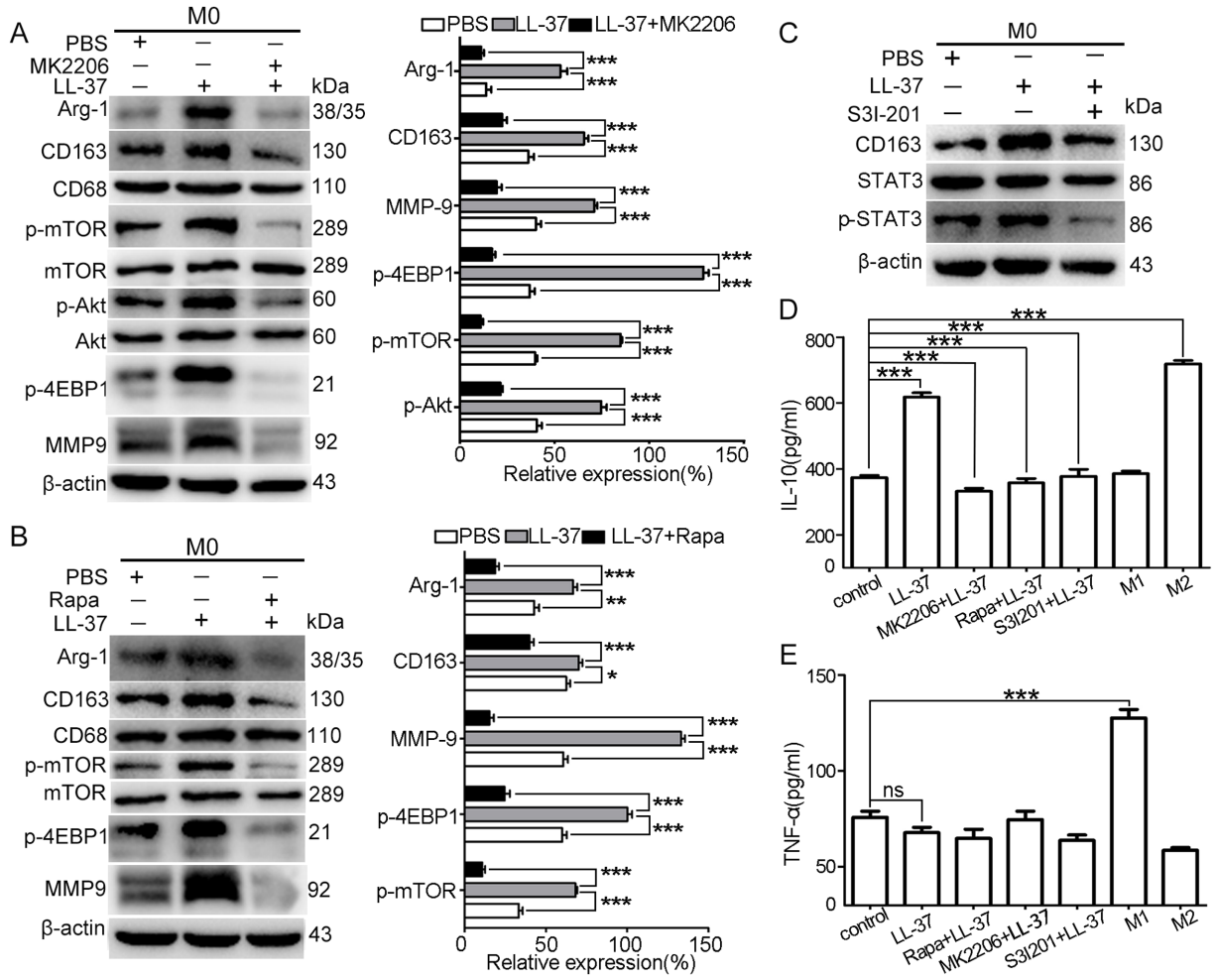


FIGURE 3
Akt/mTOR and STAT3 signals during LL-37-stimulated macrophage M2 polarization. After treatment of M0 macrophages with LL-37 (2 μM) for 48 h, western blot analysis of M2 markers (Arg-1, CD163, CD68) and phosphorylation levels (p-mTOR, p-Akt, p-4EBP1) in LL-37-treated M0 macrophages with pathway-specific inhibitors: **(A)** p-Akt inhibitor MK2206, **(B)** p-mTOR inhibitor Rapa, and **(C)** p-STAT3 inhibitor S3I201. ELISA quantification of anti-inflammatory (IL-10) and pro-inflammatory (TNF-$\alpha$) cytokines secreted by LL-37-stimulated macrophages. Data are mean ± SEM of 4–6 different experiments. ns, no significance, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

## 3.5 Suramin enhances the anticancer activity of 1,25(OH)$_2$D$_3$ in HCC/macrophage co-culture model

To evaluate suramin's synergistic effects on 1,25(OH)$_2$D$_3$-mediated anticancer activity, we performed proliferation, clonogenic, and invasion assays in HCC/macrophage co-culture systems. Co-culture with M0 macrophages (upper layer) significantly enhanced the proliferation of HCC cells (PLC/PRF-5 and Huh7, bottom layer) by 40 ~ 50% compared to HCC monoculture ($p < 0.001$) (Figure 5A), as evidenced by an increase in EdU-positive cells. Notably, 1,25(OH)$_2$D$_3$ mono-treatment inhibited the proliferation by 40 ~ 50%, while suramin co-treatment synergistically reduced proliferation to < 5% ($p < 0.001$). In M2-type macrophage-derived conditioned medium (M2$^{CM}$) assays, suramin further enhanced 1,25(OH)$_2$D$_3$'s inhibitory effects on colony formation ($p < 0.001$) (Figure 5B), demonstrating enhanced suppression of malignant potential. While M0 macrophages (bottom chamber) promoted HCC cell (upper chamber) invasion by ~ 40% ($p < 0.001$), 1,25(OH)$_2$D$_3$ reduced this invasion by 20% to Huh7 and 45% to PLC/PRF-5, respectively. Importantly, suramin combination treatment achieved 70% invasion inhibition compared to 1,25(OH)$_2$D$_3$ alone ($p < 0.001$) (Figure 5C). These findings confirm that suramin potently enhances 1,25(OH)$_2$D$_3$'s anticancer efficacy through synergistic suppression of proliferation, clonogenicity, and invasion in HCC/macrophage microenvironments.

## 3.6 Suramin enhances the antitumor effect of 1,25(OH)$_2$D$_3$ in HCC/macrophage co-xenografts

We established subcutaneous PLC/PRF-5 xenografts and HCC/macrophage co-xenografts (Balb/c nude mice injected with PLC/PRF-5 or PLC/PRF-5 + M0 cells). As shown in Supplementary Figure S1D, body weight remained stable across groups. Co-injection with M0 macrophages accelerated tumor growth, yielding double larger volumes (1,208 ± 90 mm$^3$ vs. 606 ± 65 mm$^3$) compared to monoculture controls ($p < 0.001$) (Figure 6A). While 1,25(OH)$_2$D$_3$ and suramin

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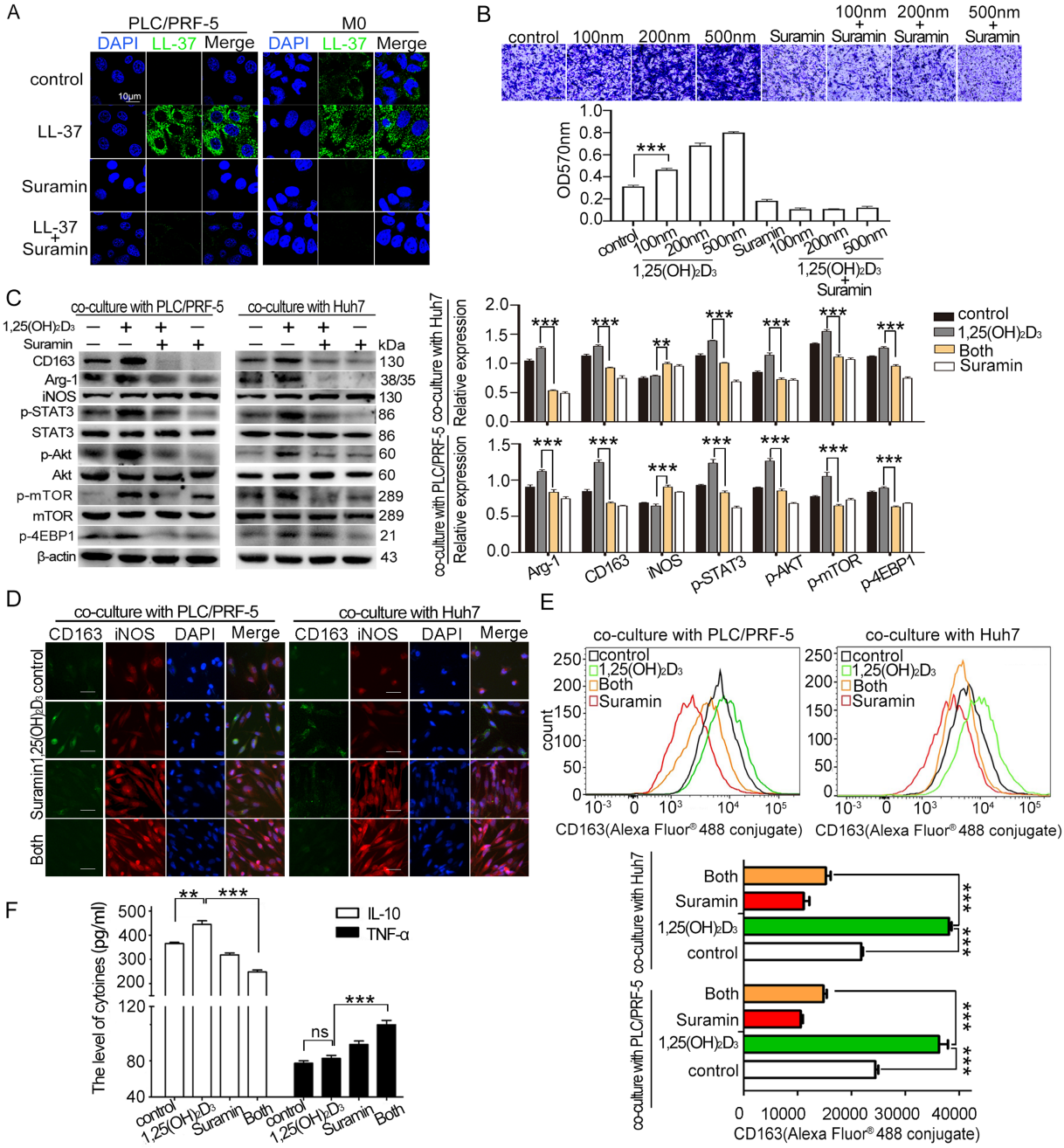


FIGURE 4
Effect of suramin on $1,25(OH)_2D_3$-induced recruitment and M2 polariation. **(A)** Confocal microscopy analysis of LL-37 distribution (FITC-green) in PLC/PRF-5/macrophage co-cultures treated with and FITC-LL-37 (1 μM) and suramin (5 μM). DAPI (blue) marks nuclei. Scale bar, 10 μm. **(B)** Transwell recruitment assay quantifying THP-1 monocyte (upper layer) recruitment to HCC cells (bottom layer) **(C)** Western blot analysis of M1/M2 markers (iNOS/CD163), Akt/mTOR (p-Akt, p-mTOR, p4EBP1), and p-STAT3 phosphorylation in co-cultured macrophages after $1,25(OH)_2D_3$ (200 nM) mono-treatment or combination with suramin (5 μM). **(D)** After incubation with anti-CD163 antibody, anti-iNOS antibody and Alexa Fluor® 488-conjugated mouse IgG, CD163 and iNOS levels in macrophages was observed by immunofluorescence assay. Scale bars, 20 μm. **(E)** After incubation with Alexa Fluor® 488-conjugated anti-CD163, flow cytometric quantification of CD163 level in macrophages. **(F)** ELISA detection of IL-10 and TNF-α in co-culture supernatants. Levels of IL-10 and TNF-α in culture supernatant of HCC/macrophage co-cultures ($n$ = 4 samples per group). Data are mean±SEM ($n$ = 4–6). ns, no significance, $^{**}p < 0.01$, $^{***}p < 0.001$.

mono-treatment inhibited co-xenograft growth by 44 and 56% ($p < 0.01$), respectively, their combination achieved synergistic suppression (75% inhibition vs. mono-treatment) ($p < 0.01$) (Figure 6B). ELISA revealed that co-xenografted mice exhibited 2.0-fold elevated serum hCAP18/LL-37 levels versus PLC/PRF-5 control ($p < 0.001$). Although $1,25(OH)_2D_3$ further increased these levels by 40%, suramin monotherapy or combination treatment reduced them by 70% ($p < 0.001$) (Figure 6C). IHC analysis demonstrated that $1,25(OH)_2D_3$ induced hCAP18/LL-37 level upregulation and M2 polarization, evidenced by $CD163^+/Arg\text{-}1^+/$

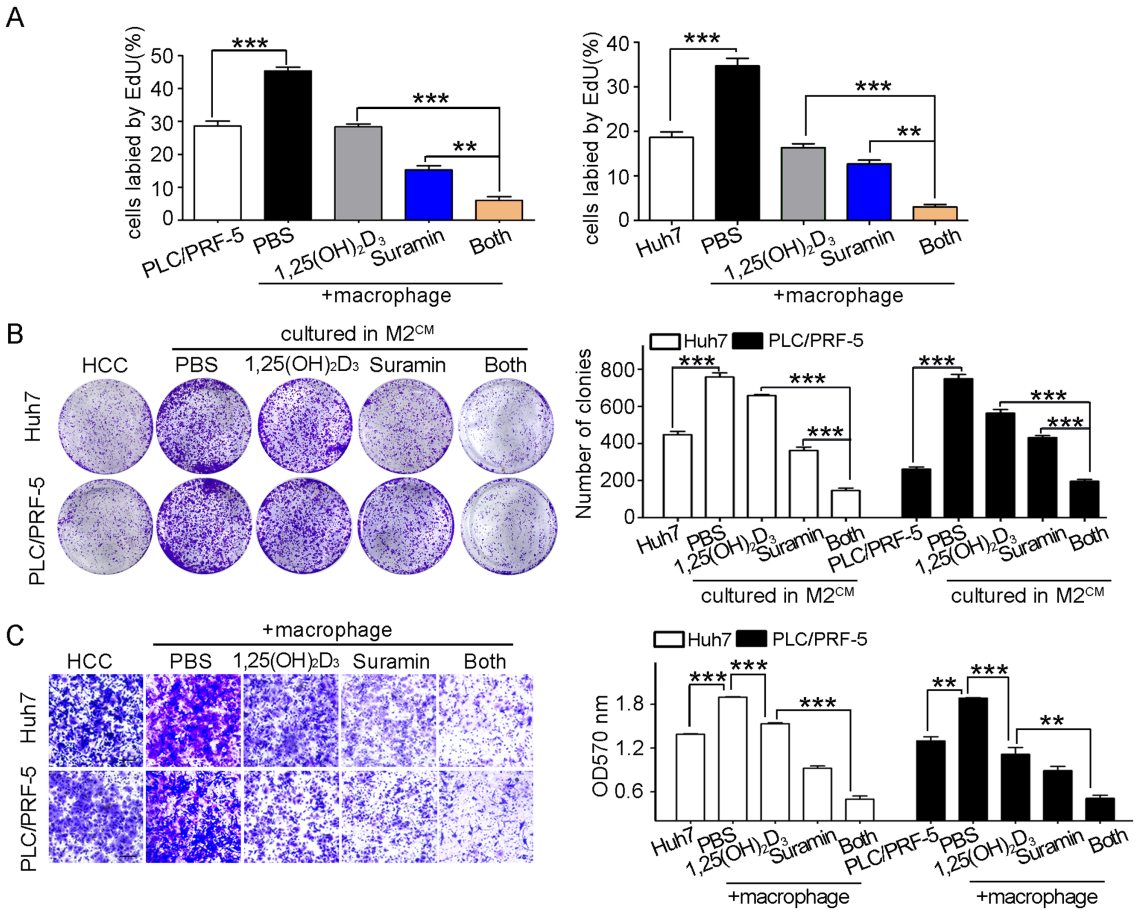


FIGURE 5
Suramin promotes the anticancer activity of $1,25(OH)_2D_3$ in HCC/macrophage co-cultures. PLC/PRF-5 or Huh7 cells (bottom layer) were co-cultured with M0 macrophages (upper layer) and then treated with $1,25(OH)_2D_3$ (200 nM) and/or suramin (5 μM). **(A)** EdU assays detected the proliferation of PLC/PRF-5 and Huh7 cells. **(B)** Colony formation assay was conducted to detect the growth and survival of PLC/PRF-5 and Huh7 cells in $M2^{CM}$. **(C)** Transwell assays detected the invasion of PLC/PRF-5 and Huh7 cells when co-culture with M0 macrophages. Scale bars, 50 μm. Data are represented as the mean±SEM of 4–6 different experiments. $^{**}p < 0.01$, $^{***}p < 0.001$.

iNOS$^-$ staining (Figure 6D). While suramin abrogated this effect, restoring M1 phenotype (iNOS$^+$/CD163$^-$) in co-treated tumors ($p < 0.001$). Flow cytometry revealed that $1,25(OH)_2D_3$ treatment significantly increased CD163 level in co-xenograft tumor ($p < 0.001$). However, co-treatment with suramin obviously reduced the CD163 level ($p < 0.01$) (Figure 6E). Western blot analysis showed that $1,25(OH)_2D_3$ upregulated p-Akt, p-mTOR, p-4EBP1 and p-STAT3. Suramin treatment completely reversed these phosphorylation activations in both mono- and combination therapies (Figure 6F). These findings demonstrate that suramin enhances the antitumor activity of $1,25(OH)_2D_3$ through reprogramming M1 macrophage polarization, and suppressing oncogenic Akt/mTOR and STAT3 signals.

## 3.7 Suramin enhances the antitumor effect of $1,25(OH)_2D_3$ in DEN/$CCl_4$-induced HCC mouse model

HCC mouse model was established after DEN/$CCl_4$ administration for 20 weeks (Figure 7A). After sacrifice (week 23), all DEN/$CCl_4$-treated mice developed HCC with typical histopathological features (data not shown), while no distant metastasis was observed. $1,25(OH)_2D_3$ (5 μg/kg, s.c.) or suramin (10 mg/kg, i.p.) treatment significantly reduced whitish macroscopic nodules compared to PBS control ($p < 0.001$) (Figures 7B–D), while combination treatment further reduced nodules and hepatic tumor load, with surface nodule count: 21.8 ± 1.69 (PBS) vs. 12.7 ± 0.97 (suramin) vs. 12.3 ± 0.65 ($1,25(OH)_2D_3$) vs. 7.9 ± 0.54 (combination). Flow cytometry analysis revealed that tumor-infiltrating macrophages (CD11b$^+$F4/80$^+$) in $1,25(OH)_2D_3$ group significantly increased from 8.13% (PBS) to 33.4% ($p < 0.01$), while significantly decreased in suramin group (21.7%, $p < 0.05$) and combination group (17.95%, $p < 0.001$) (Figure 7E). Additionally, tumor-infiltrating macrophages (CD11b$^+$F4/80$^+$) showed distinct immunophenotypic changes: M2-like (CD11b$^+$F4/80$^+$/CD206$^+$/CD86$^-$) macrophages decreased from 27.07 ± 2.72% ($1,25(OH)_2D_3$) to 15.88 ± 0.78% (combination; $p < 0.01$); and M1-like (CD11b$^+$F4/80$^+$/CD86$^+$/CD206$^-$) macrophages increased from 10.51 ± 2.50% ($1,25(OH)_2D_3$) to 25.96 ± 3.72% (combination; $p < 0.001$). Further analysis revealed that CD86$^+$/CD206$^+$ ratio increased 1.7-fold ($p < 0.001$) with combination therapy versus $1,25(OH)_2D_3$ alone. These findings demonstrate that suramin enhances $1,25(OH)_2D_3$ anticancer activity partly via inhibiting monocyte/macrophages infiltration and reprogramming TAMs from protumorigenic M2 to antitumoral M1 phenotype in HCC mouse model.

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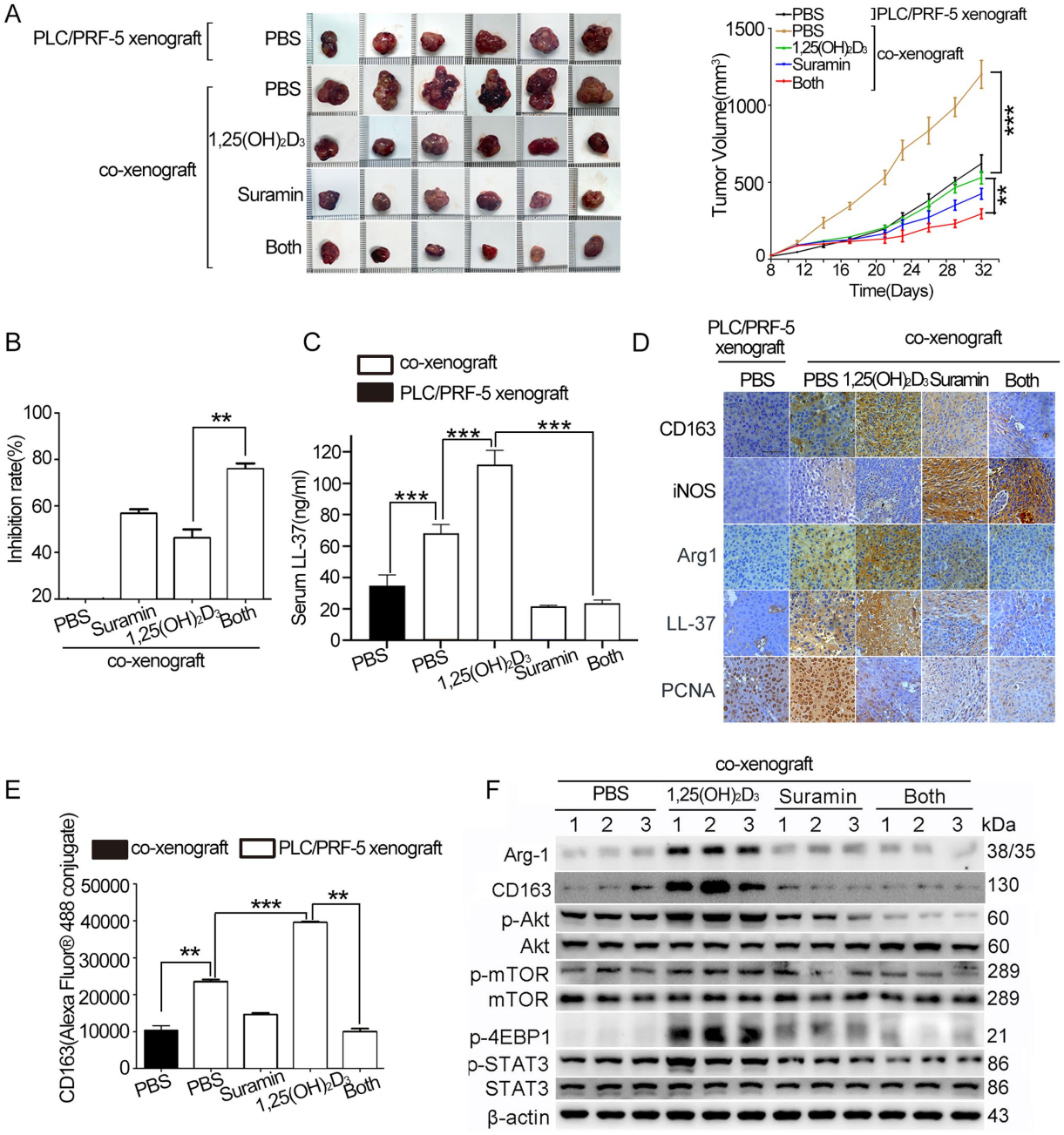


FIGURE 6
Suramin enhances the antitumor efficacy of 1,25(OH)$_2$D$_3$ in PLC/PRF-5/macrophage co-xenografts. Four to 6 weeks-old nude mice were subcutaneously injected with PLC/PRF-5 cells (6 × 10$^6$) or a mixture of PLC/PRF-5 cells (6 × 10$^6$) plus M0 macrophages (1.5 × 10$^6$). About 4 weeks later, the mice were euthanized and tumor tissue dissected for analysis. Tumor volume **(A)** was determined every 4 days and tumor growth curves were illustrated. Tumor images of each group at the end of treatment were shown. **(B)** The tumor growth inhibition rate was calculated from tumor weights. **(C)** Serum hCAP18/LL-37 levels quantified by ELISA assay. **(D)** Paraffin sections were prepared for IHC staining using anti-CD163, anti-Arg-1, anti-hCAP18/LL-37, and anti-PCNA antibodies, respectively. Representative images from each group are shown. Scale bar, 50 μm. **(E)** After incubation with anti-CD163 and secondary antibody (Alexa Fluor® 488-conjugated mouse IgG), flow cytometry was performed to detect the CD163 level. **(F)** Tumor tissues were disassociated to collect cells. Cells were lysed with RIPA lysis buffer, and the supernatants were collected for western blot analysis using indicated antibodies. Data are mean±SEM. $^{**}p < 0.01$, $^{***}p < 0.001$.

## 4 Discussion

The recruitment of macrophages to the TME precedes their transformation into TAMs, while the density of TAMs is closely associated with a poor prognosis for solid tumor patients (23). Cathelicidin hCAP18/LL-37 has demonstrated multifaceted tumorigenic properties across malignancies. Beyond its direct oncogenic effects on cancer cells, emerging evidence underscores its immunomodulatory role within the TME. In prostate cancer, overexpressed LL-37 chemo-attracts immature myeloid progenitors to the TME (24). Similarly, murine CRAMP (the functional homolog of LL-37) drives colon cancer progression by recruiting inflammatory

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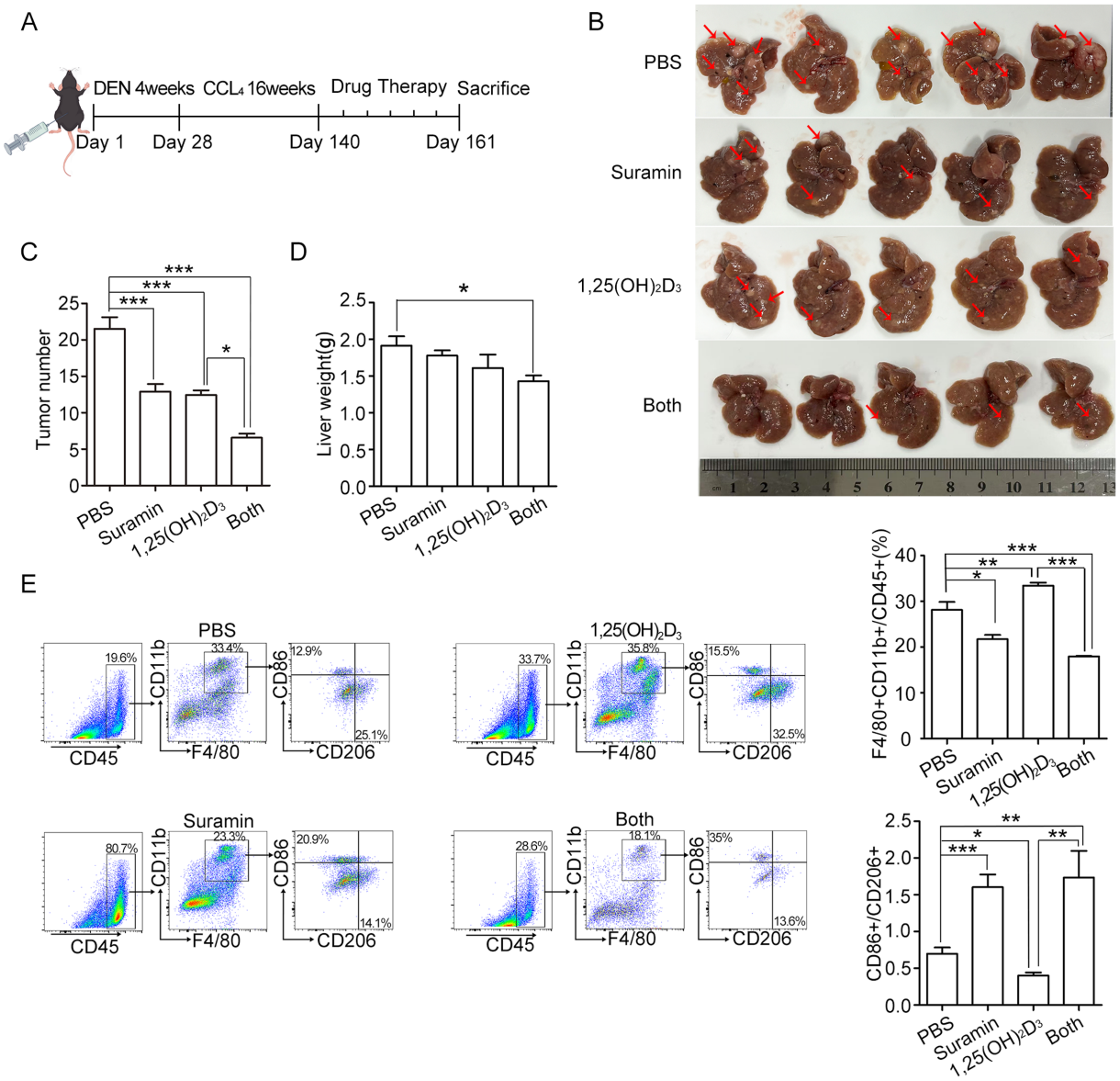


FIGURE 7
Suramin enhances the antitumor effect of $1,25(OH)_2D_3$ in DEN/$CCl_4$-induced HCC model. **(A)** the schedule of DEN/$CCl_4$-induced HCC modeling and administration of primary liver cancer mice. About 20 weeks later, the mice were randomly divided into 4 groups ($n$ = 8 per group): (1) PBS group, (2) $1,25(OH)_2D_3$ group (a dose of 0.5 μg/kg per day), (3) suramin group (10 mg/kg twice a week), and (4) $1,25(OH)_2D_3$ plus suramin group. All treatments were initiated post-model confirmation and continued for 3 weeks. Mice were euthanized and tumor tissue dissected for analysis. **(B)** The images of the mice liver were recorded, the red arrows indicate the location of partial tumors. **(C)** Mice tumor number and **(D)** liver weight were analyzed. **(E)** Flow cytometry was performed on mouse liver tumors to detect the levels of CD45, F4/80, CD11b, M1 marker CD86 and M2 marker CD206. Macrophages infiltration were analysized by both $CD11b^+$ and $F4/80^+$ in the tumor tissue. The CD86/CD206 ratio was calculated to assess the polarization status TAMs. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

cells, particularly macrophages, into the TME (25). Our current investigations reveal that LL-37 overexpression significantly enhances macrophage migration toward HCC cells *in vitro*. This finding aligns with reports demonstrating that supraphysiological concentrations of LL-37 act as potent chemoattractants for phagocytes (monocytes, macrophages, and neutrophils), recruiting them to infection sites either directly or via chemokine induction (26, 27). Notably, such chemotactic gradients may originate not only from infection sites but also from the TME itself, where they persist and create permissive niches for sustained macrophage infiltration into tumors.

Our *in vivo* studies revealed that HCC/macrophage co-xenografts exhibited accelerated growth with enhanced M2 polarization, evidenced by an increased number of $CD163^+$/$Arg\text{-}1^+$ macrophages in tumor. This finding aligns with established mechanisms where HCC cells recruit and trigger M2 polarization through exosomal delivery or by secreting cytokines (IL-4, IL-13, CSF-1) (3, 28). Here we demonstrate that both exogenous LL-37 addition and endogenous overexpression significantly promotiate M2 polarization in THP-1-derived macrophages, whether they were cultured alone or co-cultured with HCC cells. Notably, hCAP18/LL-37 expression showed

significant upregulation in M2-type macrophage, HCC/macrophage co-culture, and HCC/macrophages co-xenograft. Establishing a paracrine loop where TAMs themselves become major sources of LL-37. LL-37 upregulationhas similarly been reported to drive M2 polarization in breast, colorectal, and prostate cancer *in vitro* models were reported previously (24, 25, 29). While STAT3 activation has been implicated in LL-37-mediated M2 polarization in prostate cancer (24, 30). Beyond STAT3 activation, our mechanistic investigations uncover a distinct Akt/mTOR-dependent pathway in HCC microenvironment. Phosphorylation analysis revealed that LL-37 stimulation induced 2-fold Akt activation and mTOR phosphorylation, which were completely abrogated by Akt inhibitor MK-2206 and mTOR inhibitor Rapa, respectively. This novel signal provides a mechanistic basis for the enhanced M2-like TAM polarization observed in co-xenograft models, suggesting that LL-37-mediated immunosuppression occurs through parallel STAT3 and Akt/mTOR pathways.

Previous studies have demonstrated that hCAP18/LL-37 attenuates the anticancer efficacy of $1,25(OH)_2D_3$ in HCC xenograft model (20). Human cathelicidin (hCAP18/LL-37) is directly regulated by vitamin D via the VDR pathway, whereas murine *CRAMP* lacks a functional VDRE in its promoter region (31, 32). To address this research gap, we established humanized co-culture and co-xenograft models using human HCC cells and macrophages. Our findings reveal that $1,25(OH)_2D_3$ treatment obviously upregulates hCAP18/LL-37 expression in HCC/macrophages co-cultures *in vitro* and in tumor tissues and serum of co-xenografted mice. This observation suggests that clinical vitamin D supplementation might inadvertently elevate LL-37 levels within HCC microenvironments. Given the established protumorigenic effects of LL-37 (24, 25, 29), our results emphasize the necessity for monitoring serum hCAP18/LL-37 dynamics during clinical trials evaluating vitamin D-based therapies for HCC patients.

Vitamin D exhibits dual effects on the immune microenvironment in HCC. While $1,25(OH)_2D_3$ demonstrates direct anticancer activity through inhibition of proliferation and colony formation, emerging evidence highlights its immunosuppressive potential. Our findings reveal that $1,25(OH)_2D_3$ promotes M2 polarization ($CD163^+/Arg\text{-}1^+$) while suppressing M1 phenotype ($iNOS^+$) in *in vitro* co-cultures, *in vivo* co-xenograft and DEN/$CCl_4$-induced HCC mouse models, resulting in increased M2: M1 ratio. This immunosuppressive polarization parallels previous observations in inflammatory bowel disease and diabetic nephropathy, where $1,25(OH)_2D_3$ enhances macrophage M2 skewing (12, 13, 33). Additionally, $1,25(OH)_2D_3$ treatment (500 nM) also significantly increased macrophage recruitment to HCC cells *in vitro.* Notably, quantitative analysis revealed a significant increase in mouse-derived $CD11b^+$macrophage infiltration within $1,25(OH)_2D_3$-treated tumors compared to PBS control in mouse co-xenograft models (data not shown). Previous study in mammary carcinoma models showed that $1,25(OH)_2D_3$ may similarly enhance M2 polarization, potentially facilitating metastasis (15). Another study reported that high expression of VDR in pancreatic cancer promotes M2 macrophage polarization and recruitment through the secretion of CCL20, which activates tumor progression (34). Overall, current research on the effect of $1,25(OH)_2D_3$ on TAMs remains limited, and the claim that $1,25(OH)_2D_3$ drives macrophage polarization toward an M2-like phenotype remains controversial. Due to the microenvironmental heterogeneity across cancer types, systematic investigations are urgently needed in the future. The immunosuppressive TME induced by $1,25(OH)_2D_3$ may counteract its therapeutic benefits, as evidenced by increased tumor inhibition rates upon combination with suramin. This observation aligns with Anisiewicz et al.'s proposition that vitamin D supplementation could adversely affect TME dynamics in oncology patients (35), particularly in HCC where LL-37-mediated macrophage activation exacerbates immunosuppression.

Our findings reveal that si-LL-37 significantly enhances $1,25(OH)_2D_3$'s anticancer efficacy in HCC/macrophage co-cultures. Co-treatment with si-LL-37 potentiated $1,25(OH)_2D_3$-mediated inhibition of proliferation and colony formation, indicating that LL-37 neutralization reverses $1,25(OH)_2D_3$'s immunosuppressive effects. Suramin, a clinically approved anti-parasitic agent (36), exhibited dual anticancer mechanisms: (1) direct inhibition of LL-37 function by blocking its membrane binding and cellular uptake in macrophages, and (2) promoting M1 polarization while suppressing M2 polarization and the Akt/mTOR signaling pathway. Notably, suramin completely abolished $1,25(OH)_2D_3$-driven macrophage recruitment and M2 polarization in co-xenografts, enhancing the tumor inhibition rates from 44% ($1,25(OH)_2D_3$ alone) to 75% (combination therapy), and significantly reducing whitish macroscopic nodules. Although clinical trials showed limited efficacy of vitamin D monotherapy in HCC (10, 37), our discovery of the $1,25(OH)_2D_3$-LL-37-TAM axis provides a mechanistic explanation for these discrepancies. The paradoxical protumorigenic mechanism of $1,25(OH)_2D_3$ via LL-37-mediated immunosuppression suggests that combining $1,25(OH)_2D_3$ with suramin may optimize therapeutic outcomes. These findings warrant clinical evaluation of serum hCAP18/LL-37 as predictive biomarkers and suramin as an adjuvant agent for HCC patients receiving vitamin D-based therapies.

Suramin, a clinically approved agent with a century-long history in trypanosomiasis, has recently gained much more attention for its numerous potential applications (36). Emerging evidence highlights its promising applications in antiviral, antidepressant, and anticancer therapies (38, 39). Our study provides the first demonstration of suramin's critical role in modulating TAMs and proposes a novel combinatorial strategy with $1,25(OH)_2D_3$ to enhance therapeutic efficacy in HCC treatment. However, the clinical translation of suramin is hindered by several limitations, including neurotoxicity, poor tissue bioavailability, and poor tissue penetration and retention (40, 41). Though a literature stated that suramin was generally safe and well tolerated in healthy Chinese volunteers at the dose of 10 mg/kg or 15 mg/kg (42). Further pharmacokinetic studies are required to optimize dosing regimens. Furthermore, advanced drug delivery systems should be explored to improve its tumor targeting and minimize off-target effects, thereby accelerating suramin's clinical application in oncology.

## 5 Conclusion

This study identifies the $1,25(OH)_2D_3$-LL-37-TAM axis as a key mechanism underlying the limited efficacy of vitamin D monotherapy in HCC (Figure 8). Specifically, hCAP18/LL-37 acts as a pivotal immunosuppressive mediator, driving monocytes/macrophages recruitment and M2 polarization via $1,25(OH)_2D_3$-induced transcriptional activation. Mechanistically, LL-37 upregulation by $1,25(OH)_2D_3$ promotes HCC progression by enhancing proliferation, migration, and invasion through TAM immunosuppressive reprogramming. Importantly, suramin

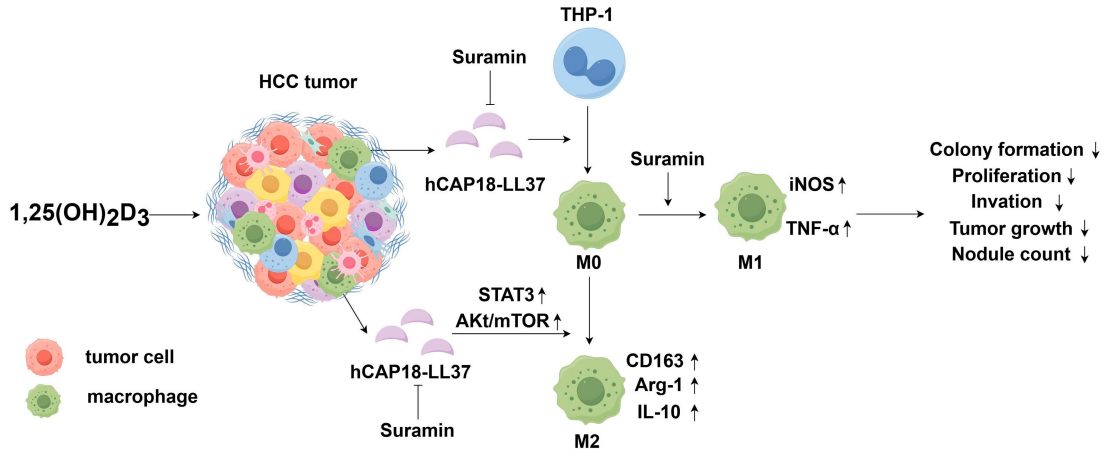


FIGURE 8
Schematic illustration showing the $1,25(OH)_2D_3$-LL-37-TAM axis affecting $1,25(OH)_2D_3$'s anti-cancer efficacy in HCC. $1,25(OH)_2D_3$ induces robust LL-37 secretion in HCC/macrophages co-cultures. Secreted LL-37 (a) enhances monocyte/macrophage migration toward HCC cells (b) activates AKt/mTOR and STAT3 pathways, and (c) drives M2 polarization, while suppressing M1 phenotype. This LL-37-mediated M2-TAM fosters an immunosuppressive environment that diminishes $1,25(OH)_2D_3$'s anticancer effects. Suramin inhibits the membrane binding and internalization of LL-37 in macrophages, blocking LL-37-mediated TAMs recruitment and M2 polarization, while promoting antitumor M1 phenotype responses. Suramin-mediated blockade of the $1,25(OH)_2D_3$-LL-37-TAM axis potentiates $1,25(OH)_2D_3$'s anticancer efficacy by potentiating the inhibition of HCC cell proliferation, colony formation, invasion, and tumor growth.

potently antagonizes this axis, blocking LL-37-mediated TAM recruitment and M2 polarization, while promoting antitumor M1 phenotypes. Our results emphasize the necessity for monitoring serum hCAP18/LL-37 dynamics during clinical trials evaluating vitamin D-based therapies for HCC patients. These findings highlight suramin as a promising adjunct to $1,25(OH)_2D_3$-based immunotherapy for HCC.

## 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

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used. The animal study was approved by Ethics Committee of Nanjing Normal University. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

HZ: Writing – review & editing, Investigation, Methodology, Writing – original draft. WX: Methodology, Writing – review & editing. WD: Writing – review & editing, Data curation. XY: Writing – review & editing, Methodology. YY: Methodology, Writing – review & editing. QC: Writing – review & editing, Data curation. YZ: Data curation, Writing – review & editing. YC: Writing – review & editing, Funding acquisition, Supervision.

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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/fnut.2025.1556533/full#supplementary-material

## References


1. Rumgay H, Arnold M, Ferlay J, Lesi O, Cabasag CJ, Vignat J, et al. Global burden of primary liver cancer in 2020 and predictions to 2040. *J Hepatol*. (2022) 77:1598–606. doi: 10.1016/j.jhep.2022.08.021

2. Mamdouh S, Aboushousha T, Abdelraouf A, Hamdy H, Seleem M, Hassan H. Molecular noninvasive diagnosis of hepatocellular carcinoma using microsatellite instability. *Asian Pac J Cancer Prev*. (2021) 22:3337–46. doi: 10.31557/APJCP.2021.22.10.3337

3. Cerreto M, Cardone F, Cerrito L, Stella L, Santopaolo F, Pallozzi M, et al. The new era of systemic treatment for hepatocellular carcinoma: from the first line to the optimal sequence. *Curr Oncol*. (2023) 30:8774–92. doi: 10.3390/curroncol30100633

4. Yang C, Zhang H, Zhang L, Zhu AX, Bernards R, Qin W, et al. Evolving therapeutic landscape of advanced hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol*. (2023) 20:203–22. doi: 10.1038/s41575-022-00704-9

5. Yuan Y, Wu D, Li J, Huang D, Zhao Y, Gao T, et al. Mechanisms of tumor-associated macrophages affecting the progression of hepatocellular carcinoma. *Front Pharmacol*. (2023) 14:1217400. doi: 10.3389/fphar.2023.1217400

6. Wu K, Lin K, Li X, Yuan X, Xu P, Ni P, et al. Redefining tumor-associated macrophage subpopulations and functions in the tumor microenvironment. *Front Immunol*. (2020) 11:1731. doi: 10.3389/fimmu.2020.01731

7. Yi Z, Wang L, Tu X. Effect of vitamin D deficiency on liver cancer risk: a systematic review and meta-analysis. *Asian Pac J Cancer Prev*. (2021) 22:991–7. doi: 10.31557/APJCP.2021.22.4.991

8. Adelani IB, Rotimi OA, Maduagwu EN, Rotimi SO. Vitamin D: possible therapeutic roles in hepatocellular carcinoma. *Front Oncol*. (2021) 11:642653. doi: 10.3389/fonc.2021.642653

9. Gaksch M, Jorde R, Grimnes G, Joakimsen R, Schirmer H, Wilsgaard T, et al. Vitamin D and mortality: individual participant data meta-analysis of standardized 25-hydroxyvitamin D in 26916 individuals from a European consortium. *PLoS One*. (2017) 12:e0170791. doi: 10.1371/journal.pone.0170791

10. Bilgen A, Kani HT, Akdeniz E, Alahdab YO, Ozdogan O, Gunduz F. Effects of vitamin D level on survival in patients with hepatocellular carcinoma. *Hepatol Forum*. (2020) 1:97–100. doi: 10.14744/hf.2020.2020.0017

11. Li P, Zhu X, Cao G, Wu R, Li K, Yuan W, et al. 1α,25 (OH)2D3 reverses exhaustion and enhances antitumor immunity of human cytotoxic T cells. *J Immunother Cancer*. (2022) 10:e003477. doi: 10.1136/jitc-2021-003477

12. Zhang X, Zhou M, Guo Y, Song Z, Liu B. 1, 25-Dihydroxyvitamin $D_3$ promotes high glucose-induced M1 macrophage switching to M2 via the VDR-PPARγ signaling pathway. *Bio Med Res Int*. (2015) 2015:157834:1–14. doi: 10.1155/2015/157834

13. Zhu X, Zhu Y, Li C, Yu J, Ren D, Qiu S, et al. 1, 25-Dihydroxyvitamin D regulates macrophage polarization and ameliorates experimental inflammatory bowel disease by suppressing mi R-125b. *Int Immunopharmacol*. (2019) 67:106–18. doi: 10.1016/j.intimp.2018.12.015

14. Cervantes JL, Oak E, Garcia J, Liu H, Lorenzini PA, Batra D, et al. Vitamin D modulates human macrophage response to *Mycobacterium tuberculosis* DNA. *Tuberculosis (Edinb)*. (2019) 116:S131–7. doi: 10.1016/j.tube.2019.04.021

15. Stachowicz-Suhs M, Łabędź N, Anisiewicz A, Banach J, Kłopotowska D, Milczarek M, et al. Calcitriol promotes M2 polarization of tumor-associated macrophages in 4T1 mouse mammary gland cancer via the induction of proinflammatory cytokines. *Sci Rep*. (2024) 14:3778. doi: 10.1038/s41598-024-54433-x

16. Koivisto O, Hanel A, Carlberg C. Key vitamin D target genes with functions in the immune system. *Nutrients*. (2020) 12:1140. doi: 10.3390/nu12041140

17. Fahy RJ, Wewers MD. Pulmonary defense and the human cathelicidin hCAP-18/LL-37. *Immunol Res*. (2005) 31:75–89. doi: 10.1385/IR:31:2:075

18. Chung C, Silwal P, Kim I, Modlin RL, Jo EK. Vitamin D-cathelicidin axis: at the crossroads between protective immunity and pathological inflammation during infection. *Immune Netw*. (2020) 20:e12. doi: 10.4110/in.2020.20.e12

19. Meca AD, Ștefănescu S, Bogdan M, Turcu-Știolică A, Nițu FM, Matei M, et al. Crosstalk between vitamin D axis, inflammation and host immunity mechanisms: a prospective study. *Exp Ther Med*. (2021) 21:608. doi: 10.3892/etm.2021.10040

20. Zhang H, Zhen J, Zhang R, Wanyan Y, Liu K, Yuan X, et al. Cathelicidin hCAP18/LL-37 promotes cell proliferation and suppresses antitumor activity of 1, 25 $(OH)_2D_3$ in hepatocellular carcinoma. *Cell Death Discov*. (2022) 8:27. doi: 10.1038/s41420-022-00816-w

21. Genin M, Clement F, Fattaccioli A, Raes M, Michiels C. M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. *BMC Cancer*. (2015) 15:577. doi: 10.1186/s12885-015-1546-9

22. Quemé-Peña M, Ricci M, Juhász T, Horváti K, Bősze S, Biri-Kovács B, et al. Old polyanionic drug suramin suppresses detrimental cytotoxicity of the host defense peptide LL-37. *ACS Pharmacol Transl Sci*. (2020) 4:155–67. doi: 10.1021/acsptsci.0c00155

23. Dehne N, Mora J, Namgaladze D, Weigert A, Brüne B. Cancer cell and macrophage cross-talk in the tumor microenvironment. *Curr Opin Pharmacol*. (2017) 35:12–9. doi: 10.1016/j.coph.2017.04.007

24. Cha HR, Lee JH, Hensel JA, Sawant AB, Davis BH, Lee CM, et al. Prostate cancer-derived cathelicidin-related antimicrobial peptide facilitates macrophage differentiation and polarization of immature myeloid progenitors to protumorigenic macrophages. *Prostate*. (2016) 76:624–36. doi: 10.1002/pros.23155

25. Quan WW, Pan XH, Wu JL, Yao YW, Li D. Effect and mechanism of antimicrobial peptide cathelicidin secreted by tumor associated macrophage on the growth of colorectal cancer in mice. *Zhonghua Zhong Liu Za Zhi*. (2018) 40:172–7. doi: 10.3760/cma.j.issn.0253-3766.2018.03.003

26. Hilchie AL, Wuerth K, Hancock RE. Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. *Nat Chem Biol*. (2013) 9:761–8. doi: 10.1038/nchembio.1393

27. Hemshekhar M, Choi KG, Mookherjee N. Host defense peptide LL-37-mediated chemoattractant properties, but not anti-inflammatory cytokine IL-1RA production, is selectively controlled by Cdc 42 rho GTPase via G protein-coupled receptors and JNK mitogen-activated protein kinase. *Front Immunol*. (2018) 9:1871. doi: 10.3389/fimmu.2018.01871

28. Hu Z, You L, Hu S, Yu L, Gao Y, Li L, et al. Hepatocellular carcinoma cell-derived exosomal mi R-21-5p promotes the polarization of tumor-related macrophages (TAMs) through SP1/XBP1 and affects the progression of hepatocellular carcinoma. *Int Immunopharmacol*. (2024) 126:111149. doi: 10.1016/j.intimp.2023.111149

29. Chen J, Shin VY, Ho JC, Siu MT, Cheuk IW, Kwong A. Functional implications of cathelicidin antimicrobial protein in breast cancer and tumor-associated macrophage microenvironment. *Biomol Ther*. (2020) 10:688. doi: 10.3390/biom10050688

30. Hashimoto S, Hashimoto A, Muromoto R, Kitai Y, Oritani K, Matsuda T. Central roles of STAT3-mediated signals in onset and development of cancers: tumorigenesis and immunosurveillance. *Cells*. (2022) 11:2618. doi: 10.3390/cells11162618

31. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1, 25-dihydroxyvitamin D3. *FASEB J*. (2005) 19:1067–77. doi: 10.1096/fj.04-3284com

32. Lowry MB, Guo C, Zhang Y, Fantacone ML, Logan IE, Campbell Y, et al. A mouse model for vitamin D-induced human cathelicidin antimicrobial peptide gene expression. *J Steroid Biochem Mol Biol*. (2020) 198:105552. doi: 10.1016/j.jsbmb.2019.105552

33. Zhang XL, Guo YF, Song ZX, Zhou M. Vitamin D prevents podocyte injury via regulation of macrophage M1/M2 phenotype in diabetic nephropathy rats. *Endocrinology*. (2014) 155:4939–50. doi: 10.1210/en.2014-1020

34. Li H, Ruan Y, Liu C, Fan X, Yao Y, Dai Y, et al. VDR promotes pancreatic cancer progression in vivo by activating CCL20-mediated M2 polarization of tumor associated macrophage. *Cell Commun Signal*. (2024) 22:224. doi: 10.1186/s12964-024-01578-x

35. Anisiewicz A, Łabędź N, Krauze I, Wietrzyk J. Calcitriol in the presence of conditioned media from metastatic breast cancer cells enhances ex vivo polarization of M2 alternative murine bone marrow-derived macrophages. *Cancers (Basel)*. (2020) 12:3485. doi: 10.3390/cancers12113485

36. Wiedemar N, Hauser DA, Mäser P. 100 years of suramin. *Antimicrob Agents Chemother*. (2020) 64:e01168–19. doi: 10.1128/AAC.01168-19

37. Negri M, Gentile A, de Angelis C, Montò T, Patalano R, Colao A, et al. Vitamin D-induced molecular mechanisms to potentiate cancer therapy and to reverse drug-resistance in cancer cells. *Nutrients*. (2020) 12:1798. doi: 10.3390/nu12061798

38. Bazan NG, Reid MM, Flores VAC, Gallo JE, Lewis W, Belayev L. Multiprong control of glioblastoma multiforme invasiveness: blockade of pro-inflammatory signaling, anti-angiogenesis, and homeostasis restoration. *Cancer Metastasis Rev*. (2021) 40:643–7. doi: 10.1007/s10555-021-09987-x

39. Boniardi I, Corona A, Basquin J, Basquin C, Milia J, Nagy I, et al. Suramin inhibits SARS-CoV-2 nucleocapsid phosphoprotein genome packaging function. *Virus Res*. (2023) 336:199221. doi: 10.1016/j.virusres.2023.199221

40. von der Ahe D, Huehnchen P, Balkaya M, Peruzzaro S, Endres M, Boehmerle W. Suramin-induced neurotoxicity: preclinical models and neuroprotective strategies. *Molecules*. (2018) 23:346. doi: 10.3390/molecules23020346

41. Su L, Bryan N, Battista S, Freitas J, Garabedian A, D'Alessio F, et al. Identification of HMGA2 inhibitors by alpha screen-based ultra-high-throughput screening assays. *Sci Rep*. (2020) 10:18850. doi: 10.1038/s41598-020-75890-0

42. Wu G, Zhou H, Lv D, Zheng R, Wu L, Yu S, et al. Phase I, single-dose study to assess the pharmacokinetics and safety of suramin in healthy chinese volunteers. *Drug Des Devel Ther*. (2023) 17:2051–61. doi: 10.2147/DDDT.S416325

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# Evaluating the predictive effect of vitamin D on clinical outcomes of infliximab-treated Crohn's disease patients

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**Backgrounds:** The aim of this study was to examine the clinical predictors of Infliximab (IFX) in Crohn's disease (CD) patients in eastern China and to support further research on the vitamin D remission rate compared to CD patients.

**Methods:** Patients with CD who were hospitalized at Xuzhou First People's Hospital between January 2020 and December 2023 were included in our retrospective analysis. Clinical information was gathered from CD patients at baseline and the endpoint (7th IFX therapy, 38 weeks). To determine the baseline variable [Crohn's Disease Activity Index (CDAI) < 150] for endpoint clinical remission in patients receiving IFX, and to examine the relationship between blood vitamin D (VIT-D) levels before starting IFX medication and CDAI at Week 38. The potential risk variables were then investigated using univariate, multivariate, and LASSO regression models.

**Results:** Included were 158 individuals with CD treated with IFX. At baseline, 18.35% of patients had a VIT-D deficit; 64.19% of patients experienced a decrease in VIT-D, and 63.29% of patients achieved clinical remission. The high Vitamin D levels at baseline were independent predictors of clinical remission after IFX therapy, according to univariate, multivariate, and LASSO regression analysis ($P < 0.05$). Receiver operating characteristic curve analysis revealed that AUC (95%CI) 0.56(0.25-0.95) was the endpoint CDAI (= 150) diagnostic value when the Vit-D level was 19.35 ng/ml. The corresponding sensitivity and specificity were 75.02% and 79.6%. Endpoint CDAI was independently predicted by male sex, age, BMI, and VIT-D levels <30 ng/ml ($P < 0.05$).

**Conclusion:** After receiving IFX therapy, CD patients in eastern China with higher VIT-D levels were more likely to achieve clinical remission, particularly those who were male, older, had a higher BMI, and had VIT-D levels below 30 ng/ml.

KEYWORDS

**Crohn's disease, vitamin D, infliximab, remission rate, gastroenterology**

# Introduction

Crohn's disease, an inflammatory condition of the gut driven by a dysregulated immune response, is one of many chronic inflammatory disorders affecting the gastrointestinal tract (1). In Asia, China reports the highest rate of CD diagnoses (3.44 per 100,000) (2). Current therapeutic goals primarily focus on promoting mucosal healing to slow disease progression (3). The complete development of the illness is likely influenced by a complex interplay of infectious and environmental factors (4). Notably, CD is associated with low levels of sunshine exposure, and both forms of CD are more common in North America and Europe as latitude increases (5). In CD, biologics targeting tumor necrosis factor-α (TNF-α) significantly increase response rates and rates of clinical response. However, clinical relapse and disease progression may result from a loss of response to anti-TNF-α therapy over time (6). It is estimated that 30% of inflammatory bowel disease (IBD) patients experience a loss of response to infliximab (IFX) during the course of therapy (7). Therefore, enhancing the efficacy and responsiveness to anti-TNFα agents is crucial.

Vitamin D3 is a nutrient essential for healthy bone production and growth (3). V Vitamin D can modulate both innate and adaptive immune responses (8). Vitamin D deficiency is associated with increased autoimmunity and heightened susceptibility to infection (9). Since immune cells in autoimmune diseases respond to the ameliorating effects of vitamin D, the benefits of supplementation in individuals with vitamin D deficiency may outweigh those on bone and calcium homeostasis (10). Vitamin D has emerged as a key regulator of the innate immune response to pathogen threat (11, 12). The hormone form of vitamin D signals through nuclear receptor transcription factors and regulates gene transcription (3). The link between vitamin D deficiency and CD is also gaining traction (1). The incidence of inflammatory bowel disease (IBD) is linked to vitamin D insufficiency, regardless of whether it results from inadequate dietary intake or a lack of sun exposure and skin synthesis (13). Vitamin D deficiency has been shown to worsen colitis progression in animal models, and vitamin D supplementation has been shown to restore epithelial integrity and inhibit inflammatory cytokine levels, thereby improving intestinal inflammation (3). Recent research has also shown that vitamin D levels in CD patients treated with IFX tend to correlate with IFX plasma concentrations. Growing evidence suggests that vitamin D plays a crucial role in regulating both innate and adaptive immunity, and could serve as a predictive factor for CD patients (1, 14). It remains unclear, however, how vitamin D functions in CD and what specific indicators may predict clinical outcomes.

While the relationship between vitamin D and IFX-treated CD disease has been reported in western China, its role in eastern China remains less understood (15). In order to determine the relationship between blood vitamin D levels and clinical remission in patients treated with CD by IFX, we first examined the clinical baseline data of CD patients treated with IFX.

# Materials and methods

According to the inclusion and exclusion criteria listed below, clinical data from patients in the Crohn's disease patient database in the Department of Gastroenterology at Xuzhou First People's Hospital was gathered for this study using a retrospective survey method between January 2020 and December 2023. The Xuzhou City First People's Medical Ethics Committee gave its approval to this research.

## Inclusion criteria

The study's inclusion criteria were as follows: 1) CD patients diagnosed using Chinese diagnostic criteria for inflammatory bowel disease (16); 2) aged between 18 and 60 years; 3) receiving Infliximab (IFX) for their first course of therapy; 4) having baseline 25-hydroxyvitamin D (25(OH)D) levels recorded before initiating IFX medication; and 5) having a recorded Crohn's Disease Activity Index (CDAI) score at the endpoint (Week 38), following the seventh IFX treatment. Exclusion criteria included: 1) individuals lacking routine follow-up; 2) individuals treated with IFX less than three months before the study start date; 3) patients with a history of glucocorticoid use within three months before initiating IFX medication; and 4) patients with severe or chronic cardiovascular, pulmonary, urinary, endocrine, reproductive, skeletal, muscular, neurological, or other systemic disorders, or those with other active infectious diseases.

## Outcome

Prior to stratifying by vitamin D status, we initially examined baseline data from CD patients receiving IFX (17). According to the Endocrine Society Clinical Practice Guidelines 2011, patients with 25(OH)D levels < 15 ng/mL were classified as deficient, those with levels > 30 ng/mL as sufficient, and those with levels between 15 and 30 ng/mL as insufficient (18).

## Clinical, and biological assessment

IFX therapeutic doses were administered ranging from 5 to 10 mg/kg as specified by IFX guidelines (100 mg/infusion). Treatment intervals followed standard protocols: weeks 0, 2, and 6 for the induction phase and every 8 weeks thereafter for the maintenance phase. At Week 38, clinical outcomes were evaluated, including clinical remission, biochemical remission, endoscopic remission, clinical response, and endoscopic response. Patients were classified into remission and non-remission groups based on clinical remission criteria. Subsequently, the association between baseline characteristics, Time-Drug Exposure (TDM), Area Under the Curve for Drug Concentration (ATI), and clinical remission status was analyzed.

Baseline clinical data collected for CD patients included disease course, symptoms, history of bowel and perianal surgery, use of combined immunosuppressants (IMM), Montreal classification, CDAI score, Simple Endoscopic Score for Crohn's Disease (SES-CD), inflammatory markers, nutritional indicators, liver and kidney function, electrolytes, fasting blood glucose (FBG), blood lipids, and antinuclear antibodies (ANA). Clinical remission was defined as a CDAI score < 150, clinical response as a CDAI reduction ≥ 70 (baseline to Week 7), biochemical remission as a C-reactive protein (CRP) < 5 mg/L, endoscopic remission as SES-CD ≤ 4, and endoscopic response as a ≥ 50% reduction in SES-CD from baseline to Week 38 (19, 20). Patients meeting these remission criteria could be considered for discharge. The IFX dosage (ranging from 5 to 10 mg/kg) adhered to the specified infusion dose (100 mg) and standard protocols for induction (weeks 0, 2, 6) and maintenance (every 8 weeks).

## Prognostic nomogram analysis

The LASSO (Least Absolute Shrinkage and Selection Operator) method was used to identify and select risk factors from the multivariate data. Variables with non-zero LASSO regression coefficients were selected for further analysis. Multiple logistic regression analysis was performed on these selected variables within the LASSO regression model to construct a predictive model. A calibration curve was created to assess the nomogram's calibration, and the Harrell c-index was computed to measure its discriminative power. Decision curve analysis was performed to evaluate the nomogram's clinical utility across different threshold probabilities and to assess net benefit. The diagnostic efficacy of the clinical factor model was assessed using the Receiver Operating Characteristic (ROC) curve (AUC) from the ROC toolbox (21).

## Statistical analysis

The statistical program SPSS version 26.0 was used to analyze the data. Normality of continuous variables was tested using the Shapiro-Wilk test. Data normally distributed are presented as mean ± standard deviation (SD); comparisons between groups for normally distributed data were conducted using independent-sample t-tests. Data not normally distributed are presented as median (Q1, Q3); comparisons between groups for non-normally distributed data were conducted using nonparametric tests. Categorical data are presented as counts (n) and percentages (%). Each variable underwent univariate analysis. Variables with a P-value < 0.1 from univariate analysis were included as candidates for multivariate logistic regression analysis. The threshold for statistical significance was set at $P < 0.05$. Subgroup analyses were conducted to investigate the variability of treatment effects and influencing variables among different patient groups. ROC curve analyses were performed comparing the endpoint CDAI results with the aforementioned subgroup factors. A P-value < 0.05 in the final multivariate model was considered significant.

# Results

## Baseline characteristic

Ten of the 185 patients we recruited were disqualified because there was insufficient information on vitamin D insufficiency, as Table 1 illustrates. Furthermore, the absence of follow-up data led to the exclusion of 17 individuals. Thus, there were 158 CD patients hospitalized in all (Supplementary Figure 1). The patients were 36.95 ± 3.65 years old on average. There are seventy-five ladies. There were 87 patients with prior surgery and 45 smokers, with a BMI score of 22.15 ± 2.48.

Second, we divided the groups based on vitamin D levels, and we discovered that 29 and 103 individuals, respectively, were in the vitamin D deficient group. Diarrhea and stomach discomfort were the primary clinical signs. Age, sex, ethnicity, disease course, history of prior surgery, perianal disease, intestinal length resection, vitamin B12 or zinc levels, history of anti-TNF use, and the site and behavior of the disease (inflammation, stricture, or penetration) did not differ among the three groups.

The highest tritile of vitamin D had the greatest percentage of patients using supplements ($p = 0.02$). We were unable to statistically examine dosage associations since most patients were using over-the-counter vitamin D pills. The usage of biologics and postoperative immunomodulators did not significantly vary across groups.

## Change from baseline data for clinical remission

The CD patients were separated into two groups, one for clinical remission (100 instances) and the other for clinical non-remission (58 cases), based on their response status after IFX therapy (CDO score <150), as shown in Table 2. We discovered that the non-remission group's levels of vitamin D, BUN, and Cr were significantly lower than those of the remission group. Second, we discovered that compared to the remission group, the non-remission group had noticeably greater levels of serum ferroglobin, ATI, HS-CRP, ESR, and CRP. Hemoglobin, white blood cell, and liver function indices did not change significantly.

## Univariate and multivariate analyses influenced the assessment of risk factors

As indicated in Table 3, univariate analysis revealed a strong correlation between poor clinical remission following IFX treatment and age, vitamin D3 levels significantly below 30 ng, and vitamin D supplementation. Subsequent multivariate analysis revealed a strong correlation between poor clinical remission following IFX treatment and age and vitamin D deficiency.

A ROC curve analysis was conducted between the endpoint CDAI results and the aforementioned subgroup factors. AUC (95% CI) 0.56(0.25-0.95) was the endpoint CDAI (= 150) diagnostic value in the male subgroup when the Vit-D level was 19.35 ng/ml. The

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TABLE 1 Baseline patient characteristics.

| Characteristic | All, N=158 | Serum vitamin D | | | |
|---|---|---|---|---|---|
| | | <15 ng/mL, N = 29 (18.35%) | 15–30 ng/mL, N = 103(65.19%) | >30 ng/mL, N = 26 (16.46%) | p value |
| Age, years (mean ± SD) | 36.95 ± 3.65 | 32.48 ± 4.95 | 37.15 ± 6.25 | 35.66 ± 3.91 | 0.28 |
| Gender, female, n (%) | 75 | 15 | 55 | 5 | 0.44 |
| Disease duration, year(mean ± SD) | 8.29 ± 1.62 | 5.61 ± 0.95 | 9.86 ± 2.61 | 9.34 ± 1.05 | 0.39 |
| **Disease location, n (%)** | | | | | |
| Ileum | 45 | 10 | 28 | 7 | |
| Colon | 30 | 2 | 25 | 3 | |
| Ileum-colon | 61 | 10 | 38 | 13 | |
| Upper GI | 22 | 7 | 12 | 3 | 0.05 |
| **Disease behavior, n (%)** | | | | | |
| Inflammatory | 36 | 12 | 20 | 4 | |
| Stricturing | 48 | 9 | 30 | 9 | |
| Penetrating | 74 | 8 | 53 | 13 | 0.58 |
| Perianal disease, n (%) | 45 | 9 | 31 | 5 | 0.55 |
| Current smoker, n (%) | 28 | 11 | 14 | 3 | 0.15 |
| Previous use of anti-TNF-α agents, n (%) | 24 | 7 | 10 | 7 | 0.25 |
| Naïve to biologics, n (%) | 31 | 9 | 12 | 10 | 0.68 |
| History of surgery, n (%) | 87 | 12 | 62 | 13 | 0.24 |
| Previous surgery, median (IQR), n | 3 | 1 | 1 | 1 | 0.09 |
| **Type of surgery, n (%)** | | | | | |
| Small bowel resection | 45 | 12 | 29 | 4 | |
| Colon resection | 63 | 12 | 41 | 10 | |
| Ileocecectomy or ileocolectomy | 50 | 5 | 33 | 12 | 0.24 |
| **Postoperative medication, n (%)** | | | | | |
| No medication | 42 | 6 | 30 | 6 | 0.25 |
| Metronidazole | 27 | 3 | 20 | 4 | 0.11 |
| Steroid | 28 | 5 | 21 | 2 | 0.24 |
| Immunomodulators | 75 | 24 | 38 | 13 | 0.62 |
| **Biologic use, n (%)** | | | | | |
| No biologic | 71 | 14 | 50 | 7 | |
| Anti-TNF-α agents | 52 | 8 | 30 | 14 | |
| Vedolizumab | 35 | 7 | 23 | 5 | 0.11 |
| Vitamin D supplement, n (%) | 62 | 7 | 49 | 6 | 0.01 |
| Vitamin B12, pg/mL (IQR) | 561.25 ± 12.62 | 385.48 ± 15.94 | 482.61 ± 21.58 | 594.55 ± 21.05 | 0.2 |
| Zinc, μg/mL (IQR) | 0.78 ± 0.08 | 0.61 ± 0.08 | 0.67 ± 0.11 | 0.72 ± 0.03 | 0.16 |

GI, gastrointestinal tract; IQR, interquartile-range.

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TABLE 2 Analysis of differences in clinical factors between the two groups.

| Analyte | Remission group (n = 100) | Non-remission group (n = 58) | OR | 95% CI | P |
|---|---|---|---|---|---|
| Vit-D | 20.98 ± 6.93 | 18.23 ± 6.99 | 0.89 | 0.32-0.89 | 0.012 |
| CRP | 12.34 ± 3.21 | 78.20 ± 9.32 | 1.23 | 0.55-1.83 | 0.011 |
| ESR | 18.22 ± 2.34 | 46.22 ± 5.21 | 1.2 | 0.99-1.92 | 0.032 |
| Hs-CRP | 28.93 ± 5.93 | 72.92 ± 6.11 | 2.34 | 1.42-3.23 | 0.016 |
| WBC | 6.03 ± 1.02 | 8.93 ± 1.42 | 1.11 | 0.63-1.63 | 0.52 |
| Hb | 129.52 ± 21.84 | 89.25 ± 25.31 | 2.93 | 1.23-4.22 | 0.113 |
| ALB | 42.61 ± 3.68 | 34.69 ± 4.69 | 1.05 | 0.68-1.58 | 0.512 |
| AST | 14.78 ± 5.62 | 12.34 ± 2.11 | 0.98 | 0.42-1.56 | 0.582 |
| ALT | 11.62 ± 1.36 | 8.26 ± 1.26 | 0.68 | 0.31-1.11 | 0.625 |
| ALP | 75.64 ± 6.35 | 74.95 ± 6.89 | 6.84 | 2.15-9.65 | 0.381 |
| TBil | 9.35 ± 0.95 | 6.42 ± 0.69 | 1.05 | 0.34-1.66 | 0.582 |
| BUN | 4.39 ± 0.95 | 3.05 ± 0.65 | 0.86 | 0.44-1.25 | 0.025 |
| Cr | 68.27 ± 12.65 | 60.11 ± 11.02 | 0.68 | 0.42-0.99 | 0.045 |
| Uric Acid | 352.61 ± 85.64 | 301.08 ± 78.95 | 1.36 | 0.89-1.99 | 0.485 |
| VB12 | 443.95 ± 25.61 | 482.61 ± 23.68 | 1.68 | 0.89-1.99 | 0.586 |
| IFX-TC | 4.25 ± 0.86 | 2.03 ± 0.98 | 0.56 | 0.14-0.99 | 0.066 |
| ATI, n (%) | 8 | 14 | 1.02 | 0.85-1.68 | 0.002 |
| Blood glucose | 4.36 ± 0.95 | 4.29 ± 0.68 | 1.25 | 0.69-1.99 | 0.625 |
| Serum Ferritin | 78.69 ± 10.25 | 199.25 ± 19.64 | 0.89 | 0.48-1.52 | 0.032 |

vit-d, Vitamin D; CRP,C-reactive protein; ESR, Erythrocyte sedimentation rate; Hs-CRP, Highly sensitive C-reactive protein; WBC, White blood cell; Hb: hemoprotein; ALB, albumin; AST, Aspartate aminotransferase; ALT, glutamic-pyruvic transaminase; ALP, alkaline phosphatase; TBil, Total Bilirubin; BUN, Urea nitrogen; Cr, creatinine; VB12, Vitamin B12; IFX-TC, infliximab trough concentration, ATI, anti-TNFα antibody; OR, odds ratio.

sensitivity and specificity were 0.75 and 0.79 (P = 0.015), respectively, suggesting a greater impact on normal-weight persons. The endpoint CDAI (= 150) diagnostic value was AUC (95%CI) 0.78 (0.68-0.89) in the normal BMI subgroup when the Vit-D level was 16.25 ng/ml. The sensitivity and specificity were 0.68 and 0.86 (P = 0.032), respectively, suggesting a greater impact on normal-weight people. When the Vit-D levels were 19.45 ng/ml, the diagnostic value of the endpoint CDAI (= 150) in the smoke-free subgroup was AUC (95% CI) 0.77 (0.61-0.85), the sensitivity was 0.85, and the specificity was 0.88 (P = 0.024). Consequently, vitamin D supplementation may be especially beneficial for this subgroup in the treatment of Crohn's disease (Table 4).

TABLE 3 Factors assessed with univariate and multivariate analysis.

| Factors | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | p value | OR | 95% CI | p value |
| Age (>35) | 2.68 | 0.85-6.25 | 0.01 | 1.18 | 0.32-2.61 | 0.03 |
| Disease vitamin D triplet (>6 years) | 1.22 | 0.25-3.69 | 0.28 | 2.68 | 0.96-3.62 | 0.09 |
| Female | 2.95 | 0.24-6.35 | 0.28 | 1.65 | 0.88-2.68 | 0.06 |
| Perianal disease | 0.35 | 0.02-4.62 | 0.95 | 2.69 | 1.25-3.66 | 0.11 |
| Anti-TNF naïve | 0.58 | 0.24-5.62 | 0.21 | 1.69 | 0.36-2.68 | 0.36 |
| Vitamin D <30 ng/mL | 0.36 | 0.08-1.25 | 0.02 | 0.89 | 0.42-2.26 | 0.03 |
| Vitamin D supplement | 0.22 | 0.18-0.95 | 0.09 | 2.69 | 0.98-4.85 | 0.12 |
| Vitamin B12 >240 pg/mL | 1.25 | 0.65-3.69 | 0.55 | 0.89 | 0.21-1.25 | 0.09 |
| Zinc >0.68 μg/mL | 1.69 | 0.98-2.26 | 0.11 | 2.61 | 1.25-4.58 | 0.16 |

OR, odds ratio.

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TABLE 4 Diagnostic efficiency of different subgroups for endpoint CDAI outcomes by ROC curve.

| Subgroup | Diagnostic value (Vit-D baseline level ng/ml) | AUC(95%CI) | Sensitivity | Specificity | P |
|---|---|---|---|---|---|
| Male | 19.35 | 0.56(0.25-0.95) | 0.75 | 0.79 | 0.015 |
| BMI: normal weight | 16.25 | 0.78(0.68-0.89) | 0.68 | 0.86 | 0.032 |
| No smoking | 19.45 | 0.77(0.61-0.85) | 0.85 | 0.88 | 0.024 |
| Disease duration (>6 years) | 18.22 | 0.78(0.65-0.85) | 0.78 | 0.77 | 0.158 |
| without Perianal disease | 16.35 | 0.77(0.58-0.85) | 0.85 | 0.81 | 0.248 |
| Without intestinal surgery | 15.64 | 0.84(0.55-0.95) | 0.79 | 0.62 | 0.258 |

CDAI, Crohn's Disease Activity Index; ROC, receiver operating characteristic; BMI, Body Mass Index; AUC, Area under the curve.

## LASSO regression and nomogram validation

Multiple linear regression models demonstrated a connection between Crohn's disease and vitamin D, as seen in Figure 1. In multivariate logistic regression models, Crohn's disease was also independently predicted by male, age, BMI, and vitamin D levels <30 ng/ml (Table 3). Fifteen variables were narrowed down to five potential predictors with nonzero coefficients in the LASSO regression model based on the 158 patients in the cohort (4:1 ratio). These included being male, being older, having a BMI, and having vitamin D levels below 30 ng/ml. The model that included the previously described independent predictors was used to produce the nomogram. The nonadherence risk nomogram's calibration curve for risk prediction in Crohn's disease, meantime, showed high consistency in this population. The model demonstrated high discrimination, as shown by the C-index for the cohort's prediction nomogram, which was 0.982 (95 percent CI, 0.25–1.03) and was confirmed to be 0.925 after bootstrapping validation. The decision curve analysis for the vit-D-linked indicators nomogram is shown in Figure 1B, and the area under the curve (AUC) was 0.968, according to Figure 1C. In summary, a vitamin D deficit might be a useful predictor of Crohn's disease outcome.

# Discussion

Human health is significantly impacted by the high frequency and poor prognostic outcomes of CD patients (22, 23). Anti-TNF treatment for CD patients, however, has limited remission impact, making improving the treatment's remission rate a crucial area of study (24, 25). Furthermore, prevalent vitamin D deficiency is observed in CD patients, with over 50% affected. Comparisons of baseline data revealed that the non-remission group had a significantly higher rate of vitamin D deficiency. Subsequent studies utilizing univariate, multivariate, and LASSO regression models further identified vitamin D insufficiency as a significant risk factor for CD patients receiving IFX intervention.

In 2006, Robert Modlin's group demonstrated that stimulating macrophages through the pattern recognition receptor toll-like receptor 2 (TLR2) results in the expression of CYP27B1, leading to endogenous production of 25-hydroxyvitamin D (25(OH)D) (26, 27). T This finding is considered a key mechanism supporting the role of 1,25-dihydroxyvitamin D (1,25D) signaling in the innate immune response. For many years, it has been recognized that vitamin D deficiency is associated with CD.

Schäffler et al. found that CD patients benefited from therapy with tumor necrosis factor-α inhibitor and exhibited significantly higher vitamin D levels than those without (28). Shema Ayadi et al. also found that Vitamin D deficiency is common in Tunisian CD patients as well as in controls and is associated with disease activity. This association is often attributed to either intestinal malabsorption of dietary vitamin D or insufficient sun exposure during active illness, particularly in high-latitude populations (1). Nonetheless, evidence from the aforementioned laboratory research strongly suggests that vitamin D insufficiency may play a role in the pathophysiology of CD, and that sufficient vitamin D supplementation may improve innate immunity, reduce inflammation, and alleviate CD symptoms. Numerous clinical trials conducted since 2010 have shown the potential therapeutic effects of potent vitamin D supplementation for CD patients. For example, a 2011 clinical study reported that women with anticipated 25(OH)D levels of >30 ng/mL (75 nM) had a multivariate-adjusted hazard ratio of 0.38 (95% CI, 0.15–0.97) compared to women with expected levels of <20 ng/mL (50 nM), suggesting that higher circulating 25(OH)D levels significantly lower the incidence of CD in this female population (29).

Vitamin D insufficiency is prevalent in the CD patient population and is independently linked to worse health-related quality of life (HRQOL) and increased disease activity, according to a retrospective cohort analysis of 403 CD patients (30). Furthermore, a retrospective analysis of pediatric populations revealed similar findings; 47% of children had vitamin D deficiency or insufficiency, and in this group, vitamin D deficiency was linked to higher exposure to corticosteroids (31, 32). Serum vitamin D levels were found to be substantially lower in children with CD compared to healthy controls, and the group of

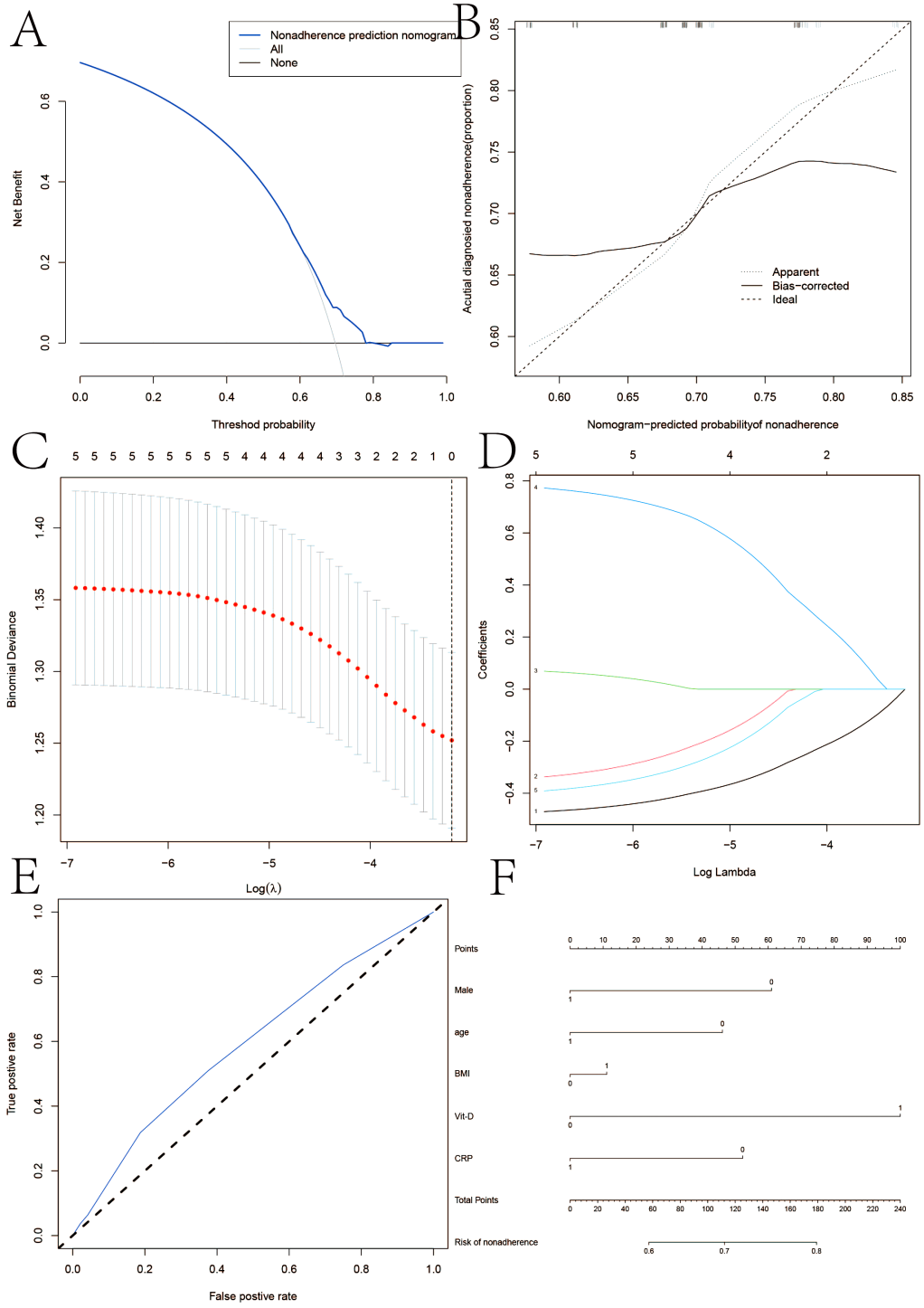


FIGURE 1
**(A)** Calibration curves of the nonadherence nomogram prediction in the cohort. **(B, C)** Decision curve analysis for the nonadherence nomogram and AUC curve. **(D)** LASSO coefficient profiles of the 20 characteristics. A coefficient profile plot was constructed against the log(lambda) sequence. **(E, F)** ertical line was drawn at the value determined using fivefold cross-validation, where optimum lambda resulted in five features with nonzero coefficients in iron homeostasis in SSNHL patients.

children recently diagnosed with IBD also exhibited high rates of vitamin D insufficiency or deficiency (31, 32). A 25(OH)D level of <20 ng/mL (50 nM) was linked to a statistically significant increase in the risk of surgery and hospitalizations due to IBD compared to individuals with levels >30 ng/mL, according to a multivariate study of over 1500 CD patients (33). In clinical research, many studies have reported the relationship between 25(OH)D levels and clinical outcomes in CD patients, as well as the effect of biologic agents (34). The lack or insufficiency of 25(OH)D is more common in CD patients. Furthermore, patients with low 25(OH)D levels are less

likely to achieve clinical remission and a poorer response to biologic agents (35, 36). In a small, double-blind, randomized, placebo-controlled trial involving 27 CD patients in remission, those assigned to vitamin D (2000 IU/d) achieved a significantly higher 25(OH)D level (at least 75 nmol/L) compared to the placebo group, although the Crohn's Disease Activity Index (CDAI) scores did not significantly decrease (37).

Only one study in western China examined the therapeutic effects of vitamin D combined with IFX. This study found that vitamin D levels in the subgroup of patients with a normal BMI, non-smoking status, and receiving immunosuppressant therapy had independent predictive value for the endpoint CDAI score ($P < 0.05$). This represents one of the relatively few studies on this specific topic. Following IFX medication, baseline vitamin D levels in CD patients—particularly those with a normal BMI, who do not smoke, and who receive IFX in addition to immunosuppressants—predict clinical remission (15). However, this research focused on the examination of vitamin D levels and IFX-induced remission in CD patients within an eastern region, likely due to significant differences in latitude and altitude between the eastern and western parts of China.

Potential underlying mechanisms linking the effectiveness of IFX and vitamin D therapy may include inhibiting VDR expression and function, increasing downstream inflammatory signaling, and enhancing the production of inflammatory cytokines (38). Some research suggests that intestinal epithelial integrity depends on Vitamin D and that these cells are closely linked to VDR expression. In experimental colitis models using VDR knockout (VDR-/-) mice, animals exhibit increased vulnerability to epithelial damage, typically characterized by disruption of epithelial integrity and loss of tight junctions, which consequently increases susceptibility to bacterial translocation (39–41).

Clinical investigations frequently demonstrate strong correlations among Vitamin D levels, clinical outcomes in CD patients, and the effects of biologics (34). Vitamin D deficiency or insufficiency is more prevalent in CD patients. Furthermore, patients with low Vitamin D levels often experience poor responses to biologics and have lower rates of clinical remission. In individuals with CD, Vitamin D status may even serve as a predictor of the long-term effectiveness of biologics, and supplementing these patients with Vitamin D can enhance biologic therapy (35, 36). Some research also indicates that individuals with Vitamin D insufficiency are more likely to discontinue IFX treatment due to poor response or the early emergence of anti-drug antibodies. Moreover, Vitamin D levels show a positive correlation with the duration of response to anti-TNF-α therapy (42).

### Limitations

A single-center retrospective research with a limited sample size is one of the study's shortcomings. Another is that prospective intervention studies are required to further establish the causal association between vitamin D and clinical remission. We tried to use statistical methods to screen out the effect of each variable on the dependent variable after univariate analysis, taking into account several potentially significant variables. This was done to analyze the influencing factors more thoroughly and more in line with the actual clinical situation, given the bias of single-center, retrospective, and small-sample studies. To minimize bias from baseline values, we also used subgroup analyses at the same time. Second, information on vitamin D, calcium, and food consumption from sun exposure is lacking. Third, HPLC-MS produced more accurate findings than radioimmunoassay when it came to detecting VIT-D levels. However, only immunoassays may be carried out in our hospital laboratory.

## Conclusion

This research examined the association between vitamin D levels and CD patients in eastern China after IFX intervention. The findings showed a strong correlation between low vitamin D levels and the poor remission rate of CD patients following IFX intervention.

## 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.

## Author contributions

YP: Conceptualization, Investigation, Methodology, Software, Supervision, Writing – original draft. JZ: Data curation, Methodology, Supervision, Writing – original draft. HG: Methodology, Project administration, Supervision, Validation, Writing – review & editing. MS: Data curation, Methodology, Supervision, Conceptualization, Formal analysis, Project administration, Writing - original draft, Writing - review & editing. YZ: Formal analysis, Project administration, Validation, Writing – review & editing. HS: Funding acquisition, Resources, Visualization, Writing – review & editing.

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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.

## Correction note

A correction has been made to this article. Details can be found at: 10.3389/fimmu.2025.1651209.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1578191/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Flow chart.

## References

1. White JH. Vitamin D deficiency and the pathogenesis of Crohn's disease. *J Steroid Biochem Mol Bio*. (2018) 175:23–8. doi: 10.1016/j.jsbmb.2016.12.015

2. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterolog*. (2012) 142:46–54.e42;quiz e30. doi: 10.1053/j.gastro.2011.10.001

3. Vernia F, Valvano M, Longo S, Cesaro N, Viscido A, Latella G. Vitamin D in inflammatory bowel diseases. Mechanisms of action and therapeutic implications. *Nutrient*. (2022) 14(2):269. doi: 10.3390/nu14020269

4. Murdaca G, Tonacci A, Negrini S, Greco M, Borro M, Puppo F, et al. Emerging role of vitamin D in autoimmune diseases: An update on evidence and therapeutic implications. *Autoimmun R*. (2019) 18:102350. doi: 10.1016/j.autrev.2019.102350

5. Ghaly S, Lawrance I. The role of vitamin D in gastrointestinal inflammation. *Expert Rev gastroenterology hepatology*. (2014) 8:909–23. doi: 10.1586/17474124.2014.925796

6. Qiu Y, Chen BL, Mao R, Zhang SH, He Y, Zeng ZR, et al. Systematic review with meta-analysis: loss of response and requirement of anti-TNFα dose intensification in Crohn's disease. *J gastroenterology*. (2017) 52:535–54. doi: 10.1007/s00535-017-1324-3

7. Bendix M, Dige A, Jørgensen SP, et al. Seven weeks of high-dose vitamin D treatment reduces the need for infliximab dose-escalation and decreases inflammatory markers in crohn's disease during one-year follow-up. *Nutrients*. (2021) 13(4):1083. doi: 10.3390/nu13041083

8. Athanassiou L, Kostoglou-Athanassiou I, Koutsilieris M, Shoenfeld Y. Vitamin D and autoimmune rheumatic diseases. *Biomolecules*. (2023) 15(4):259. doi: 10.3390/biom13040709

9. Durá-Travé T, Gallinas-Victoriano F. Autoimmune thyroiditis and vitamin D. *Int J Mol Sc*. (2024) 25(6):3154. doi: 10.3390/ijms25063154

10. Giampazolias E, Pereira da Costa M, Lam KC, Lim KHJ, Cardoso A, Piot C, et al. Vitamin D regulates microbiome-dependent cancer immunity. *Sci (New York NY)*. (2024) 384:428–37. doi: 10.1126/science.adh7954

11. Basson A. Vitamin D. and Crohn's disease in the adult patient: a review. *JPEN J parenteral enteral nutrition*. (2014) 38:438–58. doi: 10.1177/0148607113506013

12. Palmese F, Del Toro R, Di Marzio G, Cataleta P, Sama MG, Domenicali M. Sarcopenia and vitamin D deficiency in patients with crohn's disease: pathological conditions that should be linked together. *Nutrients*. (2021) 13(4):1378. doi: 10.3390/nu13041378

13. Aggeletopoulou I, Marangos M, Assimakopoulos SF, Mouzaki A, Thomopoulos K, Triantos C. Vitamin D and microbiome: molecular interaction in inflammatory bowel disease pathogenesis. *Am J pathology*. (2023) 193:656–68. doi: 10.1016/j.ajpath.2023.02.004

14. Raftery AL, Tsantikos E, Harris NL, Hibbs ML. Links between inflammatory bowel disease and chronic obstructive pulmonary disease. *Front Immuno*. (2020) 11:2144. doi: 10.3389/fimmu.2020.02144

15. Song X, Zhang H, Song J, Wang H, Guo H, Zhou X. Evaluating the predictive effect of vitamin D on clinical outcomes of infliximab-treated Crohn's disease patients in western China. *Clin Exp medicine*. (2024) 24:237. doi: 10.1007/s10238-024-01483-0

16. Chinese consensus on diagnosis and treatment in inflammatory bowel disease (2018, Beijing). *J digestive Di*. (2021) 22:298–317. doi: 10.1111/1751-2980.12994

17. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Meta*. (2011) 96:1911–30. doi: 10.1210/jc.2011-0385

18. Lőrinczy K, Lakatos PL, Tóth M, Salamon Á, Nemes A, Csontos ÁA, et al. Vitamin D level in Hungarian patients with inflammatory bowel diseases. *Orvosi hetilap*. (2013) 154:1821–8. doi: 10.1556/oh.2013.29750

19. van Hoeve K, Dreesen E, Hoffman I, Van Assche G, Ferrante M, Gils A, et al. Higher infliximab trough levels are associated with better outcome in paediatric patients with inflammatory bowel disease. *J Crohn's colitis*. (2018) 12:1316–25. doi: 10.1093/ecco-jcc/jjy111

20. Best WR, Becktel JM, Singleton JW, Kern FJr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology*. (1976) 70:439–44. doi: 10.1016/S0016-5085(76)80163-1

21. Guan Z, Jin X, Guan Z, Liu S, Tao K, Luo L. The gut microbiota metabolite capsiate regulate SLC2A1 expression by targeting HIF-1α to inhibit knee osteoarthritis-induced ferroptosis. *Aging Cel*. (2023) 22:e13807. doi: 10.1111/acel.13807

22. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet (London England)*. (2017) 389:1741–55. doi: 10.1016/s0140-6736(16)31711-1

23. Veauthier B, Hornecker JR. Crohn's disease: diagnosis and management. *Am Family physician*. (2018) 98:661–9.

24. Ballester Ferré MP, Boscá-Watts MM, Mínguez Pérez M. Crohn's disease. *Medicina clinica*. (2018) 151:26–33. doi: 10.1016/j.medcli.2017.10.036

25. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet (London England)*. (2012) 380:1590–605. doi: 10.1016/s0140-6736(12)60026-9

26. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Sci (New York NY)*. (2006) 311:1770–3. doi: 10.1126/science.1123933

27. Liu PT, Krutzik SR, Modlin RL. Therapeutic implications of the TLR and VDR partnership. *Trends Mol medicine*. (2007) 13:117–24. doi: 10.1016/j.molmed.2007.01.006

28. Schäffler H, Schmidt M, Huth A, Reiner J, Glass Ä, Lamprecht G. Clinical factors are associated with vitamin D levels in IBD patients: A retrospective analysis. *J digestive diseases*. (2018) 19:24–32. doi: 10.1111/1751-2980.12565

29. Ananthakrishnan AN, Khalili H, Higuchi LM, White JH, et al. Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. *Gastroenterology*. (2012) 142:482–9. doi: 10.1053/j.gastro.2011.11.040

30. Ananthakrishnan AN, Khalili H, Higuchi LM, Bao Y, Korzenik JR, Giovannucci EL, et al. Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life. *JPEN J parenteral enteral nutrition*. (2011) 35:308–16. doi: 10.1177/0148607110381267

31. El Amrousy D, El Ashry H, Hodeib H, Hassan S. Vitamin D in children with inflammatory bowel disease: A randomized controlled clinical trial. *J Clin gastroenterology*. (2021) 55:815–20. doi: 10.1097/mcg.0000000000001443

32. Rigterink T, Appleton L, Day AS. Vitamin D therapy in children with inflammatory bowel disease: A systematic review. *World J Clin pediatrics*. (2019) 8:1–14. doi: 10.5409/wjcp.v8.i1.1

33. Ananthakrishnan AN, Cheng SC, Cai T, Rigterink T, Appleton L, Day AS, et al. Association between reduced plasma 25-hydroxy vitamin D and increased risk of cancer in patients with inflammatory bowel diseases. *Clin gastroenterology hepatology: Off Clin Pract J Am Gastroenterological Association*. (2014) 12:821–7. doi: 10.1016/j.cgh.2013.10.011

34. Mechie NC, Mavropoulou E, Ellenrieder V, Kunsch S, Cameron S, Amanzada A. Distinct association of serum vitamin D concentration with disease activity and trough levels of infliximab and adalimumab during inflammatory bowel disease treatment. *Digestion*. (2020) 101:761–70. doi: 10.1159/000502515

35. Mechie NC, Mavropoulou E, Ellenrieder V, Kunsch S, Cameron S, Amanzada A, et al. Influence of vitamin D3 supplementation on infliximab effectiveness in chinese patients with crohn's disease: A retrospective cohort study. *Front nutrition*. (2021) 8:739285. doi: 10.3389/fnut.2021.739285

36. Song X, Zhang H, Wang H, Li Z, Zhou X, Guo H. Correlation between treatment outcomes and serum vitamin D levels as well as infliximab trough concentration among chinese patients with crohn's disease. *Gastroenterology Res practice*. (2023) 2023:6675401. doi: 10.1155/2023/6675401

37. Song X, Zhang H, Wang H, Li Z, Zhou X, Guo H, et al. Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: Results from a randomised double-blind placebo-controlled study. *United Eur gastroenterology*. (2015) 3:294–302. doi: 10.1177/2050640615572176

38. Raftery T, Martineau AR, Greiller CL, Ghosh S, McNamara D, Bennett K, et al. Pleiotropic effects of vitamin D in patients with inflammatory bowel diseases. *J Clin medicine*. (2022) 3(3):294–302. doi: 10.3390/jcm11195715

39. Wu S, Yoon S, Zhang YG, et al. Vitamin D receptor pathway is required for probiotic protection in colitis. *Am J Physiol Gastrointestinal liver Physio*. (2015) 309: G341–9. doi: 10.1152/ajpgi.00105.2015

40. Wu S, Zhang YG, Lu R, et al. Intestinal epithelial vitamin D receptor deletion leads to defective autophagy in colitis. *Gut*. (2015) 64:1082–94. doi: 10.1136/gutjnl-2014-307436

41. Abdo J, Rai V, Agrawal DK. Interplay of immunity and vitamin D: interactions and implications with current IBD therapy. *Curr medicinal Che*. (2017) 24:852–67. doi: 10.2174/0929867323666161026124951

42. Kabbani TA, Koutroubakis IE, Schoen RE, et al. Association of vitamin D level with clinical status in inflammatory bowel disease: A 5-year longitudinal study. *Am J gastroenterology*. (2016) 111:712–9. doi: 10.1038/ajg.2016.53

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# Effect of vitamin D supplementation on COVID-19 outcomes: an umbrella review of systematic reviews

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**Background:** Vitamin D is suggested as a supportive therapy to reduce the severity of COVID-19 due to its immunomodulatory and anti-inflammatory effects. However, its effect on critical outcomes, such as ICU admissions and mortality, shows significant variation across randomized clinical trials and meta-analyses.

**Objectives:** To summarize the influence of vitamin D supplementation on ICU admissions and mortality among COVID-19 patients.

**Methods:** Overall, 21 eligible studies were retrieved using a comprehensive search from Scopus, PubMed, and Web of Science. A citation matrix was developed, revealing a Corrected Covered Area (CCA) of 0.54, indicating moderate overlap. Fixed-effects models were applied to data with low heterogeneity (ICU admissions: Q = 10.87, $p$ = 0.33), while random-effects models were used for mortality outcomes (Q = 27.23, $p$ = 0.006). Pooled odds ratios (OR) with 95% confidence intervals (CI) quantified the overall effects.

**Results:** Vitamin D supplementation was associated with a significant 38% reduction in ICU admissions (OR = 0.62; 95% CI: 0.54–0.71) and a 33% reduction in mortality risk (OR = 0.67; 95% CI: 0.56–0.79). The benefit was pronounced in vitamin D-deficient populations, although heterogeneity in mortality outcomes highlighted variability across studies.

**Conclusion:** While these findings suggest that vitamin D supplementation may help reduce ICU admissions and mortality among COVID-19 patients—particularly in those with vitamin D deficiency—the results should be interpreted with caution. The observed variability and potential confounding factors underscore the need for further large-scale, randomized controlled trials with standardized dosing protocols before definitive clinical recommendations can be made.

KEYWORDS

**COVID-19, vitamin D supplementation, mechanical ventilation, hospital stay, clinical outcomes, immune modulation, randomized controlled trials**

# 1 Introduction

The COVID-19 pandemic began in December 2019 in Wuhan, China, with the emergence of a novel coronavirus known as SARS-CoV-2. As declared by the World Health Organization (WHO), by March 2020, the virus had spread worldwide, reaching a critical global scale (1). This situation undoubtedly escalated into a public health emergency of international concern due to the virus's high contagiousness (2). Initially, scientists and healthcare professionals thought that COVID-19 mainly impacted the respiratory system, resulting in interstitial pneumonia and acute respiratory distress syndrome (3–5). However, subsequent research has shown that, in addition to respiratory complications, COVID-19 can lead to a broad spectrum of disorders affecting various organs, either directly or indirectly associated with the infection (6–8).

The symptoms of COVID-19 vary from asymptomatic and mild cases, which do not require special medical care, to moderate and severe cases that necessitate hospitalization, respiratory support, and even intensive care unit (ICU) treatment (9–11). Several risk factors are associated with the progression of the disease (12, 13). For example, elderly individuals are at the greatest risk of adverse outcomes and complications. Moreover, the likelihood of complications increases in patients with comorbidities such as cardiovascular diseases, diabetes, cancer, or obesity (14–18). Other studies have shown that factors like age, sex, race, obesity, diabetes, and hypertension play significant roles in triggering an uncontrolled release of cytokines, leading to disease exacerbation and an unbalanced immune response (19–24).

Since viral infections primarily spread through close social contact and large gatherings, nearly every country implemented social distancing measures to reduce the transmission of SARS-CoV-2 (25, 26). These measures aimed to limit the frequency of social interactions and increase physical distance between individuals, thereby decreasing the risk of human-to-human transmission (27). It has been demonstrated that staying at home for extended periods made people more prone to physical inactivity, unhealthy eating habits, and limited sunlight exposure, which could contribute to the development of vitamin D deficiency or insufficiency (28–30).

The active form of vitamin D [1,25(OH)2D3 — calcitriol] is a fat-soluble hormone that possesses numerous biological properties (endocrine, paracrine, and intracrine) in the human body. Its paracrine and intracrine functions have garnered significant interest, particularly due to the almost ubiquitous expression of the vitamin D receptor (VDR) by immune cells, highlighting its role in regulating acute and chronic inflammatory responses (31).

Several studies have demonstrated a correlation between vitamin D levels, age, oxygen therapy needs, and mortality (32–34). Daneshkhah et al. showed that COVID-19 mortality was highest in Italy, Spain, and France, European countries with the highest rates of severe vitamin D deficiency (35). Langlois et al. found that low vitamin D levels were associated with an increased frequency of infections, sepsis, and mortality (36). Other studies have established connections between inflammatory markers and disease progression. For instance, Ai-Ping Yang et al. demonstrated that white blood cell count, lymphocyte count, neutrophil count, C-reactive protein (CRP) levels, and ratios such as neutrophil-to-lymphocyte, lymphocyte-to-monocyte, and platelet-to-lymphocyte were statistically higher in patients with severe cases than in those with mild cases (37). Guohui et al. investigated novel serological markers in COVID-19 and, through multivariate logistic regression analysis, found that the CRP-to-albumin and CRP-to-prealbumin high-sensitivity ratios correlated with the risk of severe COVID-19 (38).

This systematic review and meta-analysis aimed to assess the effects of vitamin D supplementation on critical COVID-19 outcomes, including ICU admissions and mortality.

# 2 Materials and methods

## 2.1 Study design and data extraction

This meta-analysis was performed in order to determine the impact of vitamin D supplementation on severe cases of critical COVID-19 (i.e., ICU admission and mortality). Eligible studies included systematic reviews and meta-analyses investigating the association between vitamin D supplementation and COVID-19 severity.

A comprehensive literature search was performed on PubMed, Scopus, and Web of Science (WoS) on 20 December 2024. The following search strategies were used:

For PubMed, the search query used was: (Vitamin D OR Vit D) AND (COVID-19 OR SARS-CoV-2 OR COVID-2019 OR 2019-nCoV OR "2019 novel coronavirus infection" OR "coronavirus disease-19" OR "coronavirus disease 2019" OR "novel coronavirus") AND (systematic review[pt] OR meta-analysis[pt] OR "systematic review"[tiab] OR "meta analysis"[tiab] OR "systematic overview"[tiab]) AND (supplementation OR supplement[tiab]).

For Scopus, the search query was: TITLE-ABS-KEY("Vitamin D" OR "Vit D") AND TITLE-ABS-KEY(COVID-19 OR SARS-CoV-2 OR COVID-2019 OR 2019-nCoV OR "2019 novel coronavirus infection" OR "coronavirus disease-19" OR "coronavirus disease 2019" OR "novel coronavirus") AND TITLE-ABS-KEY ("systematic review" OR "meta analysis" OR "systematic overview") AND TITLE-ABS-KEY(supplementation OR supplement).

For Web of Science (WoS), the search query was: TS = ("Vitamin D" OR "Vit D") AND TS = (COVID-19 OR SARS-CoV-2 OR COVID-2019 OR 2019-nCoV OR "2019 novel coronavirus infection" OR "coronavirus disease-19" OR "coronavirus disease 2019" OR "novel coronavirus") AND TS = ("systematic review" OR "meta analysis" OR "systematic overview") AND TS = (supplementation OR supplement). Data extraction was performed independently by two reviewers using a standardized data collection form. Extracted data included the number of studies within each systematic review, the total sample size, primary outcomes (ICU admissions and mortality), and effect size measures such as odds ratios (OR) with corresponding 95% confidence intervals (CI). Any disagreements were resolved through discussion or by consulting a third reviewer.

## 2.2 Inclusion and exclusion criteria

For this umbrella review, only systematic reviews and meta-analyses that quantitatively synthesized the effect of vitamin D supplementation on COVID-19 outcomes were included. Eligible reviews evaluated individuals diagnosed with COVID-19, regardless of disease severity, and incorporated data from randomized controlled

trials (RCTs) as well as reviews that combined RCTs with observational studies. Studies had to report primary outcome data for ICU admissions and/or mortality; when available, baseline vitamin D status was also considered.

Reviews were excluded if they did not focus on vitamin D supplementation or if they lacked a quantitative synthesis (e.g., narrative reviews or single observational studies). Additionally, any reviews that did not report data on ICU admissions or mortality, or were published in languages other than English, were excluded. In cases where overlapping primary studies were present across multiple reviews, all eligible reviews were initially considered, with overlapping data noted and carefully managed during analysis.

The review by Rawat et al (82) was excluded from the sensitivity analysis because it had a limited sample size—incorporating only five studies—and exhibited methodological inconsistencies that produced effect sizes significantly different from those in other reviews. Including this review risked disproportionately skewing the overall pooled estimates. Sensitivity analyses confirmed that excluding (82). improved the consistency of the results without altering the overall direction of the effect.

## 2.3 Quality assessment of included reviews

To assess the quality of the included reviews, the AMSTAR 2 (A Measurement Tool to Assess Systematic Reviews) was used (39, 40). This tool assesses methodological rigor based on several criteria, including the comprehensiveness of the search strategy, clear inclusion criteria, robust data extraction methods, and an adequate assessment of risk of bias. Reviews were classified as "high," "moderate," or "low" quality based on these assessments.

## 2.4 Statistical analysis

Pooled odds ratios (OR) and 95% confidence intervals (CI) were computed for ICU admissions and mortality to determine the overall effect of vitamin D supplementation. For ICU admissions, heterogeneity was low ($Q = 10.87$, $p = 0.33$), so a fixed-effects model was applied using the inverse-variance method. In contrast, for mortality, significant heterogeneity ($Q = 27.23$, $p = 0.006$) warranted a random-effects model (DerSimonian and Laird) to account for between-study variability.

### 2.4.1 Heterogeneity assessment

Heterogeneity among the studies was evaluated using Cochran's Q-statistic and quantified by the $I^2$ statistic, which represents the percentage of total variation across studies attributable to heterogeneity rather than chance. An $I^2$ value of 25, 50, and 75% was interpreted as low, moderate, and high heterogeneity, respectively.

### 2.4.2 Meta-regression analysis

A meta-regression analysis was performed using the logarithm of the odds ratio (log_OR) as the dependent variable and the publication year as the independent variable. The weighted least squares (WLS) method was employed, where inverse variance weighting was used to account for differences in study precision. Bubble plots were generated to visually examine the relationship between study year and effect size. The size of each bubble represents the weight assigned to each study, reflecting the study's precision (inverse of variance).

### 2.4.3 Calculation of pooled estimates

For each outcome of interest, we calculated pooled odds ratios (ORs) using a weighted average of individual study estimates, with weights assigned based on the inverse of each study's variance. The tau-squared ($\tau^2$) statistic was used to quantify between-study variance, while statistical significance of the pooled ORs was assessed via Z-tests ($p < 0.05$ was considered significant).

To evaluate the stability of our results, we conducted a sensitivity analysis using a leave-one-out approach. In this procedure, each systematic review was removed one at a time, and the overall effect was recalculated. We compared these new pooled estimates, along with heterogeneity metrics (Q and $I^2$), to the original values to determine whether any single review had a disproportionate impact on the findings.

One review, conducted by Rawat et al. (82) and Meng et al. (21), was flagged as an outlier due to its small sample size and methodological inconsistencies. Its effect estimates deviated substantially from those of the other reviews. When Rawat et al. (82) was excluded, the recalculated ORs for ICU admission and mortality remained consistent, suggesting that our main results are robust and not unduly affected by individual studies.

### 2.4.4 Software and visualization

All statistical analyses were conducted using Python (v3.9) and relevant libraries (numpy, scipy, and matplotlib). Forest plots were generated to visually represent the pooled effect sizes and confidence intervals. The shaded area on the forest plot reflects the 95% confidence interval for the pooled OR, while vertical lines indicate the null effect (OR = 1).

# 3 Results

## 3.1 Study selection

The systematic search yielded a total of 402 records from Scopus ($n = 213$), PubMed ($n = 82$), and Web of Science ($n = 107$). After the removal of 213 duplicate records, 189 unique records proceeded to title and abstract screening. Following this stage, 65 records were excluded based on irrelevance to the research question or failure to meet the inclusion criteria. Full-text retrieval was attempted for the remaining 124 studies, with 10 records unavailable despite comprehensive efforts to obtain them.

A detailed eligibility assessment was conducted for 114 full-text articles, resulting in the exclusion of 93 studies for the following reasons: Ultimately, 21 studies met the predefined eligibility criteria and were included in the final systematic review and meta-analysis (Figure 1; Table 1).

## 3.2 Risk of bias

The risk of bias (RoB) across the included studies was evaluated using the AMSTAR 2 (A Measurement Tool to Assess Systematic

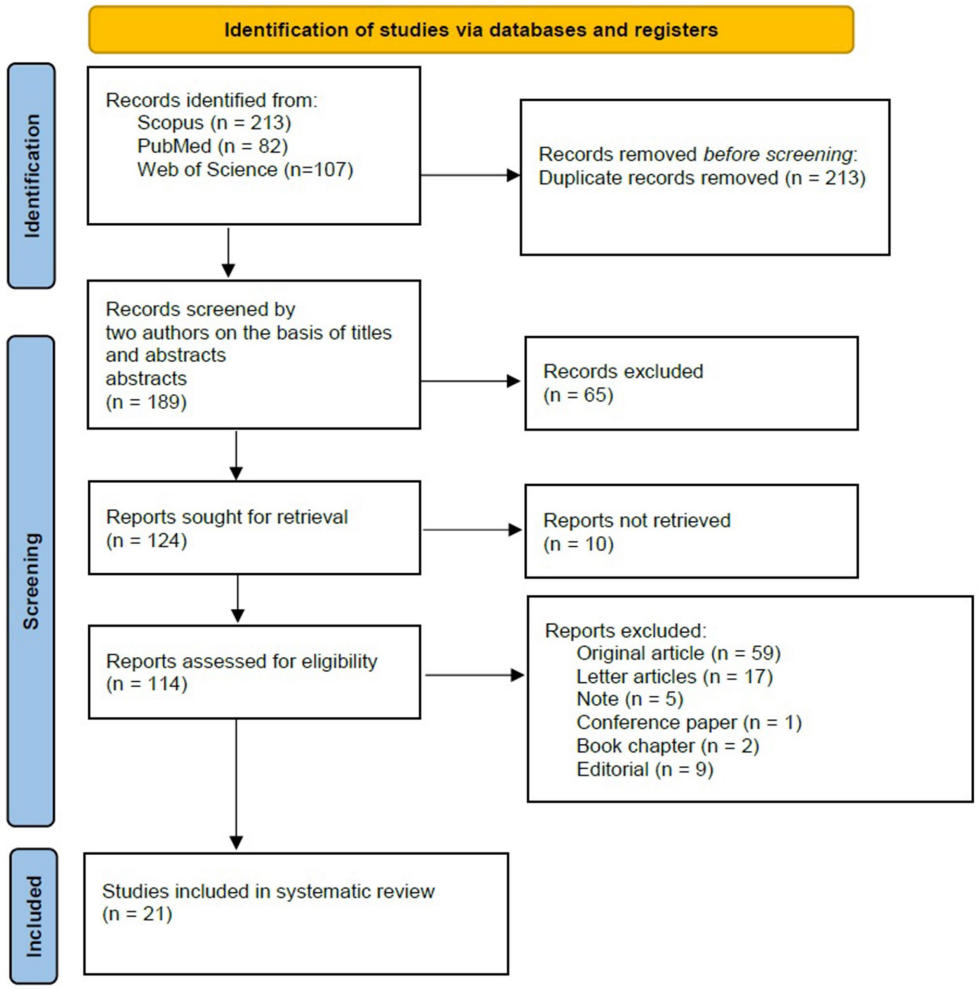


FIGURE 1
RISMA flow diagram for study selection. This flow diagram outlines the process of study identification, screening, eligibility assessment, and inclusion in the systematic review and meta-analysis. A total of 402 records were initially identified through database searches. After the removal of duplicates and irrelevant records, 21 studies met the inclusion criteria and were analyzed. Reasons for exclusion at each stage are detailed, including non-retrievable reports and studies that did not meet eligibility criteria.

Reviews) framework. The overall quality of the included studies ranged from moderate to high, with the majority demonstrating rigorous methodological approaches and adherence to best practices in systematic review conduct.

Among the 21 studies included in the umbrella analysis, 15 demonstrated a low risk of bias in at least 80% of the assessed areas, indicating the high quality of the systematic reviews included in the studies (Figure 2).

## 3.3 ICU admissions

This meta-analysis included data from 10 studies examining the effect of vitamin D on ICU admissions among COVID-19 patients. Due to low heterogeneity among the studies ($Q = 10.87$, $p = 0.33$), a fixed-effects model was applied. The pooled odds ratio (OR) for ICU admissions was calculated at 0.62 (95% CI: 0.54–0.71), suggesting that vitamin D supplementation is associated with a 38% reduction in the likelihood of ICU admission.

Odds ratios in the systematic reviews ranged from 0.33 to 0.80, with all but one study showing an OR below 1, indicating that vitamin D supplementation may reduce the rate of ICU admission (Figure 3).

## 3.4 Mortality

Fifteen studies were included in the analysis assessing the effect of vitamin D supplementation on mortality rates in COVID-19 patients. Unlike ICU admissions, the mortality data showed significant heterogeneity ($Q = 27.23$, $p = 0.006$). Therefore, a random-effects model was used. The pooled OR for mortality was 0.67 (95% CI: 0.56–0.79), indicating a 33% reduction in the risk of death associated with vitamin D supplementation.

The individual study ORs for mortality ranged from 0.19 to 1.13, with several studies demonstrating CIs crossing the null line, indicating non-significant results. For instance, Adil et al. (42) and Sirbu et al. (73) reported ORs of 0.91 (95% CI: 0.67–1.23) and 0.96

TABLE 1 Characteristics of included studies.

| References | Search strategy | No. of studies included | Total no. of participants | Dose regimen and period | Outcomes | Results |
|---|---|---|---|---|---|---|
| Sartini et al. (43) | PubMed/MEDLINE, Scopus, Cochrane, and Google Scholar | 29 | Not specified | Vitamin D dosages varied considerably, including daily, weekly, and monthly doses. Example regimens: 80,000 IU/day, 21,280 IU/day initial dose followed by 10,640 IU/day maintenance dose, or 200,000 IU/day. | Mortality, ICU admissions, intubation rates, and hospital length of stay (LOS). | Significant reduction in ICU admissions (OR = 0.55, 95% CI: 0.37–0.79), intubation rates (OR = 0.50, 95% CI: 0.27–0.92). Mortality reduction in analytical studies (OR = 0.45, 95% CI: 0.24–0.86). LOS showed non-significant reduction (−0.62 days). Subgroup effects in older and severe COVID-19 cases. |
| Zhang et al. (47) | PubMed, Embase, Web of Science, and Cochrane | 21 | 4,553 | Single-dose vs. continuous-dose; total intake within 14 days (≥100,000 IU vs. <100,000 IU). Baseline serum Vitamin D levels compared (deficient vs. non-restricted). | Mortality, ICU admissions, intubation rates, LOS. | Continuous dosing and lower doses (<100,000 IU) were more effective. Mortality and ICU admission rates were significantly reduced in the deficient group. No significant effect on LOS. |
| Adil et al. (42) | PubMed, Cochrane, CINAHL, EMBASE, and Google Scholar | 14 | 2,165 | Dosages ranged from 5,000 IU to 500,000 IU, administered daily for 1 day to 2 weeks. | ICU admissions, mechanical ventilation, mortality, hospital stay, oxygen requirement. | ICU admissions and need for mechanical ventilation reduced. No significant impact on mortality, hospital stay length, or oxygen requirement. |
| Ghoreshi et al. (53) | PubMed, Scopus, Web of Science, Embase, and Cochrane | 16 | The sample size in the evaluated studies ranged from 40 to 237 patients. | Dosage varied from 2000 to 500,000 IU. | Hospital LOS, CRP, ferritin, D-dimer, Hb, lymphocyte counts. | Reduced LOS (MD = −1.16; $p = 0.033$), especially with ≤10,000 IU doses. Significant CRP reduction in older adults. No significant changes in other inflammatory markers. |
| Yang et al. (50) | Cochrane Library, PubMed, Web of Science, and Embase | 19 | 2,345 participants | Single-dose vs. multiple-dose administration. | ICU admission, mechanical ventilation (MV), LOS, mortality, inflammatory markers. | Multiple-dose regimens showed greater reductions in ICU admissions (OR 0.39), MV (OR 0.18), and LOS. No significant mortality or inflammatory marker changes. |
| Sobczak and Pawliczak (48) | PubMed, Web of Science, Embase, and Cochrane Central Register of Controlled Trials | 13 | Not specified | Not specified. | ICU admissions, mortality. | ICU admissions reduced (RR 0.73; $p = 0.02$). Mortality significantly lowered (RR 0.56; $p = 0.02$). |
| Jamilian et al. (72) | Web of Science, PubMed, Scopus, and Embase | 13 | Not specified | Not specified. | Mortality, infection risk, disease severity. | Reduced mortality in intervention studies (ES = 0.42) and observational studies (ES = 1.99). Vitamin D deficiency increased infection risk and severity. |
| Meng et al. (49) | PubMed, Cochrane Library, Embase, Web of Science, and Google Scholar | 29 | 8,128 | Ranging from 800 IU to 5,000 IU | ICU admissions, mechanical ventilation, mortality, SARS-CoV-2 infection prevention. | ICU admissions and MV rates reduced. No conclusive preventive effect against SARS-CoV-2 infection. |

*(Continued)*





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TABLE 1 (Continued)

| References | Search strategy | No. of studies included | Total no. of participants | Dose regimen and period | Outcomes | Results |
|---|---|---|---|---|---|---|
| Sîrbu et al. (73) | PubMed, Embase | 13 | Not specified | Not specified. | Length of hospital stay (LOS), ICU admissions, mortality | High-dose vitamin D showed potential benefits in reducing LOS and ICU admissions. No significant effect on mortality. |
| Cao et al. (74) | PubMed, Embase, Web of Science, Cochrane Library, Google Scholar | Not specified. | Not specified. | Not specified. | Mortality | All-cause mortality decreased (RR = 0.54, 95% CI: 0.33–0.88). |
| Zhang et al. (75) | PubMed, Embase, Cochrane Library, CBM, CNKI, VIP, and WanFang | 16 | 3,359 | Not specified. | Mortality, ICU admissions, LOS. | No significant reduction in mortality in RCTs (RR = 0.94, 95% CI: 0.69–1.29). Positive impact on mortality in cohort studies (RR = 0.33, 95% CI: 0.23–0.47). No significant differences in ICU admission or MV rates. |
| Zaazouee et al. (76) | PubMed, Embase, Scopus, Web of Science, and Cochrane Library | 9 | 1,586 | Not specified. | ICU admissions, inflammatory markers | Reduced ICU admissions. |
| Kümmel et al. (51) | PubMed, Embase, Scopus, Web of Science, The Cochrane Library, medRxiv, Cochrane COVID-19 Study Register, and ClinicalTrial.gov | 8 | 657 | Not specified. | Mortality, ICU admissions. | No significant effects, but a trend for reduced mortality (OR = 0.74, 95% CI: 0.32–1.71) and reduced ICU admissions (OR = 0.41, 95% CI: 0.15–1.12). |
| D'Ecclesii et al. (77) | PubMed, Ovid Medline, EMBASE, and ISI Web of Science | 38 | Not specified | Not specified | ICU admissions, mechanical ventilation, LOS. | Vitamin D supplementation associated with lower risk of severe COVID-19 (SRR = 0.38, 95% CI: 0.20–0.72) and mortality (SRR = 0.35, 95% CI: 0.17–0.70). Older individuals and higher latitudes showed greater reduction in mortality. |
| Hosseini et al. (44) | PubMed, Cochrane, CINAHL, and EMBASE | 5 | 1548 (RCT) and 586,841 (NRIS) | Not specified. | COVID-19 infection risk, hospital admission, ICU admission, mortality | Reduced ICU admission (RR = 0.35, 95% CI: 0.20–0.62) and mortality (RR = 0.46, 95% CI: 0.30–0.70). No significant effect on infection risk |
| Beran et al. (52) | PubMed/MEDLINE, Embase, and Cochrane | Not specified. | Not specified. | Not specified. | ICU admissions, LOS. | Vitamin D did not reduce mortality (RR 0.75, 95% CI 0.49–1.17) but reduced intubation rate (RR = 0.55, 95% CI 0.32–0.97) and LOS (MD = −1.26; 95% CI − 2.27 to −0.25). |
| Tentolouris et al. (78) | Med, Google Scholar, Embase, Web of Science, and medRxiv | 9 | 2078 | Not specified. | Mortality, ICU admissions, mechanical ventilation | No significant effect on mortality (OR = 0.60, 95% CI: 0.32–1.12). ICU admissions significantly reduced (OR = 0.33, 95% CI: 0.15–0.71). |
| Stroehlein et al. (79) | Cochrane Library and PubMed | 3 | 645 | Not specified. | Mortality, LOS. | No significant effect on mortality. Limited data on LOS. |

*(Continued)*





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TABLE 1 (Continued)

| References | Search strategy | No. of studies included | Total no. of participants | Dose regimen and period | Outcomes | Results |
|---|---|---|---|---|---|---|
| Szarpak et al. (80) | PubMed, EMBASE, Web of Science, Cochrane, Scopus | 8 | 2,322 | Not specified. | ICU admissions, mechanical ventilation. | The use of vitamin D was associated with:<br>- Lower 14-day mortality<br>- Lower in-hospital mortality<br>- Fewer ICU admissions<br>- Fewer cases of mechanical ventilation |
| Pal et al. (81) | PubMed/MEDLINE, Scopus, Web of Science | 13 | 2,933 | Not specified. | Mortality, ICU Admissions, Length of Stay (LOS) | Pooled analysis of unadjusted data showed that vitamin D use was significantly associated with reduced ICU admission/mortality and also reduced the risk of adverse outcomes when pooled risk estimates were adjusted Subgroup analysis showed benefits only for those receiving vitamin D post-COVID-19 diagnosis |
| Rawat et al. (82) | PubMed, Embase, Scopus | 5 | 467 | Not specified. | Mortality, ICU admission rates, Need for invasive ventilation | Vitamin D did not reduce:<br>- Mortality (RR 0.55; 95% CI: 0.22–1.39; $p = 0.21$)<br>- ICU admission rates (RR 0.20; 95% CI: 0.01–4.26; $p = 0.3$)<br>- Need for invasive ventilation (RR 0.24; 95% CI: 0.01–7.89; $p = 0.42$) |

(95% CI: 0.57–1.52), respectively, suggesting no statistically significant effect. However, the majority of studies, including Sartini et al. (43) (OR: 0.45, 95% CI: 0.24–0.86) and Hosseini et al. (44) (OR: 0.46, 95% CI: 0.30–0.70), reported significant reductions in mortality risk (Figure 4).

## 3.5 Meta-regression analysis

The regression analysis for ICU admissions yielded an R-squared value of 0.129, indicating that publication year explains 12.9% of the variation in log_OR. The coefficient for year was 0.0909 ($p = 0.309$), suggesting a small positive trend over time, though this effect was not statistically significant.

For mortality, the model had an R-squared value of 0.038, meaning that only 3.8% of the variability in log_OR was explained by publication year. The coefficient for year was 0.0585 ($p = 0.504$), indicating no significant effect.

The results indicate that publication year is not a significant predictor of the odds ratios for ICU admissions or mortality in COVID-19 patients receiving Vitamin D supplementation. This suggests that the reported effects of Vitamin D on COVID-19 outcomes have remained relatively stable over time, without clear evidence of publication bias or time-dependent changes in treatment efficacy (Figure 5).

## 3.6 Primary study overlap and corrected covered area

To quantify the degree of overlap between primary studies included in the 21 meta-analyses assessed, we constructed a citation matrix comparing each pair of reviews. The number in each cell indicates how many randomized controlled trials (RCTs) were shared between the corresponding pair of systematic reviews.

The extent of redundancy between reviews was further summarized using the Corrected Covered Area (CCA), a validated metric of overlap: $CCA = \frac{N - R}{r(c-1)}$, where: N = total number of included instances of primary studies across reviews (i.e., sum of all overlaps); R = number of unique primary studies; r, $c$= number of included reviews.

In our analysis, assuming approximately 300 unique RCTs across all reviews, the calculated CCA was 0.543 (54.3%), indicating moderate-to-high overlap. This level of redundancy is expected in umbrella reviews focusing on an emergent topic with intense publication activity, such as COVID-19 (Figure 6).

# 4 Discussion

Over 5 years have passed since the start of the COVID-19 pandemic. Since then, many RCT trials, systematic reviews, and other research studies have been conducted. Here, we present a summary of the findings regarding the potential benefits of vitamin D supplementation on common COVID-19 outcomes.

The most common forms of vitamin D supplementation are cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), which are

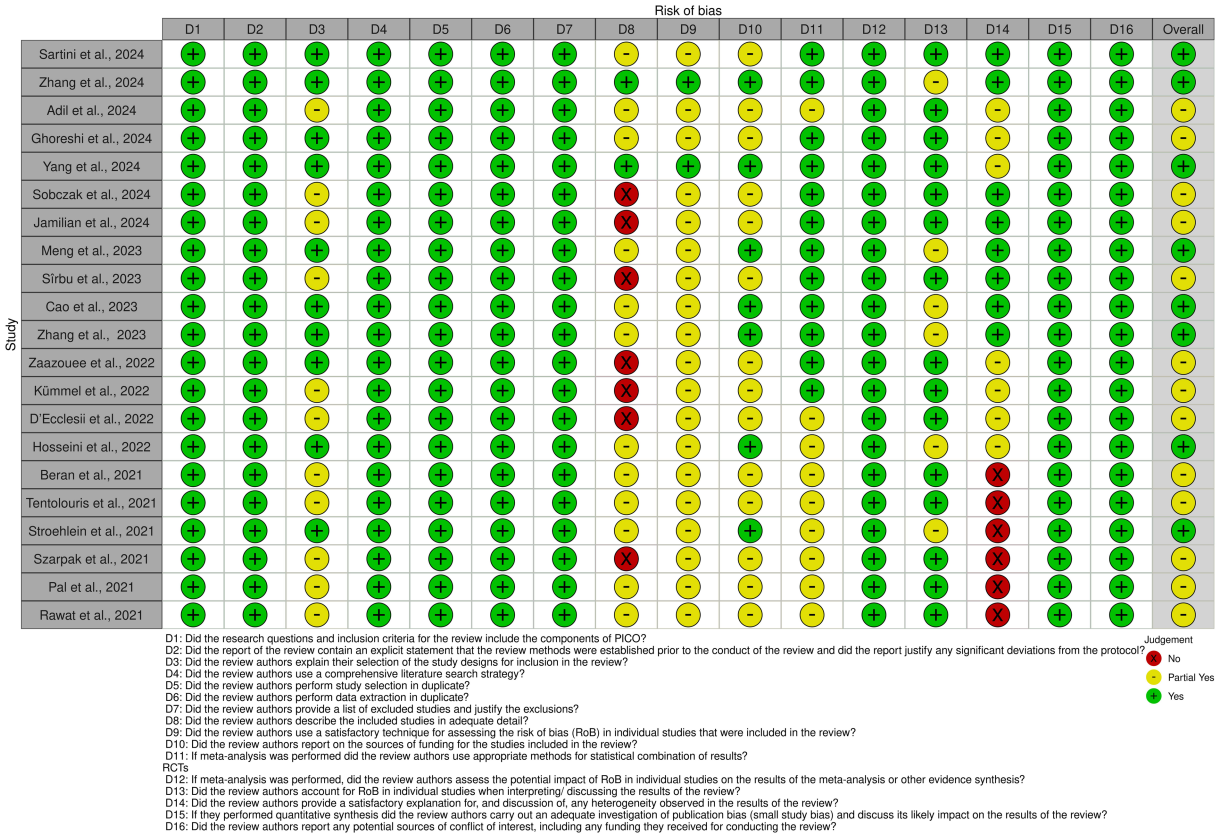


FIGURE 2
Risk of bias assessment for included studies. The figure displays the risk of bias (RoB) assessment for each included study based on the AMSTAR 2 criteria. Each row represents a study, while columns correspond to specific domains of bias. Green circles indicate low risk of bias, yellow circles represent partial fulfillment (moderate risk), and red circles denote high risk of bias.

precursors of 1,25-dihydroxyvitamin D3, the active form of vitamin D. In addition to its classical biological functions, such as regulating bone metabolism and maintaining calcium and phosphorus balance, vitamin D also plays a role in immune modulation, lung and muscle function, cardiovascular health, and the prevention of infectious diseases.

Much research, particularly during the first wave of the pandemic, has suggested an association between vitamin D deficiency and the risk of SARS-CoV-2 infection, the incidence and severity of COVID-19, and mortality (45). Speculative observations about the higher prevalence of hypovitaminosis D in European countries, along with the high rates of SARS-CoV-2 and COVID-19 infections, especially in northern regions, have linked the two events. However, these observations have not verified the causal relationship or ruled out causality.

Importantly, it should be noted that most studies on this topic have been retrospective in nature. This raises the possibility that decreased vitamin D levels may be a consequence of acute illness rather than a predisposing factor, leading to concerns about reverse causality. This issue has been widely discussed and remains a subject of debate. However, subsequent studies adopting a prospective methodology have provided further evidence supporting the role of vitamin D deficiency in predicting negative outcomes in COVID-19 patients (45). Some studies have found that total serum calcium levels measured at admission are inversely related to proinflammatory biomarkers associated with severe COVID-19. Additionally, serum calcium may serve as a useful marker for risk stratification, helping to better predict adverse in-hospital outcomes (46).

In this umbrella analysis, we summarized recent findings from systematic reviews and meta-analyses on two critical outcomes: ICU admissions and mortality. Our results indicated that vitamin D supplementation significantly reduces both ICU admissions and mortality.

The pooled analysis for ICU admissions showed a significant reduction (OR = 0.62, 95% CI: 0.54–0.71). This effect was consistent across multiple studies (43, 47, 48). The low heterogeneity (Q = 10.87, $p = 0.33$) suggests that despite differences in dosage and study design, the overall effect size remained stable across diverse populations. Sartini et al. (43) reported a similar reduction in ICU admissions (OR = 0.55, 95% CI: 0.37–0.79), consistent with the findings of Hosseini et al. (44) and Meng et al. (49).

Subgroup analyses provided further insights. For instance, Zhang et al. (47) found that continuous high-dose regimens were more effective than single-dose interventions (RR = 0.44 vs. 0.79). Additionally, Yang et al. (50) observed a more significant reduction in ICU admission rates among patients with moderate to severe COVID-19 (OR = 0.43, 95% CI: 0.23–0.80).

Populations with vitamin D deficiency consistently showed more pronounced reductions in ICU admissions (RR = 0.63, 95% CI: 0.42–0.93), supporting the hypothesis that correcting vitamin D deficiency offers greater immunological and anti-inflammatory benefits.

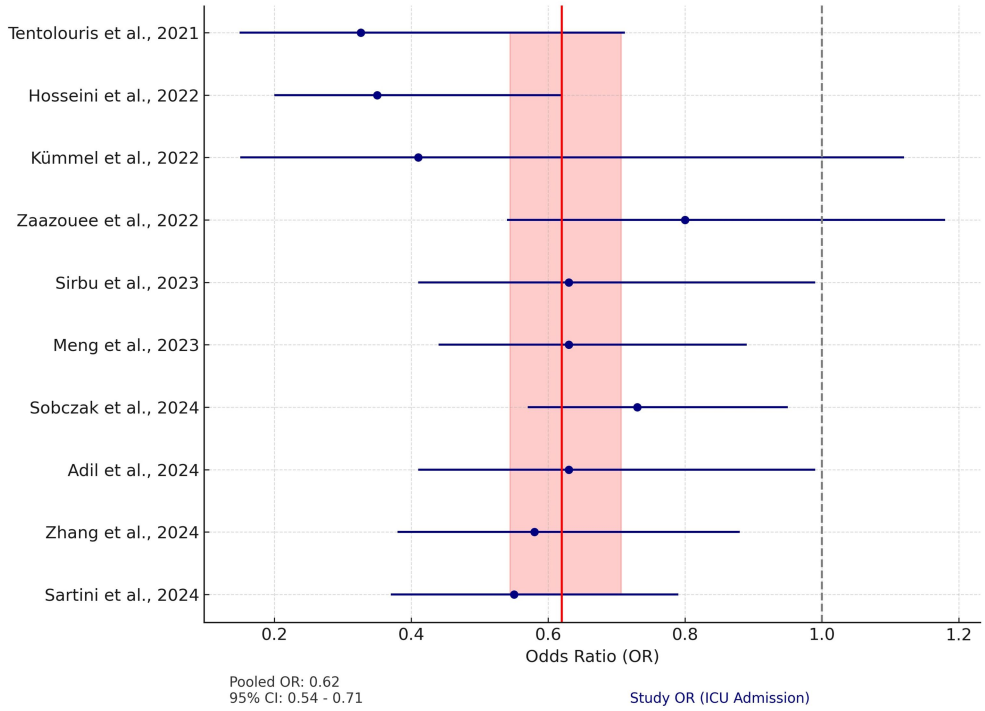


FIGURE 3
Forest plot for ICU admission. The plot displays the odds ratios (OR) and 95% confidence intervals (CI) for individual studies assessing the effect of vitamin D on ICU admission rates in COVID-19 patients. The red vertical line represents the pooled OR of 0.62 (95% CI: 0.54–0.71), calculated using a fixed-effects model. The shaded red area indicates the 95% CI of the pooled OR. The dashed gray line at OR = 1 represents the null effect. Blue circles represent the OR of each study, with horizontal lines depicting the 95% CI.

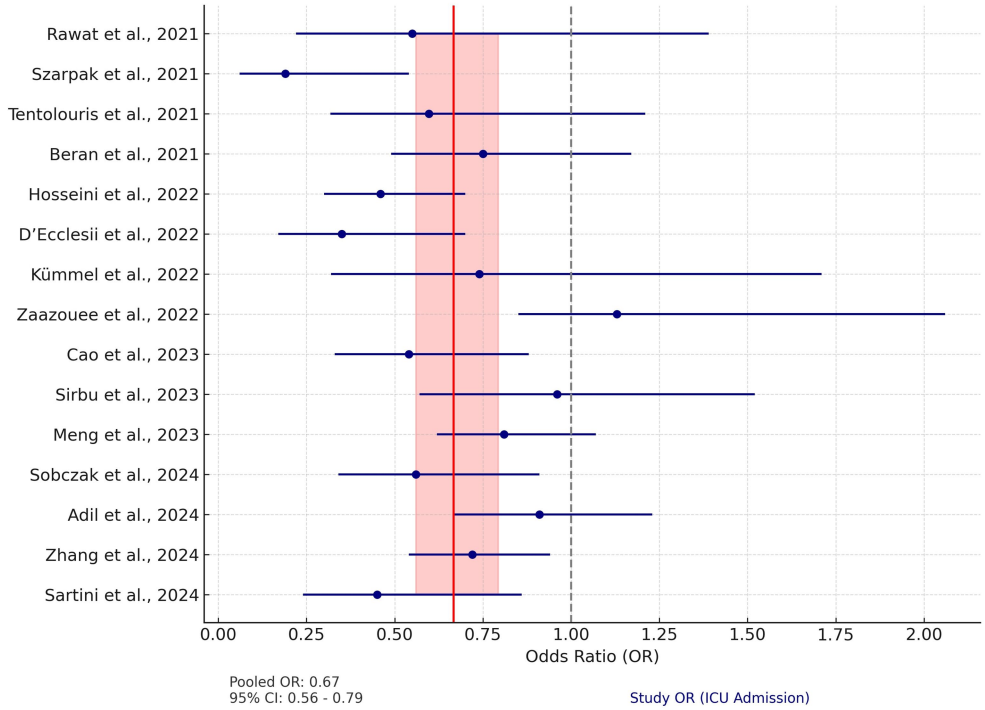


FIGURE 4
Forest plot for mortality. This plot illustrates the OR and 95% CI for studies analyzing the impact of vitamin D on mortality in COVID-19 patients. The pooled OR is 0.67 (95% CI: 0.56–0.79), calculated using a random-effects model to account for significant heterogeneity (Q = 27.23, $p$ = 0.006). The red vertical line represents the pooled OR, and the shaded red area indicates its 95% CI. The dashed gray line at OR = 1 denotes the null effect. Blue circles and horizontal lines represent the OR and 95% CI for each study, respectively.

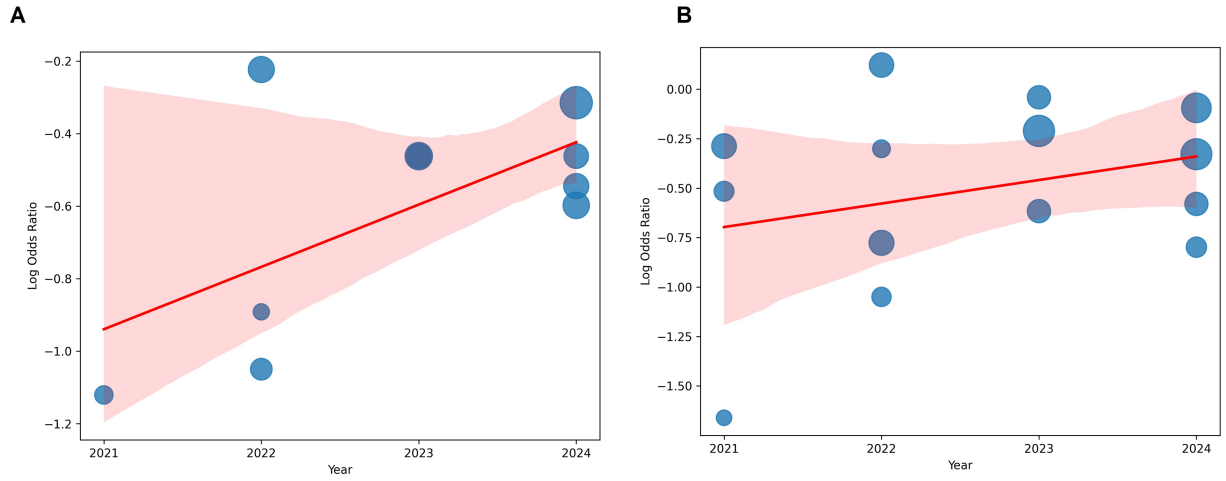


FIGURE 5
Bubble plot analysis. **(A)** ICU admissions. **(B)** Mortality. The x-axis represents the publication year. The y-axis represents the log-transformed odds ratio. The size of each bubble corresponds to the weight assigned to the study. A fitted regression line illustrates the overall trend. Despite minor fluctuations in effect sizes over time, no clear pattern or significant trend was observed, supporting the statistical findings.

Regarding the effect of vitamin D supplementation on mortality, the analysis showed a significant reduction in mortality (OR = 0.67, 95% CI: 0.56–0.79); however, substantial heterogeneity was observed across studies (Q = 27.23, $p = 0.006$). This variability could be due to differences in dosing protocols, patient populations, baseline vitamin D levels, and comorbidities, all of which may influence the differences in mortality reduction rates.

Adil et al. (42) and Hosseini et al. (44) reported significant reductions in mortality, with Adil's analysis highlighting the effect of high-dose vitamin D in reducing death rates. Zhang et al. (47) found that the mortality benefit was confined to vitamin D-deficient patients (RR = 0.73, 95% CI: 0.59–0.89), with no significant reductions in non-deficient populations. This suggests that vitamin D supplementation may be most effective as a corrective intervention rather than a prophylactic measure for individuals with adequate vitamin D levels.

In contrast, studies by Kümmel et al. (51) and Beran et al. (52) reported non-significant trends toward mortality reduction, with ORs around 0.74. This may be due to limited sample sizes, short follow-up periods, or underlying comorbidities that affect the therapeutic effect of vitamin D. However, the authors suggest that these trends still support the idea that vitamin D supplementation may offer a protective role, even if the effect is not universally statistically significant.

The analysis also revealed a modest, non-significant reduction in length of hospital stay (LOH) (MD = −1, 95% CI: −2.16 to 0.16, $p = 0.13$). Ghoreshi et al. (53) and Sartini et al. (43) reported similar findings, with reductions in LOH mainly observed in elderly populations or those receiving lower daily doses (≤10,000 IU). This suggests that while vitamin D supplementation may not drastically shorten hospitalization, it could contribute to a gradual improvement in recovery.

Mechanical ventilation outcomes showed mixed results. Yang et al. (50) and Meng et al. (49) reported significant reductions in mechanical ventilation requirements (OR = 0.44, 95% CI: 0.27–0.72), while other studies, such as Zhang et al. (47) and Adil et al. (42), found non-significant differences. These discrepancies may also stem from the complex interaction between patient condition, baseline vitamin D levels, and the timing of supplementation.

Patients with COVID-19 may benefit from vitamin D supplementation as both a preventive and therapeutic agent. Vitamin D binds to its receptor and influences two primary pathways: first, it inhibits pro-inflammatory cytokines by interfering with the TNF-induced NFκB1 pathway, and second, it activates the Jak–Stat pathway by inducing the expression of interferon-stimulating genes (54, 55).

1,25-dihydroxyvitamin D has explicitly antimicrobial properties, inducing the expression of cathelicidin and $\beta$-defensin 2, which exhibit direct and indirect antimicrobial effects. These effects include stimulating immune cell chemotaxis and pro-inflammatory cytokine expression, leading to removing infected cells in the respiratory tract. Vitamin D also stimulates the expression of β-defensin via nucleotide-binding oligomerization domain-containing protein 2 (NOD2) (56). Additionally, 1,25(OH)2D inhibits hepcidin expression, allowing for increased iron export from infected cells and reducing iron availability for microbial growth (57). Vitamin D's antimicrobial effects also extend to promoting intestinal and alveolar epithelial barrier function, enhancing the production of reactive oxygen species (ROS), and supporting neutrophil function and macrophage activities, including phagocytosis and autophagy (58–61).

In adaptive immunity, calcitriol limits T lymphocyte activation, induces the expression of regulatory T cells (Tregs), and helps shift immune responses from pro-inflammatory Th1/Th17 to regulatory Th2, thus supporting immune tolerance (62). The effectiveness of vitamin D depends on its receptor (VDR), and variations in the VDR gene, particularly SNPs, have been linked to immune dysfunctions. For example, the TT genotype of the FokI polymorphism has been associated with an increased risk of respiratory syncytial virus infections (63).

Vitamin D may also influence the viral replication process in human cells. SARS-CoV-2 enters host cells through the angiotensin-converting enzyme-2 (ACE2) receptor, leading to severe pathology

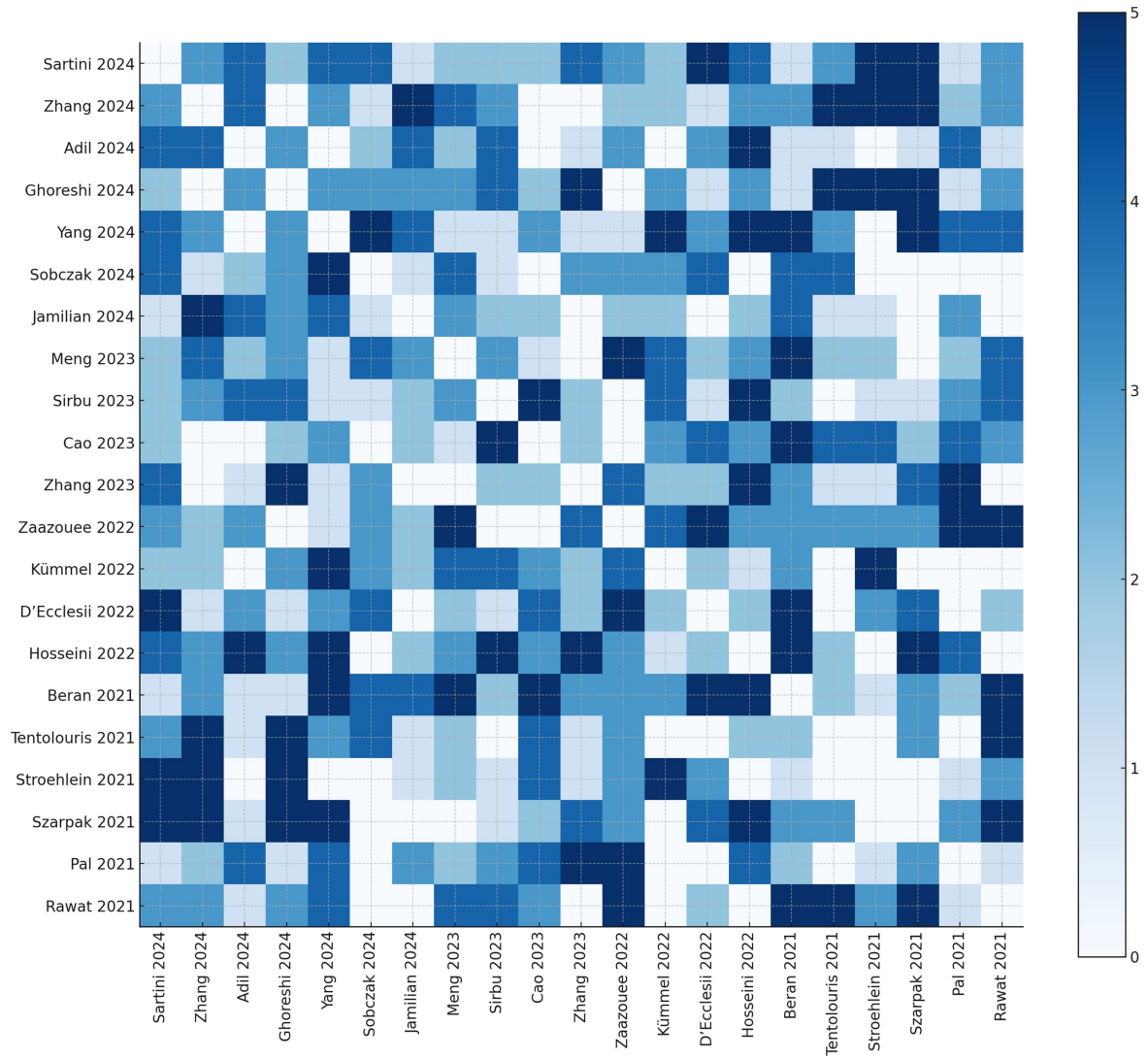


FIGURE 6
Heatmap of primary study overlap across included systematic reviews Each cell shows the number of shared primary studies between the corresponding pair of reviews. Darker shades represent a higher degree of overlap. The diagonal has been set to zero by definition.

and cell death. The virus modulates the renin-angiotensin system (RAS), causing excess angiotensin II (Ang-II) production, which activates the cytokine storm and downregulates the immune system. Vitamin D has been proposed to help prevent acute respiratory distress syndrome (ARDS) by downregulating Ang-II production and enhancing the ACE2/Ang-(1–7)/Mas receptor axis, providing protective effects against tissue damage and inflammation (64–68).

In severe COVID-19 cases, the inflammatory response can cause significant damage, particularly to the lungs and heart, with cytokine levels such as IL-6 elevated significantly in more severe cases (69, 70). Vitamin D may reduce this cytokine storm by promoting the expression of anti-inflammatory mediators like IL-10, IL-4, and TGFβ and shifting the immune response toward T-regs. This modulation of the immune response may help reduce the inflammatory damage seen in severe COVID-19 cases (71).

Overall, the findings of this umbrella review point to a potentially beneficial role of vitamin D supplementation in reducing the severity of COVID-19 outcomes, particularly in relation to ICU admission and mortality. These effects appear more pronounced in individuals with low baseline vitamin D levels, which aligns with earlier observational and mechanistic evidence. Although current data do not warrant routine high-dose supplementation in all COVID-19 patients, maintaining sufficient vitamin D status—through moderate supplementation or lifestyle measures—may be a low-cost and low-risk approach worth considering, especially in populations where deficiency is common. In hospital settings, evaluating vitamin D levels could be clinically justified in selected patients, such as the elderly or those with comorbidities, though universal screening may not be practical or necessary. Given the observed variability in dosing strategies and study populations, further well-designed trials are essential to clarify when, for whom, and how vitamin D supplementation can be most effectively applied in the context of respiratory infections like COVID-19.

## 5 Limitations and future directions

This umbrella review has several limitations that should be acknowledged.

First, baseline vitamin D status was not consistently reported across the included reviews. This inconsistency hinders our ability to determine whether the observed benefits are predominantly confined to individuals with vitamin D deficiency or extend to those with sufficient levels. In addition, differences in how deficiency was defined or measured across studies may have introduced variability into the pooled estimates.

Second, significant heterogeneity was observed in mortality outcomes ($Q = 27.23$, $p = 0.006$). Although we explored potential sources of this heterogeneity—such as variations in dosing regimens, study design, and patient demographics—the available data did not always permit detailed subgroup or meta-regression analyses. As a result, unmeasured factors (e.g., differences in SARS-CoV-2 variants, concomitant treatments like corticosteroids or antivirals, and variations in population health status) may have influenced the reported effects.

Third, many of the studies included in the reviews were retrospective, which raises the possibility of reverse causality. It is plausible that severe COVID-19 itself might lead to lower vitamin D levels, rather than low vitamin D predisposing patients to severe disease. Although more recent prospective studies suggest an independent role of vitamin D deficiency in predicting adverse outcomes, the potential for reverse causality remains a concern.

Fourth, confounding factors—such as overall health status, preexisting comorbidities, and socioeconomic conditions—were not uniformly controlled across the included reviews. This limitation may bias the association between vitamin D supplementation and improved outcomes, as these factors are known to influence COVID-19 severity.

Fifth, some meta-analyses incorporated overlapping primary studies, raising concerns about the double counting of data. While we noted these overlaps and attempted to address them during our analysis, this issue could lead to an overestimation of the pooled effect sizes.

Sixth, optimal dosing strategies for vitamin D supplementation remain uncertain. Although several reviews suggested that continuous high-dose regimens might yield stronger benefits compared to single-dose protocols, the available data were insufficient to establish standardized dosing recommendations. The recent findings from Minasi et al., which underscore the role of hypocalcemia in adverse COVID-19 outcomes, further highlight the complex interplay between vitamin D, calcium homeostasis, and clinical outcomes.

Future research should focus on large-scale, multicenter randomized controlled trials with standardized dosing protocols, consistent baseline vitamin D measurements, and robust control for confounding variables.

## 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.

## Author contributions

PP: Data curation, Investigation, Validation, Visualization, Writing – original draft. IK: Formal analysis, Writing – review & editing. IH: Formal Analysis, Visualization, Writing – original draft. OK: Formal analysis, Supervision, Validation, 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



## References

1. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Biomedi.* (2020) 91:157–60. doi: 10.23750/abm.v91i1.9397

2. Hadizadeh F. Supplementation with vitamin D in the COVID-19 pandemic? *Nutr Rev.* (2021) 79:200–8. doi: 10.1093/nutrit/nuaa081

3. Ball L, Silva P, Giacobbe D, Bassetti M, Zubieta-Calleja G, Rocco P, et al. Understanding the pathophysiology of typical acute respiratory distress syndrome and severe COVID-19. *Expert Rev Respir Med.* (2022) 16:1–10. doi: 10.1080/17476348.2022.2057300

4. Buchynskyi M, Oksenych V, Kamyshna I, Budarna O, Halabitska I, Petakh P, et al. Genomic insight into COVID-19 severity in MAFLD patients: a single-center prospective cohort study. *Front Genet.* (2024) 15:1460318. doi: 10.3389/fgene.2024.1460318

5. Buchynskyi M, Oksenych V, Kamyshna I, Vorobets I, Halabitska I, Kamyshnyi O. Modulatory roles of AHR, FFAR2, FXR, and TGR5 gene expression in metabolic-associated fatty liver disease and COVID-19 outcomes. *Viruses.* (2024) 16:985. doi: 10.3390/v16060985

6. Fernandes DM, Oliveira CR, Guerguis S, Eisenberg R, Choi J, Kim M, et al. Severe acute respiratory syndrome coronavirus 2 clinical syndromes and predictors of disease severity in hospitalized children and youth. *J Pediatr*. (2021) 230:23–31.e10. doi: 10.1016/j.jpeds.2020.11.016

7. Petakh P, Griga V, Mohammed IB, Loshak K, Poliak I, Kamyshnyiy A. Effects of metformin, insulin on hematological parameters of COVID-19 patients with type 2 diabetes. *Med Arch*. (2022) 76:329–32. doi: 10.5455/medarh.2022.76.329-332

8. Petakh P, Isevych V, Mohammed I, Loshak K, Poliak I, Kamyshnyiy A. Association between use of metformin and insulin with hematological parameters in COVID-19 patients with type 2 diabetes: a single center, cross-sectional study. *Clin Diabetol*. (2022) 11:432–3. doi: 10.5603/DK.a2022.0055

9. Campi I, Gennari L, Merlotti D, Mingiano C, Frosali A, Giovanelli L, et al. Vitamin D and COVID-19 severity and related mortality: a prospective study in Italy. *BMC Infect Dis*. (2021) 21:566. doi: 10.1186/s12879-021-06281-7

10. Petakh P, Kamyshna I, Oksenych V, Kainov D, Kamyshnyi A. Metformin therapy changes gut microbiota alpha-diversity in COVID-19 patients with type 2 diabetes: the role of SARS-CoV-2 variants and antibiotic treatment. *Pharmaceuticals*. (2023) 16:904. doi: 10.3390/ph16060904

11. Buchynskyi M, Kamyshna I, Lyubomirskaya K, Moshynets O, Kobyliak N, Oksenych V, et al. Efficacy of interferon alpha for the treatment of hospitalized patients with COVID-19: a meta-analysis. *Front Immunol*. (2023) 14:1069894. doi: 10.3389/fimmu.2023.1069894

12. Martono M, Fatmawati F, Mulyanti S. Risk factors associated with the severity of COVID-19. *Malays J Med Sci*. (2023) 30:84–92. doi: 10.21315/mjms2023.30.3.7

13. Petakh P, Kobyliak N, Kamyshnyi A. Gut microbiota in patients with COVID-19 and type 2 diabetes: a culture-based method. *Front Cell Infect Microbiol*. (2023) 13:1142578. doi: 10.3389/fcimb.2023.1142578

14. Annweiler C, Beaudenon M, Gautier J, Simon R, Dubée V, Gonsard J, et al. COvid-19 and high-dose VITamin D supplementation TRIAL in high-risk older patients (COVIT-TRIAL): study protocol for a randomized controlled trial. *Trials*. (2020) 21:1031. doi: 10.1186/s13063-020-04928-5

15. Bassatne A, Basbous M, Chakhtoura M, El Zein O, Rahme M, El-Hajj FG. The link between COVID-19 and VItamin D (VIVID): a systematic review and meta-analysis. *Metab Clin Exp*. (2021) 119:154753. doi: 10.1016/j.metabol.2021.154753

16. Petakh P, Kamyshna I, Oksenych V, Kamyshnyi O. Metformin alters mRNA expression of FOXP3, RORC, and TBX21 and modulates gut microbiota in COVID-19 patients with type 2 diabetes. *Viruses*. (2024) 16:281. doi: 10.3390/v16020281

17. Repchuk Y, Sydorchuk LP, Sydorchuk AR, Fedonyuk LY, Kamyshnyi O, Korovenkova O, et al. Linkage of blood pressure, obesity and diabetes mellitus with angiotensinogen gene (AGT 704T>C/rs699) polymorphism in hypertensive patients. *Bratislavske Lekarske Listy*. (2021) 122:715–20. doi: 10.4149/BLL_2021_114

18. Halabitska I, Petakh P, Kamyshna I, Oksenych V, Kainov DE, Kamyshnyi O. The interplay of gut microbiota, obesity, and depression: insights and interventions. *Cell Mol Life Sci*. (2024) 81:443. doi: 10.1007/s00018-024-05476-w

19. Lima-Martínez MM, Carrera Boada C, Madera-Silva MD, Marín W, Contreras M. COVID-19 and diabetes: a bidirectional relationship. *Clin Invest Arterioscl*. (2021) 33:151–7. doi: 10.1016/j.arteri.2020.10.001

20. Mathur R, Rentsch CT, Morton CE, Hulme WJ, Schultze A, MacKenna B, et al. Ethnic differences in SARS-CoV-2 infection and COVID-19-related hospitalisation, intensive care unit admission, and death in 17 million adults in England: an observational cohort study using the OpenSAFELY platform. *Lancet*. (2021) 397:1711–24. doi: 10.1016/S0140-6736(21)00634-6

21. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora VM, Solway J. Association of Vitamin D Levels, race/ethnicity, and clinical characteristics with COVID-19 test results. *JAMA Netw Open*. (2021) 4:e214117. doi: 10.1001/jamanetworkopen.2021.4117

22. Mauvais-Jarvis F. Aging, male sex, obesity, and metabolic inflammation create the perfect storm for COVID-19. *Diabetes*. (2020) 69:1857–63. doi: 10.2337/dbi19-0023

23. Tal Y, Adini A, Eran A, Adini I. Racial disparity in Covid-19 mortality rates - a plausible explanation. *Clin Immunol*. (2020) 217:108481. doi: 10.1016/j.clim.2020.108481

24. Cozier YC, Castro-Webb N, Hochberg NS, Rosenberg L, Albert MA, Palmer JR. Lower serum 25(OH)D levels associated with higher risk of COVID-19 infection in U.S. black women. *PLoS One*. (2021) 16:e0255132. doi: 10.1371/journal.pone.0255132

25. Fong MW, Gao H, Wong JY, Xiao J, Shiu EYC, Ryu S, et al. Nonpharmaceutical measures for pandemic influenza in nonhealthcare settings-social distancing measures. *Emerg Infect Dis*. (2020) 26:976–84. doi: 10.3201/eid2605.190995

26. Wong SCY, Kwong RT, Wu TC, Chan JWM, Chu MY, Lee SY, et al. Risk of nosocomial transmission of coronavirus disease 2019: an experience in a general ward setting in Hong Kong. *J Hosp Infect*. (2020) 105:119–27. doi: 10.1016/j.jhin.2020.03.036

27. Bell D, Nicoll A, Fukuda K, Horby P, Monto A, Hayden F, et al. Non-pharmaceutical interventions for pandemic influenza, international measures. *Emerg Infect Dis*. (2006) 12:81–7. doi: 10.3201/eid1201.051370

28. Wang G, Zhang Y, Zhao J, Zhang J, Jiang F. Mitigate the effects of home confinement on children during the COVID-19 outbreak. *Lancet*. (2020) 395:945–7. doi: 10.1016/S0140-6736(20)30547-X

29. Nowson CA, McGrath JJ, Ebeling PR, Haikerwal A, Daly RM, Sanders KM, et al. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust*. (2012) 196:686–7. doi: 10.5694/mja11.10301

30. Kamyshna I, Kamyshnyi O, Pavlovych L, Malyk I. 25-OH vitamin D blood serum linkage with VDR gene polymorphism (rs2228570) in thyroid pathology patients in the west-Ukrainian population. *J Med Life*. (2021) 14:549–56. doi: 10.25122/jml-2021-0101

31. Aranow C. Vitamin D and the immune system. *J Invest Med*. (2011) 59:881–6. doi: 10.2310/JIM.0b013e31821b8755

32. D'Avolio A, Avataneo V, Manca A, Cusato J, De Nicolò A, Lucchini R, et al. 25-Hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2. *Nutrients*. (2020) 12:1359. doi: 10.3390/nu12051359

33. Ünsal YA, Gül Ö, Cander S, Ersoy C, Aydemir E, Ateş C, et al. Retrospective analysis of vitamin D status on ınflammatory markers and course of the disease in patients with COVID-19 infection. *J Endocrinol Investig*. (2021) 44:2601–7. doi: 10.1007/s40618-021-01566-9

34. Marik PE, Kory P, Varon J. Does vitamin D status impact mortality from SARS-CoV-2 infection? *Med Drug Discov*. (2020) 6:100041. doi: 10.1016/j.medidd.2020.100041

35. Daneshkhah A, Agrawal V, Eshein A, Subramanian H, Roy HK, Backman V. Evidence for possible association of vitamin D status with cytokine storm and unregulated inflammation in COVID-19 patients. *Aging Clin Exp Res*. (2020) 32:2141–58. doi: 10.1007/s40520-020-01677-y

36. Langlois PL, D'Aragon F, Manzanares W. Vitamin D in the ICU: more sun for critically ill adult patients? *Nutrition*. (2019) 61:173–8. doi: 10.1016/j.nut.2018.11.001

37. Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *Int Immunopharmacol*. (2020) 84:106504. doi: 10.1016/j.intimp.2020.106504

38. Xue G, Gan X, Wu Z, Xie D, Xiong Y, Hua L, et al. Novel serological biomarkers for inflammation in predicting disease severity in patients with COVID-19. *Int Immunopharmacol*. (2020) 89:107065. doi: 10.1016/j.intimp.2020.107065

39. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both (2017) 358:j4008. doi: 10.1136/bmj.j4008

40. Smith V, Devane D, Begley CM, Clarke M. Methodology in conducting a systematic review of systematic reviews of healthcare interventions. *BMC Med Res Methodol*. (2011) 11:15. doi: 10.1186/1471-2288-11-15

41. Meng Z, Wang J, Lin L, Wu C. Sensitivity analysis with iterative outlier detection for systematic reviews and meta-analyses. *Stat Med*. (2024) 43:1549–63. doi: 10.1002/sim.10008

42. Adil M, Saleem MM, Vijay S, Ehsan M, Atiq I, Anwar E, et al. Efficacy of vitamin D supplementation in the treatment of patients with COVID-19: a systematic review and meta-analysis of randomized controlled trials. *Ann Med Surg* (2012). (2024) 86:6079–90. doi: 10.1097/MS9.0000000000002445

43. Sartini M, Del Puente F, Carbone A, Schinca E, Ottria G, Dupont C, et al. The effect of vitamin D supplementation post COVID-19 infection and related outcomes: a systematic review and Meta-analysis. *Nutrients*. (2024) 16:3794. doi: 10.3390/nu16223794

44. Hosseini B, El Abd A, Ducharme FM. Effects of vitamin D supplementation on COVID-19 related outcomes: a systematic review and meta-analysis. *Nutrients*. (2022) 14:2134. doi: 10.3390/nu14102134

45. di Filippo L, Uygur M, Locatelli M, Nannipieri F, Frara S, Giustina A. Low vitamin D levels predict outcomes of COVID-19 in patients with both severe and non-severe disease at hospitalization. *Endocrine*. (2023) 80:669–83. doi: 10.1007/s12020-023-03331-9

46. Minasi A, Andreadi A, Maiorino A, Giudice L, De Taddeo S, D'Ippolito I, et al. Hypocalcemia is associated with adverse outcomes in patients hospitalized with COVID-19. *Endocrine*. (2023) 79:577–86. doi: 10.1007/s12020-022-03239-w

47. Zhang X, Wu J, Dong H, Shang N, Li Y, Zhang Y, et al. The impact of supplementing vitamin D through different methods on the prognosis of COVID-19 patients: a systematic review and meta-analysis. *Front Nutr*. (2024) 11:1441847. doi: 10.3389/fnut.2024.1441847

48. Sobczak M, Pawliczak R. Effect of vitamin D3 supplementation on severe COVID-19: a Meta-analysis of randomized clinical trials. *Nutrients*. (2024) 16:1402. doi: 10.3390/nu16101402

49. Meng J, Li X, Liu W, Xiao Y, Tang H, Wu Y, et al. The role of vitamin D in the prevention and treatment of SARS-CoV-2 infection: a meta-analysis of randomized controlled trials. *Clin Nutr*. (2023) 42:2198–206. doi: 10.1016/j.clnu.2023.09.008

50. Yang Y, Sun W, Yang F, Zhang G, Li X, Sun S, et al. Therapeutic effects of vitamin D supplementation on COVID-19 aggravation: a systematic review and meta-analysis of randomized controlled trials. *Front Pharmacol*. (2024) 15:1367686. doi: 10.3389/fphar.2024.1367686

51. Kümmel LS, Krumbein H, Fragkou PC, Hünerbein BL, Reiter R, Papathanasiou KA, et al. Vitamin D supplementation for the treatment of COVID-19: a systematic review and meta-analysis of randomized controlled trials. *Front Immunol*. (2022) 13:1023903. doi: 10.3389/fimmu.2022.1023903

52. Beran A, Mhanna M, Srour O, Ayesh H, Stewart JM, Hjouj M, et al. Clinical significance of micronutrient supplements in patients with coronavirus disease 2019: a

comprehensive systematic review and meta-analysis. *Clin Nutr ESPEN*. (2022) 48:167–77. doi: 10.1016/j.clnesp.2021.12.033

53. Ghoreshi ZA, Charostad J, Arefinia N, Nakhaie M, Rezaei Zadeh Rukerd M, Salajegheh F. Effect of vitamin D supplementation on clinical outcomes in adult patients with COVID-19: a GRADE-assessed systematic review and meta-analysis of randomized controlled trials. *Pharmacol Res Perspect*. (2024) 12:e70013. doi: 10.1002/prp2.70013

54. Zdrenghea MT, Makrinioti H, Bagacean C, Bush A, Johnston SL, Stanciu LA. Vitamin D modulation of innate immune responses to respiratory viral infections. *Rev Med Virol*. (2017) 27:27(1). doi: 10.1002/rmv.1909

55. Arboleda JF, Urcuqui-Inchima S. Vitamin D supplementation: a potential approach for coronavirus/COVID-19 therapeutics? *Front Immunol*. (2020) 11:1523. doi: 10.3389/fimmu.2020.01523

56. Wang TT, Dabbas B, Laperriere D, Bitton AJ, Soualhine H, Tavera-Mendoza LE, et al. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. *J Biol Chem*. (2010) 285:2227–31. doi: 10.1074/jbc.C109.071225

57. Bacchetta J, Zaritsky JJ, Sea JL, Chun RF, Lisse TS, Zavala K, et al. Suppression of iron-regulatory hepcidin by vitamin D. *J Am Soc Nephrol*. (2014) 25:564–72. doi: 10.1681/ASN.2013040355

58. Kong J, Zhang Z, Musch MW, Ning G, Sun J, Hart J, et al. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol Gastrointest Liver Physiol*. (2008) 294:G208–16. doi: 10.1152/ajpgi.00398.2007

59. Shi YY, Liu TJ, Fu JH, Xu W, Wu LL, Hou AN, et al. Vitamin D/VDR signaling attenuates lipopolysaccharide-induced acute lung injury by maintaining the integrity of the pulmonary epithelial barrier. *Mol Med Rep*. (2016) 13:1186–94. doi: 10.3892/mmr.2015.4685

60. Subramanian K, Bergman P, Henriques-Normark B. Vitamin D promotes pneumococcal killing and modulates inflammatory responses in primary human neutrophils. *J Innate Immun*. (2017) 9:375–86. doi: 10.1159/000455969

61. Bhutia SK. Vitamin D in autophagy signaling for health and diseases: insights on potential mechanisms and future perspectives. *J Nutr Biochem*. (2022) 99:108841. doi: 10.1016/j.jnutbio.2021.108841

62. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. *J Pharmacol Exp Ther*. (2008) 324:23–33. doi: 10.1124/jpet.107.127209

63. Laplana M, Royo JL, Fibla J. Vitamin D receptor polymorphisms and risk of enveloped virus infection: a meta-analysis. *Gene*. (2018) 678:384–94. doi: 10.1016/j.gene.2018.08.017

64. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. (2005) 436:112–6. doi: 10.1038/nature03712

65. Wösten-van Asperen RM, Lutter R, Specht PA, Moll GN, van Woensel JB, van der Loos CM, et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. *J Pathol*. (2011) 225:618–27. doi: 10.1002/path.2987

66. Grace JA, Klein S, Herath CB, Granzow M, Schierwagen R, Masing N, et al. Activation of the MAS receptor by angiotensin-(1-7) in the renin-angiotensin system mediates mesenteric vasodilatation in cirrhosis. *Gastroenterology*. (2013) 145:874–84.e5. doi: 10.1053/j.gastro.2013.06.036

67. Xu J, Yang J, Chen J, Luo Q, Zhang Q, Zhang H. Vitamin D alleviates lipopolysaccharide-induced acute lung injury via regulation of the renin-angiotensin system. *Mol Med Rep*. (2017) 16:7432–8. doi: 10.3892/mmr.2017.7546

68. Song Y, Qayyum S, Greer RA, Slominski RM, Raman C, Slominski AT, et al. Vitamin D3 and its hydroxyderivatives as promising drugs against COVID-19: a computational study. *J Biomol Struct Dyn*. (2022) 40:11594–610. doi: 10.1080/07391102.2021.1964601

69. Petakh P, Kamyshna I, Nykyforuk A, Yao R, Imbery J, Oksenych V, et al. Immunoregulatory intestinal microbiota and COVID-19 in patients with type two diabetes: a double-edged sword. *Viruses*. (2022) 14:477. doi: 10.3390/v14030477

70. Hawerkamp H, Dyer A, Patil N, McElheron M, O'Dowd N, O'Doherty L, et al. Characterisation of the pro-inflammatory cytokine signature in severe COVID-19. *Front Immunol*. (2023) 14:14. doi: 10.3389/fimmu.2023.1170012

71. Ulivieri FM, Banfi G, Camozzi V, Colao A, Formenti AM, Frara S, et al. Vitamin D in the Covid-19 era: a review with recommendations from a G.I.O.S.E.G. expert panel. *Endocrine*. (2021) 72:597–603. doi: 10.1007/s12020-021-02749-3

72. Jamilian A, Ghalichi F, Hamedi Kalajahi F, Radkhah N, Jourabchi N, Musazadeh V, et al. The role of vitamin D in outcomes of critical care in COVID-19 patients: evidence from an umbrella meta-analysis of interventional and observational studies. *Public Health Nutr*. (2024) 27:e127. doi: 10.1017/S1368980024000934

73. Sîrbu AC, Sabin O, Bocșan IC, Vesa ȘC, Buzoianu AD. The effect of vitamin D supplementation on the length of hospitalisation, intensive care unit admission, and mortality in COVID-19-a systematic review and Meta-analysis. *Nutrients*. (2023) 15:3470. doi: 10.3390/nu15153470

74. Cao M, He C, Gong M, Wu S, He J. The effects of vitamin D on all-cause mortality in different diseases: an evidence-map and umbrella review of 116 randomized controlled trials. *Front Nutr*. (2023) 10:1132528. doi: 10.3389/fnut.2023.1132528

75. Zhang Y, Li J, Yang M, Wang Q. Effect of vitamin D supplementation on COVID-19 patients: a systematic review and meta-analysis. *Front Nutr*. (2023) 10:1131103. doi: 10.3389/fnut.2023.1131103

76. Zaazouee MS, Eleisawy M, Abdalalaziz AM, Elhady MM, Ali OA, Abdelbari TM, et al. Hospital and laboratory outcomes of patients with COVID-19 who received vitamin D supplementation: a systematic review and meta-analysis of randomized controlled trials. *Naunyn Schmiedeberg's Arch Pharmacol*. (2023) 396:607–20. doi: 10.1007/s00210-022-02360-x

77. D'Ecclesiis O, Gavioli C, Martinoli C, Raimondi S, Chiocca S, Miccolo C, et al. Vitamin D and SARS-CoV2 infection, severity and mortality: a systematic review and meta-analysis. *PLoS One*. (2022) 17:e0268396. doi: 10.1371/journal.pone.0268396

78. Tentolouris N, Samakidou G, Eleftheriadou I, Tentolouris A, Jude EB. The effect of vitamin D supplementation on mortality and intensive care unit admission of COVID-19 patients. A systematic review, meta-analysis and meta-regression. *Diabetes Metab Res Rev*. (2022) 38:e3517. doi: 10.1002/dmrr.3517

79. Stroehlein JK, Wallqvist J, Iannizzi C, Mikolajewska A, Metzendorf MI, Benstoem C, et al. Vitamin D supplementation for the treatment of COVID-19: a living systematic review. *Cochrane Database Syst Rev*. (2021) 2021. doi: 10.1002/14651858.CD015043

80. Szarpak L, Filipiak KJ, Gasecka A, Gawel W, Koziel D, Jaguszewski MJ, et al. Vitamin D supplementation to treat SARS-CoV-2 positive patients Evidence from meta-analysis. *Cardiol J*. (2022) 29:188–96. doi: 10.5603/CJ.a2021.0122

81. Pal R, Banerjee M, Bhadada SK, Shetty AJ, Singh B, Vyas A. Vitamin D supplementation and clinical outcomes in COVID-19: a systematic review and meta-analysis. *J Endocrinol Investig*. (2022) 45:53–68. doi: 10.1007/s40618-021-01614-4

82. Rawat D, Roy A, Maitra S, Shankar V, Khanna P, Baidya DK. Vitamin D supplementation and COVID-19 treatment: a systematic review and meta-analysis. *Diabete Metab Syndr*. (2021) 15:102189. doi: 10.1016/j.dsx.2021.102189

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# High-dose Vitamin D supplementation for immune recalibration in autoimmune diseases

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**high-dose, vitamin D, immune, autoimmune disease, VDR**

## 1 Autoimmune disease pathophysiology and immune dysregulation

Hileman et al. reported that ADs arise from a complex interplay of genetic susceptibility and environmental triggers, with viral infections playing a central role (1). Viruses activate innate immunity by inducing type I interferon (IFN-α/β) production and stimulating neutrophil extracellular trap (NET) release, thereby enhancing dendritic cell maturation and antigen presentation. In genetically predisposed individuals, these events promote adaptive immune activation, leading to B- and T-cell expansion, autoantibody generation, and T-cell dysregulation.

Robinson et al. further proposed that Epstein-Barr virus (EBV) contributes to autoimmunity through multiple mechanisms: molecular mimicry of human autoantigens, reprogramming of B-cell function, and binding of EBV nuclear antigen 2 (EBNA2) to host super-enhancer regions associated with autoimmune-susceptibility genes (2). Together, these actions disrupt normal gene regulation and perpetuate chronic immune activation.

Taken together, these studies underscore the pivotal role of viral infections in both initiating and exacerbating ADs and highlight potential antiviral or immunomodulatory targets for therapeutic intervention.

## 2 Vitamin D deficiency in autoimmune diseases and its immunomodulatory potential

According to the recent estimation, nearly 15 million Americans live with at least one ADs. Increasing evidence suggests that vitamin D insufficiency is highly prevalent in these patients and correlates with immune dysregulation, higher disease activity, and more frequent flares (3). Vitamin D supplementation was important to prevent and treat deficiency-related conditions like rickets. However, in vitamin D-replete adults, large-scale randomized trials and Mendelian randomization studies consistently showed no significant benefit for preventing cancer, cardiovascular disease, diabetes, or fractures. High-dose supplementation may even pose risks. Thus, routine use in the general population is not supported, except to correct deficiency or in specific at-risk groups (4).

Epidemiological studies have linked low serum 25-hydroxyvitamin D [25(OH)D] levels (<20 ng/mL) to elevated risk for ADs such as psoriasis, T1D, and multiple sclerosis (MS) (5). Vitamin D contributes to immune homeostasis by promoting innate defenses, enhancing macrophage and dendritic cell function, while modulating adaptive responses through suppression of Th1- and Th17-mediated inflammation and upregulation of regulatory T cells (6).

In MS specifically, vitamin D influences lymphocyte activation, T-helper cell polarization, and cytokine production. It decreases pro-inflammatory cytokines (e.g., IFN-γ, IL-17) and increases anti-inflammatory mediators (e.g., IL-10), thereby shifting the immune milieu toward tolerance (7). Randomized trials of supplementation (e.g., 4,000 IU/day cholecalciferol) have demonstrated significant reductions in relapse rates and Magnetic Resonance Imaging (MRI) lesion burden in relapsing–remitting MS, particularly in patients with baseline 25(OH)D <30 ng/mL (8).

Taken together, these findings support a therapeutic role for vitamin D in ADs, especially MS, by rebalancing innate and adaptive immunity and modulating key cytokines such as IL-10 and IL-17. Future large-scale trials are warranted to define optimal dosing, target serum levels, and long-term safety profiles.

## 3 Relevance to vitamin D receptor signaling and immune regulation (T-cell modulation and cytokine suppression)

The hormonally active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], exerts its immunomodulatory functions primarily by binding the vitamin D receptor (VDR), a nuclear transcription factor expressed across diverse immune cell types (5). Upon ligand engagement, VDR heterodimerizes with retinoid X receptor and associates with vitamin D response elements in target gene promoters, thereby regulating transcription. This VDR-mediated gene regulation underlies vitamin D's capacity to shape both innate and adaptive immune responses.

In the innate arm, 1,25(OH)2D3–VDR signaling promotes monocyte-to-macrophage differentiation, enhances expression of antimicrobial peptides such as cathelicidin, and upregulates HLA-DR and co-stimulatory molecules on dendritic cells, facilitating more effective antigen presentation (5). Concomitantly, VDR activation modulates cytokine and chemokine profiles within the innate compartment, fostering an environment that supports pathogen clearance while limiting excessive inflammation.

Within adaptive immunity, VDR signaling exerts a potent suppressive effect on Th1- and Th17-mediated inflammation by directly downregulating transcription of IFN-γ and IL-17, respectively. At the same time, it promotes the expansion and functional stability of regulatory T cells (Tregs), enhancing IL-10 production and strengthening peripheral tolerance. (5, 9) B-cell activity is similarly restrained: VDR activation inhibits plasma cell differentiation and autoantibody secretion, thereby reducing humoral autoimmunity (10). Figure 1 revealed the relationship between vitamin D and immune modulation.

Beyond direct effects on immune cells, Sîrbe et al. (5) have reported that VDR signaling contributes to the maintenance of gut barrier integrity and the modulation of microbiota composition, mechanisms increasingly implicated in the pathogenesis of ADs. Clinical investigations echo these molecular insights. Zhao et al. (11) and Manousaki et al. (12) observed that vitamin D supplementation was associated with lower incidence rates and reduced disease severity in SLE and T1D cohorts. Taken together, these findings highlight VDR-dependent pathways as promising targets for therapeutic strategies aiming to recalibrate immune homeostasis in autoimmune disorders.

## 4 The efficacy and safety of high-dose vitamin D supplementation in modulating immune profiles in autoimmune disease

High-dose cholecalciferol is increasingly investigated to overcome VDR sensitivity loss in genetically predisposed individuals, where pathogen-mediated VDR downregulation, chronic glucocorticoid exposure, environmental toxins, low UVB exposure, and aging all contribute to impaired vitamin D signaling and elevated autoimmune risk (13). However, the efficacy of high-dose vitamin D remains debated (14–19). Table 1 summarizes clinical trials of high-dose vitamin D in autoimmune diseases.

Several studies have revealed high-dose vitamin $D_3$ supplementation in autoimmune diseases but reported no

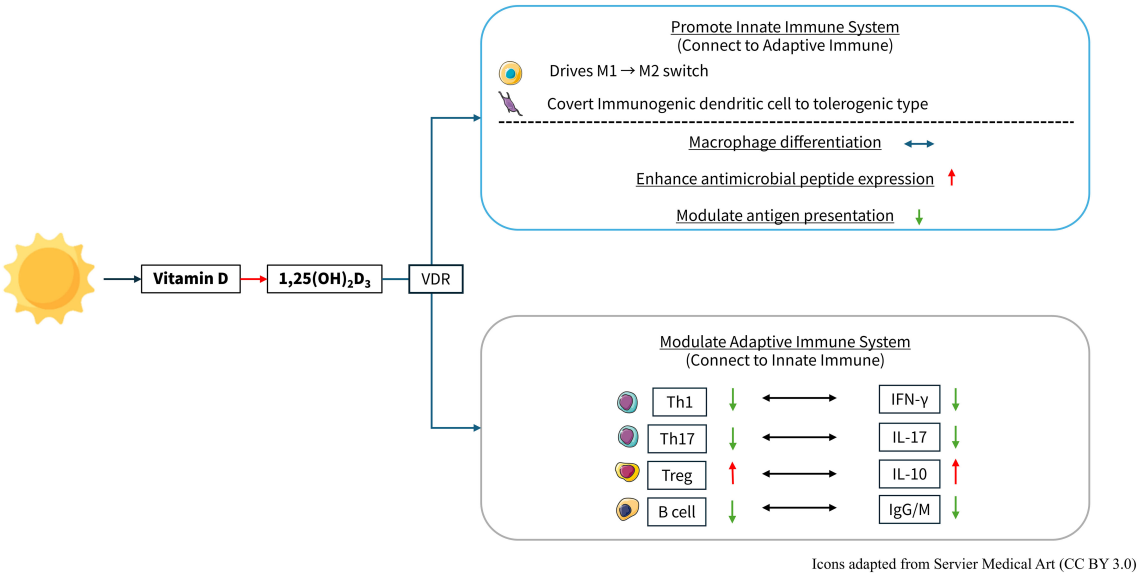


FIGURE 1
The active form of vitamin D, 1,25-dihydroxyvitamin $D_3$, exerts immunomodulatory effects on both the innate and adaptive immune systems. Vitamin D and Immune Regulation. 1,25-$(OH)_2D_3$: vitamin D, 1,25-dihydroxyvitamin $D_3$; VDR, Vitamin D receptor; Th, T helper cell; IFN-γ, interferon-γ; IL, interleukin; Treg, regulatory T cells; IgG, immunoglobulin G; IgM, immunoglobulin M. https://smart.servier.com/.

significant clinical benefits. However, many of these studies have important methodological limitations that may affect the interpretation of their findings. For instance, the study by Cassard SD et al. (14) involved a relatively small sample size, lacked a placebo control, and was conducted only in the United States, limiting its generalizability. Similarly, the trial by Aranow C et al. (15) showed patients with SLE, was also restricted by a small participant pool, a short follow-up period of only 12 weeks, and a predominantly female population with varied baseline disease activity, introducing potential heterogeneity in treatment response. The study by Grove-Laugesen D et al. (16) was also limited to a single country (Denmark), and included mostly female participants, raising concerns about gender representation and external validity. In the trial by Nwosu BU. et al. (17), the limitations included a small sample size, a narrow age range, a single-country of Denmark. Lastly, the study conducted by Fernandes AL et al. (19) assessed the effects of a single high-dose intervention (200,000 IU) and faced challenges such as a limited sample size for 1 year analysis and reliance on self-reported symptoms, which may compromise the reliability of the outcome assessment.

Brustad et al. (20) conducted a systematic review and meta-analysis of 32 randomized trials (n = 8,400 children, doses 1,200–10,000 IU/day; bolus up to 600,000 IU) and found no increase in serious adverse events, including hypercalcemia or nephrolithiasis. In France, Thouvenot et al. (21) treated 316 early MS patients with 100,000 IU cholecalciferol biweekly for 24 months, observing reduced relapse rates and MRI lesion accumulation in clinically isolated syndrome and relapsing–remitting MS. Bendix et al. (22) administered 20,000 IU/day for seven weeks to 40 Crohn's disease patients, reporting a 25% decrease in the need for infliximab dose escalation.

These data suggest that high-dose vitamin D can safely modulate immune profiles, decrease disease activity, and potentiate existing therapies in autoimmune disorders. Nonetheless, optimal dosing regimens, especially for individuals with profound deficiency or specific disease phenotypes, require further large randomized trials to balance maximal immunomodulation against potential toxicity.

## 5 Future research and study design

Future research should focus on well designed, randomized controlled trials that enroll patients based on their baseline 25(OH) D levels, VDR related genetic variants, and specific autoimmune phenotypes. Such trials ought to include dose finding phases to identify effective yet safe vitamin D regimens, serial immunological assessments (e.g., Th1/Th17 cytokines, Treg frequencies, autoantibody titers), and disease specific clinical endpoints (relapse rates, imaging markers, or activity indices). Close monitoring for hypercalcemia and renal effects will ensure safety, while stratified analyses will reveal which patient subgroups derive the greatest benefit from high dose supplementation.

TABLE 1 Summary of high dose vitamin D for autoimmune disease.

| Author | Participants | Vitamin D dose | Disease | Age | Sex | Study location | Results | Study design | Level of evidence |
|---|---|---|---|---|---|---|---|---|---|
| Cassard S.D. et al. (14) | 172 | 600 IU/day (LDVD) or 5000 IU/ day (HDVD) | Relapsing- remitting multiple sclerosis | 18–50 years | Not specified | 16 neurology clinics in the US | HDVD did not reduce disease activity | RCT | Level I |
| Aranow C. et al. (15) | 57 | 2,000 or 4,000 IU/day | Systemic lupus erythematosus | 36–39 years | Female 94.4% | 8 centers in US | Failed to diminish the IFN signature in vitamin D–deficient Systemic lupus erythematosus | RCT | Level I |
| Grove-Laugesen D. et al. (16) | 278 | 2800 IU/day | Graves' Disease | 44 ± 14 years | Female 79% | 7 endocrine clinics, Denmark | High-dose vitamin D does not recommend for Graves' Disease | RCT | Level I |
| Nwosu B.U. et al. (17) | 36 | 50000 IU/ week (2 months), then Q2W (10 months) | Type 1 diabetes | 10–21 years | Not specified | Not specified | Partial clinical remission | RCT | Level I |
| Finamor D.C. et al. (18) | 9 psoriasis and 16 vitiligo | vitamin D3 35,000 IU/day for 6 months | Psoriasis and vitiligo | 47.8 years old | Female 28% | 2 clinics, Brazil | Effective and safe for vitiligo and psoriasis patients | Retrospective | Level II |
| Fernandes A.L. et al. (19) | 144 | vitamin D3 200,000 IU | moderate to severe COVID-19 | 54.3 years old | Female 46.5% | São Paulo,and Ibirapuera Field Hospital, Brazil | not support symptoms control | RCT | Level I |
| Thouvenot E. et al. (21) | 316 | oral cholecalciferol 100–000 IU/2 weeks for 24 months. | Isolated syndrome and early relapsing remitting multiple sclerosis | 18–55 years | Female 69.6% | 36 Multiple sclerosis centers, France | Reduced disease activity in Isolated syndrome and early relapsing remitting multiple sclerosis | RCT | Level I |
| Bendix M. et al. (22) | 40 | 20,000 IU/day for seven weeks and infliximab infusion with 5 mg/kg | Crohn's Disease | Not specified | Not specified | Aarhus University Hospital | Reduced infliximab dose escalation | RCT | Level I |

(LDVD), Low dose vitamin D3; (HDVD), high dose vitamin D3; RCT, Randomized Controlled Trial; US, United States; IFN, interferon; Q2W, every other week.



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## 6 Conclusion

Vitamin D is essential for immune balance, and its deficiency contributes to autoimmunity. High-dose vitamin D can rebalance Th1/Th17 versus Treg activity, lessen disease flares, and boost standard therapies without raising serious safety concerns. Tailoring supplementation to patients' baseline levels and genetics offers a promising adjunct in managing autoimmune diseases.

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## References

1. Hileman CO, Malakooti SK, Patil N, Singer NG, McComsey GA. New-onset autoimmune disease after COVID-19. *Front Immunol*. (2024) 15:1337406. doi: 10.3389/fimmu.2024.1337406

2. Robinson WH, Younis S, Love ZZ, et al. Epstein–Barr virus as a potentiator of autoimmune diseases. *Nat Rev Rheumatol*. (2024) 20:729–40. doi: 10.1038/s41584-024-01167-9

3. Abend AH, He I, Bahroos N, et al. Estimation of prevalence of autoimmune diseases in the United States using electronic health record data. *J Clin Invest*. (2025) 135:e178722. doi: 10.1172/JCI178722

4. Bouillon R, Manousaki D, Rosen C, et al. The health effects of vitamin D supplementation: evidence from human studies. *Nat Rev Endocrinol*. (2022) . 18:96–110. doi: 10.1038/s41574-021-00593-z

5. Sîrbe C, Rednic S, Grama A, et al. An update on the effects of vitamin D on the immune system and autoimmune diseases. *Int J Mol Sci*. (2022) 23:9784. doi: 10.3390/ijms23179784

6. Daryabor G, Gholijani N, Rezaei Kahmini F. A review of the critical role of vitamin D axis on the immune system. *Exp Mol Pathol*. (2023) 132–133:104866. doi: 10.1016/j.yexmp.2023.104866

7. Artusa P, White JH. Vitamin D and its analogs in immune system regulation. *Pharmacol Rev*. (2025) 77:100032. doi: 10.1016/j.pharmrev.2024.100032

8. Hupperts R, Smolders J, Vieth R, Holmøy T, Marhardt K, Schluep M, et al. Randomized trial of daily high-dose vitamin $D_3$ in patients with RRMS receiving subcutaneous interferon β-1a. *Neurology*. (2019) 93:e1906–16. doi: 10.1212/WNL.0000000000008445

9. Khosravi-Largani M, Pourvali-Talatappeh P, Rousta AM, et al. A review on potential roles of vitamins in incidence, progression, and improvement of multiple sclerosis. *eNeurologicalSci*. (2018) 10:37–44. doi: 10.1016/j.ensci.2018.01.007

10. Feng W, Ma XN, Wu Q, et al. Serum 25-hydroxyvitamin D levels and dermatomyositis: A 2-sample mendelian randomization study. *Int J Rheum Dis*. (2024) 27:e15204. doi: 10.1111/1756-185X.15204

11. Zhao SS, Mason A, Gjekmarkaj E, et al. Associations between vitamin D and autoimmune diseases: Mendelian randomization analysis. *Semin Arthritis Rheum*. (2023) 62:152238. doi: 10.1016/j.semarthrit.2023.152238

12. Manousaki D, Harroud A, Mitchell RE, et al. Vitamin D levels and risk of type 1 diabetes: A Mendelian randomization study. *PLoS Med*. (2021) 18:e1003536. doi: 10.1371/journal.pmed.1003536

13. Lemke D, Klement RJ, Schweiger F, et al. Vitamin D resistance as a possible cause of autoimmune diseases: A hypothesis confirmed by a therapeutic high-dose vitamin D protocol. *Front Immunol*. (2021) 12:655739. doi: 10.3389/fimmu.2021.655739

14. Cassard SD, Fitzgerald KC, Qian P, et al. High-dose vitamin D3 supplementation in relapsing-remitting multiple sclerosis: A randomized clinical trial. *eClinicalMedicine*. (2023) 59:101957. doi: 10.1016/j.eclinm.2023.101957

15. Aranow C, Kamen DL, Dall'Era M, et al. Randomized, double-blind, placebo-controlled trial of the effect of vitamin D3 on the interferon signature in patients with systemic lupus erythematosus. *Arthritis Rheumatol*. (2015) 67:1848–57. doi: 10.1002/art.39108

16. Grove-Laugesen D, Ebbehoj E, Watt T, Riis AL, Østergaº rd T, Bruun BJ, et al. Effect of vitamin D supplementation on Graves' disease: The DAGMAR trial. *Thyroid*. (2023) 33:1110–8. doi: 10.1089/thy.2023.0111

17. Nwosu BU. Guidance for high-dose vitamin D supplementation for prolonging the honeymoon phase in children and adolescents with new-onset type 1 diabetes. *Front Endocrinol*. (2022) 13:974196. doi: 10.3389/fendo.2022.974196

18. Finamor DC, Sinigaglia-Coimbra R, Neves LCM, et al. A pilot study assessing the effect of prolonged administration of high daily doses of vitamin D on the clinical course of vitiligo and psoriasis. *Dermatoendocrinol*. (2013) 5:222–34. doi: 10.4161/derm.23385

19. Fernandes AL, Sales LP, Santos MD, Caparbo VF, Murai IH, Pereira RMR. Persistent or new symptoms 1 year after a single high dose of vitamin D3 in patients with moderate to severe COVID-19. *Front Nutr*. (2022) 9:979667. doi: 10.3389/fnut.2022.979667

20. Brustad N, Yousef S, Stokholm J, et al. Safety of high-dose vitamin D supplementation among children aged 0 to 6 years: A systematic review and meta-analysis. *JAMA Netw Open*. (2022) 5:e227410. doi: 10.1001/jamanetworkopen.2022.7410

21. Thouvenot E, Laplaud D, Lebrun-Frenay C, Derache N, Page EL, Maillart M, et al. High-dose Vitamin D in clinically isolated syndrome typical of multiple sclerosis: the D-LayMS randomized clinical trial. *JAMA*. (2025) 333(16):1413–22. doi: 10.1001/jama.2025.1604

22. Bendix M, Dige A, Jørgensen SP, et al. Seven weeks of high-dose vitamin D treatment reduces the need for infliximab dose-escalation and decreases inflammatory markers in Crohn's disease during one-year follow-up. *Nutrients*. (2021) 13:1083. doi: 10.3390/nu13041083

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# Passive physical barrier modulates UVB-induced METosis-related MPO expression and activity, 25-hydroxyvitamin D3-1alpha-hydroxylase, and the shift of tissue-resident macrophages toward M1-associated iNOS

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**Background:** This study investigated the role of UVB radiation and the influence of a simulated passive barrier on the enzymatic conversion of 25-hydroxyvitamin D3 (25(OH)$D_3$) by 1-alpha hydroxylase and its effects on the functional activity of tissue-resident macrophages.

**Methods:** Murine peritoneal tissue-resident macrophages (PRMφs) were exposed to three conditions: (1) Baseline (Control group), with no light exposure; (2) UVB+/RF- group, exposed to UVB rays without passive barrier simulation; (3) UVB+/RF+ group, UVB exposure with a thin layer of rat fur to mimic the passive barrier on the skin.

**Results:** UVB exposure did not significantly alter 25$OHD_3$ levels across groups but led to a marked downregulation of 1-alpha hydroxylase, particularly with the

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simulated barrier. UVB slightly enhanced phagocytosis and significantly increased nitric oxide (NO) and hydrogen peroxide ($H_2O_2$) production. Moreover, hypochlorous acid (HOCl) levels were significantly upregulated in the UVB-exposed PRMφ group, whereas they returned to baseline levels in the UVB+/RF + group. Furthermore, both MPO expression and activity were markedly upregulated after UVB exposure and downregulated in UVB+/RF+ group, suggesting that the overall effect of UVB on METosis-related MPO activity was substantially attenuated by the simulated barrier (for both comparisons, $p < 0.001$ by ANOVA test). Additionally, UVB exposure shifted PRMφs toward M1-phenotype, as evidenced by decreased ARG1 activity and increased iNOS activity and $M1_{(iNOS)}$-to-$M2_{(ARG1)}$ ratio. Additionally, UVB downregulated catalase (CAT) activity and intracellular glucose ($_{i}$GLU) levels, with a stronger effect in the barrier group. While UVB increased total cellular cholesterol content ($_{tcc}$CHOL), this effect was mitigated by the barrier. Finally, intracellular free calcium ion ($_{if}Ca^{2+}$) levels remained unaffected by UVB but showed a slight increase with the barrier.

**Conclusions:** UVB exposure enhances tissue-resident macrophage function in a preclinical rat model, increasing respiratory burst, phagocytosis, and M1-like polarization. The simulated barrier modulates these effects, notably by reducing MPO expression and METosis-related activity, which suggests a potential attenuation of excessive inflammation. These findings provide valuable insights relevant to human immune modulation and support further translational research. Future studies should investigate the role of circadian rhythms and other cell types in UVB- and vitamin D-mediated immune modulation.

KEYWORDS

**25-hydroxyvitamin D3-1alpha-hydroxylase, physical barrier simulation, peritoneal tissue-resident macrophages, METosis-related MPO expression and activity, UVB exposure, M1 macrophage-associated iNOS activity**

# 1 Introduction

Vitamin D (VD), recognized both as a vitamin and pre-hormone, assumes a crucial role in diverse physiological processes. Its active form, 1,25-dihydroxyvitamin $D_3$ (1,25(OH)$_2D_3$), is integral for bone health and calcium balance (1, 2). Although conventionally linked to calcium metabolism and skeletal well-being, recent studies have uncovered its role as an immunomodulator (3).Various immune cells, including macrophages (4), express the vitamin D receptor (VDR), a nuclear receptor and ligand-activated transcription factor belonging to the superfamily of nuclear receptors (5). Notably, macrophages not only respond to vitamin D but also possess the ability to produce bioactive vitamin D (6).

The circulating form of vitamin D, 25-hydroxyvitamin D (25 [OH]D), is predominantly bound to the vitamin D-binding protein (DBP) in the bloodstream, while 10–15% is bound to albumin, and less than 1% of circulating vitamin D exists in an unbound form (7). This binding stabilizes 25(OH)D, prolongs its half-life, and facilitates its delivery to target cells. The DBP25(OH)D complex interacts with specific receptors on the cell surface, such as megalin and cubilin, enabling endocytosis and internalization of the complex (8, 9). Inside the cell, 1,25(OH)$_2D_3$ binds to the VDR, located in either the cytosol or the nucleus. The activated VDR then forms a heterodimer with the retinoid-X receptor (RXR), which binds to DNA, thereby stimulating the production of antibiotic peptides like cathelicidin and β-defensin (10). Moreover, it is important to note that, unlike other steroid hormones in the body, which are synthesized directly from cholesterol, vitamin D synthesis requires both the 7-dehydrocholesterol precursor and UVB rays (290–320 nm) (11). In the absence of this reaction, humans must rely on dietary vitamin D intake, available in two forms: vitamin $D_2$ (ergocalciferol) and vitamin $D_3$ (cholecalciferol) (12, 13).

UVB at the Earth's surface is influenced by various physical and temporal factors, including latitude, altitude (14), season (12), and weather conditions. Biological factors, such as skin melanin (13), and personal, cultural, and behavioral factors including clothing

(15), holiday habits (16), and sunscreen use (17) as well as the extent of exposed body surface area (18), also affect the efficiency of UVB penetration, its interaction with skin cells for vitamin D synthesis, and the subsequent bioconversion process.

Moreover, it is of great importance to note that based on association studies, it has been reported that vitamin D intake, as well as circulating levels of 25(OH)D, are not systematically altered in certain pathological conditions, including autoimmune diseases, allergies, and other immune-related disorders. Conversely, insufficient vitamin D intake or reduced circulating levels have been detected in individuals who appear to be in good health. These observations emphasize that circulating 25(OH)D levels, although commonly used as standard indicators, may not be sufficient to fully assess the status of vitamin D and its role in both pathological and healthy contexts. These variations suggest that additional factors, such as differences in sun exposure, diet, or genetic capacity to metabolize vitamin D, as well as the possible presence of compensatory mechanisms, may influence the utilization and metabolism of vitamin D, including its conversion into the bioactive form, *i.e.*, $1,25(OH)_2D_3$. It is likely that these compensatory mechanisms function normally in healthy conditions but may be altered in pathological contexts.

All these observations highlight the need to study the 'active reservoir' form of vitamin D, which is ready to be utilized (when necessary) in response to external stimuli, such as activation by inflammatory factors, rather than focusing exclusively on its circulating levels. This vitamin D form could correspond to 'non-circulating vitamin D (tissue $25(OH)D_3$ levels)', produced locally in keratinocytes under the influence of UVB rays and directly used by local immune cells, such as tissue-resident macrophages. In this way, it would play an essential and direct role in modulating the immune response, compensating for the effect of circulating vitamin D deficiency. This form is not found in the bloodstream, but could be crucial for the local functions of immune cells. Here, we hypothesize that tissue-resident macrophages could be influenced by UVB exposure. This hypothesis is based on two aspects: first, their capacity to reprogram their cholesterol metabolism upon activation and exposure to inflammatory signals (19); and second, their involvement in the synthesis of bioactive molecules, including vitamin D metabolites, serving as a source of extrarenal production of $1,25(OH)_2D_3$ (20). Based on the aforementioned, this pioneering study aims to evaluate the impact of UVB radiation and passive physical barrier on both the intracellular conversion of $25(OH)D_3$ and the overall functional activities of tissue-resident macrophages.

# 2 Materials and methods

This study aims to assess the impact of UVB radiation and the potential influence of passive physical barriers on the enzymatic conversion activity of $25(OH)D_3$ and the overall functional activities of tissue-resident macrophages, as illustrated in the flowchart (Figure 1). Assays were conducted on whole cells, supernatants, or whole cell lysates.

## 2.1 Cell-based experimental model

In our study, we used murine peritoneal tissue-resident macrophages (PRMφs) as an experimental model, given their ability to metabolize $25(OH)D_3$ to $1,25(OH)_2D_3$ (21), their higher stability (22), their high responsiveness to inflammatory signals, their capacity to migrate to the skin (23) along avascular routes under inflammatory conditions (24), their role in regulating inflammation (25), their ease of accessibility, their remarkable functional plasticity, and their well-established involvement in immune responses comparable to tissue-resident macrophages in other organs. Additionally, their structural and developmental similarities with other tissues, such as the skin—including comparable surface area, mesodermal origin (26), and a shared embryonic coelomic cavity—further support their relevance in studying local immune modulation. Moreover, it is not merely because normal skin lacks a sufficient number of macrophages for planned assays, but more specifically because PRMφs are commonly used for *in vitro* assays, as they are distinguished by their maturity compared to other macrophage populations, characterized by enhanced stability in their phenotype and functionality (27).

Although the tissue was sourced from *Wistar* rats, a nocturnal species (28) that naturally displays circadian variations in metabolic and hormonal activity, the *in vitro* nature of our model allows us to minimize systemic circadian influences and focus on direct cellular responses to UVB exposure. Nonetheless, we acknowledge that macrophages possess intrinsic circadian clockworks that can remain functional even *ex vivo* (29, 30), suggesting that some degree of circadian imprinting might persist at the cellular level. While this influence is expected to be limited under controlled culture conditions, it remains an important factor to consider and explore in future *in vivo* studies.

## 2.2 PRMφs collection and preparation

The experiments were performed on primary PRMφs isolated from healthy 8-week-old female *Wistar rats* weighing 60–70 grams, with four independent repetitions (n = 12 per group), each conducted in duplicate or triplicate. To obtain PRMφs, peritoneal exudate cells (PECs) were collected from animals (31) in a sterile manner from the peritoneal cavity at different time intervals through lavage using a minimum of 10 mL sterile ice-cold phosphate-buffered saline (PBS) twice. This was achieved by intraperitoneal injection, followed by gentle abdominal massage, in accordance with established procedures as outlined elsewhere (32–35). PEC were seeded at $2 \times 10^6$ cells/mL in cell culture media and incubated for 2 h at 37°C in a humidified atmosphere containing 5% $CO_2$. The culture medium was changed regularly to maintain cell health. Non-adherent cells were removed by washing vigorously three times with warm RPMI-1640 medium. To activate PRMφs, adherent cells were incubated with 10 ng/mL LPS (*Escherichia coli* O26:B6, Sigma-Aldrich, St. Louis, MO, USA) in RPMI-1640 culture medium, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cell

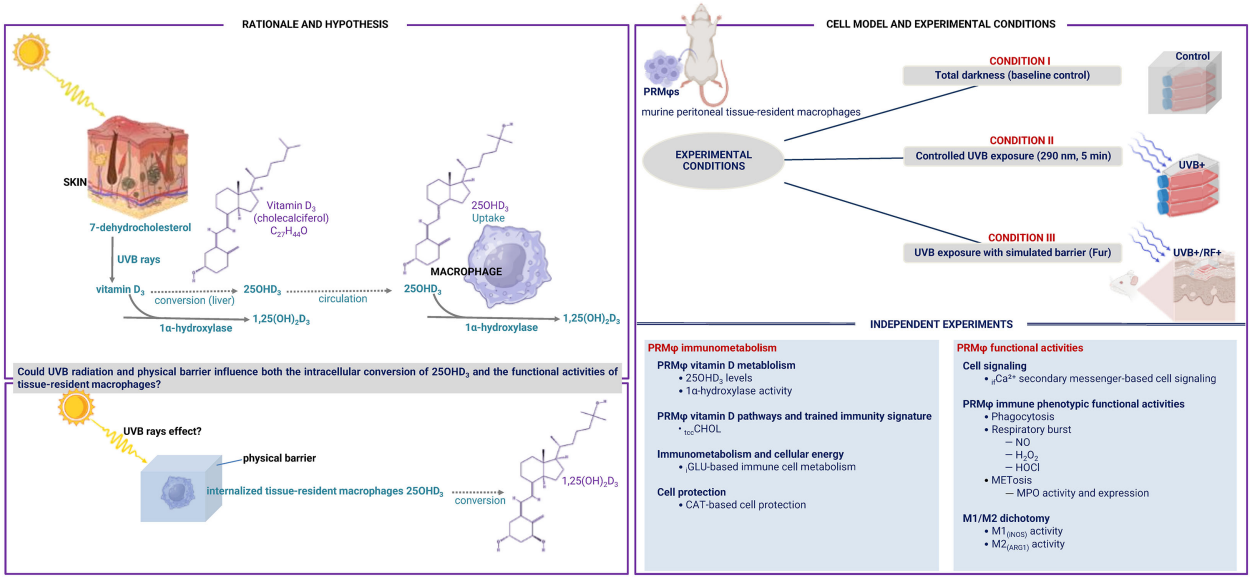


FIGURE 1
Flowchart of the current study. This study investigated the role of UVB radiation and the potential influence of passive physical barriers, such as simulated clothing, in preventing UVB exposure, on the enzymatic conversion of 25-hydroxyvitamin D3 (25(OH)D3) by 1-alpha hydroxylase, and assessed their effects on the phenotypic functional activities of tissue-resident macrophages. Experiments were conducted using murine peritoneal tissue-resident macrophages under three conditions: (i) Baseline (Control group), (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group).

population density and viability were evaluated using the trypan blue exclusion test (TBET) with a hemocytometer under photonic microscopy (Zeiss, Jena, Germany) (36). Cell viability percentage was calculated using the standard formula: $\text{Viability}(\%) = \left(\frac{\text{Number of viable cells}}{\text{Total number of cells}}\right) \times 100$. After the adhesion step, the purity of PRMφs was analyzed using a Floid Cell Imaging Station (Thermo Fisher Scientific, MA, USA), routinely achieving a purity greater than 90%.

## 2.3 Experimental groups and light and barrier conditions

The experiments were conducted under three conditions:

1. **Control group:** This group was used to assess baseline levels of 25(OH)$D_3$, its conversion by 25-hydroxyvitamin D3-1α-hydroxylase, and overall PRMφ functional activities in the absence of light exposure.
2. **Controlled exposure to UVB rays (UVB+ group):** This group underwent a 5-min exposure to UVB radiation to mimic sunlight exposure (37), using a UV lamp calibrated to emit biologically relevant wavelengths at 290 nm.
3. **UVB exposure with a simulated physical barrier (RF+ group):** In this group, a thin layer of rat fur was placed above the cell culture system to partially mimic the passive barrier on the skin, assessing the impact of clothing-like coverage on UVB-mediated vitamin D metabolism and its effects on macrophage activity.

## 2.4 Rationale for the selected passive physical barrier

The use of *Wistar* rat fur was selected due to its unique physical characteristics, making it a more suitable model for our experimentation. Unlike humans, rats, as animals, do not wear clothing in their natural environment, rendering their fur a functional equivalent for protection against external elements, including UV rays. Furthermore, the choice of *Wistar* rat fur was specifically aligned with the source of the studied macrophages, which were derived from *Wistar* rats, ensuring biological consistency in the experimental setup.

Moreover, *Wistar* rat fur, due to its density and texture, could provide a relatively homogeneous and passive physical barrier that might realistically simulate skin protection against UVB rays. Unlike textiles, which vary widely in composition (e.g., synthetic *vs.* natural fibers), weave density, and industrial treatments (38–42), rat fur offers a reproducible, endogenous, and uniform material. Its fibrous structure could provide a consistent and quantifiable light-attenuating interface that partially mimics some clothing while avoiding confounding factors introduced by textile variability. This natural barrier might possess light-filtering properties akin to clothing, with the added advantage of being more easily quantifiable and manipulable in a controlled environment. Its fibrous composition also allows partial diffusion of UVB rays, which could be essential for studying the impact of reduced sun exposure on vitamin D metabolism and macrophage activity. Furthermore, the structure of the fur might replicate an effective physical barrier comparable to that of some clothing while being

less susceptible to variations inherent in different fabric types, as would be the case with conventional garments. Notably, since rats (including albino *Wistar* strains) are nocturnal (43, 44) and not naturally exposed to prolonged sunlight, their fur may incidentally limit UV radiation penetration. However, this is likely a byproduct of its primary functions (e.g., insulation) rather than a specific photoprotective adaptation—similar to how human hair (which provides both physical shading and an additional effect due to pigmentation that enhances UV absorption) can passively protect against both UVB and UVA radiation (45). Taken together, these considerations underscore the relevance of using *Wistar* rat fur as a passive physical barrier in our experimental model. Its consistent structure, endogenous origin, and natural light-attenuating properties provide a biologically and mechanically appropriate means to simulate reduced exposure to environmental stressors—including UV radiation—while ensuring reproducibility and relevance to the source of the studied macrophages.

## 2.5 Immune cell lysis assay

Cells were lysed for protein, $_{tcc}$CHOL, $H_2O_2$, and arginase activity assays. Briefly, cells were detached from culture plates, and the cell pellets were lysed using 500 µL of 0.1% Triton X-100 and incubated for 30 min. A mixture of Tris-HCl and $MnCl_2$ was added to stop the reaction, and the lysate was collected (46).

## 2.6 Total protein assay

Protein concentration in the cell lysates was spectrophotometrically measured at 540 nm using a commercial kit (Thermo Fisher Scientific, Inc., Middletown, CT).

## 2.7 25(OH)$D_3$ assay

Cellular vitamin D 25(OH)$D_3$ levels were measured using a chemiluminescent microparticle immunoassay (CMIA) on the ARCHITECT 8200 autoanalyzer (ABBOTT®) at the Biochemistry Department of Tlemcen University Hospital Center. Due to the low volume of the PRMφ culture supernatant, a volume-adjusted serum with a known concentration of vitamin D was used to ensure detectability, and the vitamin D concentration in the supernatant alone was then determined by subtracting the serum concentration from the total measured concentration.

## 2.8 1α-hydroxylase activity based-25(OH)$D_3$ bioconversion assay

To measure PRMφ 1α-hydroxylase activity, we incubated cell lysates with serum containing a known concentration of 25(OH)$D_3$ for two distinct time periods—30 min and 2 h, using the two-point technique (47, 48). After each incubation period, we measured the concentration of 25(OH)$D_3$ in the mixture. The activity of 1α-hydroxylase was calculated by determining the change in 25(OH)$D_3$ concentration over time and normalizing this change by the amount of protein in the sample and the time interval, using the following formula: $1\alpha - hydroxylase\ activity = \frac{[25(OH)D_3]_{initial} - [25(OH)D_3]_{final}}{t \times P}$, where $t$ represents the incubation time and $P$ the amount of protein in the sample (in milligrams). This approach assumes that the decrease in 25(OH)$D_3$ correlates with the production of 1,25(OH)$_2$$D_3$, providing an estimate of the enzyme activity based on its effect on the substrate concentration.

## 2.9 PRMφs functional phenotypic activities

The functional phenotypic activity of PRMφs was assessed by phagocytosis, respiratory burst activity, and METosis-related MPO expression and activity.

### 2.9.1 ROS-dependent NBT-based functional phagocytosis assay

The phagocytosis activity was evaluated using the nitro-blue tetrazolium (NBT, Sigma-Aldrich, Germany) assay, as previously described (49, 50). This semi-quantitative test measures the ability of phagocytic cells to reduce NBT to formazan, a black-blue crystal precipitate, reflecting the production of superoxide ($O_2^-$) during phagocytosis (51). To perform the assay, 100 µL of cell suspension was mixed with 100 µL of NBT solution, followed by incubation for 15 min at 37°C and an additional 15 min at room temperature, allowing the formation of formazan crystals within phagocytes. The extent of phagocytosis was quantified by measuring the reduction of soluble yellow NBT dye to insoluble black-blue formazan crystals within each PRMφs, using the Optika Microscope Camera and the Java-based image analysis software Fiji/ImageJ2 (NIH, USA). The level of phagocytosis was expressed as the percentage of NBT-positive cells.

### 2.9.2 Respiratory burst assay

Oxidative/respiratory burst was performed by measuring NO production, $H_2O_2$ levels (52), and HOCl levels (53).

**a) NO assay**

NO production levels were spectrophotometrically determined on supernatants based on the sensitive colorimetric Griess reaction measuring the accumulation of oxidative metabolites (NOx, nitrite [$NO_2$-] and nitrate [$NO_3$-]). The assay involved the use of vanadium chloride (III) (VCIII), and Griess reagent (Sigma-Aldrich, St. Louis, MO, USA). First, 50 µL of supernatants from the PRMφs culture was seeded into 96-well microtiter plates with 50 µL VCIII (8 mg/mL) and 25 µL of Griess reagent. The absorbance was read at 540 nm using the ELISA plate reader (Biochrom Anthos 2020, Cambridge, UK). After 30 min incubation at 37°C, concentrations of NOx were determined from a linear standard curve established by 0–150 µmol/L sodium nitrite ($NaNO_2$).

**b) $H_2O_2$ assay**

$H_2O_2$ levels were measured by the adapted method of Pick and Keisari. This method consists of the use of a buffered Phenol Red

Solution (PRS), which contains a peroxide assay buffer (PAB) (5.0 mM $K_2HPO_4$, 1.0 mM $KH_2PO_4$, 140 mM NaCl, 0.5 mM glucose adjusted to pH 7.4), 0.28 mM (0.1 g/L) of phenol red (phenolsulfonphthalein) and 8.5 U/L (50 µg/mL) of horseradish peroxidase (HRPO, EC 1.11.1.7). The PRS solution was prepared immediately prior to the assay, by adding phenol red and HRPO to 2.1 mL of PAB at a final concentration of 0.46 mM and 0.046 U/mL, respectively. The supernatant was added to the assay mixture at a ratio of 1 to 4 and then incubated for 30 min at 37°C. To stop the reaction, 10 µL of 1 N NaOH was added. The $H_2O_2$ levels were measured spectrophotometrically at 610 nm against a blank containing buffered PRS and NaOH at the appropriate concentrations. A standard curve was prepared by the use of sequential dilutions of 30% $H_2O_2$. Concentration of $H_2O_2$ was expressed as nmol per $2 \times 10^5$ cells per mL (54).

**c) HOCl assay**

The levels of HOCl were determined in cell supernatants by measuring the decomposition of $H_2O_2$ based on the *in vitro* oxidation of bromide (Br) by HOCl produced by the activated cells in the presence of chloride anion ($Cl^-$) released into the extracellular medium. Therefore, 10 µL of cell supernatant in PBS was added to 10 µL of 22 mM sodium bromide (NaBr). The concentration of HOBr was determined by measuring its absorbance at 330 nm immediately after 30 min and 1 h of incubation (pH 12, $\varepsilon_{330}$ = 332 $M^{-1}cm^{-1}$) (55). The level of HOCl was estimated using a standard prepared under the same conditions by adding 10 µL of 22 mM NaBr to 10 µL of 20 mM HOCl in PBS (pH 7.4) (56–58).The values were obtained using a standard curve created by serial dilutions of 5.25% (w/v) HOCl in PBS at pH 7.4.

### 2.9.3 METosis-related MPO expression and activity assays

Myeloperoxidase (MPO, E.C.1.11.1.7) activity was assessed as a critical marker for the creation of macrophage extracellular traps (METs) (58). It was quantified using both a direct catalytic activity and immunofluorescence assays. For the catalytic activity assay, HOCl values were normalized to total protein content (59), with one unit of MPO activity defined as the amount catalyzing 1 µmol of HOCl per milligram of protein per 60 min and expressed percentage active chlorine per milligram of protein per one hour. The MPO expression was assessed by immunofluorescence assay based on the use of an anti-MPO antibody labeled with FITC (clone 5B8, Ms IgG1, BD Biosciences) for direct detection, performed on the Floid Cell Imaging Station (Thermo Fisher, MA, USA). CellProfiler 4.2.6 (Broad Institute, USA) was used to visualize the input image, delineate the contours of the MPO-marked fluorescent cell, segment the entire fluorescent cell, measure pixel intensity, and then calculate the area and perimeter of the MPO-marked cell. To ensure accurate comparisons and account for differences in cell size, normalized fluorescence intensity (NFI) was calculated as the weighted mean intensity (WMI) divided by the cell area ($\mu m^2$), providing a measure of fluorescence intensity per unit area, and expressed as a.u./$\mu m^2$.

## 2.10 M1/M2 dichotomy

The $M1_{(iNOS)}/M2_{(ARG1)}$ dichotomy was determined mathematically by measuring the M1-to-M2 ratio (60, 61).

**a) $M1_{(iNOS)}$ activity assay**

iNOS (EC 1.14.13.39) activity was determined by normalizing the concentration of NO to the amount of protein per well, and the results were expressed as pmol per mg of protein per 30 min (62).

**b) $M2_{(ARG1)}$ activity assay**

Arginase 1 activity (ARG1, EC 3.5.3.1) was assessed by a spectrophotometric assay based on evaluating the concentration of urea in PRMφs lysates after the addition of L-arginine (63). Firstly, 25 µL of cell lysates were inactivated by heating for 10 min at 56°C, then mixed with 200 µL aliquot of arginine buffer (10 mM L-arg, pH 6.4), and incubated at 37°C for 1 h. The reaction was stopped by adding acetic acid. The concentration of urea generated after arginine catabolism by arginase was measured at 600 nm (64). ARG1 activity was expressed as nanomoles of urea released per mg of proteins per 1 h (46).

## 2.11 CAT-based cell protection assay

CAT (EC 1.11.1.6) activity was determined by spectrophotometric analysis of $H_2O_2$ decomposition. Fifty microliters of cell lysates were combined with 50 µL of $H_2O_2$ and 50 µL of physiological saline. After vortexing and a 5-min incubation, 500 µL of titanium sulfate ($TiSO_4$) was added and vortexed. Absorbance at 420 nm was then measured, using a blank of physiological saline and $TiSO_4$.

## 2.12 Trained immunity activation-based $_{tcc}$CHOL signature assay

$_{tcc}$CHOL levels were measured spectrophotometrically in cell lysates by cholesterol oxidation. Free cholesterol was obtained from cholesterol esters using cholesterol ester hydrolase (EC 3.1.1.1.13), and the resulting $H_2O_2$ was detected at 505 nm with a chromogenic reagent (4-AP) in the presence of peroxidase (Trinder's reaction). Results were expressed as µg $_{tcc}$CHOL per mg protein.

## 2.13 $_i$GLU-based immune cell metabolism assay

Intracellular glucose ($_i$GLU) levels were determined spectrophotometrically in cell lysates, following supernatant removal, based on the oxidation of glucose by glucose oxidase (Gox, EC 1.1.3.4), which produces gluconic acid ($C_6H_{12}O_7$) and $H_2O_2$, detected at 505 nm using Trinder's method (65). The $_i$GLU concentration was calculated by comparing the absorbance to a glucose standard curve and expressed as nanomoles per mg of protein.

## 2.14 $_{if}Ca^{2+}$ secondary messenger-based cell signaling assay

Intracellular free calcium ions ($_{if}Ca^{2+}$) levels were measured in cell culture lysates after the removal of supernatants using the ortho-cresolphthalein complexone (o-CPC) method as described in detail (63, 66, 67), and expressed as μg/mg of protein.

## 2.15 Statistical analyses

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were conducted using SPSS software version 26.0 for Windows (SPSS Inc., Chicago, IL, USA). The Kruskal-Wallis test, a non-parametric method appropriate for non-normally distributed data, was used for comparisons involving more than two groups. For normally distributed data, one-way ANOVA was applied (68). Additionally, pairwise comparisons were conducted using the Student's *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

# 3 Results

This study investigates the impact of UVB radiation and passive physical barriers on PRMφs, focusing on their effects on the enzymatic conversion of 25(OH)$D_3$ into its active form, 1,25(OH)$_2D_3$, mediated by 1-alpha hydroxylase, as well as their broader influence on the global functional activities of these macrophages.

## 3.1 UVB exposure has no effect on 25(OH)$D_3$ levels, but downregulates 1-alpha hydroxylase activity with the greatest impact in the simulated barrier

The results presented in Figure 2 reveal that there were no significant differences in the levels of 25(OH)D3 across the three experimental groups, irrespective of whether the PRMφs were exposed to UVB radiation alone or UVB radiation with a simulated barrier (RF + group). In contrast, Figure 3 highlights a significant downregulation in the activity of 1-alpha hydroxylase following UVB exposure. Notably, this downregulation was most pronounced in the simulated barrier group (UVB+/RF+) (for all comparisons, $p < 0.001$).

## 3.2 UVB increases NO production, $H_2O_2$, METosis-related MPO expression, and HOCl levels, with a moderate effect on phagocytosis, while the simulated barrier downregulates HOCl levels

As shown in Figure 4, UVB exposure led to a minimal increase in phagocytic activity in PRMφs, and did not reach statistical significance. In contrast, UVB exposure—whether with or without the simulated barrier—resulted in a significant upregulation of both NO and $H_2O_2$ levels relative to the control group ($p < 0.05$ and $p < 0.01$, respectively). Moreover, the levels of HOCl were significantly upregulated in the UVB-exposed PRMφ group ($p < 0.001$); whereas, in the RF+ group, where UVB exposure was combined with a simulated barrier, HOCl levels returned to baseline levels, showing no significant difference when compared to the control group (for the comparison among the three groups using ANOVA, $p < 0.001$).

Figure 5 further extends our analyses by specifically exploring MPO activity and expression. The analysis of MPO fluorescence intensity highlighted significant differences across the three experimental groups, underscoring the impact of UVB exposure on PRMφ activation. In contrast to the control group, where the fluorescence intensity corresponded to minimal MPO expression levels, the PRMφs group exposed to UVB displayed the highest fluorescence intensity, corresponding to the significant upregulation of MPO expression, likely triggered by UVB-induced stimulation. Lastly, in the RF+ group, where a simulated barrier limited UVB exposure, the fluorescence intensity remained similar to baseline levels. This suggests that while fluorescence intensity increased per unit area, the overall effect of UVB on METosis-related MPO activity was substantially attenuated by the simulated barrier. These findings were consistent across normalized fluorescence intensity ($p < 0.001$ by ANOVA test), area ($p < 0.001$ by Kruskal-Wallis test), perimeter ($p < 0.01$ by Kruskal-Wallis test), and normalized weighted mean intensity of MPO-marked cells ($p < 0.001$ by ANOVA test), further corroborated by the direct enzymatic activity analysis of MPO ($p < 0.001$ by ANOVA test).

## 3.3 UVB upregulates iNOS activity and shifts $M1_{(iNOS)}$/$M2_{(ARG1)}$ towards M1 functional phenotype

Figure 6 illustrates the effects of UVB exposure on the enzymatic activity of iNOS and ARG1 in PRMφs, revealing distinct modulatory patterns. Unlike iNOS activity, ARG1 activity was significantly downregulated in PRMφs exposed to UVB radiation ($p < 0.01$). The passive physical barrier similarly reduced ARG1 activity, though this effect was less pronounced compared to UVB exposure ($p < 0.05$). In contrast, neither UVB radiation nor the physical barrier significantly altered iNOS activity. Finally, both the UVB-exposed group and the RF+ group exhibited a significant increase in the $M1_{(iNOS)}$-to-$M2_{(ARG1)}$ ratio, indicating a shift toward a more M1-like phenotype ($p < 0.01$ by ANOVA test).

## 3.4 UVB downregulates CAT activity, specifically in simulated barrier condition

As shown in Figure 7, UVB exposure induced a significant downregulation of CAT activity in PRMφs. This activity decreased even further in the PRMφ group with the simulated barrier (RF+

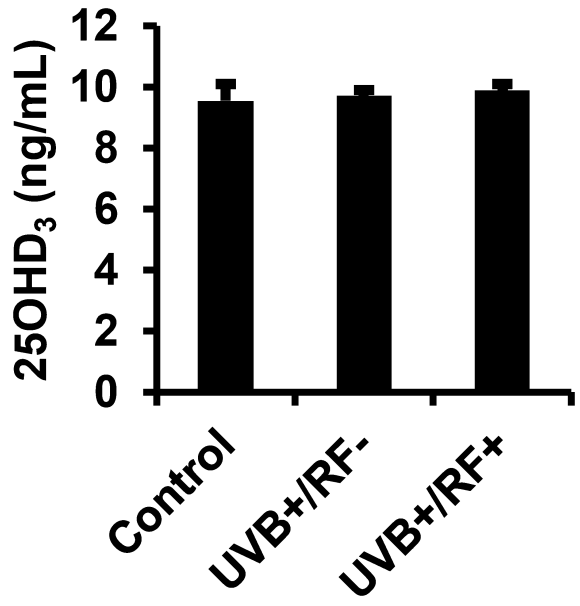


FIGURE 2
Effects of UVB radiation and passive physical barrier on 25(OH)$D_3$ content of PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). Levels of 25(OH)$D_3$ were measured using a chemiluminescent microparticle immunoassay. Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). No significant differences were observed between groups using one-way ANOVA.

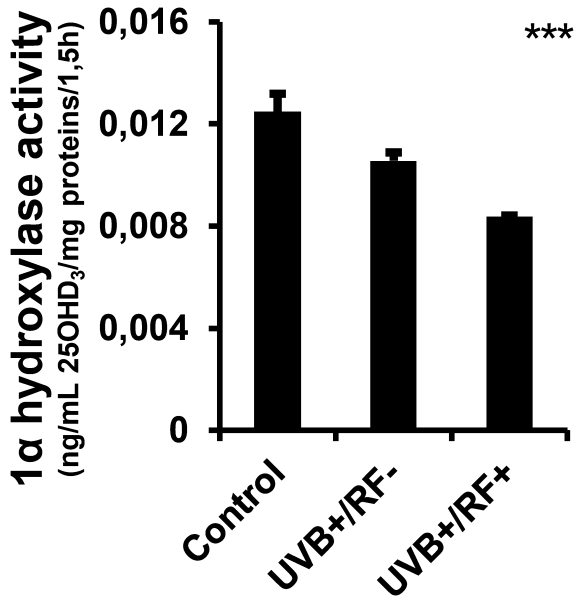


FIGURE 3
Effects of UVB radiation and passive physical barrier on 25-hydroxyvitamin $D_3$-1alpha-hydroxylase of PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). The activity of 1α-hydroxylase assay was based on the two-point technique, determining the change in 25(OH)$D_3$ concentration over time and normalizing this change by the amount of protein in the sample and the time interval. Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). Significant differences are indicated by an asterisk. Statistical analyses were performed using one-way ANOVA. ***$p < 0.001$.

group) compared to the control group. For multiple comparisons using ANOVA test, $p$-value was less than 0.001.

### 3.5 Simulated barrier attenuates the UVB-induced increase in $_{tcc}$CHOL

As shown in Figure 8, $_{tcc}$CHOL levels were upregulated in the UVB-exposed PRMφ group compared to the control PRMφ group, although the difference was not statistically significant. However, in the RF+ group, where UVB exposure was combined with a simulated barrier (rat fur), $_{tcc}$CHOL levels returned to near baseline values. For all comparisons between the three groups using the Kruskal-Wallis test, the $p$-value was less than 0.05.

### 3.6 UVB decreases $_{i}$GLU levels with a more pronounced effect under the simulated barrier condition

Figure 9 illustrates that UVB radiation, regardless of whether combined with the simulated barrier (UVB+/RF+ group) or not (UVB+/RF- group), led to a significant downregulation in $_{i}$GLU levels compared to the control PRMφ group. Notably, the decrease in $_{i}$GLU levels was more pronounced in the UVB+/RF+ group ($p$-value was less than 0.001 using ANOVA test), suggesting that the presence of the simulated barrier may enhance or reinforce this effect.

### 3.7 UVB exposure alone does not alter $_{if}Ca^{2+}$ levels, but a mild increase is observed with the simulated barrier

As shown in Figure 10, there were no significant differences in $_{if}Ca^{2+}$ levels among the three experimental groups. However, the UVB-exposed PRMφ group with the simulated barrier (UVB+/RF+) showed an upregulation in $_{if}Ca^{2+}$ levels, although this increase was not statistically significant, suggesting a subtle enhancement of calcium signaling by the simulated barrier.

## 4 Discussion

Tissue macrophages act as immune sentinels due to their strategic positioning and ability to initiate and modulate immune responses during infection or injury, while maintaining tissue homeostasis (69–71). Among these, peritoneal macrophages are well-characterized in terms of development, biology, and inflammation-related responses (72–76).

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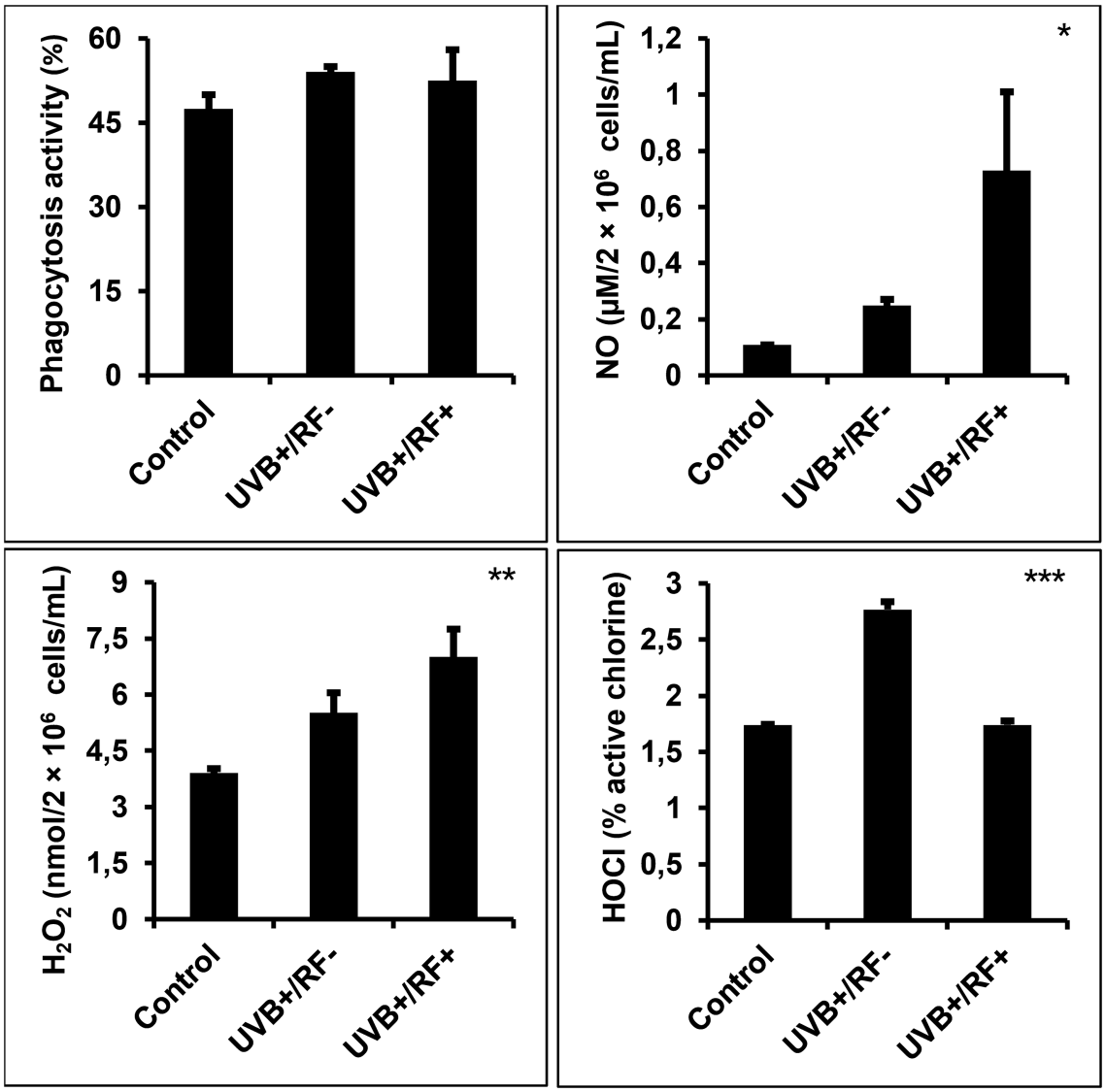


FIGURE 4
Effects of UVB radiation and passive physical barrier on phagocytosis and respiratory burst activity of PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). Phagocytosis activity was evaluated using the NBT method, based on its reduction to formazan by superoxide anions produced during the respiratory burst in phagocytic cells. Oxidative/respiratory burst was performed by measuring the levels of NO production, $H_2O_2$ levels, and HOCl. $H_2O_2$ levels were spectrophotometrically determined using a phenol red-based assay. NO production levels were spectrophotometrically determined using the sensitive colorimetric Griess method. HOCl levels were determined by measuring the decomposition of $H_2O_2$ through *in vitro* bromide oxidation by HOCl generated by activated cells in the presence of chloride released into the extracellular medium. Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). $H_2O_2$: hydrogen peroxide, HOCL: hypochlorous acid, NBT: nitro-blue tetrazolium, NO: nitric oxide. Significant differences are indicated by an asterisk. Statistical analyses were performed using one-way ANOVA. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

While UVB exposure from natural or artificial sources is known to modulate immunity locally and systemically, typically reducing cellular responses (77–79), our study is, to our knowledge, the first to examine how UVB radiation and passive physical barriers affect both intracellular $25OHD_3$ conversion and functional characteristics of tissue-resident macrophages under the studied conditions.

Although it might initially seem intuitive that introducing a passive physical barrier would simply attenuate UVB-induced inflammation by reducing exposure intensity, our results suggest a more nuanced and biologically significant phenomenon. The presence of a fur-based barrier did not merely lower UVB energy input, but qualitatively modulated key aspects of the macrophage response. Specifically, we observed changes in macrophage polarization (M1/M2), enzymatic activity (iNOS, MPO, Arg1), phagocytic function, and the expression of local 1α-hydroxylase involved in vitamin D activation. These findings indicate that the passive barrier influenced not only the total UVB dose but also its spectral and spatial properties—through wavelength-dependent filtering consistent with Beer–Lambert principles and modulation by light scattering. While the Beer–Lambert framework provides a

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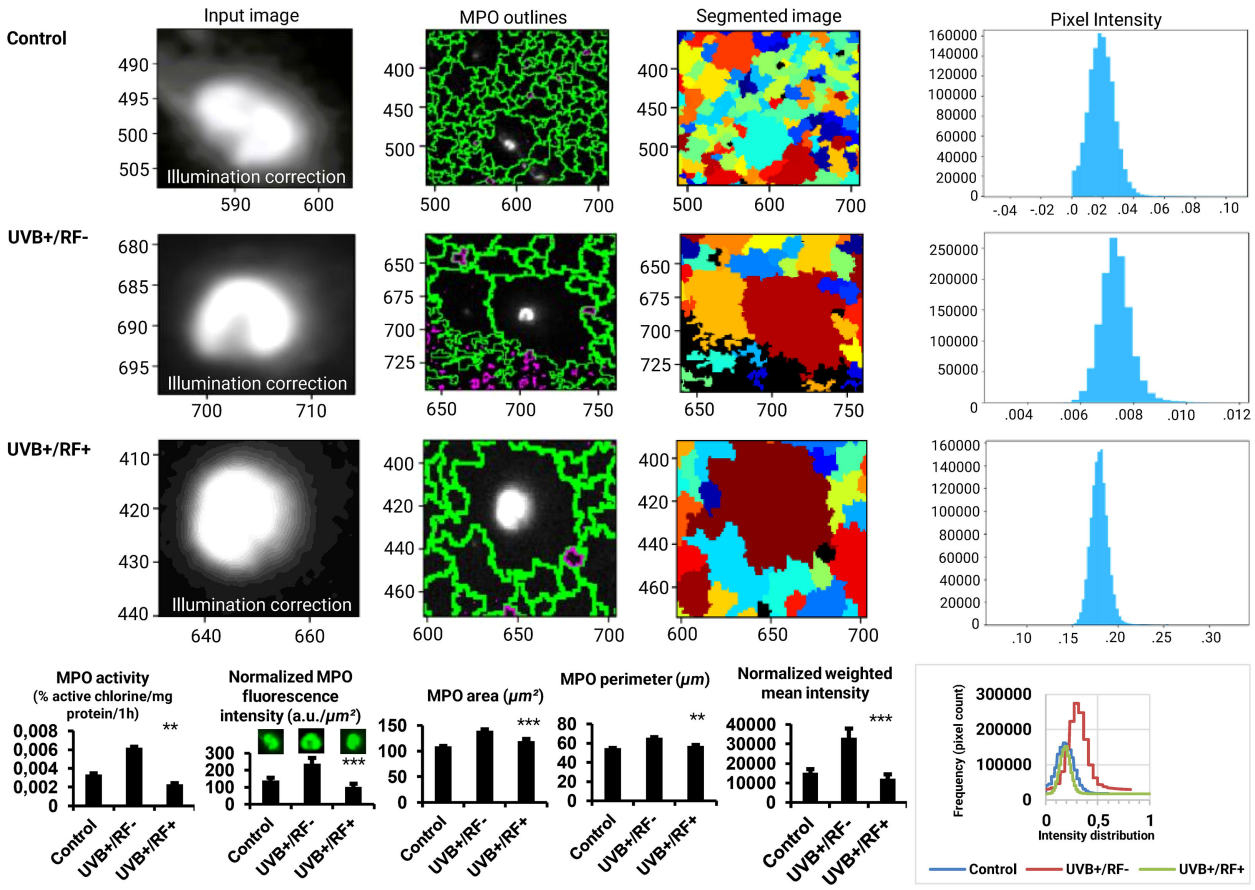


FIGURE 5
Effects of UVB radiation and passive physical barrier on MPO expression and activity of PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). MPO activity was quantified by directly measuring HOCl levels, normalized to total protein content, and expressed as percentage active chlorine per milligram of protein per one hour. MPO expression was assessed *via* immunofluorescence imaging, analyzed using CellProfiler software (v4.2.6, Broad Institute, USA). For imaging analysis, object dimensions were converted from pixels to micrometers ($\mu m$) to ensure accurate quantification. This conversion is based on an estimated image resolution of 0.58 $\mu m$ per pixel, derived from an approximate field of view of 750 $\mu m$ for an image width of 1296 pixels. The resolution was calculated as: $Resolution = \frac{Field\ of\ view\ width\ (\mu m)}{Image\ width\ (pixels)} = \frac{750\ \mu m}{1296\ pixels} \approx 0.5787 \mu m/pixel$. This estimation aligns with the theoretical resolution limit of the device (~0.5 $\mu m$). To convert area values, the square of the resolution (0.58 $\mu m$/pixel)$^2$ was applied to pixel measurements, resulting in approximate areas in $\mu m^2$. For perimeter values, a linear conversion of 0.58 $\mu m$/pixel was applied. Therefore, the reported values should be considered approximate. The intensity measurements were normalized using a min-max scaling approach to ensure comparability across images with differing intensity ranges. This normalization method adjusts the raw pixel intensities (I) to a scale between 0 and 1, calculated as: $I_{normalized} = \frac{I - I_{min}}{I_{max} - I_{min}}$, where $I_{min}$ and $I_{max}$ represent the minimum and maximum intensity values in the dataset, respectively. This process preserves the relative differences between pixel intensities while standardizing their range aligning with the quantitative expectations of the theoretical resolution limits and ensuring that the observed patterns are not influenced by varying dynamic ranges of the original images. The normalization was performed independently for each group, allowing for direct comparisons of intensity distributions across experimental conditions. Finally, the weighted mean intensity (WMI) values were calculated to account for the normalized intensity distributions across the analyzed regions of interest (ROIs). These values were derived as: $WMI = \frac{\sum_i w_i \times I_i}{\sum_i w_i}$, where $I_i$ represents the intensity value of a given pixel, and W denotes the pixel area. This method ensures that the calculated intensity reflects the contribution of each pixel proportionally to its area within the ROI. Overlaid histograms representing the fluorescence intensity distribution for the three experimental groups. Intensities were normalized to ensure comparability, and the x-axis represents fluorescence intensities (a.u.), while the y-axis indicates the pixel count. For histogram data, values are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). HOCl: hypochlorous acid, MPO: myeloperoxidase, RF: rat fur, UVB: ultraviolet B. Significant differences are indicated by an asterisk. Statistical analyses were performed using the Kruskal-Wallis test for non-normally distributed data (area and perimeter) or one-way ANOVA for normally distributed data (normalized fluorescence intensity, normalized weighted mean intensity, and MPO activity). $^{**}p < 0.01$, $^{***}p < 0.001$.

basis for understanding absorption-driven attenuation, additional tissue-relevant factors such as anisotropy, optical heterogeneity, and multiple scattering—known to deviate from ideal Beer–Lambert conditions in biological systems—likely contribute to the altered UVB distribution and biological effects (80, 81). Such selective attenuation may result in change in signaling cascades within skin-resident immune cells.

In line with previous observations in tissue-specific immunomodulation, our results reinforce the notion that the local photophysical environment—including the presence of

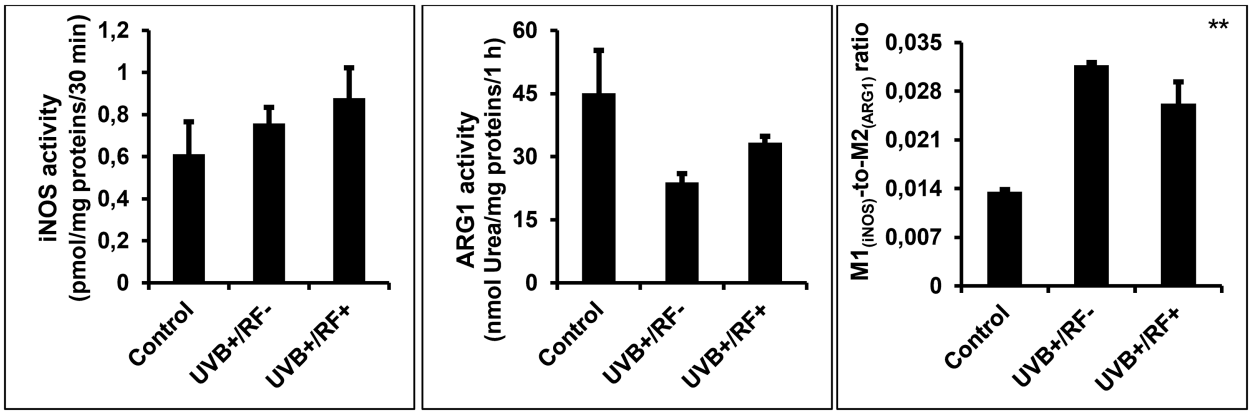


FIGURE 6
Effects of UVB radiation and passive physical barrier on the M1/M2 dichotomy. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). The $M1_{(iNOS)}/M2_{(ARG1)}$ dichotomy was determined mathematically by measuring the M1-to-M2 ratio. M1 activity was determined by measuring iNOS (EC 1.14.13.39) activity through the quantification of NO production normalized to protein content, whereas M2 activity was assessed by measuring the amount of urea generated by ARG1 (EC 3.5.3.1). Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). iNOS: inducible nitric oxide synthase, ARG1: arginase 1. Significant differences are indicated by an asterisk. Statistical analyses were performed using one-way ANOVA. $^{**}p < 0.01$.

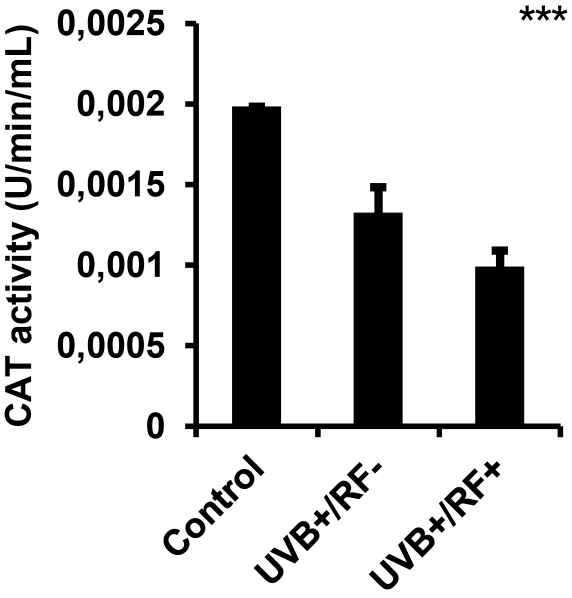


FIGURE 7
Effects of UVB radiation and passive physical barrier on catalase-based cell protection activity of PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). CAT (EC 1.11.1.6)-based cell protection activity was spectrophotometrically assessed by measuring $H_2O_2$ decomposition through titanium sulfate-based detection. Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). CAT: catalase activity, $H_2O_2$: hydrogen peroxide. Significant differences are indicated by an asterisk. Statistical analyses were performed using one-way ANOVA. $^{***}p < 0.001$.

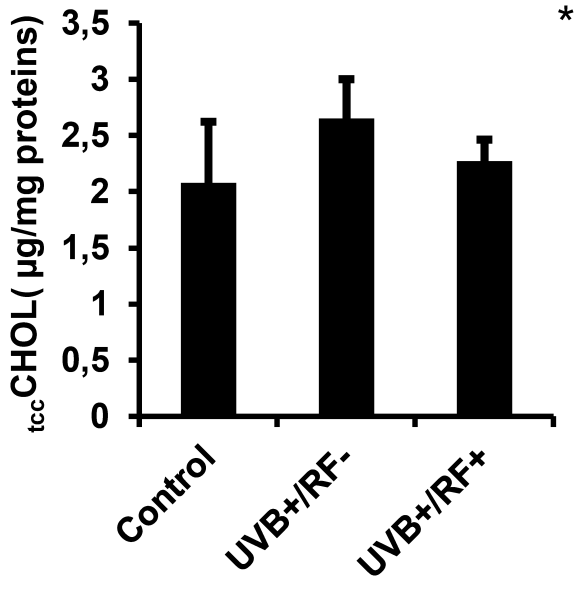


FIGURE 8
Effects of UVB radiation and passive physical barrier on trained immunity activation-based $_{tcc}$CHOL signature of PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). Trained immunity activation-based $_{tcc}$CHOL signature was measured spectrophotometrically through Trinder's reaction. Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). $_{tcc}$CHOL: total cellular cholesterol content. Significant differences are indicated by an asterisk. Statistical analyses were performed using one-way ANOVA. $^{*}p < 0.05$.

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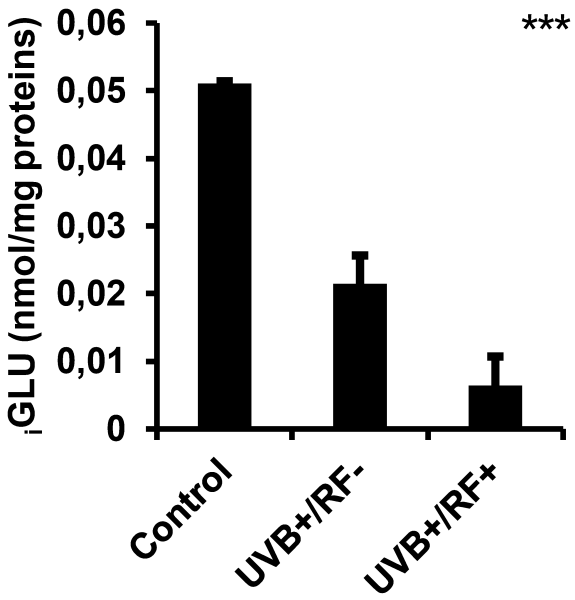


FIGURE 9
Effects of UVB radiation and passive physical barrier on ¡GLU-based immune cell metabolism of PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). ¡GLU-based immune cell metabolism was assessed spectrophotometrically by measuring $H_2O_2$ produced during glucose oxidation by glucose oxidase. Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). ¡GLU: intracellular glucose. Significant differences are indicated by an asterisk. Statistical analyses were performed using one-way ANOVA.
***$p < 0.001$.

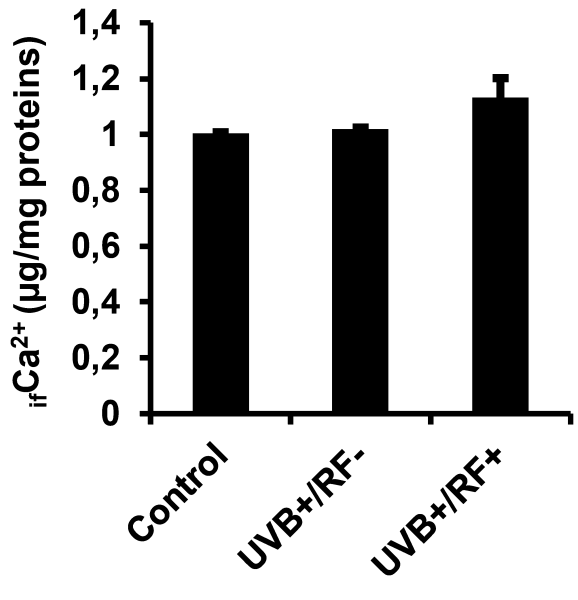


FIGURE 10
Effects of UVB radiation and passive physical barrier on free $Ca^{2+}$ levels in PRMφs. Experiments were conducted on murine peritoneal tissue-resident macrophages (PRMφs) under three conditions: (i) Control group, (ii) Controlled exposure to UVB rays (UVB+ group), and (iii) UVB exposure with a simulated physical barrier (RF+ group). $_{if}Ca^{2+}$ levels were assessed using the ortho-cresolphthalein complexone (oCPC) method. Data are presented as mean ± standard error of the mean (SEM) from four independent repetitions (n = 12 per group). No significant differences were observed between groups using one-way ANOVA. $_{if}Ca^{2+}$, intracellular free calcium ions.

partial UVB barriers such as clothing or natural photoprotective substances (82–84)—can shape immunological outcomes in a non-linear, context-dependent manner. This distinction is clinically relevant: in autoimmune or chronic inflammatory diseases, a controlled modulation of UVB-induced macrophage activation could help limit deleterious inflammation, while in oncology or infectious contexts, preserving specific UVB wavelengths might enhance beneficial immune responses. Notably, this could extend to inflammatory bowel diseases (IBD), where systemic and local macrophage dysregulation is a known factor (85), and where skin-mediated photomodulation might exert systemic effects. Moreover, our findings may also be relevant in the context of skin inflammatory disorders such as psoriasis and atopic dermatitis, where dysregulated macrophage activation contributes to disease pathogenesis (86–90). By demonstrating that UVB exposure modulates tissue-resident macrophage function—both enhancing and restraining oxidative activity depending on barrier conditions—our study suggests that careful manipulation of UVB exposure could offer therapeutic avenues for these conditions. Further investigations in preclinical and clinical models are warranted to fully elucidate these translational possibilities.

## 4.1 Effects of UVB radiation and passive physical barrier on 25-hydroxyvitamin $D_3$-1alpha-hydroxylase of PRMφs

Our findings show that UVB significantly downregulates 1α-hydroxylase activity. This may stem from the non-enzymatic UV-driven conversion of 7-dehydrocholesterol (7-DHC) to previtamin $D_3$, bypassing enzymatic steps (91, 92). This aligns with the concept that metabolic pathways, including vitamin D conversion, adapt dynamically to physiological needs rather than being solely driven by circadian rhythms (93).

Supporting evidence also links vitamin D to sleep regulation: its receptor and metabolizing enzymes are expressed in sleep-regulatory brain regions (94), and vitamin D promotes melatonin synthesis—a hormone critical for circadian alignment and nocturnal physiological processes (95, 96). A systematic review in youth populations reported a link between vitamin D deficiency and disorders like insomnia, obstructive sleep apnea (OSA), and restless legs syndrome (RLS), highlighting its involvement in serotonergic/dopaminergic pathways vital to sleep (97).

Our data also show that the simulated rat barrier dampens UVB penetration, markedly reducing 1α-hydroxylase activity in the UVB +/Rat fur+ condition (18). This reinforces the concept that physical

obstructions can limit local UVB-induced vitamin D metabolism by restricting UVB access.

## 4.2 Effects of UVB radiation and passive physical barrier on phagocytosis and respiratory burst activity of PRMφs

Our study revealed a modest increase in phagocytic activity of PRMφs upon direct UVB exposure. In contrast, previous *in vivo* studies reported impaired macrophage function following systemic UV irradiation, including reduced phagocytosis, bacterial clearance, and NO production (98, 99). These discrepancies likely stem from differences in experimental models—namely, our direct *in vitro* exposure versus whole-body UVB irradiation, which introduces systemic and indirect immune effects. By isolating macrophages *ex vivo*, our approach reveals a cell-intrinsic enhancement of phagocytic function, avoiding confounding influences from other immune or stromal cells.

Similarly, another study (99) showed diminished phagocytosis and active oxygen production in macrophages after UVB exposure, though neutrophils were unaffected. Our approach isolated the direct impact of UVB on PRMφs, excluding systemic factors or indirect effects mediated by other cell types. This may explain why we observed a cell-autonomous increase in phagocytic activity, whereas *in vivo* studies reported broader functional impairments due to whole-body irradiation. Furthermore, while our study did not assess other immune cells, including neutrophils, the differential effects of UVB across innate immune cell types suggest that tissue-resident macrophages may be particularly sensitive to UVB-induced modulation.

Differences in UVB doses and functional assays may also contribute to conflicting results in the literature. While other studies emphasized microbial killing, we focused on NBT-based assay to assess phagocytosis, highlighting UVB-induced ROS production during this process. Notably, phagocytosis in the UVB+/RF+ group remained higher than in the control but slightly lower than in the UVB+/RF– group, suggesting that the barrier dampens—but does not abolish—UVB effects.

The respiratory burst is central to macrophage antimicrobial defense, involving ROS and RNS generation. UVB exposure increased NO and $H_2O_2$ levels in PRMφs, even with partial UVB attenuation by fur, supporting its capacity to stimulate innate immune responses. Our NO data are consistent with reports of UVB-induced NO production in murine PRMφs (100) and human vitiligo skin (101), but differ from studies where therapeutic UVB inhibited NOS2 expression in keratinocytes and macrophages (102). Similarly, $H_2O_2$ production in response to UVB mirrors findings in neutrophils (103), mouse serum (104), and even algae (105), underscoring the evolutionary conservation of this oxidative stress response.

## 4.3 Effects of UVB radiation and passive physical barrier on MPO expression and activity of PRMφs

Our findings show that UVB exposure significantly enhances HOCl production in PRMφs, supporting our previous results on the respiratory burst. This increase is accompanied by higher MPO expression, suggesting activation of myeloperoxidase-driven oxidative mechanisms, potentially indicating METosis-like activity. The presence of rat fur, mimicking some clothing, partially attenuated this effect, highlighting its role as a passive UVB barrier—consistent with evidence that garments reduce UVB impact on skin and immune responses (106).

Elevated MPO activity, though crucial for microbial killing, is also implicated in chronic inflammatory diseases. For example, in atherosclerosis—a persistent immunoinflammatory condition—MPO-derived oxidants contribute to tissue damage, oxidized LDL accumulation, and lesion progression (107–111). Similarly, in Crohn's disease, characterized by intestinal inflammation and immune dysregulation (112, 113), UVB-induced MPO activity may intensify oxidative stress, underlining the need to limit UVB exposure in such contexts.

Conversely, increased MPO activity in malignant conditions may support macrophage-mediated antitumor responses (114), with some studies linking higher MPO levels to reduced tumor growth and improved survival (115). This dual role suggests the context-dependent outcomes of UVB-induced MPO activation.

While rat fur differs from human fabric, it provides a consistent UVB-shielding surface. Its light-filtering properties parallel those of clothing, which varies in UV modulation based on material thickness, porosity, fiber composition, and moisture (116–120). The partial barrier function of fur may thus modulate UVB-induced inflammation without fully suppressing macrophage activity.

To assess the translational potential of our findings, future *in vivo* studies are needed to examine the systemic consequences of UVB-induced MPO activation and its modulation by passive physical barriers in disease settings. We acknowledge that our *ex vivo* model, while providing tight experimental control, does not fully recapitulate the complexity of organismal physiology. Extension to relevant *in vivo* models of chronic inflammation, cardiovascular disease, or cancer may reveal novel therapeutic applications for UVB and vitamin D–mediated modulation of macrophage function.

## 4.4 Effects of UVB radiation and passive physical barrier on the M1/M2 dichotomy

iNOS and ARG1 compete for L-arginine, their shared substrate, in activated macrophages. iNOS uses L-arginine to produce NO, whereas ARG1 converts it into L-ornithine and urea. Elevated

ARG1 activity can therefore limit L-arginine availability for iNOS, reducing NO production (121).

Classically activated M1 macrophages are typically associated with high iNOS expression and NO production, representing a proinflammatory phenotype (122), whereas M2 macrophages display higher ARG1 activity, contributing to immunoregulation and wound healing (123). Thus, the iNOS/ARG1 balance shapes macrophage polarization and function (124, 125).

Our results confirm that UVB exposure enhances iNOS activity in PRMφs, consistent with previous studies in immune and skin cells (100, 126–128). Simultaneously, we observed decreased ARG1 activity, aligning with reports showing reduced M2-like macrophages after moderate UVB irradiation (129, 130). This shift in the iNOS/ARG1 ratio indicates a tendency toward an M1-like polarization. This is further supported by Karisola et al. (131), who demonstrated that UVB radiation promotes proinflammatory M1 macrophages. A potential mechanism for this polarization is UVB-induced cellular stress, which may activate proinflammatory signaling pathways, such as NF-κB (132). Additionally, UVB exposure has been shown to upregulate toll-like receptor (TLR) signaling, further driving M1 polarization (133–135).

Interestingly, the presence of a simulated barrier (RF+) further enhances iNOS activity, suggesting that the barrier may influence PRMφs beyond simple UV filtration, possibly through additional mechanical or environmental cues. Further studies are needed to determine whether this shift is transient or sustained and to elucidate the underlying molecular pathways.

## 4.5 Effects of UVB radiation and passive physical barrier on CAT activity in PRMφs

CAT is a key antioxidant enzyme that decomposes $H_2O_2$ into water and oxygen, shielding macrophages from oxidative damage (136). In our study, CAT activity peaked when PRMφs were fully shielded from UVB, emphasizing its frontline role in defending against oxidative stress. Conversely, direct UVB exposure markedly reduced CAT activity, even when partially filtered by the passive physical barrier, revealing the enzyme's sensitivity to UVB-induced oxidative stress.

Beyond its role in neutralizing ROS, CAT is essential for maintaining redox homeostasis, which supports macrophage viability and function (137, 138). The observed suppression of CAT activity after UVB exposure indicates a weakened antioxidant defense, potentially impairing macrophage responsiveness and survival. Interestingly, the intermediate CAT activity observed in partially shielded cells suggests a threshold effect, where suboptimal UVB levels may not fully activate protective mechanisms yet still cause cellular damage.

These findings point to the need for further investigation into the molecular mechanisms underlying UVB-induced CAT alteration, including the potential involvement of redox-sensitive signaling pathways or epigenetic regulation. A deeper understanding of how environmental stress modulates macrophage antioxidant capacity could inform strategies for preserving immune cell function under oxidative conditions.

## 4.6 Effects of UVB radiation and passive physical barrier on trained immunity activation-based $_{tcc}$CHOL signature of PRMφs

Our findings show a slight increase in $_{tcc}$CHOL levels in UVB-exposed PRMφs, suggesting that UVB influences cholesterol metabolism, likely *via* oxidative stress and metabolic reprogramming. UVB-generated ROS (139–141) are known to affect enzymes like HMG-CoA reductase (142) and cholesterol efflux transporters such as ABCG1 (143), potentially disrupting cholesterol homeostasis. Interestingly, $_{tcc}$CHOL levels returned to near baseline in the RF+ group, indicating that the passive physical barrier mitigated UVB effects.

Changes in $_{tcc}$CHOL levels are also relevant to trained immunity—a long-lasting innate immune adaptation driven by metabolic and epigenetic reprogramming (144, 145). While moderate ROS can enhance trained immunity (146), excessive cholesterol accumulation may provoke dysfunction (147). Cholesterol and its metabolites shape key metabolic pathways and epigenetic remodeling, notably *via* LXR-alpha activation (148, 149), mTOR signaling (150, 151), and regulation of DNA methylation and histone acetylation. Additionally, cholesterol is an integral component of lipid rafts, which cluster PRRs like TLRs and potentiate immune signaling (152–154). Consequently, alterations in $_{tcc}$CHOL levels could impact raft integrity and PRMφ responsiveness to secondary challenges—though this remains hypothetical and warrants experimental validation.

Finally, cholesterol's role in modulating mitochondrial function and ROS production adds another layer of immune regulation (155). The normalization of $_{tcc}$CHOL in the RF+ group suggests that passive physical barriers may help maintain a balance between the beneficial aspects of trained immunity and the potentially harmful effects of oxidative stress.

## 4.7 Effects of UVB radiation and passive physical barrier on $_{i}$GLU-based immune cell metabolism of PRMφs

Glucose is the primary energy substrate supporting key macrophage functions (156). While its role in immune activation has long been recognized, its impact on cellular metabolism—particularly in linking energy production to immune responses—

has only recently been fully appreciated. In macrophages, glucose not only fuels ATP production *via* glycolysis and oxidative phosphorylation (OXPHOS) but, also supports essential biosynthetic processes by providing NADPH for ROS generation, glycerol 3-phosphate for lipid synthesis, and ribose for RNA synthesis required for cytokine production (157–159).

In our study, UVB exposure led to a marked decrease in $_{i}$GLU levels in PRMφs, likely reflecting an upregulation of oxidative metabolism and increased metabolic demand. This aligns with the observed rise in oxidative stress markers—including NO, $H_2O_2$, and HOCl—highlighting the interplay between energy metabolism and immune activation under photonic stress.

Interestingly, the simulated physical barrier further intensified the decrease in $_{i}$GLU levels. Rather than mitigating the UVB effect, the passive barrier appeared to modulate glucose metabolism—possibly by altering glucose uptake, transporter expression, or by promoting alternative metabolic pathways activated under dual stress conditions. This unexpected outcome suggests that the barrier may function not only as a physical shield that limits UVB radiation exposure, but also as a biological modulator influencing cellular metabolism in tissue-resident macrophages in PRMφs, revealing a complex interaction between environmental stressors and immune cell bioenergetics. Further investigation is needed to elucidate the underlying mechanisms and to determine whether this effect is beneficial or detrimental to macrophage function and survival.

## 4.8 Effects of UVB radiation and passive physical barrier on $_{if}Ca^{2+}$ levels in PRMφs

$_{if}Ca^{2+}$ serves as a pivotal second messenger controlling various macrophage functions, including phagocytosis and oxidative burst in macrophages (160–162). In our study, UVB exposure alone did not significantly alter $_{if}Ca^{2+}$ levels in PRMφs. However, a mild, non-significant increase was observed in the UVB+/RF+ group, suggesting that partial attenuation and spectral modulation by the simulated barrier may subtly enhance calcium signaling.

This subtle enhancement may reflect wavelength-dependent changes in receptor- or ion channel–mediated calcium influx, as well as possible involvement of intracellular stores through endoplasmic reticulum channels like inositol 1,4,5-trisphosphate receptor ($IP_3R$) and ryanodine receptor (RyR) (163), which are sensitive to ROS and calcium dynamics (164).

Additionally, mitochondrial calcium uptake *via* the mitochondrial calcium uniporter-associated channel (MiCa) channel may stimulate ATP production and ROS generation (165, 166). Since longer wavelengths can enhance mitochondrial ROS output (167–170), the spectral shift induced by the passive physical barrier—likely absorbing short UVB (292 nm) and transmitting longer wavelengths—may influence these interconnected pathways. These interlinked mechanisms are coherent with our findings of elevated NO and $H_2O_2$ levels, suggesting that barrier-modulated UVB exposure may fine-tune calcium signaling and oxidative stress responses in PRMφs. Future studies will be needed to clarify whether these calcium dynamics translate into functional changes under photonic stress.

## 4.9 Synthesis: integrated effects of UVB radiation and biological barrier on PRMφs function – spectral, energetic, and functional modulation

Our findings highlight the multifaceted impact of UVB radiation on PRMφ function, revealing that the presence of a biological barrier—simulated by rat fur—modulates both the intensity and quality of UVB reaching the cells. The barrier's partial absorption of photons not only reduces UVB intensity but also shifts the average wavelength toward longer values, thereby decreasing photon energy, as predicted by Planck's relation $E = \frac{h \times c}{\lambda}$, where $h$ is Planck's constant, $c$ the speed of light, and $\lambda$ the wavelength (171, 172). Lower-energy photons are less effective at exciting biomolecular electrons, such as those in proteins and lipids, which dampens cellular responses to photonic stress. According to the Grotthuss-Draper law, only absorbed photons can initiate photochemical changes (173–175), implying that reduced photon energy directly modulates PRMφ activity.

Beyond simple shielding, UVB photons interact with sensitive molecular targets like transient receptor potential (TRP) channels, rhodopsins (a member of the G protein-coupled receptor [GPCR] family), and flavins (176–179), thereby influencing intracellular signaling cascades. These effects involve complex phenomena at the crossroads of optics, quantum physics (e.g., photon scattering and interference), and molecular biology. The passive barrier's selective absorption and scattering alter photon-cell interactions, potentially explaining how the barrier increases $_{if}Ca^{2+}$ levels while dampening the excitation of other pathways.

Overall, this integrated analysis underscores the dual importance of UVB intensity and spectral composition in shaping PRMφ functions. A deeper understanding of how passive physical barriers modulate photonic stress could inform strategies to preserve immune cell resilience under environmental stressors.

# 5 Conclusions and future prospects

Our study highlights the significant effects of UVB exposure on PRMφ, enhancing their functionality and shifting them toward a pro-inflammatory phenotype by increasing the $M1_{(iNOS)}$-to-$M2_{(ARG1)}$ ratio. UVB also induced metabolic adjustments, including downregulation of 1α-hydroxylase, catalase activity, and $_{tcc}$CHOL—a well-established signature of trained immunity. Notably, UVB exposure led to a marked increase in MPO activity—indicative of METosis-like oxidative responses in PRMφs, but this effect was significantly reversed by the simulated physical barrier (rat fur), with MPO levels returning close to baseline. This underscores MPO's dual role in inflammation: while it aids host defense by producing reactive oxidants—most notably HOCl, a potent antimicrobial—it can also cause collateral tissue damage when excessively or chronically activated. Attenuating MPO activity may therefore help reduce oxidative stress and limit inflammation; by contrast, broad or unregulated inhibition without protective mechanisms could compromise immune competence. These

findings highlight the need for a targeted, context-aware approach to MPO modulation—one that maximizes therapeutic benefit while minimizing unintended harm.

These findings emphasize PRMφ's role in extrarenal vitamin D metabolism and support the concept of tissue-resident macrophages serving as local "active reservoirs" of vitamin D. This non-circulating form of vitamin D, likely produced under UVB influence, could be directly utilized by immune cells to modulate localized immune responses. Our results highlight the need to go beyond circulating 25-hydroxyvitamin D levels as the sole indicator of vitamin D status and to consider tissue-specific dynamics, particularly in contexts where compensatory mechanisms are likely to influence vitamin D metabolism and utilization.

Future studies should focus on the role of tissue-resident macrophages as local vitamin D reservoirs, the long-term impact of UVB exposure on macrophage function, and the influence of passive physical barriers such as fabric or other UV-blocking materials. Additionally, extending these findings to disease models may uncover therapeutic opportunities for modulating macrophage function and vitamin D metabolism in immune-related conditions. Moreover, circadian rhythms should not be overlooked, as they can influence both macrophage reactivity and UVB-induced signaling. Incorporating temporal aspects into future experimental designs may offer deeper insights into the dynamics of immune modulation under photobiological conditions.

Beyond the insights gained from macrophage-specific effects, it is also necessary to consider the broader cellular context within which these interactions take place. Therefore, although our study centered on macrophage responses, it is important to recognize that UVB radiation and vitamin D signaling likely exert effects on multiple cell populations within the tissue microenvironment. In particular, epithelial and stromal cells are known to contribute to local immune regulation and may respond differentially to photobiological stimuli. Future studies should also investigate how these findings in a preclinical rat model could be translated to human health, with a particular focus on additional cell types involved in UVB- and vitamin D-mediated immune modulation.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## Ethics statement

The study was conducted in compliance with Good Laboratory Practices (GLP) and approved by the Faculty Scientific Council/Institutional Ethics Committee of Tlemcen University. Measures were implemented to prevent and alleviate any potential pain or distress, in alignment with ethical principles that emphasize minimizing animal suffering whenever feasible, while ensuring the scientific justification of procedures (180).

## Author contributions

FSM: Writing – original draft, Writing – review & editing, Data curation, Investigation, Methodology. SZ: Writing – review & editing, Data curation, Investigation, Methodology. NEB: Writing – review & editing, Data curation, Investigation, Methodology. RM: Writing – review & editing, Data curation, Investigation, Methodology. FB: Data curation, Investigation, Methodology, Writing – review & editing. CE: Formal analysis, Investigation, Methodology, Writing – review & editing. NBN: Formal analysis, Writing – review & editing. ZM: Formal analysis, Investigation, Methodology, Writing – review & editing. SB: Formal analysis, Investigation, Methodology, Writing – review & editing. FJDM: Writing – review & editing, Resources. CT: Writing – review & editing, Resources. XL: Conceptualization, Writing – review & editing, Supervision, Resources. AB: Conceptualization, Writing – review & editing, Supervision, Resources. MA: Writing – original draft, Conceptualization, Writing – review & editing, Supervision, Methodology, Formal analysis, Resources.

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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 Generative AI was used in the creation of this manuscript, exclusively to enhance English language editing. The authors thoroughly reviewed all AI-assisted content to ensure its accuracy and relevance to the study.

## Publisher's note



## References

1. Carmeliet G, Dermauw V, Bouillon R. Vitamin D signaling in calcium and bone homeostasis: A delicate balance. *Best Pract Res Clin Endocrinol Metab*. (2015) 29:621–31. doi: 10.1016/j.beem.2015.06.001

2. Janoušek J, Pilařová V, Macáková K, Nomura A, Veiga-Matos J, Silva DDD, et al. Vitamin D: sources, physiological role, biokinetics, deficiency, therapeutic use, toxicity, and overview of analytical methods for detection of vitamin D and its metabolites. *Crit Rev Clin Lab Sci*. (2022) 59(8):517–54. doi: 10.1080/10408363.2022.2070595

3. Aribi M, Mennechet FJD, Touil-Boukoffa C. Editorial: The role of vitamin D as an immunomodulator. *Front Immunol*. (2023) 14:1186635. doi: 10.3389/fimmu.2023.1186635

4. Nouari W, Ysmail-Dahlouk L, Aribi M. Vitamin D3 enhances bactericidal activity of macrophage against Pseudomonas aeruginosa. *Int Immunopharmacol*. (2016) 30:94–101. doi: 10.1016/j.intimp.2015.11.033

5. Chun RF, Shieh A, Gottlieb C, Yacoubian V, Wang J, Hewison M, et al. Vitamin D binding protein and the biological activity of vitamin D. *Front Endocrinol*. (2019) 10:718. doi: 10.3389/fendo.2019.00718

6. Lopez DV, Al-Jaberi FAH, Woetmann A, Ødum N, Bonefeld CM, Kongsbak-Wismann M, et al. Macrophages control the bioavailability of vitamin D and vitamin D-regulated T cell responses. *Front Immunol*. (2021) 12:722806. doi: 10.3389/fimmu.2021.722806

7. Yousefzadeh P, Shapses SA, Wang X. Vitamin D binding protein impact on 25-hydroxyvitamin D levels under different physiologic and pathologic conditions. *Int J Endocrinol*. (2014) 2014:981581. doi: 10.1155/2014/981581

8. Rowling MJ, Kemmis CM, Taffany DA, Welsh J. Megalin-mediated endocytosis of vitamin D binding protein correlates with 25-hydroxycholecalciferol actions in human mammary cells. *J Nutr*. (2006) 136:2754–9. doi: 10.1093/jn/136.11.2754

9. Nykjaer A, Fyfe JC, Kozyraki R, Leheste JR, Jacobsen C, Nielsen MS, et al. Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D (3). *Proc Natl Acad Sci U S A*. (2001) 98:13895–900. doi: 10.1073/pnas.241516998

10. Ao T, Kikuta J, Ishii M. The effects of vitamin D on immune system and inflammatory diseases. *Biomolecules*. (2021) 11:1624. doi: 10.3390/biom11111624

11. Marwaha RK, Sreenivas V, Talwar D, Yenamandra VK, Challa A, Lakshmy R, et al. Impact of solar ultraviolet B radiation (290–320 nm) on vitamin D synthesis in children with type IV and V skin. *Br J Dermatol*. (2015) 173:604–6. doi: 10.1111/bjd.13887

12. O'Neill C, Kazantzidis A, Ryan M, Barber N, Sempos C, Durazo-Arvizu R, et al. Seasonal changes in vitamin D-effective UVB availability in Europe and associations with population serum 25-hydroxyvitamin D. *Nutrients*. (2016) 8:533. doi: 10.3390/nu8090533

13. Fajuyigbe D, Lwin SM, Diffey BL, Baker R, Tobin DJ, Sarkany RPE, et al. Melanin distribution in human epidermis affords localized protection against DNA photodamage and concurs with skin cancer incidence difference in extreme phototypes. *FASEB J*. (2018) 32:3700–6. doi: 10.1096/fj.201701472R

14. Tsiaras W, Weinstock M. Factors influencing vitamin D status. *Acta Derm Venerol*. (2011) 91:115–24. doi: 10.2340/00015555-0980

15. Passeron T, Bouillon R, Callender V, Cestari T, Diepgen TL, Green AC, et al. Sunscreen photoprotection and vitamin D status. *Br J Dermatol*. (2019) 181:916–31. doi: 10.1111/bjd.17992

16. Lips P, van Schoor NM, de Jongh RT. Diet, sun, and lifestyle as determinants of vitamin D status. *Ann NY Acad Sci*. (2014) 1317:92–8. doi: 10.1111/nyas.12443

17. Berry EG, Bezecny J, Acton M, Sulmonetti TP, Anderson DM, Beckham HW, et al. Slip versus slop: A head-to-head comparison of UV-protective clothing to sunscreen. *Cancers*. (2022) 14:542. doi: 10.3390/cancers14030542

18. Neville JJ, Palmieri T, Young AR. Physical determinants of vitamin D photosynthesis: A review. *JBMR Plus*. (2021) 5(1):e10460. doi: 10.1002/jbm4.10460

19. Lee M-S, Bensinger SJ. Reprogramming cholesterol metabolism in macrophages and its role in host defense against cholesterol-dependent cytolysins. *Cell Mol Immunol*. (2022) 19:327–36. doi: 10.1038/s41423-021-00827-0

20. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev*. (2016) 96:365–408. doi: 10.1152/physrev.00014.2015

21. Shany S, Rapoport J, Zuili I, Gavriel A, Lavi N, Chaimovitz C. Metabolism of 25-OH-vitamin D3 by peritoneal macrophages from CAPD patients. *Kidney Int*. (1991) 39:1005–11. doi: 10.1038/ki.1991.127

22. De Jesus A, Pusec CM, Nguyen T, Keyhani-Nejad F, Gao P, Weinberg SE, et al. Optimized protocol to isolate primary mouse peritoneal macrophage metabolites. *STAR Protoc*. (2022) 3:101668. doi: 10.1016/j.xpro.2022.101668

23. Song L, Papaioannou G, Zhao H, Luderer HF, Miller C, Dall'Osso C, et al. The vitamin D receptor regulates tissue resident macrophage response to injury. *Endocrinology*. (2016) 157:4066–75. doi: 10.1210/en.2016-1474

24. Rehermann B. Mature peritoneal macrophages take an avascular route into the injured liver and promote tissue repair. *Hepatology*. (2017) 65:376–9. doi: 10.1002/hep.28883

25. García-Peñarrubia P, Ruiz-Alcaraz AJ, Ruiz-Ballester M, Ramírez-Pávez TN, Martínez-Esparza M. Recent insights into the characteristics and role of peritoneal macrophages from ascites of cirrhotic patients. *World J Gastroenterol*. (2021) 27:7014–24. doi: 10.3748/wjg.v27.i41.7014

26. Ardavín C, Alvarez-Ladrón N, Ferriz M, Gutiérrez-González A, Vega-Pérez A. Mouse tissue-resident peritoneal macrophages in homeostasis, repair, infection, and tumor metastasis. *Adv Sci (Weinh)*. (2023) 10:e2206617. doi: 10.1002/advs.202206617

27. Liu T, Liu F, Peng L-W, Chang L, Jiang Y-M. The peritoneal macrophages in inflammatory diseases and abdominal cancers. *Oncol Res*. (2018) 26:817–26. doi: 10.3727/096504017X15130753659625

28. Hu K, Scheer FAJL, Buijs RM, Shea SA. The circadian pacemaker generates similar circadian rhythms in the fractal structure of heart rate in humans and rats. *Cardiovasc Res*. (2008) 80:62–8. doi: 10.1093/cvr/cvn150

29. Shirato K, Sato S. Macrophage meets the circadian clock: implication of the circadian clock in the role of macrophages in acute lower respiratory tract infection. *Front Cell Infect Microbiol*. (2022) 12:826738. doi: 10.3389/fcimb.2022.826738

30. Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk H-D, et al. A circadian clock in macrophages controls inflammatory immune responses. *Proc Natl Acad Sci U.S.A*. (2009) 106:21407–12. doi: 10.1073/pnas.0906361106

31. Fukuta K. Collection of body fluids. In: *The Laboratory Mouse*. Amsterdam, Netherlands: Elsevier (2012). p. 727–38. doi: 10.1016/B978-0-12-382008-2.00031-3

32. Dai JH, Iwatani Y, Ishida T, Terunuma H, Kasai H, Iwakula Y, et al. Glycyrrhizin enhances interleukin-12 production in peritoneal macrophages. *Immunology*. (2001) 103:235–43. doi: 10.1046/j.1365-2567.2001.01224.x

33. Aribi M, Meziane W, Habi S, Boulatika Y, Marchandin H, Aymeric J-L. Macrophage Bactericidal Activities against Staphylococcus aureus Are Enhanced *In Vivo* by Selenium Supplementation in a Dose-Dependent Manner. *PLoS One*. (2015) 10:e0135515. doi: 10.1371/journal.pone.0135515

34. Lu R, Sampathkumar NK, Benayoun BA. Measuring phagocytosis in bone marrow-derived macrophages and peritoneal macrophages with aging. *Methods Mol Biol*. (2020) 2144:161–70. doi: 10.1007/978-1-0716-0592-9_14

35. Okerblom JJ, Schwarz F, Olson J, Fletes W, Ali SR, Martin PT, et al. Loss of CMAH during Human Evolution Primed the Monocyte-Macrophage Lineage toward a More Inflammatory and Phagocytic State. *J Immunol*. (2017) 198:2366–73. doi: 10.4049/jimmunol.1601471

36. Piccinini F, Tesei A, Arienti C, Bevilacqua A. Cell counting and viability assessment of 2D and 3D cell cultures: expected reliability of the trypan blue assay. *Biol Proced Online*. (2017) 19:8. doi: 10.1186/s12575-017-0056-3

37. Kift RC, Webb AR. Globally estimated UVB exposure times required to maintain sufficiency in vitamin D levels. *Nutrients*. (2024) 16:1489. doi: 10.3390/nu16101489

38. Aguilera J, Navarrete-de Gálvez E, Sánchez-Roldán C, Herrera-Ceballos E, de Gálvez MV. Sun-protective properties of technical sportswear fabrics 100% Polyester: the influence of moisture and sweat on protection against different biological effects of ultraviolet (UV) radiation. *Photochem Photobiol*. (2023) 99:184–92. doi: 10.1111/php.13679

39. Park K, Frey MW. Designing an effective and scalable UV-protective cooling textile with nanoporous fibers. *Nano Lett*. (2023) 23:10398–405. doi: 10.1021/acs.nanolett.3c03055

40. Aguilera J, de Gálvez MV, Sánchez-Roldán C, Herrera-Ceballos E. New advances in protection against solar ultraviolet radiation in textiles for summer clothing. *Photochem Photobiol*. (2014) 90:1199–206. doi: 10.1111/php.12292

41. Gambichler T, Laperre J, Hoffmann K. The European standard for sun-protective clothing: EN 13758. *J Eur Acad Dermatol Venereol*. (2006) 20:125–30. doi: 10.1111/j.1468-3083.2006.01401.x

42. Gambichler T, Rotterdam S, Altmeyer P, Hoffmann K. Protection against ultraviolet radiation by commercial summer clothing: need for standardised testing and labelling. *BMC Dermatol.* (2001) 1:6. doi: 10.1186/1471-5945-1-6

43. Khvatov IA, Ganza PN, Kharitonov AN, Samuleeva MV. Wistar Male Rats (Rattus norvegicus domestica) Are Aware of Their Dimensions. *Animals.* (2024) 14:3384. doi: 10.3390/ani14233384

44. Peña-Escudero C, Priego-Fernández S, Caba M, Rodríguez-Alba JC, Corona-Morales AA, García-García F. Effect of a hedonic stimulus on the sleep architecture of male wistar rats. *Sleep Sci.* (2023) 16:e329–34. doi: 10.1055/s-0043-1773788

45. de Gálvez MV, Aguilera J, Bernabó J-L, Sánchez-Roldán C, Herrera-Ceballos E. Human hair as a natural sun protection agent: A quantitative study. *Photochem Photobiol.* (2015) 91:966–70. doi: 10.1111/php.12433

46. Miliani M, Nouar M, Paris O, Lefranc G, Mennechet F, Aribi M. Thymoquinone potently enhances the activities of classically activated macrophages pulsed with necrotic jurkat cell lysates and the production of antitumor Th1-/M1-related cytokines. *J Interferon Cytokine Res.* (2018) 38:539–51. doi: 10.1089/jir.2018.0010

47. Bisswanger H. Enzyme assays. *Perspect Sci.* (2014) 1:41–55. doi: 10.1016/j.pisc.2014.02.005

48. Smith AF, Taylor RH. Comparison of two-point (AutoAnalyzer II) with kinetic methods for transaminase assay. *J Clin Pathol.* (1973) 26:42–7. doi: 10.1136/jcp.26.1.42

49. Chauhan AK, Jakhar R, Paul S, Kang SC. Potentiation of macrophage activity by thymol through augmenting phagocytosis. *Int Immunopharmacol.* (2014) 18:340–6. doi: 10.1016/j.intimp.2013.11.025

50. Segal AW. Nitroblue-tetrazolium tests. *Lancet.* (1974) 2:1248–52. doi: 10.1016/s0140-6736(74)90758-2

51. Sim Choi H, Woo Kim J, Cha Y, Kim C. A quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells. *J Immunoassay Immunochemistry.* (2006) 27:31–44. doi: 10.1080/15321810500403722

52. Aribi M. Macrophage bactericidal assays. In: Rousselet G, editor. *Macrophages. Methods in Molecular Biology.* Springer New York, New York, NY (2018). p. 135–49. doi: 10.1007/978-1-4939-7837-3_14

53. Thomas DC. The phagocyte respiratory burst: Historical perspectives and recent advances. *Immunol Lett.* (2017) 192:88–96. doi: 10.1016/j.imlet.2017.08.016

54. Meziane W, Mekkaoui Z, Hai I, Kacimi K, Djilali K, Touil-Boukoffa C, et al. Combination of metformin with sodium selenite induces a functional phenotypic switch of human GM-CSF monocyte-derived macrophages. *Int Immunopharmacol.* (2019) 73:212–24. doi: 10.1016/j.intimp.2019.05.004

55. Kumar K, Margerum DW. Kinetics and mechanism of general-acid-assisted oxidation of bromide by hypochlorite and hypochlorous acid. *Inorg Chem.* (1987) 26:2706–11. doi: 10.1021/ic00263a030

56. Harwood DT, Kettle AJ, Winterbourn CC. Production of glutathione sulfonamide and dehydroglutathione from GSH by myeloperoxidase-derived oxidants and detection using a novel LC-MS/MS method. *Biochem J.* (2006) 399:161–8. doi: 10.1042/BJ20060978

57. Waters JW, Froidevaux L, Harwood RS, Jarnot RF, Pickett HM, Read WG, et al. The Earth observing system microwave limb sounder (EOS MLS) on the aura Satellite. *IEEE Trans Geosci Remote Sens.* (2006) 44:1075–92. doi: 10.1109/TGRS.2006.873771

58. Pulli B, Ali M, Forghani R, Schob S, Hsieh KLC, Wojtkiewicz G, et al. Measuring myeloperoxidase activity in biological samples. *PloS One.* (2013) 8:e67976. doi: 10.1371/journal.pone.0067976

59. Farcas MT, Kisin ER, Menas AL, Gutkin DW, Star A, Reiner RS, et al. Pulmonary exposure to cellulose nanocrystals caused deleterious effects to reproductive system in male mice. *J Toxicol Environ Health A.* (2016) 79:984–97. doi: 10.1080/15287394.2016.1211045

60. Mills CD. M1 and M2 macrophages: oracles of health and disease. *Crit Rev Immunol.* (2012) 32:463–88. doi: 10.1615/critrevimmunol.v32.i6.10

61. Rath M, Müller I, Kropf P, Closs EI, Munder M. Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. *Front Immunol.* (2014) 5:532. doi: 10.3389/fimmu.2014.00532

62. Nouar M, Miliani M, Belhassena I, Fatmi A, Aribi M. Sodium selenite modulates global activation of proinflammatory M1-like macrophages, necroinflammation and M1-like/M2-like dichotomy at the onset of human type 1 diabetes. *Endocr Metab Immune Disord Drug Targets.* (2023) 23:1104–17. doi: 10.2174/1871530323666230201135916

63. Dahou S, Smahi MC-E, Nouari W, Dahmani Z, Benmansour S, Ysmail-Dahlouk L, et al. L-Threoascorbic acid treatment promotes S. aureus-infected primary human endothelial cells survival and function, as well as intracellular bacterial killing, and immunomodulates the release of IL-1β and soluble ICAM-1. *Int Immunopharmacol.* (2021) 95:107476. doi: 10.1016/j.intimp.2021.107476

64. Aribi M. Macrophage bactericidal assays. *Methods Mol Biol.* (2018) 1784:135–49. doi: 10.1007/978-1-4939-7837-3_14

65. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst.* (1972) 97:142–5. doi: 10.1039/an9729700142

66. McBurney RN, Neering IR. The measurement of changes in intracellular free calcium during action potentials in mammalian neurones. *J Neurosci Methods.* (1985) 13:65–76. doi: 10.1016/0165-0270(85)90044-5

67. Chutipongtanate S, Thongboonkerd V. Establishment of a novel colorimetric assay for high-throughput analysis of calcium oxalate crystal growth modulation. *Analyst.* (2010) 135:1309–14. doi: 10.1039/B927046A

68. Dwivedi AK, Mallawaarachchi I, Alvarado LA. Analysis of small sample size studies using nonparametric bootstrap test with pooled resampling method. *Stat Med.* (2017) 36:2187–205. doi: 10.1002/sim.7263

69. Taylor PR, Gordon S. Monocyte heterogeneity and innate immunity. *Immunity.* (2003) 19:2–4. doi: 10.1016/S1074-7613(03)00178-X

70. Gordon S. The macrophage: Past, present and future. *Eur J Immunol.* (2007) 37: S9–S17. doi: 10.1002/eji.200737638

71. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature.* (2013) 496:445–55. doi: 10.1038/nature12034

72. Cassado ADA, D'Império Lima MR, Bortoluci KR. Revisiting mouse peritoneal macrophages: heterogeneity, development, and function. *Front Immunol.* (2015) 6:225. doi: 10.3389/fimmu.2015.00225

73. Murray HW, Spitalny GL, Nathan CF. Activation of mouse peritoneal macrophages *in vitro* and *in vivo* by interferon-gamma. *J Immunol.* (1985) 134:1619–22. doi: 10.4049/jimmunol.134.3.1619

74. Hayashi M, Shimba S, Tezuka M. Characterization of the molecular clock in mouse peritoneal macrophages. *Biol Pharm Bull.* (2007) 30:621–6. doi: 10.1248/bpb.30.621

75. Gurusmatika S, Ishida M, Nishi K, Sugahara T. Exploring the anti-inflammatory effect of clove water extract in lipopolysaccharide-stimulated RAW264.7 cells and mouse peritoneal macrophages. *J Food Bioactives.* (2024) 25:72–80. doi: 10.31665/JFB.2024.18373

76. Balog S, Jeong S, Asahina K. Recruitment of large peritoneal macrophages to capsular fibrosis developed on the liver surface. *FASEB J.* (2024) 38:e23327. doi: 10.1096/fj.202301187R

77. Ullrich SE, Kripke ML, Ananthaswamy HN. Mechanisms underlying UV-induced immune suppression: implications for sunscreen design. *Exp Dermatol.* (2002) 11:13–6. doi: 10.1034/j.1600-0625.11.s.1.4.x

78. Slominski RM, Chen JY, Raman C, Slominski AT. Photo-neuro-immuno-endocrinology: How the ultraviolet radiation regulates the body, brain, and immune system. *Proc Natl Acad Sci U S A.* (2024) 121:e2308374121. doi: 10.1073/pnas.2308374121

79. Yu Z, Zheng M, Fan H, Liang X, Tang Y. Ultraviolet (UV) radiation: a double-edged sword in cancer development and therapy. *Mol BioMed.* (2024) 5:49. doi: 10.1186/s43556-024-00209-8

80. Oshina I, Spigulis J. Beer-Lambert law for optical tissue diagnostics: current state of the art and the main limitations. *J BioMed Opt.* (2021) 26:100901. doi: 10.1117/1.JBO.26.10.100901

81. Baranoski GVG, Krishnaswamy A. *Light & skin interactions: simulations for computer graphics applications.* Amsterdam: Morgan Kaufmann (2010).

82. Kostyuk V, Potapovich A, Albuhaydar AR, Mayer W, De Luca C, Korkina L. Natural substances for prevention of skin photoaging: screening systems in the development of sunscreen and rejuvenation cosmetics. *Rejuvenation Res.* (2018) 21:91–101. doi: 10.1089/rej.2017.1931

83. Hawk JLM. Safe, mild ultraviolet-B exposure: An essential human requirement for vitamin D and other vital bodily parameter adequacy: A review. *Photodermatol Photoimmunol Photomed.* (2020) 36:417–23. doi: 10.1111/phpp.12584

84. Norval M, Lucas RM, Cullen AP, de Gruijl FR, Longstreth J, Takizawa Y, et al. The human health effects of ozone depletion and interactions with climate change. *Photochem Photobiol Sci.* (2011) 10:199–225. doi: 10.1039/c0pp90044c

85. Fletcher J, Bishop EL, Harrison SR, Swift A, Cooper SC, Dimeloe SK, et al. Autoimmune disease and interconnections with vitamin D. *Endocr Connect.* (2022) 11: e210554. doi: 10.1530/EC-21-0554

86. Sieminska I, Pieniawska M, Grzywa TM. The immunology of psoriasis-current concepts in pathogenesis. *Clin Rev Allergy Immunol.* (2024) 66:164–91. doi: 10.1007/s12016-024-08991-7

87. Sajja AP, Joshi AA, Teague HL, Dey AK, Mehta NN. Potential immunological links between psoriasis and cardiovascular disease. *Front Immunol.* (2018) 9:1234. doi: 10.3389/fimmu.2018.01234

88. Clark RA, Kupper TS. Misbehaving macrophages in the pathogenesis of psoriasis. *J Clin Invest.* (2006) 116:2084–7. doi: 10.1172/JCI29441

89. Liu S-H, Zhang J, Zuo Y-G. Macrophages in inflammatory skin diseases and skin tumors. *Front Immunol.* (2024) 15:1430825. doi: 10.3389/fimmu.2024.1430825

90. Kasraie S, Werfel T. Role of macrophages in the pathogenesis of atopic dermatitis. *Mediators Inflammation.* (2013) 2013:942375. doi: 10.1155/2013/942375

91. Tian XQ, Holick MF. Catalyzed thermal isomerization between previtamin D3 and vitamin D3 via β-cyclodextrin complexation. *J Biol Chem.* (1995) 270:8706–11. doi: 10.1074/jbc.270.15.8706

92. Delrue C, Speeckaert MM. Vitamin D and vitamin D-binding protein in health and disease. *IJMS.* (2023) 24:4642. doi: 10.3390/ijms24054642

93. Masood T, Kushwaha RS, Singh R, Sailwal S, Pandey H, Varma A, et al. Circadian rhythm of serum 25 (OH) vitamin D, calcium and phosphorus levels in

the treatment and management of type-2 diabetic patients. *DD&T*. (2015) 9:70–4. doi: 10.5582/ddt.2015.01002

94. Stephenson A, Mamo JCL, Takechi R, Hackett MJ, Lam V. Genetic, environmental and biomarker considerations delineating the regulatory effects of vitamin D on central nervous system function. *Br J Nutr*. (2020) 123:41–58. doi: 10.1017/S000711451900268X

95. Romano F, Muscogiuri G, Di Benedetto E, Zhukouskaya VV, Barrea L, Savastano S, et al. Vitamin D and sleep regulation: is there a role for vitamin D? *CPD*. (2020) 26:2492–6. doi: 10.2174/1381612826666200310145935

96. Huiberts LM, Smolders KCHJ. Effects of vitamin D on mood and sleep in the healthy population: Interpretations from the serotonergic pathway. *Sleep Med Rev*. (2021) 55:101379. doi: 10.1016/j.smrv.2020.101379

97. Prono F, Bernardi K, Ferri R, Bruni O. The role of vitamin D in sleep disorders of children and adolescents: A systematic review. *IJMS*. (2022) 23:1430. doi: 10.3390/ijms23031430

98. Jeevan A, Bucana CD, Dong Z, Dizon VV, Thomas SL, Lloyd TE, et al. Ultraviolet radiation reduces phagocytosis and intracellular killing of mycobacteria and inhibits nitric oxide production by macrophages in mice. *J Leukocyte Biol*. (1995) 57:883–90. doi: 10.1002/jlb.57.6.883

99. Kasahara S, Aizawa K, Okamiya M, Kazuno N, Mutoh S, Fugo H, et al. Uvb irradiation suppresses cytokine production and innate cellular immune functions in mice. *Cytokine*. (2001) 14:104–11. doi: 10.1006/cyto.2001.0849

100. Sethi G, Sodhi A. *In vitro* activation of murine peritoneal macrophages by ultraviolet B radiation: upregulation of CD18, production of NO, proinflammatory cytokines and a signal transduction pathway. *Mol Immunol*. (2004) 40:1315–23. doi: 10.1016/j.molimm.2004.01.001

101. Vaccaro M, Irrera N, Cutroneo G, Rizzo G, Vaccaro F, Anastasi G, et al. Differential Expression of Nitric Oxide Synthase Isoforms nNOS and iNOS in Patients with Non-Segmental Generalized Vitiligo. *IJMS*. (2017) 18:2533. doi: 10.3390/ijms18122533

102. Sur R, Heck DE, Mariano TM, Jin Y, Murphy WJ, Laskin JD. UVB light suppresses nitric oxide production by murine keratinocytes and macrophages. *Biochem Pharmacol*. (2002) 64:1469–81. doi: 10.1016/S0006-2952(02)01419-3

103. Inaba I, Hiramoto K, Yamate Y, Morita A, Tsutsumi T, Yasuda H, et al. Inhibiting neutrophil extracellular traps protects against ultraviolet B-induced skin damage: effects of Hochu-ekki-to and DNase I. *Int J Mol Sci*. (2024) 25:1723. doi: 10.3390/ijms25031723

104. Zhou X, Du H-H, Long X, Pan Y, Hu J, Yu J, et al. β-Nicotinamide mononucleotide (NMN) administrated by intraperitoneal injection mediates protection against UVB-induced skin damage in mice. *JIR*. (2021) 14:5165–82. doi: 10.2147/JIR.S327329

105. Xue S, Zang Y, Chen J, Shang S, Gao L, Tang X. Ultraviolet-B radiation stress triggers reactive oxygen species and regulates the antioxidant defense and photosynthesis systems of intertidal red algae Neoporphyra haitanensis. *Front Mar Sci*. (2022) 9:1043462. doi: 10.3389/fmars.2022.1043462

106. Luther CA, Lim HW. Solar Angioedema: A report of a patient and a review of literature. *Photoderm Photoimm Photomed*. (2019) 35:187–9. doi: 10.1111/phpp.12445

107. Fan J, Watanabe T. Atherosclerosis: known and unknown. *Pathol Int*. (2022) 72:151–60. doi: 10.1111/pin.13202

108. Falk E. Pathogenesis of atherosclerosis. *J Am Coll Cardiol*. (2006) 47:C7–C12. doi: 10.1016/j.jacc.2005.09.068

109. Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. *Am J Cardiol*. (2003) 91:12–6. doi: 10.1016/S0002-9149(02)03145-4

110. McMillen TS, Heinecke JW, LeBoeuf RC. Expression of human myeloperoxidase by macrophages promotes atherosclerosis in mice. *Circulation*. (2005) 111:2798–804. doi: 10.1161/CIRCULATIONAHA.104.516278

111. Sugiyama S, Okada Y, Sukhova GK, Virmani R, Heinecke JW, Libby P. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. *Am J Pathol*. (2001) 158:879–91. doi: 10.1016/S0002-9440(10)64036-9

112. Veauthier B, Hornecker JR. Crohn's disease: diagnosis and management. *Am Fam Physician*. (2018) 98:661–9.

113. Ballester Ferré MP, Boscá-Watts MM, Mínguez Pérez M. Enfermedad de crohn. *Medicina Clínica*. (2018) 151:26–33. doi: 10.1016/j.medcli.2017.10.036

114. Liu Y, Liu L. The pro-tumor effect and the anti-tumor effect of neutrophils extracellular traps. *BST*. (2019) 13:469–75. doi: 10.5582/bst.2019.01326

115. Ali M, Fulci G, Grigalavicius M, Pulli B, Li A, Wojtkiewicz GR, et al. Myeloperoxidase exerts anti-tumor activity in glioma after radiotherapy. *Neoplasia*. (2022) 26:100779. doi: 10.1016/j.neo.2022.100779

116. Islam M, Akter T, Ferdush J, Kamrunnahar K. Impact of fabric density, color and composition of plain weave fabric on ultraviolet protective factor. *GJRE*. (2019) 19:13–5. doi: 10.34257/GJREJVOL19IS1PG13

117. Saravanan D. Uv protection textile materials. *AUTEX Res J*. (2007) 7:53–62. doi: 10.1515/aut-2007-070106

118. Rahman N, Dafader NC. Radiation technology for the modification of textiles. In: *Fundamentals of Natural Fibres and Textiles*. Amsterdam, Netherlands: Elsevier (2021). p. 407–38. doi: 10.1016/B978-0-12-821483-1.00003-6

119. Davis S, Capjack L, Kerr N, Fedosejcvs R. Clothing as protection from ultraviolet radiation: which fabric is most effective? *Int J Dermatol*. (1997) 36:374–9. doi: 10.1046/j.1365-4362.1997.00046.x

120. Khan A, Nazir A, Rehman A, Naveed M, Ashraf M, Iqbal K, et al. A review of UV radiation protection on humans by textiles and clothing. *IJCST*. (2020) 32:869–90. doi: 10.1108/IJCST-10-2019-0153

121. Koti RS, Tsui JC, Lobos E, Yang W, Seifalian AM, Davidson BR. Nitric oxide synthase distribution and expression with ischemic preconditioning of the rat liver. *FASEB J*. (2005) 19:1155–7. doi: 10.1096/fj.04-3220fje

122. Hu X, Li Y, Cao Y, Shi F, Shang L. The role of nitric oxide synthase/nitric oxide in infection-related cancers: Beyond antimicrobial activity. *Biochim Biophys Acta (BBA) - Rev Cancer*. (2024) 1879:189156. doi: 10.1016/j.bbcan.2024.189156

123. Dzik JM. Evolutionary roots of arginase expression and regulation. *Front Immunol*. (2014) 5:544. doi: 10.3389/fimmu.2014.00544

124. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS–) vs. Alternatively Activated Macrophages. *Front Immunol*. (2019) 10:1084. doi: 10.3389/fimmu.2019.01084

125. Lee KY. M1 and M2 polarization of macrophages: a mini-review. *Med Biol Sci Eng*. (2019) 2:1–5. doi: 10.30579/mbse.2019.2.1.1

126. Abbas S, Alam S, Pal A, Kumar M, Singh D, Ansari KM. UVB exposure enhanced benzanthrone-induced inflammatory responses in SKH-1 mouse skin by activating the expression of COX-2 and iNOS through MAP kinases/NF-κB/AP-1 signalling pathways. *Food Chem Toxicol*. (2016) 96:183–90. doi: 10.1016/j.fct.2016.07.034

127. Kuhn A, Fehsel K, Lehmann P, Krutmann J, Ruzicka T, Kolb-Bachofen V. Aberrant timing in epidermal expression of inducible nitric oxide synthase after UV irradiation in cutaneous lupus erythematosus. *J Invest Dermatol*. (1998) 111:149–53. doi: 10.1046/j.1523-1747.1998.00253.x

128. Chang H-R, Tsao D-A, Wang S-R, Yu H-S. Expression of nitric oxide synthases in keratinocytes after UVB irradiation. *Arch Dermatol Res*. (2003) 295:293–6. doi: 10.1007/s00403-003-0433-4

129. Park G, Cui Y, Yang S, Sun M, Wilkinson E, Li H, et al. Moderate low UVB irradiation modulates tumor-associated macrophages and dendritic cells and promotes antitumor immunity in tumor-bearing mice†. *Photochem Photobiol*. (2023) 99:850–6. doi: 10.1111/php.13684

130. Hafeez BB, Alvarado EW, Kim DJ. Therapeutic implications of UVB irradiation in cancer by enhancing anti-tumor immunity†. *Photochem Photobiol*. (2023) 99:874–7. doi: 10.1111/php.13729

131. Karisola P, Nikkola V, Joronen H, Ylianttila L, Grönroos M, Partonen T, et al. Narrow-band UVB radiation triggers diverse changes in the gene expression and induces the accumulation of M1 macrophages in human skin. *J Photochem Photobiol B: Biol*. (2024) 253:112887. doi: 10.1016/j.jphotobiol.2024.112887

132. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. *Sig Transduct Target Ther*. (2017) 2:17023. doi: 10.1038/sigtrans.2017.23

133. Bi Z. Increased MAPK. and NF-κB expression of Langerhans cells is dependent on TLR2 and TLR4, and increased IRF-3 expression is partially dependent on TLR4 following UV exposure. *Mol Med Rep*. (2011) 4:541–6. doi: 10.3892/mmr.2011.450

134. Park G, Qian W, Zhang M-J, Chen Y-H, Ma L-W, Zeng N, et al. Platelet-rich plasma regulating the repair of ultraviolet B–induced acute tissue inflammation: adjusting macrophage polarization through the activin receptor–follistatin system. *Bioengineered*. (2021) 12:3125–36. doi: 10.1080/21655979.2021.1944026

135. Sun Q, Hu S, Lou Z, Gao J. The macrophage polarization in inflammatory dermatosis and its potential drug candidates. *Biomedicine Pharmacotherapy*. (2023) 161:114469. doi: 10.1016/j.biopha.2023.114469

136. Bratovcic A. Antioxidant enzymes and their role in preventing cell damage. *Act Scie Nutr*. (2020) 4:01–7. doi: 10.31080/ASNH.2020.04.0659

137. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. Oxford, United Kingdom: Oxford University Press (2015). doi: 10.1093/acprof:oso/9780198717478.001.0001

138. Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol*. (2017) 11:613–9. doi: 10.1016/j.redox.2016.12.035

139. Guo C, Ning X, Zhang J, Zhang C, Wang J, Su L, et al. Ultraviolet B radiation induces oxidative stress and apoptosis in human lens epithelium cells by activating NF-κB signaling to down-regulate sodium vitamin C transporter 2 (SVCT2) expression. *Cell Cycle*. (2023) 22:1450–62. doi: 10.1080/15384101.2023.2215084

140. McGlone CL, Christian L, Schmeusser B, Liu L, Chalfant CE, Stephensen DJ, et al. Evidence for systemic reactive oxygen species in UVB-mediated microvesicle formation. *Photochem Photobiol*. (2022) 98:242–7. doi: 10.1111/php.13494

141. de Jager TL, Cockrell AE, Du Plessis SS. Ultraviolet light induced generation of reactive oxygen species. *Adv Exp Med Biol*. (2017) 996:15–23. doi: 10.1007/978-3-319-56017-5_2

142. Sitaula S, Burris TP. Cholesterol and other steroids. In: *Encyclopedia of Cell Biology*. Amsterdam, Netherlands: Elsevier (2016). p. 173–9. doi: 10.1016/B978-0-12-394447-4.10021-5

143. Sun Y, Wang J, Long T, Qi X, Donnelly L, Elghobashi-Meinhardt N, et al. Molecular basis of cholesterol efflux via ABCG subfamily transporters. *Proc Natl Acad Sci U.S.A*. (2021) 118:e2110483118. doi: 10.1073/pnas.2110483118

144. Sherwood ER, Burelbach KR, McBride MA, Stothers CL, Owen AM, Hernandez A, et al. Innate immune memory and the host response to infection. *J Immunol*. (2022) 208:785–92. doi: 10.4049/jimmunol.2101058

145. Saeed S, Quintin J, Kerstens HHD, Rao NA, Aghajanirefah A, Matarese F, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science*. (2014) 345:1251086. doi: 10.1126/science.1251086

146. Drummer C, Saaoud F, Shao (邵颖) Y, Sun (孙宇) Y, Xu (徐克曼) K, Lu (路一凡) Y, et al. Trained immunity and reactivity of macrophages and endothelial cells. *Arterioscler Thromb Vasc Biol*. (2021) 41:1032–46. doi: 10.1161/ATVBAHA.120.315452

147. Rauchbach E, Zeigerman H, Abu-Halaka D, Tirosh O. Cholesterol induces oxidative stress, mitochondrial damage and death in hepatic stellate cells to mitigate liver fibrosis in mice model of NASH. *Antioxidants (Basel)*. (2022) 11:536. doi: 10.3390/antiox11030536

148. Bekkering S, Arts RJW, Novakovic B, Kourtzelis I, van der Heijden CDCC, Li Y, et al. Metabolic induction of trained immunity through the mevalonate pathway. *Cell*. (2018) 172:135–46.e9. doi: 10.1016/j.cell.2017.11.025

149. Netea MG, Joosten LAB, Latz E, Mills KHG, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. *Science*. (2016) 352:aaf1098. doi: 10.1126/science.aaf1098

150. Cheng S-C, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, et al. mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science*. (2014) 345:1250684. doi: 10.1126/science.1250684

151. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nat Rev Immunol*. (2015) 15:104–16. doi: 10.1038/nri3793

152. Pike LJ. Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. *J Lipid Res*. (2006) 47:1597–8. doi: 10.1194/jlr.E600002-JLR200

153. Peng J, Luo F, Ruan G, Peng R, Li X. Hypertriglyceridemia and atherosclerosis. *Lipids Health Dis*. (2017) 16:233. doi: 10.1186/s12944-017-0625-0

154. Benghalem I, Meziane W, Hadjidj Z, Ysmail-Dahlouk L, Belamri A, Mouhadjer K, et al. High-density lipoprotein immunomodulates the functional activities of macrophage and cytokines produced during ex vivo macrophage-CD4 + T cell crosstalk at the recent-onset human type 1 diabetes. *Cytokine*. (2017) 96:59–70. doi: 10.1016/j.cyto.2017.03.001

155. Goicoechea L, Conde de la Rosa L, Torres S, García-Ruiz C, Fernández-Checa JC. Mitochondrial cholesterol: Metabolism and impact on redox biology and disease. *Redox Biol*. (2023) 61:102643. doi: 10.1016/j.redox.2023.102643

156. Zhang Q, Wang J, Yadav DK, Bai X, Liang T. Glucose metabolism: the metabolic signature of tumor associated macrophage. *Front Immunol*. (2021) 12:702580. doi: 10.3389/fimmu.2021.702580

157. Curi R, De Siqueira Mendes R, De Campos Crispin LA, Norata GD, Sampaio SC, Newsholme P. A past and present overview of macrophage metabolism and functional outcomes. *Clin Sci*. (2017) 131:1329–42. doi: 10.1042/CS20170220

158. Newsholme P, Curi R, Gordon S, Newsholme EA. Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem J*. (1986) 239:121–5. doi: 10.1042/bj2390121

159. Newsholme P, Gordon S, Newsholme EA. Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages. *Biochem J*. (1987) 242:631–6. doi: 10.1042/bj2420631

160. Desai BN, Leitinger N. Purinergic and calcium signaling in macrophage function and plasticity. *Front Immunol*. (2014) 5:580. doi: 10.3389/fimmu.2014.00580

161. Nunes P, Demaurex N. The role of calcium signaling in phagocytosis. *J Leukocyte Biol*. (2010) 88:57–68. doi: 10.1189/jlb.0110028

162. Forman HJ, Torres M. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. *Am J Respir Crit Care Med*. (2002) 166:S4–8. doi: 10.1164/rccm.2206007

163. Clapham DE. Calcium signaling. *Cell*. (2007) 131:1047–58. doi: 10.1016/j.cell.2007.11.028

164. Görlach A, Bertram K, Hudecova S, Krizanova O. Calcium and ROS: A mutual interplay. *Redox Biol*. (2015) 6:260–71. doi: 10.1016/j.redox.2015.08.010

165. Malli R, Graier WF. Mitochondrial Ca2+ channels: Great unknowns with important functions. *FEBS Lett*. (2010) 584:1942–7. doi: 10.1016/j.febslet.2010.01.010

166. Bertero E, Maack C. Calcium signaling and reactive oxygen species in mitochondria. *Circ Res*. (2018) 122:1460–78. doi: 10.1161/CIRCRESAHA.118.310082

167. Yoshida A, Yoshino F, Makita T, Maehata Y, Higashi K, Miyamoto C, et al. Reactive oxygen species production in mitochondria of human gingival fibroblast induced by blue light irradiation. *J Photochem Photobiol B*. (2013) 129:1–5. doi: 10.1016/j.jphotobiol.2013.09.003

168. Golovynska I, Golovynskyi S, Qu J. Comparing the impact of NIR, visible and UV light on ROS upregulation via photoacceptors of mitochondrial complexes in normal, immune and cancer cells. *Photochem Photobiol*. (2023) 99:106–19. doi: 10.1111/php.13661

169. Sommer AP. Mitochondrial cytochrome c oxidase is not the primary acceptor for near infrared light-it is mitochondrial bound water: the principles of low-level light therapy. *Ann Transl Med*. (2019) 7:S13. doi: 10.21037/atm.2019.01.43

170. Cheng G, Zielonka M, Dranka B, Kumar SN, Myers CR, Bennett B, et al. Detection of mitochondria-generated reactive oxygen species in cells using multiple probes and methods: Potentials, pitfalls, and the future. *J Biol Chem*. (2018) 293:10363–80. doi: 10.1074/jbc.RA118.003044

171. Eisberg RM, Resnick R. *Quantum physics of atoms, molecules, solids, nuclei, and particles. 2nd ed*. New York: Wiley (1985). 1 p.

172. Griffiths DJ, Schroeter DF. Introduction to quantum mechanics. *Third edition*. Cambridge ; New York, NY: Cambridge University Press (2018). 495 p.

173. Rennie R ed. *A dictionary of chemistry. Seventh edition*. Oxford: Oxford University Press (2016). 594 p.

174. Wisniak J. John william draper. *Educación Química*. (2013) 24:215–23. doi: 10.1016/S0187-893X(13)72465-0

175. Menzel JP, Noble BB, Blinco JP, Barner-Kowollik C. Predicting wavelength-dependent photochemical reactivity and selectivity. *Nat Commun*. (2021) 12:1691. doi: 10.1038/s41467-021-21797-x

176. Moore C, Cevikbas F, Pasolli HA, Chen Y, Kong W, Kempkes C, et al. UVB radiation generates sunburn pain and affects skin by activating epidermal TRPV4 ion channels and triggering endothelin-1 signaling. *Proc Natl Acad Sci U S A*. (2013) 110:E3225–3234. doi: 10.1073/pnas.1312933110

177. Zhang M, Ma Y, Ye X, Zhang N, Pan L, Wang B. TRP (transient receptor potential) ion channel family: structures, biological functions and therapeutic interventions for diseases. *Signal Transduct Target Ther*. (2023) 8:261. doi: 10.1038/s41392-023-01464-x

178. Wicks NL, Chan JW, Najera JA, Ciriello JM, Oancea E. UVA phototransduction drives early melanin synthesis in human melanocytes. *Curr Biol*. (2011) 21:1906–11. doi: 10.1016/j.cub.2011.09.047

179. Fiedorczuk K, Letts JA, Degliesposti G, Kaszuba K, Skehel M, Sazanov LA. Atomic structure of the entire mammalian mitochondrial complex I. *Nature*. (2016) 538:406–10. doi: 10.1038/nature19794

180. Carbone L. Pain in laboratory animals: the ethical and regulatory imperatives. *PLoS One*. (2011) 6:e21578. doi: 10.1371/journal.pone.0021578

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# Evaluation of vitamin D status, vitamin D receptor expression, and innate immune mediators in COVID-19

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**Background and objectives:** The Coronavirus disease 2019 (COVID-19) pandemic underscored the importance of identifying host factors that influence susceptibility to infection. Vitamin D signaling, mediated via its receptor (*VDR*), along with innate immune mediators such as antimicrobial peptides (e.g., *DEFA1-3*) and inflammatory chemokines (e.g., *CCL20*), plays a critical role in antiviral defense. This study aimed to determine how serum vitamin D status and gene expression of *VDR*, *DEFA1-3*, and *CCL20* associate with COVID-19 risk in a Lebanese cohort.

**Methods:** This prospective observational study assessed serum vitamin D concentrations and nasopharyngeal gene expression in Lebanese participants tested for SARS-CoV-2 between January and March 2024. We enrolled 264 patients undergoing RT-qPCR (targeting *ORF1*, *N*, and *E* genes) and quantified serum 25-hydroxyvitamin D [25(OH)D]. In a subset of 70 individuals stratified by COVID-19 status, we measured *VDR*, *DEFA1-3*, *CCL20*, and *GAPDH* expression by RT-qPCR. Multiple logistic regression and Pearson correlation analyses were performed.

**Results:** Serum vitamin D levels and *CCL20* expression were not significantly associated with COVID-19 status. Elevated *VDR* expression in nasopharyngeal tissue correlated with lower COVID-19 risk (OR = 0.40, $p = 0.05$) and inversely with 25(OH)D levels (r = −0.61, $p = 0.04$). Higher *DEFA1–3* expression reduced COVID-19 risk by 81.6% (OR = 0.184, $p = 0.012$). Among COVID-19 negatives, *VDR* correlated with *CCL20* (r = 0.59, $p < 0.01$); among positives, *VDR* correlated with *DEFA1-3* (r = 0.45, $p < 0.05$).

**Conclusion:** Our findings reveal a complex interplay between systemic vitamin D status, local VDR expression, and innate inflammatory mediators in COVID-19. They support a model in which both micronutrient levels and tissue-specific vitamin D signaling modulate host susceptibility and disease severity.

KEYWORDS

**COVID-19, vitamin D, *VDR*, innate immunity, inflammatory biomarkers**

# Introduction

The Coronavirus disease 2019 (COVID-19) pandemic has prompted extensive investigation into host factors that influence susceptibility and disease severity (1). Among these factors, vitamin D has garnered considerable attention due to its immunomodulatory properties (2–6). Early observational studies highlighted a potential protective role for vitamin D, as deficiency was frequently associated with increased disease severity, hospitalization rates, and mortality in COVID-19 patients (7, 8). Vitamin D can impact numerous pathways in the host immune response, promoting an appropriate inflammatory reaction while suppressing an excessive one (5). Vitamin D's immunomodulatory role is significant in COVID-19, where severe cases involve excessive innate immune activation and lung immunothrombosis (9). Its effects are mediated through the vitamin D receptor (*VDR*), expressed on macrophages, dendritic cells, T-cells, and respiratory epithelial cells (4, 10, 11). Activation of *VDR* by active vitamin D modulates gene transcription, enhancing both innate and adaptive immune responses to strengthen antimicrobial defense (4, 9, 10).

While systemic vitamin D status is commonly assessed via circulating serum 25-hydroxyvitamin D [25(OH)D] concentrations, recent research has suggested that local tissue responsiveness—reflected by *VDR* expression—may be equally, if not more, relevant for immune protection (12). Indeed, local receptor expression levels potentially indicate the ability of tissues to mount effective vitamin D-dependent immune responses better than serum vitamin D concentrations alone. However, the relationship between local *VDR* expression and systemic vitamin D status remains inadequately explored in the context of viral infections such as COVID-19.

In Lebanon, vitamin D deficiency remains widespread despite plentiful sunshine (13, 14). This is exacerbated by cultural clothing that limits sun exposure and by low dietary intake of vitamin D–rich foods (13, 14). Lebanon's COVID-19 clinical management protocol, aligned with the World Health Organization's 2023 guidelines, did not recommend routine vitamin D supplementation as part of standard treatment during the study period.

Innate inflammatory mediators such as the alpha-defensins (*DEFA1-3*), produced by neutrophils and mucosal cells, exhibit antiviral activity by disrupting viral membranes and blocking entry (15, 16), and are linked to reduced respiratory infections (17, 18). They also maintain immune homeostasis and epithelial integrity during infections (16, 19). Chemokines like *CCL20* recruit immune cells to infected tissues (20, 21). Elevated *CCL20* levels are associated with severe COVID-19 outcomes, including acute respiratory distress syndrome (ARDS) and multisystem inflammatory syndrome in children (MIS-C), indicating a pathogenic role (22–24).

This prospective observational study aimed to assess how serum 25(OH)D concentrations and nasopharyngeal expression of *VDR*, *DEFA1-3*, and *CCL20* are associated with COVID-19 status in Lebanese participants tested between January and March 2024.

**Abbreviations:** 25(OH)D, 25-Hydroxyvitamin D; ARDS, Acute Respiratory Distress Syndrome; BMI, Body Mass Index; CCL20, C-C Motif Chemokine Ligand 20; CI, Confidence Interval; COVID-19, Coronavirus Disease 2019; Ct, Cycle Threshold; DEFA1-3, Defensin Alpha 1–3; GAPDH, Glyceraldehyde 3-Phosphate Dehydrogenase; IRB, Institutional Review Board; OR, Odds Ratio; RT-qPCR, Reverse Transcription Quantitative Polymerase Chain Reaction; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SD, Standard Deviation; VDR, Vitamin D Receptor 1.

# Materials and methods

## Study design and participants

The study was a prospective observational analysis conducted between January and March 2024, involving 264 adult participants who presented for measurement of serum 25(OH)D levels and/or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) testing. Eligible participants were consecutively enrolled after meeting the inclusion and exclusion criteria at Lebanese Hospital Geitaoui, a tertiary center in Lebanon, during the Omicron BA.5 wave. Serum 25(OH)D levels were measured for all participants during the winter season (year 2024) to minimize the effect of seasonal variation on vitamin D concentrations. Participants were enrolled using a consecutive sampling strategy during the study period. A history of vitamin D supplementation within the past three months was recorded. In a subset of 70 patients, nasopharyngeal tissue was collected for gene expression analysis. Exclusion criteria included chronic autoimmune diseases, active malignancy, uncontrolled diabetes mellitus, chronic renal disease, and acute infections other than COVID-19.

## Ethical considerations

This study was conducted in full accordance with ethical guidelines and was approved by the Institutional Review Board (IRB) of Lebanese Hospital Geitaoui-UMC under protocol code 2024-IRB-010. Written informed consent was obtained from the study participants. No personally identifiable information was included in the analyses or subsequent reporting, ensuring that all data were anonymized and handled with the utmost confidentiality.

## Data collection and laboratory analyses

### Serum vitamin D measurement

Venous blood samples were collected, and total serum 25(OH)D concentrations were quantified using the Roche Elecsys™ Vitamin D Total Assay. Participants were subsequently classified as vitamin D deficient (<20 ng/mL), insufficient (20–30 ng/mL), or sufficient (≥30 ng/mL) based on the Endocrine Society Clinical Practice Guidelines (25). The coefficient of variation for 25(OH)D measurement assays was between 3% to 8%.

### RNA extraction and cDNA synthesis

Nasopharyngeal swab samples from a subset of 70 patients were processed for RNA extraction using the ANDiS Viral RNA Auto

Extraction & Purification Kit in conjunction with the ANDiS 350 Automated Nucleic Acids Extraction System. In this automated protocol, viral particles were first lysed to release RNA, which was then captured on magnetic beads. Following washes to remove impurities, the RNA was eluted into a clean solution for further analysis. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific), ensuring A260/A280 ratios between 1.8 and 2.0. The extracted RNA was quantified, stored at −80°C, and subsequently reverse-transcribed into complementary DNA (cDNA) using a commercial reverse transcription kit, following the manufacturer's instructions.

### SARS–CoV–2 detection

SARS-CoV-2 detection was performed using the ANDiS FAST SARS-CoV-2 Detection Kit (3D Biomedicine Science & Technology Co., Limited), targeting the *ORF1*, *N*, and *E* genes. Samples were collected 1–3 days post-symptom onset, between January and March 2024, during which the Omicron BA.5 variant predominated in Lebanon. Although the detection assay targeted conserved SARS-CoV-2 genes (ORF1, N, E), variant-specific genotyping was not performed. Each reaction contained 15 ng of extracted RNA, Positive and negative controls were included to validate the assay. Amplification was conducted on the Bio-Rad CFX96 Real-Time PCR System using the following thermal cycling conditions: reverse transcription at 50°C for 10 minutes, initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 30 seconds. Cycle threshold (Ct) values were determined for each gene target, with a Ct ≤40 in at least two of the three targets (*ORF1*, *N*, or *E*) considered positive. All individuals with positive RT-PCR tests also presented with clinical symptoms (fever, cough, myalgia, anosmia), and were considered as having symptomatic COVID-19. As for the control group (non-COVID-19), they were also tested due to the presence of similar respiratory symptoms, but their test results were negative for SARS-CoV-2.

### Quantitative real–time PCR for gene expression

RT–qPCR was performed to quantify the expression of *VDR* and the inflammatory genes *DEFA1–3* and *CCL20* in nasopharyngeal tissue samples. Gene–specific primers were designed and validated for efficiency and specificity. Each 20 µL reaction mixture contained SYBR Green Supermix, optimized concentrations of forward and reverse primers, and 70 ng of cDNA template, with *GAPDH* serving as the housekeeping gene for normalization. The thermal cycling protocol commenced with an initial enzyme activation and denaturation step at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and a combined annealing/extension step at 60°C for 30 seconds. All reactions were executed in duplicate to ensure reproducibility and accuracy.

### Statistical analysis

Analyses were performed using IBM SPSS Statistics. Continuous variables are expressed as the mean ± standard deviation, and categorical variables are presented as frequencies and percentages. Normality of continuous variables was assessed using the Kolmogorov-Smirnov test prior to applying parametric tests. Independent samples t-tests and Chi-square tests were used to compare continuous and categorical variables, respectively, between patients with and without COVID-19. The Ct values were used solely to define SARS-CoV-2 positivity as a binary variable. Quantitative Ct data were not included in the downstream correlation or regression analyses. The sample size was calculated using G*Power software based on a moderate effect size (Cohen's d = 0.6), with $\alpha = 0.05$ and power = 80%, resulting in a minimum of 45 participants per group.

For the entire cohort, a multiple logistic regression analysis was conducted, adjusting for age, sex, and BMI, to identify independent predictors of COVID-19 disease. The results are reported as odds ratios and 95% confidence intervals.

For the subset of 70 patients with gene expression data, separate logistic regression models, adjusted for age, sex, and BMI, were used to evaluate the association between the normalized expression of *VDR*, *DEFA1-3*, and *CCL20* and COVID-19 status. For each gene, a median value was calculated and further used as a cut-off to classify the gene expression as high or low. Pearson correlation analysis was used to assess the relationship between serum 25(OH)D concentrations and *VDR* expression, as well as the association between inflammatory biomarker levels and SARS-CoV-2 viral gene expression in COVID-19-positive patients. A two-tailed *p*-value ≤ 0.05 was considered statistically significant.

## Results

The study cohort consisted of 264 patients, comprising 148 individuals with COVID-19 and 116 individuals without COVID-19 (Table 1). Although the data presentation compares COVID-19-positive and -negative groups, the study was conducted prospectively with no prior matching or retrospective case selection. The mean age was similar between groups (58.11 ± 22.29 years in COVID-positive vs. 57.84 ± 17.47 years in COVID-negative; $p = 0.57$). Likewise, the sex distribution did not differ significantly between the two groups, with males representing 38.5% of COVID-positive patients and 38.8% of COVID-negative patients ($p = 1.00$).

Analysis based on BMI categories revealed a significantly lower proportion of COVID-positive patients with a normal BMI (37.2%) compared to those with a negative test result (47.4%; $p = 0.05$). However, the proportions of overweight and obese participants were comparable between groups. Serum 25(OH)D concentrations

TABLE 1 Clinical and demographic characteristics of the study participants.

| Characteristic | All Samples | COVID-19 Status | | *P* |
|---|---|---|---|---|
| | | COVID-19 positive (n = 148) | COVID-19 negative (n = 116) | |
| Characteristic | | Mean ± SD | Mean ± SD | |
| Age (years) | 57.9 ± 19.7 | 58.11 ± 22.29 | 57.84 ± 17.47 | 0.57 |
| Sex N(%) | | | | |
| Males | 102 (39%) | 57 (39%) | 45 (39%) | 1 |
| Females | 162 (61%) | 91 (61%) | 71 (61%) | |
| BMI Category N (%) | | | | |
| Normal (18.5 – 24.9 kg/m$^2$) | 110 (42%) | 55 (37%) | 55 (47%) | 0.05* |
| Overweight (25 – 29.9 kg/m$^2$) | 101 (38%) | 58 (39%) | 43 (37%) | |
| Obese (>30 kg/m$^2$) | 53 (20) | 35 (24%) | 18 (16%) | |
| **Vitamin D (ng/mL)** | 25.9 ± 13.8 | 25.32 ± 13.27 | 26.51 ± 14.70 | 0.77 |
| Vitamin D Status N(%) | | | | |
| Deficiency (<20 ng/mL) | 71 (35%) | 42 (34%) | 28 (34%) | 0.95 |
| Insufficiency (20–30 ng/mL) | 72 (35%) | 40 (33%) | 29 (34%) | |
| Sufficient (>30 ng/mL) | 62 (30%) | 40 (33%) | 26 (32%) | |
| Vitamin D Supplements N(%) | | | | |
| No | 120 (45%) | 69 (47%) | 51 (44%) | 0.71 |
| Yes | 144 (55%) | 79 (53%) | 65 (56%) | |

Data are presented as mean ± standard deviation for continuous variables and as number (percentage) for categorical variables. *P*-values were calculated using independent samples t-tests or Chi-square tests, with $p \leq 0.05$ considered statistically significant.

did not significantly differ between COVID-positive (25.32 ± 13.27 ng/mL) and COVID-negative patients (26.51 ± 14.70 ng/mL; $p$ = 0.77). Similarly, vitamin D status categories (deficiency, insufficiency, sufficiency) and vitamin D supplement use showed no significant differences ($p$> 0.05).

Multivariate logistic regression analysis identified age ≥60 years as a significant predictor of increased COVID-19 disease risk (OR = 1.90, 95% CI: 1.02–3.55, $p$ = 0.04). Conversely, sex, BMI categories, and vitamin D status did not independently predict COVID-19 disease ($p$ > 0.05) (Table 2).

In the subset of 70 patients evaluated for gene expression (Table 3), higher *VDR* expression in nasopharyngeal samples was significantly associated with a reduced likelihood of COVID-19 disease (OR = 0.40, 95% CI: 0.15–1.06, $p$ = 0.05). Likewise, elevated *DEFA1–3* mRNA expression exhibited strong protective effects, significantly reducing COVID-19 disease risk by 81.6% (OR = 0.184, 95% CI: 0.035–0.97, $p$ = 0.012). Conversely, *CCL20* expression did not differ significantly between COVID-positive and COVID-negative patients ($p$ = 0.294).

The gene expression comparison according to the COVID-19 disease status showed that the normalized *VDR, DEFA1–3 and CCL20* expression is significantly higher in the negative group than in the positive group (Figure 1, $P$<0.05).

Pearson correlation analysis demonstrated a significant inverse relationship between serum 25(OH)D concentrations and *VDR* expression (r = -0.61, $p$ = 0.04). Interestingly, In the COVID-19 negative group, *VDR* expression was positively correlated with *CCL20* expression (r = 0.59, $p$ < 0.01), while no significant correlation was observed between *VDR* and *DEFA1-3* (r = –0.15) or between *CCL20* and *DEFA1-3* (r = –0.04). In the COVID-19 positive group, *VDR* expression showed a significant positive correlation with *DEFA1-3* (r = 0.45, $p$ < 0.05). The correlation between *CCL20* and *DEFA1–3* was weak and nonsignificant (r = 0.16) in the positive group (Table 4). When pooling samples, *VDR* expression remained significantly correlated with *CCL20* (r = 0.45, $p$ < 0.01).

Correlation analysis among COVID-19-positive patients showed no significant associations between *DEFA1–3* and *CCL20* expression or with SARS-CoV-2 replication genes *(ORF1*, *N*, *E)* (all $p$ >0.05). However, strong correlations were observed among the viral replication genes themselves (*ORF1*, *N*, and *E*; all r >0.99, $p$ <0.001), validating their use as reliable markers of viral replication (Supplementary Table 1).

## Discussion

Our integrated analysis, combining clinical data from a cohort with detailed gene expression profiling in a representative subset, provides critical insights into the roles of vitamin D and innate

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TABLE 2 Multivariate logistic regression analysis of predictors of COVID-19 disease: clinical parameters.

| Characteristic | COVID-19 disease | | |
|---|---|---|---|
| | OR | 95% C.I. | *P* |
| **Age** | | | |
| <60 | 1 | | 0.04* |
| ≥60 | 1.90 | (1.02 – 3.55) | |
| **Sex** | | | |
| Male | 1 | | 0.25 |
| Female | 1.48 | (0.77 - 2.76) | |
| **BMI** | | | |
| Normal (18.5 – 24.9 kg/m$^2$) | 1 | | |
| Overweight (25 – 29.9 kg/m$^2$) | 0.48 | (0.21 – 1.09) | 0.08 |
| Obese (>30 kg/m$^2$) | 0.57 | (0.25 – 1.29) | 0.18 |
| **Vitamin D status** | | | |
| Deficiency (<20 ng/mL) | 1 | | |
| Insufficiency (20–30 ng/mL) | 0.75 | (0.36 – 1.56) | 0.44 |
| Sufficient (>30 ng/mL) | 0.76 | (0.37 – 1.58) | 0.45 |

Odds ratios (OR), 95% confidence intervals (CI), and *p*-values are provided for age, sex body mass index (BMI), and vitamin D status, along with reference categories.

TABLE 3 Multivariate logistic regression analysis with COVID-19 disease predictors: gene expression data.

| Characteristics | COVID-19 Disease | | |
|---|---|---|---|
| | OR | 95% C.I. | *P* |
| **Age** | | | |
| <60 | 1 | | |
| ≥60 | 5.250 | 1.547-17.821 | 0.008* |
| **Sex** | | | |
| Male | 1 | | |
| Female | 3.359 | 0.891-12.658 | 0.073 |
| **BMI** | | | |
| Normal | 1 | | |
| Overweight | 1.151 | 0.314-4.219 | 0.832 |
| Obese | 1.891 | 0.490-6.881 | 0.491 |
| **Normalized DEFA1–3 Expression** | | | |
| Low | 1 | | |
| High | 0.184 | 0.035-0.960 | 0.012* |
| **Normalized CCL20 Expression** | | | |
| Low | 1 | | |
| High | 0.532 | 0.163-1.732 | 0.294 |
| **Normalized *VDR* Expression** | | | |
| Low | 1 | | |
| High | 0.4 | (0.15 - 1.06) | 0.05* |

Gene expression levels were classified as low or high based on their median value.
Odds ratios (OR), 95% confidence intervals (CI), and *p*-values for age, sex, BMI, and gene expression levels of defensin alpha 1-3 (*DEFA1-3*), chemokine (C-C motif) ligand 20 (*CCL20*), and vitamin D receptor-1 (*VDR-1*). *indicates statistical significance ($p \leq 0.05$).

immune responses in COVID-19 susceptibility. Despite similar serum 25(OH)D concentrations between COVID-19-positive and negative patients, this finding suggests that circulating vitamin D concentrations alone may inadequately reflect the full immunomodulatory potential of vitamin D. Rather, local tissue responsiveness—as represented by *VDR* expression in nasopharyngeal tissues—emerges as a crucial determinant of protective immunity. Higher *VDR* expression correlated with a significant 60% reduction in COVID-19 disease risk, highlighting the importance of receptor-mediated signaling within local mucosal environments.

Additionally, analysis of inflammatory biomarkers indicated that elevated *DEFA1–3* expression significantly reduces COVID-19 susceptibility by 81.6% (OR: 0.184, $p = 0.012$). *DEFA1–3* peptides

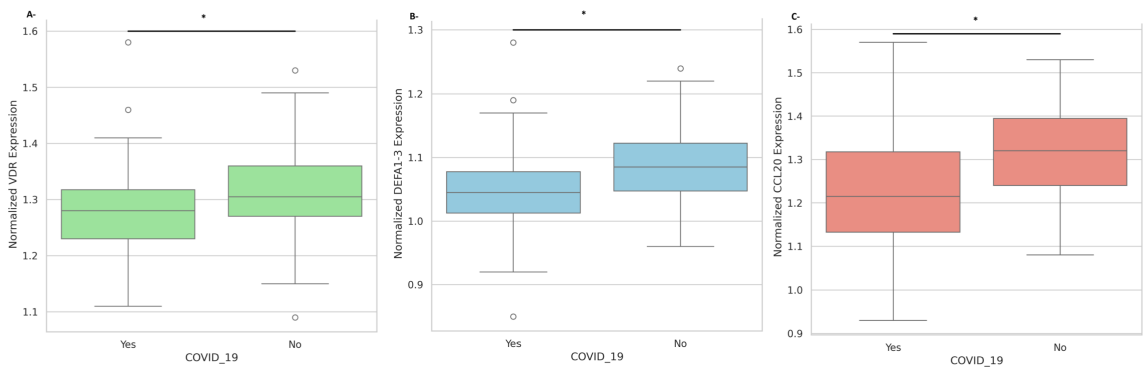


FIGURE 1
Nasopharyngeal expression of innate immune mediators according to COVID-19 disease status. **(A)** Normalized expression levels of *VDR* mRNA in COVID-19 negative versus COVID-19 positive participants. **(B)** Normalized expression levels of *DEFA1–3* mRNA in COVID-19 negative versus COVID-19 positive participants. **(C)** Normalized expression levels of *CCL20* mRNA in COVID-19 negative versus COVID-19 positive participants. All expression values are normalized to *GAPDH*. Data are presented as mean ± standard deviation. Statistical significance is indicated (*$p < 0.05$).

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TABLE 4 Pearson correlation analysis of normalized *VDR*, *CCL20*, and *DEFA1–3* expression stratified by COVID-19 disease status.

| | *VDR* | *CCL20* | *DEFA1-3* |
|---|---|---|---|
| COVID-19 NEGATIVE (n=40) | | | |
| *VDR* | - | r = 0.59, $p < 0.01$ | r = –0.15, $p$ = N.S. |
| *CCL20* | r = 0.59, $p < 0.01$ | - | r = –0.04, $p$ = N.S. |
| *DEFA1-3* | r = –0.15, $p$ = N.S. | r = –0.04, $p$ = N.S. | - |
| COVID-19 POSITIVE (n=30) | | | |
| *VDR* | - | r = 0.28, $p$ = N.S. | r = 0.45, $p < 0.05$ |
| *CCL20* | r = 0.28, $p$ = N.S. | – | r = 0.16, $p$ = N.S. |
| *DEFA1-3* | r = 0.45, $p < 0.05$ | r = 0.16, $p$ = N.S. | - |
| All Samples (n=70) | | | |
| *VDR* | - | r = 0.45, $p < 0.01$ | r = 0.15, $p$ = N.S. |
| *CCL20* | r = 0.45, $p < 0.01$ | - | r = 0.15, $p$ = N.S. |
| *DEFA1-3* | r = 0.15, $p$ = N.S. | r = 0.15, $p$ = N.S. | - |

*N.S., Not Significant.

are known for their potent antiviral activities, including disruption of viral membranes, inhibition of viral entry, and modulation of local immune responses (17, 26). Their protective role was further supported by our finding of higher *DEFA1–3* expression in COVID-19-negative individuals, consistent with reduced viral susceptibility. This aligns with observations by Idris et al. (27), who reported significant downregulation of *DEFA1–3* during active SARS-CoV-2 infection, suggesting a possible viral evasion mechanism through suppression of host antimicrobial peptides. This suppression may partly explain the increased susceptibility to secondary infections observed in severe COVID-19 cases (27).

However, the role of *DEFA1–3* extends beyond direct antiviral activity. Alpha-defensins, including *DEFA1-3*, have complex functions that involve immune modulation and inflammatory responses. Elevated alpha-defensin levels have been associated with thrombotic complications in COVID-19 through interactions with fibrinogen and interleukin-6, highlighting their dual roles in both protective immunity and pathology (28). *DEFA1–3* peptides facilitate neutrophil recruitment and cytokine production, potentially driving beneficial inflammation for pathogen clearance; however, excessive or dysregulated activity could lead to tissue damage and exacerbated

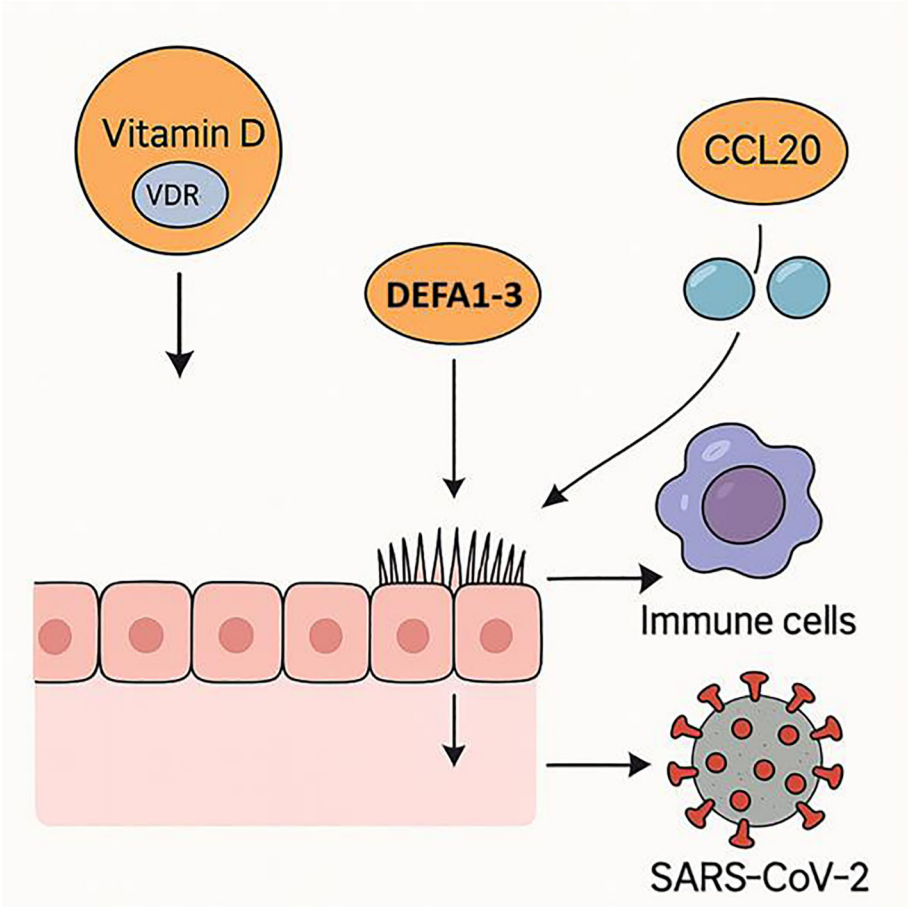


FIGURE 2
Proposed mechanism of vitamin D in reducing SARS-CoV-2 susceptibility. Vitamin D signaling via *VDR* in respiratory epithelium enhances antimicrobial defenses through *DEFA1–3* upregulation and facilitates immune cell recruitment by modulating *CCL20* expression, collectively lowering the risk of SARS-CoV-2 infection.

pathology (15, 28). This delicate balance underscores the importance of cautious interpretation and further investigation into the role of defensins during SARS-CoV-2 infection.

In our study, *DEFA1–3* expression showed no significant correlations with SARS-CoV-2 viral replication genes (*ORF1*, *N*, and *E*), suggesting that defensin activity operates independently of viral replication dynamics and is likely influenced predominantly by host factors (29). Consequently, *DEFA1–3* expression may serve as a valuable biomarker for assessing susceptibility to COVID-19. While elevated baseline defensin levels may reflect robust innate immunity, they could also signal immune dysregulation or exhaustion (28). Measuring defensin levels clinically enhances risk assessment and patient management strategies.

In contrast to *DEFA1-3*, *CCL20* expression did not differ significantly between infected and non-infected individuals, suggesting variability in the contribution of inflammatory mediators to COVID-19 susceptibility (22–24). However, our results suggest that the role of *CCL20* in disease susceptibility may be context-dependent, warranting further investigation.

The correlation patterns suggest that vitamin D signaling through *VDR* is functionally linked to immune cell recruitment (via *CCL20*) under non-infectious conditions. However, this relationship appears to be disrupted during COVID-19 disease, where vitamin D signaling may shift toward enhancing antimicrobial peptide production (*DEFA1-3*) as part of the host defense mechanism. The loss of *VDR-CCL20* correlation during infection could reflect immune system dysregulation or a shift in immune response priorities under infectious stress.

Our study has several limitations. Although our findings highlight reduced 25(OH)D levels in COVID-19 cases compared to controls, clinical severity data were not collected, precluding stratified analysis. Previous reports have shown that vitamin D status may prospectively predict COVID-19 severity and outcomes (30, 31). The study design limits our ability to establish causality and to assess longitudinal changes in vitamin D status, *VDR* expression, and inflammatory biomarkers. Additionally, although the overall cohort was sizable (n = 264), the subset for gene expression analysis was relatively limited, which may have limited its representativeness and generalizability. Potential confounders, including seasonal variations in vitamin D levels, nutritional status, and other environmental factors, were not fully controlled. Another limitation is the lack of data on participants' COVID-19 vaccination status, as it was not available at the time of data collection.

Given the high prevalence of vitamin D deficiency in Lebanon and its apparent link to COVID-19 susceptibility, future interventional trials should evaluate the efficacy and optimal dosing of vitamin D supplementation in reducing infection risk and disease severity. We recommend systematic screening for 25 (OH)D levels in at-risk populations (e.g., individuals with limited sun exposure) followed by randomized controlled studies to determine whether correcting deficiency can improve clinical outcomes in SARS-CoV-2 infection.

## Conclusion

In summary, our findings demonstrate that the protective effects of vitamin D in COVID-19 are more closely associated with local *VDR* expression and innate antimicrobial pathways, particularly DEFA1-3, than with systemic 25(OH)D concentrations alone (Figure 2). These results support a model in which both micronutrient status and tissue-specific vitamin D signaling modulate host susceptibility and disease severity (Figure 2). Future longitudinal and interventional studies are warranted to validate these associations and explore the therapeutic potential of enhancing local vitamin D responsiveness in respiratory viral infections.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

## Ethics statement

This study was reviewed and approved by the Institutional Review Board (IRB) at Lebanese Hospital Geitaoui-UMC (Protocol code: 2024-IRB-010). All participants provided written informed consent prior to their involvement in the study. The research was conducted following the principles outlined in the Declaration of Helsinki, and all participant data were anonymized and handled confidentially.

## Author contributions

FM: Formal Analysis, Investigation, Software, Writing – original draft. DM: Formal Analysis, Investigation, Software, Writing – original draft. ES-S: Conceptualization, Data curation, Project administration, Writing – review & editing. SK: Writing – review & editing. HF: Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. SE: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, 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/fendo.2025.1600623/full#supplementary-material

## References

1. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med*. (2020) 26:1017–32. doi: 10.1038/s41591-020-0968-3

2. Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, et al. Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. *Nutrients*. (2020) 12:988. doi: 10.3390/nu12040988

3. Greiller CL, Martineau AR. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients*. (2015) 7:4240–70. doi: 10.3390/nu7064240

4. Aranow C. Vitamin D and the immune system. *J Invest Med*. (2011) 59:881–6. doi: 10.2310/JIM.0b013e31821b8755

5. Fakhoury HM, Kvietys PR, Shakir I, Shams H, Grant WB, Alkattan K. Lung-centric inflammation of COVID-19: potential modulation by vitamin D. *Nutrients*. (2021) 13:2216. doi: 10.3390/nu13072216

6. Hamza FN, Daher S, Fakhoury HM, Grant WB, Kvietys PR, Al-Kattan K. Immunomodulatory properties of vitamin D in the intestinal and respiratory systems. *Nutrients*. (2023) 15:1696. doi: 10.3390/nu15071696

7. Wang Z, Joshi A, Leopold K, Jackson S, Christensen S, Nayfeh T, et al. Association of vitamin D deficiency with COVID-19 infection severity: Systematic review and meta-analysis. *Clin Endocrinol*. (2022) 96:281–7. doi: 10.1111/cen.14540

8. Kaya MO, Pamukçu E, Yakar B. The role of vitamin D deficiency on COVID-19: a systematic review and meta-analysis of observational studies. *Epidemiol Health*. (2021) 43:e2021074. doi: 10.4178/epih.e2021074

9. Kvietys PR, Fakhoury HM, Kadan S, Yaqinuddin A, Al-Mutairy E, Al-Kattan K. COVID-19: lung-centric immunothrombosis. *Front Cell Infect Microbiol*. (2021) 11:679878. doi: 10.3389/fcimb.2021.679878

10. Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1α, 25 (OH) 2vitamin D3: Genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab*. (2011) 25:543–59. doi: 10.1016/j.beem.2011.05.010

11. Wang T-T, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1, 25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol*. (2004) 173:2909–12. doi: 10.4049/jimmunol.173.5.2909

12. Carlberg C. Vitamin D and its target genes. *Nutrients*. (2022) 14:1354. doi: 10.3390/nu14071354

13. Harkous D, Ghorayeb N, Gannagé-Yared MH. Prevalence and predictors of vitamin D deficiency in Lebanon: 2016-2022, before and during the COVID-19 outbreak. *Endocrine*. (2023) 82:654–63. doi: 10.1007/s12020-023-03483-8

14. Arabi A, Chamoun N, Nasrallah MP, Tamim HM. Vitamin D deficiency in lebanese adults: prevalence and predictors from a cross-sectional community-based study. *Int J Endocrinol*. (2021) 2021:3170129. doi: 10.1155/2021/3170129

15. Wilson SS, Wiens ME, Smith JG. Antiviral mechanisms of human defensins. *J Mol Biol*. (2013) 425:4965–80. doi: 10.1016/j.jmb.2013.09.038

16. Xu C, Wang A, Marin M, Honnen W, Ramasamy S, Porter E, et al. Human defensins inhibit SARS-CoV-2 infection by blocking viral entry. *Viruses*. (2021) 13:1246. doi: 10.3390/v13071246

17. López-Bermejo A, Chico-Julia B, Castro A, Recasens M, Esteve E, Biarnés J, et al. Alpha defensins 1, 2, and 3: potential roles in dyslipidemia and vascular dysfunction in humans. *Arterioscler Thromb Vasc Biol*. (2007) 27:1166–71. doi: 10.1161/ATVBAHA.106.138594

18. Shrivastava S, Chelluboina S, Jedge P, Doke P, Palkar S, Mishra AC, et al. Elevated levels of neutrophil activated proteins, alpha-defensins (DEFA1), calprotectin (S100A8/A9) and myeloperoxidase (MPO) are associated with disease severity in COVID-19 patients. *Front Cell Infect Microbiol*. (2021) 11:751232. doi: 10.3389/fcimb.2021.751232

19. Christensen HM, Frystyk J, Faber J, Schou M, Flyvbjerg A, Hildebrandt P, et al. α-Defensins and outcome in patients with chronic heart failure. *Eur J Heart Fail*. (2012) 14:387–94. doi: 10.1093/eurjhf/hfs021

20. Lee AY, Eri R, Lyons AB, Grimm MC, Korner H. CC chemokine ligand 20 and its cognate receptor CCR6 in mucosal T cell immunology and inflammatory bowel disease: odd couple or axis of evil? *Front Immunol*. (2013) 4:194. doi: 10.3389/fimmu.2013.00194

21. Skovdahl HK, Damås JK, Granlund A, Østvik AE, Doseth B, Bruland T, et al. CC motif ligand 20 (CCL20) and CC motif chemokine receptor 6 (CCR6) in human peripheral blood mononuclear cells: dysregulated in ulcerative colitis and a potential role for CCL20 in IL-1β release. *Int J Mol Sci*. (2018) 19:3257. doi: 10.3390/ijms19103257

22. Chi Y, Ge Y, Wu B, Zhang W, Wu T, Wen T, et al. Serum cytokine and chemokine profile in relation to the severity of coronavirus disease 2019 in China. *J Infect Dis*. (2020) 222:746–54. doi: 10.1093/infdis/jiaa363

23. Gruber CN, Patel RS, Trachtman R, Lepow L, Amanat F, Krammer F, et al. Mapping systemic inflammation and antibody responses in multisystem inflammatory syndrome in children (MIS-C). *Cell*. (2020) 183:982–995.e14. doi: 10.1016/j.cell.2020.09.034

24. Hue S, Beldi-Ferchiou A, Bendib I, Surenaud M, Fourati S, Frapard T, et al. Uncontrolled innate and impaired adaptive immune responses in patients with COVID-19 acute respiratory distress syndrome. *Am J Respir Crit Care Med*. (2020) 202:1509–19. doi: 10.1164/rccm.202005-1885OC

25. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. (2011) 96:1911–30. doi: 10.1210/jc.2011-0385

26. Kudryashova E, Zani A, Vilmen G, Sharma A, Lu W, Yount JS, et al. Inhibition of SARS-CoV-2 infection by human defensin HNP1 and retrocyclin RC-101. *J Mol Biol*. (2022) 434:167225. doi: 10.1016/j.jmb.2021.167225

27. Idris MM, Banu S, Siva AB, Nagaraj R. Down regulation of defensin genes during SARS-CoV-2 infection. *Acta Virol*. (2022) 66:249–53. doi: 10.4149/av_2022_306

28. Xu D, Lu W. Defensins: a double-edged sword in host immunity. *Front Immunol*. (2020) 11:3389/fimmu.2020.00764. doi: 10.3389/fimmu.2020.00764

29. Fu J, Zong X, Jin M, Min J, Wang F, Wang Y. Mechanisms and regulation of defensins in host defense. *Signal Transduct Target Ther*. (2023) 8:300. doi: 10.1038/s41392-023-01553-x

30. Asla MM, Nawar AA, Elsayed E, Farahat RA, Abdulgadir A, Alsharabasy MA, et al. Vitamin D on COVID-19 patients during the pandemic, 2022. A systematic review and meta-analysis. *Curr Res Nutr Food Sci*. (2023) 11. doi: 10.12944/CRNFSJ.11.1.3

31. Di Filippo L, Uygur M, Locatelli M, Nannipieri F, Giustina A. Low vitamin D levels predict outcomes of COVID-19 in patients with both severe and non-severe disease at hospitalization. *Endocrine*. (2023) 80:669–83. doi: 10.1007/s12020-023-03331-9

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