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Vascular injury in systemic diseases: current concepts and future perspectives

Edited by
Panagiota Anyfanti, Allegra Battistoni and Theodoros Dimitroulas

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Vascular injury in systemic diseases: current concepts and future perspectives

Topic editors

Panagiota Anyfanti — Aristotle University of Thessaloniki, Greece

Allegra Battistoni — Sapienza University of Rome, Italy

Theodoros Dimitroulas — Aristotle University of Thessaloniki, Greece

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DeLisa Fairweather,
Mayo Clinic Florida, United States

*CORRESPONDENCE
Panagiota Anyfanti
✉ panyfan@hotmail.com

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Editorial: Vascular injury in systemic diseases: current concepts and future perspectives

Allegra Battistoni[1], Theodoros Dimitroulas[2] and Panagiota Anyfanti[3]*

[1]Clinical and Molecular Medicine Department, Sapienza University of Rome, Rome, Italy, [2]Fourth Department of Internal Medicine, Hippokration Hospital, Thessaloniki, Greece, [3]3rd Department of Internal Medicine, Papageorgiou Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

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Editorial on the Research Topic
Vascular injury in systemic diseases: current concepts and future perspectives

The vasculature is no longer viewed simply as a passive conduit for the circulation of blood, but rather as a dynamic and highly responsive system that reflects and shapes the status of systemic health (1). In recent years, vascular injury has emerged not merely as a downstream consequence of chronic disease but as a crucial driver of multi-organ pathology (2, 3). Whether through immune activation, metabolic dysregulation, chronic inflammation, or environmental stressors, systemic diseases frequently converge on the vascular system, compromising its integrity and function at various levels and with diverse clinical consequences (Figure 1).

The articles collected in this Research Topic, *Vascular Injury in Systemic Diseases*, illuminate how this convergence manifests in both common and rare scenarios, and how the cardiovascular system, whether at the level of capillaries, major vessels, or the heart itself responds to these multifaceted challenges.

One major contribution of this collection is the emphasis on the complexity of vascular remodeling in chronic systemic diseases. For example, systemic sclerosis and cirrhotic portal hypertension, both classically studied for their fibrotic or hemodynamic component, were here reframed in vascular terms. In the contribution by Heilmeier et al. on the systemic sclerosis, low-grade inflammation and persistent immune activation seem to promote atherogenesis through chronic endothelial stress, challenging clinicians to recognize the vascular burden in what is often mischaracterized as purely connective tissue disease (Heilmeier et al.). Similarly, the pathophysiology of portal hypertension is enriched by a focus on intra- and extrahepatic vascular dysregulation, offering new perspectives on the interplay between liver disease and systemic vascular remodeling in the paper by Li et al.

Genetic approaches further deepen this perspective. Indeed, Mendelian randomization analysis can provide a powerful tool in addressing the limitations of observational studies and reveal causal effects. A Mendelian randomization study by Deng et al. included in this Topic explores the vascular implications of insomnia, an increasingly prevalent condition often overlooked as a cardiovascular risk factor. By demonstrating that genetically

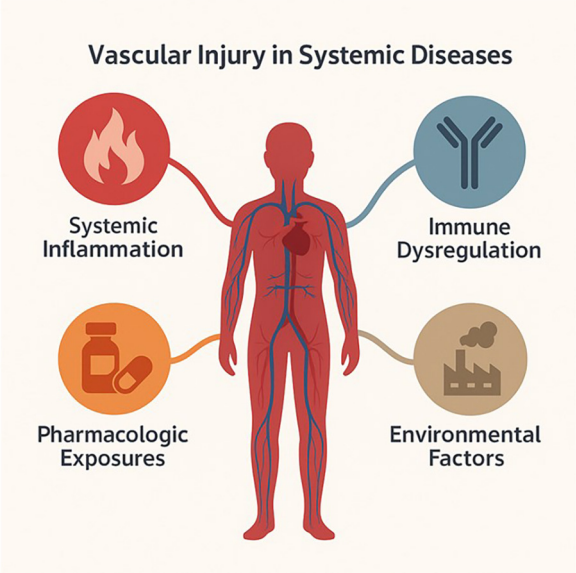

FIGURE 1
Complex and diverse factors interact and contribute to vascular injury in systemic diseases, including immune activation, metabolic dysregulation, chronic inflammation, pharmacological exposures, and/or environmental stressors.

predicted insomnia increases the risk of myocardial infarction through modifiable intermediaries such as smoking and body mass index, the authors highlight the need to expand our understanding of behavioral contributors to vascular injury (Deng et al.). Another study by Lin et al. aims to fill the gap in research concerning the relationship between lupus and cardiac structure and function using similar methodology. The conclusion, supporting a causal relationship between lupus traits and alterations in cardiac structure and function, brings fresh insight into how autoimmune diseases may exert subclinical but progressive cardiovascular damage (Lin et al.).

The role of infection and immune activation in provoking vascular injury is also a recurring theme. Huang et al. evaluate the emergence of anti-neutrophil cytoplasmic antibodies following SARS-CoV-2 infection, pointing to the virus's capacity to induce autoimmune vascular responses even in the absence of overt vasculitis. Though the pandemic's acute effects may be receding, its immunological sequelae remain a fertile ground for understanding vascular reactivity in post-infectious contexts (Huang et al.).

Several clinical case reports in this Topic serve as reminders of how vascular involvement may dominate or complicate systemic illnesses. Teng et al. striking example details a case of granulomatosis with polyangiitis mimicking a mass in the aortic root, a rare but instructive instance of how large-vessel vasculitis can masquerade as neoplastic disease (Teng et al.). In such cases, misdiagnosis can carry fatal consequences, underlining the importance of maintaining a high index of suspicion for immune-mediated vascular pathology.

Other cases document severe, therapy-related vascular and hematological complications, including heparin-induced thrombocytopenia after plasmapheresis by Zhou et al. and propylthiouracil-induced ANCA-associated vasculitis complicated by granulocytopenia and hemophagocytosis by Chen et al. These reports reveal how therapeutic interventions, especially in immunologically fragile individuals, may act as unintentional vascular stressors.

Even within the pediatric population, the consequences of microvascular injury might be severe. A rare but illuminating case of livedoid vasculopathy in children is presented by Qu et al., underscoring how microcirculatory disorders, though often underdiagnosed, can lead to painful and debilitating outcomes (Qu et al.). Such contributions expand the conversation around vascular injury beyond adult cardiovascular risk, into realms where early recognition may alter long-term prognosis.

What ties these studies together is not a single anatomical structure or disease mechanism, but a shared recognition that vascular injury is a central, actionable pathway through which systemic diseases evolve and express their severity. The endothelium, the vessel wall, the cardiac muscle, and the immune interface all represent sites where systemic disease can either be intercepted or exacerbated. This requires a shift in both research and clinical practice from viewing vascular damage as a late-stage complication to treating it as a primary target for prevention and intervention.

We hope that this collection of articles will serve not only as a scientific contribution but also as a call to shift our clinical and research focus toward the vasculature—not simply as a passive conduit but as a dynamic, vulnerable, and actionable player in systemic disease. By placing vascular injury at the center of multidisciplinary discourse, we may unlock new strategies to predict, prevent, and treat the complications of systemic illness.

Author contributions

AB: Writing – review & editing, Writing – original draft. TD: Writing – original draft, Writing – review & editing. PA: Writing – original draft, Writing – review & editing.

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. Gavriilaki E, Anyfanti P. Editorial: endotheliopathies: current concepts and importance in clinical practice. *Front Med.* (2023) 10:1162121. doi: 10.3389/fmed.2023.1162121

2. Anyfanti P, Gavriilaki E, Douma S, Gkaliagkousi E. Endothelial dysfunction in patients with rheumatoid arthritis: the role of hypertension. *Curr Hypertens Rep.* (2020) 22:56. doi: 10.1007/s11906-020-01064-y

3. Gavriilaki E, Anyfanti P, Gavriilaki M, Lazaridis A, Douma S, Gkaliagkousi E. Endothelial dysfunction in COVID-19: lessons learned from coronaviruses. *Curr Hypertens Rep.* (2020) 22:63. doi: 10.1007/s11906-020-01078-6

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Yingjian Deng,
Xiamen University, China
Eleni Gavriilaki,
Aristotle University of Thessaloniki, Greece
Lina Su,
China-Japan Friendship Hospital, China

*CORRESPONDENCE
Jian He
✉ xiangyahejian1993@163.com
Yanfang Xu
✉ xuyanfang99@hotmail.com

†These authors have contributed equally to this work

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Impact of systemic lupus erythematosus on cardiovascular morphologic and functional phenotypes: a Mendelian randomization analysis

Zishan Lin[1,2,3†], Wenfeng Wang[1,2,3†], Bingjing Jiang[1,2,3], Jian He[4*] and Yanfang Xu[1,2,3*]

[1]Department of Nephrology, Blood Purification Research Center, The First Affiliated Hospital, Fujian Medical University, Fuzhou, China, [2]Research Center for Metabolic Chronic Kidney Disease, The First Affiliated Hospital, Fujian Medical University, Fuzhou, China, [3]Department of Nephrology, National Regional Medical Center, Binhai Campus of the First Affiliated Hospital, Fujian Medical University, Fuzhou, China, [4]Department of Gastroenterology, Nanfang Hospital, Southern Medical University, Guangzhou, China

Background: Previous studies have established a correlation between systemic lupus erythematosus (SLE) and cardiovascular health, but the potential causal effects of SLE on heart function and structure remain poorly understood. Cardiovascular magnetic resonance imaging (CMR), a novel non-invasive technique, provides a unique assessment of cardiovascular structure and function, making it an essential tool for evaluating the risk of heart disease. In this study, we performed a Mendelian randomization analysis to determine the causal relationship between SLE and CMR traits.

Methods: Genetic variants independently linked to SLE were selected from a genome-wide association study (GWAS) containing 5,201 cases and 9,066 controls as instrumental variables. A set of 82 CMR traits was obtained from a recent GWAS, serving as preclinical indicators and providing preliminary insights into the morphology and function of the four cardiac chambers and two aortic segments. Primary analysis employed a two-sample Mendelian randomization study using the inverse-variance weighted method. Heterogeneity testing, sensitivity analyses, and instrumental variable strength assessments confirmed the robustness of the findings.

Results: SLE exhibited a correlation with increased stroke volume ($\beta_{LVSV} = 0.007$, $P = 0.045$), regional peak circumferential strain ($\beta_{Ecc\_AHA\_9} = 0.013$, $P = 0.002$; $\beta_{Ecc\_AHA\_12} = 0.009$, $P = 0.043$; $\beta_{Ecc\_AHA\_14} = 0.013$, $P = 0.006$), and global peak circumferential strain of the LV ($\beta_{Ecc\_global} = 0.010$, $P = 0.022$), as well as decreased regional radial strain ($\beta_{Err\_AHA\_11} = -0.010$, $P = 0.017$).

Conclusions: This research presents evidence of a potential causal association between traits of SLE and alterations in cardiac function, guiding cardiac examinations and disease prevention in lupus patients.

KEYWORDS

systemic lupus erythematosus, cardiovascular magnetic resonance imaging, cardiovascular structure and function, Mendelian randomization, cardiac function

1 Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that commonly affects multiple organs and is associated with high prevalence and mortality. Several observational studies have indicated a relationship between SLE and the development of heart disease, such as cardiovascular diseases, myocarditis, valvular heart diseases, and heart failure (1–4). It is well-documented that structural changes in the heart may precede the onset of cardiac conditions. Notably, individuals with lupus who manifest heart disease often exhibit alterations in heart structure or function in clinical settings (1, 4). These alterations in lupus patients may be associated with the aforementioned cardiac conditions. However, these findings may be influenced by biases arising from residual confounding factors and reverse causality, due to the inherent limitations of observational studies (5). Therefore, caution is advised when interpreting causality in these correlations, and randomized controlled trials or advanced statistical methods are required to validate these findings and minimize biases and confounding factors.

Cardiac and aortic structures, crucial for maintaining normal physiological functions, can manifest abnormalities even before overt disease symptoms present. The utilization of cardiovascular magnetic resonance imaging (CMR) allows for the comprehensive integration of morphological and functional assessments, enabling precise tissue characterization of myocardial changes (6). This capability provides detailed insights comparable to pathological observations of various myocardial abnormalities, including edema, necrosis, fibrosis, and more (7, 8). As a result, CMR is widely recognized as the gold standard for the non-invasive assessment of cardiovascular structure and function. The attributes obtained from CMR serve as recognized endophenotypes, playing a crucial role as key risk indicators (9, 10). The ongoing exploration of gene repositories has facilitated the availability of genome-wide association studies (GWAS) associated with CMR phenotypes, supporting in-depth research enhanced by pertinent GWAS data.

As an evolving epidemiological research approach, Mendelian randomization (MR) analysis employs genetic variants as instrumental variables (IVs) to evaluate the causal relationships between exposures and outcomes. In conventional observational studies, confirming the causal relationship between SLE and heart structure and function presents challenges due to the complexities of reverse causality and residual confounders. In contrast, IVs in MR analysis have unique advantages. Alleles are randomly assigned from parents to offspring according to Mendel's laws of inheritance, ensuring genetic variation largely independent of confounding factors. Additionally, MR analysis adheres to the natural order of causation, as genetic variation precedes both exposure and outcome (11). Therefore, we conducted a two-sample MR study to examine the potential causal relationships between SLE and these CMR traits.

2 Methods

2.1 Study design

Figure 1 displays an overview of the research methodology. A two-sample MR study was conducted to assess causal associations linking SLE and CMR characteristics, with SLE as the exposure and CMR features as the outcomes. This investigation was underpinned by three pivotal assumptions to obtain a persuasive conclusion from MR analysis: (1) IVs demonstrate a robust correlation with SLE; (2) IVs are independent of potential confounders; and (3) IVs predominantly influence outcomes through the exposure pathway. The data utilized were exclusively sourced from the publicly available GWAS catalog, and ethical approvals as well as informed consent were obtained in all original papers.

2.2 Data source

The single nucleotide polymorphisms (SNPs) associated with SLE were sourced from the study conducted by Bentham J. et al., which was published in 2015 (12). This investigation involved a newly conducted GWAS, a meta-analysis, and a replication study, encompassing 14,267 individuals of European ancestry (5,201 cases and 9,066 controls). For the outcome datasets, 82 CMR features were primarily sourced from a study conducted by Zhao et al. in 2023, including four left atrium (LA) traits, 64 left ventricle (LV) traits, four right atrium (RA) traits, four right ventricle (RV) traits, three ascending aorta (AAO) traits, and three descending aorta (DAO) traits. This research involved a cohort of 40,000 participants and employed the analytical pipelines developed by Bai et al. for extracting imaging traits from raw brain and cardiac MRI images (13). The datasets for SLE and CMR traits were limited to individuals of European ancestry to minimize potential bias. The summary of the GWAS data used in our study is provided in Table 1. Further details regarding the exposure and results data, such as trait names and categories can be found in Supplementary Table S1, S2.

2.3 Genetic instrument selection

The criteria for selecting IVs required SNPs to be significantly correlated with exposures, achieving genome-wide significance ($P < 5 \times 10^{-8}$). Additionally, linkage disequilibrium clumping procedures were applied with a threshold set at $r^2 < 0.001$ and a maximum clump distance of <10,000 kb to ensure the independence of SNPs. All of these SNPs were searched for secondary phenotypes using the PhenoScanner tool (http://www.phenoscanner.medschl.cam.ac.uk/).log to exclude potential pleiotropic effects. SNPs corresponding to phenotypes directly related to the outcome or associated with confounders ($P < 5 \times 10^{-8}$), including cardiovascular disease, hypertension, and diabetes were removed. Only genetic instruments with an

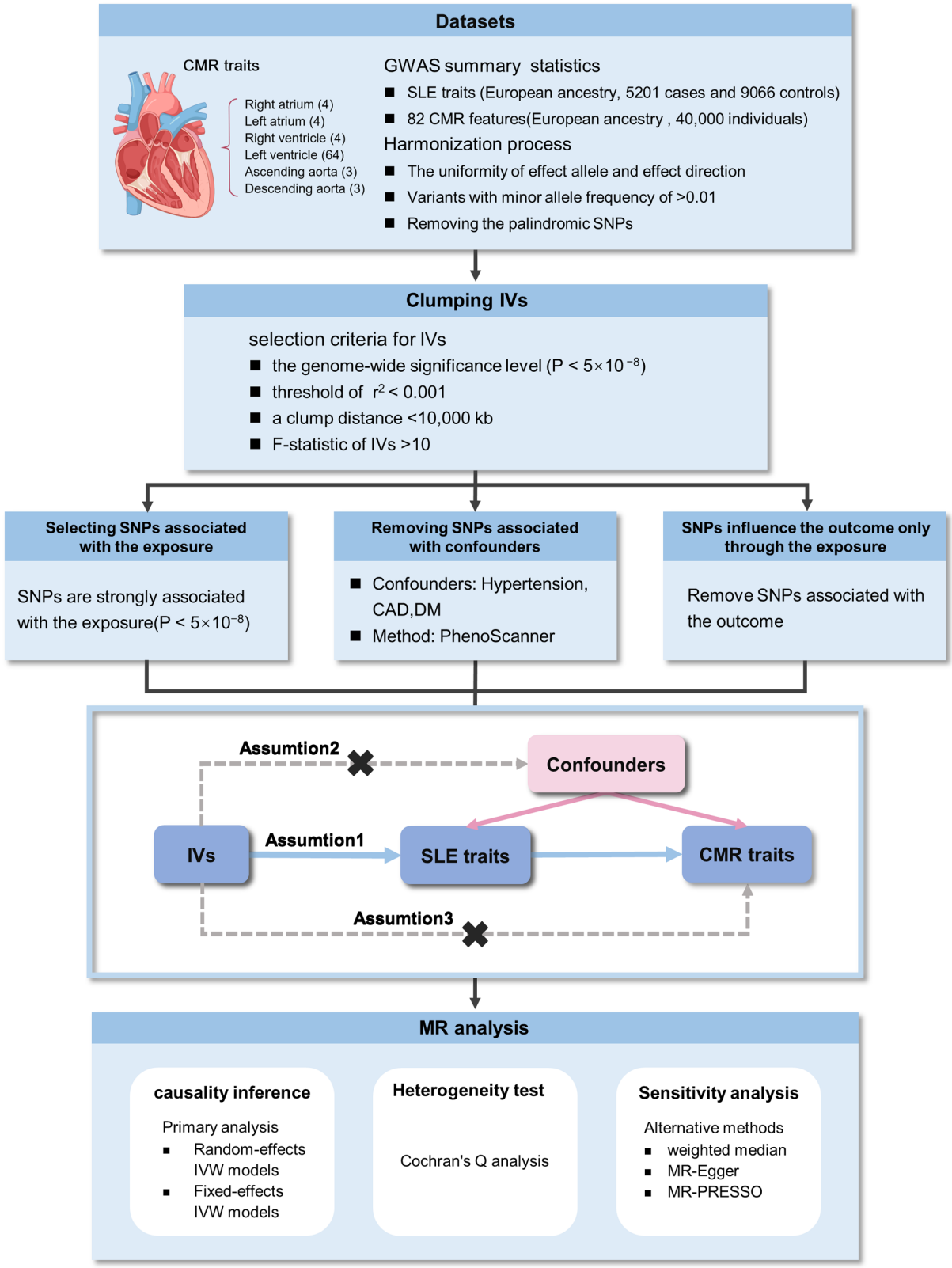

FIGURE 1
Overview of the study design and analyses. CMR, cardiovascular magnetic resonance imaging; GWAS, genome-wide association studies; SLE, systemic lupus erythematosus; SNPs, single nucleotide polymorphisms; IVW, inverse-variance weighted; IVs, instrumental variables.

TABLE 1 Studies and datasets included in the mendelian randomization analyses.

Traits	Data source	Sample size (cases/controls)	Year	Ancestry	PMID
Exposure					
Systemic lupus erythematosus	Bentham J. et al.	5,201/9,066	2015	European	26502338
Outcome					
Cardiovascular magnetic resonance imaging	Zhao et al.	40,000	2023	European	37262162

F-statistic greater than 10 were retained for further analysis to eliminate weak instruments. To assess the robustness of each genetic instrument, the F-statistic was calculated using the specified formula: $F = \left(\frac{\hat{\beta}_X}{Se(\hat{\beta}_X)}\right)^2$ (5).

2.4 Statistical analyses

Before initiating the MR analysis, the exposure and outcome datasets were harmonized to remove palindromic SNPs. The primary analytical approach for the MR analysis involved using the inverse-variance weighted (IVW) method, which assumes that all SNPs function as valid IVs to achieve the most accurate estimates. Heterogeneity was tested using Cochran's Q analysis. A *P*-value greater than 0.05 led to the adoption of the fixed-effects IVW approach under the assumption of homogeneity. Conversely, the random-effects IVW method was employed in scenarios where heterogeneity was detected.

2.5 Sensitivity analyses

Additional sensitivity analyses were performed using the weighted median, MR-Egger, and MR-PRESSO. Specifically, the weighted median approach posits that a valid causal inference can still be made if at least 50% of the weights from the IVs are valid. The MR-Egger test provides a valid result even in cases where all IVs are invalid. Although both the weighted median and MR-Egger methods have less statistical power compared to the IVW method, consistent results in the same direction across all three methods strengthen the reliability of the causal estimates from the primary analysis. Furthermore, the MR-PRESSO method was utilized to identify and address horizontal pleiotropy introduced by outlier SNPs, which were subsequently identified, removed, and re-analyzed (5). The results of the study were presented as effect value (β) with 95% confidence interval (CI), setting the threshold for significance at α=0.05. All statistical analyses were carried out using Rstudio (version 4.3.1) with the TwoSampleMR package (version 0.5.8).

3 Results

3.1 Identifying genetic instruments for SLE traits

Following the predefined screening criteria, 39–41 significant ($P < 5 \times 10^{-8}$) and independent ($r^2 < 0.001$, 10,000-kb) SNPs were carefully selected as IVs from the SLE GWAS (Supplementary Table S3). No SNPs directly associated with CMR traits were identified, and SNPs associated with confounds were excluded (Supplementary Table S4). Each F-statistic associated with the instrumental exposure exceeded 10 (ranging from 30 to 461), thereby successfully reducing the influence of weak IVs on the study outcomes.

3.2 SLE and CMR traits

The main results are presented in Figure 2. In the comprehensive analysis of all outcome data, a genetically predicted SLE exhibited a correlation ($P < 0.05$) with 6 CMR traits. Among these, SLE showed a positive association with left ventricular stroke volume (LVSV) ($\beta_{LVSV} = 0.007$, 95% CI 0.001–0.015, $P = 0.045$). Furthermore, SLE was positively correlated with three regional peak circumferential strains of LV from the 16 pre-defined American Heart Association (AHA) segments, as well as global peak circumferential strain. The specific values for each are as follows: Ecc_AHA_9 ($\beta_{Ecc\_AHA\_9} = 0.013$; 95% CI 0.005–0.022; $P = 0.002$), Ecc_AHA_12 ($\beta_{Ecc\_AHA\_12} = 0.009$, 0.001–0.018; $P = 0.043$), Ecc_AHA_14 ($\beta_{Ecc\_AHA\_14} = 0.013$; 95% CI 0.003–0.023; $P = 0.006$), and global peak circumferential strain ($\beta_{Ecc\_global} = 0.010$, 95% CI 0.002–0.019, $P = 0.022$). In contrast, SLE was negatively associated with a regional radial strain of LV from the 16 pre-defined AHA segments: Err_AHA_11 ($\beta_{Err\_AHA\_11} = -0.010$, 95% CI −0.019 to −0.002, $P = 0.017$). There was a notable trend toward significance between SLE and increased right ventricular end-diastolic volume (RVEDV) ($\beta_{RVEDV} = 0.006$, $P = 0.060$), although the findings were not statistically significant between SLE and any of AAO, DAO, LA, RA, or RV (Supplementary Table S5).

3.3 Sensitivity analyses of MR

Most of the results remained directionally consistent with those from the IVW method, thereby enhancing the robustness of the findings. No statistical associations were found between SLE and CMR traits using the MR-Egger and weighted median methods. No evidence of heterogeneity or horizontal pleiotropy was observed among the statistically significant CMR traits. Some outlier SNPs were identified and removed using the MR-PRESSO method, but their exclusion did not affect the stability of the main findings (Supplementary Table S5).

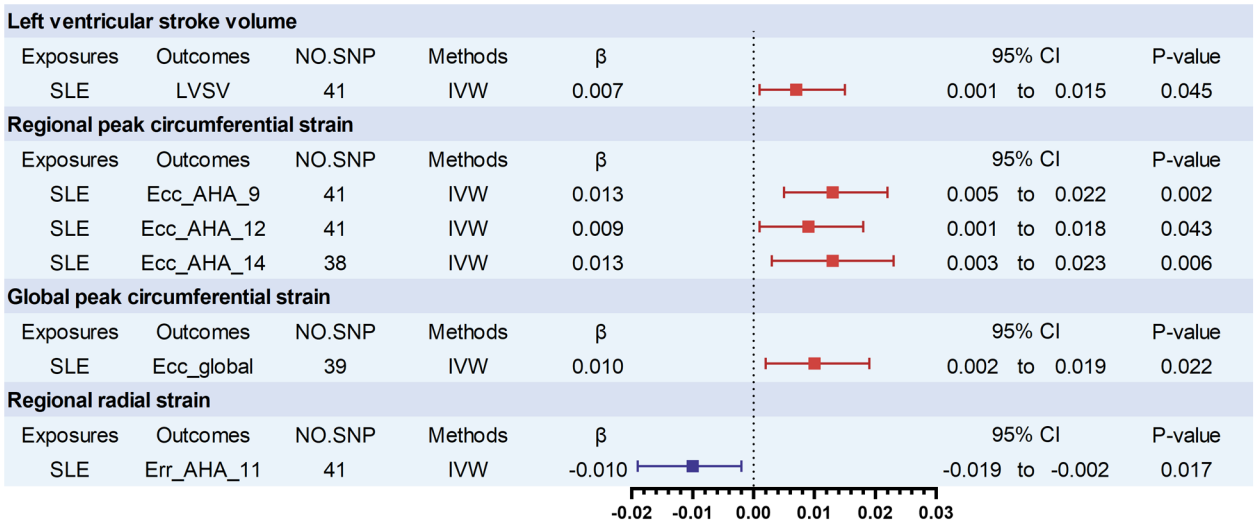

FIGURE 2
Significant associations of genetically predicted SLE traits with cardiovascular magnetic resonance imaging traits. LVSV, left ventricular stroke volume; Ecc_AHA, regional peak circumferential strain, segments 9, 12, and 14 of the 16 predefined segments by the American Heart Association (AHA); Ecc_global, global peak circumferential strain. Err_AHA, regional radial strain, segment 11 of the 16 predefined segments by the American Heart Association (AHA); SLE, systemic lupus erythematosus.

4 Discussion

SLE is a complex autoimmune disorder that can affect various organs, including cardiovascular system. The 2017 European Society of Cardiology Consensus Document by Caforio et al. emphasized the prevalence and severity of cardiac involvement in patients with SLE. The study also highlighted the crucial role of non-invasive diagnostic methods, including CMR, in detecting early cardiac structural changes in these patients (14). Extensive studies have already indicated a possible association between SLE and heart diseases, such as cardiomyopathy, heart failure, valvular heart diseases, and cardiovascular diseases (1–4). Despite advancements in medical technology that have improved the longevity and quality of life for individuals with lupus, mortality linked to cardiovascular events has increased (15, 16). As a major source of early damage in lupus patients, cardiovascular system impairment has garnered considerable scholarly attention in recent years (17). A cohort study involving 252,676 patients with SLE and 758,034 matched controls in the United States revealed an increased risk of CAD linked to SLE (adjusted OR 1.42; 95% CI 1.40–1.44) (18). Furthermore, a long-term observational study of 3,411 SLE patients demonstrated an elevated risk of heart failure and other cardiovascular outcomes in comparison to matched control subjects (19). In 2022, Gao et al. conducted an MR study that confirmed the potential causal relationship between SLE and an elevated risk of heart failure (20). Similarly, Kain et al. leveraged MR and pathway analysis in the same year to identify shared genetic risk factors for SLE and CAD (21). Despite these findings, there remains a gap in research concerning the relationship between lupus and cardiac structure and function, and whether these heart changes observed in lupus patients are solely attributed to SLE is uncertain.

In this investigation, we utilized the latest large-scale GWAS summary-level data to examine the association between SLE and a comprehensive set of 82 CMR traits. These traits were systematically classified into six categories: RA, RV, LA, LV, AAO, and DAO. This study is the first to apply MR to systematically explore the potential causal relationships between SLE and both heart structure and function. The main discovery of our research suggests a potential causal relationship between SLE traits on LV. The findings are summarized as follows: (1) SLE was linked to greater LVSV; (2) SLE was correlated with three increased regional peak circumferential strains from the 16 pre-defined AHA segments (Ecc_AHA_9, 12, 14); (3) SLE was associated with higher global circumferential strain; (4) SLE was related to decreased left ventricular regional radial strain (Err_AHA_11).

In our study, left ventricular function changes associated with SLE were identified. These findings are consistent with several observational studies. For instance, in an observational study of 79 patients with SLE, Myhr et al. identified a higher prevalence of myocardial fibrosis and noted structural changes in the LV compared to the control group. Notably, LVSV was found to be increased in SLE patients compared to normal controls ($P = 0.03$), corroborating our findings (2). As a critical method for evaluating cardiac function, myocardial strain offers detailed insights into myocardial mechanics and functional status (22, 23). A meta-analysis by Di Minno et al. demonstrated a significant reduction in left ventricular radial strain in SLE patients compared to non-SLE controls (95% CI: −13.819 to −8.241, $P < 0.001$), consistent with our study outcomes (24). In this study, we identified an association between SLE and increased left ventricular regional and global peak circumferential strains, which appears to diverge from the findings of previous

study (24). It is possible that similar to myocardial injury caused by other etiologies, SLE-induced myocardial fibrosis or endothelial dysfunction varies in severity across different regions of the myocardium. Some areas might initially affect radial strain, while other regions, either unaffected or experiencing compensatory enhancement, could show increased circumferential strain. Therefore, the observed increase in myocardial circumferential strain may reflect a compensatory mechanism of the heart aimed at preserving overall cardiac function. Another possible explanation could be the ethnic differences caused by the fact that our database included only European samples. Larger studies with more diverse populations are needed to determine the association between these factors.

Previous studies have frequently suggested a close association between SLE and right ventricular (25, 26). In our study, although no statistically causal relationship was established, a trend was observed indicating some degree of correlation between SLE and RVEDV ($\beta = 0.006$, $P = 0.060$). It is recognized that enlargement of the RV in SLE patients is typically attributed to pulmonary hypertension, which is caused by inflammation and vascular damage. However, a study by Deidda et al. demonstrated that subclinical RV dysfunction can be observed in SLE patients free of pulmonary hypertension using echocardiographic screening (25). Similarly, our study identified a trend towards statistical significance between SLE and increased RVEDV, suggesting that SLE could directly impact RV function. Several possible explanations may account for the lack of a positive result in our study. It is possible that in clinical practice, the use of medications and the presence of various comorbidities in patients contribute to the differences in outcomes, or that SLE induces changes in RV structure and function through alternative mechanisms, without a direct causal relationship between SLE and RV. Another possibility is the limited availability of data on RV within the GWAS data we included, which may explain the absence of positive findings (5).

As a chronic condition, SLE can have a persistent impact on cardiac and aortic structures and function, leading to gradual deterioration over time. These changes may eventually reach a critical threshold, triggering a transition from a subclinical to a symptomatic phase of heart disease. CMR, as an advanced tool, can detect these cardiac structural and functional changes at an earlier stage, making it valuable for early diagnosis and intervention. While previous research has demonstrated an association between SLE and cardiovascular disease, our findings may offer more direct evidence that SLE not only correlates with cardiovascular disease but may also directly impair cardiovascular function, which could be reflected in CMR measurements. Moreover, the CMR features associated with SLE could be further investigated as potential biomarkers for SLE-related cardiovascular damage. This could facilitate clinical monitoring and management of cardiovascular health in SLE patients, enabling earlier detection and intervention before clinical symptoms arise.

Our study showcases several strengths. First, MR was employed for the first time to explore the relationship between SLE and both cardiac structure and function using the latest CMR data. Second, MR effectively reduces the impact of residual confounding and reverse causality. By using sensitivity analyses and assessing the strength of IVs, the results were verified, thereby bolstering the robustness of the causal evidence. Third, since our findings predominantly rely on aggregated data from individuals of European descent, the potential bias due to population stratification is minimized.

Our study has some limitations. First, the scale and scope of genetic association studies on SLE have been limited, with constrained power and genomic coverage. To maximize power and coverage, we utilized the largest available GWAS of SLE. Second, as the prevalence and mortality of SLE exhibit variations across ethnicities, the exclusive inclusion of European participants in this MR analysis complicates the extrapolation of the potential causal relationship between SLE and CMR traits to other populations. Third, it is also worth noting that certain risk factors leading to different phenotypic outcomes, such as SLE and cardiac structure, may be influenced by environmental factors that cannot be fully explained by genetics. Our MR study can only address the genetically related components, and cannot fully account for the impact of environmental and non-genetic factors on these phenotypes. Lastly, the β value was relatively low and should be interpreted carefully.

In conclusion, we explored the influence of SLE traits on cardiac and aortic remodeling. Our findings suggest that SLE contributes to left ventricular remodeling by increasing LVSV, and both regional and global peak circumferential strain, while also leading to the reduction of regional radial strain. These findings may indicate a potential risk of cardiac function changes in SLE patients, aiding in the understanding of how SLE affects the cardiovascular system and providing guidance for cardiac examinations and disease prevention.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: the manuscript and Supplementary Materials contain all necessary data for evaluating its findings. For additional data related to this study, one can request it from the corresponding author upon reasonable request.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

ZL: Writing – original draft, Funding acquisition, Data curation, Conceptualization. WW: Formal Analysis, Writing – original draft, Data curation. BJ: Visualization, Writing – original draft, Software. JH: Methodology, Writing – review & editing. YX: Funding acquisition, Writing – review & editing, 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2024.1454645/full#supplementary-material

References

1. Ajeganova S, Hafström I, Frostegård J. Patients with SLE have higher risk of cardiovascular events and mortality in comparison with controls with the same levels of traditional risk factors and intimamedia measures, which is related to accumulated disease damage and antiphospholipid syndrome: a case-control study over 10 years. *Lupus Sci Med*. (2021) 8(1):e000454. doi: 10.1136/lupus-2020-000454

2. Myhr KA, Zinglersen AH, Pecini R, Jacobsen S. Myocardial fibrosis associates with lupus anticoagulant in patients with systemic lupus erythematosus. *Int J Cardiovasc Imaging*. (2023) 40:127–37. doi: 10.1007/s10554-023-02970-3

3. Vivero F, Gonzalez-Echavarri C, Ruiz-Estevez B, Maderuelo I, Ruiz-Irastorza G. Prevalence and predictors of valvular heart disease in patients with systemic lupus erythematosus. *Autoimmun Rev*. (2016) 15:1134–40. doi: 10.1016/j.autrev.2016.09.007

4. Dhakal BP, Kim CH, Al-Kindi SG, Oliveira GH. Heart failure in systemic lupus erythematosus. *Trends Cardiovasc Med*. (2018) 28:187–97. doi: 10.1016/j.tcm.2017.08.015

5. Burgess ST. *Mendelian Randomization: Methods for Causal Inference Using Genetic Variants*. 2nd edn. Boca Raton: Chapman and Hall/CRC (2021). p. 224. doi: 10.1201/9780429324352

6. Russo V, Lovato L, Ligabue G. Cardiac MRI: technical basis. *Radiol Med*. (2020) 125:1040–55. doi: 10.1007/s11547-020-01282-z

7. Nakou E, Patel RK, Fontana M, Bucciarelli-Ducci C. Cardiovascular magnetic resonance parametric mapping techniques: clinical applications and limitations. *Curr Cardiol Rep*. (2021) 23:185. doi: 10.1007/s11886-021-01607-y

8. Zhao S. Letter to the editor: is it time for imaging to level with pathology? *Int J Cardiovasc Imaging*. (2020) 36:2249–50. doi: 10.1007/s10554-020-01936-z

9. Demirkiran A, Everaars H, Amier RP, Beijnink C, Bom MJ, Gotte MJW, et al. Cardiovascular magnetic resonance techniques for tissue characterization after acute myocardial injury. *Eur Heart J Cardiovasc Imaging*. (2019) 20:723–34. doi: 10.1093/ehjci/jez094

10. Li Z, Xiong J, Guo Y, Tang H, Guo B, Wang B, et al. Effects of diabetes mellitus and glycemic traits on cardiovascular morpho-functional phenotypes. *Cardiovasc Diabetol*. (2023) 22:336. doi: 10.1186/s12933-023-02079-w

11. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, 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

12. Bentham J, Morris DL, Cunninghame Graham DS, Pinder CL, Tombleson P, Behrens TW, et al. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet*. (2015) 47:1457–64. doi: 10.1038/ng.3434

13. Zhao B, Li T, Fan Z, Yang Y, Shu J, Yang X, et al. Heart-brain connections: phenotypic and genetic insights from magnetic resonance images. *Science*. (2023) 380:abn6598. doi: 10.1126/science.abn6598

14. Caforio ALP, Adler Y, Agostini C, Allanore Y, Anastasakis A, Arad M, et al. Diagnosis and management of myocardial involvement in systemic immune-mediated diseases: a position statement of the European Society of Cardiology working group on myocardial and pericardial disease. *Eur Heart J*. (2017) 38:2649–62. doi: 10.1093/eurheartj/ehx321

15. Moghaddam B, Marozoff S, Li L, Sayre EC, Zubieta JAA. All-cause and cause-specific mortality in systemic lupus erythematosus: a population-based study. *Rheumatology (Oxford)*. (2021) 61:367–76. doi: 10.1093/rheumatology/keab362

16. McMahon M, Seto R, Skaggs BJ. Cardiovascular disease in systemic lupus erythematosus. *Rheumatol Immunol Res*. (2021) 2:157–72. doi: 10.2478/rir-2021-0022

17. Harden OC, Hammad SM. Sphingolipids and diagnosis, prognosis, and organ damage in systemic lupus erythematosus. *Front Immunol*. (2020) 11:586737. doi: 10.3389/fimmu.2020.586737

18. Katz G, Smilowitz NR, Blazer A, Clancy R, Buyon JP, Berger JS. Systemic lupus erythematosus and increased prevalence of atherosclerotic cardiovascular disease in hospitalized patients. *Mayo Clin Proc*. (2019) 94:1436–43. doi: 10.1016/j.mayocp.2019.01.044

19. Yafasova A, Fosbøl EL, Schou M, Baslund B, Faurschou M, Docherty KF, et al. Long-term cardiovascular outcomes in systemic lupus erythematosus. *J Am Coll Cardiol*. (2021) 77:1717–27. doi: 10.1016/j.jacc.2021.02.029

20. Gao N, Kong M, Li X, Wei D, Zhu X, Hong Z, et al. Systemic lupus erythematosus and cardiovascular disease: a Mendelian randomization study. *Front Immunol*. (2022) 13:908831. doi: 10.3389/fimmu.2022.908831

21. Kain J, Owen KA, Marion MC, Langefeld CD, Grammer AC, Lipsky PE. Mendelian randomization and pathway analysis demonstrate shared genetic associations between lupus and coronary artery disease. *Cell Rep Med*. (2022) 3(11):100805. doi: 10.1016/j.xcrm.2022.100805

22. Scatteia A, Baritussio A, Bucciarelli-Ducci C. Strain imaging using cardiac magnetic resonance. *Heart Fail Rev*. (2017) 22:465–76. doi: 10.1007/s10741-017-9621-8

23. Halliday BP, Senior R, Pennell DJ. Assessing left ventricular systolic function: from ejection fraction to strain analysis. *Eur Heart J*. (2021) 42:789–97. doi: 10.1093/eurheartj/ehaa587

24. Di Minno MND, Forte F, Tufano A, Buonauro A, Rossi FW, De Paulis A, et al. Speckle tracking echocardiography in patients with systemic lupus erythematosus: a meta-analysis. *Eur J Intern Med*. (2020) 73:16–22. doi: 10.1016/j.ejim.2019.12.033

25. Deidda M, Noto A, Firinu D, Piras C, Cordeddu W, Depau C, et al. Right ventricular subclinical dysfunction in SLE patients correlates with metabolomic fingerprint and organ damage. *Metabolites*. (2023) 13(7):781. doi: 10.3390/metabo13070781

26. Rakha S, Hammad A, Elmarsafawy H, Korkor MS, Eid R. A deeper look into the functions of right ventricle using three-dimensional echocardiography: the forgotten ventricle in children with systemic lupus erythematosus. *Eur J Pediatr*. (2023) 182:2807–19. doi: 10.1007/s00431-023-04936-y

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Athanasia Dara,
Hippokration General Hospital, Greece
Theodoros Dimitroulas,
Aristotle University of Thessaloniki, Greece
Eleni Pagkopoulou,
Hippokration Hospital of Thessaloniki, Greece

*CORRESPONDENCE
Stephanie Finzel
✉ Stephanie.Finzel@uniklinik-freiburg.de

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Chronic low-grade inflammation in patients with systemic sclerosis is associated with increased risk for arteriosclerotic cardiovascular disease

Ursula Heilmeier[1,2], Daria Feldmann[1,3], Andrew Leynes[2], Magdalena Seng[1,4], Ilona Jandova[1], Marius Keute[5], Florian Kollert[1,6,7], Reinhard Edmund Voll[1] and Stephanie Finzel[1]*

[1]Department of Rheumatology and Clinical Immunology, Faculty of Medicine, Medical Center–University of Freiburg, University of Freiburg, Freiburg im Breisgau, Germany, [2]Musculoskeletal Quantitative Imaging Research Group, University of California San Francisco, San Francisco, CA, United States, [3]Department of Anesthesiology, Krankenhaus Porz am Rhein, Cologne, Germany, [4]Department of Radiology and Nuclear Medicine, University of Basel, Basel, Switzerland, [5]Institute for Neuromodulation and Neurotechnology, University of Tübingen, Tübingen, Germany, [6]Department of Rheumatology and Immunology, University Hospital Bern, University of Bern, Bern, Switzerland, [7]Department of Rheumatology, University Hospital Basel, Basel, Switzerland

Background: Vasculopathy is a hallmark of systemic sclerosis (SSc) putting patients at an increased risk of cardiovascular disease. Approximately 20–25% of all SSc patients show prolonged elevated C-reactive protein (CRP) levels and thus signs of chronic low-grade inflammation. While CRP–positivity is an independent predictor of cardiovascular disease in non-SSc populations, the relationship between CRP–positivity and cardiovascular health/atherosclerosis in SSc patients is only incompletely understood. Here, we aimed to assess (1) which general, SSc disease-specific and cardiovascular parameters are associated with CRP–positivity in a cohort of SSc patients with prolonged CRP elevations (CRP+ SSc group) relative to SSc patients without CRP elevations (CRP– SSc group). In addition (2), we aimed to investigate whether prolonged CRP–positivity in SSc patients is associated with a higher cardiovascular risk and an increased atherosclerotic burden. We also aimed to (3) identify via random forest classification modeling which combined cardiovascular and/or SSc-specific parameters could differentiate best between SSc patients with elevated CRP levels (the so-called "inflammatory SSc subtype") and SSc patients without increased CRP levels.

Methods: Sixty-five SSc patients were recruited and assigned to the CRP+ SSc group ($n = 20$) if their CRP levels were > 5 mg/L in at least three half-yearly visits within 2 years before enrolment or to the CRP– SSc group ($n = 45$), respectively. All patients underwent an anamnesis, physical examination, blood draw, and bilateral carotid ultrasound in order to assess arteriosclerotic burden including the presence, number and height of plaques, and carotid intima–media thickness (CIMT) as well as lipid profiles. 10-year ASCVD risk was estimated via the ASCVD risk estimator plus. Statistical evaluation included Spearman's correlations, logistic regression and random forest modeling under 5-fold cross-validation, and permutation testing to determine combinations of cardiovascular variables highly discriminatory for CRP–positivity.

Results: SSc groups showed comparable mean age, height, and extent of SSc organ involvement. Regarding cardiovascular health, CRP+ SSc patients exhibited a significantly altered HDL-, LDL-, and triglyceride profile ($0.001 \leq p \leq 0.017$) and a significantly higher 10-year ASCVD risk ($p = 0.047$), relative to CRP− SSc patients. Additionally, within the subgroup of CRP+ SSc patients, positive correlations between CRP levels and CIMT right ($\rho = 0.657$, $p = 0.002$) and mean CIMT left and right ($\rho = 0.497$, $p = 0.026$) were seen. Combined ROC models identified the four lipid components (HDL, LDL, total cholesterol, and triglycerides) or the SSc duration and ASCVD category to differentiate with high cross-validated ROC-AUCs (AUC: 0.83 ± 0.15, and AUC: 0.86 ± 0.09, $p < 0.001$) for prolonged CRP−positivity among SSc patients.

Conclusion: Our data indicate that persistent CRP−positivity and thus chronic low-grade inflammation in SSc patients enhance the risk for arteriosclerotic-cardiovascular disease significantly beyond the ASCVD risk observed for our SSc patients without CRP elevations. It seems to be along with a disrupted lipid profile the hallmark of a distinct "inflammatory" subgroup of SSc patients. However, large population-based studies and clinical trials in patients with SSc are needed to validate our findings in a prospective or interventional setting.

KEYWORDS

systemic sclerosis, CRP, arteriosclerotic cardiovascular risk, carotid ultorasonography, inflammatory systemic sclerosis, Framingham score, ASCVD risk score, SCORE (systemic coronary risk evaluation)

1 Introduction

Systemic sclerosis (SSc) is an autoimmune disease characterized by excessive deposition of extracellular matrix resulting in distinct progressive fibrosis of the skin and of internal organs, such as the lungs, and in vasculopathy (1). SSc is linked to one of the highest mortality rates among all rheumatic diseases (2). Clinical diagnosis, treatment, and research are challenging due to the phenotypic heterogeneity of SSc. Efforts have been undertaken to identify biomarkers that can help predict SSc organ involvement and severity, SSc progression, and mortality and may therefore be utilized for risk stratification and personalized treatment.

C-reactive protein (CRP) is an easily accessible and cost-efficient serum marker of inflammation in daily clinical routine. To date, several studies have shown that approximately 23.2–41.5% of patients with SSc have elevated CRP levels (3, 4). In approximately 18% of SSc patients, CRP elevations are even persistent (5, 6). More importantly, prospective studies and a meta-analysis carried out in this particular "inflammatory subtype" of SSc patients have linked the presence of elevated CRP levels (cutoff >5 mg/L) to worse health outcomes such as an increased risk of pulmonary hypertension (7), progressive fibrosis, and higher mortality (8, 9). In addition, elevated CRP levels have been found associated with the increasing severity of skin and joint involvement (9, 10).

Microvascular and macrovascular diseases are additional hallmarks of SSc, putting SSc patients at a significantly increased risk of cardiovascular events compared to the general population (11, 12). In a recent meta-analysis, pooled hazard ratios in SSc patients ranged from 2.36 (95% CI 1.97–2.81) for cardiovascular disease (CVD) or myocardial infarction (95% CI 1.71–3.25) to a hazard ratio of HR 5.27 (95% CI 4.27–6.51) for peripheral vascular disease compared to the general population (12). For most inflammatory rheumatic diseases, an increased cardiovascular risk has been demonstrated with the most detailed data available for rheumatoid arthritis (RA). Analyzing carotid intima–media thickness (CIMT) and pulse wave velocity, Dimitroulas et al. (13) found comparable magnitudes of subclinical atherosclerosis in patients with SSc when compared with patients with RA, highlighting the importance and need for cardiovascular risk assessment and management also for SSc patients (13). While CRP elevation has long been established as a strong predictor and risk factor for carotid arteriosclerosis (14), future cardiovascular events (15), and poor prognosis after CV events (16, 17) in non-SSc patients, the relationship between CRP levels and cardiovascular health/arteriosclerosis in SSc patients has to date only been incompletely (18) and inconsistently (19–23) investigated as studies so far either assessed CRP–cardiovascular correlations only as a (bystander) secondary outcome (19) or their study design and sample size did not allow for or foresee more in-depth analyses (such as stratification by prolonged CRP status) (13, 22–25).

Therefore, the aims of this cross-sectional observational study were 3-fold: (1) to determine in a cohort of SSc patients with prolonged CRP elevations (CRP+ SSc group) relative to SSc patients without CRP elevations (CRP− SSc group) which general, and/or SSc disease-specific, and/or cardiovascular parameters are associated with a prolonged CRP elevation in systemic sclerosis. Furthermore, we aimed (2) to investigate whether prolonged CRP−positivity in SSc patients is associated with a higher cardiovascular risk as determined via the Framingham risk and arteriosclerotic cardiovascular disease (ASCVD) risk scores and with an increased atherosclerotic burden as determined by carotid ultrasound. We also aimed to (3) identify via random forest classification modeling, which combined cardiovascular and/or SSc-specific parameters could differentiate best between SSc patients with elevated CRP levels (the

"inflammatory SSc subtype") and SSc patients without increased CRP levels.

2 Materials and methods

2.1 Patient selection

In this cross-sectional, observational study, 65 patients with SSc from the outpatient clinic at the Department of Rheumatology and Clinical Immunology, University Medical Center, Freiburg, Germany, were enrolled. All patients fulfilled the ACR/EULAR 2013 classification criteria for SSc at the time of enrollment (26). Inclusion and exclusion criteria for this study as well as the group assignment into CRP−positive and CRP−negative groups have been published elsewhere (10). As described (10), 20 of the 65 patients exhibited elevated CRP levels >5 mg/L in at least three half-yearly visits within 2 years prior to enrolment and were assigned to the CRP−positive group (CRP+ SSc group), while 45 SSc patients exhibited normal CRP concentrations for at least 1.5 consecutive years before enrollment and were assigned to the CRP−negative group (CRP− SSc group). The Freiburg Institutional Review Board granted ethical approval for the study (386/17). Prior to any study-related measures, all patients provided written informed consent. The study was carried out in accordance with the principles of Good Clinical Practice (GCP) as developed by the International Conference of Harmonization (ICH) and set forth by the Declaration of Helsinki.

2.2 Study visits and clinical measurements

At the time of the study visit, all patients underwent a structured interview that assessed traditional risk factors for arteriosclerosis (such as the history of smoking, number of pack years, presence of arterial hypertension, type 2 diabetes mellitus, chronic kidney disease, and a positive family history with respect to the cardiovascular event [any cardiovascular event before the age of 65 years]). Information on the duration and extent of SSc and current medication use was extracted from the patient charts. In addition, all patients underwent a physical examination. This included apart from height, weight, heart rate, and blood pressure, the assessment of the acra for the presence of digital ulcerations, Raynaud's syndrome, and a tender and swollen joint count, which were recorded in a standardized way.

2.3 Laboratory measurements

All patients underwent a blood draw at the time of the study visit, and serum antibody and CRP concentrations were measured at the Department of Rheumatology and Clinical Immunology University Medical Center Freiburg laboratory (10). In addition, data on fasting serum levels of total cholesterol, HDL cholesterol, LDL cholesterol, total triglycerides, and eGFR levels were retrieved from the patients' laboratory charts, which all had been measured by the University Medical Center Freiburg's Department of Clinical Chemistry and Laboratory Medicine laboratory according to the quality standards of routine clinical diagnostic laboratory measures.

2.4 Ultrasound evaluation of carotid arteries

At the study visit, bilateral carotid ultrasound was carried out at the common and external carotid arteries using an 18-MHz linear array transducer on the same Esaote MyLab Twice ultrasound scanner (Esaote, Genoa, Italy) by the same sonographer (SF) with 9-year experience in ultrasound at the time of examination. Carotid intima–media thickness (CIMT) was measured in millimeters as a well-established marker of arteriosclerosis and determinant of cardiovascular disease (27, 28). CIMT measurements were performed by the American Society of Echocardiography's consensus scanning protocol (29). Common and external carotid arteries were scanned and evaluated in both the longitudinal and axial plane for the presence, height, and number of arteriosclerotic plaques at standardized locations as suggested by the American Society for Echocardiography. An arteriosclerotic plaque was therefore defined as a focal thickening of the arterial wall that was at least 50% thicker than the surrounding vessel walls and/or a focal thickening of the arterial wall protruding into the vessel lumen and being bigger than 1.5 mm (29). In order to not overlook cholesterol plaques, which are difficult to visualize in B-mode, color Doppler ultrasound scanning at 5.6 MHz was utilized. In total, the following arteriosclerotic ultrasound parameters were acquired for the left and right carotid arteries separately: carotid intima–media thickness (CIMT) in millimeters of the left and right common carotid arteries, the maximum plaque height in millimeters, and the number of plaques (n). In addition, the total number of plaques was calculated as a summary variable summarizing the number of all plaques of both sides. A mean CIMT of left and right carotid arteries was calculated as the sum of left and right CIMT divided by two and reported in millimeters.

2.5 Estimation of arteriosclerotic cardiovascular risk via Framingham, ASCVD, and SCORE 2019 risk scores

In order to estimate the arteriosclerotic cardiovascular risk in both SSc groups, three different risk calculators were utilized. For the individual 10-year risk of hard coronary heart disease (individual FRS 10-year risk of HCDH) and the average 10-year risk of hard coronary heart disease (average FRS 10-year risk of HCDH), we used the Framingham Risk Score for Hard Coronary Heart Disease risk calculator/outcome model, which is freely available online under the URLs https://www.mdcalc.com/calc/38/framingham-risk-score-hard-coronary-heart-disease and the https://www.framinghamheartstudy.org/fhs-risk-functions/hard-coronary-heart-disease-10-year-risk/ which is based on risk equations published by Wilson et al. (30). Outcome is the 10-year risk of hard coronary heart disease (=defined as any myocardial infarction or coronary death). The model is intended for use in patients aged 30–79 years with no prior history of coronary heart disease or intermittent claudication.

To estimate a patient's 10-year risk for atherosclerotic cardiovascular disease (ASCVD), we utilized the ASCVD Risk Estimator Plus provided by the American Heart Association and the American College of Cardiology and freely accessible online under the URL https://tools.acc.org/ascvd-risk-estimator-plus/#!/calculate/estimate/. Atherosclerotic cardiovascular disease (ASCVD) is hereby

defined as coronary death or non-fatal myocardial infarction, or fatal or non-fatal stroke, and the calculator is based on the pooled cohort equations and on the study published by Goff et al. (31) and others (32, 33). The input information needed to estimate ASCVD risk includes age, sex, race, total cholesterol, HDL cholesterol, systolic blood pressure, blood pressure-lowering medication use, diabetes status, and smoking status. Depending on their calculated ASCVD 10-year risk, all SSc patients were stratified in line with the recommendations of the American College of Cardiology into one of the following four ASCVD risk groups: low ASCVD risk group (containing SSc patients with <5% calculated ASCVD 10-year risk score), borderline ASCVD risk group (containing SSc patients with a 5–7.4% calculated ASCVD 10-year risk score), intermediate ASCVD risk group (containing SSc patients with a 7.5–19.9% calculated ASCVD 10-year risk score), and high ASCVD risk group (containing SSc patients with a ≥ 20% calculated ASCVD 10-year risk score).

To estimate a patient's 10-year risk of fatal cardiovascular disease (CVD), we utilized the Systematic Coronary Risk Evaluation (SCORE) cardiovascular risk chart (SCORE 2019) as provided and developed by the European Society of Cardiology and as published and updated by Mach et al. (34). We employed the low-risk chart as Germany is considered a low-risk country (34). For the SCORE 2019 risk calculation, the input information encompassed the following risk factors: age, gender, smoking, systolic blood pressure, and total cholesterol levels.

2.6 Statistical evaluation

The normal distribution of data was visually verified via Q-Q plots and statistically tested using Kolmogorov–Smirnov and Shapiro–Wilk testing. For normally distributed data, differences between CRP+ and CRP− groups were assessed via independent *t*-tests, Pearson's chi-squared tests, or Fisher's exact tests, as appropriate. For non-normally distributed data, Mann–Whitney *U*-tests were utilized. Spearman's rank order correlations were performed in order to assess the associations between CRP levels and cardiovascular risk factors, risk scores, and cardiovascular carotid ultrasound parameters in the overall cohort and the CRP+ and CRP− groups. Due to the relatively small patient sample, we could not test all variables in a common statistical model. Instead, we used binary logistic regression models to test the associations between the dependent variable (CRP positive yes/no) and the general, cardiovascular, and arteriosclerotic carotid ultrasound parameters. An odds ratio (OR) >1 indicates that a higher value in the respective candidate predictor variable (or response "yes" for categorical candidate predictor variables) is associated with a higher probability of belonging to the group CRP+. Subsequently, we used Random Forest classification along with a greedy feature selection procedure (35) with a 5-fold cross-validation and permutation test with 1,000 permutations in order to determine the optimal combination of cardiovascular variables associated with a positive CRP status. The binary classification area under the receiver operating curve (ROC-AUC) was employed as the scoring metric for feature selection. K-nearest neighbor imputation was performed ahead of any missing values. The AUCs between combined ROC models across the 5-fold cross-validation were compared using the Wilcoxon signed-rank test. Data analysis was carried out in Python using the scikit-learn 1.3.0 package and using SPSS statistics IBM, version 25. Statistical significance was defined as $p < 0.05$.

3 Results

3.1 Patients' characteristics

Patient demographics, SSc disease characteristics, and information on SSc-related medication usage as well as laboratory measures are summarized in Tables 1A,B and have in part been previously published by Feldmann et al. (10). In brief, CRP+ and CRP− groups showed similar mean age and height and did not significantly differ with respect to their age of SSc onset and gender proportions and with respect to the extent of their SSc organ involvement. Particularly, the proportions of SSc patients suffering from SSc-related vascular complications such as digital ulcerations, Raynaud's syndrome, or pulmonary arterial hypertension were comparable among both CRP− groups, as were the proportions of vasodilatory drug users ($p > 0.05$). However, CRP+ SSc patients had a significantly higher BMI (28.2 vs. 25.8 kg/m^2, $p = 0.037$), used significantly more often glucocorticoids ($p = 0.023$), significantly less often hydroxychloroquine ($p = 0.012$) and showed a statistical trend toward having on average an almost 2-year shorter SSc disease duration. As outlined previously (10), CRP+ SSc patients had a higher frequency of being SCL70-antibody positive ($p = 0.041$), whereas the CRP− SSc patients showed significantly more often positive anti-nucleosome antibodies ($p = 0.013$).

With respect to cardiovascular health, CRP+ SSc patients showed a significantly altered HDL-, LDL, and triglyceride profile (Table 1B: $0.001 \leq p \leq 0.017$) as well as a significantly higher proportion of type 2 diabetes compared to their CRP− SSc peers ($p = 0.049$). In addition, the estimation of cardiovascular risk scores revealed that CRP+ SSc patients had a significantly higher 10-year risk for ASCVD ($p = 0.047$) than the CRP− SSc patients. Risk stratification of SSc patients by ASCVD risk into a low-ASCVD SSc risk group (<5% ASCVD 10-year risk) and into a combined intermediate-to-high risk ASCVD risk group (IMH risk group, ≥7.5% or > 20% 10y-ASCVD risk estimated) confirmed significantly higher CRP levels in the IMH-risk SSc group compared to low-risk SSc individuals ($p = 0.024$) (Supplementary Table 1). Calculation and intergroup comparisons of SCORE 2019 risk scores demonstrated that the calculated 10-year risk for fatal cardiovascular disease was significantly higher in the CRP+ SSc group relative to the CRP− SSc group ($p = 0.040$). The individual Framingham risk scores for hard coronary heart disease were numerically higher in the CRP+ SSc group but did not reach statistical significance. Average Framingham risk scores for hard coronary heart disease and other cardiovascular risk factors such as the presence of arterial hypertension, chronic kidney disease, smoking history, and positive family history for a cardiovascular event below the age of 65 years were comparable between both groups, as were the atherosclerotic quantitative ultrasound measures of left and right carotid arteries and estimated glomerular filtration rates.

TABLE 1 Patient characteristics of all 65 study participants with systemic sclerosis (SSc), presented by CRP status.

(A)			
	Means ± SD		
	CRP+ group ($n = 20$)	CRP− group ($n = 45$)	CRP+ vs. CRP− p-value
Demographics			
Age [years]*	59.3 ± 13.0	58.0 ± 13.9	0.984
BMI [kg/m^2]	28.2 ± 5.3	25.8 ± 4.9	**0.037**
Height (m)	1.7 ± 0.1	1.7 ± 0.10	0.772
Weight [kg]	79.6 ± 19.1	71.7 ± 15.5	*0.085*
Sex, female n [%]*	13 [65.0]	38 [84.4%]	0.105
SSc characteristics			
Time since SSc diagnosis [months]*	107.2 ± 114.6	130.9 ± 91.6	*0.093*
Age of SSc diagnosis [years]*	50.4 ± 12.3	47.1 ± 14.43	0.453
Type of SSc Limited n [%]*	9 [45.0]	28 [62.2]	0.278
Type of SSc Diffuse n [%]*	11 [55.0]	17 [37.8]	0.278
Overall SSc organ involvement Ł n [%]*	15 [75.0]	27 [60.0]	0.276
Heart involvement n [%]*	1 [5.0]	1 [2.2]	0.524
Lung involvement n [%]*	10 [50.0]	16 [35.6]	0.289
Presence of PAH n [%]	6 [30.0]	10 [22.2]	0.542
Presence of SSc-related interstitial lung disease [%]	7 [35.0]	9 [20.0]	0.223
Esophagus involvement n [%]*	10 [50.0]	19 [42.2]	0.598
Gastrointestinal involvement n [%]*	1 [5.0]	3 [6.7]	1.000
Kidney involvement n [%]*	0 [0.0]	2 [2.2]	1.000
Presence of digital ulcerations n [%]	5 [25.0]	8 [17.8]	0.517
History of digital ulcerations n [%]	6 [30.0]	14 [31.1]	0.547
Presence of Raynaud's syndrome	20 [100.0]	42 [93.3]	1.000
Medication usage			
Usage of immunosuppressants			
glucocorticoids n [%]*	8 [40.0]	6 [13.3]	**0.023**
hydroxychloroquine n [%]*	0 [0.0]	12 [26.7]	**0.012**
methotrexate n [%]*	2 [10.0]	2 [4.4]	0.581
mycophenolate mofetil n [%]*	3 [15.0]	8 [17.8]	1.000
azathioprine n [%]*	1 [5.0]	1 [2.2]	0.524
cyclophosphamide n [%]*	1 [5.0]	0 [0.0]	0.308
Usage of vasodilatory medication	14 [70.0]	29 [64.4]	0.379
endothelin receptor antagonists n [%]	4 [20.0]	5 [11.1]	0.262
PDE-5 inhibitors n [%]	2 [10.0]	2 [4.4]	0.571
calcium antagonists n [%]	12 [60.0]	25 [55.6]	0.573
Antihypertensive medication usage ~	9 [45.0]	21 [46.7]	0.515
Lipid-lowering agents[†]	0 [0.0]	6 [13.3]	0.120

Ł organ involvement was considered present if any of the following organs (esophagus, lung, heart, GI system, or kidney) were affected by SSc. *Reprinted with permission as in Feldmann et al. (10) PAH, pulmonary arterial hypertension. ~ includes antihypertensive classes beta blockers, thiazide diuretics, loop diuretics, sartans, ACE-inhibitors. [†]Includes statins, fibrates, and ezetimibe. Shown are (unadjusted) means with standard deviations (SD). Significant p-values ($p < 0.05$) are marked in bold print, and statistical trends are printed in italics. CRP status was considered positive or negative, respectively, if at least 75% of the highly sensitive CRP values were positive (> 5 mg/L) or negative (≤ 5 mg/L) in at least three half-yearly visits within the past 2 years prior to the start of the study.

(Continued)

TABLE 1 (Continued)

(B)	Means ± SD		
	CRP+ group ($n = 20$)	CRP− group ($n = 45$)	CRP+ vs. CRP− p-value
Serum laboratory measures			
CRP levels [mg/l]	11.7 ± 8.0	3.5 ± 0.5	**<0.001**
Anti-Scl-70 antibody positive n [%]*	10 [50.0]	10 [22.2]	0.041
Anti-centromere antibody positive n [%] *	7 [35.0]	31 [68.9]	0.013
eGFR (estimated glomerular filtration rate) [ml/min/1.73m^2]	80.5 [61.1–87.1]	77.0 [73.6–85.3]	0.439
Total cholesterol [mg/dl]	216.7 ± 34.0	206.4 ± 41.0	0.330
HDL cholesterol [mg/dl]	50.7 ± 16.8	67.4 ± 16.4	**<0.001**
LDL cholesterol [mg/dl]	152.9 ± 28.4	129.3 ± 37.9	**0.017**
Triglycerides [mg/dl]	186.1 ± 91.1	123.4 ± 54.1	**0.003**
Cardiovascular risk factors			
Arterial hypertension n [%]	10 [50.0]	21 [46.7]	0.804
History of smoking n [%]	11 [55.0]	18 [40.0]	0.262
Pack years [years]	9.3 ± 15.0	7.5 ± 13.8	0.365
Diabetes mellitus type 2 n [%]	4 [20.0]	2 [4.4]	**0.049**
Chronic kidney disease n [%]	4 [20.0]	6 [13.3]	0.492
Positive family history of cardiovascular event below the age of 65 years n [%]	5 [25.0]	9 [20.0]	0.651
Individual FRS 10-year risk of HCHD [%]	9.5 ± 8.6	6.4 ± 6.9	0.200
Average FRS 10-year risk of HCHD [%]	7.6 ± 5.2	7.8 ± 6.0	0.762
ASCVD 10-year risk [%]	15.0 ± 12.0	9.3 ± 10.3	**0.047**
SCORE 2019 10-year risk of fatal CVD [%]	3.3 ± 3.4	1.9 ± 2.7	**0.040**
Arteriosclerotic assessment by carotid US			
Right-side carotid arteries	(n= 20)	(n=44)	
CIMT right [mm]	0.65 ± 0.19	0.60 ± 0.20	0.212
proportion of patients with right plaques n [%]	12 [60.0]	18 [40.9]	0.156
number of plaques right n	0.70 ± 0.66	0.48 ± 0.63	0.171
maximal plaque diameter right [mm]	1.35 ± 1.40	0.83 ± 1.11	0.138
Left-side carotid arteries	(n = 19)	(n =44)	
CIMT left [mm]	0.68 ± 0.29	0.63 ± 0.26	0.349
proportion of patients with left plaques n [%]	11 [57.9]	17 [38.6]	0.312
number of plaques left n	0.58 ± 0.51	0.43 ± 0.59	0.232
maximal plaque diameter left [mm]	1.22 ± 1.25	1.00 ± 1.55	0.270
Total number of plaques left and right n	1.92 ± 0.64	1.67 ± 0.76	0.240
Mean CIMT left and right carotids art. [mm]	0.67 ± 0.19	0.61 ± 0.21	0.313

FRS, Framingham risk score; HCDH, Hard coronary heart disease defined as either myocardial infarction or coronary death; ASCVD, Arteriosclerotic cardiovascular disease; CIMT, Carotid intima–media thickness measured via ultrasound; CVD, cardiovascular disease; SCORE, Systematic cardiovascular risk evaluation. *Reprinted with permission as in Feldmann et al. (10). eGFR is expressed as median [25th–75th percentile].

3.2 Associations between SSc-specific and demographic parameters, cardiovascular parameters, and CRP status

When assessing the associations between SSc disease-specific, demographic, and cardiovascular parameters with CRP positivity, we found that suffering from type 2 diabetes mellitus increased the risk of having a prolonged CRP−positive status by 5.3-fold (OR: 5.25 [0.87–31.52] $p = 0.070$) (Table 2). Moreover, a 10 mg/dL increase in HDL levels was associated with a 47% reduced risk of having a prolonged positive CRP status (OR: 0.53 [0.36, 0.79] $p = 0.002$) and a 10 mg/dL increase in LDL and triglyceride levels showed a significant 22% or 13% risk increase for being CRP−positive, respectively ($p = 0.022$, and $p = 0.005$). Moreover, a point increase in the 10-year

TABLE 2 Results of binary logistic regression analyses were carried out in all $n = 65$ SSc patients to assess associations between SSc disease-specific and demographic parameters, cardiovascular parameters, and CRP−positivity status.

	OR	95% CI of OR	*p*-value
SSc characteristics and demographics			
Time since SSc diagnosis [months]	1.00	[0.99; 1.00]	0.373
Age of SSc diagnosis [years]	1.02	[0.98; 1.06]	0.378
Age [years]	1.01	[0.97, 1.05]	0.729
Height (m)	2.35	[0.01, 680.0]	0.768
Weight [kg]	1.03	[1.00, 1.06]	*0.089*
BMI [kg/m^2]	1.10	[0.99, 1.22]	*0.088*
Sex (female)	2.92	[0.86; 9.92]	*0.085*
Cardiovascular risk factors			
Total cholesterol [mg/dl]	1.01	[0.99; 1.02]	0.326
HDL cholesterol [10 mg/dL]	0.53	[0.36; 0.79]	**0.002**
LDL cholesterol [10 mg/dL]	1.22	[1.03; 1.44]	**0.022**
Triglycerides [10 mg/dL]	1.13	[1.04; 1.24]	**0.005**
Arterial hypertension *n* [%]	1.14	[0.40, 3.31]	0.804
History of smoking *n* [%]	1.83	[0.64, 5.43]	0.264
Pack years [years]	1.01	[0.97, 1.05]	0.626
Diabetes mellitus type 2 *n* [%]	5.25	[0.87, 31.52]	*0.070*
Chronic kidney disease *n* [%]	1.62	[0.37, 6.49]	0.494
Positive family history of cardiovascular event below the age of 65 years *n* [%]	1.33	[0.36, 4.56]	0.651
Individual FRS 10-year risk of HCHD~ [%]	1.06	[0.98, 1.14]	0.178
Average FRS 10-year risk of HCHD ~ [%]	1.00	[0.89, 1.11]	0.926
ASCVD 10-year risk [%]	1.05	[1.00, 1.10]	*0.078*
SCORE 2019 10-year risk of fatal CVD [%]	1.54	[0.94, 1.47]	0.154

ASCVD, Arteriosclerotic cardiovascular disease; CVD, Cardiovascular disease; FRS, Framingham risk score; ~HCDH, Hard coronary heart disease defined as either myocardial infarction or coronary death; HDL cholesterol, High-density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; SSc, Systemic sclerosis. SCORE, Systematic cardiovascular risk evaluation. CRP status was considered positive or negative respectively, if at least 75% of the highly sensitive CRP values were positive (> 5 mg/L) or negative (≤ 5 mg/L) in at least three half-yearly visits within the past 2 years prior to study start. The odds ratios (OR) along with its 95% confidence intervals are given. Significant *p*-values ($p < 0.05$) are marked in bold print, and statistical trends are printed in italics.

ASCVD risk score resulted in a statistical trend for a 5% risk increase in experiencing sustained CRP−positivity. We also observed statistical trends for weight (OR: 1.03 [1.00–1.06] $p = 0.089$) and BMI (OR: 1.10 [0.99–1.22] $p = 0.088$) to be predictors for CRP−positivity, while other cardiovascular risk factors such as the presence of arterial hypertension or smoking, FRS risk scores and arteriosclerotic ultrasound parameters such as carotid intima–media thickness were not predictive of CRP−positive status (see Supplementary Table 2).

3.3 Correlations between baseline CRP levels and cardiovascular risk factors, risk scores, and cardiovascular carotid ultrasound parameters

With respect to clinical and ultrasound correlations (Table 3), we found that in the overall SSc-cohort CRP levels were significant, although weakly, positively correlated with several indicators of cardiovascular health such as HDL cholesterol, LDL cholesterol, triglycerides, the 10-year risk of ASCVD, and the 10-year risks of fatal cardiovascular disease (SCORE 2019) ($0.283 < \rho < 0.339$, $p \leq 0.041$). In addition, we noted in the overall SSc cohort a weak positive correlation between CRP levels and the right carotid intima–media thickness (CIMT right) ($\rho = 0.283$, $p = 0.041$) and an almost significant correlation between CRP levels and the mean CIMT left and right ($\rho = 0.227$, $p = 0.071$). When stratifying by the CRP group, both quantitative ultrasound parameters turned into moderate-to-strong positive correlations within the subgroup of CRP+ SSc patients (CIMT right $\rho = 0.657$, $p = 0.002$, mean CIMT left and right $\rho = 0.497$, $p = 0.026$). Additionally, moderate positive correlations for ultrasound markers of arteriosclerotic burden, such as the CIMT left and a number of plaques at the right carotid arteries, were found in the CRP+ SSc group, both approaching statistical significance ($0.406 < \rho < 0.429$, $p \leq 0.076$). In the CRP− group, CRP levels were not correlated with any of the SSc or cardiovascular or ultrasound-specific parameters (data not shown).

3.4 Classification modeling

To assess the performance and diagnostic accuracy of clinical, cardiovascular, and ultrasound parameters and to identify interesting

TABLE 3 Spearman's rank-order correlations showing the associations between baseline CRP levels and cardiovascular risk factors, risk scores, and cardiovascular carotid ultrasound parameters in all SSc participants, and for the CRP+ SSc group, respectively.

	All participants ($n = 65$)		CRP+ group ($n = 20$)	
	Correlation coefficient ρ	*P*-value	Correlation coefficient ρ	*P*-value
Age [years]	0.081	0.521	0.399*	*0.081*
Weight [kg]	0.179	0.154	−0.296	0.204
BMI [kg/m^2]	0.211*	*0.091*	*−0.227*	*0.336*
Total cholesterol [mg/dl]	0.196	0.124	0.175	0.462
HDL cholesterol [mg/dl]	0.293**	0.020	0.288	0.219
LDL cholesterol [mg/dl]	0.326**	0.009	0.172	0.470
Triglycerides [mg/dl]	0.335**	0.008	0.002	0.992
Individual FRS 10-year risk of HCHD [%]	0.118	0.404	−0.011	0.969
Average FRS 10-year risk of HCHD [%]	0.080	0.600	−0.082	0.771
ASCVD 10-year risk [%]	0.274**	0.041	0.086	0.735
SCORE 2019 10-year risk of fatal CVD [%]	0.339**	*0.024*	−0.413	0.112
CIMT right [mm]	0.283**	0.023	0.657**	0.002
number of plaques right *n*	0.185	0.142	*0.406**	*0.076*
maximal plaque diameter right [mm]	0.197	0.125	0.236	0.316
CIMT left [mm]	0.114	0.373	*0.429**	*0.067*
number of plaques left *n*	0.167	0.192	0.389	0.100
maximal plaque diameter left [mm]	0.151	0.237	0.261	0.281
Total number of plaques left and right *n*	0.140	0.409	−0.310	0.303
Mean CIMT left and right carotids art. [mm]	*0.227**	*0.071*	0.497**	0.026

ASCVD, Arteriosclerotic cardiovascular disease; CVD, Cardiovascular disease; CIMT, carotid intima–media thickness measured via ultrasound; FRS, Framingham risk score; HCDH, Hard coronary heart disease defined as either myocardial infarction or coronary death; HDL cholesterol, High-density lipoprotein cholesterol; LDL cholesterol, Low-density lipoprotein cholesterol; SSc, Systemic sclerosis; SCORE, Systematic cardiovascular risk evaluation. Significant *p*-values ($p < 0.05$) are marked in bold print, and statistical trends are printed in italics. One asterisk is used to highlight correlations of trending significance. Two asterisks are used to highlight correlations with a significance level of $p < 0.05$.

potential combinations of clinical, cardiovascular, and imaging parameters (ROC-models) with high diagnostic accuracy and discriminatory ability between CRP+ and CRP− groups, we next carried out random forest classification modeling with a greedy feature selection procedure under 5-fold cross-validation. We then performed permutation testing with 1,000 permutations to calculate the *p*-value of the ROC-AUC scores. Results are summarized in Table 4. As single ROC-models, SSc duration, total cholesterol, and HDL cholesterol demonstrated each a cross-validated AUC of 0.73, 0.75, and 0.76, respectively ($0.005 \leq p \leq 0.011$) and therefore showed good discriminatory ability to differentiate CRP−positivity in SSc patients. LDL cholesterol and ASCVD group assignment alone showed an AUC of 0.67 and 0.65, respectively, both approaching statistical significance ($0.057 \leq p \leq 0.068$). When looking at possible combinations of clinical and cardiovascular parameters, the greedy feature search procedure using the random forest classification model identified several combined ROC models with very high and significant cross-validated AUCs. These included a combined ROC model consisting of the four lipids: total cholesterol, HDL cholesterol, LDL cholesterol, and total triglycerides resulting in an AUC of 0.83 (model 1, $p < 0.001$). Moreover, combining SSc duration and ASCVD group into a combined ROC model yielded a significant AUC of 0.86 (model 2, $p < 0.001$). When the presence of type 2 diabetes mellitus was added, the significant cross-validated AUC of model 2 amounted to 0.85 (model 3, $p < 0.001$). All three combined ROC models showed a similarly high AUC. Combined ROC models using ultrasound parameters or the SCORE 2019 risk score did not reach comparably high AUC values (data not shown).

4 Discussion

In this cross-sectional observational study, we aimed to explore and assess which general, SSc-specific, and cardiovascular parameters are associated with persistent CRP elevation and thus with chronic low-grade inflammation in SSc. In addition, we also investigated whether persistent CRP elevation in SSc patients is associated with a higher cardiovascular risk as determined using the Framingham risk and ASCVD risk scores and an increased atherosclerotic burden as determined via carotid ultrasound. Finally, we applied random forest classification modeling with cross-validation and permutation testing in order to identify combinations of cardiovascular and SSc-specific parameters that could best differentiate the two groups.

One of the main findings of our study is that a persistent CRP−positivity (of >5 mg/L for at least 1.5 consecutive years), and thus, chronic low-grade inflammation in SSc patients is associated with a significantly higher estimated 10-year risk for ASCVD risk than SSc patients without CRP elevations. The ASCVD risk estimation is based

TABLE 4 Area under the ROC curve (AUC) values for different ROC models including combined ROC models.

Single ROC models	5-fold cross-validation AUC ± SD	Permutation test *p*-value
Age [years]	0.46 ± 0.07	0.625
Weight [kg]	0.46 ± 0.10	0.660
BMI [kg/m^2]	0.64 ± 0.20	0.119
Sex, female n [%]	0.60 ± 0.15	0.101
SSc duration [months]	0.73 ± 0.12	**0.011**
Age at SSc diagnosis [years]	0.37 ± 0.12	0.915
Overall SSc organ involvement ł *n* [%]	0.58 ± 0.15	0.265
Diabetes mellitus type 2 (T2DM) *n*	0.58 ± 0.13	0.072
Total cholesterol [mg/dl]	0.75 ± 0.11	**0.010**
HDL cholesterol [mg/dl]	0.76 ± 0.15	**0.005**
LDL cholesterol [mg/dl]	0.67 ± 0.16	*0.057*
Triglycerides [mg/dl]	0.52 ± 0.12	0.412
Individual FRS 10-year risk of HCHD [%]	0.45 ± 0.25	0.647
Average FRS 10-year risk of HCHD [%]	0.60 ± 0.06	0.180
ASCVD 10-year risk [%]	0.47 ± 0.10	0.612
ASCVD risk group	0.65 ± 0.21	*0.068*
SCORE 2019 10-year risk of fatal CVD [%]	0.49 ± 0.13	0.550
CIMT right [mm]	0.46 ± 0.08	0.675
number of plaques right n	0.54 ± 0.06	0.352
maximal plaque diameter right [mm]	0.49 ± 0.18	0.380
CIMT left [mm]	0.37 ± 0.10	0.899
number of plaques left *n*	0.54 ± 0.23	0.391
maximal plaque diameter left [mm]	0.46 ± 0.12	0.667
Total number of plaques left and right n	0.54 ± 0.06	0.353
Mean CIMT left and right carotids art. [mm]	0.45 ± 0.14	0.621
Combined ROC models		
Model 1: Total cholesterol +HDL+ LDL + total triglycerides	0.83 ± 0.15	**<0.001**
Model 2: SSc duration +ASCVD group	0.86 ± 0.09	**<0.001**
Model 3: SSc duration + T2DM + ASCVD group	0.85 ± 0.05	**<0.001**

ASCVD, Arteriosclerotic cardiovascular disease; CVD, cardiovascular disease; CIMT, carotid intima–media thickness measured via ultrasound; FRS, Framingham risk score; HCDH, Hard coronary heart disease defined as either myocardial infarction or coronary death; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, Low-density lipoprotein cholesterol; SSc, systemic sclerosis; T2DM, diabetes mellitus type 2; SCORE, Systematic cardiovascular risk evaluation. ASCVD risk groups encompassing the following four categories: low ASCVD risk group (containing SSc patients with < 5% calculated 10-year ASCVD risk score), borderline ASCVD risk group (containing SSc patients with a 5–7.4% calculated 10-year ASCVD risk score), intermediate ASCVD risk group (containing SSc patients with a 7.5–19.9% calculated 10-year ASCVD risk score), and high ASCVD risk group (containing SSc patients with a ≥ 20% calculated 10-year ASCVD risk score). Significant *p*-values ($p < 0.05$) are marked in bold print, and statistical trends are printed in italics.

on sex- and age-specific Pooled Cohort Equations validated in Caucasian asymptomatic individuals (33, 36) and is therefore a rather conservative estimate. In a recent population-based cohort study, Kurmann et al. (37) demonstrated that for SSc patients in general (no stratification by CRP status yet), the real-world 10-year-risk for suffering a cardiovascular event was 5 times higher than what was predicted by their respective ASCVD risk scores (37), concluding that the currently recommended ASCVD risk calculator heavily underestimates the ASCVD risk in SSc. The mean ASCVD risk scores reported in Kurmann's study was approximately 8.9% for all SSc patients (37), a magnitude comparable to the ASCVD-risk score magnitude reported in our study for our CRP− group (see Table 1). Given that Kurmann et al. (37) only looked at all SSc patients (without stratifying by CRP−positivity) and that our reported ASCVD risk for the CRP+ SSc patient group ranged with a mean risk of 15% even significantly above Kurmann's SSc patient cohort ASCVD risk, it seems very likely that the significant relationship between prolonged CRP−positivity and 10-year arteriosclerotic-cardiovascular risk observed in our study may even be underestimated. Along these lines, Kurmann et al. (37) also found the FRS score to strongly underestimate CV risk in SSc patients by approximately 4-fold (37). Although we did not detect any significant association between FRS scores and CRP−positivity, it seems possible that our FRS-risk estimations for the CRP+ SSc group may have been overly conservative representations of hard coronary heart disease risk and thus may have obscured a potentially significant association with CRP levels in this specific

patient group. Larger prospective population-based SSc studies stratified by persistent CRP positivity are therefore needed to validate and elucidate our findings further.

The mechanisms underlying the higher ASCVD risk in CRP+ SSc patients remain unclear but may be attributable in part to their chronic low-grade inflammatory state (38–40) and their disrupted lipid profile, higher BMI, and a more frequent glucocorticoid usage. Chronic inflammation (41) and particularly chronic-low-grade inflammation (42) has widely been accepted as important drivers of atherosclerosis in both non-SSc and SSc settings, leading to activation of leucocytes, oxidative stress, and endothelial dysfunction, triggering a cascade of events culminating in the disruption of atherosclerotic plaques (43–46). Particularly, CRP has been shown in a multitude of studies in non-SSc populations to predict the risk of (cardio)vascular disease independent of all traditional risk factors (47–49) and has emerged as a validated measure of cardiovascular inflammation of similar rank and importance as blood pressure or cholesterol (39). Despite this, CRP has been surprisingly underutilized in the field of primary care and cardiology as a determinant for vascular risk (39). In addition, in the rheumatologic field, SSc studies stratifying by elevated CRP status have been very scarce to date and have been limited so far to the analyses by Muangchan et al. (3) and Jha et al. (7) using data from the Canadian Scleroderma Research Group (3, 7). Although both authors did not focus on arteriosclerotic cardiovascular disease as the main outcome, Jha et al. (7) clearly demonstrated a significantly increased risk of death and pulmonary arterial hypertension in the presence of abnormally elevated CRP levels for SSc patients. Similar to our study, Muangchan et al. (3) observed a significantly higher BMI and more frequent corticosteroid usage in SSc patients with sustained CRP levels above 8 mg/L (3). Overall, both reports reinforce our current and previous (5) notion that chronic low-grade inflammation seems to be an important risk factor in SSc patients and that SSc patients with sustained CRP elevations should be classified as a separate "inflammatory" SSc phenotype.

Other conditions such as dyslipidemia, diabetes, and obesity have also been attributed to trigger and sustain inflammatory responses and thus contribute to atherosclerosis and ASCVD risk (41, 42). Interestingly, our CRP+ SSc group displayed a disrupted atherogenic lipid profile compared to their CRP− SSc peers and to recommended reference values. This altered lipid profile was characterized by increased borderline high to high levels of triglycerides, significantly reduced and borderline-low HDL cholesterol levels, and significantly increased borderline high to high LDL cholesterol levels. Moreover, combining all 4 lipids (HDL, LDL, total cholesterol, and triglycerides) into a classification ROC model was highly discriminatory to CRP− positivity. Abnormal lipid profiles have been described in SSc patients versus controls before (20, 50) and have been partly attributed to a reduced HDL cholesterol efflux capacity in SSc macrophages and thus an impaired ability of SSc patients to clear off cholesterol from the body (51). However, this is the first study to identify a strongly disrupted lipid profile as one of the main differentiating features of the inflammatory SSc phenotype. What drives the alteration in lipid profile in the CPR+ SSc group remains unknown. However, more and more experimental and clinical evidence in non-SSc patients hints toward a close interplay and a series of pathways linking inflammatory molecules such as CRP with lipid metabolism (41, 52, 53) which can both be pharmacologically targeted. Several non-SSc studies have demonstrated that, for example, statins are highly effective not only in lowering lipids but also in reducing CRP serum levels independently of effects on lipids (54, 55). As our patients in the CRP+ SSc group exhibited both elevated CRP levels and an abnormal lipid profile, one might speculate that this specific patient group may particularly benefit from the dual anti-inflammatory and lipid-lowering effects of lipid-lowering therapy. However, additional clinical trials of lipid-lowering agents in SSc patients are needed to prove the potential benefit of statins for this specific patient group.

Apart from the findings above, we also observed moderate-to-strong correlations between prolonged CRP−positivity in SSc patients with several carotid ultrasound parameters, especially with the left or/and right CIMT and with the number of arteriosclerotic plaques at the right carotid, suggesting an increasing subclinical atherosclerotic burden in inflammatory SSc patients. Our results extend to the exploratory observations by Gamal et al. (21) who reported CRP−positivity as a potential risk factor for atherosclerosis in SSc patients compared to controls (21). However, Gamal's study neither reported the magnitude of CRP levels nor stratified by prolonged CRP status as CRP measurements were taken only at one time point (21). In line with our study, Schiopu et al. (19) and Sedky et al. (20) also observed in their SSc cohorts a significant association between CRP levels and carotid plaques number (19) and CIMT levels, respectively (20). However, unlike our study, both studies did not stratify their cohorts by CRP status due to limitations in the study design including the lack of serial CRP measurements (19).

Our study has several strengths such as a relatively large and well-characterized SSc patient cohort. In addition, our study design and patient selection were based on serial CRP measurements in order to identify an "inflammatory subtype" with a prolonged and thus chronic inflammation. During patient selection, we were also able to track patients for incurring infections and were able to exclude patients with elevated CRP levels due to concomitant infections.

Our study has also several limitations. The first limitation of our study pertains to the cross-sectional study design which only allowed for ASCVD risk estimation but did not provide any information on observed prospective ASCVD outcomes such as myocardial infarction. Second, a higher proportion of corticosteroid users and type 2 diabetics among the CRP+ SSc group may also have contributed to the higher amount of inflammation and subsequently the higher ASCVD risk noted in the CRP+ SSc group. However, due to the limited sample size further subgroup analyses, adjustments for confounding factors, especially further stratification by immunosuppressive medication usage, were not possible. To minimize these drawbacks, we tried to be as transparent as possible by calculating correlations separately for the groups, by reporting extensively group characteristics and group comparisons on all variables of interest, and by cautiously interpreting our findings. Third, we were only able to assess the atherosclerotic burden at the carotid artery level via ultrasound and did not have the means to quantify the extent of atherosclerosis at other ASCVD predilection sites such as the coronary arteries (via calcium-scoring). Moreover, unlike Pagkopoulou et al. (56), we did not have nail fold video capillaroscopy at disposition at our study visit and therefore could not assess morphological markers of microcirculatory dysregulation. Therefore, our study might have underestimated the true extent of microvascular damage present in our SSc patients. However, we collected information on the presence and history of

digital ulcerations, PAH, and Raynaud's syndrome through visual inspection and physical examination during our study visits and by chart review. As those standard vascular parameters showed comparable proportions across both CRP groups, we are confident that the significant association between ASCVD risk and CRP positivity is not mainly driven by a higher vascular dysfunction in the CRP+ group.

5 Conclusion

While having an autoimmune disease such as SSc alone increases the risk of experiencing a CV event (37) and for coronary heart disease by 3-fold (57) compared to the general population, our study provides first hints that suffering from SSc and having additionally a chronic inflammation as seen in our CRP+ SSc group may enhance the estimated risk for arteriosclerotic cardiovascular disease beyond the risk of CRP− SSc patients. Prolonged CRP−positivity may act as an additional important modifiable risk-enhancing factor for atherosclerotic-cardiovascular disease in patients with systemic sclerosis and seems to be along with a disrupted lipid profile the hallmark of a distinct "inflammatory" subgroup of SSc patients who are more prone to suffer from musculoskeletal involvement as well as to a higher cardiovascular risk. SSc patients might therefore benefit from serial tracking of inflammatory markers such as CRP and from lipid markers as part of their standard surveillance and follow-up regimen. Due to the dual anti-inflammatory and lipid-lowering effect of statins, those SSc patients may even benefit from aggressive lipid-lowering and anti-inflammatory pharmacological therapies. However, large population-based studies and clinical trials in patients with SSc are needed to validate our findings in a prospective or interventional setting.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to SF, stephanie.finzel@uniklinik-freiburg.de.

Ethics statement

The studies involving humans were approved by the Freiburg Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

UH: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. DF: Data curation, Investigation, Writing – original draft, Writing – review & editing. AL: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. MS: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. IJ: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. MK: Formal analysis, Methodology, Writing – review & editing. FK: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. RV: Conceptualization, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing. SF: Conceptualization, Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

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References

1. Volkmann ER, Andréasson K, Smith V. Systemic sclerosis. *Lancet*. (2023) 401:304–18. doi: 10.1016/S0140-6736(22)01692-0

2. Bournia VK, Fragoulis GE, Mitrou P, Mathioudakis K, Tsolakidis A, Konstantonis G, et al. All-cause mortality in systemic rheumatic diseases under treatment compared with the general population, 2015-2019. *RMD Open*. (2021) 7:e001694. doi: 10.1136/rmdopen-2021-001694

3. Muangchan C, Harding S, Khimdas S, Bonner A, Canadian Scleroderma Research groupBaron M, et al. Association of C-reactive protein with high disease activity in systemic sclerosis: results from the Canadian scleroderma research group. *Arthritis Care Res*. (2012) 64:1405–14. doi: 10.1002/acr.21716

4. Ohtsuka T. Relation between elevated high-sensitivity C-reactive protein and anti-mitochondria antibody in patients with systemic sclerosis. *J Dermatol*. (2008) 35:70–5. doi: 10.1111/j.1346-8138.2008.00418.x

5. Mitev A, Christ L, Feldmann D, Binder M, Möller K, Kanne AM, et al. Inflammatory stays inflammatory: a subgroup of systemic sclerosis characterized by high morbidity and inflammatory resistance to cyclophosphamide. *Arthritis Res Ther*. (2019) 21:262. doi: 10.1186/s13075-019-2057-x

6. Mitev A, Feldmann D, Binder M, Möller K, Kanne AM, Hügle T, et al. (2023). Inflammatory stays inflammatory: a subgroup of systemic sclerosis characterized by high morbidity and inflammatory resistance to cyclophosphamide. ACR Meeting Abstracts. Available at: https://acrabstracts.org/abstract/inflammatory-stays-inflammatory-a-subgroup-of-systemic-sclerosis-characterized-by-high-morbidity-and-inflammatory-resistance-to-cyclophosphamide/ (Accessed November 22, 2023).

7. Jha M, Wang M, Steele R, Baron M, Fritzler MJ, Canadian Scleroderma Research Groupet al. NT-proBNP, hs-cTnT, and CRP predict the risk of cardiopulmonary outcomes in systemic sclerosis: findings from the Canadian scleroderma research group. *J Scleroderma Relat Disord*. (2022) 7:62–70. doi: 10.1177/23971983211040608

8. Pokeerbux MR, Giovannelli J, Dauchet L, Mouthon L, Agard C, Lega JC, et al. Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. *Arthritis Res Ther*. (2019) 21:86. doi: 10.1186/s13075-019-1867-1

9. Liu X, Mayes MD, Pedroza C, Draeger HT, Gonzalez EB, Harper BE, et al. Does C-reactive protein predict the long-term progression of interstitial lung disease and survival in patients with early systemic sclerosis? *Arthritis Care Res*. (2013) 65:1375–80. doi: 10.1002/acr.21968

10. Feldmann D, Jandova I, Heilmeier U, Kollert F, Voll RE, Finzel S. Inflammatory arthritis in systemic sclerosis is associated with elevated C-reactive protein and requires musculoskeletal ultrasound for reliable detection. *Front Med*. (2022) 9:933809. doi: 10.3389/fmed.2022.933809

11. Aviña-Zubieta JA, Man A, Yurkovich M, Huang K, Sayre EC, Choi HK. Early cardiovascular disease after the diagnosis of systemic sclerosis. *Am J Med*. (2016) 129:324–31. doi: 10.1016/j.amjmed.2015.10.037

12. Cen X, Feng S, Wei S, Yan L, Sun L. Systemic sclerosis and risk of cardiovascular disease. *Medicine*. (2020) 99:e23009. doi: 10.1097/MD.0000000000023009

13. Dimitroulas T, Baniotopoulos P, Pagkopoulou E, Soulaidopoulos S, Nightingale P, Sandoo A, et al. Subclinical atherosclerosis in systemic sclerosis and rheumatoid arthritis: a comparative matched-cohort study. *Rheumatol Int*. (2020) 40:1997–2004. doi: 10.1007/s00296-020-04677-3

14. Blackburn R, Giral P, Bruckert E, André JM, Gonbert S, Bernard M, et al. Elevated C-reactive protein constitutes an independent predictor of advanced carotid plaques in dyslipidemic subjects. *Arterioscler Thromb Vasc Biol*. (2001) 21:1962–8. doi: 10.1161/hq1201.099433

15. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. (2003) 107:363–9. doi: 10.1161/01.CIR.0000053730.47739.3C

16. VanGilder RL, Davidov DM, Stinehart KR, Huber JD, Turner RC, Wilson KS, et al. C-reactive protein and long-term ischemic stroke prognosis. *J Clin Neurosci*. (2014) 21:547–53. doi: 10.1016/j.jocn.2013.06.015

17. Liu S, Jiang H, Dhuromsingh M, Dai L, Jiang Y, Zeng H. Evaluation of C-reactive protein as predictor of adverse prognosis in acute myocardial infarction after percutaneous coronary intervention: a systematic review and meta-analysis from 18,715 individuals. *Front Cardiovasc Med*. (2022) 9:1013501. doi: 10.3389/fcvm.2022.1013501

18. Frerix M, Stegbauer J, Kreuter A, Weiner SM. Atherosclerotic plaques occur in absence of intima-media thickening in both systemic sclerosis and systemic lupus erythematosus: a duplexsonography study of carotid and femoral arteries and follow-up for cardiovascular events. *Arthritis Res Ther*. (2014) 16:R54. doi: 10.1186/ar4489

19. Schiopu E, Au KM, McMahon MA, Kaplan MJ, Divekar A, Singh RR, et al. Prevalence of subclinical atherosclerosis is increased in systemic sclerosis and is associated with serum proteins: a cross-sectional, controlled study of carotid ultrasound. *Rheumatology*. (2014) 53:704–13. doi: 10.1093/rheumatology/ket411

20. Sedky Abdou MM, El Desouky SM, Helmy El Kaffas KM, Ahmed Hassan AM. Premature atherosclerosis in systemic sclerosis patients: its relation to disease parameters and to traditional risk factors. *Int J Rheum Dis*. (2017) 20:383–9. doi: 10.1111/1756-185X.12987

21. Gamal RM, Abozaid HSM, Zidan M, Abdelmegid MAKF, Abdel-Razek MR, Alsayed SAR, et al. Study of MRI brain findings and carotid US features in systemic sclerosis patients, relationship with disease parameters. *Arthritis Res Ther*. (2019) 21:95. doi: 10.1186/s13075-019-1877-z

22. Mok MY, Lau CS, Chiu SSH, Tso AWK, Lo Y, Law LSC, et al. Systemic sclerosis is an independent risk factor for increased coronary artery calcium deposition. *Arthritis Rheum*. (2011) 63:1387–95. doi: 10.1002/art.30283

23. Hettema ME, Zhang D, de Leeuw K, Stienstra Y, Smit AJ, Kallenberg CGM, et al. Early atherosclerosis in systemic sclerosis and its relation to disease or traditional risk factors. *Arthritis Res Ther*. (2008) 10:R49. doi: 10.1186/ar2408

24. Bartoloni E, Pucci G, Cannarile F, Battista F, Alunno A, Giuliani M, et al. Central hemodynamics and arterial stiffness in systemic sclerosis. *Hypertension*. (2016) 68:1504–11. doi: 10.1161/HYPERTENSIONAHA.116.08345

25. Ozen G, Inanc N, Unal AU, Korkmaz F, Sunbul M, Ozmen M, et al. Subclinical atherosclerosis in systemic sclerosis: not less frequent than rheumatoid arthritis and not detected with cardiovascular risk indices. *Arthritis Care Res*. (2016) 68:1538–46. doi: 10.1002/acr.22852

26. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European league against rheumatism collaborative initiative. *Arthritis Rheum*. (2013) 65:2737–47. doi: 10.1002/art.38098

27. del Sol AI, Moons KG, Hollander M, Hofman A, Koudstaal PJ, Grobbee DE, et al. Is carotid intima-media thickness useful in cardiovascular disease risk assessment? *Study Stroke*. (2001) 32:1532–8. doi: 10.1161/01.STR.32.7.1532

28. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, et al. Association of Coronary Heart Disease Incidence with carotid Arterial Wall thickness and major risk factors: the atherosclerosis risk in communities (ARIC) study, 1987–1993. *Am J Epidemiol*. (1997) 146:483–94. doi: 10.1093/oxfordjournals.aje.a009302

29. Stein JH, Korcarz CE, Post WS. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: summary and discussion of the American Society of Echocardiography consensus statement. *Prev Cardiol*. (2009) 12:34–8. doi: 10.1111/j.1751-7141.2008.00021.x

30. Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation*. (1998) 97:1837–47. doi: 10.1161/01.CIR.97.18.1837

31. Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk. *J Am Coll Cardiol*. (2014) 63:2935–59. doi: 10.1016/j.jacc.2013.11.005

32. Lloyd-Jones DM, Huffman MD, Karmali KN, Sanghavi DM, Wright JS, Pelser C, et al. Estimating longitudinal risks and benefits from cardiovascular preventive therapies among Medicare patients. *J Am Coll Cardiol*. (2017) 69:1617–36. doi: 10.1016/j.jacc.2016.10.018

33. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Circulation*. (2019) 140:e596–646. doi: 10.1161/CIR.0000000000000678

34. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: the task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European atherosclerosis society (EAS). *Eur Heart J*. (2020) 41:111–88. doi: 10.1093/eurheartj/ehz455

35. Ferri FJ, Pudil P, Hatef M, Kittler J. Comparative study of techniques for large-scale feature selection In: ES Gelsema and LS Kanal, editors. Machine Intelligence and Pattern Recognition. North-Holland: Elsevier (1994). 403–13.

36. Emdin CA, Khera AV, Natarajan P, Klarin D, Baber U, Mehran R, et al. Evaluation of the pooled cohort equations for prediction of cardiovascular risk in a contemporary prospective cohort. *Am J Cardiol*. (2017) 119:881–5. doi: 10.1016/j.amjcard.2016.11.042

37. Kurmann RD, Sandhu AS, Crowson CS, Matteson EL, Osborn TG, Warrington KJ, et al. Cardiovascular risk factors and atherosclerotic cardiovascular events among incident cases of systemic sclerosis: results from a population-based cohort (1980-2016). *Mayo Clin Proc*. (2020) 95:1369–78. doi: 10.1016/j.mayocp.2019.12.015

38. Ridker PM. A test in context: high-sensitivity C-reactive protein. *J Am Coll Cardiol*. (2016) 67:712–23. doi: 10.1016/j.jacc.2015.11.037

39. Ridker PM, Koenig W, Kastelein JJ, Mach F, Lüscher TF. Has the time finally come to measure hsCRP universally in primary and secondary cardiovascular prevention? *Eur Heart J*. (2018) 39:4109–11. doi: 10.1093/eurheartj/ehy723

40. Emerging Risk Factors CollaborationKaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. (2010) 375:132–40. doi: 10.1016/S0140-6736(09)61717-7,

41. Libby P. The changing landscape of atherosclerosis. *Nature*. (2021) 592:524–33. doi: 10.1038/s41586-021-03392-8

42. Amezcua-Castillo E, González-Pacheco H, Sáenz-San Martín A, Méndez-Ocampo P, Gutierrez-Moctezuma I, Massó F, et al. C-reactive protein: the quintessential marker of systemic inflammation in coronary artery disease-advancing toward precision medicine. *Biomedicines*. (2023) 11:2444. doi: 10.3390/biomedicines11092444

43. Tabas I, Bornfeldt KE. Intracellular and intercellular aspects of macrophage Immunometabolism in atherosclerosis. *Circ Res*. (2020) 126:1209–27. doi: 10.1161/CIRCRESAHA.119.315939

44. Ketelhuth DFJ, Lutgens E, Bäck M, Binder CJ, Van den Bossche J, Daniel C, et al. Immunometabolism and atherosclerosis: perspectives and clinical significance: a position paper from the working group on atherosclerosis and vascular biology of the European society of cardiology. *Cardiovasc Res*. (2019) 115:1385–92. doi: 10.1093/cvr/cvz166

45. Frostegård J. Atherosclerosis in patients with autoimmune disorders. *Arterioscler Thromb Vasc Biol*. (2005) 25:1776–85. doi: 10.1161/01.ATV.0000174800.78362.ec

46. Gerasimova EV, Shayakhmetova RU, Gerasimova DA, Popkova TV, Ananyeva LP. Systemic sclerosis and atherosclerosis: potential cellular biomarkers and mechanisms. *Front Biosci*. (2023) 15:16. doi: 10.31083/j.fbs1504016

47. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. (1997) 336:973–9. doi: 10.1056/NEJM199704033361401

48. Koenig W, Sund M, Fröhlich M, Fischer HG, Löwel H, Döring A, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (monitoring trends and determinants in cardiovascular disease) Augsburg cohort study, 1984 to 1992. *Circulation*. (1999) 99:237–42. doi: 10.1161/01.CIR.99.2.237

49. Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med*. (2012) 367:1310–20. doi: 10.1056/NEJMoa1107477

50. Tsifetaki N, Georgiadis AN, Alamanos Y, Fanis S, Argyropoulou MI, Drosos AA. Subclinical atherosclerosis in scleroderma patients. *Scand J Rheumatol*. (2010) 39:326–9. doi: 10.3109/03009741003605648

51. Ferraz-Amaro I, Delgado-Frías E, Hernández-Hernández V, Sánchez-Pérez H, de Armas-Rillo L, Armas-González E, et al. HDL cholesterol efflux capacity and lipid profile in patients with systemic sclerosis. *Arthritis Res Ther*. (2021) 23:62. doi: 10.1186/s13075-021-02443-9

52. Pilely K, Fumagalli S, Rosbjerg A, Genster N, Skjoedt MO, Perego C, et al. C-reactive protein binds to cholesterol crystals and co-localizes with the terminal complement complex in human atherosclerotic plaques. *Front Immunol*. (2017) 8:1040. doi: 10.3389/fimmu.2017.01040

53. Hansen SEJ, Madsen CM, Varbo A, Nordestgaard BG. Low-grade inflammation in the association between mild-to-moderate hypertriglyceridemia and risk of acute pancreatitis: a study of more than 115000 individuals from the general population. *Clin Chem*. (2019) 65:321–32. doi: 10.1373/clinchem.2018.294926

54. Albert MA, Danielson E, Rifai N, Ridker PM, Investigators P. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA*. (2001) 286:64–70. doi: 10.1001/jama.286.1.64

55. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The cholesterol and recurrent events (CARE) Investigators. *Circulation*. (1999) 100:230–5. doi: 10.1161/01.CIR.100.3.230

56. Pagkopoulou E, Soulaidopoulos S, Triantafyllidou E, Arvanitaki A, Katsiki N, Loutradis C, et al. Peripheral microcirculatory abnormalities are associated with cardiovascular risk in systemic sclerosis: a nailfold video capillaroscopy study. *Clin Rheumatol*. (2021) 40:4957–68. doi: 10.1007/s10067-021-05795-4

57. Ngian GS, Sahhar J, Proudman SM, Stevens W, Wicks IP, Van Doornum S. Prevalence of coronary heart disease and cardiovascular risk factors in a national cross-sectional cohort study of systemic sclerosis. *Ann Rheum Dis*. (2012) 71:1980–3. doi: 10.1136/annrheumdis-2011-201176

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Xinpei Wang,
The University of Chicago, United States
Xintian Cai,
People's Hospital of Xinjiang Uygur Autonomous Region, China

*CORRESPONDENCE
Qian Xu
dz13256916857@163.com

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

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Genetically predicted smoking and body mass index mediate the relationship between insomnia and myocardial infarction

Limei Deng[1†], Yuan Gao[2†], Dongmei Wan[1], Zheng Dong[1], Yuming Shao[2], Jing Gao[1], Wenji Zhai[1] and Qian Xu[1*]

[1]The Second Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang, China, [2]Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang, China

Objective: This study aimed to investigate the causal relationship between insomnia and the risk of myocardial infarction (MI) and explore potential mediators such as smoking initiation, alcohol consumption and body mass index (BMI) using mendelian randomization (MR) analysis.
Methods: Data from 1,207,228 individuals of European ancestry were obtained from the UK Biobank and 23andMe for insomnia-related genetic associations. Genetic instruments for MI, smoking initiation, alcohol consumption, and BMI were derived from large-scale genome-wide association studies. Univariate MR analysis mainly utilized the inverse variance weighting method, and multivariable MR analysis assessed the mediation effects of smoking initiation and BMI.
Results: The univariate MR analysis revealed a 96% increased risk of MI in individuals with insomnia [odds ratio (OR) = 1.96; 95% CI: 1.67, 2.31]. Smoking initiation and BMI were identified as potential mediators. The multivariable MR analysis indicated smoking initiation accounted for 29% of the total effect (95% CI: 13%, 61%), while BMI accounted for 15% (95% CI: 7%, 27%), with a combined mediation proportion of 54% (95% CI: 31%, 91%).
Conclusions: The results of this MR analysis demonstrate that insomnia increases the risk of MI. Quitting smoking and losing weight may reduce this risk; however, there is still a portion of the impact of insomnia on MI that cannot be explained. Therefore, further investigation into other potentially modifiable intermediate factors is necessary.

KEYWORDS

insomnia, myocardial infarction, smoking, body mass index, alcohol consumption, Mendelian randomization

1 Introduction

Myocardial infarction (MI) is a serious cardiovascular disease, typically caused by the blockage of coronary arteries, leading to myocardial ischemia and necrosis (1). With the changes in social lifestyles and the increase in unhealthy habits, the incidence of MI is gradually rising, exerting a significant impact on public health, prompting scientists to

Abbreviations
MI, myocardial infarction; BMI, body mass index; MR, Mendelian randomized; IV, instrumental variable; GWAS, Genome-wide association studies; SNP, single nucleotide polymorphism; MAF, minimum allele frequency; IVW, inverse variance weighting; UVMR, univariate mendelian randomization; MVMR, multivariable mendelian randomization; OR, odds ratio; CI, confidence interval; β regression coefficient; SE, standard error; FDR, false discovery rate.

delve into its potential influencing factors (2). Epidemiological studies have revealed high-risk factors for MI, including hypertension (3), hyperlipidemia (4), diabetes (5) and so on. In recent years, research on the relationship between insomnia and MI has gradually attracted widespread attention in the academic community.

Insomnia is the most common sleep disorder, known to have a negative impact on the overall health and quality of life of the population (6). Recent research reports indicated a high prevalence of insomnia in the United States, ranging from 10% to 15% (7). An increasing number of studies suggested a close association between insomnia and MI (8, 9), but the limitations of observational studies cannot be ignored. Additionally, lifestyle factors such as smoking (10, 11), alcohol consumption (12, 13), and body mass index (BMI) (14, 15) have also been confirmed to be associated with insomnia. However, these relationships are often limited by observational studies, requiring more in-depth research methods to reveal potential causal effects.

Mendelian randomization (MR) analysis provides a powerful tool in addressing the limitations of observational studies and revealing causal effects. By using genetic variations as instrumental variables (IVs) to assess the causal impact of exposure on outcomes, it effectively mitigates the potential influence of confounding factors, enhancing the reliability of study results (16, 17). This study aims to use MR methods to delve into whether smoking, alcohol consumption, and BMI mediate the causal effects of insomnia on MI, providing a scientific basis for developing more effective cardiovascular health interventions. Through this research, we hope to gain a more comprehensive understanding of the intricate relationship between lifestyle and cardiac health, offering more precise guidance for the prevention and treatment of MI.

2 Materials and methods

2.1 Data sources

The study exclusively involved individuals of European ancestry as other ancestral groups were not universally available for all features of interest, and due to genetic population structure, the mixing of ancestries could introduce confounding. Genetic associations related to insomnia come from the UK Biobank and 23andMe (18), with this analysis incorporating a large sample of 1,207,228 individuals of European ancestry, making it one of the largest genome-wide association study (GWAS) studies on insomnia to date. To explore the genetic associations with MI, we acquired data from another GWAS analysis, encompassing 395,795 participants and 10,290,368 SNPs (19). Genetic instruments for smoking initiation and alcohol consumption are derived from statistical data in a large-scale publicly available genome-wide association meta-analysis (20). In this study, a standardized analysis was conducted across 34 individual cohorts, considering adjustments for age, age squared, gender, and genetic ancestry principal components. The effects sizes of individual single nucleotide polymorphisms (SNPs) on smoking and alcohol consumption, along with their respective standard errors, were assessed. In addition, data related to BMI came from a GWAS involving 806,834 individuals of European ancestry (21). BMI values were obtained through measurements of standing height and weight during the initial assessment center visit. Table 1 presented a summary of the studied GWAS information.

2.2 Genetic instrument selection

In our MR analysis, we utilized SNPs as IVs to estimate the overall impact of insomnia on MI. When the genome-wide significance threshold was $P < 5 \times 10^{-8}$, SNPs associated with each exposure were selected as potential IVs. To ensure the independence of genetic variations used as IVs, we set the linkage disequilibrium threshold for grouping to $R^2 < 0.01$, with a window size of 1,000 kb. If suitable replacement SNPs were not available, the SNP was discarded. Simultaneously, we set a minimum allele frequency (MAF) of 0.3 to ensure SNP commonality (22, 23). Additionally, we standardized genetic variations from various studies based on their effects, excluding any palindromic variations. To assess the strength of the instruments, we calculated the F-statistic. We consider an F-statistic greater than 10 as an indicator of robust instrument strength. The F-statistic was calculated using the formula $((N-K-1)/K \times [R^2/(1-R^2)])$, where R^2 was the variance explained by each SNP for each exposure, N was the sample size of the exposure GWAS, K was the number of SNPs, and the R^2 formula was expressed as $R^2 = 2 \times MAF \times (1 - MAF) \times [\beta^2/(SE^2 \times N)]$, where MAF represented the minor allele frequency, β was the effect size of the exposure, and SE denoted the standard error of the effect size (24).

2.3 Statistical analysis

2.3.1 Univariate MR analysis

In our study, we employed the Inverse Variance Weighting (IVW) method as the primary approach for univariate analysis. This technique utilized a random-effects meta-analysis approach to combine results obtained from individual SNPs. To ensure consistency in the causal direction, we also conducted additional analyses using other methods: MR Egger, Weighted Median, Weighted Mode, and Simple Mode. To ensure the reliability of the results, significance in the IVW method results was required, and the results from the other four methods should be directionally consistent with the IVW results. To control for the type I error rate, the Benjamini–Hochberg method was used to adjust for multiple testing. The false discovery rate (FDR) threshold was set at 0.05 for significance. After the MR analysis, Cochran's Q test was used to assess the heterogeneity of the ratio estimates of the IVs related to the exposure on the outcome risk (25). When test results indicated heterogeneity ($P < 0.05$), we introduced the MR-PRESSO method to remove IVs with heterogeneity and then reanalyzed those not identified as heterogeneous IVs (26). We also employed the MR-Egger intercept method to assess evidence of horizontal pleiotropy within selected SNPs (the presence of horizontal pleiotropy was speculated for $P < 0.05$).

TABLE 1 Summarized information for GWASs included.

Study	Authors	PMID	Sample size	No. of SNPs	R^2	F
Insomnia	Watanabe et al.	35835914	1,207,228	429	1.65E-05	19.87
Smoking initiation	Liu et al.	30643251	1,232,091	77	1.49E-05	18.33
Alcohol consumption	Liu et al.	30643251	941,280	34	2.48E-05	23.38
BMI	Pulit et al.	30239722	806,834	60	3.03E-05	24.48
Myocardial infarction	Hartiala et al.	33532862	395,795	NA	NA	NA

GWAS, Genome-wide association studies, BMI, body mass index; SNP: single nucleotide polymorphism.

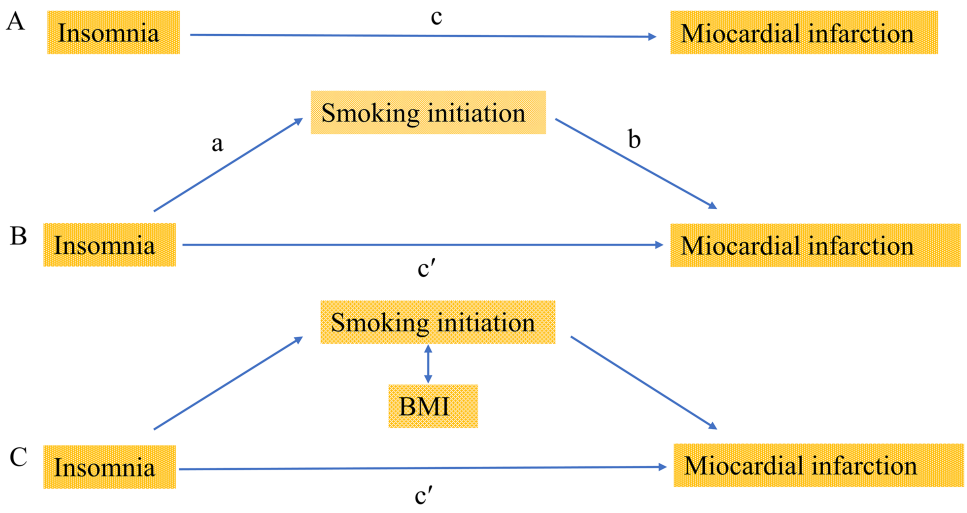

FIGURE 1
Diagrams illustrating associations examined in this study. **(A)** The total effect of insomnia on miocardial infarction (MI), c, was derived using univariable MR (i.e. genetically predicted insomnia as exposure and miocardial infarction as outcome). **(B)** The total effect was decomposed into: (i) indirect effect using a two-step approach (where a is the total effect of insomnia on smoking initiation, and b is the effect of smoking initiation on MI) and the product method (a × b) and (ii) direct effect (c′ = c − a × b). The same process applied to mediation analysis of body mass index (BMI). **(C)** For mediation by both smoking initiation and BMI combined (arrows represent their bidirectional causal relationship), the indirect effect was derived using the difference method (c − c′). Proportion mediated was the indirect effect divided by the total effect.

2.3.2 Multivariable MR analysis

In our study, we utilized multivariable MR to investigate the causal relationship between insomnia and MI risk, with smoking initiation and BMI as mediators. The specific methods were follows: firstly, the estimated value of the mediating effect of insomnia (a in Figure 1B) was multiplied by the estimated value of the mediating effect on MI (b in Figure 1B) to obtain the estimate of the indirect effect. Then, the indirect effect was divided by the total causal effect of insomnia on MI (c in Figure 1A) to obtain the proportion of mediation. In the presence of multiple mediators, the difference method was employed, subtracting the direct effect from the total causal effect (c' in Figure 1C) to obtain the indirect effect. Then, we further divide the indirect effect by the total causal effect to determine the proportion of mediated mediation.

All statistical analyses in this study were conducted using the "TwoSampleMR" package in R software (version 4.1.0). All presented *P*-values were two-sided, and statistical significance was set at the 5% level. We adhered to the guidance of the STROBE-MR guidelines when reporting MR studies (27).

3 Results

3.1 Instrumental variables

Summary data of SNP-phenotype associations were obtained from GWAS for each phenotype (Supplementary Tables S1–S4). A total of 429 SNPs were chosen as IVs for insomnia, with an F-statistic of 19.87. For smoking initiation, alcohol consumption, and BMI, we extracted 77 SNPs (F = 18.33), 34 SNPs (F = 23.38), and 60 SNPs (F = 24.48), respectively, ensuring the absence of weak IVs.

3.2 Effects of insomnia and potential mediators on MI

In the univariate mendelian randomization (UVMR) analysis, the risk of MI increased by 96% in patients with insomnia, with an odds ratio (OR) of 1.96 (95% CI: 1.67, 2.31). For the three potential mediators analyzed, alcohol consumption was not associated with MI (OR = 0.89; 95% CI: 0.72, 1.12). The unadjusted associations of smoking initiation and BMI with MI

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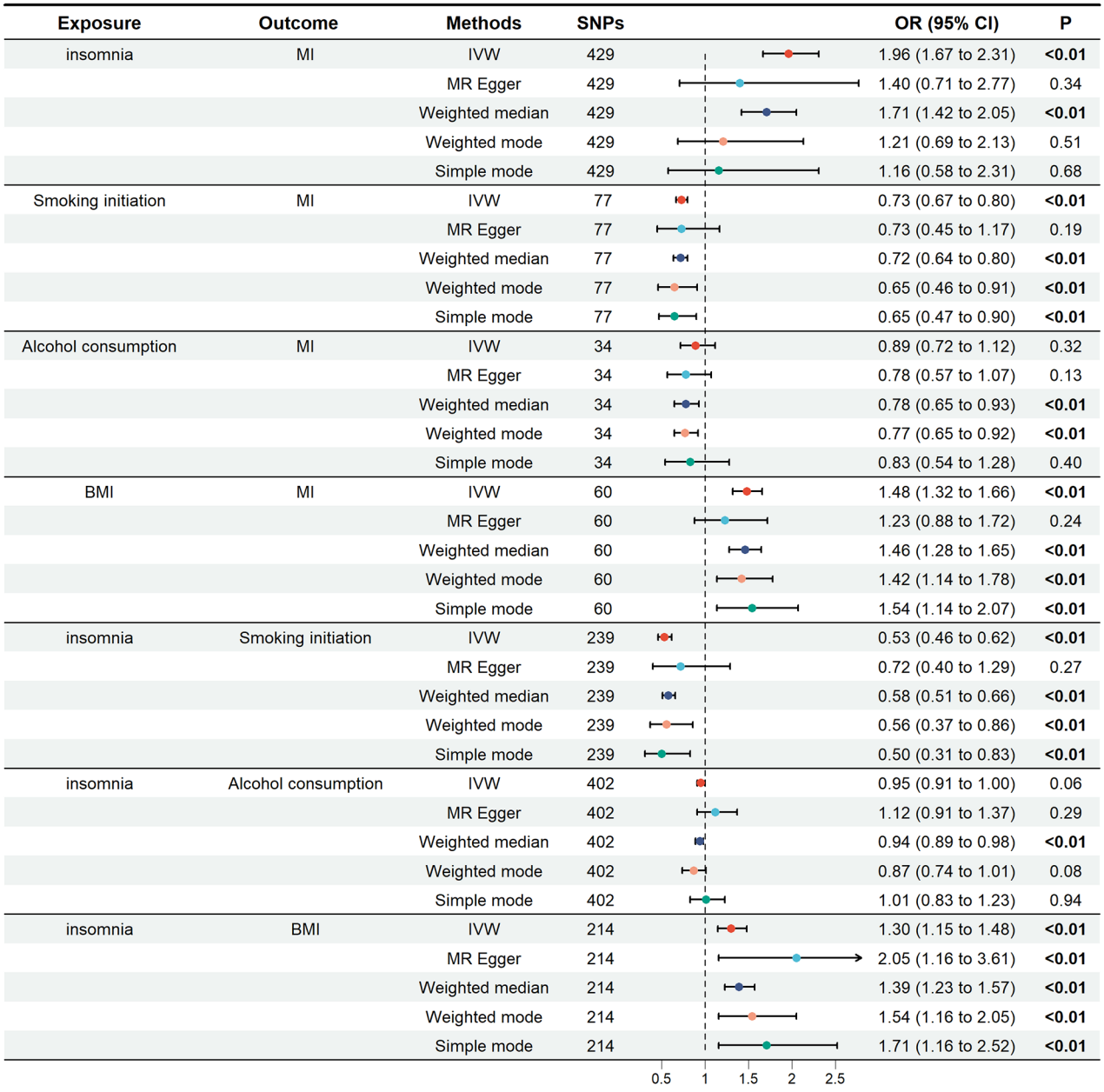

FIGURE 2
Univariate Mendelian randomization analysis. The figure showed the impact of insomnia and potential mediators on MI, as well as the effects of insomnia on potential mediators. Potential mediators include smoking initiation, alcohol consumption and BMI. MI, miocardial infarction; BMI, body mass index; SNPs, single nucleotide polymorphisms; OR, odds ratio; IVW, inverse variance weighting.

were 0.73 (95% CI: 0.67–0.80) and 1.48 (95% CI: 1.32–1.66), respectively. The impact of insomnia on smoking initiation was (OR = 0.53; 95% CI: 0.46, 0.62), as well as on alcohol consumption (OR = 0.95; 95% CI: 0.91, 1.00) and BMI (OR = 1.30; 95% CI: 1.15, 1.48). These findings were depicted in Figure 2.

3.3 Mediation analysis

In the UVMR analysis, alcohol consumption had no effect on MI and was therefore excluded from subsequent multivariable mendelian randomization (MVMR) analysis. When screening potential mediators, we constructed three models, each adjusting for different mediators, as shown in Figure 3. Consistent results indicated a direct impact of insomnia on MI, with no evidence of complete mediation. In Model 1, the adjusted causal effect of smoking initiation on MI was OR = 0.76 (95% CI: 0.69, 0.85). In Model 2, the adjusted causal effect of BMI on MI was OR = 1.34 (95% CI: 1.18, 1.51). In Model 3, the adjusted causal effects of smoking initiation and BMI on MI were OR = 0.83 (95% CI: 0.73, 0.94) and OR = 1.39 (95% CI: 1.22, 1.58), respectively.

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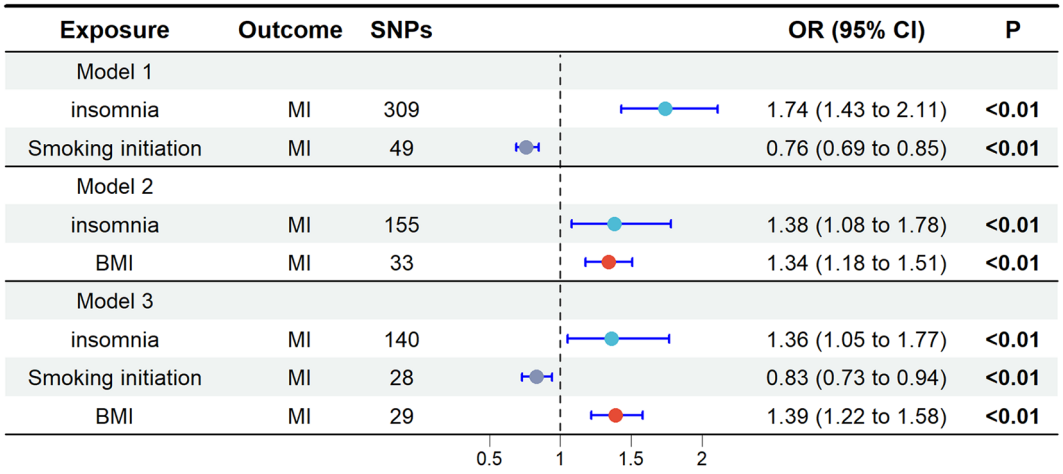

FIGURE 3
Multivariate Mendelian randomization analysis. The OR is derived from the method of inverse variance weighting. BMI, body mass index; MI, miocardial infarction; OR, odds ratio; SNPs, single nucleotide polymorphisms.

TABLE 2 Estimate of the effect of insomnia on MI explained by each mediator and by both combined.

Methods	Exposures	Outcome	β (95% CI)	p-value
UVMR	Insomnia	MI	0.674（0.512, 0.836）	<0.001
	Insomnia	Smoking initiation	−0.626（−0.771, −0.481）	<0.001
	Insomnia	BMI	0.264（0.137, 0.390）	<0.001
	Smoking initiation	MI	−0.317 (−0.405, −0.229)	<0.001
	BMI	MI	0.391 (0.276, 0.506)	<0.001
MVMR	Insomnia	MI	0.309 (0.045, 0.573)	0.022

UVMR, univariate mendelian randomization; MVMR, multivariable mendelian randomization; MI, myocardial infarction; BMI, body mass index.

In the MR analysis, the total effect of insomnia on MI was 0.674 (95% CI: 0.512, 0.836). The direct effect in the MR analysis was 0.309 (95% CI: 0.045, 0.573). Mediation analysis showed that in the MR study, smoking initiation accounted for 29% of the total effect (95% CI: 13%, 61%), while BMI accounted for 15% (95% CI: 7%, 27%), with a combined mediation proportion of 54% (95% CI: 31%, 91%) see Table 2 for details.

3.4 Sensitivity analysis

In the MR analysis, heterogeneity tests revealed significant heterogeneity among the selected IVs ($P < 0.05$). Given this, the IVW random-effects model was used in all MR analyses (Table 3). MR-Egger_intercept analyses did not detect potential horizontal pleiotropy ($P > 0.05$), indicating that IVs did not significantly affect the outcome through pathways outside the exposure (Table 4).

4 Discussion

Our study results support a potential causal relationship between genetically predicted insomnia and MI risk. Compared to the general population, insomnia patients have a 96% increased risk of MI. Of this impact, 29% is mediated by smoking, and 15% is mediated by BMI. These two major risk factors for MI account for 54% of the total effect of insomnia. To our knowledge, this is the first application of MR mediation analysis to study mediators of insomnia and MI risk.

Our study results were consistent with previous study on the impact of insomnia on MI. Hu et al.'s meta-analysis found that in individuals with no prior MI, insomnia was associated with a higher risk of MI (9). Similar results were also found in another meta-analysis (28). The study found that patients with insomnia, compared to healthy individuals, experience changes in the hypothalamic-pituitary-adrenal axis, with a significant increase in adrenocortical hormones and cortisol levels (29). Elevated cortisol was associated with MI, as evidenced by a significant increase in cortisol levels in patients with acute MI one month before the onset of the disease (30). Animal experiments suggest that chronic stress and elevated cortisol can accelerate atherosclerosis, potentially leading to MI (31). Insomnia patients, due to insufficient sleep, suffer from chronic stress and elevated cortisol, exacerbating their risk of MI.

The mediation analysis results emphasize that in the causal relationship between insomnia and MI, smoking and BMI serve as key intermediate variables. This finding was consistent with past research results, highlighting the close association between insomnia and higher rates of smoking and obesity, both identified as factors increasing the risk of MI (15, 32, 33). Smoking, as a behavioral habit, was not only closely associated with insomnia but has also been confirmed as an independent risk factor for MI (34). This suggests that insomnia may increase the risk of MI by promoting smoking behavior. Meanwhile, BMI, as an indicator of physical health, plays a crucial mediating role between insomnia and MI. There is an association between obesity and insomnia, and higher BMI has been confirmed to be closely related to heart disease (35). Therefore, insomnia may

TABLE 3 Heterogeneity tests for UVMR analysis.

Exposure	Outcome	Methods	Q	Q-df	Q-pval
Insomnia	MI	MR Egger	892.61	427	4.56E-35
Insomnia	MI	IVW	894.69	428	3.83E-35
Smoking initiation	MI	MR Egger	112.51	75	3.30E-03
Smoking initiation	MI	IVW	112.51	76	4.15E-03
Alcohol consumption	MI	MR Egger	91.29	32	1.31E-07
Alcohol consumption	MI	IVW	95.39	33	5.65E-08
BMI	MI	MR Egger	125.78	58	6.45E-07
BMI	MI	IVW	128.62	59	4.35E-07
Insomnia	Smoking initiation	MR Egger	987.44	237	3.52E-92
Insomnia	Smoking initiation	IVW	991.69	238	1.43E-92
Insomnia	Alcohol consumption	MR Egger	1,114.66	400	1.06E-68
Insomnia	Alcohol consumption	IVW	1,121.40	401	2.03E-69
Insomnia	BMI	MR Egger	552.51	212	3.42E-32
Insomnia	BMI	IVW	559.31	213	6.66E-33

UVMR, univariate Mendelian randomization; MI, myocardial infarction; BMI, body mass index; IVW, inverse variance weighting. Q, Cochran's Q statistic in IVW and MR-Egger; df, (number of) degrees of freedom; Q-pval, the null hypothesis (H0) for Q-pval is that there is no difference between each SNP.

TABLE 4 Horizontal pleiotropy test for UVMR analysis.

Exposure	Outcome	Egger-intercept	SE	*p*-value
Insomnia	MI	0.002	0.002	0.319
Smoking initiation	MI	<0.001	0.006	1.000
Alcohol consumption	MI	0.004	0.004	0.239
BMI	MI	0.005	0.005	0.257
Insomnia	Smoking initiation	−0.002	0.002	0.314
Insomnia	Alcohol consumption	−0.001	0.001	0.121
Insomnia	BMI	−0.003	0.002	0.108

UVMR, univariate Mendelian randomization; MI, myocardial infarction; BMI, body mass index. Egger-intercept represents the intercept computed using the MR-Egger method. The *p*-value associated with Egger-intercept refers to the hypothesis test conducted on the intercept, where the null hypothesis is that the intercept is equal to zero.

trigger complex and interwoven effects in the pathogenesis of MI by influencing smoking and BMI. The results of this study provide valuable insights for the management and prevention of heart disease. When formulating intervention strategies, it is essential to not only focus on insomnia itself but also comprehensively consider the associated behaviors and physiological factors, especially smoking and BMI. Taking these intermediate variables into account may contribute to the development of more effective measures to reduce MI risk.

Another interesting aspect of our study was the finding that smoking and BMI jointly mediate 54% of the impact of insomnia on MI, while there was still a portion of the impact that remains unexplained. It is well known that insomnia may trigger depression and emotional fluctuations, and depression itself is considered an independent risk factor for MI (36, 37). Under depressive states, biological changes may lead to an imbalance in hormone levels, such as increased release of cortisol and overactivity of the autonomic nervous system (38, 39). These physiological changes are closely related to the health of the cardiovascular system, thereby increasing the risk of MI. Furthermore, factors such as the triglyceride glucose index-waist circumference (40), hepatic steatosis index (41), plasma aldosterone concentration (42), and weight-adjusted-waist index (43) may also serve as potential risk factors for MI. These indices reflect metabolic and hormonal disturbances that can exacerbate cardiovascular issues, particularly in individuals experiencing insomnia and depression. Additionally, depression may lead to unhealthy lifestyle choices, such as lack of exercise and poor dietary habits, further aggravating heart health issues. Therefore, insomnia indirectly influences the risk of MI by triggering depression, highlighting the intricate relationship between sleep, mental health, biology, and heart disease. Consequently, these non-lifestyle factors deserve further investigation as mediators of the impact of insomnia on MI.

This study has several notable strengths. Firstly, despite previous MR studies on insomnia and MI (44, 45), we are the first to use the mediation MR method to study the causal relationship between insomnia and MI and discover the mediating roles of smoking and BMI. Secondly, we utilized summary-level data from recent extensive sample studies in GWAS, covering a more comprehensive set of SNPs, thereby enhancing the statistical power of the data. Finally, employing the mediation MR method helped reduce biases caused by confounding between exposure factors, mediators, outcomes, and measurement errors.

However, we should not overlook several limitations. Firstly, although smoking and BMI largely explain the impact of insomnia on the risk of MI, the remaining portion of the effect still needs further exploration. Secondly, despite some heterogeneity in this study, our data came from public databases, and specific factor sub-analyses (such as age, gender, etc.) to explore the source of heterogeneity were not feasible. Additionally, the overlap in samples from the UK Biobank for both insomnia and MI may also impact our findings, but quantifying this overlap was not possible due to data source limitations. Future research on the correlation between insomnia and MI should consider the overall impact of these factors. Finally, GWAS summary data primarily came from European populations, so there may be some potential biases if extrapolated to non-European ancestries. These limitations emphasize the need for further research to refine the evidence in this field.

5 Conclusions

In summary, we demonstrated the hazardous effect of insomnia on the risk of MI. Quitting smoking and losing weight may help reduce this risk, especially crucial for those at higher risk of MI. However, nearly half of the impact of insomnia on MI remains difficult to explain. To comprehensively reduce the risk, efforts to improve lifestyle

habits need to be strengthened, and further study is required to investigate other potential influencing factors not controlled by lifestyle factors.

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 Ethics Committee of the Genome-Wide Association Studies. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LD: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. YG: Data curation, Methodology, Writing – original draft. DW: Formal Analysis, Investigation, Software, Writing – review & editing. ZD: Conceptualization, Formal Analysis, Investigation, Writing – review & editing. YS: Data curation, Investigation, Methodology, Writing – review & editing. JG: Investigation, Visualization, Writing – original draft. WZ: Conceptualization, Formal Analysis, Visualization, Writing – original draft. QX: Funding acquisition, Project administration, 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 association that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2024.1456918/full#supplementary-material

References

1. Lindahl B, Baron T, Albertucci M, Prati F. Myocardial infarction with non-obstructive coronary artery disease. *EuroIntervention*. (2021) 17(11):e875–e87. doi: 10.4244/EIJ-D-21-00426

2. Li S, Gao X, Yang J, Xu H, Wang Y, Zhao Y, et al. Number of standard modifiable risk factors and mortality in patients with first-presentation ST-segment elevation myocardial infarction: insights from China acute myocardial infarction registry. *BMC Med*. (2022) 20(1):217. doi: 10.1186/s12916-022-02418-w

3. Liu X, Song Q, Wu S, Zhou W, Wang X. Prediabetes and risk for myocardial infarction by hypertension status in a Chinese population: a prospective cohort study. *J Hypertens*. (2021) 39(1):77–83. doi: 10.1097/HJH.0000000000002607

4. Chen P, Zhang M, Zhang Y, Su X, Chen J, Xu B, et al. Economic burden of myocardial infarction combined with dyslipidemia. *Front Public Health*. (2021) 9:648172. doi: 10.3389/fpubh.2021.648172

5. Plakht Y, Elkis Hirsch Y, Shiyovich A, Abu Tailakh M, Liberty IF, Gilutz H. Heterogenicity of diabetes as a risk factor for all-cause mortality after acute myocardial infarction: age and sex impact. *Diabetes Res Clin Pract*. (2021) 182:109117. doi: 10.1016/j.diabres.2021.109117

6. Darchia N, Oniani N, Sakhelashvili I, Supatashvili M, Basishvili T, Eliozishvili M, et al. Relationship between sleep disorders and health related quality of life-results from the Georgia SOMNUS study. *Int J Environ Res Public Health*. (2018) 15(8):1588. doi: 10.3390/ijerph15081588

7. Kaur H, Spurling BC, Bollu PC. *Chronic Insomnia*. Treasure Island (FL): StatPearls (2024).

8. He Q, Zhang P, Li G, Dai H, Shi J. The association between insomnia symptoms and risk of cardio-cerebral vascular events: a meta-analysis of prospective cohort studies. *Eur J Prev Cardiol*. (2017) 24(10):1071–82. doi: 10.1177/2047487317702043

9. Hu S, Lan T, Wang Y, Ren L. Individual insomnia symptom and increased hazard risk of cardiocerebral vascular diseases: a meta-analysis. *Front Psychiatry*. (2021) 12:654719. doi: 10.3389/fpsyt.2021.654719

10. Hagg SA, Ljunggren M, Janson C, Holm M, Franklin KA, Gislason T, et al. Smokers with insomnia symptoms are less likely to stop smoking. *Respir Med*. (2020) 170:106069. doi: 10.1016/j.rmed.2020.106069

11. Cohen A, Szepsenwol O, Haimov I. Insomnia severity mediates the association between COVID-19 related anxiety and increase in tobacco smoking during the COVID-19 pandemic among adults. *Behav Sleep Med*. (2023) 21(5):608–19. doi: 10.1080/15402002.2022.2147934

12. Verlinden JJ, Moloney ME, Whitehurst LN, Weafer J. Sex differences in the association between poor sleep quality and alcohol-related problems among heavy drinkers with insomnia. *Front Behav Neurosci*. (2022) 16:875168. doi: 10.3389/fnbeh.2022.875168

13. Miller MB, Carpenter RW, Freeman LK, Dunsiger S, McGeary JE, Borsari B, et al. Effect of cognitive behavioral therapy for insomnia on alcohol treatment outcomes among US veterans: a randomized clinical trial. *JAMA Psychiatry*. (2023) 80(9):905–13. doi: 10.1001/jamapsychiatry.2023.1971

14. Kauffman BY, Kotov R, Garey L, Ruggero CJ, Luft BJ, Zvolensky MJ. The association between body mass Index and anxious arousal, depressive, and insomnia symptoms among world trade center responders. *Health Behav Res*. (2022) 5(1):3. doi: 10.4148/2572-1836.1107

15. Johnson KA, Gordon CJ, Chapman JL, Hoyos CM, Marshall NS, Miller CB, et al. The association of insomnia disorder characterised by objective short sleep duration with hypertension, diabetes and body mass index: a systematic review and meta-analysis. *Sleep Med Rev*. (2021) 59:101456. doi: 10.1016/j.smrv.2021.101456

16. Zhao SS, Mackie SL, Zheng J. Why clinicians should know about Mendelian randomization. *Rheumatology (Oxford)*. (2021) 60(4):1577–9. doi: 10.1093/rheumatology/keab007

17. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *Br Med J*. (2018) 362:k601. doi: 10.1136/bmj.k601

18. Watanabe K, Jansen PR, Savage JE, Nandakumar P, Wang X, Me Research T, et al. Genome-wide meta-analysis of insomnia prioritizes genes associated with metabolic and psychiatric pathways. *Nat Genet*. (2022) 54(8):1125–32. doi: 10.1038/s41588-022-01124-w

19. Hartiala JA, Han Y, Jia Q, Hilser JR, Huang P, Gukasyan J, et al. Genome-wide analysis identifies novel susceptibility loci for myocardial infarction. *Eur Heart J*. (2021) 42(9):919–33. doi: 10.1093/eurheartj/ehaa1040

20. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat Genet*. (2019) 51(2):237–44. doi: 10.1038/s41588-018-0307-5

21. Pulit SL, Stoneman C, Morris AP, Wood AR, Glastonbury CA, Tyrrell J, et al. Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Hum Mol Genet*. (2019) 28(1):166–74. doi: 10.1093/hmg/ddy327

22. Lu W, Pu B, Wang S, Li M, An Y, Lian J, et al. Bidirectional two-sample Mendelian randomization analysis identifies causal associations between cardiovascular diseases and frozen shoulder. *J Orthop Surg Res*. (2024) 19(1):116. doi: 10.1186/s13018-024-04600-7

23. Pu B, Gu P, Luo L, Yue D, Xin Q, Zeng Z, et al. Causal effects of tea intake on multiple types of fractures: a two-sample Mendelian randomization study. *Medicine (Baltimore)*. (2023) 102(22):e33542. doi: 10.1097/MD.0000000000033542

24. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol*. (2011) 40 (3):755–64. doi: 10.1093/ije/dyr036

25. Bowden J, Del Greco MF, Minelli C, Zhao Q, Lawlor DA, Sheehan NA, et al. Improving the accuracy of two-sample summary-data Mendelian randomization: moving beyond the NOME assumption. *Int J Epidemiol*. (2019) 48(3):728–42. doi: 10.1093/ije/dyy258

26. Verbanck M, Chen CY, 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(5):693–8. doi: 10.1038/s41588-018-0099-7

27. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA*. (2021) 326(16):1614–21. doi: 10.1001/jama.2021.18236

28. Dean YE, Shebl MA, Rouzan SS, Bamousa BAA, Talat NE, Ansari SA, et al. Association between insomnia and the incidence of myocardial infarction: a systematic review and meta-analysis. *Clin Cardiol*. (2023) 46(4):376–85. doi: 10.1002/clc.23984

29. Vgontzas AN, Tsigos C, Bixler EO, Stratakis CA, Zachman K, Kales A, et al. Chronic insomnia and activity of the stress system: a preliminary study. *J Psychosom Res*. (1998) 45(1):21–31. doi: 10.1016/S0022-3999(97)00302-4

30. Faresjo T, Stromberg S, Jones M, Stomby A, Karlsson JE, Ostgren CJ, et al. Elevated levels of cortisol in hair precede acute myocardial infarction. *Sci Rep*. (2020) 10(1):22456. doi: 10.1038/s41598-020-80559-9

31. Okutsu M, Lira VA, Higashida K, Peake J, Higuchi M, Suzuki K. Corticosterone accelerates atherosclerosis in the apolipoprotein E-deficient mouse. *Atherosclerosis*. (2014) 232(2):414–9. doi: 10.1016/j.atherosclerosis.2013.11.076

32. Laugsand LE, Vatten LJ, Platou C, Janszky I. Insomnia and the risk of acute myocardial infarction: a population study. *Circulation*. (2011) 124(19):2073–81. doi: 10.1161/CIRCULATIONAHA.111.025858

33. Hu N, Wang C, Liao Y, Dai Q, Cao S. Smoking and incidence of insomnia: a systematic review and meta-analysis of cohort studies. *Public Health*. (2021) 198:324–31. doi: 10.1016/j.puhe.2021.07.012

34. Chen LJ, Steptoe A, Chen YH, Ku PW, Lin CH. Physical activity, smoking, and the incidence of clinically diagnosed insomnia. *Sleep Med*. (2017) 30:189–94. doi: 10.1016/j.sleep.2016.06.040

35. Yoshida N, Ogawa M, Nakai M, Kanaoka K, Sumita Y, Emoto T, et al. Impact of body mass index on in-hospital mortality for six acute cardiovascular diseases in Japan. *Sci Rep*. (2022) 12(1):18934. doi: 10.1038/s41598-022-23354-y

36. Aw PY, Pang XZ, Wee CF, Tan NHW, Peck EW, Teo YN, et al. Co-prevalence and incidence of myocardial infarction and/or stroke in patients with depression and/or anxiety: a systematic review and meta-analysis. *J Psychosom Res*. (2023) 165:111141. doi: 10.1016/j.jpsychores.2022.111141

37. Wang M, Cheng L, Gao Z, Li J, Ding Y, Shi R, et al. Investigation of the shared molecular mechanisms and hub genes between myocardial infarction and depression. *Front Cardiovasc Med*. (2023) 10:1203168. doi: 10.3389/fcvm.2023.1203168

38. Druzhkova TA, Yakovlev AA, Rider FK, Zinchuk MS, Guekht AB, Gulyaeva NV. Elevated Serum cortisol levels in patients with focal epilepsy, depression, and comorbid epilepsy and depression. *Int J Mol Sci*. (2022) 23(18):10414. doi: 10.3390/ijms231810414

39. Teo CH, Wong ACH, Sivakumaran RN, Parhar I, Soga T. Gender differences in cortisol and cortisol receptors in depression: a narrative review. *Int J Mol Sci*. (2023) 24(8):7129. doi: 10.3390/ijms24087129

40. Hu J, Cai X, Li N, Zhu Q, Wen W, Hong J, et al. Association between triglyceride glucose index-waist circumference and risk of first myocardial infarction in Chinese hypertensive patients with obstructive sleep apnoea: an observational cohort study. *Nat Sci Sleep*. (2022) 14:969–80. doi: 10.2147/NSS.S362101

41. Wen W, Cai X, Zhu Q, Hu J, Hong J, Zhang X, et al. Linear relationship between hepatic steatosis Index and Major adverse cardiovascular events in hypertensive patients with obstructive sleep apnea: a real-world cohort study from China. *Rev Cardiovasc Med*. (2023) 24(10):280. doi: 10.31083/j.rcm2410280

42. Cai X, Song S, Hu J, Zhu Q, Shen D, Yang W, et al. Association of the trajectory of plasma aldosterone concentration with the risk of cardiovascular disease in patients with hypertension: a cohort study. *Sci Rep*. (2024) 14(1):4906. doi: 10.1038/s41598-024-54971-4

43. Zhao J, Cai X, Hu J, Song S, Zhu Q, Shen D, et al. J-Shaped relationship between weight-adjusted-Waist Index and cardiovascular disease risk in hypertensive patients with obstructive sleep apnea: a cohort study. *Diabetes Metab Syndr Obes*. (2024) 17:2671–81. doi: 10.2147/DMSO.S469376

44. Yang Y, Fan J, Shi X, Wang Y, Yang C, Lian J, et al. Causal associations between sleep traits and four cardiac diseases: a Mendelian randomization study. *ESC Heart Fail*. (2022) 9(5):3160–6. doi: 10.1002/ehf2.14016

45. Arora N, Bhatta L, Skarpsno ES, Dalen H, Asvold BO, Brumpton BM, et al. Investigating the causal interplay between sleep traits and risk of acute myocardial infarction: a Mendelian randomization study. *BMC Med*. (2023) 21(1):385. doi: 10.1186/s12916-023-03078-0

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Luisa Ricciardi,
University of Messina, Italy
Hajar Arfaoui,
University of Hassan II Casablanca, Morocco

*CORRESPONDENCE
Shusheng Liao
lshsh303@sina.com

†These authors have contributed equally to this work

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Case report: Granulomatosis with polyangiitis patient presented with a mass in the aortic root

Yinyan Teng[1†], Mei Chen[2†], Zhongwei Chen[3], Xiaowei Zhang[1], Zhongyun Li[1] and Shusheng Liao[1*]

[1]Department of Ultrasound, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China, [2]Department of Ultrasonography, The First Affiliated Hospital of Ningbo University, Ningbo, Zhejiang, China, [3]Department of Radiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

Granulomatosis with polyangiitis (GPA) is an autoimmune inflammatory disease that affects small- and medium-sized blood vessels in the body, representing a rare entity. Cardiac involvement was identified as an independent risk factor for death in GPA patients, yet it has not been systematically elucidated in previous literature. Cardiac lesions in patients with GPA can manifest in various ways, such as pericarditis, myocarditis, coronary vasculitis, valvular abnormalities, conduction system abnormalities, and heart failure. Herein, we report a 55-year-old woman with GPA; she had a 2-year history of recurrent episodes of headache, accompanying sickness, and fatigue, which have been aggravated for the past half-month. The main manifestation is presenting as a mass in the aortic root, which was successfully diagnosed by multimodality imaging (including two-dimensional echocardiography, contrast-enhanced ultrasound, and computed tomography). After treatment with methylprednisolone and cyclophosphamide, the patient's symptoms significantly improved and she remained asymptomatic over 6 months of follow-up. This article will enrich our knowledge about cardiac involvement in GPA patients and highlights the value of imaging, especially ultrasound, in the diagnosis and post-treatment follow-up of this condition.

KEYWORDS

granulomatosis with polyangiitis, diagnosis, heart, echocardiography, therapy

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a kind of systemic necrotizing vasculitis. The main pathological features of AAV are fibrous necrosis and inflammation in the walls of neutrophil and small vessels. Clinical types include granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), and microscopic polyangiitis (MPA) (1). Symptoms of AAV patients are non-

specific, such as arthralgia, weight loss, malaise, and fatigue, approximately 30%–45% of whom manifested as fever (2). Cardiovascular involvement is identified as an independent risk factor for death in AAV patients. According to the study by McGeoch et al. (3), 3.3% of patients with GPA show cardiovascular involvement. Cardiac lesions in patients with GPA are characterized by pericarditis, myocarditis, coronary vasculitis, valvular abnormalities, myocardial infarction, conduction system abnormalities, and heart failure (4–6). Presenting as a cardiac mass is very rare in this disease, but it can be easily misdiagnosed as other diseases such as infection and a malignant tumor, leading to inappropriate treatment. We reported a case of GPA presenting as a mass in the aortic root that was successfully diagnosed by multimodality imaging and timely treated. It will enrich our knowledge of this disease, reduce clinical misdiagnosis, and improve the efficiency of clinical treatment.

Case presentation

A 55-year-old woman was admitted to our hospital with a 2-year history of recurrent episodes of headache and accompanying dizziness, sickness, and fatigue, which have been aggravated for the past half-month. She also had a history of sinusitis and otitis media for more than 2 years. She denied the history of arthritis and there were no cardiovascular risk factors, such as system hypertension, coronary artery disease, and diabetes mellitus. This time, her clinical symptoms were accompanied by a fever with temperature of 38.7°C and chest distress. Laboratory tests showed dramatic elevated inflammatory blood markers, with an erythrocyte sedimentation rate (ESR) of 160 mm/h (normal range <20 mm/h) and C-reactive protein (CRP) 157 mg/L (normal range <6 mg/L). WBC (5.20×10^9/L) and platelet (285×10^9/L) counts were within the normal range. The level of NT-proBNP was higher at 1,069 ng/L (normal range <125 ng/L) and hs-TNT was within the normal range. The level of creatinine was at 49 μmol/L and within the normal range. Diagnostic workup revealed positive pANCA, and the anti-myeloperoxidase (MPO) antibody was increased at 5.2 AI (normal range within 0–0.99 AI). Testing for anti-nuclear antibodies (ANA) was positive with a titer of 1:320; however, anti-double-stranded DNA (dsDNA), anti-SSA, anti-SSB, anti-Scl-70, anti-Jo-1, and anti-Ro52 antibody yielded negative results. The phospholipid antibodies and lupus anticoagulants also showed negative results. Serum IgG4 was at 1.05 g/L, within the normal range. The level of D-dimer was slightly higher at 1.72 mg/L (normal range <0.05 mg/L). Further tests showed positive anti-protein C antibody and 4G/5G plasminogen activator inhibitor-1 (PAI-1) gene. In addition, the results of lymphocyte differential antigen testing analysis reveal a normal ratio of CD4+/CD8+ (of T lymphocytes) at 1.0.

The electrocardiogram showed a first-degree conduction block (Figure 1E). Her head MRI revealed meninges and cavernous sinus thickening in both anterior and middle cranial fossa with abnormal signal, and diffuse signal abnormality in both nasopharynx walls, parapharyngeal space, bilateral long cephalic muscle, medial pterygoid muscle, and lateral pterygoid muscle (Figures 1A, B). Nasopharyngeal tissue biopsy was performed, and histopathology

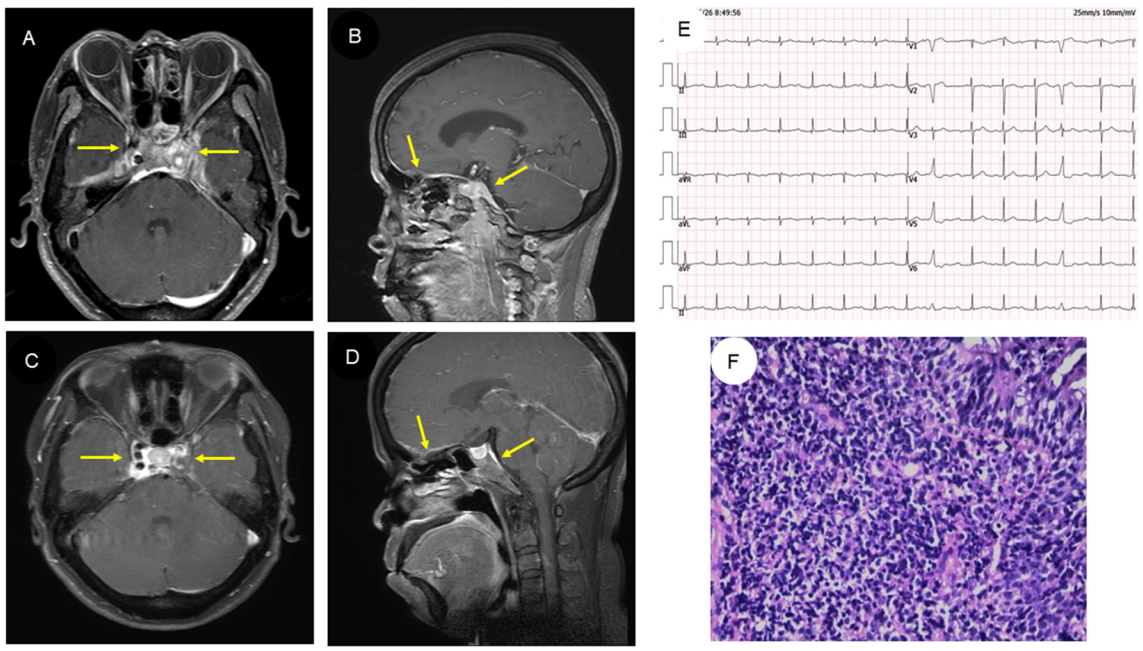

FIGURE 1
MRI findings, electrocardiogram, and histopathologic results of the patient. **(A, B)** Head MRI of the patient revealed abnormal signals in both the anterior and middle cranial fossa and nasopharynx wall. **(C, D)** Subsequent head MRI showed that the abnormal signal had reduced in size after treatment. **(E)** The electrocardiogram revealed first-degree atrioventricular block. **(F)** Histopathologic examination of the nasopharynx suggests chronic inflammation of the mucosa with reactive lymphoid hyperplasia (HE staining, original magnification ×200).

showed consistency with chronic inflammation of the mucosa with reactive hyperplasia of lymphoid tissue (Figure 1F). Her chest computed tomography (CT) scan revealed an irregular low-density mass in the aortic root (Supplementary Figures S1A, B); upon enhancement scanning, the lesion displayed low enhancement (Supplementary Figures S1D, E). In addition, a few pericardial effusion and bilateral pleural effusion were found.

The patient underwent transthoracic echocardiography and presented with an iso-echoic mass of approximately 38 × 42 × 27 mm around the aortic root, with a clear boundary, irregular shape, and uneven internal echo without calcification (Figures 2A–C, Supplementary Movies 1–3). Color Doppler demonstrated mild-to-moderate aortic regurgitation. In addition, a small of amount of pericardial effusion was found. However, her cardiac function was preserved with a normal LVEF at 66%. To further understand the perfusion characteristics of the lesions, the patient underwent contrast-enhanced ultrasound (CEUS). The CEUS demonstrated that the mass showed a low enhancement pattern, with less enhancement than the surrounding myocardium, and washed out earlier than the myocardium. There was no necrotic area in the mass (Figures 3A–C, Supplementary Movies 4–6). Scanning of the carotid artery revealed thrombosis in the left internal carotid artery (Supplementary Figures S2A–D). In addition, the examination of bilateral superficial temporal artery (STA) was also carried out, and no abnormalities were found on ultrasound (Supplementary Figures S2E–J).

The initial diagnosis considered for the patient was ANCA-associated vasculitis and GPA. Meanwhile, she was also considered to have thrombophilia. During hospitalization, therapy with methylprednisolone was started at 1,000 mg daily, resulting in a good clinical and biochemical response. Subsequently, the methylprednisolone was gradually reduced to 40 mg daily. Following this corticoid tapering schedule, cyclophosphamide was administered at 600 mg for immune suppression. In addition, considering that this patient had thrombophilia, heparin (0.4 mL) was used daily as anticoagulation therapy for 1 week, which was subsequently changed to rivaroxaban at 10 mg daily. Antiplatelet therapy with clopidogrel (75 mg daily) was also arranged for her. After 6 weeks of medicine treatment, the patient's hearing improved, although tinnitus persisted and headache significantly improved. A follow-up MRI showed a significant reduction in the lesion (Figures 1C, D). Echocardiographic follow-up revealed a significant reduction in the mass in the aortic root compared to the initial presentation (Figures 2D–F, 3D–F, Supplementary Movies 7–12). After 3 months of therapy, a chest CT scan demonstrated the disappearance of the lesion in the aortic root (Supplementary Figures S1C, F). Carotid artery ultrasound examination showed regression of the thrombosis in the left internal carotid artery. The patient was followed up for 6 months as outpatient, and laboratory tests showed negative pANCA and anti-MPO antibody, and normal levels of ESR (11 mm/h), CRP (2.2 mg/L), and NT-proBNP (164 ng/L), indicating a stable condition (Figure 4).

Discussion

GPA (formerly known as Wegner's granulomatosis) is an autoimmune inflammatory disease that affects small- and medium-sized blood vessels in the body. The disease is

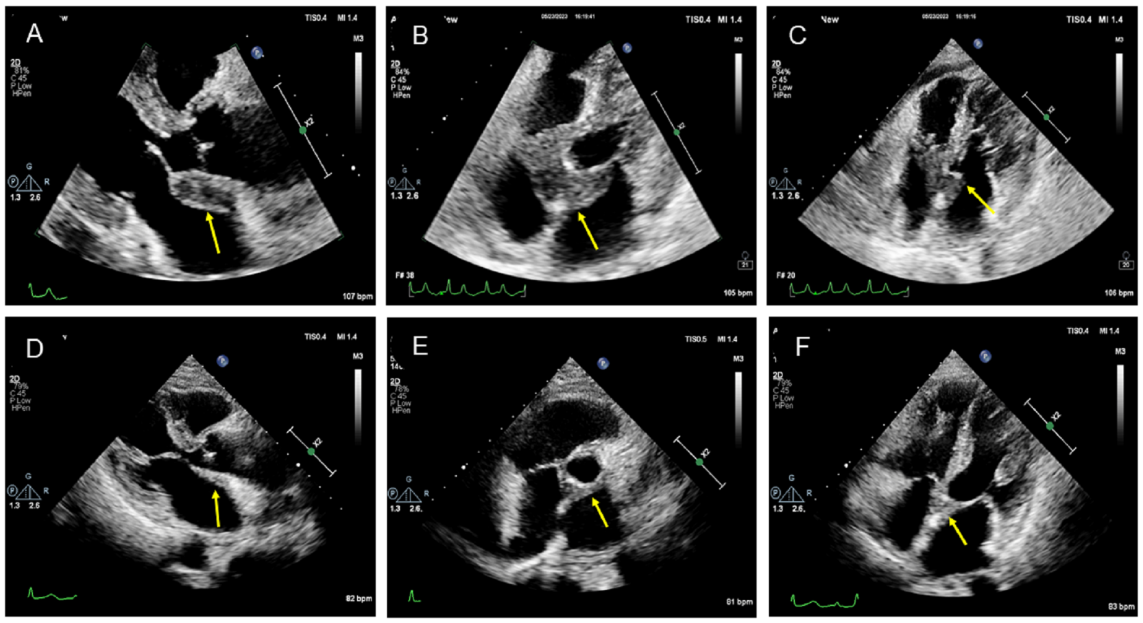

FIGURE 2
Two-dimensional transthoracic echocardiographic manifestation of the lesion in the aortic root. **(A–C)** Two-dimensional transthoracic echocardiography revealed an isoechoic mass around the aortic root with a clear boundary, irregular shape, uneven internal echo, and no calcification. **(D–F)** Transthoracic echocardiography showed that the mass around the aortic root was smaller after treatment.

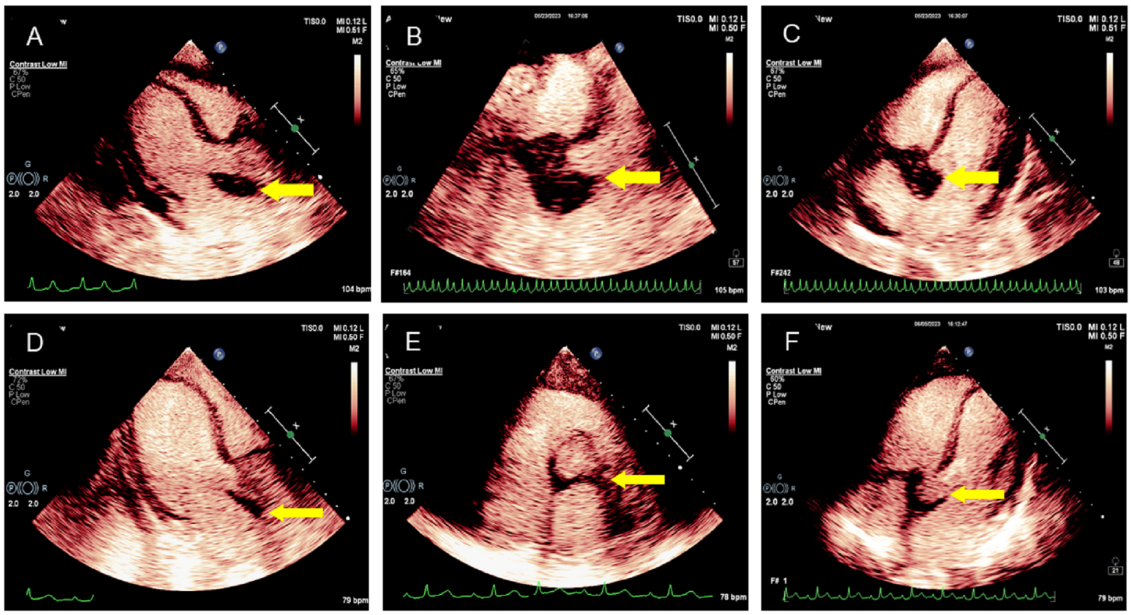

FIGURE 3
CEUS manifestation of the lesion in the aortic root. **(A–C)** CEUS demonstrated the mass surrounding the aortic root with a low enhancement pattern; the degree of enhancement was lower than that of the surrounding myocardium. No necrotic area was found in the mass. **(D–F)** CEUS showed that the lesion in the aortic root was smaller after treatment.

uncommon, with a reported incidence of 5–10 cases per million population (7). It primarily affects people aged 40–60 years; our case falls within this age range. GPA can affect every organ in the body, primarily involving the respiratory tract, lung, and kidney, while cardiac involvement is relatively rare and mostly subclinical. According to previous literature reports, cardiac involvement with clinical symptoms was observed in 3.3%–10.3% of patients with GPA (8, 9). At present, the mechanism of GPA-induced cardiac damage has not been accurately reported, but it may be related to the acceleration of arteriosclerosis, vascular inflammatory response, and related complications. Studies have suggested that myocardial injury in GPA patients may be caused by vasculitis, leading to

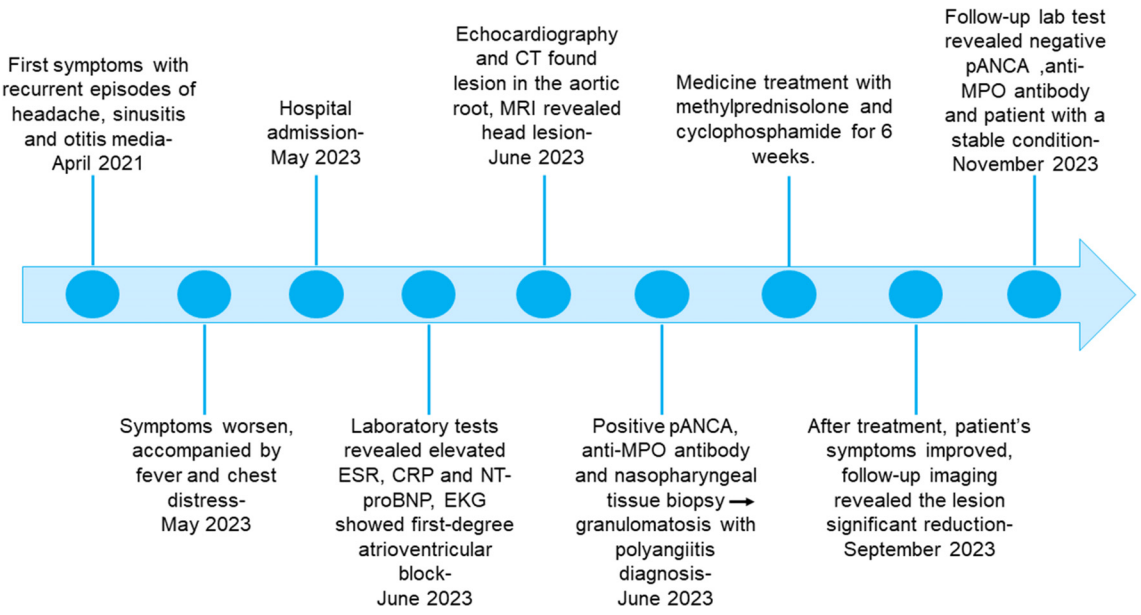

FIGURE 4
Timeline.

coronary arteritis and occlusion of coronary arteries. Alternatively, it may be caused by eosinophil granulocyte infiltration in the myocardium and endocardium, and activation and release of cytotoxic granular protein and lipid mediators, which are directly caused by myocardial injury (10). Cardiac lesions in patients with GPA are commonly characterized by valvular abnormalities, pericarditis, myocarditis, coronary vasculitis, heart conduction system abnormalities, and intracardiac mass. The most common cardiac manifestations in GPA patients include pericarditis, aortic regurgitation, and conduction system abnormalities (3). In this case, a first-degree atrioventricular block was observed. Complete atrioventricular block can also be found in some conditions (11–13). In recent years, literature reports indicate that cardiac valvular involvement in GPA has become more common. Among these cases, the aortic valve is most commonly affected, followed by the mitral valve and combined aortic and mitral valve disease. Valve regurgitation is the most common valvular lesion. Our case also presented mild-to-moderate aortic regurgitation. Histopathologic examination of valvular lesion in GPA patients shows fibrinoid connective tissue damage and fragmentation of the collagen fibrils, suggesting ANCA-induced endothelial injury through the activation of neutrophils (14). If the GPA patient presented cardiovascular signs and symptoms, the cardiac involvement should be suspected (Supplementary Figure S3).

GPA is a complex and potentially fatal disease with high mortality if not treated promptly. Early detection and prompt treatment can improve prognosis and reduce mortality (15). Conventional ultrasound, as a noninvasive means of imaging examination, has the advantages of being economical and simple and causing no radiographic damage, making it easily accessible. It is widely used in clinical practice and is often the initial examination method for the evaluation of cardiac involvements in this disease. Two-dimensional echocardiography can identify structural abnormalities of the heart and pericardial effusion, while Doppler echocardiography can assess the cardiac valvular function.

As a new imaging technology, CEUS is simple, reliable, and safe. It enables dynamic observation of the microcirculation inside the mass in real time and has unique value in the diagnosis and differential diagnosis of the lesion. The presence of an aortic root space-occupying lesion in this patient is an extremely rare manifestation of cardiac involvement in GPA patients. Only a few reports in the literature have described such findings. Two middle-age patients with GPA presented with a mass involving the aortic outflow tract or intracardiac septum and mitral valve (16, 17), and two pediatric patients presented with an intracardiac mass in the left ventricle (18, 19). To the best of our knowledge, no CEUS findings of GPA have been reported in the previous literature, and our patient showed that the performance of GPA on CEUS has certain characteristics. This article provided strong evidence of cardiac involvement through two-dimensional echocardiography and CEUS, offering definitive imaging evidence for the follow-up of patients after treatment. Moreover, the chest CT scan of our patient further corroborated the imaging information regarding cardiac involvement. Generally, corticosteroid therapy remains the first drug of choice in GPA, and immunosuppressants should be added in patients with cardiac involvement. For our case, two therapeutic approaches were simultaneously employed, leading to a satisfactory outcome during a 6-month follow-up. After treatment, symptoms of the patient were alleviated, and both the cardiac lesion and thrombosis in the left internal carotid artery showed a significant improvement. In addition, immunobiological therapy (such as rituximab) may be considered for GPA patients with conduction system abnormalities.

Conclusion

GPA is a rare disease, and it can affect virtually any organ. The heterogeneous manifestations of GPA with cardiac involvement represent a significant challenge in the diagnosis of this condition. The present case showed that cardiac imaging, especially echocardiography, is useful for the diagnosis of cardiac impairment caused by GPA. This case report enriched our understanding of the imaging findings of cardiac involvement in patients with GPA. Cardiac involvement in GPA often indicates that the disease is active. However, if diagnosed promptly and accurately, and treated actively with corticosteroids and immunosuppressants, it can effectively alleviate symptoms and improve the prognosis of patients.

Ethics statement

This study was approved by Ethics Committees in Clinical Research of First Affiliated Hospital of Wenzhou Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YT: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. MC: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. ZC: Data curation, Formal analysis, Methodology, Writing – review & editing. XZ: Data curation, Investigation, Software, Writing – review & editing. ZL: Methodology, Resources, Software, Writing – review & editing. SL: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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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/fimmu.2024.1373769/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Chest CT manifestation of the lesion in the aortic root. **(A, B)** Chest CT scan revealed a low density lesion in the aortic root. **(D, E)** CT enhanced scan demonstrated the lesion with low enhancement. **(C, F)** Chest CT scan showed that the lesion was not found after treatment.

SUPPLEMENTARY FIGURE S2
Ultrasound examination of left internal carotid artery and bilateral superficial temporal artery (STA). **(A, B)** Two-dimensional ultrasound revealed thrombosis in the left internal carotid artery. **(C)** CDFI showed changes in blood flow within the carotid artery lumen. **(D)** CEUS indicated the thrombosis in internal left carotid artery showed no contrast agent perfusion. **(E, F)** CDFI demonstrated normal blood flow within the right superficial temporal artery. **(G)** Pulse-wave(PW) spectral Doppler interrogation revealed the normal velocity of right superficial temporal artery. **(H, I)** CDFI demonstrated normal blood flow within the left superficial temporal artery. **(J)** PW interrogation revealed the normal velocity of left superficial temporal artery.

SUPPLEMENTARY FIGURE S3
The diagnostic algorithm for patients with susceptive GPA (of whom cardiac involvement).

References

1. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of vasculitides. *Arthritis Rheum*. (2013) 65:1–11. doi: 10.1002/art.37715

2. Puéchal X, Iudici M, Pagnoux C, Cohen P, Hamidou M, Aouba A, et al. Comparative study of granulomatosis with polyangiitis subsets according to ANCA status: data from the French Vasculitis Study Group Registry. *RMD Open*. (2022) 8: e002160. doi: 10.1136/rmdopen-2021-002160

3. McGeoch L, Carette S, Cuthbertson D, Hoffman GS, Khalidi N, Koening CL, et al. Cardiac involvement in granulomatosis with polyangiitis. *J Rheumatol*. (2015) 42:1209–12. doi: 10.3899/jrheum.141513

4. Czaplińska M, Dorniak K, Lizakowski S, Grzybowski A, Król E, Dębska-Ślizień A, et al. Cardiac involvement as a fatal complication of granulomatosis with polyangiitis. *Pol Arch Intern Med*. (2021) 131:73–4. doi: 10.20452/pamw.15730

5. Saghabalyan D, Schiller D, Lerch S. A 60-year-old with heart failure symptoms due to 1st degree AV-Block caused by granulomatosis with polyangiitis (Wegener's granulomatosis). *J Cardiol cases*. (2019) 21:114–8. doi: 10.1016/j.jccase.2019.11.005

6. Maya-Piña LV, Barbosa-Cobos RE, Lugo-Zamudio GE, Cassaigne-Guasco ME, Nicolás-Mendoza R, Godínez-Baca LE. Acute myocardial infarction, as a rare manifestation in granulomatosis with polyangiitis. *Cir Cir*. (2020) 88:112–5. doi: 10.24875/CIRU.20001764

7. Lutalo PM, D'Cruz DP. Diagnosis and classification of granulomatosis with polyangiitis (aka Wegener's granulomatosis). *J Autoimmun*. (2014) 48-49:94–8. doi: 10.1016/j.jaut.2014.01.028

8. Pugnet G, Gouya H, Puéchal X, Terrier B, Kahan A, Legmann P, et al. Cardiac involvement in granulomatosis with polyangiitis: a magnetic resonance imaging study of 31 consecutive patients. *Rheumatol (Oxford)*. (2017) 56:947–56. doi: 10.1093/rheumatology/kew490

9. Walsh M, Flossmann O, Berden A, Westman K, Höglund P, Stegeman C, et al. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*. (2012) 64:542–8. doi: 10.1002/art.33361

10. Furuta S, Iwamoto T, Nakajima H. Update on eosinophilic granulomatosis with polyangiitis. *Allergol Int*. (2019) 68:430–6. doi: 10.1016/j.alit.2019.06.004

11. Karthik Raman R, Ramanathan S, Ram Ganesh VR, Nagaprabu VN, Rao SVP, Gopalakrishnan S. Granulomatosis polyangiitis of the heart—an unusual presentation: a case report and review of literature. *Indian J Thorac Cardiovasc Surgery*. (2022) 38:312–6. doi: 10.1007/s12055-021-01263-5

12. Santos LP, Bomfim VG, Bezerra CF, Costa NV, Carvalho RB, Carvalho RS, et al. Heart conduction system defects and sustained ventricular tachycardia complications in a patient with granulomatosis with polyangiitis. A case report and literature review. *Rev Bras Terapia Intensiva*. (2017) 29:386–90. doi: 10.5935/0103-507X.20170052

13. Rogaczewska M, Puszczewicz M, Stopa M. Exclusively ocular and cardiac manifestation of granulomatosis with polyangiitis – a case report. *BMC Ophthalmol*. (2019) 19:139. doi: 10.1186/s12886-019-1148-4

14. Yates M, Watts R. ANCA-associated vasculitis. *Clin Med (Lond)*. (2017) 17:60–4. doi: 10.7861/clinmedicine.17-1-6

15. Kubaisi B, Abu Samra K, Foster CS. Granulomatosis with polyangiitis (Wegener's disease): an updated review of ocular disease manifestations. *Intractable Rare Dis Res*. (2016) 5:61–9. doi: 10.5582/irdr.2016.01014

16. Herbst A, Padilla MT, Prasad AR, Morales MC, Copeland JG. Cardiac Wegener's granulomatosis masquerading as left atrial myxoma. *Ann Thorac Surg*. (2003) 75:1321–3. doi: 10.1016/s0003-4975(02)04662-3

17. Oliveira GHM, Seward JB, Tsang TSM, Specks U. Echocardiographic findings in patients with Wegener granulomatosis. *Mayo Clin Proc*. (2005) 80:1435–40. doi: 10.4065/80.11.1435

18. Kosovsky PA, Ehlers KH, Rafal RB, Williams WM, O'Loughlin JE, Markisz JA. MR imaging of cardiac mass in Wegener granulomatosis. *J Comput Assist Tomogr*. (1991) 15:1028–30. doi: 10.1097/00004728-199111000-00024

19. Harris JG, Salvay DM, Klein-Gitelman MS. Asymptomatic intracardiac mass in a 14-year-old girl with granulomatosis with polyangiitis: Case report. *Pediatr Rheumatol Online J*. (2012) 10:9. doi: 10.1186/1546-0096-10-9

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Philipp Douschan,
Medical University of Graz, Austria
Ayman Geddawy,
Prince Sattam Bin Abdulaziz University, Saudi Arabia

*CORRESPONDENCE
Ying Feng
✉ fengying@ccmu.edu.cn
Xianbo Wang
✉ wangxb@ccmu.edu.cn

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Advances in intrahepatic and extrahepatic vascular dysregulations in cirrhotic portal hypertension

Yanqiu Li, Bingbing Zhu, Ke Shi, Yu Lu, Xuanwei Zeng, Yongqi Li, Qun Zhang, Ying Feng* and Xianbo Wang*

Center for Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China

Cirrhotic portal hypertension, the most prevalent and clinically significant complication of liver cirrhosis, manifests as elevated portal venous pressure and is associated with severe complications. Although much research on the mechanisms of portal hypertension has focused on liver fibrosis, less attention has been given to the role of intrahepatic and extrahepatic vascular dysfunction, particularly with respect to extrahepatic vasculature. While the role of hepatic fibrosis in cirrhotic portal hypertension is undeniable, the underlying mechanisms involving intrahepatic and extrahepatic vasculature are highly complex. Sinusoidal capillarization and endothelial dysfunction contribute to increased intrahepatic vascular resistance. Hemodynamic changes in the extrahepatic circulation, including splanchnic vasodilation and hyperdynamic circulation, play a significant role in the development of portal hypertension. Additionally, therapeutic strategies targeting these vascular mechanisms are diverse, including improvement of sinusoidal microcirculation, therapies targeting hepatic stellate cells activation, and pharmacological modulation of systemic vascular tone. Therefore, in this review, we will discuss the vascular-related mechanisms and treatment progress of portal hypertension in cirrhosis to provide a new theoretical basis and practical guidance for clinical treatment.

KEYWORDS

cirrhotic portal hypertension, sinusoidal capillarization, endothelial dysfunction, splanchnic vasodilation, hyperdynamic circulation, nitric oxide

1 Introduction

Liver cirrhosis is pathologically characterized by hepatocyte necrosis, fibrous tissue proliferation, and intrahepatic vascular remodeling (1). Portal hypertension is one of the most common and severe complications of cirrhosis, significantly impacting patients' quality of life and prognosis (2). Except for fibrosis, the mechanisms underlying portal hypertension are complex, involving both intrahepatic and extrahepatic factors. The development of portal hypertension is primarily attributed to increased intrahepatic vascular resistance and increased portal venous inflow. Increased intrahepatic vascular resistance arises from structural changes induced by hepatic fibrosis and regenerative nodule formation. Additionally, the activation of hepatic stellate cells (HSCs) and the excessive proliferation of myofibroblasts play crucial roles (3). The activation of these cells not only elevates intrahepatic vascular resistance but also promotes further fibrosis through the release of various cytokines and growth factors (4). Moreover, endothelial dysfunction and microvascular structural alterations significantly impact hepatic hemodynamics (5). The anticoagulant properties of liver sinusoidal endothelial cells are diminished, promoting thrombosis, which further exacerbates portal hypertension (6). Extrahepatically, vascular remodeling of the portal venous system and the formation of

portosystemic collaterals are important compensatory mechanisms (7). While these vascular changes can partially alleviate pressure within the portal system, they can also lead to serious complications such as esophageal and gastric varices and gastrointestinal bleeding.

However, the intrahepatic and extrahepatic vascular regulatory mechanisms governing portal hypertension are complex and a comprehensive understanding remains elusive. Current therapeutic options, while capable of reducing portal pressure and preventing bleeding to some extent, have limited efficacy and are associated with side effects. Therefore, this review aims to systematically summarize the intrahepatic and extrahepatic vascular mechanisms of portal hypertension in liver cirrhosis, analyze the progress of existing research and explore potential therapeutic strategies and future research directions. Through a comprehensive understanding of the pathophysiological mechanisms of portal hypertension, we expect to provide a theoretical basis and novel insights for clinical management, thereby improving the prognosis and quality of life for patients with cirrhosis.

2 Intrahepatic vascular changes in cirrhotic portal hypertension

During the process of cirrhosis, the liver's internal vasculature undergoes significant structural changes, mainly manifested as sinusoidal remodeling and capillarization. In terms of functional changes, endothelial cell damage leads to reduced nitric oxide (NO) synthesis, imbalance of systolic and diastolic vascular factors, and increased blood flow resistance, ultimately leading to the occurrence and development of portal hypertension (Figure 1).

2.1 Intrahepatic structural changes

During the development of liver cirrhosis, the intrahepatic vascular system undergoes significant structural changes. Major structural changes include hepatic sinus remodeling and capillarization. Hepatic sinuses are special capillary-like structures. Liver sinus endothelial cells (LSECs) in hepatic sinuses have fenestrae that allow direct contact between blood and hepatocyte, facilitating the exchange of substances (5). In liver cirrhosis, hepatic sinus remodeling and capillary vascularization are key pathophysiological changes and affect intrahepatic hemodynamics. Hepatic sinus remodeling involves LSECs dysfunction, capillarization, and activation of Kupffer cells and HSCs (Figure 2).

LSECs dysfunction and capillarization play a role in hepatic sinus remodeling. LSECs maintain important physiological functions in healthy liver, including regulation of liver blood flow, substance exchange, and immune surveillance (5, 8). In liver cirrhosis, LSECs lose

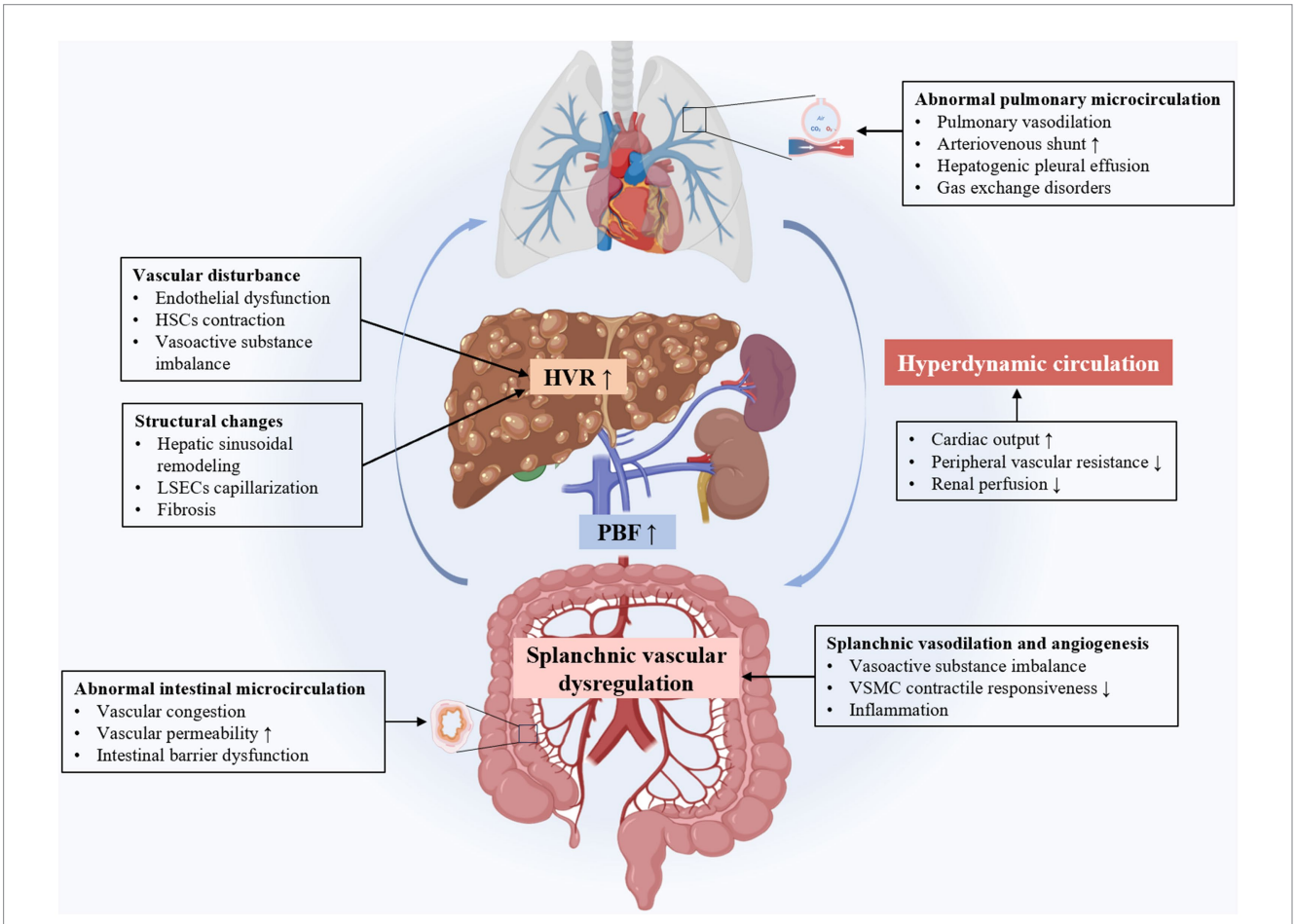

FIGURE 1
The intrahepatic and extrahepatic mechanisms of cirrhotic portal hypertension. LSECs, liver sinus endothelial cells; HSCs, hepatic stellate cells; HVR, hepatic vascular resistance; PBF, portal blood flow; VSMC, vascular smooth muscle cell.

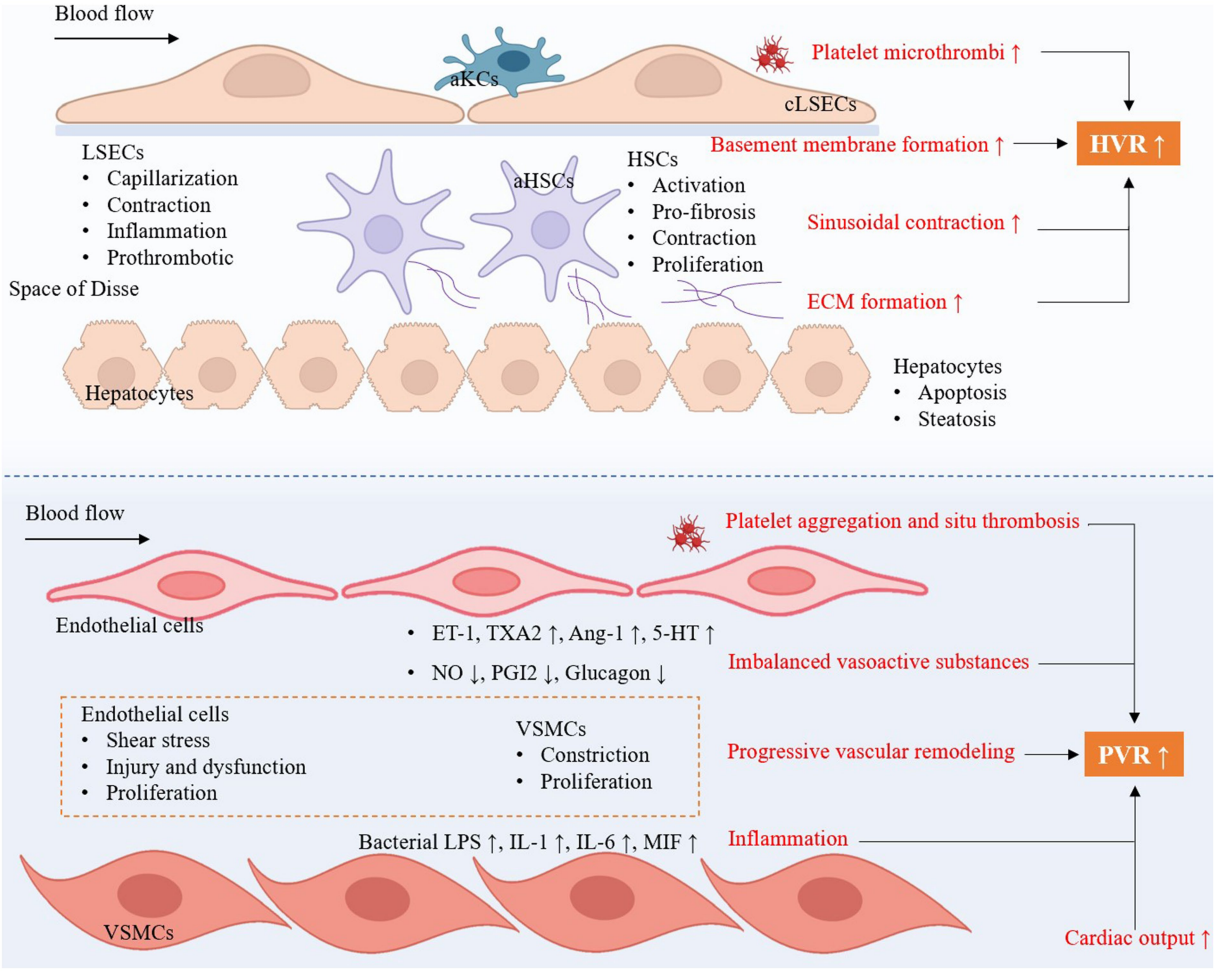

FIGURE 2
The key intrahepatic cellular changes occur in cirrhotic portal hypertension and the development of portopulmonary hypertension. HVR, hepatic vascular resistance; PVR, pulmonary vascular resistance; aKCs, activated Kupffer cells; LSECs, liver sinus endothelial cells; HSCs, hepatic stellate cells; aHSCs, activated hepatic stellate cells; cLSECs, capillarized liver sinus endothelial cells; ECM, extracellular matrix; ET-1, endothelin-1; TXA2, thromboxane A2; Ang-1, angiotensin-1; 5-HT, 5-Hydroxytryptamine; NO, nitric oxide; PGI2, prostaglandins I2; VSMCs, vascular smooth muscle cells; LPS, lipopolysaccharides; IL-1, interleukin-1; IL-6, interleukin-6; MIF, macrophage migration inhibitory factor.

their normal fenestrae structure and form a continuous basement membrane, a process known as hepatic sinusoidal capillarization (4, 5). Capillary vascularization leads to increased blood flow resistance and increased portal vein pressure. The formation of basement membrane is another key link in the hepatic sinusoidal capillarization. Dysfunctional LSECs secrete large amounts of basement membrane components, such as type IV collagen and laminin (9). These components are deposited beneath the LSECs, forming a continuous basement membrane (5). This basement membrane increases blood flow resistance and impedes the exchange of substances. Besides, dedifferentiation of LSECs means LSECs lost their characteristic fenestrated structure (10). This change causes the hepatic sinuses to lose their high permeability, obstructing material exchange and increasing blood flow resistance (11). The reasons for the loss of fenestration include endothelial cell damage, chronic inflammatory response and persistent cytokine stimulation.

Activation of HSCs play a central role in hepatic sinus remodeling and capillary vascularization. In liver cirrhosis, HSCs change from a resting state to an active state, similar to myofibroblasts, producing large amounts of extracellular matrix (ECM) (12, 13). These ECM components further promote the formation of basement membrane and hepatic sinusoidal capillarization. HSCs also release a variety of pro-fibrotic factors, such as transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF), which further promotes the fibrotic process and hepatic sinus remodeling (14).

Kupffer cells are resident macrophages in liver that play important immune and clearance functions in the hepatic sinuses (15). In liver cirrhosis, Kupffer cells are activated and release multiple inflammatory mediators and chemokines, such as tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and reactive oxygen species (ROS) (16). These inflammatory mediators not only cause local inflammatory response, but also promote the activation of HSCs and the dysfunction of LSECs (17). It also causes hepatocytes apoptosis and necrosis by releasing ROS and cytokines, and exacerbating hepatic sinus remodeling and fibrosis.

2.2 Intrahepatic functional changes

2.2.1 Endothelial dysfunction

Endothelial cell damage occurs in the early stages of cirrhosis and portal hypertension. Chronic inflammation and oxidative stress are the main factors leading to endothelial cell damage in liver cirrhosis (18). Chronic hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) and other causes can cause persistent inflammation of liver, this chronic inflammation will lead to endothelial cell damage and dysfunction. Oxidative stress is caused by excessive production of ROS or insufficient antioxidant capacity, and this oxidative stress environment further damages endothelial cells (19).

In cirrhotic portal hypertension, there is a reduction in NO synthesis and release. Liver endothelial cells synthesize NO via endothelial nitric oxide synthase (eNOS) in healthy liver, a potent vasodilator that maintains vascular tone and normal blood flow (20). However, eNOS expression and activity are significantly reduced in cirrhosis, leading to reduced synthesis and release of NO (20, 21). The reduced NO production and decreased NO bioavailability directly leads to a decrease in the diastolic ability of intrahepatic blood vessels (21, 22). The blood vessels show continuous contraction, increasing portal system resistance. NO is not only a vasodilator, but also has the effect of inhibiting platelet adhesion and aggregation (23). The reduction of NO causes platelets to adhere to and aggregate on the damaged endothelial surface, forming tiny thrombi, further blocking blood vessels and aggravating microcirculatory disorders (6). The microthrombi formation not only increases the intrahepatic blood flow resistance, but may also lead to local ischemia and further tissue damage (24).

In contrast to the decreased NO, vasoconstrictors are significantly increased in liver cirrhosis. Endothelial dysfunction is also manifested by excessive release of vasoconstrictive factors such as endothelin-1 (ET-1). ET-1 is a potent vasoconstriction factor secreted by endothelial cells (13), inducing contraction of vascular smooth muscle cells (VSMCs) and increasing vascular resistance by binding to its receptor (13). In liver cirrhosis, due to endothelial cell damage and stimulation of inflammatory mediators, the expression and release of ET-1 are significantly increased (25), resulting in continuous vasoconstriction and further aggravating of portal hypertension (26).

2.2.2 Contraction of HSCs

Activated HSCs have contractile function similar to smooth muscle cells, which is mainly achieved through cytoskeletal remodeling and actin (α-SMA) expression (27). Contractile HSCs can directly increase hepatic sinusoidal resistance and impede blood flow in the portal system (28). α-SMA is a typical myofibroblast marker, and its expression level is closely related to the contractility of HSCs. Through the regulation of intracellular calcium concentration and the interaction of actin-myosin system (29, 30), contractile HSCs can significantly increase the intrahepatic vascular resistance and further lead to portal hypertension (31). Activated HSCs not only have contractile function, but also secrete a variety of vasoconstrictor factors, such as ET-1 and angiotensin II (AngII) (13). These factors act on intrahepatic blood vessels through autocrine and paracrine pathways, further promoting vasoconstriction and fibrosis (32, 33).

2.2.3 Vasoactive substance imbalance

There is an imbalance between NO and ET-1 in cirrhotic portal hypertension. The reduction of NO and the increase of ET-1 in patients with cirrhosis are typical manifestations of vasoactive substance imbalance (34). The decrease of NO leads to weakened vasodilation, while the increase of ET-1 leads to enhanced vasoconstriction (32). This imbalance between vasodilation and contraction directly leads to increased intrahepatic vascular resistance and increased portal pressure (35).

The expression of prostacyclin (PGI2) and cyclooxygenase-2 (COX-2) is also decreased in liver cirrhosis. PGI2 is a potent vasodilator that inhibits the VSMCs contraction by activating adenylate cyclase (AC) to produce cyclic adenosine phosphate (cAMP) (36). In cirrhosis, the synthesis and release of PGI2 are reduced, resulting in reduced vasodilation (37, 38). COX-2 is a key enzyme in the PGI2 synthesis, and its reduced expression directly affects PGI2 production, further weakening the vasodilation ability (39).

The role of AngII and Angiotensin-converting enzyme (ACE) in the pathophysiology of cirrhotic portal hypertension should not be overlooked. ACE is a key enzyme for AngII production, and its increased activity leads to increased AngII levels (40). AngII is a potent vasoconstriction that causes VSMCs to contract and increase vascular resistance by binding to AngII receptor (41). In addition, AngII also has a pro-fibrotic effect, further aggravating liver fibrosis and portal hypertension by stimulating the activation of HSCs and the production of ECM (42). Inhibiting AngII expression can decrease collagen synthesis (42, 43).

The compensation of vasodilators is obviously insufficient. In cirrhosis, although the levels of certain vasodilator factors such as adrenomedullin (AM) and brain natriuretic peptide (BNP) are elevated to certain extent in an attempt to counteract the overexpression of vasoconstrictors (44, 45), their compensatory effects are often insufficient to maintain normal vascular tone and balance. AM has a strong vasodilatory effect by increased cAMP generation (46). BNP inhibits VSMCs contraction by increasing the production of cyclic guanosine phosphate (cGMP) (47). However, in cirrhosis, the compensatory mechanisms of these vasodilator factors are unable to fully offset the overexpression of vasoconstrictor, resulting in vascular tone imbalance and increased portal pressure.

3 Extrahepatic vascular changes

Portal hypertension in cirrhosis leads to major changes in the extrahepatic vascular and systemic circulation. These changes include increased portal blood flow, splanchnic vasodilation, portal-systemic collateral formation, hyperdynamic circulation, and abnormalities in the intestinal and pulmonary microcirculation. Complex molecular mechanisms involve angiogenesis, vasodilation, and oxidative stress (Figure 1).

3.1 Extrahepatic portal vascular changes

Portal hypertension is one of the core pathological changes in liver cirrhosis. It is caused by many factors, among which changes of extrahepatic portal vein are particularly critical. These changes mainly include a significant increase in portal blood flow and the formation of portosystemic collateral circulation.

Extrahepatic vascular changes in liver cirrhosis include portal blood flow and systemic hemodynamics. Due to the dilation of visceral blood vessels, especially in the gastrointestinal and splenic region, splanchnic blood flow increases, eventually leading to increased blood flow to portal vein (48–50). In addition, changes in systemic hemodynamics are particularly critical. Patients with liver cirrhosis are usually accompanied by a hypovolemic state and reduced effective blood volume in the systemic circulation (51). This state activates the adrenal glands and the sympathetic nervous system, causing systemic vasoconstriction, especially in the renal and splenic vessels, thereby reducing blood flow elsewhere and increasing portal blood flow (52). At the same time, activation of the renin-angiotensin system by the kidney increases the release of vasoconstrictor factors, which further promotes an increase in portal blood flow. Alterations in splanchnic vasculature and systemic hemodynamics in cirrhosis interact to result in a significant increase in portal blood flow (49).

As portal vein pressure continues to rise, portosystemic collaterals are formed to relieve the pressure (53). Although this compensatory mechanism helps to reduce portal vein pressure in the short term, its long-term consequences can lead to a series of complications, including esophageal and gastric varices, hypersplenism, etc. (54). The vascular regulation mechanisms in this process involves are complex. Firstly, one of the core mechanisms of portosystemic collateral circulation is angiogenesis. In cirrhotic portal hypertension, multiple factors induce overexpression of angiogenic factors such as vascular endothelial growth factor (VEGF) and PDGF (55–57). These factors initiate the formation of new collateral circulation by promoting the proliferation and migration of vascular endothelial cells (56). Hypoxia-inducible factor (HIF-1α) is another key regulator. HIF-1α, stimulated by hypovolemia, not only induces VEGF expression, but also promotes other related angiogenic factors production, further accelerating the generation of collateral circulation (58, 59). Secondly, remodeling of existing blood vessels is also a mechanism that cannot be ignored during the formation of portosystemic collateral circulation. Especially in the esophagus and fundus of the stomach, the original microvascular network expands and remodels driven by portal pressure, forming functional varicose veins (60). Smooth muscle cells and collagen deposition in the blood vessel wall increase, which enhances the capacity of the blood vessel and allows greater blood flow to pass through (61). At the same time, this vascular remodeling process is accompanied by thinning of the vessel wall, increasing the risk of varicose vein rupture bleeding (57, 61). In addition, inflammatory factors not only promote angiogenesis, but also accelerate local blood vascular remodeling (57). At the same time, leukocyte infiltration will also accelerate blood vessels dilation and the formation of collateral circulation.

3.2 Changes in systemic circulation

Splanchnic vasodilation is one of the core features of systemic circulatory changes in patients with cirrhotic portal hypertension (62). Splanchnic vasodilation causes a series of adverse consequences, including hypovolemia, ascites and so on (7). In cirrhosis, visceral vascular endothelial cells are dysfunctional, and the levels of vasodilators such as NO, PGI2, and carbon monoxide (CO) are significantly increased (63–65). These factors are mainly produced by endothelial cells. Increased production of vasodilator synthase promotes vascular smooth muscle relaxation and leads to vasodilation (63, 66). In addition, vasoconstrictor factors are relatively reduced. Although vasoconstrictor factors such as ET-1 also increase, their effects are offset by a large number of vasodilator factors. Moreover, the responsiveness of vascular smooth muscle to contractile stimuli decreases (67). Long-term exposure to high concentrations of vasodilator factors reduces the sensitivity of VSMCs to normal contractile stimulation (68). Inflammatory responses also participate in visceral vasodilation and increase vascular permeability, leading to ascites and tissue edema.

Hyperdynamic circulation is another important feature of cirrhotic patients, which is closely related to splanchnic vasodilation and the formation of portosystemic collateral circulation (49). The main manifestations of hyperdynamic circulation include increased cardiac output, decreased peripheral vascular resistance and decreased renal blood flow (69, 70). The occurrence of hyperdynamic circulation involves multiple mechanisms. The first is increased systemic NO production. NO not only causes splanchnic vasodilation, but also reduces arteriolar and capillary resistance through systemic vasodilation, thereby increasing cardiac output (71). This compensatory mechanism is to maintain the oxygen supply requirements of peripheral tissues. Secondly, the sympathetic nervous system and renin-angiotensin system activation are also involved in this process (72). In response to the decrease in systemic vascular resistance, the sympathetic nervous system and renin-angiotensin system are activated to increase blood volume by constricting blood vessels and retaining sodium and water (73). However, this compensatory mechanism has limited effect under the action of NO and other vasodilator factors, and instead aggravates vasodilation and reduce renal blood flow (74). In addition, the role of systemic inflammation in this process cannot be ignored (70). Inflammatory factors promote fluid exudation by increasing vascular permeability, leading to aggravation of ascites. Finally, the compensatory response of the heart will cause some adverse consequences. Long-term compensatory load increase may lead to cirrhotic cardiomyopathy, in which the heart is unable to maintain normal function under increased load (75, 76).

3.3 Microcirculation changes

The microcirculation, as the most subtle component of the vascular system, includes small arteries, capillaries and small veins, responsible for the transport of oxygen and nutrients and the discharge of waste. Cirrhotic portal hypertension has a profound impact on the extrahepatic microcirculation such as intestinal tract and lung through complex mechanisms.

The abnormalities of intestinal microcirculation in patients with cirrhosis are mainly manifested in intestinal ischemia, obvious vascular congestion in the intestinal wall, increased vascular permeability, and resulting in intestinal wall edema (77–79). It affects the absorption of nutrients and aggravates the malnutrition of patients (78). In addition, portal hypertension aggravates local intestinal inflammatory response, destroy intestinal barrier function, increase the risk of bacterial translocation, and thus induce systemic inflammatory response syndrome (SIRS) (80). It also promotes the entry of intestinal endotoxins into the portal vein system, aggravating liver inflammation and fibrosis (81). The mechanism of abnormal

intestinal microcirculation involves multiple aspects. Firstly, portal hypertension causes intestinal venous congestion, vasodilation, and slow blood flow, leading to insufficient microcirculatory perfusion (79). Secondly, there is an imbalance of vasoactive substances. Increased vasoconstrictor factors, such as ET-1, lead to intestinal microvascular spasm and further aggravate ischemia (82). The decreased bioavailability of NO leads to vascular endothelial dysfunction and further deteriorates intestinal microcirculation (83). In addition, the oxidative stress response is enhanced in cirrhosis, leading to endothelial damage, weakening the dilation ability of blood vessels, and ultimately affecting microcirculatory function (84).

Changes in pulmonary microcirculation are a common but easily overlooked complication in patients with cirrhotic portal hypertension. In terms of pulmonary microcirculation, the main manifestations are hepatopulmonary syndrome and hepatogenic pleural effusion. Hepatopulmonary syndrome is characterized by abnormal dilation of pulmonary blood vessels and redistribution of intrapulmonary blood flow (85), leading to oxygenation dysfunction (86); while hepatogenic pleural effusion is caused by obstruction of lymphatic drainage. In contrast to hepatogenic pleural effusion, hepatic hydrothorax develops when ascitic fluid moves from the peritoneal cavity into the pleural space through diaphragmatic defects (87), which is unrelated to pulmonary microcirculatory disorders. The mechanism of pulmonary microcirculation changes involves multiple aspects. The first is that pulmonary vasodilation occurs primarily at the level of alveolar capillaries. In liver cirrhosis, excessive synthesis of NO will cause abnormal expansion of pulmonary capillaries, increasing pulmonary blood flow, but decreasing oxygen diffusion efficiency, resulting in hypoxemia (88). In addition, the exchange time of oxygen between the alveoli and blood is insufficient, resulting in gas exchange disorder (89, 90). Increased arteriovenous shunting also leads to alveolar ventilation-blood flow imbalance (91). The chronic inflammatory response and oxidative stress will increase the permeability of pulmonary capillaries, leading to pulmonary edema and further aggravate the damage of lung function (90, 92, 93). Recent studies have revealed that increased pulmonary expression of placental growth factor (PlGF) and VEGF-A plays a central role in pathological angiogenesis (94). The von Willebrand factor-angiopoietin axis activation and altered circadian rhythm proteins, particularly BMAL1, significantly affect hypoxic responses and vascular remodeling (88, 95). Additionally, bacterial translocation and endotoxemia contribute to pulmonary inflammation through recruitment of intravascular monocytes that produce proangiogenic factors (96). These molecular mechanisms create extensive pulmonary microvascular alterations, including capillary dilatation, arteriovenous malformations, and altered vascular reactivity. In contrast, portopulmonary hypertension (POPH) represents a distinct entity characterized by pulmonary arterial hypertension in the setting of portal hypertension (97). The pathophysiology involves pulmonary vasoconstriction, vascular remodeling, and *in situ* thrombosis. Key molecular pathways include endothelial dysfunction with decreased NO and prostacyclin production, upregulation of ET-1 and serotonin pathways, and proliferation of pulmonary arterial smooth muscle cells (98). BMP9 is a sensitive and specific biomarker of POPH, which could predict transplant-free survival and the presence of pulmonary arterial hypertension in liver disease (99). The mechanical stress from increased pulmonary blood flow in the hyperdynamic circulatory state may trigger endothelial injury, initiating these pathological cascades.

4 Pharmacological interventions based on intrahepatic vascular changes

Intrahepatic vascular changes play a role in liver cirrhosis. And therapeutic strategies targeting intrahepatic vessels mainly focus on anti-fibrotic treatment, improvement of hepatic sinusoidal microcirculation, and treatment targeting HSCs (Table 1).

4.1 Antifibrotic therapy

Antifibrotic therapy is one of the cornerstones of cirrhotic portal hypertension treatment. Liver fibrosis leads to structural remodeling and functional abnormalities of intrahepatic blood vessels, thereby causing increased portal pressure. Therefore, inhibiting and reversing the process of liver cirrhosis has become a key strategy to reduce portal pressure.

Firstly, inhibiting excessive deposition of ECM. The main pathological feature of liver cirrhosis is the massive ECM deposition (100). Antifibrotic treatments aim to reduce or reverse the accumulation of ECM (3). Many drugs and molecular targets can intervene in this process, including blocking liver fibrosis formation by inhibiting the TGF-β signaling pathway (14). TGF-β is an important profibrotic factor in liver fibrosis, and inhibiting its activity can significantly reduce the degree of fibrosis. Activators of matrix metalloproteinases (MMPs) can promote ECM degradation (101); while inhibitors of tissue inhibitors of metalloproteinases (TIMPs) can reduce ECM deposition (102). Therefore, MMP/TIMP balance is a potential therapeutic target for regulating the extracellular matrix (102, 103). Activation of HSCs can lead to imbalances in MMP2/TIMP2 and MMP9/TIMP1, aggravating fibrosis (104, 105).

Secondly, applying antioxidant and anti-inflammatory treatment. Oxidative stress and inflammatory responses also play a key role in the process of liver cirrhosis. Therefore, antioxidants and anti-inflammatory drugs are used to alleviate oxidative stress damage to liver cells. Common antioxidants include vitamin E, lipoic acid, etc., which improve liver fibrosis by reducing the ROS production (106, 107). Wang Q, et al. found that glycyrrhizic acid inhibited oxidative stress injury through targeting AKR7A2 in HSCs, reduced the activated HSCs proliferation and reversed hepatic fibrosis (108). In addition, anti-inflammatory drugs such as glucocorticoids and certain immunomodulators can reduce the chronic inflammatory response and progression of liver cirrhosis. Qin BF, et al. found that specnuezhenide inhibited inflammatory response via SIRT6-P2X7R/NLRP3 pathway and improve fibrosis (109).

Targeting HSCs activation is important in pharmacological treatments for patients with cirrhotic portal hypertension. Activated HSCs are the main effector cells of liver cirrhosis. By inhibiting the activation of HSCs or promoting their apoptosis, ECM production can be effectively reduced (110). Some drugs, such as retinoic acid receptor gamma agonists (such as retinoic acid) and peroxisome proliferator-activated receptor gamma (PPARγ) agonists, have shown the potential to inhibit HSCs activation (111). Benedicto AM, et al. have shown that interference with mitochondrial function could target HSCs to inhibit fibrosis (110). Tung HC, et al. demonstrated that inhibition of heme-thiolate monooxygenase CYP1B1 could decrease HSCs activation and fibrosis (12).

TABLE 1 Evidence for pharmacological therapy targeting intrahepatic and extrahepatic vascular dysregulations in portal hypertension.

	Categories	Agent	Model	Research subjects	Direct target	Mechanism
Pun et al. (143)	Clinical drugs	Fructooligos-accharides	BDL rats	Intrahepatic vessels	ROS/eNOS	Ameliorate of dysbiosis and oxidative stress
Asada et al. (144)		Tofogliflozin	CCl_4 rats	LSECs, HSCs	SGLT 2 inhibitors	Improving endothelial dysfunction
Fan et al. (57)		Cediranib	BDL rats	Intrahepatic and extrahepatic vessels	VEGFR-2	Improving vascular remodeling and contractility
Vairappan et al. (145)		Candesartan cilexetil	CCl_4 mice	HUVECs	Nostrin-eNOS-NO	Improving endothelial dysfunction
Tai et al. (146)		Celecoxib	TAA rats	LSECs	eNOS	NO homeostasis
Noah et al. (147)		Empagliflozin	CCl4 rats	LSECs, HSCs	Gal-1/NRP-1	Suppression of angiogenesis
Zheng et al. (148)		Telmisartan	CCl4, BDL rats	Liver and mesenteric tissue	KLF-4, eNOS	Reducing angiogenesis and vascular remodeling
Zhu et al. (67)	Small molecular agents	8-OH-DPAT	TAA, BDL, PPVL rats	VSMCs	5-HT receptor 1A	Inducing the contraction of portal vein
Zhao et al. (61)		Imperatorin	CCl_4 rats	HSCs	TGF-β	Reducing hepatic fibrosis and vascular remodeling
Li et al. (149)		Urolithin A	CCl_4, BDL mice	HSCs	Glutaminas-e1	Inhibiting fibrogenesis and HSCs contraction
Gunarathne et al. (150)		MrgD	BDL, PPVL, CCl_4 rats	Splanchnic vessels	Mas receptor	Mesenteric Vasodilation
Wang et al. (151)		DPP4i	CCl_4 rats	Mesenteric arterioles	Nox4	Normalizing arterial hypocontractility
Pun et al. (152)		Glycyrrhizin	BDL rats	Mesenteric vessels	VEGF	Attenuating portosystemic collateral shunting
Boyer-Diaz et al. (153)		Lanifibranor	TAA, BDL rats	HSCs, LSECs	Pan-PPAR agonist	Ameliorating hepatic microvascular function
Brusilovskaya et al. (154)		TADA	BDL rats	Intrahepatic vessels	PDE-5 inhibitor	Reducing sinusoidal vascular resistance
Tsai et al. (155)		Obeticholic acid	BDL rats	Intrahepatic vessels	Farnesoid X receptor agonist	Inhibiting vasoconstriction
Hu et al. (156)		AICAR	BDL, PPVL, CCl_4 rats	LSECs	AMPK/NO	Improving NO bioavailability
Castillo (157)		PHIN-156	BDL rats	Vasopressin receptor	V1a partial agonists	Reducing portal blood flow
Jones (158)		BI 685509	TAA rats	Portosystemic shunting	sGC, cGMP	NO-independent sGC activator
Zhao et al. (39)		PTUPB	CCl4 rats	Intrahepatic and extrahepatic vessels	sEH/COX-2/TGF-β	Inhibiting intra-or extrahepatic angiogenesis and vascular remodeling

BDL, bile duct ligation; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; CCl_4, Carbon tetrachloride; LSECs, liver sinus endothelial cells; HSCs, hepatic stellate cells; SGLT2, Sodium glucose transferase 2; VEGFR-2, vascular endothelial growth factor receptor 2; HUVECs, human umbilical vein endothelial cells; NO, Nitric oxide; TAA, thioacetamide; Gal-1, galactin-1; NRP-1, Neuropilin-1; KLF-4, Krüppel-like factor-4; PPVL, partial portal vein ligation; VSMCs, vascular smooth muscle cells; 5-HT, 5-hydroxytryptamine; TGF-β, Transforming growth factor-β; MrgD, Mas-related G protein-coupled receptor type D; DPP4i, Dipeptidyl peptidase-4 inhibitor; Nox4, NADPH oxidase 4; VEGF, vascular endothelial growth factor; Pan-PPAR, pan-peroxisome proliferator-activated receptor; TADA, Soluble guanylyl cyclase stimulation and phosphodiesterase-5; PDE-5, phosphodiesterase-5; AICAR, 5-aminoimidazole-4-carboxyamide ribonucleoside; AMPK, Adenosine 5′-monophosphate-activated protein kinase; V1a, vasopressin 1a; sGC, soluble guanylyl cyclase; cGMP, cyclic guanosine monophosphate; PTUPB, 4-(5-phenyl-3-57-pyrazol-1-yl) -benzenesulfonamide; sEH, soluble epoxide hydrolase; COX-2, cyclooxygenase-2.

Promoting hepatocyte regeneration can improve liver function and indirectly reduce fibrosis. Growth factors such as hepatocyte growth factor (HGF) and epidermal growth factor (EGF) have been shown to promote liver regeneration and reduce fibrosis. Wang P, et al. have identified hepatic Snai1 and Snai2 as key transcriptional regulators of liver regeneration and fibrosis (112). Novel stem cell therapies, such as mesenchymal stem cells (MSCs), possess immunomodulatory and anti-inflammatory capabilities that make them an attractive approach for promoting liver regeneration (113). The primary mechanism involves promoting apoptosis of HSCs and subsequently stimulating hepatocyte proliferation, thereby replacing damaged hepatocytes and reducing liver fibrosis (114).

4.2 Improvement of hepatic sinusoidal microcirculation

Hepatic sinusoidal microcirculation disorder is one of the important mechanisms for portal hypertension. It can directly reduce intrahepatic vascular resistance, thereby reducing portal pressure.

Statins, the most widely used lipid-lowering drugs, have been found in recent years to have the potential to improve liver cirrhosis and hepatic sinusoidal microcirculation (115, 116). Statins increase the expression and activity of eNOS, promoting the production of NO, and reducing hepatic sinusoidal resistance (116). In addition, statins can inhibit the production of inflammatory factors, such as TNF-α and IL-6, thereby reducing microcirculation disorders caused by inflammation (117). Statins can also protect LSECs and maintain their normal function by reducing the ROS production (117, 118). Statins can also reduce hepatic sinusoidal contraction and improve microcirculation by inhibiting Rho kinase activity (119). Some studies have shown that simvastatin and atorvastatin can significantly reduce portal pressure and improve the prognosis of patients with cirrhosis (120, 121). However, it should be noted that statins should be used with caution in patients with advanced cirrhosis to avoid potential hepatotoxicity (122). Statins represent the most clinically advanced antifibrotic therapy, with multiple Phase III trials demonstrating their potential in portal hypertension. Simvastatin and atorvastatin have shown particular promise, with data supporting their safety in compensated cirrhosis. However, their use in advanced cirrhosis requires careful monitoring.

In liver cirrhosis, NO production is reduced due to endothelial cell dysfunction, leading to increased vasoconstriction. Therefore, exogenous NO donors, such as nitrates, can improve the expansion ability of liver sinuses and reduce intrahepatic vascular resistance (32). In addition, NO donors can inhibit the contraction of HSCs and reduce their compression on hepatic sinusoids, thereby improving microcirculation (20). Villanueva C, et al. have found that isosorbide mononitrate (ISMN) can significantly reduce portal pressure and prevent variceal rebleeding, especially during acute application (123). However, long-term use may lead to the reduced tolerance and effectiveness of treatment. To overcome tolerance issues, researchers are exploring intermittent dosing regimens and novel NO donors. For example, NCX-1000 is a liver-targeted NO donor that can specifically release NO, promising to improve therapeutic efficacy and reduce systemic side effects (124). In addition, combination treatment strategies combining statins and nitrates also show good promise. This combination can work synergistically through different mechanisms to improve hepatic sinusoidal microcirculation more effectively. Nicorandil and atorvastatin may alleviate hepatic sinusoidal microcirculatory disorders by improving liver function, anti-inflammation and anti-oxidation (125).

Endothelin (ET) receptor antagonist is another choice of hepatic sinusoidal microcirculation improvement. There is an observed up-regulation of the ET-1 gene accompanied by a compensatory down-regulation of the ET A receptor (ETAR) gene in the human portal vein (25). Blocking the ET-1/ETAR pathway using selective ETAR antagonists (ERAs) represents a promising therapeutic strategy for liver cirrhosis treatment (26). Ten Hove M, et al. have demonstrated that engineered SPIONs functionalized with ETAR antagonist had improved liver fibrosis through the inhibition of HSCs activation (13). A selective ET-A antagonists, such as BQ 123 and Ambrisentan, decrease the portal pressure in cirrhotic patients (126).

4.3 Treatment targeting the contractile function of HSCs

HSCs play a central role in the development of portal hypertension. Activated HSCs are not only the main ECM producers that lead to liver fibrosis, but also have contractile properties and are directly involved in the regulation of hepatic sinusoidal resistance. Therefore, therapeutic strategies targeting HSCs have become a hot topic in recent years. HSCs have contractile properties and are directly involved in the regulation of liver sinusoidal resistance (127). ET-1 is a potent vasoconstrictor that can cause HSCs to contract. The use of ET-1 receptor antagonists can reduce HSCs contraction and reduce liver sinusoidal resistance (25). In addition, AngII can promote HSCs contraction and proliferation (128). AngII receptor antagonists, such as losartan, can reduce HSCs contraction and improve liver sinusoidal microcirculation (42). Moreover, Nanotechnology can be used to achieve targeted drug delivery, improve therapeutic effects and reduce side effects. Vitamin A-modified liposomes can specifically deliver drugs to HSCs because HSCs are the main vitamin A storage cells in liver. This strategy can be used to deliver anti-fibrotic drugs, siRNA or gene therapy vectors. Kaili Wang et al. constructed hyaluronic acid (HA) modified liposomes co-delivering all-trans retinoic acid (RA) and L-arginine (L-arg) to reverse hepatic fibrosis (129). Lingfeng Zhang et al. designed chondroitin sulfate-modified and vismodegib-loaded nanoparticles (CS-NPs/VDG) to efficiently normalize the fenestrae phenotype of LSECs and restore HSCs to quiescent state by inhibiting Hedgehog signaling pathway (130). Additionally, stem cell therapy is a treatment method targeting HSCs that has attracted much attention in recent years. MSCs have multidirectional differentiation potential and immunomodulatory functions, and can inhibit HSCs activation by secreting various anti-inflammatory and anti-fibrotic factors (131, 132). Preliminary clinical studies have shown that stem cell therapy is effective in reducing liver fibrosis and improving liver function (131). These have inspired new ways of thinking about treating liver fibrosis.

5 Pharmacological interventions based on changes in extrahepatic vessels

Extrahepatic vascular dilation, increased blood flow, and changes in peripheral vascular resistance exacerbate the portal pressure. Therefore, treatment strategies based on changes in extrahepatic blood vessels aim to reduce portal blood flow, regulate vascular tension, and improve systemic hemodynamic balance (Table 1). The clinical development of therapies targeting extrahepatic vascular changes shows a clear stratification in terms of evidence and approval status. Non-selective beta blockers (NSBBs) and vasoconstrictors represent the current standard of care, supported by Class I, Level A evidence. Propranolol, carvedilol, and nadolol are FDA-approved and widely used in clinical practice. For acute complications, terlipressin and octreotide have established roles in management protocols. Beyond these approved therapies, several novel approaches are in various stages of clinical development. Understanding this therapeutic hierarchy is essential for optimal clinical decision-making and future research directions.

5.1 NSBBs

NSBBs are classic drugs for cirrhotic portal hypertension (133). The main mechanisms include reducing cardiac output by blocking β1 receptors, and causing splanchnic vasoconstriction and reducing portal blood flow by blocking β2 receptors. NSBBs can effectively reduce the burden on portal system, and are especially suitable for preventing esophageal and gastric variceal bleeding (134). Propranolol and Carvedilol are commonly used NSBBs. These drugs are widely used for the primary prevention of portal hypertension, which is to prevent bleeding from varicose veins that are not yet bleeding (135). For patients who have already suffered bleeding, NSBBs are also used for secondary prevention to reduce the risk of rebleeding (136). Although NSBBs are effective in reducing portal pressure and preventing variceal rupture, not all patients can tolerate these drugs, especially those with hypotension or severe cardiac dysfunction (137). In addition, NSBBs may interact with other medications, so they should be used with caution.

5.2 Vasoconstrictors

Vasoconstrictors reduce portal pressure by constricting the visceral arterial system and reducing blood flow to the portal vein. Their main target is the visceral vascular smooth muscle, directly or indirectly regulating its contractile function. They are usually used to treat acute complications of portal hypertension, such as gastric variceal. Terlipressin, one of the most commonly used vasoconstrictors clinically, reduces portal vein blood flow by selectively acting on V1 receptors in visceral blood vessels (138). Long-term continuous infusion of terlipressin can significantly increase cardiac reserve and attenuate a hyperdynamic state (139). Octreotide have similar effect for the management of variceal bleeding (140). The main side effects of vasoconstrictors include increased blood pressure, myocardial ischemia, and impaired renal function. Therefore, patients with cardiovascular disease or renal insufficiency should be used with extreme caution and closely monitored. In addition, long-term use of these drugs may lead to decreased renal perfusion and increase the risk of AKI (141).

5.3 Angiogenesis inhibition therapy

An important feature of cirrhotic portal hypertension is abnormal angiogenesis in the visceral vascular system, especially in the spleen and intestinal areas. These abnormal neovascularization structures are unstable and permeable, leading to increased portal vein pressure. Therefore, targeted treatment strategies to inhibit abnormal angiogenesis have gradually become the focus of research. Cediranib may ameliorate extrahepatic hyperdynamic circulation by targeting angiogenesis. This is achieved through the inhibition of vascular endothelial growth factor receptor 2 (VEGFR-2) signaling, thereby reducing both portal collateral vessel formation and eNOS-mediated vasodilation and vascular remodeling (57). Hydroxysafflor yellow A is a multi-target tyrosine kinase inhibitor that inhibits the VEGF and PDGF signaling pathways, thereby inhibiting abnormal angiogenesis (142). Although anti-angiogenic therapy has great potential in theory, its clinical application is still being explored. These drugs may cause systemic side effects such as hypertension, bleeding, and delayed wound healing, so they should still be used with caution.

6 Future prospective

Future research will focus on further elucidating the intricate intrahepatic and extrahepatic vascular regulatory mechanisms underlying portal hypertension in cirrhosis.

- Nanotechnology for Targeted Therapies

Emerging nanotechnology offers new possibilities for the treatment of portal hypertension. Utilizing nanocarriers enables precise drug delivery to specific cells or tissues, such as HSCs or LSECs. This not only enhances drug concentration at the site of action but also minimizes off-target effects on healthy tissues. This strategy holds significant promise for improving both the efficacy and safety of therapeutic interventions. However, nanotechnology approaches are currently in preclinical development and require additional safety data.

- The Promise of Stem Cell Therapy

Stem cell therapy, a burgeoning therapeutic modality, has demonstrated potential in early clinical trials for reducing liver fibrosis. MSCs, through their immunomodulatory and anti-inflammatory properties, can suppress HSC activation and reduce ECM production, thereby slowing the progression of fibrosis. Future research will further investigate the long-term efficacy of stem cell therapy in individuals with cirrhosis and explore strategies to enhance stem cell functionality through gene editing techniques. Stem cell therapies, while showing promise in animal studies, are still in early development phases. MSCs has progressed to Phase I trials, focusing primarily on safety assessments in cirrhotic patients.

- Optimization of Existing Drugs and Development of Novel Agents

Future research will focus on optimizing the efficacy and safety of existing drugs. For example, while statins have shown promise in improving sinusoidal microcirculation and reducing portal pressure, their long-term safety requires further validation. The development of novel agents targeting pathological mechanisms like angiogenesis and

vasodilation will also be a priority. Although anti-angiogenic drugs, such as VEGF inhibitors, are effective, they can cause systemic side effects like hypertension. Therefore, research will focus on improving drug targeting and minimizing adverse reactions. The safety and preliminary efficacy of novel NO donors are in the early clinical development stage. New small molecules targeting specific pathways require toxicology studies. VEGF inhibitors, obeticholic acid, and rifaximin combined with statins have entered the clinical research stage.

7 Conclusion

Cirrhotic portal hypertension involves complex intrahepatic and extrahepatic vascular mechanisms. Comprehensive treatments such as improving microcirculation and regulating vascular tension can effectively reduce portal pressure, alleviate complications, and improve patient prognosis. More research is needed in the future to validate drugs targeting intrahepatic and extrahepatic vascular disorders in order to improve treatment of portal hypertension.

Author contributions

YaL: Writing – original draft, Writing – review & editing. BZ: Writing – review & editing. KS: Writing – review & editing. YuL: Conceptualization, Writing – review & editing. XZ: Visualization, Writing – review & editing. YoL: Visualization, Writing – review & editing. QZ: Formal analysis, Writing – review & editing. YF: Supervision, Writing – review & editing. XW: Supervision, Writing – review & editing.

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Conflict of interest

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

Generative AI statement

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

Publisher's note

References

1. Dong Z, Wang Y, Jin W. Liver cirrhosis: molecular mechanisms and therapeutic interventions. *MedComm (2020)*. (2024) 5:e721. doi: 10.1002/mco2.721

2. Guixé-Muntet S, Quesada-Vázquez S, Gracia-Sancho J. Pathophysiology and therapeutic options for cirrhotic portal hypertension. *Lancet Gastroenterol Hepatol*. (2024) 9:646–63. doi: 10.1016/S2468-1253(23)00438-7

3. Akkız H, Gieseler RK, Canbay A. Liver fibrosis: from basic science towards clinical Progress, focusing on the central role of hepatic stellate cells. *Int J Mol Sci*. (2024) 25:7873. doi: 10.3390/ijms25147873

4. Gao J, Lan T, Kostallari E, Guo Y, Lai E, Guillot A, et al. Angiocrine signaling in sinusoidal homeostasis and liver diseases. *J Hepatol*. (2024) 81:543–61. doi: 10.1016/j.jhep.2024.05.014

5. Qu J, Wang L, Li Y, Li X. Liver sinusoidal endothelial cell: an important yet often overlooked player in the liver fibrosis. *Clin Mol Hepatol*. (2024) 30:303–25. doi: 10.3350/cmh.2024.0022

6. Hon WM, Lee KH, Khoo HE. Nitric oxide in liver diseases: friend, foe, or just passerby? *Ann N Y Acad Sci*. (2002) 962:275–95. doi: 10.1111/j.1749-6632.2002.tb04074.x

7. Mauro E, Gadano A. What's new in portal hypertension? *Liver Int*. (2020) 40:122–7. doi: 10.1111/liv.14366

8. Poisson J, Lemoinne S, Boulanger C, Durand F, Moreau R, Valla D, et al. Liver sinusoidal endothelial cells: physiology and role in liver diseases. *J Hepatol*. (2017) 66:212–27. doi: 10.1016/j.jhep.2016.07.009

9. Lehmann J, Praktiknjo M, Nielsen MJ, Schierwagen R, Meyer C, Thomas D, et al. Collagen type IV remodelling gender-specifically predicts mortality in decompensated cirrhosis. *Liver Int*. (2019) 39:885–93. doi: 10.1111/liv.14070

10. Huang R, Deng J, Zhu C-P, Liu SQ, Cui YL, Chen F, et al. Sulodexide attenuates liver fibrosis in mice by restoration of differentiated liver sinusoidal endothelial cell. *Biomed Pharmacother*. (2023) 160:114396. doi: 10.1016/j.biopha.2023.114396

11. DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. *Hepatology*. (2015) 61:1740–6. doi: 10.1002/hep.27376

12. Tung H-C, Kim J-W, Zhu J, Li S, Yan J, Liu Q, et al. Inhibition of heme-thiolate monooxygenase CYP1B1 prevents hepatic stellate cell activation and liver fibrosis by accumulating trehalose. *Sci Transl Med*. (2024) 16:eadk8446. doi: 10.1126/scitranslmed.adk8446

13. Ten Hove M, Smyris A, Booijink R, Wachsmuth L, Hansen U, Alic L, et al. Engineered SPIONs functionalized with endothelin a receptor antagonist ameliorate liver fibrosis by inhibiting hepatic stellate cell activation. *Bioact Mater*. (2024) 39:406–26. doi: 10.1016/j.bioactmat.2024.05.034

14. Ye L, Huang J, Liang X, Guo W, Sun X, Shao C, et al. Jiawei Taohe Chengqi decoction attenuates CCl4 induced hepatic fibrosis by inhibiting HSCs activation via TGF-β1/CUGBP1 and IFN-γ/Smad 7 pathway. *Phytomedicine*. (2024) 133:155916. doi: 10.1016/j.phymed.2024.155916

15. Guillot A, Tacke F. Liver macrophages revisited: the expanding universe of versatile responses in a spatiotemporal context. *Hepatol Commun*. (2024) 8:e0491. doi: 10.1097/HC9.0000000000000491

16. Wang Z, Du K, Jin N, Tang B, Zhang W. Macrophage in liver fibrosis: identities and mechanisms. *Int Immunopharmacol*. (2023) 120:110357. doi: 10.1016/j.intimp.2023.110357

17. Sauer J, Steixner-Kumar AA, Gabler S, Motyka M, Rippmann JF, Brosa S, et al. Diverse potential of secretome from natural killer cells and monocyte-derived macrophages in activating stellate cells. *Front Immunol*. (2024) 15:1232070. doi: 10.3389/fimmu.2024.1232070

18. Kumar S, Duan Q, Wu R, Harris EN, Su Q. Pathophysiological communication between hepatocytes and non-parenchymal cells in liver injury from NAFLD to liver fibrosis. *Adv Drug Deliv Rev*. (2021) 176:113869. doi: 10.1016/j.addr.2021.113869

19. Pu Y, Zhang S, Zhou R, Huang N, Li H, Wei W, et al. IL-17A up-regulates expression of endothelial tissue factor in liver cirrhosis via the ROS/p 38 signal pathway. *Biochem Biophys Res Commun*. (2016) 470:41–7. doi: 10.1016/j.bbrc.2015.12.093

20. Perramón M, Navalón-López M, Fernández-Varo G, Moreno-Lanceta A, García-Pérez R, Faneca J, et al. Liver-targeted nanoparticles delivering nitric oxide reduce portal hypertension in cirrhotic rats. *Biomed Pharmacother*. (2024) 171:116143. doi: 10.1016/j.biopha.2024.116143

21. Jung E, Baek EB, Hong E-J, Kang JH, Park S, Park S, et al. TXNIP in liver sinusoidal endothelial cells ameliorates alcohol-associated liver disease via nitric oxide production. *Int J Biol Sci*. (2024) 20:606–20. doi: 10.7150/ijbs.90781

22. Vukotic R, Di Donato R, Roncarati G, Simoni P, Renzulli M, Gitto S, et al. 5-MTHF enhances the portal pressure reduction achieved with propranolol in patients with cirrhosis: A randomized placebo-controlled trial. *J Hepatol*. (2023) 79:977–88. doi: 10.1016/j.jhep.2023.06.017

23. Albornoz L, Bandi JC, Otaso JC, Laudanno O, Mastai R. Prolonged bleeding time in experimental cirrhosis: role of nitric oxide. *J Hepatol*. (1999) 30:456–60. doi: 10.1016/S0168-8278(99)80105-6

24. Ding J, Zhao F, Cui J, Zhu M, Zhao W. Platelet activation is upregulated in cirrhotic patients with portal vein thrombosis. *Thromb Haemost*. (2023) 124:739–52. doi: 10.1055/a-2190-8705

25. Owen NE, Williams TL, Maguire JJ, Kuc RE, Davenport EE, Davenport AP. Microarray analysis demonstrates up-regulation of the endothelin-1 gene with compensatory down-regulation of the ETA receptor gene in human portal vein. *Biosci Rep*. (2024) 44:BSR20240528. doi: 10.1042/BSR20240528

26. Owen T, Carpino G, Chen L, Kundu D, Wills P, Ekser B, et al. Endothelin receptor-A inhibition decreases Ductular reaction, liver fibrosis, and angiogenesis in a model of cholangitis. *Cell Mol Gastroenterol Hepatol*. (2023) 16:513–40. doi: 10.1016/j.jcmgh.2023.06.005

27. Ortiz C, Klein S, Reul WH, Magdaleno F, Gröschl S, Dietrich P, et al. Neprilysin-dependent neuropeptide Y cleavage in the liver promotes fibrosis by blocking NPY-receptor 1. *Cell Rep*. (2023) 42:112059. doi: 10.1016/j.celrep.2023.112059

28. Wu Y, Li Z, Wang S, Xiu A, Zhang C. Carvedilol inhibits angiotensin II-induced proliferation and contraction in hepatic stellate cells through the rho A/rho-kinase pathway. *Biomed Res Int*. (2019) 2019:7932046. doi: 10.1155/2019/7932046

29. van Dijk F, Teekamp N, Post E, Schuppan D, Kim YO, Zuidema J, et al. The antifibrotic potential of a sustained release formulation of a PDGFβ-receptor targeted rho kinase inhibitor. *J Control Release*. (2019) 296:250–7. doi: 10.1016/j.jconrel.2018.12.039

30. Li Z, Ding Q, Ling L-P, Wu Y, Meng DX, Li X, et al. Metformin attenuates motility, contraction, and fibrogenic response of hepatic stellate cells in vivo and in vitro by activating AMP-activated protein kinase. *World J Gastroenterol*. (2018) 24:819–32. doi: 10.3748/wjg.v24.i7.819

31. Wang F, Jia Y, Li M, Wang L, Shao J, Guo Q, et al. Blockade of glycolysis-dependent contraction by oroxylin a via inhibition of lactate dehydrogenase-a in hepatic stellate cells. *Cell Commun Signal*. (2019) 17:11. doi: 10.1186/s12964-019-0324-8

32. Duong HTT, Dong Z, Su L, Boyer C, George J, Davis TP, et al. The use of nanoparticles to deliver nitric oxide to hepatic stellate cells for treating liver fibrosis and portal hypertension. *Small*. (2015) 11:2291–304. doi: 10.1002/smll.201402870

33. Tièche S, De Gottardi A, Kappeler A, Shaw S, Sägesser H, Zimmermann A, et al. Overexpression of endothelin-1 in bile duct ligated rats: correlation with activation of hepatic stellate cells and portal pressure. *J Hepatol*. (2001) 34:38–45. doi: 10.1016/S0168-8278(00)00031-3

34. Abdel-Razik A, Mousa N, Abdelsalam M, Abdelwahab A, Tawfik M, Tawfik AM, et al. Endothelin-1/nitric oxide ratio as a predictive factor of response to therapy with Terlipressin and albumin in patients with Type-1 Hepatorenal syndrome. *Front Pharmacol*. (2020) 11:9. doi: 10.3389/fphar.2020.00009

35. Tung H-C, Hsu S-J, Tsai M-H, Lin TY, Hsin IF, Huo TI, et al. Homocysteine deteriorates intrahepatic derangement and portal-systemic collaterals in cirrhotic rats. *Clin Sci (Lond)*. (2017) 131:69–86. doi: 10.1042/CS20160470

36. Sacerdoti D, Pesce P, Di Pascoli M, Brocco S, Cecchetto L, Bolognesi M. Arachidonic acid metabolites and endothelial dysfunction of portal hypertension. *Prostaglandins Other Lipid Mediat*. (2015) 120:80–90. doi: 10.1016/j.prostaglandins.2015.05.008

37. Skill NJ, Theodorakis NG, Wang YN, Wu JM, Redmond EM, Sitzmann JV. Role of cyclooxygenase isoforms in prostacyclin biosynthesis and murine prehepatic portal hypertension. *Am J Physiol Gastrointest Liver Physiol*. (2008) 295:G953–64. doi: 10.1152/ajpgi.00013.2008

38. Rosado E, Rodríguez-Vilarrupla A, Gracia-Sancho J, Monclús M, Bosch J, García-Pagán JC. Interaction between NO and COX pathways modulating hepatic endothelial cells from control and cirrhotic rats. *J Cell Mol Med*. (2012) 16:2461–70. doi: 10.1111/j.1582-4934.2012.01563.x

39. Zhao Z, Zhang C, Lin J, Zheng L, Li H, Qi X, et al. COX-2/sEH dual inhibitor PTUPB alleviates CCl 4-induced liver fibrosis and portal hypertension. *Front Med (Lausanne)*. (2021) 8:761517. doi: 10.3389/fmed.2021.761517

40. Mastoor Z, Diz-Chaves Y, González-Matías LC, Mallo F. Renin-angiotensin system in liver metabolism: gender differences and role of incretins. *Meta*. (2022) 12:411. doi: 10.3390/metabo12050411

41. Grace JA, Herath CB, Mak KY, Burrell LM, Angus PW. Update on new aspects of the renin-angiotensin system in liver disease: clinical implications and new therapeutic options. *Clin Sci (Lond)*. (2012) 123:225–39. doi: 10.1042/CS20120030

42. Yu FJ, Dong PH, Fan XF, Lin Z, Chen YP, Li J. Down-regulation of angiotensin II by sh RNA reduces collagen synthesis in hepatic stellate cells. *Int J Mol Med*. (2010) 25:801–6. doi: 10.3892/ijmm_00000407

43. Abd El-Rahman SS, Fayed HM. Targeting Ang II/AT1R signaling pathway by perindopril inhibits ongoing liver fibrosis in rat. *J Tissue Eng Regen Med*. (2019) 13:2131–41. doi: 10.1002/term.2940

44. Araujo T, Vohra I, Palacios P, Katiyar V, Flores E, Randhawa T, et al. B-type natriuretic peptide (BNP) predicts 90-day mortality and need for paracentesis in cirrhotic patients without systolic heart failure. *Sci Rep*. (2021) 11:1697. doi: 10.1038/s41598-020-78946-3

45. Fábrega E, Casafont F, Crespo J, De la Peña J, San Miguel G, De las Heras G, et al. Plasma adrenomedullin levels in patients with hepatic cirrhosis. *Am J Gastroenterol*. (1997) 92:1901–4.

46. Elnagdy M, Barve S, McClain C, Gobejishvili L. cAMP signaling in pathobiology of alcohol associated liver disease. *Biomol Ther*. (2020) 10:1433. doi: 10.3390/biom10101433

47. Holditch SJ, Schreiber CA, Harris PC, LaRusso NF, Ramirez-Alvarado M, Cataliotti A, et al. B-type natriuretic peptide overexpression ameliorates hepatorenal fibrocystic disease in a rat model of polycystic kidney disease. *Kidney Int*. (2017) 92:657–68. doi: 10.1016/j.kint.2017.02.017

48. Pun CK, Huang H-C, Chang C-C, Hsu SJ, Chuang CL, Huang YH, et al. Low-dose alcohol exacerbates hyperdynamic circulation and shunting in non-alcoholic cirrhotic rats. *Biosci Rep*. (2024) 44:BSR20240354. doi: 10.1042/BSR20240354

49. Kotani K, Kawada N. Recent advances in the pathogenesis and clinical evaluation of portal hypertension in chronic liver disease. *Gut Liver*. (2024) 18:27–39. doi: 10.5009/gnl230072

50. Simonetto DA, Singal AK, Garcia-Tsao G, Caldwell SH, Ahn J, Kamath PS. ACG clinical guideline: disorders of the hepatic and mesenteric circulation. *Am J Gastroenterol*. (2020) 115:18–40. doi: 10.14309/ajg.0000000000000486

51. Téllez L, Albillos A. Non-selective beta-blockers in patients with ascites: the complex interplay among the liver, kidney and heart. *Liver Int*. (2022) 42:749–61. doi: 10.1111/liv.15166

52. McGrath MS, Wentworth BJ. The renin-angiotensin system in liver disease. *Int J Mol Sci*. (2024) 25:5807. doi: 10.3390/ijms25115807

53. Juncu S, Minea H, Girleanu I, Huiban L, Muzica C, Chiriac S, et al. Clinical implications and Management of Spontaneous Portosystemic Shunts in liver cirrhosis. *Diagnostics (Basel)*. (2024) 14:1372. doi: 10.3390/diagnostics14131372

54. Simón-Talero M, Roccarina D, Martínez J, Lampichler K, Baiges A, Low G, et al. Association between portosystemic shunts and increased complications and mortality in patients with cirrhosis. *Gastroenterology*. (2018) 154:1694–1705.e4. doi: 10.1053/j.gastro.2018.01.028

55. Mariotti V, Fiorotto R, Cadamuro M, Fabris L, Strazzabosco M. New insights on the role of vascular endothelial growth factor in biliary pathophysiology. *JHEP Rep*. (2021) 3:100251. doi: 10.1016/j.jhepr.2021.100251

56. Gana JC, Serrano CA, Ling SC. Angiogenesis and portal-systemic collaterals in portal hypertension. *Ann Hepatol*. (2016) 15:303–13. doi: 10.5604/16652681.1198799

57. Fan Q, Wu G, Chen M, Luo G, Wu Z, Huo H, et al. Cediranib ameliorates portal hypertensive syndrome via inhibition of VEGFR-2 signaling in cirrhotic rats. *Eur J Pharmacol*. (2024) 964:176278. doi: 10.1016/j.ejphar.2023.176278

58. Yang X, Wang Z, Kai J, Wang F, Jia Y, Wang S, et al. Curcumol attenuates liver sinusoidal endothelial cell angiogenesis via regulating Glis-PROX1-HIF-1α in liver fibrosis. *Cell Prolif*. (2020) 53:e12762. doi: 10.1111/cpr.12762

59. Wang Q, Zhang F, Lei Y, Liu P, Liu C, Tao Y. micro RNA-322/424 promotes liver fibrosis by regulating angiogenesis through targeting CUL2/HIF-1α pathway. *Life Sci*. (2021) 266:118819. doi: 10.1016/j.lfs.2020.118819

60. Maruyama H, Shiina S. Collaterals in portal hypertension: anatomy and clinical relevance. *Quant Imaging Med Surg*. (2021) 11:3867–81. doi: 10.21037/qims-20-1328

61. Zhao Z, Fan Q, Zhang C, Zheng L, Lin J, Chen M, et al. Imperatorin attenuates CCl4-induced cirrhosis and portal hypertension by improving vascular remodeling and

profibrogenic pathways. *Eur J Pharmacol*. (2024) 980:176833. doi: 10.1016/j.ejphar.2024.176833

62. Turco L, Reiberger T, Vitale G, la Mura V. Carvedilol as the new non-selective beta-blocker of choice in patients with cirrhosis and portal hypertension. *Liver Int*. (2023) 43:1183–94. doi: 10.1111/liv.15559

63. Lluch P, Segarra G, Medina P. Asymmetric dimethylarginine as a mediator of vascular dysfunction in cirrhosis. *World J Gastroenterol*. (2015) 21:9466–75. doi: 10.3748/wjg.v21.i32.9466

64. Bech JN, Aagaard NK, Pedersen RS, Sorensen TB, Vilstrup H, Pedersen EB. Renal effects of NO-inhibition in patients with cirrhosis vs. healthy controls: a randomized placebo-controlled crossover study. *Liver Int*. (2014) 34:211–9. doi: 10.1111/liv.12233

65. Serna E, Mauricio MD, Lluch P, Segarra G, Cortina B, Lluch S, et al. Basal release of nitric oxide in the mesenteric artery in portal hypertension and cirrhosis: role of dimethylarginine dimethylaminohydrolase. *J Gastroenterol Hepatol*. (2013) 28:880–6. doi: 10.1111/jgh.12119

66. Reiberger T, Payer BA, Schwabl P, Hayden H, Horvatits T, Jäger B, et al. Nebivolol treatment increases splanchnic blood flow and portal pressure in cirrhotic rats via modulation of nitric oxide signalling. *Liver Int*. (2013) 33:561–8. doi: 10.1111/liv.12101

67. Zhu C-P, Liu S-Q, Wang K-Q, Xiong HL, Aristu-Zabalza P, Boyer-Díaz Z, et al. Targeting 5-Hydroxytryptamine receptor 1A in the portal vein to decrease portal hypertension. *Gastroenterology*. (2024) 167:993–1007. doi: 10.1053/j.gastro.2024.06.007

68. Kondo R, Furukawa N, Deguchi A, Kawata N, Suzuki Y, Imaizumi Y, et al. Downregulation of Ca2+-activated cl-Channel TMEM16A mediated by angiotensin II in cirrhotic portal hypertensive mice. *Front Pharmacol*. (2022) 13:831311. doi: 10.3389/fphar.2022.831311

69. Miceli G, Calvaruso V, Casuccio A, Pennisi G, Licata M, Pintus C, et al. Heart rate variability is associated with disease severity and portal hypertension in cirrhosis. *Hepatol Commun*. (2023) 7:e0050. doi: 10.1097/HC9.0000000000000050

70. Ripoll C, Ibáñez-Samaniego L, Neumann B, Vaquero J, Greinert R, Bañares R, et al. Evaluation of the definition of hyperdynamic circulation in patients with cirrhosis and ascites. *Hepatol Commun*. (2022) 6:3528–38. doi: 10.1002/hep4.2102

71. Caracuel L, Sastre E, Llévenes P, Prieto I, Funes T, Aller MÁ, et al. Acute-on-chronic liver disease enhances phenylephrine-induced endothelial nitric oxide release in rat mesenteric resistance arteries through enhanced PKA, PI3K/AKT and cGMP signalling pathways. *Sci Rep*. (2019) 9:6993. doi: 10.1038/s41598-019-43513-y

72. Møller S, Nørgaard A, Henriksen JH, Frandsen E, Bendtsen F. Effects of tilting on central hemodynamics and homeostatic mechanisms in cirrhosis. *Hepatology*. (2004) 40:811–9. doi: 10.1002/hep.1840400410

73. Gao Y, Liu X, Gao Y, Duan M, Hou B, Chen Y. Pharmacological interventions for cirrhotic ascites: from challenges to emerging therapeutic horizons. *Gut Liver*. (2024) 18:934–48. doi: 10.5009/gnl240038

74. Tandon P, Abraldes JG, Berzigotti A, Garcia-Pagan JC, Bosch J. Renin-angiotensin-aldosterone inhibitors in the reduction of portal pressure: a systematic review and meta-analysis. *J Hepatol*. (2010) 53:273–82. doi: 10.1016/j.jhep.2010.03.013

75. Gananandan K, Wiese S, Møller S, Mookerjee RP. Cardiac dysfunction in patients with cirrhosis and acute decompensation. *Liver Int*. (2024) 44:1832–41. doi: 10.1111/liv.15896

76. Liu H, Naser JA, Lin G, Lee SS. Cardiomyopathy in cirrhosis: from pathophysiology to clinical care. *JHEP Rep*. (2024) 6:100911. doi: 10.1016/j.jhepr.2023.100911

77. Maksan S-M, Ryschich E, Ulger Z, Gebhard MM, Schmidt J. Disturbance of hepatic and intestinal microcirculation in experimental liver cirrhosis. *World J Gastroenterol*. (2005) 11:846–9. doi: 10.3748/wjg.v11.i6.846

78. Rochoń J, Kalinowski P, Szymanek-Majchrzak K, Grąt M. Role of gut-liver axis and glucagon-like peptide-1 receptor agonists in the treatment of metabolic dysfunction-associated fatty liver disease. *World J Gastroenterol*. (2024) 30:2964–80. doi: 10.3748/wjg.v30.i23.2964

79. Rusticeanu MA, Zimmer V. Alterations in intestinal mucosal barrier visualized by confocal laser Endomicroscopy in liver cirrhosis: A pilot trial (AMBIC). *Diagnostics (Basel)*. (2024) 14:1606. doi: 10.3390/diagnostics14151606

80. Zhu X, Zhou Z, Pan X. Research reviews and prospects of gut microbiota in liver cirrhosis: a bibliometric analysis (2001-2023). *Front Microbiol*. (2024) 15:1342356. doi: 10.3389/fmicb.2024.1342356

81. Gioia S, Carnevale R, Tavano D, Overi D, Ridola L, Nardelli S, et al. Association between gut-derived endotoxins and Porto-sinusoidal vascular disorder with portal hypertension. *Aliment Pharmacol Ther*. (2023) 58:1205–16. doi: 10.1111/apt.17727

82. Nagasue N, Dhar DK, Yamanoi A, Emi Y, Udagawa J, Yamamoto A, et al. Production and release of endothelin-1 from the gut and spleen in portal hypertension due to cirrhosis. *Hepatology*. (2000) 31:1107–14. doi: 10.1053/he.2000.6596

83. Du Plessis J, Vanheel H, Janssen CEI, Roos L, Slavik T, Stivaktas PI, et al. Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol*. (2013) 58:1125–32. doi: 10.1016/j.jhep.2013.01.038

84. Pijls KE, Jonkers DMAE, Elizalde M, Drittij-Reijnders MJ, Haenen GR, Bast A, et al. Is intestinal oxidative stress involved in patients with compensated liver cirrhosis? *Ann Hepatol*. (2016) 15:402–9. doi: 10.5604/16652681.1198816

85. Zhang J, Luo B, Tang L, Wang Y, Stockard CR, Kadish I, et al. Pulmonary angiogenesis in a rat model of hepatopulmonary syndrome. *Gastroenterology*. (2009) 136:1070–80. doi: 10.1053/j.gastro.2008.12.001

86. Rodríguez-Roisin R. Hepatopulmonary syndrome: A forgotten liver-induced lung vascular disorder. *Arch Bronconeumol*. (2023) 59:137–8. doi: 10.1016/j.arbres.2022.12.007

87. Chin A, Bastaich DR, Dahman B, Kaplan DE, Taddei TH, John BV. Refractory hepatic hydrothorax is associated with increased mortality with death occurring at lower MELD-Na compared to cirrhosis and refractory ascites. *Hepatology*. (2024) 79:844–56. doi: 10.1097/HEP.0000000000000577

88. Dandavate V, Bolshette N, Van Drunen R, Manella G, Bueno-Levy H, Zerbib M, et al. Hepatic BMAL1 and HIF1α regulate a time-dependent hypoxic response and prevent hepatopulmonary-like syndrome. *Cell Metab*. (2024) 36:2038–2053.e5. doi: 10.1016/j.cmet.2024.07.003

89. Douschan P, Kovacs G, Sassmann T, Stadlbauer V, Avian A, Foris V, et al. Pulmonary vascular disease and exercise hemodynamics in chronic liver disease. *Respir Med*. (2022) 202:106987. doi: 10.1016/j.rmed.2022.106987

90. Zhu J, Qiu J, Chen K, Wang W, Zheng S. Tea polyphenols and levofloxacin alleviate the lung injury of hepatopulmonary syndrome in common bile duct ligation rats through endotoxin-TNF signaling. *Biomed Pharmacother*. (2021) 137:111263. doi: 10.1016/j.biopha.2021.111263

91. Raevens S, Boret M, Fallon MB. Hepatopulmonary syndrome. *JHEP Rep*. (2022) 4:100527. doi: 10.1016/j.jhepr.2022.100527

92. Nascimento ESP, Nunes WMC, Guerra EM, da Roza MR, Silva-Costa S, Machado-Silva W, et al. Combined exercise training improved exercise capacity and lung inflammation in rats with hepatopulmonary syndrome. *Life Sci*. (2021) 287:120112. doi: 10.1016/j.lfs.2021.120112

93. Mangoura SA, Ahmed MA, Hamad N, Zaka AZ, Khalaf KA. Hepatoprotective effects of vildagliptin mitigates lung biochemical and histopathological changes in experimental hepatopulmonary syndrome model in rat. *Int Immunopharmacol*. (2024) 143:113254. doi: 10.1016/j.intimp.2024.113254

94. Li Y-J, Wu X-F, Wang D-D, Li P, Liang H, Hu XY, et al. Serum soluble vascular endothelial growth factor receptor 1 as a potential biomarker of Hepatopulmonary syndrome. *J Clin Transl Hepatol*. (2023) 000–1160. doi: 10.14218/JCTH.2022.00421

95. Horvatits T, Drolz A, Roedl K, Herkner H, Ferlitsch A, Perkmann T, et al. Von Willebrand factor antigen for detection of hepatopulmonary syndrome in patients with cirrhosis. *J Hepatol*. (2014) 61:544–9. doi: 10.1016/j.jhep.2014.04.025

96. Zhang Z-J, Yang C-Q. Progress in investigating the pathogenesis of hepatopulmonary syndrome. *Hepatobiliary Pancreat Dis Int*. (2010) 9:355–60.

97. Krowka MJ, DuBrock HM, Raevens S, Fallon MB. Unmet needs in diagnosis and management of portopulmonary hypertension: how far have we reached? *Hepatology*. (2024). doi: 10.1097/HEP.0000000000001218

98. Khoramzadeh M, Dehghanian A, Ketabchi F. Roles of endothelin B receptors and endothelial nitric oxide synthase in the regulation of pulmonary hemodynamic in cirrhotic rats. *J Cardiovasc Pharmacol*. (2019) 73:178–85. doi: 10.1097/FJC.0000000000000650

99. Nikolic I, Yung L-M, Yang P, Malhotra R, Paskin-Flerlage SD, Dinter T, et al. Bone morphogenetic protein 9 is a mechanistic biomarker of Portopulmonary hypertension. *Am J Respir Crit Care Med*. (2019) 199:891–902. doi: 10.1164/rccm.201807-1236OC

100. Luangmonkong T, Parichatikanond W, Olinga P. Targeting collagen homeostasis for the treatment of liver fibrosis: opportunities and challenges. *Biochem Pharmacol*. (2023) 215:115740. doi: 10.1016/j.bcp.2023.115740

101. Geervliet E, Karkdijk E, Bansal R. Inhibition of intrahepatic monocyte recruitment by Cenicriviroc and extracellular matrix degradation by MMP1 synergistically attenuate liver inflammation and fibrogenesis in vivo. *Sci Rep*. (2024) 14:16897. doi: 10.1038/s41598-024-67926-6

102. Shan L, Wang F, Zhai D, Meng X, Liu J, Lv X. Matrix metalloproteinases induce extracellular matrix degradation through various pathways to alleviate hepatic fibrosis. *Biomed Pharmacother*. (2023) 161:114472. doi: 10.1016/j.biopha.2023.114472

103. Xie H, Su D, Zhang J, Ji D, Mao J, Hao M, et al. Raw and vinegar processed Curcuma wenyujin regulates hepatic fibrosis via bloking TGF-β/Smad signaling pathways and up-regulation of MMP-2/TIMP-1 ratio. *J Ethnopharmacol*. (2020) 246:111768. doi: 10.1016/j.jep.2019.01.045

104. Robert S, Gicquel T, Bodin A, Fautrel A, Barreto E, Victoni T, et al. Influence of inflammasome pathway activation in macrophages on the matrix metalloproteinase expression of human hepatic stellate cells. *Int Immunopharmacol*. (2019) 72:12–20. doi: 10.1016/j.intimp.2019.03.060

105. Ren J-J, Huang T-J, Zhang Q-Q, Zhang HY, Guo XH, Fan HQ, et al. Insulin-like growth factor binding protein related protein 1 knockdown attenuates hepatic fibrosis via the regulation of MMPs/TIMPs in mice. *Hepatobiliary Pancreat Dis Int*. (2019) 18:38–47. doi: 10.1016/j.hbpd.2018.08.008

106. Panera N, Braghini MR, Crudele A, Smeriglio A, Bianchi M, Condorelli AG, et al. Combination treatment with Hydroxytyrosol and vitamin E improves NAFLD-related fibrosis. *Nutrients*. (2022) 14:3791. doi: 10.3390/nu14183791

107. Yaqub F, Latief N, Butt H, Naseer N, Riazuddin S. Alpha lipoic acid priming enhances the hepatoprotective effect of adipose derived stem cells in CCl4 induced

hepatic injury in-vitro. *Eur J Pharmacol*. (2021) 906:174201. doi: 10.1016/j.ejphar.2021.174201

108. Wang Q, Lu T, Song P, Dong Y, Dai C, Zhang W, et al. Glycyrrhizic acid ameliorates hepatic fibrosis by inhibiting oxidative stress via AKR7A2. *Phytomedicine*. (2024) 133:155878. doi: 10.1016/j.phymed.2024.155878

109. Qin B-F, Zhang J-J, Feng Q-Y, Guo X, Sun HM, Song J. Specnuezhenide ameliorates hepatic fibrosis via regulating SIRT6-mediated inflammatory signaling cascades. *J Ethnopharmacol*. (2024) 335:118646. doi: 10.1016/j.jep.2024.118646

110. Benedicto AM, Lucantoni F, Fuster-Martínez I, Diaz-Pozo P, Dorcaratto D, Muñoz-Forner E, et al. Interference with mitochondrial function as part of the antifibrogenic effect of Rilpivirine: A step towards novel targets in hepatic stellate cell activation. *Biomed Pharmacother*. (2024) 178:117206. doi: 10.1016/j.biopha.2024.117206

111. Lv M, Chen S, Shan M, Si Y, Huang C, Chen J, et al. Arctigenin induces activated HSCs quiescence via AMPK-PPARγ pathway to ameliorate liver fibrosis in mice. *Eur J Pharmacol*. (2024) 974:176629. doi: 10.1016/j.ejphar.2024.176629

112. Wang P, Kang Q, Wu W-S, Rui L. Hepatic Snai 1 and Snai 2 promote liver regeneration and suppress liver fibrosis in mice. *Cell Rep*. (2024) 43:113875. doi: 10.1016/j.celrep.2024.113875

113. Yao L, Hu X, Yuan M, Liu P, Zhang Q, Wang Z, et al. Human umbilical cord-derived mesenchymal stromal cells alleviate liver cirrhosis through the hippo/YAP/Id1 pathway and macrophage-dependent mechanism. *Int Immunopharmacol*. (2023) 123:110456. doi: 10.1016/j.intimp.2023.110456

114. Yadav P, Singh SK, Rajput S, Allawadhi P, Khurana A, Weiskirchen R, et al. Therapeutic potential of stem cells in regeneration of liver in chronic liver diseases: current perspectives and future challenges. *Pharmacol Ther*. (2024) 253:108563. doi: 10.1016/j.pharmthera.2023.108563

115. Tapper EB, Zhao Z, Henderson J. Statins for the prevention of cirrhosis complications: an American emulation of the stat liver trial. *Hepatol Commun*. (2024) 8:e0530. doi: 10.1097/HC9.0000000000000530

116. Gil M, Khouri L, Raurell I, Rafael D, Andrade F, Abasolo I, et al. Optimization of statin-loaded delivery nanoparticles for treating chronic liver diseases by targeting liver sinusoidal endothelial cells. *Pharmaceutics*. (2023) 15:2463. doi: 10.3390/pharmaceutics15102463

117. Kezer CA, Schmidt KA, Shah VH. Statin the course: navigating unchartered territory in cirrhosis. *Hepatol Commun*. (2024) 8:e0456. doi: 10.1097/HC9.0000000000000456

118. Abdelhamid AM, Saber S, Hamad RS, Abdel-Reheim MA, Ellethy AT, Amer MM, et al. STA-9090 in combination with a statin exerts enhanced protective effects in rats fed a high-fat diet and exposed to diethylnitrosamine and thioacetamide. *Front Pharmacol*. (2024) 15:1454829. doi: 10.3389/fphar.2024.1454829

119. Trebicka J, Schierwagen R. Statins, rho GTPases and KLF2: new mechanistic insight into liver fibrosis and portal hypertension. *Gut*. (2015) 64:1349–50. doi: 10.1136/gutjnl-2014-308800

120. Pose E, Solà E, Lozano JJ, Juanola A, Sidorova J, Zaccherini G, et al. Treatment with simvastatin and Rifaximin restores the plasma Metabolomic profile in patients with decompensated cirrhosis. *Hepatol Commun*. (2022) 6:1100–12. doi: 10.1002/hep4.1881

121. Kronborg TM, Schierwagen R, Trošt K, Gao Q, Moritz T, Bendtsen F, et al. Atorvastatin for patients with cirrhosis. A randomized, placebo-controlled trial. *Hepatol Commun*. (2023) 7:e0332. doi: 10.1097/HC9.0000000000000332

122. Muñoz AE, Pollarsky FD, Marino M, Cartier M, Vázquez H, Salgado P, et al. Addition of statins to the standard treatment in patients with cirrhosis: safety and efficacy. *World J Gastroenterol*. (2021) 27:4639–52. doi: 10.3748/wjg.v27.i28.4639

123. Villanueva C, Aracil C, Colomo A, Lopez-Balaguer JM, Piqueras M, Gonzalez B, et al. Clinical trial: a randomized controlled study on prevention of variceal rebleeding comparing nadolol + ligation vs. hepatic venous pressure gradient-guided pharmacological therapy. *Aliment Pharmacol Ther*. (2009) 29:397–408. doi: 10.1111/j.1365-2036.2008.03880.x

124. Berzigotti A, Bellot P, De Gottardi A, Garcia-Pagan JC, Gagnon C, Spénard J, et al. NCX-1000, a nitric oxide-releasing derivative of UDCA, does not decrease portal pressure in patients with cirrhosis: results of a randomized, double-blind, dose-escalating study. *Am J Gastroenterol*. (2010) 105:1094–101. doi: 10.1038/ajg.2009.661

125. Abdel-Sattar AR, Abo-Saif AA, Aboyoussef AM. Nicorandil and atorvastatin attenuate carbon tetrachloride – induced liver fibrosis in rats. *Immunopharmacol Immunotoxicol*. (2020) 42:582–93. doi: 10.1080/08923973.2020.1830104

126. Zipprich A, Gittinger F, Winkler M, Dollinger MM, Ripoll C. Effect of ET-A blockade on portal pressure and hepatic arterial perfusion in patients with cirrhosis: A proof of concept study. *Liver Int*. (2021) 41:554–61. doi: 10.1111/liv.14757

127. Zhang F, Wang F, He J, Lian N, Wang Z, Shao J, et al. Regulation of hepatic stellate cell contraction and cirrhotic portal hypertension by Wnt/β-catenin signalling via interaction with Gli 1. *Br J Pharmacol*. (2021) 178:2246–65. doi: 10.1111/bph.15289

128. Zhang Y, Xing M, Meng F, Zhu L, Huang Q, Ma T, et al. The mechanical mechanism of angiotensin II induced activation of hepatic stellate cells promoting portal hypertension. *Eur J Cell Biol*. (2024) 103:151427. doi: 10.1016/j.ejcb.2024.151427

129. Wang K, Chen H, Zheng J, Chen J, Chen Y, Yuan Y. Engineered liposomes targeting hepatic stellate cells overcome pathological barriers and reverse liver fibrosis. *J Control Release*. (2024) 368:219–32. doi: 10.1016/j.jconrel.2024.02.022

130. Zhang L-F, Deng W-Q, Huang Q-W, Zhang JJ, Wang Y, Zhou TJ, et al. Vicious cycle-breaking lipid nanoparticles remodeling multicellular crosstalk to reverse liver fibrosis. *Adv Mater*. (2024) 36:e2311474. doi: 10.1002/adma.202311474

131. Xu Y, Wang X-S, Zhou X-L, Lu WM, Tang XK, Jin Y, et al. Mesenchymal stem cell therapy for liver fibrosis need "partner": results based on a meta-analysis of preclinical studies. *World J Gastroenterol*. (2024) 30:3766–82. doi: 10.3748/wjg.v30.i32.3766

132. Ji G, Zhang Z, Wang X, Guo Q, Zhang E, Li C. Comprehensive evaluation of the mechanism of human adipose mesenchymal stem cells ameliorating liver fibrosis by transcriptomics and metabolomics analysis. *Sci Rep*. (2024) 14:20035. doi: 10.1038/s41598-024-70281-1

133. Calès P, Bertrais S, Boursier J, Fouchard I, Oberti FSNIFF 16 group. Non-selective beta-blockers increase overall and liver mortality in alcoholic cirrhosis with MELD ≥ 12 over 5 years of follow-up. *Liver Int*. (2021) 41:168–79. doi: 10.1111/liv.14674

134. Ranjan A, Jindal A, Maiwall R, Vashishtha C, Vijayaraghavan R, Arora V, et al. Midodrine plus propranolol versus propranolol alone in preventing first bleed in patients with cirrhosis and severe ascites: a randomized controlled trial. *Hepatol Int*. (2024) 18:1261–70. doi: 10.1007/s12072-024-10687-1

135. Villanueva C, Sapena V, Lo G-H, Seo YS, Shah HA, Singh V, et al. Improving primary prophylaxis of variceal bleeding by adapting therapy to the clinical stage of cirrhosis. A competing-risk meta-analysis of individual participant data. *Aliment Pharmacol Ther*. (2024) 59:306–21. doi: 10.1111/apt.17824

136. Balcar L, Mandorfer M, Hernández-Gea V, Procopet B, Meyer EL, Giráldez Á, et al. Predicting survival in patients with "non-high-risk" acute variceal bleeding receiving β-blockers+ligation to prevent re-bleeding. *J Hepatol*. (2024) 80:73–81. doi: 10.1016/j.jhep.2023.10.007

137. Gillespie S-L, Hanrahan TP, Rockey DC, Majumdar A, Hayes PC. Review article: controversies surrounding the use of carvedilol and other beta blockers in the management of portal hypertension and cirrhosis. *Aliment Pharmacol Ther*. (2023) 57:454–63. doi: 10.1111/apt.17380

138. Terbah R, Testro AG, Hoermann R, Majumdar A, Chapman B, Gow PJ, et al. Continuous home terlipressin infusion increases handgrip strength and reduces ascites-A prospective randomized crossover study. *Hepatology*. (2024) 80:605–20. doi: 10.1097/HEP.0000000000000820

139. Terbah R, Koshy AN, Majumdar A, Vaz K, Testro A, Sinclair M. Long-term continuous Terlipressin infusion improves cardiac Reserve in Patients with Decompensated Cirrhosis. *Clin Gastroenterol Hepatol*. (2024). doi: 10.1016/j.cgh.2024.08.010

140. Rehman H, Rehman ST, Zulfiqar S, Awan S, Abid S. Real-world comparison of terlipressin vs. octreotide as an adjuvant treatment in the management of variceal bleeding. *Sci Rep*. (2024) 14:6692. doi: 10.1038/s41598-024-56873-x

141. Garcia-Tsao G, Abraldes JG, Rich NE, Wong VWS. AGA clinical practice update on the use of vasoactive drugs and intravenous albumin in cirrhosis: expert review. *Gastroenterology*. (2024) 166:202–10. doi: 10.1053/j.gastro.2023.10.016

142. Xue X, Li Y, Zhang S, Yao Y, Peng C, Li Y. Hydroxysafflor yellow A exerts anti-fibrotic and anti-angiogenic effects through mi R-29a-3p/PDGFRB axis in liver fibrosis. *Phytomedicine*. (2024) 132:155830. doi: 10.1016/j.phymed.2024.155830

143. Pun CK, Huang H-C, Chang C-C, Chuang CL, Hsu SJ, Hou MC, et al. Fructooligosaccharides reverses hepatic vascular dysfunction and dysbiosis in rats with liver cirrhosis and portal hypertension. *Eur J Clin Investig*. (2024) 54:e14287. doi: 10.1111/eci.14287

144. Asada S, Kaji K, Nishimura N, Koizumi A, Matsuda T, Tanaka M, et al. Tofogliflozin delays portal hypertension and hepatic fibrosis by inhibiting sinusoidal Capillarization in cirrhotic rats. *Cells*. (2024) 13:538. doi: 10.3390/cells13060538

145. Vairappan B, Wright G, Sundhar M, Ravikumar TS. Candesartan cilexetil ameliorates NOSTRIN-NO dependent portal hypertension in cirrhosis and ACLF. *Eur J Pharmacol*. (2023) 958:176010. doi: 10.1016/j.ejphar.2023.176010

146. Tai Y, Zhao C, Zhang L, Tang S, Jia X, Tong H, et al. Celecoxib reduces hepatic vascular resistance in portal hypertension by amelioration of endothelial oxidative stress. *J Cell Mol Med*. (2021) 25:10389–402. doi: 10.1111/jcmm.16968

147. Noah AA, El-Mezayen NS, El-Ganainy SO, Darwish IE, Afify EA, et al. Reversal of fibrosis and portal hypertension by Empagliflozin treatment of CCl4-induced liver fibrosis: emphasis on gal-1/NRP-1/TGF-β and gal-1/NRP-1/VEGFR2 pathways. *Eur J Pharmacol*. (2023) 959:176066. doi: 10.1016/j.ejphar.2023.176066

148. Zheng L, Zhao Z, Lin J, Li H, Wu G, Qi X, et al. Telmisartan relieves liver fibrosis and portal hypertension by improving vascular remodeling and sinusoidal dysfunction. *Eur J Pharmacol*. (2022) 915:174713. doi: 10.1016/j.ejphar.2021.174713

149. Li R, Liu Z, Huang W, Guo Y, Xie C, Wu H, et al. Microbial-derived Urolithin A targets GLS1 to inhibit Glutaminolysis and attenuate cirrhotic portal hypertension. *Cell Mol Gastroenterol Hepatol*. (2024) 18:101379. doi: 10.1016/j.jcmgh.2024.101379

150. Gunarathne LS, Rajapaksha IG, Casey S, Qaradakhi T, Zulli A, Rajapaksha H, et al. Mas-related G protein-coupled receptor type D antagonism improves portal hypertension in cirrhotic rats. *Hepatol Commun*. (2022) 6:2523–37. doi: 10.1002/hep4.1987

151. Wang X, Gu H, Li K, Lin J, Zhu Y, Deng W. DPP4 inhibitor reduces portal hypertension in cirrhotic rats by normalizing arterial hypocontractility. *Life Sci*. (2021) 284:119895. doi: 10.1016/j.lfs.2021.119895

152. Pun CK, Huang H-C, Chang C-C, Chuang CL, Yen CH, Hsu SJ, et al. Glycyrrhizin attenuates portal hypertension and collateral shunting via inhibition of extrahepatic angiogenesis in cirrhotic rats. *Int J Mol Sci*. (2021) 22:7662. doi: 10.3390/ijms22147662

153. Boyer-Diaz Z, Aristu-Zabalza P, Andrés-Rozas M, Robert C, Ortega-Ribera M, Fernández-Iglesias A, et al. Pan-PPAR agonist lanifibranor improves portal hypertension and hepatic fibrosis in experimental advanced chronic liver disease. *J Hepatol*. (2021) 74:1188–99. doi: 10.1016/j.jhep.2020.11.045

154. Brusilovskaya K, Königshofer P, Lampach D, Szodl A, Supper P, Bauer D, et al. Soluble guanylyl cyclase stimulation and phosphodiesterase-5 inhibition improve portal hypertension and reduce liver fibrosis in bile duct-ligated rats. *United European Gastroenterol J*. (2020) 8:1174–85. doi: 10.1177/2050640620944140

155. Tsai Y-L, Liu C-W, Hsu C-F, Huang CC, Lin MW, Huang SF, et al. Obeticholic acid ameliorates hepatorenal syndrome in ascitic cirrhotic rats by down-regulating the renal 8-iso-PGF2α-activated COX-TXA2 pathway. *Clin Sci (Lond)*. (2020) 134:2055–73. doi: 10.1042/CS20200452

156. Hu L, Su L, Dong Z, Wu Y, Lv Y, George J, et al. AMPK agonist AICAR ameliorates portal hypertension and liver cirrhosis via NO pathway in the BDL rat model. *J Mol Med (Berl)*. (2019) 97:423–34. doi: 10.1007/s00109-019-01746-4

157. Castillo GM, Yao Y, Guerra RE, Jiang H, Nishimoto-Ashfield A, Lyubimov AV, et al. Subcutaneous therapy for portal hypertension: PHIN-214, a partial vasopressin receptor 1A agonist. *Biomed Pharmacother*. (2024) 171:116068. doi: 10.1016/j.biopha.2023.116068

158. Jones AK, Chen H, Ng KJ, Villalona J, McHugh M, Zeveleva S, et al. Soluble guanylyl cyclase activator BI 685509 reduces portal hypertension and portosystemic shunting in a rat Thioacetamide-induced cirrhosis model. *J Pharmacol Exp Ther*. (2023) 386:70–9. doi: 10.1124/jpet.122.001532

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Abhimanyu Chand,
National Academy of Medical Sciences, Nepal
Tangia Muquith,
United Hospital Limited, Bangladesh

*CORRESPONDENCE
Shasha Fu
✉ fssxiami100@163.com
Zongcun Chen
✉ chenzongcun@muhn.edu.cn

†These authors have contributed equally to this work

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Propylthiouracil-induced ANCA-associated vasculitis complicated by granulocytopenia and hemophagocytosis: a case report

Huilin Zhou[1†], Shuchang Lai[1†], Jinyi Chen[1†], Yi Wang[1], Shasha Fu[2*] and Zongcun Chen[1*]

[1]Department of Endocrinology, The Second Affiliated Hospital of Hainan Medical University, Hainan, China, [2]Department of Respiratory and Critical Care Medicine, Haikou Affiliated Hospital of Central South University Xiangya School of Medicine (Haikou People's Hospital), Hainan, China

Objective: To analyze a rare case of ANCA-associated vasculitis (AAV) complicated by hemophagocytosis and granulocytopenia induced by long-term propylthiouracil (PTU) therapy, providing insights for clinical diagnosis and management.

Methods: A retrospective analysis was conducted on the clinical data and treatment course of a patient who developed AAV with hemophagocytosis and granulocytopenia after prolonged PTU use.

Results: Upon admission, granulocytopenia secondary to PTU was suspected. Despite transient recovery of leukocyte counts with anti-infective therapy and granulocyte colony-stimulating factor (G-CSF), recurrent leukopenia and intermittent fever persisted. Bone marrow aspiration revealed hemophagocytic cells, while serologic testing showed positivity for both PR3-ANCA and MPO-ANCA. A definitive diagnosis of PTU-induced AAV was established. Glucocorticoid therapy normalized body temperature and restored leukocyte levels. Follow-up demonstrated resolution of thyrotoxicosis, stabilized leukocyte counts, and afebrile status.

Conclusion: Long-term PTU therapy may trigger AAV accompanied by hemophagocytosis. Clinicians should consider screening for hemophagocytic lymphohistiocytosis (HLH) in such cases to guide timely immunosuppressive intervention.

KEYWORDS

ANCA-associated vasculitis, propylthiouracil, hemophagocytosis, hyperthyroidism, granulocytopenia

Background

Propylthiouracil (PTU) and methimazole (MMI) are frequently employed in the management of hyperthyroidism. These medications predominantly act by suppressing the activity of thyroid peroxidase, thereby curtailing the synthesis of thyroid hormones. Unlike methimazole (MMI), PTU also inhibits the peripheral conversion of thyroxine (T4) to triiodothyronine (T3) (1). The common adverse effects of antithyroid drugs encompass rash, gastrointestinal manifestations, hepatic function derangements, arthralgia, myalgia,

leukopenia, and, in severe cases, granulocytopenia (2–4). Antineutrophil cytoplasmic antibody (ANCA) - associated vasculitis represents a relatively infrequent adverse reaction associated with PTU (5, 6). The coexistence of hemophagocytosis in this context is exceptionally rare. Herein, we present a case of ANCA - associated vasculitis, concurrent with hemophagocytosis and granulocytopenia, subsequent to long - term PTU administration. This case offers valuable insights for the clinical diagnosis and treatment of similar patients.

Case presentation

A 58-year-old man with a decade-long history of hyperthyroidism was admitted on September 19, 2024, complaining of recurrent hand tremors and 5 days of fever. More than ten years prior, the patient experienced the onset of hand tremors, palpitations, and asthenia without an apparent etiology. These symptoms were accompanied by heat intolerance, diaphoresis, and mild exophthalmos, but diplopia was absent. He was diagnosed with "hyperthyroidism" at a local medical facility and had been on an irregular, long-term regimen of "propylthiouracil" (manufactured by Herbrand Pharma Chemicals GmbH, Germany). Three months prior to admission, the patient transitioned to a different brand of PTU, taking 50 mg twice daily, produced by Jinghua Pharmaceutical Group. Five days before admission, the patient developed paroxysmal episodes of cough and dyspnea without an obvious precipitating factor. The cough was non-productive, and these symptoms were accompanied by chest tightness and fever, with the body temperature peaking at 38.6°C.

On admission, vital signs included a temperature of 38°C, heart rate of 115 bpm, respiratory rate of 22 breaths per minute, and a blood pressure of 102/65 mmHg. The patient manifested an acute febrile facies, maintained an autonomous posture, and was fully conscious. Bilateral eyeballs exhibited no remarkable exophthalmos. Auscultation of both lungs revealed coarse breath sounds, with no audible dry or wet rales. The heart rate remained at 115 beats per minute, the cardiac rhythm was regular, and no cardiac murmurs were detected upon auscultation. The bilateral thyroid glands were enlarged to grade II, with a medium - consistency texture, were freely movable, without audible vascular murmurs, and no nodules were palpable. When the patient extended both hands horizontally, fine tremors were observable.

Upon admission, the patient manifested leukopenia and neutropenia accompanied by fever. To augment the white blood cell count, a regimen comprising human granulocyte - colony stimulating factor injection, Diyu Shengbai Capsules, adenosine phosphate tablets, and batyl alcohol tablets was promptly instituted. Subsequently, the patient developed intermittent fever. Serial hematologic tests demonstrated prolonged pancytopenia. Chest imaging revealed scattered patchy and cord-like opacities bilaterally, raising suspicion of infection. Consequently, empirical antimicrobial therapy was initiated with piperacillin-sulbactam followed by meropenem. Concurrently, blood cultures, influenza A/B viral testing, and bone marrow biopsy were performed. No pathogenic organisms were detected in blood cultures, and viral tests were negative. Despite anti-infective therapy, the patient's fever persisted. Given suspected immune-mediated pathology, methylprednisolone sodium succinate was initiated for immunosuppression, administered once daily at 40 mg. This treatment was terminated on September 25, 2024. Throughout the treatment course, the white blood cell and neutrophil counts initially exhibited a transient elevation but subsequently underwent a rapid decline (Figure 1), concomitantly with persistent intermittent fever, with the body temperature peaking at 39.5°C.On September 29, 2024, the bone marrow aspiration findings revealed active proliferation of all three hematopoietic lineages, with discernible hemophagocytic histiocytes (Figure 2). Given the patient's protracted fever (body temperature > 38.5°C), pancytopenia, and the abdominal computed tomography (CT) - detected splenomegaly, the possibility of hemophagocytic lymphohistiocytosis could not be discounted. A re - assessment of the complete blood count on October 2, 2024, disclosed a white blood cell count of 2.36×10^9/L, signifying a recurrence of leukopenia. Consequently, the therapeutic interventions aimed at leukocytosis promotion and anti - infection were continued. Owing to the indeterminate etiology of the patient's long - standing fever, leukopenia, and neutropenia, additional diagnostic investigations were pursued. The results of anti - blood cell antibody (quantitative) assays were as follows: Urinalysis: Urine occult blood (2+) was detected, anti - proteinase 3 antibody PR3 - IgG measured 400.00 AU/mL, and anti - myeloperoxidase antibody MPO - IgG was 286.30 AU/mL. The ferritin level was determined to be 813 ng/mL (reference range: 24–425 ng/mL). The results of all laboratory data are shown in Tables 1, 2.

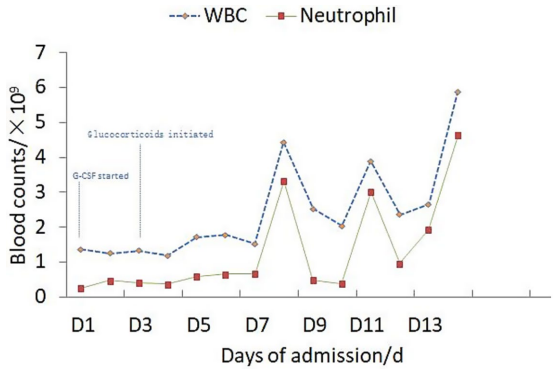

FIGURE 1
WBC and neutrophil trends with treatment timeline.

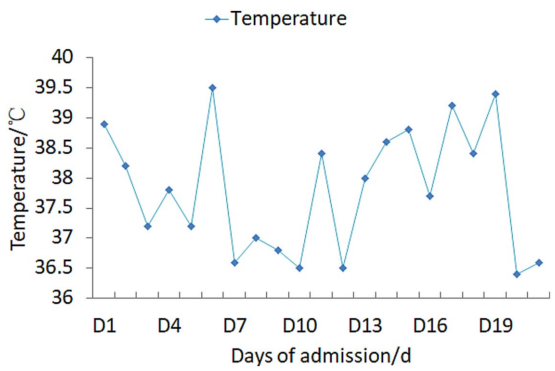

FIGURE 2
Body temperature profile during treatment.

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TABLE 1 Laboratory data.

Examination items	Test results	Normal range
White blood cells ($x10^9$/L)	1.36	3.5–9.5
Neutrophils ($x10^9$/L)	0.24	1.8–6.3
Red blood cells ($x10^{12}$/L)	3.91	4.3–5.8
Platelets ($x10^9$/L)	103	100–300
Hemoglobin (g/L)	109	130–175
Interleukin – 6 (pg/mL)	128	0–7
C - Reactive Protein (mg/L)	110.4	0–10
Procalcitonin (ng/L)	0.99	0–0.5
Erythrocyte sedimentation rate (mm/L)	61	0–15
Triiodothyronine (ng/mL)	2.53	0.75–2.1
Total Thyroxine (ng/mL)	148	50–130
Free Triiodothyronine (pg/mL)	4.39	2–4.2
Free Thyroxine (pg/mL)	29.60	9–17.5
Thyroid - Stimulating hormone (μIU/mL)	<0.001	0.3–4.5
Anti - thyroid peroxidase antibody (IU/mL)	18.60	0–10
Anti - thyroglobulin antibody (IU/mL)	2004.00	0–95
Thyroglobulin (ng/mL)	<0.02	3.5–77
Thyrotropin receptor antibody (IU/L)	26.40	0–1.5
Aspartate transaminase (U/L)	21	15–40
Alanine transaminase (U/L)	32	9–50
serum creatinine (μmol/L)	66	57–97
blood urea nitrogen (mmol/L)	4.35	2.9–8.0
Triglycerides (mmol/L)	1.52	0.56–1.70
Lactate dehydrogenase (U/L)	215	135–225
Aspartate transaminase(U/L)	21	15–40
Alanine transaminase (U/L)	32	9–50
Serum creatinine (μmol/L)	66	57–97
Blood urea nitrogen (mmol/L)	4.35	2.9–8.0
Triglycerides (mmol/L)	1.52	0.56–1.70
Lactate dehydrogenase(U/L)	215	135–225
Fibrinogen (g/L)	5.06	2–4
D – dimer (μg/mL)	1.22	0–0.55
Ferritin (ng/mL)	813	24–425

TABLE 2 Immunological and serological tests.

Examination items	Test results	Normal range
Anti - glomerular basement membrane antibody GBM – IgG(AU/ml)	2.00	0–24
Anti - proteinase 3 antibody PR3 – IgG(AU/ml)	400.00	0–24
Anti - myeloperoxidase antibody MPO – IgG(AU/ml)	286.30	0–24
Antinuclear antibody	Negative	
Antinuclear antibody extract	Negative	
Rheumatoid factor	Negative	
Immunoglobulin G content determination (g/L)	20.27	8.6–17.4
Immunoglobulin M content determination (g/L)	2.499	0.3–2.2
κ Light chain (g/L)	5.250	1.38–3.75
λ Light chain (g/L)	2.650	0.93–2.42
Gastrin 17(pmol/L)	19.2	1.7–7.6
Carbohydrate antigen 125 (U/mL)	46.3	0–35
Pepsinogen I	Negative	
Pepsinogen II	Negative	
PGI/PGII	Negative	
Prostate Tumor Markers	Negative	
COVID - 19 nucleic acid test	Negative	
Influenza A - IGM	Negative	
Influenza B - IGM	Negative	
Fungal (1–3)-β - D glucan detection (pg/mL)	278.03	0–100
Respiratory pathogen joint detection	Negative	
TORCH	Negative	
Mycobacterium tuberculosis gamma - interferon Release assay	Negative	
Blood culture	Negative	
Sputum culture	Negative	
Widal test	Negative	
Weil - felix reaction	Negative	
Plasmodium	Negative	

The administration of propylthiouracil (PTU) to the patient was promptly discontinued. Subsequently, a sequential anti - infectious regimen was initiated, commencing with meropenem followed by piperacillin - sulbactam. Concurrently, pharmacological interventions aimed at augmenting the white blood cell count were implemented, including the use of human granulocyte - colony stimulating factor (G - CSF) injection, Diyu Shengbai Capsules, adenosine phosphate tablets, and batyl alcohol tablets. Intravenous methylprednisolone sodium succinate (40 mg daily) was initiated as anti-inflammatory therapy. Throughout the treatment period, the outcomes of the leukocytosis - promoting therapies, as evidenced by the white blood cell and neutrophil counts, were suboptimal (refer to Figure 1 for detailed data).After approximately two weeks, the antibiotic therapy was terminated, while the administration of methylprednisolone sodium succinate was continued. Twenty days following this adjustment, the patient's body temperature normalized (Figure 3). A subsequent complete blood count demonstrated the restoration of the white blood cell count to within the normal range, signifying an improvement in the patient's clinical condition. Subsequently, the patient opted for radioactive iodine - 131 treatment. Post - discharge, the patient continued with an oral prednisone tablet regimen. One-month follow-up assessment revealed normalization of body temperature, leukocyte count, neutrophil count, and erythrocyte sedimentation rate. Notably, the urine occult blood test turned negative. Thyroid function reassessment showed elevated free thyroxine (FT4) at 20 pg./mL (reference range: 9–17.5 pg./mL), free

triiodothyronine (FT3) within the upper limit of normal (4.16 pg./mL; reference: 2–4.2 pg./mL), and markedly suppressed thyroid-stimulating hormone (TSH) at 0.002 μIU/mL (reference: 0.3–4.5 μIU/mL). At the 4-month follow-up, ANCA serology dynamics demonstrated persistent elevation of anti-proteinase 3 antibody (PR3-IgG: 400 AU/mL) alongside a decline in anti-myeloperoxidase antibody (MPO-IgG: 227.80 AU/mL).

Discussion

In the context of this hyperthyroidism case, subsequent to the substitution of Propylthiouracils (PTUs) from different manufacturers, manifestations such as fever, granulocyte deficiency, and ANCA - associated vasculitis with hemophagocytosis emerged. Reports of similar cases remain scarce, and there persist several contentious issues within the diagnostic and therapeutic processes.

Upon admission, the patient was found to have leukopenia and agranulocytosis (granulocyte count $<0.5 \times 10^9$/L). Agranulocytosis is one of the severe adverse drug reactions of PTU. The occurrence of agranulocytosis is related to the type, dose and application time of antithyroid drugs. Yoshimura et al. (7) discovered that both Methimazole (MMI) and Propylthiouracil (PTU), two medications used in the treatment of thyroid - related conditions, exhibited a dose - dependent elevation in the incidence of granulocyte deficiency. Specifically, when considering equipotent dosages in terms of thyroid hormone synthesis inhibition, PTU was found to have a significantly higher propensity to induce granulocyte deficiency compared to MMI. Granulocytopenia characteristically manifests within the initial three - month period subsequent to the commencement of antithyroid drug (ATD) therapy (8). Nevertheless, instances have been documented wherein the onset transpired following an exposure duration exceeding 10 years (9). The underlying pathogenesis of propylthiouracil (PTU)-induced agranulocytosis remains incompletely elucidated. It is potentially associated with the inhibition of nucleic acid metabolism in bone marrow granulocytes. PTU has the ability to trigger the generation of anti - neutrophil cytoplasmic antibodies (ANCA) within the body, thereby inciting an autoimmune reaction. Once neutrophils are sensitized and the antigens translocate to the cell membrane, ANCA can bind to neutrophil antigens, including protease - 3 (PR3), myeloperoxidase (MPO), and cathepsin G, which leads to neutrophil degranulation (10).In the present case, granulocyte colony - stimulating factor was administered to elevate the white blood cell count, meropenem, piperacillin sodium, and sulbactam sodium were utilized for anti - infection purposes, and hormones were applied for a short - term. However, the white blood cell count increased transiently and then decreased again, indicating suboptimal treatment efficacy. PTU - induced agranulocytosis typically manifests 3–6 months after the initiation of drug use, and it can also occur during long - term administration. Nevertheless, in most cases, the white blood cell count can be restored to normal levels following leukocyte - elevating treatment. In this particular patient, the poor response to leukocyte - elevating treatment is presumably related to other diseases that cause leukopenia, such as autoimmune disorders and hematological malignancies.

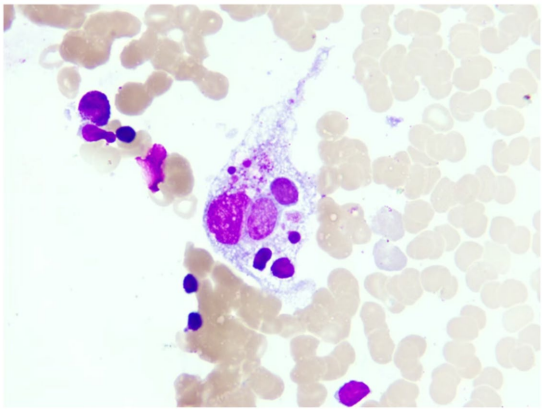

FIGURE 3
This is the macrophage phagocytosis phenomenon observed under the microscope in the patient's bone marrow image (magnification 1,000X).

The patient had no antecedent medical history of renal or pulmonary disorders and exhibited symptoms such as fever, weight loss, and cough. The urine occult blood test demonstrated a positive outcome, and the pulmonary imaging depicted scattered patchy and cord-like regions of elevated density in both lungs. Both PR3-IgG and MPO-IgG were strongly positive. In light of the history of Propylthiouracil (PTU) administration, ANCA-associated vasculitis induced by PTU was suspected. AAV encompasses a spectrum of autoimmune small-vessel vasculitides characterized histologically by fibrinoid necrosis and serologically by circulating ANCAs targeting neutrophil cytoplasmic antigens. This encompasses granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA). BALAVOINE et al. conducted a comprehensive review of the case reports of 260 patients afflicted with antithyroid drug-related ANCA-associated vasculitis (AAV). It was discovered that 75% of these cases were correlated with propylthiouracil, while 25% were associated with methimazole. Juvenile age and extended treatment duration constitute the primary risk factors for ANCA positivity (11). There exists an interaction between Propylthiouracil (PTU) and ANCA-targeted antigens (primarily proteinase 3 (PR3) and myeloperoxidase (MPO)). PTU is capable of inducing the generation of ANCA, and ANCA further facilitates the over-activation of neutrophils, which subsequently release inflammatory cytokines, reactive oxygen species, and proteases, thereby inflicting damage on vascular endothelial cells and resulting in AAV (12). Furthermore, the hyperactivation of neutrophils mediated by ANCA has the potential to induce the formation of neutrophil extracellular traps (NETs). NETs exert a cytotoxic influence on vascular endothelial cells and are mainly degraded by deoxyribonuclease I (DNase I) in the serum. Propylthiouracil (PTU) is able to inhibit the activity of DNase I. As a result, this inhibition gives rise to the accumulation of NETs in the body, which further impairs vascular endothelial cells, eventually leading to the development of ANCA - associated vasculitis (AAV) (13). Indirect immunofluorescence assay can identify two distinct subtypes of ANCA: cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA). For cANCA, the principal target antigen is proteinase 3 (PR3), while for pANCA, it is predominantly myeloperoxidase (MPO). In cases of Propylthiouracil (PTU)-induced ANCA - associated vasculitis (AAV), a double - positive profile is frequently observed, signifying the concurrent detection of both PR3 - ANCA and MPO - ANCA (14).

The clinical presentations of drug - induced ANCA - associated vasculitis bear resemblance to those of primary vasculitis. Virtually any

organ system may be affected. Oftentimes, the inaugural symptoms manifest as fever or cutaneous manifestations, with the lungs and kidneys being the most commonly implicated organs (13). In cases where the pulmonary system is affected, common clinical manifestations typically encompass cough and dyspnea. In severe instances, this condition may progress to pulmonary hemorrhage. From a radiological perspective, findings commonly include patchy, mottled, or linear opacities with increased density; alternatively, extensive areas of consolidation or ground - glass opacities may be observed. When renal involvement occurs, it is usually characterized by hematuria and proteinuria. Regarding therapeutic management, the immediate cessation of Propylthiouracil (PTU) is imperative. In certain patients, symptom alleviation may ensue following the discontinuation of PTU. Nevertheless, for individuals presenting with vital organ involvement, immunosuppressive therapy, encompassing the administration of corticosteroids and immunosuppressants, becomes requisite. In cases of refractory ANCA - associated vasculitis (AAV), plasmapheresis and biological agents may be employed (15). The patient presented in this case had an extensive history of Propylthiouracil (PTU) intake with concomitant involvement of both the pulmonary and renal systems. A dual - positive status for Proteinase 3 - Anti - Neutrophil Cytoplasmic Antibody (PR3 - ANCA) and Myeloperoxidase - Anti - Neutrophil Cytoplasmic Antibody (MPO-ANCA) was detected. Following a comprehensive assessment, the patient's clinical condition demonstrated improvement subsequent to the administration of corticosteroid pulse therapy.In patients with a protracted course of PTU utilization who develop leukopenia and exhibit an inadequate response to leukocytosis - promoting interventions, the potential presence of ANCA - associated vasculitis should be thoroughly evaluated.

The results of the patient's bone marrow aspiration revealed the presence of hemophagocytic histiocytes. Clinicians should be vigilant about the potential development of hemophagocytic lymphohistiocytosis (HLH). Hemophagocytic lymphohistiocytosis (HLH) represents a syndrome characterized by an excessive inflammatory response. From a clinical perspective, it typically presents with fever, pancytopenia, hepatosplenomegaly, and the identification of activated macrophages within hematopoietic organs. Prognostically, HLH generally portends a poor outcome, and in severe instances, it may culminate in death. HLH can be subclassified into primary and secondary forms. Primary HLH, more frequently encountered in pediatric patients, is an autosomal or X - linked recessive genetic condition. In contrast, secondary HLH is more commonly observed in adult populations and may arise secondary to a diverse array of etiologies, including infections caused by viruses, bacteria, and parasites, as well as rheumatologic and immunological disorders, metabolic derangements, and neoplasms.

At present, the HLH - 2004 criteria formulated by the Histiocyte Society are widely adopted internationally as the diagnostic standards for hemophagocytic lymphohistiocytosis (HLH). Specifically, a diagnosis of HLH can be established if either of the following two conditions is satisfied:

1 Identification of molecular - genetic abnormalities associated with HLH;
2 Meeting 5 out of the following 8 diagnostic criteria:
   (1) Persistent fever for more than 7 days;
   (2) Enlargement of the spleen (splenomegaly);
   (3) Cytopenia affecting two or three hematopoietic lineages: Hemoglobin level below 90 g/L (in infants younger than 4 weeks, below 100 g/L), platelet count less than 100×10^9/L, and neutrophil count lower than 1.0×10^9/L;
   (4) The presence of both hypertriglyceridemia and/or hypofibrinogenemia, characterized by fasting triglyceride levels exceeding 3.0 mmol/L and fibrinogen levels below 1.5 g/L;
   (5) Visualization of hemophagocytosis in bone - marrow aspirates, splenic tissue, or lymph - node specimens;
   (6) Diminished or absent natural killer (NK) cell activity;
   (7) Serum ferritin levels greater than 500 μg/L;
   (8) Elevated plasma levels of soluble CD25 (SIL - 2R) above 2,400 U/mL or an increased lactate dehydrogenase level.

In the present case, the patient fulfilled five criteria within the second category, specifically including fever, splenomegaly, pancytopenia, the detection of hemophagocytosis in bone marrow specimens, and a ferritin level exceeding 500 μg/L. Consequently, the diagnostic benchmarks for hemophagocytic lymphohistiocytosis (HLH) were met. Nonetheless, contemporary research posits that hemophagocytosis is no longer regarded as a requisite and conclusive condition for the diagnosis of HLH (16, 17). Additionally, hemophagocytosis can manifest in autoimmune disorders. In comparison to hemophagocytosis, aberrant liver function, ferritin concentrations, and natural killer (NK) cell activity carry greater diagnostic significance for HLH (16). The HLH - 2004 diagnostic guidelines specify a serum ferritin level surpassing 500 ng/mL. Nevertheless, investigations have indicated that a serum ferritin level exceeding 2000 ng/mL is commonly regarded as raising suspicion for HLH, and a level exceeding 10,000 ng/mL substantially enhances the diagnostic sensitivity and specificity for HLH, particularly in adult patients (17, 18).

The patient exhibited normal liver and kidney functions as well as triglyceride levels, with an insignificant elevation in ferritin. Taking into account the clinical presentations of fever and pancytopenia, in conjunction with the patient's pulmonary imaging findings, a stronger association with ANCA - associated vasculitis was deemed more probable. As a result, the diagnosis of hemophagocytic lymphohistiocytosis was temporarily excluded. The subsequent follow - up outcomes of the patient further precluded the possibility of HLH. In individuals afflicted with autoimmune diseases, autoantibodies may play a mediatory role, and circulating immune complexes can deposit on bone - marrow hematopoietic cells. This deposition augments the susceptibility of phagocytes, potentially culminating in the onset of hemophagocytic lymphohistiocytosis (HLH). A retrospective analysis encompassing the medical records, diagnostic algorithms, and therapeutic trajectories of 55 patients definitively diagnosed with autoimmune - disease - associated hemophagocytic syndrome (AAHS) reveals that a diverse spectrum of autoimmune disorders possess the propensity to precipitate hemophagocytic syndrome (HPS) (19). In the present case, the hemophagocytosis detected in the patient's bone - marrow specimens cannot be excluded as being attributable to ANCA - associated vasculitis. Nevertheless, to date, there have been no documented reports of HLH precipitated by ANCA - associated vasculitis.

Autoimmune disorders have the potential to induce hemophagocytosis. In the absence of early recognition, this phenomenon may advance to hemophagocytic lymphohistiocytosis (HLH). When patients diagnosed with autoimmune diseases exhibit manifestations

such as high - grade fever, cytopenia, splenomegaly, and impairment of hepatic and renal functions, the likelihood of HLH should be taken into account. HLH is a life-threatening hyperinflammatory syndrome characterized by cytokine storm, which may complicate diverse inflammatory conditions. Characterized by a rapid progression, early identification and appropriate management of HLH are of paramount importance in averting organ failure and mortality (18).

In conclusion, we present a case of a patient with hyperthyroidism who developed agranulocytosis concurrent with ANCA - associated vasculitis and hemophagocytosis subsequent to long - term administration of propylthiouracil (PTU). Presently, the body of literature regarding such cases remains limited.

This case serves as a reminder that when patients exhibit leukopenia, particularly pancytopenia, subsequent to PTU intake (encompassing both prolonged use and switching between different pharmaceutical manufacturers), and the therapeutic interventions aimed at leukocytosis promotion and anti - infection prophylaxis yield suboptimal results, it is imperative to promptly conduct comprehensive evaluations for hematological disorders, connective tissue diseases, and autoimmune pathologies to elucidate the underlying etiology. ANCA - associated vasculitis and hemophagocytic lymphohistiocytosis (HLH) manifest overlapping clinical features, including fever, leukopenia, and involvement of the integumentary and mucosal surfaces. Hence, meticulous differentiation between these two entities is essential. Moreover, hemophagocytosis can also be observed in the context of autoimmune diseases. Therefore, during the diagnostic work - up of ANCA - associated vasculitis, screening for HLH should be incorporated into the assessment protocol.

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

Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

HZ: Data curation, Writing – original draft, Project administration. SL: Writing – original draft, Data curation, Project administration. JC: Data curation, Project administration, Writing – original draft. YW: Writing – original draft, Supervision. SF: Writing – original draft, Writing – review & editing. ZC: Writing – review & editing, 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.

Generative AI statement

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

Publisher's note

References

1. Manna D, Roy G, Mugesh G. Antithyroid drugs and their analogues: synthesis, structure, and mechanism of action. *Acc Chem Res*. (2013) 46:2706–15. doi: 10.1021/ar4001229

2. Chahed F, Ben Fredj N, Ben Romdhane H, Chadli Z, Chaabane A, Fadhel NB, et al. Methimazole-induced urticaria in hyperthyroid patients: a safe re-administration protocol. *Therapie*. (2024) 79:559–63. doi: 10.1016/j.therap.2023.12.006

3. Heidari R, Niknahad H, Jamshidzadeh A, Abdoli N. Factors affecting drug-induced liver injury: antithyroid drugs as instances. *Clin Mol Hepatol*. (2014) 20:237–48. doi: 10.3350/cmh.2014.20.3.237

4. Yu W, Wu N, Li L, Wang J, OuYang H, Shen H. Side effects of PTU and mmi in the treatment of hyperthyroidism: a systematic review and META-analysis. *Endocr Pract*. (2020) 26:207–17. doi: 10.4158/ep-2019-0221

5. Han JY, Lee JM, Jung SY, Kim MS, Lee SW, Kronbichler A, et al. Comparison of agranulocytosis and anti-neutrophil cytoplasmic antibody-associated vasculitis caused by two antithyroid drugs: a pharmacovigilance study using the WHO international database. *Fundam Clin Pharmacol*. (2024) 38:780–8. doi: 10.1111/fcp.12991

6. Arai M, Tsuno T, Konishi H, Nishiyama K, Terauchi Y, Inoue R, et al. A disproportionality analysis of the adverse effect profiles of Methimazole and Propylthiouracil in patients with hyperthyroidism using the Japanese adverse drug event report database. *Thyroid*. (2023) 33:804–16. doi: 10.1089/thy.2023.0030

7. Yoshimura Noh J, Inoue K, Suzuki N, Yoshihara A, Fukushita M, Matsumoto M, et al. Dose-dependent incidence of agranulocytosis in patients treated with methimazole and propylthiouracil. *Endocr J*. (2024) 71:695–703. doi: 10.1507/endocrj.EJ24-0135

8. Nakamura H, Miyauchi A, Miyawaki N, Imagawa J. Analysis of 754 cases of antithyroid drug-induced agranulocytosis over 30 years in Japan. *J Clin Endocrinol Metab*. (2013) 98:4776–83. doi: 10.1210/jc.2013-2569

9. Mutharasan P, Oatis W, Kwaan H, Molitch M. Delayed anithyroid drug-induced agranulocytosis. *Endocr Pract*. (2012) 18:e69–72. doi: 10.4158/ep11339.Cr

10. Vicente N, Cardoso L, Barros L, Carrilho F. Antithyroid drug-induced agranulocytosis: state of the art on diagnosis and management. *Drugs R D*. (2017) 17:91–6. doi: 10.1007/s40268-017-0172-1

11. Balavoine AS, Glinoer D, Dubucquoi S, Wémeau JL. Antineutrophil cytoplasmic antibody-positive small-vessel Vasculitis associated with Antithyroid drug therapy: how significant is the clinical problem? *Thyroid*. (2015) 25:1273–81. doi: 10.1089/thy.2014.0603

12. Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. *Nat Rev Rheumatol*. (2019) 15:91–101. doi: 10.1038/s41584-018-0145-y

13. Culerrier J, Nguyen Y, Karadag O, Yasar Bilge S, Yildrim TD, Ogut TS, et al. Characteristics and outcome of ANCA-associated vasculitides induced by anti-thyroid drugs: a multicentre retrospective case-control study. *Rheumatology (Oxford)*. (2024) 63:999–1006. doi: 10.1093/rheumatology/kead319

14. Chen M, Gao Y, Guo XH, Zhao MH. Propylthiouracil-induced antineutrophil cytoplasmic antibody-associated vasculitis. *Nat Rev Nephrol*. (2012) 8:476–83. doi: 10.1038/nrneph.2012.108

15. Magri SJ, Ugarte-Gil MF, Brance ML, Flores-Suárez LF, Fernández-Ávila DG, Scolnik M, et al. Pan American league of associations for rheumatology guidelines for the treatment of ANCA-associated vasculitis. *Lancet Rheumatology*. (2023) 5:e483–94. doi: 10.1016/S2665-9913(23)00128-5

16. Zhang YL, Hao JN, Sun MM, Xing XY, Qiao SK. Etiology, clinical characteristics and prognosis of secondary Hemophagocytic syndrome. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. (2024) 32:1230–7. doi: 10.19746/j.cnki.issn.1009-2137.2024.04.040

17. Canna SW, Marsh RA. Pediatric hemophagocytic lymphohistiocytosis. *Blood*. (2020) 135:1332–43. doi: 10.1182/blood.2019000936

18. Shakoory B, Geerlinks A, Wilejto M, Kernan K, Hines M, Romano M, et al. The 2022 EULAR/ACR points to consider at the early stages of diagnosis and management of suspected haemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS). *Ann Rheum Dis*. (2023) 82:1271–85. doi: 10.1136/ard-2023-224123

19. Huang S, Liu Y, Yan W, Zhang T, Wang P, Zhu M, et al. Single center clinical analysis of macrophage activation syndrome complicating juvenile rheumatic diseases. *Pediatr Rheumatol Online J*. (2024) 22:58. doi: 10.1186/s12969-024-00991-3

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Jan Heckelmann,
University Hospital RWTH Aachen, Germany
Sanja Gluscevic,
Clinical Center of Montenegro, Montenegro

*CORRESPONDENCE
Wenbin Wan
✉ doctorwwb@163.com

†These authors have contributed equally to this work

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Case Report: Heparin-induced thrombocytopenia following double filtration plasmapheresis in a patient with anti-GAD65 autoimmune encephalitis

Ying Chen†, Wanwan Li†, Liping Ni, Yufang Mei, Yan Zhou and Wenbin Wan*

Department of Neurology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Autoimmune encephalitis (AE) is a group of disorders characterized by antibodies targeting neuronal cell surface, intracellular structures and synapse antigens. Treatment for AE involves reducing antibody levels and suppressing immune-mediated inflammation using intravenous immunoglobulin, plasma exchange (PE), and immune-modulating agents. PE is commonly used in autoimmune neurological diseases, but the safety issues of PE are worth continuous attention. This case report describes a 28-year-old patient who was diagnosed with anti-GAD65 AE and underwent treatments including double filtration plasmapheresis (DFPP), steroids, and immunosuppressive agents. However, complications arose when the patient developed thrombosis and was diagnosed with type II heparin-induced thrombocytopenia (HIT). He was treated with an oral anticoagulant and eventually recovered. One month later, follow-up examinations showed no presence of emboli and his epilepsy remained well controlled. There is a risk of HIT, a potentially dangerous adverse reaction to heparin during treatment of PE. The current case highlights the importance of monitoring for HIT during PE and the need for alternative anticoagulants.

KEYWORDS

heparin-induced thrombocytopenia, autoimmune encephalitis, double filtration plasmapheresis, heparin, platelet, thrombus

Introduction

Autoimmune encephalitis (AE) is a group of disorders characterized by antibodies targeting antigens on the neuronal cell surface and synapse. The clinical presentation of AE can vary widely, with symptoms including memory impairment, epileptic seizures, abnormal mental and behavioral functions, and specific subtype-related features (1, 2). Symptoms may develop suddenly or progress slowly over time, posing diagnostic challenges due to the diverse changes in clinical presentation.

Various types of AE antibodies have been identified, including anti-N-Methyl-D-Aspartate (NMDA) receptor antibody, anti-γ-Aminobutyric acid sub-type A (GABAA) receptor antibody, anti-GABA sub-type B (GABAB) receptor antibody, and anti-glutamic acid decarboxylase (GAD) antibody (2). These antibodies directly or indirectly

cause inflammatory damage to the central nervous system (CNS), leading to AE development. Treatment strategies for AE focus on reducing antibody levels and suppressing immune-mediated inflammatory damage (3). Commonly used treatments include intravenous immunoglobulin (IVIG), plasma exchange (PE), and immune-modulating agents (4). PE is effective in removing disease mediators from the body and has been widely used in autoimmune neurological diseases. Recent evidence suggests that PE is a suitable option for AE, especially when steroids or other immunosuppressive therapies are not effective or contraindicated in the short term (5, 6). According to the guidelines for the use of therapeutic apheresis in clinical practice (7), PE is recommended as the initial treatment for NMDAR encephalitis, with a recommendation of Category I (7).

Administration of an anticoagulant is essential during PE to prevent clotting within the circuit and ensure optimal treatment efficacy (8). Unfractionated heparin (UFH) is commonly used as an anticoagulant due to its clinical safety. However, it is crucial to be aware of heparin-induced thrombocytopenia (HIT) as a rare but potentially life-threatening adverse drug reaction to heparin in patients undergoing PE (9). Our current case report documents a patient who developed HIT type II and extensive thrombosis after undergoing double filtration plasmapheresis (DFPP) to treat anti-GAD65 AE.

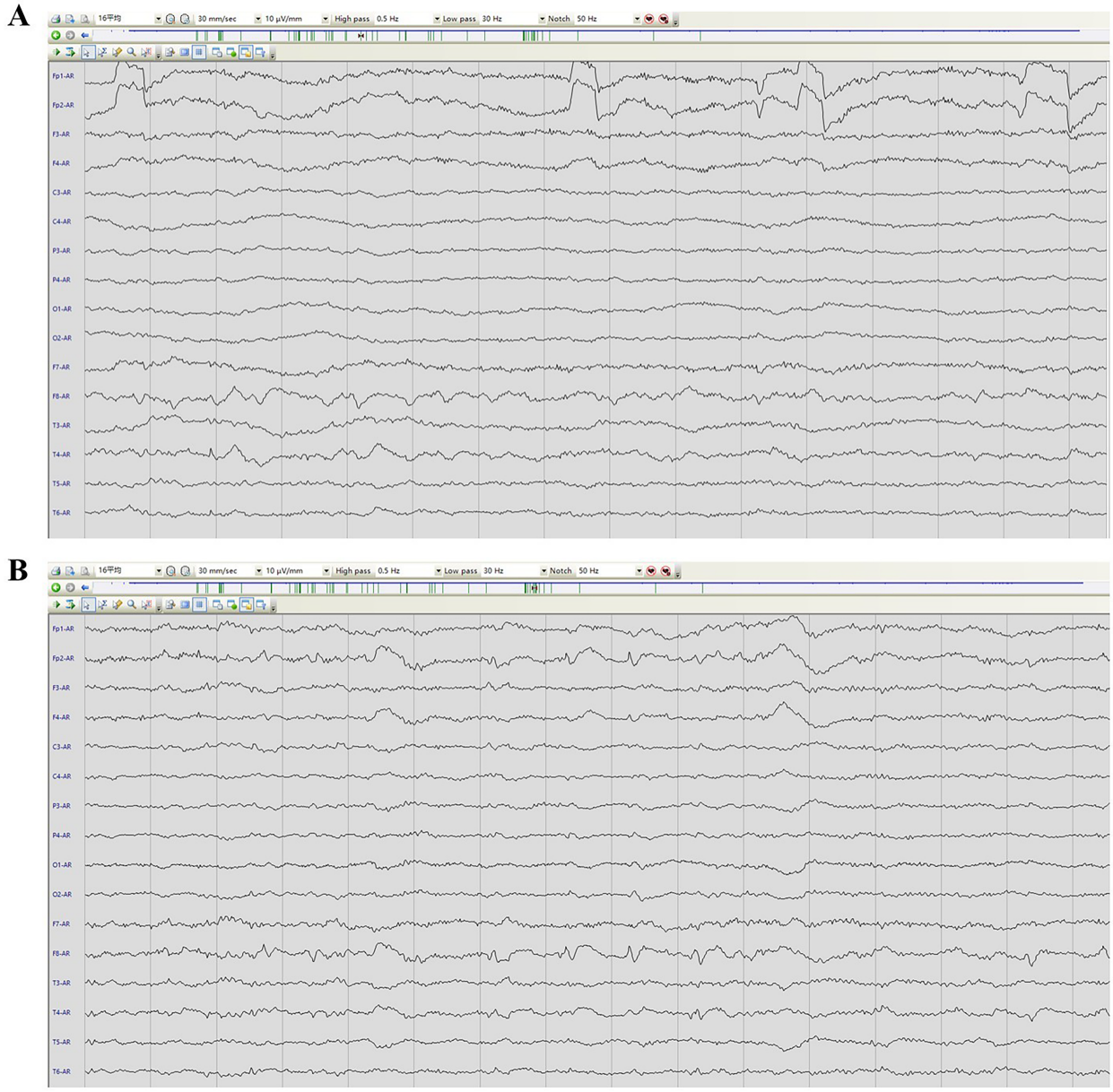

FIGURE 1
EEG image of the patient. **(A)** presented an awake EEG with sharp wave slow wave. **(B)** showed a sleep EEG with sharp waves and slow waves.

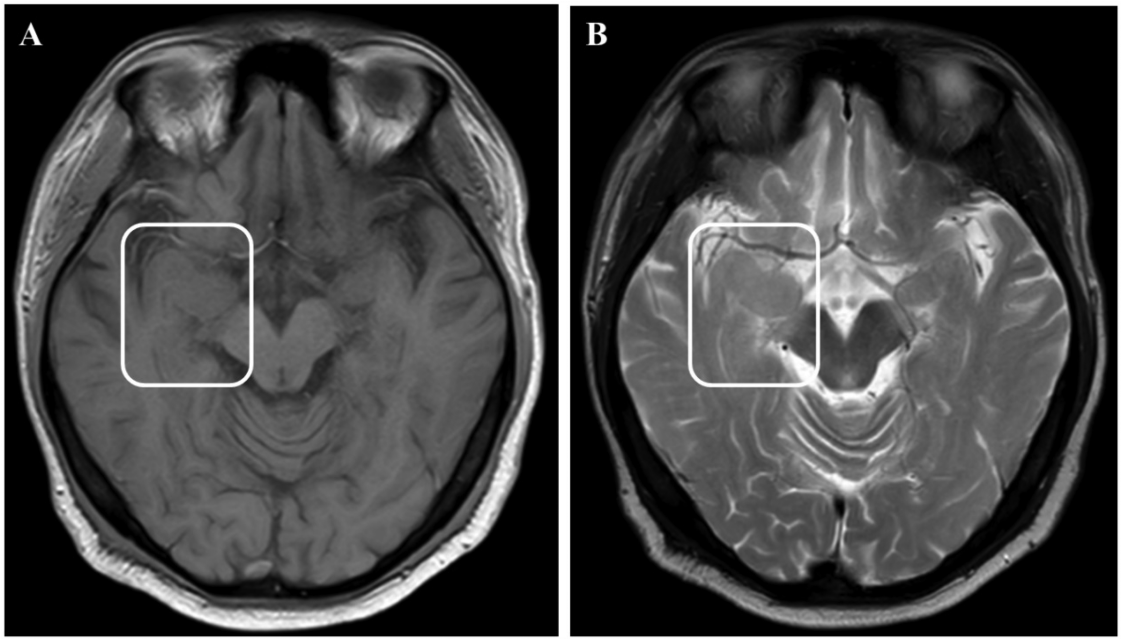

FIGURE 2
MRI images showed enlargement of the right amygdala and hippocampi. **(A)** showed T1 weight image. **(B)** showed T2 weight image.

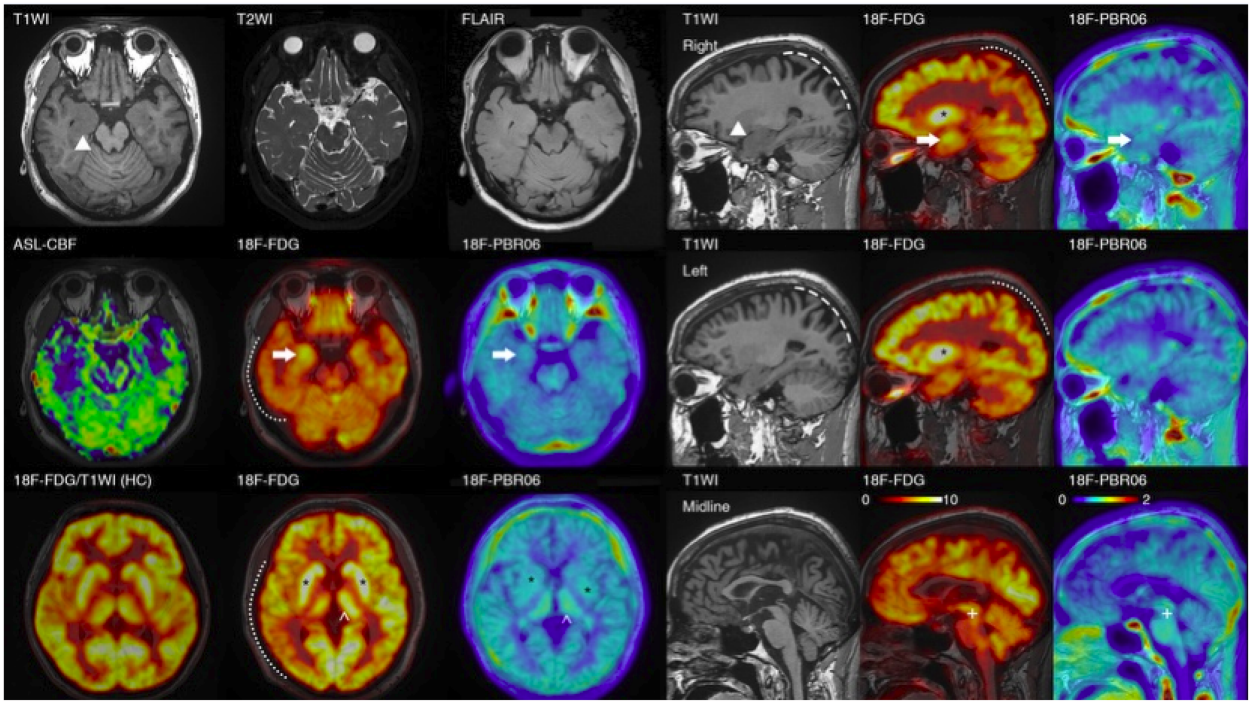

FIGURE 3
PET/MRI images of the patient. The PET/MRI imaging displays decreased FDG absorption in the cortex of the right hemisphere and the left parietal lobe (indicated by a dotted line). It also shows atrophy in both parietal lobes (marked by a dashed line) in the patient with encephalitis. Regarding focal changes, the volume of the hippocampus on both sides has increased (▲), with heightened FDG and TSPO uptake (→), particularly on the right. However, no noticeable abnormalities were detected in T2\FLAIR\ASL scans. Additionally, there is increased FDG and TSPO uptake in the bilateral basal ganglia (*), and the left thalamus (^). (In comparison with the bottom-left control: the uptake in the cortex and basal ganglia are essentially equivalent, but the patient's basal ganglia-to-cortex ratio is markedly elevated); the FDG\TSPO uptake in the midbrain is also enhanced (+).

Case report

This case report has been approved by the Ethics Committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University. In April 2023, a 28-year-old man with a history of limb convulsions and impaired consciousness presented to our institution's Department of Neurology. These symptoms had been occurring for the past 2 years and were accompanied by upward eye rolling. The seizures lasted for about 1 min and could end on their own. Two years ago, when the symptoms first appeared, he was diagnosed with epilepsy at another medical institution and was prescribed sodium valproate and perampanel. During that time, abnormal signals were found in the right frontal lobe and thalamus on a brain MRI, but no abnormalities were found in the cerebrospinal fluid (CSF) examination. Despite the medication, his epilepsy remained poorly controlled. The patient had been in good health previously, without chronic diseases such as diabetes and hypertension, and without any autoimmune diseases.

Physical examination during his hospitalization revealed no abnormal findings in his cognitive function. CSF tests showed normal cell count, protein, glucose, and chloride levels. Long-term electroencephalogram (EEG) examination revealed epileptiform electrical discharges in the right frontal and anterior temporal areas, and slowing waves in the right frontal and the temporal areas (Figure 1). Magnetic resonance imaging (MRI) of the brain showed enlargement of the right amygdala and hippocampi (Figure 2). To further evaluate changes in the brain, positron emission tomography/magnetic resonance imaging (PET/MRI) scans using 18F-FDG and TSPO were performed. These scans showed increased volume, metabolism, and TSPO uptake in the medial temporal lobes. Increased metabolism was also observed in the basal ganglia, left thalamus, left temporoparietal junction, midbrain, and localized cerebellum on the right. In contrast, decreased metabolism was observed in the right cerebral hemisphere and left parietal lobe. Additionally, increased TSPO uptake was detected in the left localized frontal lobe, bilateral insular lobes, and both medial temporal lobes (Figure 3). The PET/MRI scans did not detect any tumors, and no tumor markers were found in the patient's serum. Anti-GAD65 IgG antibodies were detected in both the serum and CSF, with antibody titers of 1:100 and 1:30, respectively (Figure 4).

Based on these findings, the patient was diagnosed with anti-GAD65 AE. He underwent treatments including double filtration plasmapheresis (DFPP) via a femoral vein, intravenous methylprednisolone (IVMP), oral steroids, and the long-term

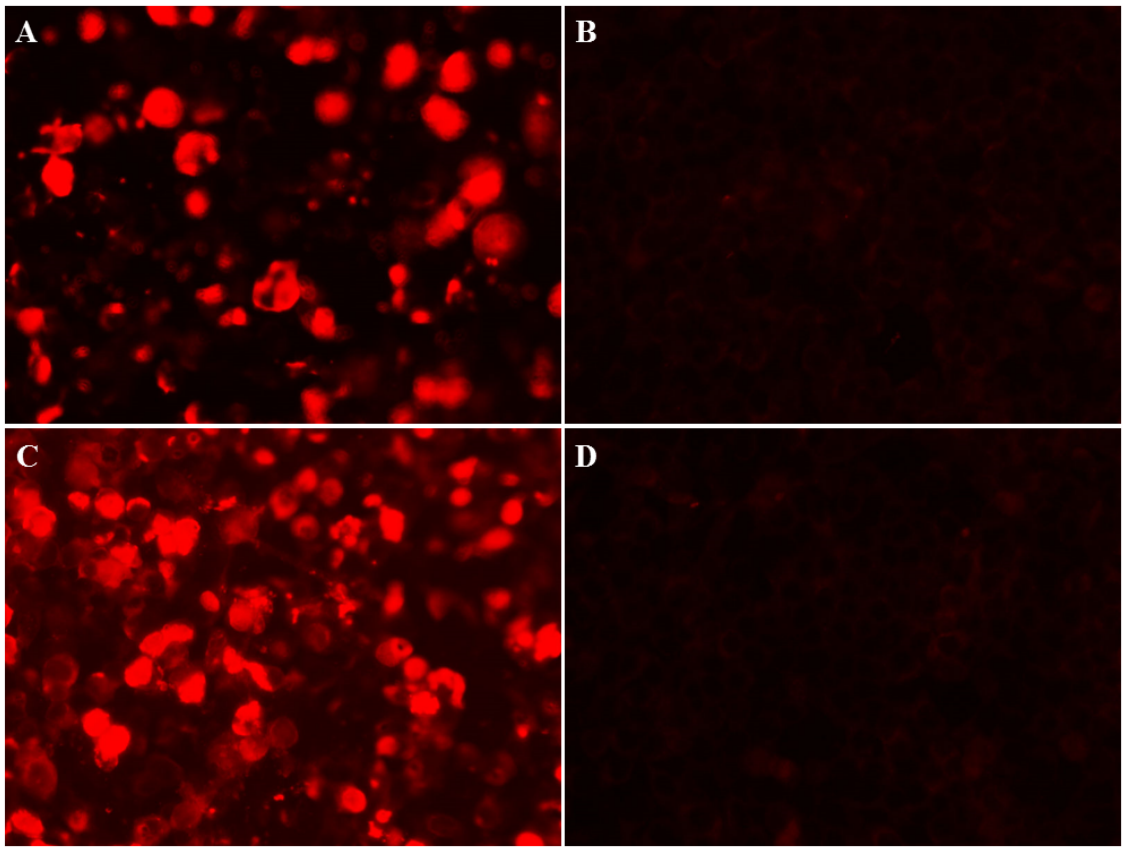

FIGURE 4
Immunofuorescence images in CSF and serum of anti-GAD65 antibody. **(A)** Positive reaction in CSF of the patient was observed in fixed HEK293 cells, with a titer of 1:30. **(B)** A negative control in CSF. **(C)** A positive reaction was detected in the serum, with a titer of 1:100. **(D)** A negative control in serum.

immunosuppressive agent mycophenolate mofetil (MMF). DFPP was performed every other day, filtering 3,300 ml of plasma per session. Membrane filtration type of DFPP was performed every other day, with a total of 3 sessions, filtering 3,300 ml of plasma per session. Plasauto Σ (Asahi Kasei Medical, Tokyo, Japan). Plasmaflo (OP-08W, Asahi Kasei Medical, Tokyo, Japan) and Cascadeflo EC (EC-20W or EC-30W, Asahi Kasei Medical, Tokyo, Japan) were used as the plasma separator and plasma component separator, respectively.

After the third session of DFPP, the patient's coagulation report indicated a lack of clotting, leading to the discontinuation of his apheresis treatment. An intraluminal thrombus was found on the wall of the right femoral vein, and the patient was subsequently treated with low molecular weight heparin (LMWH). However, one week later, the patient developed a high fever (38.8°C) and his platelet count dropped dramatically from 202×10^9/L to 27×10^9/L (Figure 5). D-dimer levels also increased during the same period Figure 5. Ultrasound

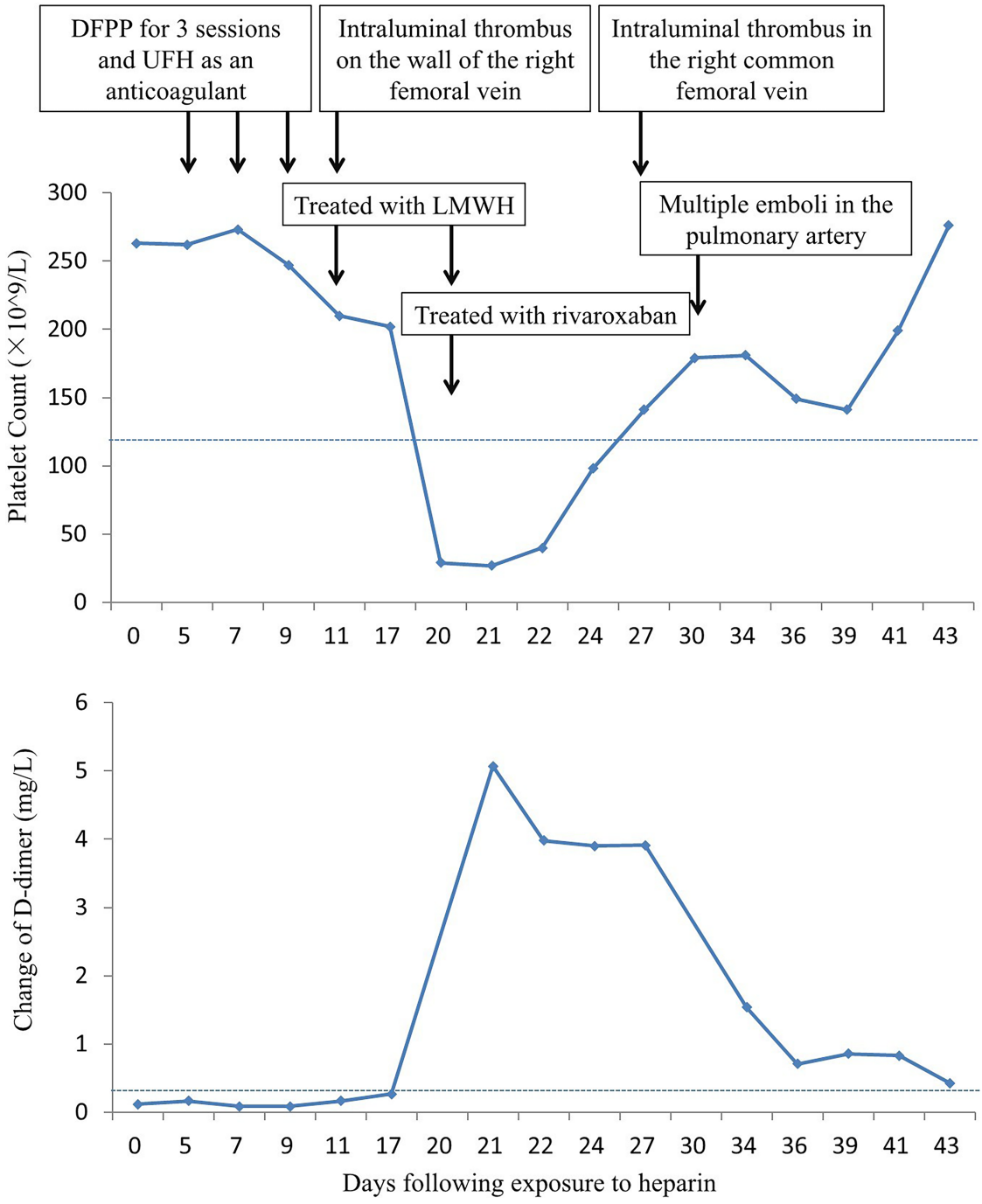

FIGURE 5
Changes of plate count and D-dimer level.

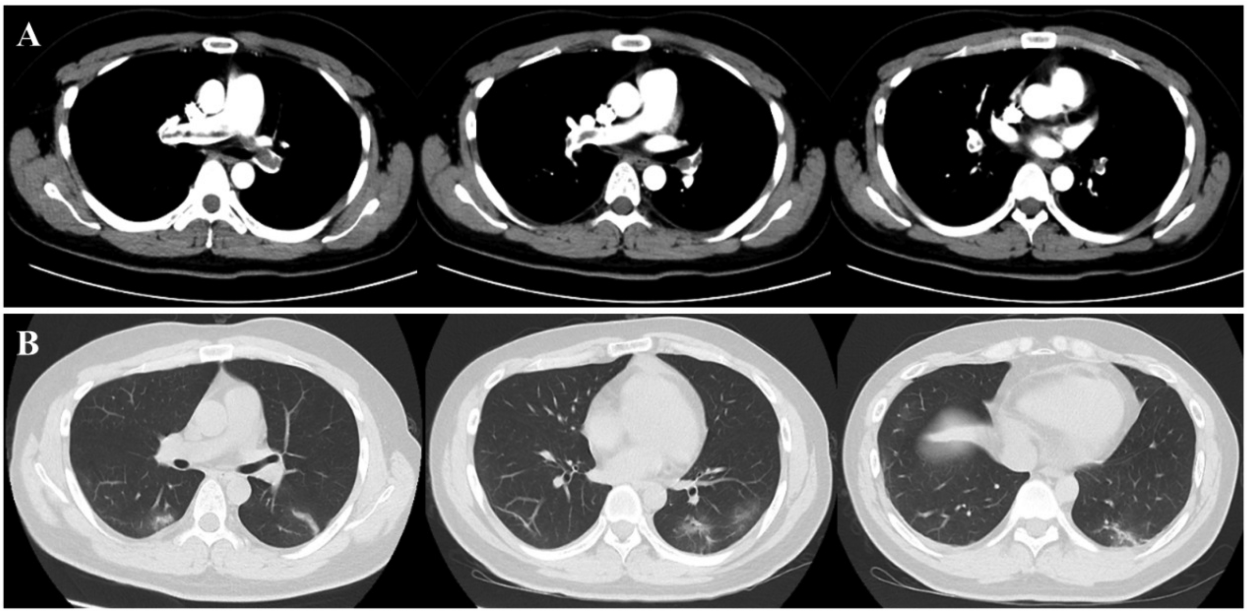

FIGURE 6
Present of thrombus in pulmonary veins and inflammatory changes in lung. The chest CT scan revealed the presence of multiple emboli in the pulmonary artery **(A)**, as well as inflammation **(B)** in the lower lobes of both lungs.

examination revealed the formation of a blood clot in the right common femoral vein, and a computed tomography (CT) scan detected multiple emboli in the pulmonary artery, as well as inflammation in the lower lobes of both lungs (Figure 6). The patient's 4 T score indicated a high probability of heparin-induced thrombocytopenia (HIT), and he tested positive for heparin-PF4 antibodies by ELISA.

As a result, the patient was prescribed the oral anticoagulant rivaroxaban to manage the thrombosis. He also received a platelet transfusion and antibiotic agents. After about a week, his platelet count returned to normal, and his fever, pulmonary inflammation, and pulmonary thrombosis gradually improved. One month later, follow-up examinations including CT and ultrasound showed no presence of emboli in his pulmonary artery or femoral vein, respectively. His epilepsy remained well controlled, and he has not experienced any seizures since then.

Discussion

HIT is a serious complication that can occur when exposed to any form or amount of heparin products. It is also the most frequent non-bleeding complication associated with exposure to UFH. For this patient, except the use during DFPP, LMWH was also used after the intraluminal thrombus on the femoral vein was found, which was almost the same time with clotting deficiency. And pulmonary inflammation and thrombocytopenia occur almost simultaneously. Considering the most characteristic feature of HIT is that it occurs between 5 and 10 days after the start of heparin therapy. We tend to think that infection and independent LMWH as the confounding factors may have facilitated the occurrence of the result, but the use in the DFPP process may play a major role. This condition is characterized by a decrease in platelet counts and an increased propensity for clotting (10). Up to 5% of patients receiving therapeutic doses of heparin may develop HIT, with up to 50% experiencing thromboembolic complications (11). HIT can lead to life-threatening blood clotting, resulting in significant morbidity and mortality rates. It is reported that the mortality rate of patients with HIT during their hospital stay is four times higher compared to those diagnosed with other causes of thrombocytopenia (12). Additionally, patients with HIT have a median length of stay in the hospital that is three times longer, and the cost of their hospitalization is four times higher (12).

The pathophysiology of HIT is complex. Type I HIT is caused by non-immune mechanisms and is more common than Type II HIT, which is an immune reaction mediated by antibodies (13, 14). In Type II HIT, the presence of platelet factor 4 (PF4) binding to heparin triggers the production of antibodies that attack the heparin-PF4 complex, leading to platelet activation via FcγRIIa and severe clot formation (15, 16). However, diagnosing HIT can still be challenging to diagnose and manage and requires the integration of clinical symptoms and laboratory testing.

The current guidelines recommend that when there is suspicion of moderate or high 4Ts score for HIT, exposure of heparin should be immediately discontinued and non-heparin alternative therapy should be used (17). Notably, alternative anticoagulant therapies should be initiated early. It is recommended that direct oral anticoagulants (DOACs), such as direct thrombin inhibitors (DTIs, e.g., argatroban) (18) or Factor Xa inhibitors (e.g., rivaroxaban) (19) could be used in

patients who are clinically stable. A recent study has shown that DOACs are safe and effective for the treatment of HIT, suggesting that the use of DOACs in patients with confirmed or suspected HIT is becoming more common (19).

PE is a crucial therapeutic strategy for acute autoimmune disorders such as AE, myasthenia gravis (MG), acute inflammatory demyelinating polyradiculoneuropathy (AIDP), and neuromyelitis optica spectrum disorder (NMOSD) (7). DFPP is a blood purification technique deriving from the TPE modality and is semi-selective in nature (20, 21). It is widely recognized in the medical community that patients who undergo cardiac surgery and receive dialysis treatment for acute kidney injury are at a significantly higher risk of developing HIT compared to the general population in a hospital setting (22). Although it has been reported in the literatures of the development of HIT in patients undergoing therapeutic plasma exchange (TPE) (23–25), no case report has been found in diseases treated with DFPP.

Accumulating evidence suggests that PE is effective and safe in clinical practice. However, striking a balance between the risks of bleeding and thrombosis is essential. Heparin is commonly used as an anticoagulant during PE procedures (26), but Type II HIT is rarely reported in this context. If HIT occurs, discontinuing heparin is crucial (27). However, if the patient's condition allows, PE can still be continued using a non-heparin anticoagulant strategy. PE is also regarded as a highly effective treatment for HIT because it successfully eliminates the immune response against the heparin-PF4 complex and stops the release of prothrombotic factors resulting from platelet aggregation. As a result, it immediately halts the formation of blood clots (18, 28). In addition to coagulation tests during PE, monitoring platelet counts is essential for timely detection of potential abnormalities.

Conclusion

PE is widely used in clinical practice, especially in neurological autoimmune diseases. Our current patient presented as a typical case of Type II HIT with new-onset thrombocytopenia and extensive venous thrombosis two weeks after heparin exposure during DFPP treatment. Clinicians should be aware of the risk of HIT and the importance of monitoring platelet counts and coagulation parameters during apheresis treatment and exercise caution.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Shanghai Jiaotong University School of Medicine, Renji Hospital Ethics Committee. 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

YC: Data curation, Writing – review & editing. WL: Writing – original draft. LN: Validation, Writing – review & editing. YM: Validation, Writing – review & editing. YZ: Validation, Writing – original draft. WW: 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.

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References

1. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol.* (2016) 15:391–404. doi: 10.1016/s1474-4422(15)00401-9

2. Braczkowski M, Soszyński D, Sierakowska A, Braczkowski R, Kufel K, Łabuz-Roszak B. Autoimmune encephalitis with antibodies: anti-NMDAR, anti-AMPAR, anti-GQ1b, anti-DPPX, anti-CASPR2, anti-LGI1, anti-RI, anti-yo, anti-hu, anti-CV2 and anti-GABAAR, in the course of psychoses, neoplastic diseases, and paraneoplastic syndromes. *Diagnostics (Basel).* (2023) 13(15):2589. doi: 10.3390/diagnostics13152589

3. Shin YW, Lee ST, Park KI, Jung KH, Jung KY, Lee SK, et al. Treatment strategies for autoimmune encephalitis. *Ther Adv Neurol Disord*. (2018) 11:1756285617722347. doi: 10.1177/1756285617722347

4. Abboud H, Probasco JC, Irani S, Ances B, Benavides DR, Bradshaw M, et al. Autoimmune encephalitis: proposed best practice recommendations for diagnosis and acute management. *J Neurol Neurosurg Psychiatry*. (2021) 92:757–68. doi: 10.1136/jnnp-2020-325300

5. Jacob S, Mazibrada G, Irani SR, Jacob A, Yudina A. The role of plasma exchange in the treatment of refractory autoimmune neurological diseases: a narrative review. *J Neuroimmune Pharmacol.* (2021) 16:806–17. doi: 10.1007/s11481-021-10004-9

6. Boedecker SC, Luessi F, Engel S, Kraus D, Klimpke P, Holtz S, et al. Immunoadsorption and plasma exchange-efficient treatment options for neurological autoimmune diseases. *J Clin Apher.* (2022) 37:70–81. doi: 10.1002/jca.21953

7. Connelly-Smith L, Alquist CR, Aqui NA, Hofmann JC, Klingel R, Onwuemene OA, et al. Guidelines on the use of therapeutic apheresis in clinical practice—evidence-based approach from the writing committee of the American society for apheresis: the ninth special issue. *J Clin Apher.* (2023) 38:77–278. doi: 10.1002/jca.22043

8. Brunetta Gavranić B, Bašić-Jukić N, Premužić V, Kes P. Membrane therapeutic plasma exchange with and without heparin anticoagulation. *J Clin Apher*. (2017) 32:479–85. doi: 10.1002/jca.21544

9. Koster A, Nagler M, Erdoes G, Levy JH. Heparin-induced thrombocytopenia: perioperative diagnosis and management. *Anesthesiology*. (2022) 136:336–44. doi: 10.1097/aln.0000000000004090

10. Hvas AM, Favaloro EJ, Hellfritzsch M. Heparin-induced thrombocytopenia: pathophysiology, diagnosis and treatment. *Expert Rev Hematol.* (2021) 14:335–46. doi: 10.1080/17474086.2021.1905512

11. Gallo T, Curry SC, Padilla-Jones A, Heise CW, Ramos KS, Woosley RL, et al. A computerized scoring system to improve assessment of heparin-induced thrombocytopenia risk. *J Thromb Haemost.* (2019) 17:383–8. doi: 10.1111/jth.14359

12. Dhakal P, Giri S, Pathak R, Bhatt VR. Heparin reexposure in patients with a history of heparin-induced thrombocytopenia. *Clin Appl Thromb Hemost.* (2015) 21:626–31. doi: 10.1177/1076029615578167

13. Nonaka T, Harada M, Sumi M, Ishii W, Ichikawa T, Kobayashi M. A case of heparin-induced thrombocytopenia that developed in the therapeutic course of anti-neutrophil cytoplasmic antibody-associated vasculitis. *Case Rep Rheumatol.* (2019) 2019:2724304. doi: 10.1155/2019/2724304

14. Bloom MB, Johnson J, Volod O, Lee EY, White T, Margulies DR. Improved prediction of HIT in the SICU using an improved model of the Warkentin 4-T system: 3-T. *Am J Surg.* (2020) 219:54–7. doi: 10.1016/j.amjsurg.2019.07.039

15. Fathi M. Heparin-induced thrombocytopenia (HIT): identification and treatment pathways. *Glob Cardiol Sci Pract.* (2018) 2018:15. doi: 10.21542/gcsp.2018.15

16. Condon AJ, Hood AJ, Willenborg KL, Kumfer K, Rose AE. Pharmacist involvement in clinical assessment and laboratory testing for heparin-induced thrombocytopenia. *J Thromb Thrombolysis.* (2020) 50:195–200. doi: 10.1007/s11239-019-02011-8

17. Arepally GM, Cines DB. Pathogenesis of heparin-induced thrombocytopenia. *Transl Res.* (2020) 225:131–40. doi: 10.1016/j.trsl.2020.04.014

18. Colarossi G, Maffulli N, Trivellas A, Schnöring H, Hatam N, Tingart M, et al. Superior outcomes with Argatroban for heparin-induced thrombocytopenia: a Bayesian network meta-analysis. *Int J Clin Pharm*. (2021) 43:825–38. doi: 10.1007/s11096-021-01260-z

19. Cirbus K, Simone P, Austin Szwak J. Rivaroxaban and apixaban for the treatment of suspected or confirmed heparin-induced thrombocytopenia. *J Clin Pharm Ther*. (2022) 47:112–8. doi: 10.1111/jcpt.13537

20. Caliskan Y, Mirioglu S, Dirim AB, Ozluk Y, Yegit O, Aksoy E, et al. A comparison of methods of plasmapheresis for the treatment of late antibody mediated rejection in kidney transplant recipients. *Ther Apher Dial.* (2023) 27:428–34. doi: 10.1111/1744-9987.13937

21. Liu L, Ji X, Zhu P, Yang L, Shi J, Zhao Y, et al. Double filtration plasmapheresis for desensitization during haploidentical stem cell transplantation in patients with positive donor-specific anti-HLA antibodies. *Blood.* (2022) 140:7594–7594. doi: 10.1182/blood-2022-165972

22. Hogan M, Berger JS. Heparin-induced thrombocytopenia (HIT): review of incidence, diagnosis, and management. *Vasc Med.* (2020) 25:160–73. doi: 10.1177/1358863X19898253

23. Arachchillage DRJ, Machin SJ, Cohen H. Heparin-induced thrombocytopenia following plasma exchange in patients with demyelinating neurological disease. *Int J Lab Hematol.* (2015) 37:e75–7. doi: 10.1111/ijlh.12316

24. Laird JH, Douglas K, Green R. Heparin-induced thrombocytopenia type II: a rare but significant complication of plasma exchange. *J Clin Apher.* (2006) 21:129–31. doi: 10.1002/jca.20073

25. Limaye K, Ravilla R, Boye B, Sahaya K, Motwani P. Heparin-induced thrombocytopenia in patients receiving plasma exchange. *Am J Med.* (2014) 127:e3–4. doi: 10.1016/j.amjmed.2014.08.004

26. Cervantes CE, Bloch EM, Sperati CJ. Therapeutic plasma exchange: core curriculum 2023. *Am J Kidney Dis.* (2023) 81:475–92. doi: 10.1053/j.ajkd.2022.10.017

27. Cuker A, Arepally GM, Chong BH, Cines DB, Greinacher A, Gruel Y, et al. American society of hematology 2018 guidelines for management of venous thromboembolism: heparin-induced thrombocytopenia. *Blood Adv.* (2018) 2:3360–92. doi: 10.1182/bloodadvances.2018024489

28. Rock G, Weber V, Stegmayr B, Therapeutic plasma exchange (TPE) as a plausible rescue therapy in severe vaccine-induced immune thrombotic thrombocytopenia. *Transfus Apher Sci*, 2021. 60: 103174. doi: 10.1016/j.transci.2021.103174

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Dragana Lazarevic,
University Clinical Center Nis, Serbia
Paulo Ricardo Criado,
Faculdade de Medicina do ABC, Brazil

*CORRESPONDENCE
Shengyou Yu
✉ eyyushengyou@scut.edu.cn

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A case report and literature review of livedoid vasculopathy in children

Jing Qu[1], Zhihong Hao[2], Wang Junli[3] and Shengyou Yu[1,2]*

[1]Department of Pediatrics, Guangzhou First People's Hospital, Guangdong Medical University, Zhanjiang, Guangdong, China, [2]Department of Pediatrics, Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong, China, [3]Department of Pediatrics, The Second Affiliated Hospital, South China University of Technology, Guangzhou, China

Background: Livedoid vasculopathy (LV) is a rare, non-inflammatory, intradermal vascular obstructive skin disorder characterized by purpuric papules and plaques with capillary dilation. These lesions typically progress to crusted ulcers and ultimately result in fixed, white, atrophic stellate scars. The condition is marked by painful ulcers that heal slowly and have a tendency to recur.
Case presentation: We report a case of a pediatric patient presenting with recurrent rashes and pain in both lower extremities. Physical examination revealed purpuric plaques with ulceration, scarring, and white atrophic healing features. Histopathological examination demonstrated intradermal thrombosis, vessel wall necrosis, and surrounding inflammatory cell infiltration with erythrocyte extravasation. Periodic acid-Schiff (PAS) staining was positive. The clinical and pathological findings were consistent with a diagnosis of LV. The patient was treated with oral rivaroxaban.
Conclusion: This case highlights the critical importance of early recognition and intervention in the management of LV. Clinicians should consider LV in the differential diagnosis when encountering patients with painful purpuric rashes. Improvement in pain following treatment with anticoagulants, such as rivaroxaban, may indirectly support the diagnosis. A skin biopsy is essential for definitive diagnosis.

KEYWORDS
livedoid vasculopathy (LV), white atrophy, rivaroxaban, capillaries, children

Introduction

Livedoid vasculopathy (LV) is a rare, non-inflammatory, intradermal vascular obstructive skin disease characterized by painful ulcers, reticular cyanotic macules, and porcelain-white atrophic scarring, predominantly affecting the distal portions of the lower extremities (1–3). Although LV was first described in the early 20th century, it was not until the revision of the International Classification of Diseases (ICD-10) in 2004 that the condition was accurately defined. LV is a chronic, relapsing disorder with a distinct seasonal pattern, typically exacerbating in the summer and remitting in the winter (4). The annual incidence of LV is approximately one case per 100,000 individuals, with a predilection for females (5). Despite its clinical significance, the international diagnostic criteria for LV remain poorly defined, with only an expert consensus published in 2013 providing guidance (6). The non-specific clinical manifestations of LV often necessitate histopathological confirmation via skin biopsy for accurate diagnosis. The most typical pathological feature of LV is focal thrombosis within the dermal capillaries (3).

Given the unique clinical and pathological characteristics of LV, differentiating it from other vasculitic diseases is crucial, making clinical diagnosis particularly challenging. Case reports of LV play a vital role in guiding clinical practice, providing valuable insights for the diagnosis and treatment of this condition. They also contribute to the development of more rational diagnostic and therapeutic strategies for similar patients.

Case presentation

Description of patient

A 13-year-old male patient from Guangdong, China, was admitted to our hospital on 23 September 2023, with a 15-day history of recurrent rash and pain in both lower extremities. The patient had been previously diagnosed with "Henoch–Schönlein purpura (HSP)" in January 2021 due to a generalized red rash. The rash began from the lower limbs and spread to the whole body, which resolved with oral cimetidine and cetirizine. He was discharged for regular checkups, during which he received methotrexate 7.5 mg once a week without any recurrence of the disease. In June 2023, the rash reappeared. The local hospital diagnosed the recurrence of allergic purpura, and the symptoms were relieved after 2 weeks of symptomatic drugs—cimetidine and methylprednisolone.

On admission, scattered old rashes were noted throughout the body, with new red eruptions on both lower limbs that did not fade with pressure, accompanied by breakouts and crusts, without pruritus (Figure 1). Hypopigmented spots with a white atrophic appearance were also observed. Ultrasound examination of the extremities performed at an outside hospital did not reveal any abnormalities in the arteries and veins of both lower extremities.

Clinical findings and diagnostic assessment

Upon admission to our hospital, a comprehensive set of laboratory tests (Table 1) was performed, including assessments of coagulation factors, D-dimer levels, vasculitis markers, humoral immunity, pre-transfusion screening, autoantibody profiles, and others. These tests did not reveal any significant abnormalities, effectively ruling out the possibility of HSP recurrence.

The skin biopsy was requested on September 26. A skin specimen measuring approximately 1 cm × 0.6 cm × 0.5 cm was taken from the right ankle for pathological examination. Microscopic examination of the specimen revealed normal-appearing squamous epithelium on the skin surface, with hyperplasia of small blood vessels in both the superficial and deep dermis, accompanied by a small number of inflammatory cells. No tumor cells were identified. Direct immunofluorescence testing showed negative results for IgG, C3, IgM, and IgA (Figures 2, 3).

After a thorough evaluation of the patient's condition, the skin specimen was reviewed twice in consultation with dermatology. Histopathological examination revealed epidermal hyperkeratosis, intravascular thrombosis, and necrosis of the vessel wall in the dermis, along with perivascular inflammatory cell infiltration and erythrocyte extravasation. Special staining techniques, such as periodic acid-Schiff (PAS) staining, were positive, further confirming the diagnosis of livedoid vasculopathy (LV) (Figure 3).

The patient, a male adolescent, presented with "rash and pain" as the primary symptoms. He had previously been diagnosed with HSP, and his symptoms had improved with treatment. On admission, physical examination revealed new erythematous eruptions on both lower limbs that did not blanch with pressure, accompanied by breakouts and crusts but no pruritus. Laboratory tests evaluated immune antibodies and coagulation function, ruling out HSP and other immune-mediated diseases. Skin biopsy findings suggested non-inflammatory vascular wall damage and fibrinoid necrosis of the vessel wall. Based on these findings, the patient was ultimately diagnosed with LV.

Therapeutic intervention

Following the diagnosis of LV, the patient was initiated on anticoagulation therapy with intravenous heparin and oral rivaroxaban (10 mg once daily). One week later, the patient's pain was significantly relieved, and he was discharged without recurrence of the rash.

Follow-up and outcomes

The patient continued to take rivaroxaban regularly for 2 months. Follow-up visits revealed a significant reduction in the rash compared with the initial presentation, with no new eruptions observed. The patient was discharged with regular follow-up appointments, and his condition showed marked improvement compared with the initial presentation.

Discussion

LV has a strong gender characteristic; the incidence of women is three times that of men, especially in the age group of 15–50 years old (7). As a disease related to coagulation disorders, it is divided into secondary and primary according to the presence or absence of primary disease. It is often associated with multiple stasis, autoimmune connective tissue diseases, tumors, and immune-related diseases (6). The disease is often exacerbated by temperature changes and a hypercoagulable state of the blood (2, 8). Therefore, clinicians must conduct a comprehensive assessment to diagnose LV accurately and manage its underlying causes. In our case, the patient had a history of Henoch–Schönlein purpura (HSP), which significantly increased the risk of vascular fragility and endothelial damage due to an autoimmune attack. This predisposition to vascular endothelial damage further heightened the likelihood of thrombosis. Upon

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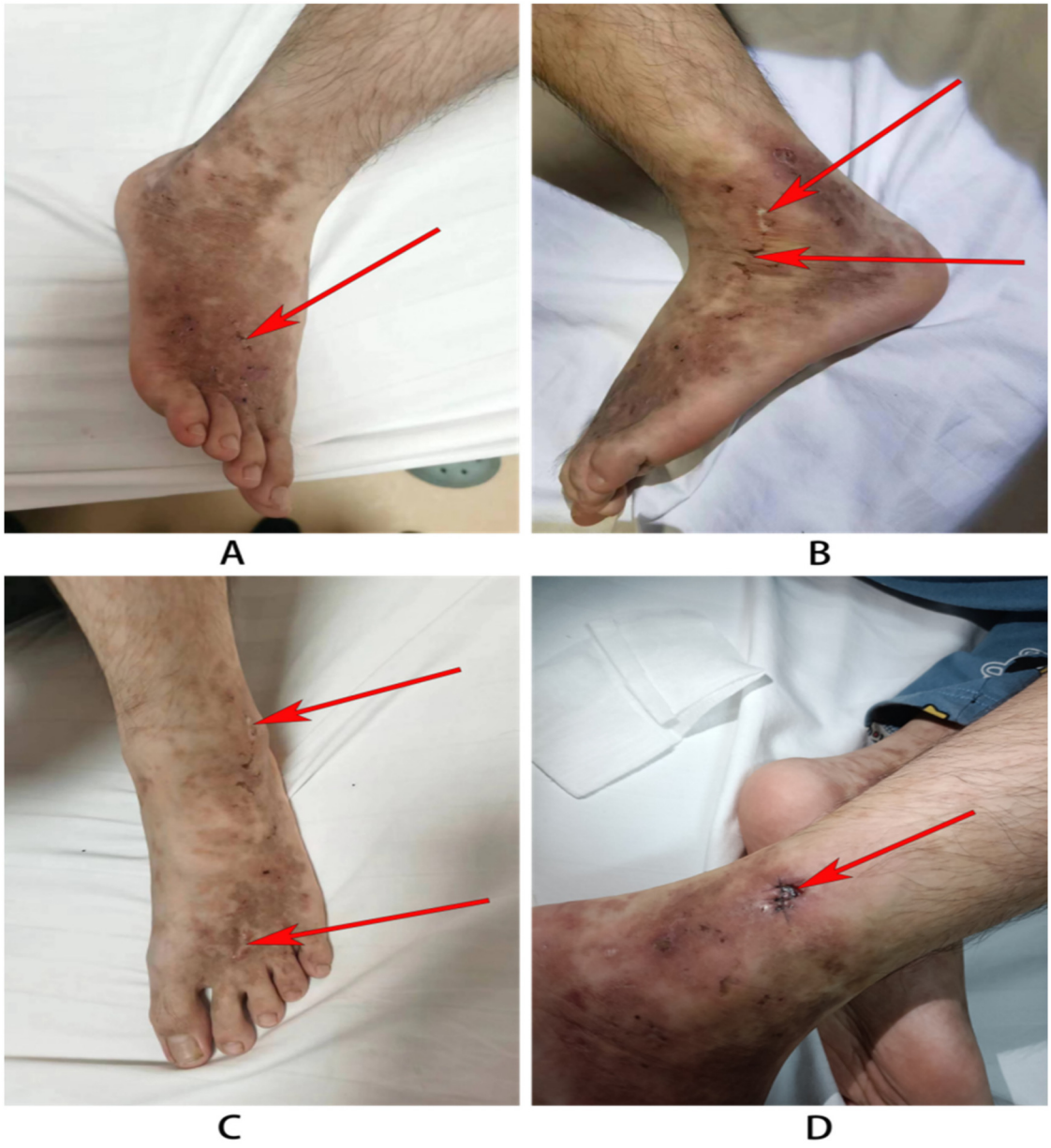

FIGURE 1
Skin rash in the child. **(A)** The left leg of the child: atrophic scarring around lateral malleoli (the red arrow). **(B,C)** The right legs: The arrow indicates porcelain-white atrophic scarring and stellate purpuric macules with telangiectasias. **(D)** Wound recovery after 3 days after dermatological pathology.

admission, we first evaluated and ruled out the recurrence of HSP based on clinical and laboratory findings.

The pathogenesis of LV remains elusive, with abnormal coagulation function being the most likely underlying mechanism. Current research primarily focuses on three aspects: flow disruption, endothelial injury, and coagulation disorders (3). This patient had suffered from the basic disease of allergic purpura, which greatly increased the possibility of vascular fragility and endothelial damage after his own immune attack. It has a high-risk factor for vascular endothelial damage, making thrombosis even easier. In pathology, the three major manifestations of LV disease are segmental hyalinization or fibrinoid degeneration of dermal vessels, proliferation of the endothelium, and intraluminal thrombosis (3, 7). The skin biopsy of this patient showed capillary telangiectasia, extravasation of red blood cells, and intraluminal capillary thrombi. Clinically, the rash presented as erythematous macules that were not significantly elevated above the skin surface and were devoid of itching or pain. The lesions progressed through various stages, including ulceration, white atrophic-like changes, and crusting. LV predominantly affects the lower limbs, with rare involvement of the upper limbs (7). Capillary embolism leads to the formation of small ulcers, which typically develop into characteristic "atrophie blanche" 3–4 months later. This

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TABLE 1 Laboratory data at the admission of the patient.

Item	Data	Experimental test method
Routine blood test		
WBC	$3.88 \times 10 \times 10^9$/L	Flow cytometry
RBC	$4.48 \times 10 \times 10^{12}$/L	Resistance method
HGB	134.00 g/L	SLS–hemoglobin test
PLT	$209.00 \times 10 \times 10^9$/L	Resistance/optical method
Neut#	$1.57 \times 10 \times 10^9$/L	Computation
Lymph#	$1.79 \times 10 \times 10^9$/L	Computation
Coagulation function		
TT	16.2 s	Magnetic bead method
PT_SEC	14.2 s	Magnetic bead method
PT_INR	1.07 INR	Computation
PT%	88%	Computation
APTT	37.9 s	Magnetic bead method
APTT_R	1.11	Computation
FIB	2.84 g/L	Magnetic bead method
D-dimer (D-D)	410 g/L	Immunoturbidimetry
Immunity		
MPO IgG	2.69 AU/ml	Chemiluminescence
PR3 IgG	<2.00 AU/ml	Chemiluminescence
ACA IgG	2.75 GPLU/ml	Chemiluminescence
ACA IgM	<2.00 MPLU/ml	Chemiluminescence
ds-DNA	–	Immunoblotting
rRNP	–	Immunoblotting
ssA	–	Immunoblotting
ssB	–	Immunoblotting
Sm	–	Immunoblotting
nRNP	–	Immunoblotting
Jo-1	–	Immunoblotting
Scl-70	–	Immunoblotting
AauA	–	Immunoblotting
CENP B	–	Immunoblotting
Histone	–	Immunoblotting
ANA	4.68 AU/ml	Chemiluminescence
IgA	1.3000 g/L	Scattering ratio turbidimetry
IgG	10.800 g/L	Scattering ratio turbidimetry
IgM	1.6800 g/L	Scattering ratio turbidimetry
IgE	19.630 ng/ml	Electrochemiluminescence

condition is often accompanied by burning pain, significantly impacting the patient's quality of life, particularly during active disease phases, affecting psychological, physical, and social aspects (9).

In terms of clinical laboratory findings, most cases of LV lack significant diagnostic markers. However, some studies have suggested an association with hypercoagulable states. For instance, a prospective study found elevated levels of hypercoagulability markers, such as fibrinopeptide A, lipoprotein (a), and plasminogen activator inhibitor-1 (10). In our case, only routine laboratory tests were performed, and more specialized studies were lacking. Currently, various therapeutic regimens are employed for LV, yet standardized treatment protocols remain limited. Among monotherapy options, anticoagulants are the most commonly used, followed by systemic glucocorticoids, antiplatelet agents, and immunomodulatory drugs (2, 11). Given the low incidence of LV, research on its treatment is relatively sparse. First-line treatments typically include antiplatelet drugs, such as aspirin and clopidogrel. Rivaroxaban has emerged as a promising therapeutic option due to its safety and ease of use. A comparative study analyzed 20 articles and 138 patients, finding that the average treatment response time was 2.4 months for rivaroxaban vs. 2.3 months for intravenous immunoglobulin (IVIG). This study further validated the efficacy of rivaroxaban (12).

Rivaroxaban is a direct Xa factor inhibitor that has been widely used to treat and prevent major thromboembolic diseases. Compared with other anticoagulants such as low molecular weight heparin and warfarin, rivaroxaban can be taken orally and does not require international normalized monitoring, making it the preferred drug. A study by Kerk et al. (13) first reported a successful case of treating LV with rivaroxaban. Rivaroxaban was gradually becoming familiar to people. In a recent review, approximately 73 patients were counted, and the therapeutic dose of lifasab was 10–20 mg/day. Approximately 82.2% (60/73) responded to the treatment, and their pain and ulcers were relieved (14). A cross-sectional study by Zhao et al. (15) found that it can monitor the active phase through coagulation factor X (IQR: 102.3–132.5 vs. IQR: 92.9–118.8, $P = 0.04$). Moreover, 73% of patients achieved complete remission within 12 weeks of rivaroxaban treatment, with low side effects (25%). It is clear from these studies that rivaroxaban is effective for LV patients. In our case, it also demonstrated that appropriate rivaroxaban use in children can effectively control the progression of LV disease. Adverse reactions were observed rarely, with heavy menstrual bleeding being the most common (13, 14).

Rivaroxaban is a direct factor Xa inhibitor widely used for treating and preventing major thromboembolic diseases. Compared to other anticoagulants, such as low molecular weight heparin and warfarin, rivaroxaban offers the advantage of oral administration without the need for international normalized ratio (INR) monitoring, making it a preferred choice (13). A recent review summarized data from 73 patients, with a therapeutic dose of rivaroxaban ranging from 10 to 20 mg/day. Approximately 82.2% (60/73) of patients responded to treatment, experiencing relief from pain and ulceration (14). A cross-sectional study demonstrated that coagulation factor X levels could be used to monitor disease activity. Moreover, 73% of patients achieved complete remission within 12 weeks of rivaroxaban treatment, with a low incidence of adverse effects (14). These studies collectively highlight the efficacy of rivaroxaban in treating LV. In our case, rivaroxaban effectively controlled the progression of LV in the pediatric patient, with minimal adverse reactions, the most common being heavy menstrual bleeding (13, 14). In addition to rivaroxaban, emerging targeted therapies show promise in treating refractory LV. For instance, tofacitinib, a pan-Janus kinase (JAK) inhibitor, has been reported to be effective in LV treatment (16). Another study demonstrated that anti-interleukin 17 A biologics can also control disease progression (17). In conclusion, the application of targeted therapies has expanded our understanding of the therapeutic mechanisms of LV, bringing the possibility of clinical cure closer to reality.

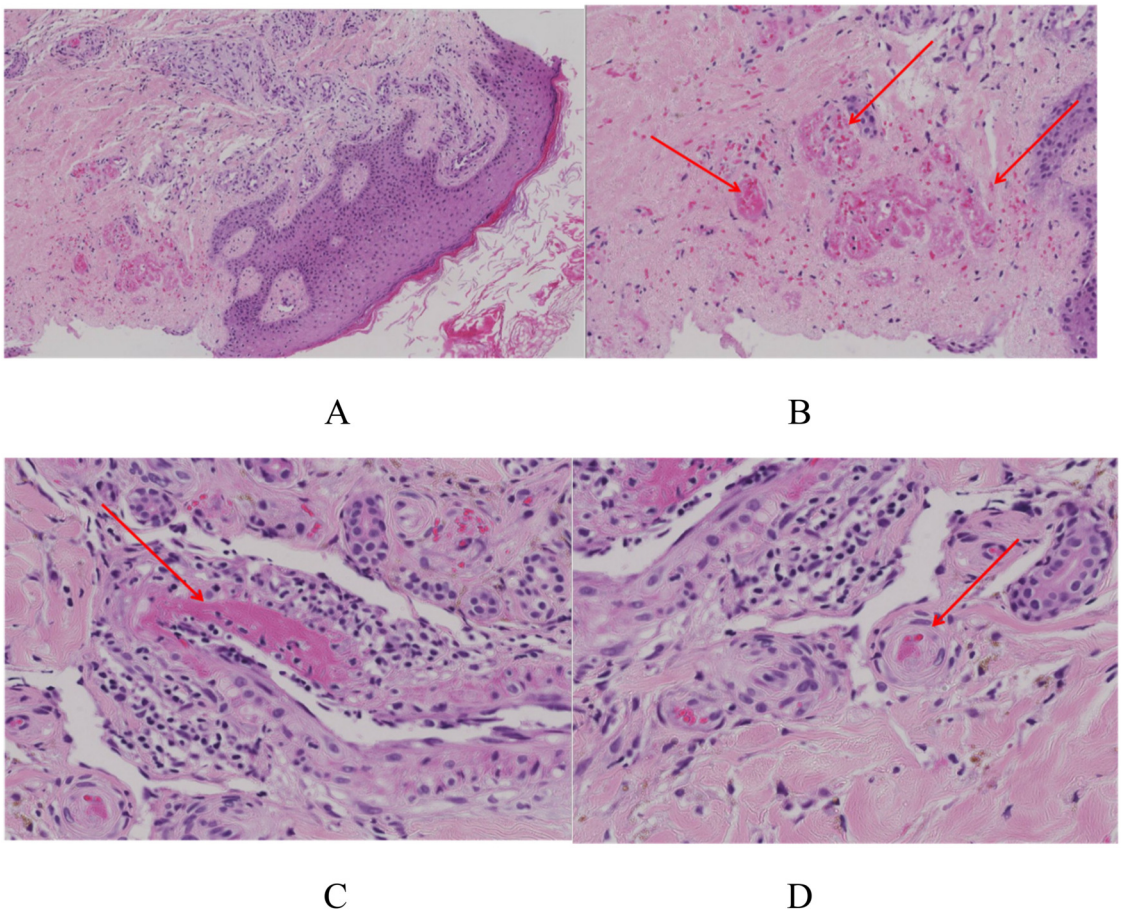

FIGURE 2
The skin biopsy of this child. **(A)** The overall picture of skin HE staining. H&E 8×. **(B)** Capillary telangiectasia, extravasation of red blood cells, and intraluminal capillary thrombi (the red arrows). H&E 10×. **(C)** Livedoid vasculopathy in a skin biopsy with an occlusive, intraluminal thrombus (the red arrows) surrounding hemorrhage. H&E 40×. **(D)** Chronic change: thickening of capillary walls with pink, glassy (hyalinized) collagen (the red arrows). H&E 40×.

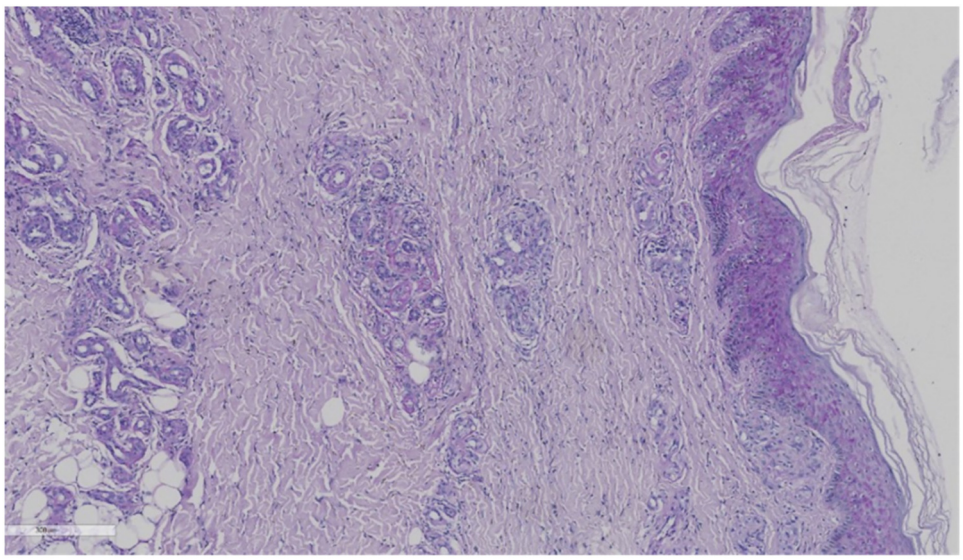

FIGURE 3
Immunofluorescence examination showing that IgG, C3, IgM, and IgA are negative.

Conclusion

When patients have a purple rash with pain, clinicians should consider the possibility of LV. It is important to first assess whether the child has a hypercoagulable state before analyzing the test results. There is no specific clinical test for this disease, and its diagnosis mainly depends on skin biopsy. However, invasive clinical examinations are difficult for family members to accept and increase the complexity of diagnosing and treating the disease. When diagnosis is challenging, anticoagulants such as rivaroxaban for treatment may be considered. If pain improves, it can indirectly confirm the condition. A skin biopsy must focus on dermal vascular occlusion, which is crucial for diagnosis.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics Committee of Guangzhou First People's Hospital. 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. 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

JQ: Investigation, Methodology, Writing – original draft. ZH: Methodology, Software, Writing – original draft. WJ: Data curation, Writing – review & editing. SY: Project administration, Resources, Writing – original draft.

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Conflict of interest

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

Generative AI statement

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

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References

1. Beato Merino MJ, Diago A, Fernandez-Flores A, Fraga J, García Herrera A, Garrido M, et al. Clinical and histopathologic characteristics of the main causes of vascular occusion—part II: coagulation disorders, emboli, and other. *Actas Dermosifiliogr (Engl Ed)*. (2021) 112(2):103–17. doi: 10.1016/j.ad.2020.10.004

2. Bilgic A, Ozcobanoglu S, Bozca BC, Alpsoy E. Livedoid vasculopathy: a multidisciplinary clinical approach to diagnosis and management. *Int J Womens Dermatol*. (2021) 7(5Part A):588–99. doi: 10.1016/j.ijwd.2021.08.013

3. Eswaran H, Googe P, Vedak P, Marston WA, Moll S. Livedoid vasculopathy: a review with focus on terminology and pathogenesis. *Vasc Med*. (2022) 27(6):593–603. doi: 10.1177/1358863X221130380

4. Gao Y, Jin H. Livedoid vasculopathy and its association with genetic variants: a systematic review. *Int Wound J*. (2021) 18(5):616–25. doi: 10.1111/iwj.13563

5. Vasudevan B, Neema S, Verma R. Livedoid vasculopathy: a review of pathogenesis and principles of management. *Indian J Dermatol Venereol Leprol*. (2016) 82(5):478–88. doi: 10.4103/0378-6323.183635

6. Alavi A, Hafner J, Dutz JP, Mayer D, Sibbald RG, Criado PR, et al. Livedoid vasculopathy: an in-depth analysis using a modified Delphi approach. *J Am Acad Dermatol*. (2013) 69(6):1033–1042.e1. doi: 10.1016/j.jaad.2013.07.019

7. Rujitharanawong C, Chularojanamontri L, Trakanwittayarak S, Pinkaew S, Nuttawong S, et al. Livedoid vasculopathy: clinical course and long-term outcome in Asian patients with a review of the literature. *Dermatol. Ther*. (2021) 34(1): e14569. doi: 10.1111/dth.14569

8. Lipsker D. Livedoid vasculopathy: how to diagnose and how to treat? *J Eur Acad Dermatol Venereol*. (2019) 33(9):1627–8. doi: 10.1111/jdv.15830

9. Polo Gascón MR, de Carvalho JF, de Souza Espinel DP, Barros AM, Alavi A, Criado PR. Quality-of-life impairment in patients with livedoid vasculopathy. *J Am Acad Dermatol*. (2014) 71(5):1024–6. doi: 10.1016/j.jaad.2014.06.030

10. Gao Y, Jin H. Plasminogen activator inhibitor-1: a potential etiological role in livedoid vasculopathy. *Int Wound J*. (2020) 17(6):1902–8. doi: 10.1111/iwj.13480

11. Wen J, Li XH, Yang QP, Yu JB. Clinical and dermoscopic features of livedoid vasculopathy. *Chin Med J (Engl)*. (2020) 133(17):2137–8. doi: 10.1097/CM9.0000000000001048

12. Ramphall S, Rijal S, Prakash V, Ekladios H, Mulayamkuzhiyil Saju J, Mandal N, et al. Comparative efficacy of rivaroxaban and immunoglobulin therapy in the treatment of livedoid vasculopathy: a systematic review. *Cureus*. (2022) 14(8): e28485. doi: 10.7759/cureus.28485

13. Kerk N, Drabik A, Luger TA, Schneider SW, Goerge T. Rivaroxaban prevents painful cutaneous infarctions in livedoid vasculopathy. *Br J Dermatol*. (2013) 168(4):898–9. doi: 10.1111/bjd.12100

14. Gao Y, Jin H. Rivaroxaban for treatment of livedoid vasculopathy: a systematic review. *Dermatol Ther*. (2021) 34(5):e15051. doi: 10.1111/dth.15051

15. Zhao C, Yu M, Cheng X, Li S, Feng S. Associations between coagulation factors and livedoid vasculopathy and the efficacy and safety of rivaroxaban in patients with livedoid vasculopathy: a retrospective study. *Clin Exp Dermatol*. (2024) 49(8):817–24. doi: 10.1093/ced/llae023

16. Jia E, Yan G, Xiao M, Geng H, Wei J, Zhang J. Refractory ulcerations associated with livedoid vasculopathy successfully treated with tofacitinib. *Dermatol Ther*. (2020) 33(6):e14470. doi: 10.1111/dth.14470

17. Qi F, Gao Y, Jin H. Anti-Interleukin 17A biologic therapy attempts on livedoid vasculopathy: a report of case series. *Clin Cosmet Investig Dermatol*. (2024) 17:1043–8. doi: 10.2147/CCID.S455903

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EDITED BY
Panagiota Anyfanti,
Aristotle University of Thessaloniki, Greece

REVIEWED BY
Mohammed Elfatih Hamida,
Orotta College of Medicine and Health Sciences, Eritrea
Dahlia N. Alsaidi,
Ibn Sina University for Medical and Pharmaceutical Sciences, Iraq

*CORRESPONDENCE
Hang Li
hang297li@126.com

†These authors have contributed equally to this work

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Correlation between severe acute respiratory syndrome coronavirus 2 infection and serum anti-neutrophil cytoplasmic antibody formation

Hao Huang†, Yu-Jie Zang†, Zheng-Guo Li, Qian Li, Chun-Yan Ma, Hai-Yuan Zhao and Hang Li*

Department of Laboratory Medicine and Key Laboratory of Tumor Marker Detection, Kaifeng Central Hospital, Kaifeng, Henan, China

Objective: To investigate the impact of SARS-CoV-2 infection on the formation of antineutrophil cytoplasmic antibodies (ANCA).

Methods: Serum samples from 154 patients infected with SARS-CoV-2 were collected and tested for ANCA and antinuclear antibodies (ANA) using indirect immunofluorescence assay (IFA). Enzyme-linked immunosorbent assay (ELISA) was used to confirm the target antigens in ANCA fluorescence-positive samples. Eighty-seven healthy individuals were selected as the control group. Statistical analysis was performed using SPSS 26.0 software, with $P < 0.05$ considered statistically significant.

Results: The ANCA fluorescence positivity rate in the control group (1.1%) was not significantly different from that in COVID-19 positive patients (2.5%) (χ^2 = 0.574, $P > 0.05$). Among COVID-19 positive patients, 4 cases (2.5%) were ANCA fluorescence positive, while 10 cases (6.4%) were ANA positive. The difference between these two rates was not statistically significant (χ^2 = 2.694, $P > 0.05$).

Conclusion: The Omicron variant of SARS-CoV-2 has minimal association with the formation of serum ANCA.

KEYWORDS

anti-neutrophil cytoplasmic antibody, antinuclear antibody, indirect immunofluorescence assay, infection, severe acute respiratory syndrome coronavirus

1 Introduction

Since December 2019, when pneumonia cases caused by the novel coronavirus (severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) were first identified both domestically and internationally, SARS-CoV-2 has become a source of widespread concern. Research has demonstrated that the serum of individuals infected with COVID-19 contains various autoantibodies, including antinuclear antibodies (ANA), anti-Sjögren's syndrome A (SSA) antibodies, anti-Ro-52 and anti-Ro-60 antibodies, antiphospholipid antibodies (APL), anti-interferon (IFN)-α and anti-IFN-1 antibodies, anti-interleukin (IL) antibodies, anti-chemokine antibodies, and anti-cardiolipin antibodies (1). Numerous studies have reported that these autoantibodies not only contribute to specific clinical manifestations in patients with COVID-19 but are also closely linked to disease severity (2–6).

Research has demonstrated that infections caused by certain viruses, bacteria, and fungi are associated with the formation of antineutrophil cytoplasmic antibodies (ANCA) (7). ANCA serves as a crucial laboratory marker for antineutrophil cytoplasmic antibody-associated vasculitis (AAV), with indirect immunofluorescence assay (IFA) being the primary detection method (8–10). Recent studies have identified multiple shared key genes between AAV and SARS-CoV-2 (11). However, there are currently no reports on ANCA formation in the serum of SARS-CoV-2-infected patients without AAV. This study employed IFA and enzyme-linked immunosorbent assay (ELISA) to detect ANCA and its spectrum in the serum of patients with SARS-CoV-2 infection to determine whether SARS-CoV-2 infection is associated with ANCA formation.

2 Participants and methods

2.1 Research participants

A random sampling method was employed to select 154 patients who tested positive for SARS-CoV-2 via oropharyngeal swab nucleic acid testing and visited Kaifeng Central Hospital between February and September 2024 as the study group (observation group). This group comprised 77 males and 77 females, with ages ranging from 17 to 98 years and a mean age of 70.7 years. The inclusion criteria were based on the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 8, Revised Edition) (12), which classified the patients into clinical categories as follows: 8 mild cases, 115 moderate cases, 14 severe cases, and 17 critical cases. Additionally, 87 healthy individuals who tested negative for SARS-CoV-2 were selected as the control group, including 26 males and 61 females, with ages ranging from 24 to 81 years and a mean age of 50.6 years. All study participants were screened to exclude those with antineutrophil cytoplasmic antibody-associated vasculitis. This study was approved by the Ethics Committee of Kaifeng Central Hospital (Approval No.: kt2024-013), and informed consent was obtained from all participants.

2.2 Methods

2.2.1 ANCA detection

The Anti-Neutrophil Cytoplasmic Antibody IgG Detection Kit (Euroimmun, Germany) using an indirect IFA was employed to detect anti-neutrophil cytoplasmic antibodies. All procedures were performed manually in strict accordance with the kit's instructions. The Eurostar III Plus fluorescence microscope (Euroimmun, Germany) was used for manual slide reading, with a titer of ≥ 1:10 considered positive. ANCA fluorescence results were classified as pANCA, cANCA, or atypical ANCA, following the relevant expert consensus (13).

2.2.2 ANA detection

The Antinuclear Antibody IgG Detection Kit (Euroimmun, Germany) using an indirect IFA was employed to detect antinuclear antibodies. All procedures were performed manually in strict accordance with the kit's instructions. The fluorescence pattern was interpreted based on the 2021 International Consensus on Antinuclear Antibody Fluorescence Pattern (ICAP) (14). The EUROSTAR III Plus fluorescence microscope (Euroimmun, Germany) was used for manual slide reading, with a titer of ≥ 1:100 considered positive.

2.2.3 ANCA spectrum detection

The ANCA Profile IgG Detection Kit (Euroimmun, Germany) using an ELISA was employed to detect the ANCA spectrum, which included proteinase 3 (PR3), lactoferrin (LF), myeloperoxidase (MPO), elastase (EL), cathepsin G (CATG), and bactericidal/permeability-increasing protein (BPI). Manual procedures were strictly performed according to the kit's instructions. Results were semi-quantitatively determined using the ratio calculation formula provided in the kit. For PR3 detection, a ratio × 1.4 < 1.0 was considered negative, 1.0 ≤ ratio × 1.4 < 2.0 was weakly positive, 2.0 ≤ ratio × 1.4 < 5.0 was positive, and ratio × 1.4 ≥ 5.0 was strongly positive. For the detection of other antigens, a ratio < 1.0 was negative, 1.0 ≤ ratio < 2.0 was weakly positive, 2.0 ≤ ratio < 5.0 was positive, and ratio ≥ 5.0 was strongly positive.

2.3 Statistical analysis

IBM SPSS Statistics 26 software was utilized for statistical analysis and description, with count data presented as case numbers or rates. Comparisons of rates were conducted using the χ^2 test or Fisher's exact probability test, with a significance threshold of $p < 0.05$. Logistic regression analysis evaluates the predictive factors for ANCA formation.

3 Results

3.1 Comparison of ANCA positivity rates among COVID-19 patients of different severities

Using IFA to test 154 COVID-19 positive serum samples, 4 cases (2.5%) were ANCA positive. In the control group of 87

individuals, 1 case (1.1%) was ANCA positive. Comparison between these two groups yielded a χ^2 value of 0.574, with P > 0.05, indicating no statistically significant difference. Additionally, when comparing the ANCA positivity rates among COVID-19 patients of different severities, the χ^2 value was 0.990, with P > 0.05, indicating no statistically significant difference (see Table 1).

3.2 IFA detection of ANCA formation in 154 COVID-19 positive serums

Among the 4 cases (2.5%) that tested positive for ANCA fluorescence, the distribution was as follows: 1 case (0.6%) was pANCA with a titer of 1:100; 1 case (0.6%) was cANCA with a titer of 1:32; 1 case (0.6%) was atypical pANCA with a titer of 1:10; and 1 case (0.6%) was atypical ANCA with a titer of 1:10. Using IFA to test COVID-19 positive serum samples, 96.1% (148/154) were ANCA negative on ethanol-fixed substrate slides, while 1.9% (3/154) were pANCA-positive, 1.2% (2/154) were cANCA-positive, and 0.6% (1/154) were atypical ANCA-positive. On formaldehyde-fixed substrate slides, 3 cases (1.9%) tested positive. The fluorescence patterns are presented in Figure 1. All serum samples with fluorescence (5.2%, 6/115) belonged to patients with moderate cases. Comparing the ANCA fluorescence positivity rates among the four groups, χ^2 = 1.345, P > 0.05, indicating no statistically significant difference.

Binary logistic regression analysis was performed to assess the predictive factors for ANCA formation in the observation group, considering variables such as age, gender, and comorbidities including diabetes mellitus, coronary artery disease, malignancy, cerebral infarction, renal insufficiency, and positive hepatitis B surface antigen. The P-values for all variables were > 0.05, indicating no significant impact (see Table 2).

3.3 Detection of ANA in 154 COVID-19 positive serum samples using IFA

Ten cases (6.4%) tested positive for antinuclear antibody fluorescence, with titers ranging from 1:100 to 1:1000 among 154 COVID-19-positive serum samples. Among these, single nuclear patterns included 0.1% (1/10) with a homogeneous nuclear pattern (AC-1), 0.1% (1/10) with a dense fine speckled nuclear pattern (AC-2), 0.2% (2/10) with a centromere pattern (AC-3), 0.1% (1/10) with a coarse speckled nuclear pattern (AC-5), 0.1% (1/10) with a dense fine speckled cytoplasmic pattern (AC-19), and 0.1% (1/10) with a NuMA-type spindle apparatus pattern (AC-26). Composite nuclear patterns included 0.1% (1/10) with a homogeneous nuclear pattern (AC-1) + fine speckled nuclear pattern (AC-4), 0.1% (1/10) with a centromere pattern (AC-3) + fine speckled nuclear pattern (AC-4), and 0.1% (1/10) with a homogeneous nuclear pattern (AC-1) + punctate nuclear membrane pattern (AC-12). The fluorescence patterns are presented in Figure 2. A comparison of the 10 cases (6.4%) testing positive for antinuclear antibody with the 4 cases (2.5%) testing positive for ANCA yielded a p value of 0.101 (> 0.05), χ^2 = 2.694, indicating no statistically significant difference. Among moderate cases, 7.8% (9/115) were antinuclear antibody positive, while among severe cases, 7.1% (1/14) were antinuclear antibody positive.

TABLE 1 Comparison of ANCA positivity rates among COVID-19 patients of different severities.

Disease classification	Positive casw(%)	Negative case(%)	χ2	P value
Mild	0(0)	8(5.2)	0.99	0.804
Moderate	4(2.5)	115(74.7)		
Severe	0(0)	14(9.1)		
Critical	0(0)	17(11.0)		
Total	4	150		
Control group	1(1.1)	86(98.9)	0.574	0.449

3.4 Confirmation of ANCA spectrum in samples positive on ethanol or formaldehyde-fixed substrate slides

An analysis of 154 COVID-19-positive serum samples identified six cases exhibiting fluorescence on ethanol-fixed or formaldehyde-fixed substrate slides. ANCA spectrum testing revealed that 16.7% (1/6) tested strongly positive for anti-PR3 antibody, 16.7% (1/6) were positive for anti-elastase antibody, and 33.3% (2/6) were weakly positive and positive for anti-BPI antibody, respectively. Anti-lactoferrin antibody, anti-MPO antibody, and anti-cathepsin G antibody were not detected. Figure 3 presents the positive ANCA spectrum results.

3.5 Confirmation of ANCA spectrum in severe and critical cases

Serum samples from 14 severe and 17 critical patients were tested for the ANCA spectrum. In the severe cases, 14.3% (2/14) tested positive for anti-bactericidal/permeability-increasing protein (BPI) antibodies, while detection rates for other antibodies remained at 0. In the critical cases, no antibodies were detected. Due to the limited number of samples in both groups, only the positivity rates were calculated without statistical analysis. The distribution of ANCA fluorescence and ANCA spectrum positivity is shown in Table 3.

4 Discussion

In the formation of ANCA, viruses such as human immunodeficiency virus, hepatitis B virus, hepatitis C virus, parvovirus B19, Epstein-Barr virus, arbovirus, and Ross River virus have all been proven to induce ANCA positivity, yet their clinical diagnostic significance is limited (7). Previous studies in

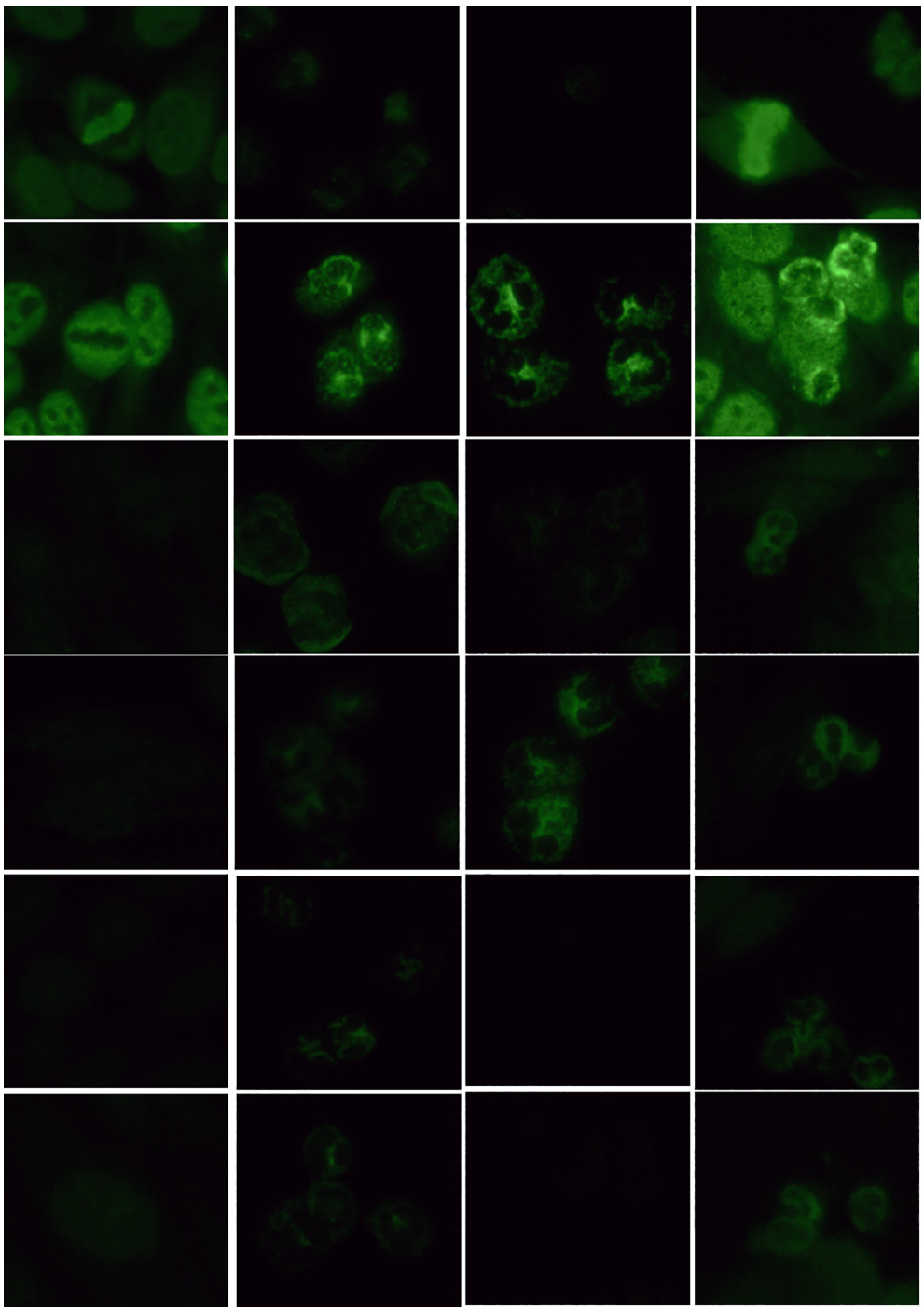

FIGURE 1
Detection of ANCA fluorescence patterns in COVID-19 nucleic acid-positive serum using IFA (40x objective lens). (1) HEp-2 cell substrate (dilution ratio 1:100); (2) granulocytes (ethanol-fixed) refers to ethanol-fixed human neutrophil granulocytes matrix (dilution ratio 1:10); (3) granulocytes (formaldehyde-fixed) refers to formaldehyde-fixed human neutrophil granulocytes matrix (dilution ratio 1:10); (4) HEp-2 + granulocytes refers to a mixed matrix of ethanol-fixed HEp-2 cells and human neutrophil granulocytes (dilution ratio 1:10).

China have found that the positivity rate of ANCA in the general population is 2.29% (15), which is comparable to the ANCA positivity rate of 2.5% observed in COVID-19 patients in our study. It is believed that the formation of ANCA during infection may involve several potential mechanisms: autoimmune reactions triggered by the complementarity of microbial peptides and self-antigens; upregulation of self-antigen genes due to epigenetic silencing and antigen complementarity; molecular mimicry between bacterial antigens and self-antigens; the formation of neutrophil extracellular traps (NETs) and the ensuing immune

TABLE 2 Logistic regression analysis of factors associated with ANCA formation.

Item	B	Standard Error	Wald	Significance	Exp(B)	95% Confidence Interval for EXP(B)	
						Lower	Upper
Age	0.007	0.038	0.034	0.854	1.007	0.935	1.085
Gender	1.212	1.213	0.998	0.318	3.359	0.312	36.208
Diabetes	-16.899	9852.771	0.000	0.999	0.000	0.000	.
Coronary Artery Disease	0.314	1.255	0.063	0.803	1.369	0.117	16.013
Malignancy	1.566	1.320	1.408	0.235	4.789	0.360	63.667
Cerebral Infarction	-17.341	8178.340	0.000	0.998	0.000	0.000	.
Renal Insufficiency	-17.695	14882.113	0.000	0.999	0.000	0.000	.
Hepatitis B Surface Antigen Positive	-15.617	15827.706	0.000	0.999	0.000	0.000	.
Constant	59.546	25218.170	0.000	0.998	725540094915593900000000000.000		

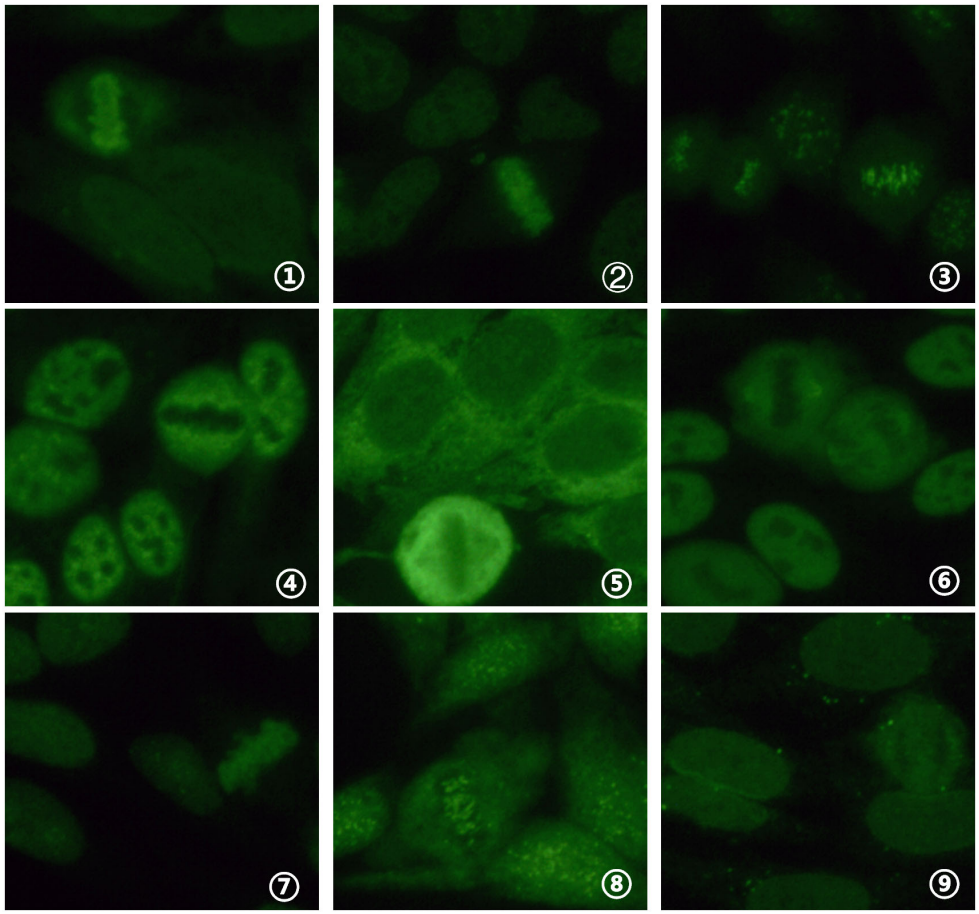

FIGURE 2
Detection of ANA fluorescence patterns in COVID-19 nucleic acid-positive serum using IFA (40x objective lens). (1) homogeneous nuclear pattern (AC-1); (2) dense fine speckled nuclear pattern (AC-2); (3) centromere pattern (AC-3); (4) coarse speckled nuclear pattern (AC-5); (5) dense fine speckled cytoplasmic pattern (AC-19); (6) NuMA-type spindle apparatus pattern (AC-26); (7) homogeneous nuclear pattern (AC-1) + fine speckled nuclear pattern (AC-4); (8) centromere pattern (AC-3) + fine speckled nuclear pattern (AC-4); (9) homogeneous nuclear pattern (AC-1) + punctate nuclear membrane pattern (AC-12).

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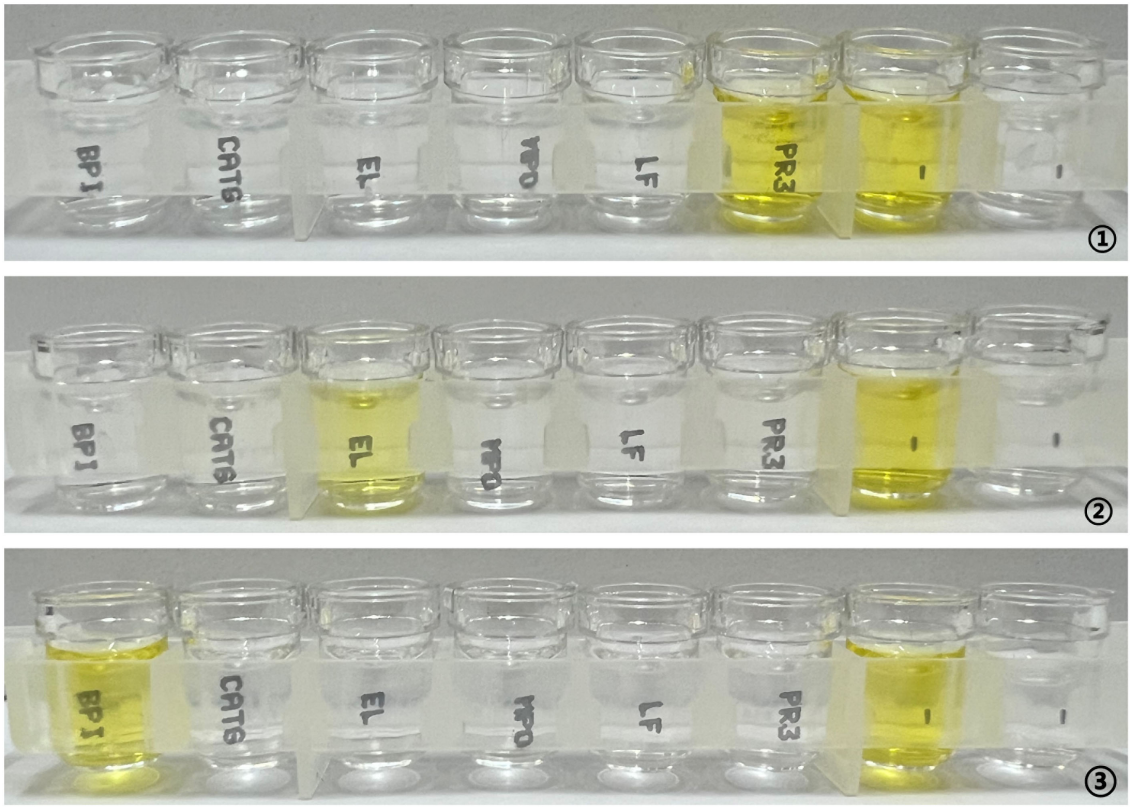

FIGURE 3
ANCA spectrum detection in samples positive for ethanol- or formaldehyde-fixed substrate slides using ELISA. (1) anti-PR3 antibody ratio strongly positive; (2) anti-elastase antibody ratio positive; (3) anti-bactericidal/permeability-increasing protein antibody ratio positive.

processes, including the production of ANCA; and the interaction between bacterial components and Toll-like receptors (TLRs), leading to the formation of immune reaction mediators that may trigger the production of ANCA (16).

Gregg E et al. (17) found that the positivity rate of ANA in the general US population is 11%–15.9%, while in China, the positivity rate of ANA in the general population ranges from 7.4% to 13.5% (18). In our study, the positivity rate of ANA in COVID-19 patients (6.4%) is not significantly different from that in the general Chinese population.One study analyzed 12 types of autoantibodies in 21 patients with severe and critical COVID-19 infections, reporting detection rates of 50% for ANA, 25% for anti-Ro-60 antibodies, 20% for anti-Ro-52 antibodies, 5% for anti-DNA topoisomerase I (Scl-70) antibodies, and 5% for anti-U1 ribonucleoprotein (U1-RNP)

TABLE 3 Distribution of ANCA spectrum positivity.

ANA fluorescence pattern	Granulocytes (Ethanol)	Granulocytes (Formaldehyde)	ANCA interpretation result	ANCA spectrum
AC-1	pANCA	negative	negative	BPI
AC-5	pANCA	cANCA	pANCA	Elastase
negative	aytpicalANCA	cANCA	aytpicalANCA	negative
negative	cANCA	cANCA	cANCA	PR3
negative	pANCA	negative	aytpicalANCA	negative
negative	cANCA	negative	negative	BPI
negative	negative	negative	negative	BPI
AC-3+AC-4	negative	negative	negative	BPI

Among the 4 samples with positive ANCA fluorescence interpretations, 2 had positive ANCA spectrum results. In the 2 samples that only showed fluorescence on ethanol-fixed substrate slides and were interpreted as ANCA negative, BPI was detected in both. Two samples had only BPI positivity with ANCA negativity.

antibodies (4). Findings from this study indicate whether ANCA formation was detected using IFA in serum samples from patients having mild, moderate, severe, and critical COVID-19. Alternatively, ELISA was used in fluorescence-patterned serum samples and severe and critical cases, and detection rates were considerably lower than those reported in prior research on other autoantibodies (4). Pascolini et al. identified autoantibodies in 33 COVID-19 patients and found 33% were ANA positive (5). In contrast, the overall ANA positivity rate in COVID-19 serum samples in this study was 6.4%, with a positivity rate of only 3.2% in severe and critical cases, differing from previous ANA detection rates in patients with COVID-19 (4, 5).

Possible reasons for these findings include differences in study periods and viral strains. The earlier studies were conducted during the first half of 2020 at the onset of the SARS-CoV-2 pandemic and examined the early wild-type strain. Research from 2023 demonstrated that changes in innate immune phenotypes and epigenetic programming of hematopoietic stem cells and progenitor cells persisted for months to a year after SARS-CoV-2 infection, influencing post-COVID immunity and recovery (19). In contrast, this study focused on the Omicron variant JN.1 from the first half of 2024 in China (20). By this time, four years had passed since the initial outbreak, most individuals had COVID antibodies, and no literature has reported the effects of COVID-19 infection on the body after 2023. The production of antibodies, recovery processes, and the virulence of variant strains may have significantly declined compared to the wild-type strain, which could contribute to the lower autoantibody production.

Additionally, studies on human leukocyte antigen (HLA) alleles in patients with COVID-19 have established links between HLA variations and disease severity (21, 22). Research indicates that different HLA types exhibit varying capacities to bind to the virus. An analysis of HLA allele frequency distribution in 99 Italian patients identified significant associations between SARS-CoV-2 susceptibility and HLA-DRB1*15:01 and HLA-DQB1*06:02 (23). Globally, only 0.63% of the population carries the HLA-B*15:03 allele, which confers relative resistance to SARS-CoV-2 infection. The prevalence of this genotype's presence in China is at a medium level on a worldwide ranking (24). Therefore, genetic factors related to HLA allele carriage may also influence autoantibody formation.

Simultaneous detection of ANCA using both IFA and ELISA can enhance the specificity of disease diagnosis and broaden its clinical application. ANCA serves as a specific serological marker for ANCA-associated vasculitis, with target antigens MPO and PR3 playing a major role in the diagnosis of microscopic polyangiitis and granulomatosis with polyangiitis, respectively. Other target antigens are rarely or never detected in autoimmune diseases (25). Our study found that among the two specimens positive by both IFA and ELISA, one case was pANCA and elastase positive, clinically diagnosed with gallbladder malignancy with liver and lymph node metastases; the other case was cANCA and PR3 positive, diagnosed with infectious fever. The application of ANCA is not limited to vasculitis but can occur due to environmental exposure, medication use, or various disease processes. Conditions associated with ANCA beyond vasculitis include chronic inflammatory diseases, other autoimmune disorders, malignancies, leukemia, and infections (7). Since both ANCA-positive cases in our study were linked to conditions unrelated to vasculitis, the findings do not indicate a direct association between ANCA formation and SARS-CoV-2 infection. Whether these two patients will later develop AAV or other autoimmune diseases requires long-term follow-up.

For the two samples in our study that were interpreted as atypical ANCA by fluorescence but did not show detectable target antigens, research has highlighted inconsistencies between ANCA detection using IFA and target antigen confirmation. These discrepancies may arise from various factors, including the choice of detection method, differences in sample processing, and changes in antibody specificity. Additionally, the presence of ANA may interfere with ANCA detection, leading to false-positive results (26). Our study employed a multi-matrix combination for ANCA detection, effectively excluding ANA interference. However, the possibility of unknown target antigens cannot be ruled out.

Studies have shown that patients with negative IFA results but positive ELISA findings are often linked to non-vascular inflammatory tissue diseases, hypertension, and heart disease (27–29). This observation suggests that these patients may have complex immune responses and inflammatory mechanisms. Chronic inflammation is common in individuals with hypertension and heart disease, potentially leading to abnormal immune system responses that influence IFA and ELISA results. BPI, a soluble protein in neutrophil granules, plays a role in defense against gram-negative bacteria. Anti-BPI antibodies are frequently detected in patients with cystic fibrosis and Pseudomonas infections (30).

In our study, only two severe cases matched the above reports, and no direct evidence confirms a link between BPI formation and COVID infection. In summary, the impact of SARS-CoV-2 on the general population has gradually weakened since its outbreak. However, whether long-term immune system abnormalities persist requires further investigation.

The limitations of this study mainly lie in the limited sample size and the single source of samples. This leads to insufficient statistical power, which may affect the accurate assessment of the association between SARS-CoV-2 infection and the formation of serum ANCA, and makes it difficult to comprehensively reflect the overall situation of patients during the prevalence of different variants in different regions, thus limiting the generalizability of the study results. Future studies should increase the sample size and cover patients from more regions and infected with different variants to enhance statistical power and verify the findings of this study.

5 Conclusion

Only two cases in severe and critical patients matched the above reports, and no direct evidence confirms a link between BPI formation and COVID infection. In summary, the impact of SARS-CoV-2 on the general population has gradually weakened

since its outbreak. However, whether long-term immune system abnormalities persist requires further investigation.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Department of Laboratory Medicine and Key Laboratory of Tumor Marker Detection Kaifeng Central Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HH: Data curation, Formal analysis, Writing – original draft. YZ: Data curation, Writing – original draft, Formal analysis. ZL: Writing – original draft, Data curation, Formal analysis. QL: Resources, Writing – review & editing, Software. CM: Writing – review & editing, Resources, Software. HZ: Resources, Writing – review & editing, Methodology. HL: Writing – review & editing, Methodology, Conceptualization.

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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.

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References

1. Macela A, Kubelkova K. Why does SARS-coV-2 infection induce autoantibody production? *Pathogens.* (2021) 10:380.

2. Zuo Y, Estes SK, Ali RA, Gandhi AA, Yalavarthi S, Shi H, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. *Sci Transl Med.* (2020) 12:eabd3876. doi: 10.1126/scitranslmed.abd3876

3. Franke C, Ferse C, Kreye J, Reincke SM, Sanchez-Sendin E, Rocco A, et al. High frequency of cerebrospinal fluid autoantibodies in COVID-19 patients with neurological symptoms. *Brain Behav Immun.* (2021) 93:415–9. doi: 10.1016/j.bbi.2020.12.022

4. Zhou Y, Han T, Chen J, Hou C, Hua L, He S, et al. Clinical and autoimmune characteristics of severe and critical cases of COVID-19. *Clin Transl Sci.* (2020) 13:1077–86. doi: 10.1111/cts.12805

5. Pascolini S, Vannini A, Deleonardi G, Ciordinik M, Sensoli A, Carletti I, et al. COVID-19 and immunological dysregulation: can autoantibodies be useful? *Clin Transl Sci.* (2021) 14:502–8. doi: 10.1111/cts.12908

6. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type IIFNs in patients with life-threatening COVID-19. *Science.* (2020) 370:eabd4585. doi: 10.1126/science.abd4585

7. Konstantinov KN, Emil SN, Barry M, Kellie S, Tzamaloukas AH. Glomerular disease in patients with infectious processes developing antineutrophil cytoplasmic antibodies. *ISRN Nephrol.* (2013) 2013:324315. doi: 10.5402/2013/324315

8. Suppiah R, Robson JC, Grayson PC, Ponte C, Craven A, Khalid S, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for microscopic polyangiitis. *Ann Rheum Dis.* (2022) 81:321–6. doi: 10.1136/annrheumdis-2021-221796

9. Robson JC, Grayson PC, Ponte C, Suppiah R, Craven A, Judge A, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for granulomatosis with polyangiitis. *Ann Rheum Dis.* (2022) 81:315–20. doi: 10.1136/annrheumdis-2021-221795

10. Grayson PC, Ponte C, Suppiah R, Robson JC, Craven A, Judge A, et al. 2022 American college of rheumatology/European alliance of associations for rheumatology classification criteria for eosinophilic granulomatosis with polyangiitis. *Ann Rheum Dis.* (2022) 81:309–14. doi: 10.1136/annrheumdis-2021-221794

11. Tou ZQ, Yang H, Zhang BE, Zhang XZ, Jin L. Identification of key expressed genes and potential therapeutic targets for ANCA-associated vasculitis and 2019 novel coronavirus infection based on bioinformatics. *Chin J Antituberculosis.* (2024) 46:9–12.

12. *Key points of the revision of the diagnosis and treatment Protocol for novel coronavirus pneumonia (trial version 8 revised edition)* Vol. 07. Beijing, China: National Health Commission of the People's Republic of China (2021). p. 4.

13. Committee of Autoantibody Detection, Rheumatology and Immunology Physicians Branch, Chinese Medical Doctor Association. Expert consensus on the clinical application of antineutrophil cytoplasmic antibody detection. *Chin J Lab Med.* (2018) 41:644–50.

14. Von Mü Hlen CA, Garcia-De La Torre I, Infantino M, Damoiseaux J, Andrade LEC, Carballo OG, et al. How to report the antinuclear antibodies (anti-cell antibodies) test on HEp-2 cells:guidelines from the ICAP initiative. *Immunol Res.* (2021) 69:594–608. doi: 10.1007/s12026-021-09233-0

15. Tan LM, Jiao AJ, Feng XJ, Xu LY, Tan FY, He SQ, et al. Anti-neutrophil cytoplasmic antibody determination for systemic vasculitis. *Lab Med.* (2018) 33(2). doi: 10.3969/j.issn.1673-8640.2018.02.002

16. Konstantinov KN, Ulff-Møller CJ, Tzamaloukas AH. Infections and antineutrophil cytoplasmic antibodies: triggering mechanisms. *Autoimmun Rev.* (2015) 14(3):201–3. doi: 10.1016/j.autrev.2014.10.020

17. Dinse GE, Parks CG, Weinberg CR, Co CA, Wilkerson J, Zeldin DC, et al. Increasing prevalence of antinuclear antibodies in the united states. *Arthritis Rheumatol*. (2022) 74(12):2032–41. doi: 10.1002/art.42330

18. Chinese Association of Integrative Medicine Laboratory Medicine Committee, and Shanghai Medical Association Laboratory Medicine Branch, Li M, Li YZ, Zheng B, Huang ZC, et al. Chinese expert consensus on the laboratory detection of antinuclear antibodies by indirect immunofluorescence assay (2023). *Chin J Lab Med*. (2023) 46 (11):1149–63. doi: 10.3760/cma.j.cn114452-20230914-00139

19. Cheong JG, Ravishankar A, Sharma S, Parkhurst CN, Grassmann SA, Wingert CK, et al. Epigenetic memory of coronavirus infection in innate immune cells and their progenitors. *Cell*. (2023) 186(18):3882–902. doi: 10.1016/j.cell.2023.07.019

20. Han TL, Lu P, Zhao IX, Liu KK, Liu YJ. Fan ryet al genetic characteristics of SARS CoV-2 in chaoyang district of beijing in 2023-2024. *7 Virol*. (2025) 1(02):1–11.

21. Mohamed Khosroshaht L, Rezaet N. Dystegulation of the immune response in coronavirus disease 2019. *Cell Biol Int*. (2021) 45:702–7. doi: 10.1002/cbin.11517

22. Wang W, Zhang W, Zhang J, et al. Distribution of HLA allele frequencies in 82 chinese individuals with coronavirus disease 2019 (COVID 19). *HLA*. (2020) 96:1916. doi: 10.1111/tan.13941

23. Novelli A, Andreani M, Mi B, et al. HUA allele frequencies and susceptibility to COVID 19 in a group of 99 italian patients. *HLA*. (2020) 96:610–4. doi: 10.1111/tan.14047

24. Ovsyannikova IG, Haralambieva IH, Crooke SN, Poland GA, Kennedy RB. The role of host genetics in the immune response to SARS-CoV-2 and COVID-19 susceptibility and severity. *Immunol Rev*. (2020) 296(1):205–19. doi: 10.1111/imr.12897

25. Rathmann J, Segelmark M, Mohammad AJ. Evaluation of the ACR/EULAR 2022 criteria for classification of ANCA-associated vasculitis in a population-based cohort from Sweden. *Rheumatol (Oxford)*. (2024) 63(7):1965–72. doi: 10.1093/rheumatology/kead516

26. Rotschild M, Elias N, Berkowitz D, Pollak S, Shinawi M, Beck R, et al. Autoantibodies against bactericidal/permeability-increasing protein (BPI-ANCA) in cystic fibrosis patients treated with azithromycin. *Clin Exp Med*. (2005) 5(2):80–5. doi: 10.1007/s10238-005-0070-7

27. Lasrado N, Yalaka B, Reddy J. Triggers of inflammatory heart disease. *Front Cell Dev Biol*. (2020) 8:192. doi: 10.3389/fcell.202000192

28. Solak Y, Afsar B, Vaziri ND, Aslan G, Yalcin CE, Covic A, et al. Hypertension as an autoimmune and inflammatory disease. *Hypertens Res*. (2016) 39(8):567–73. doi: 10.1038/hr.2016.35

29. Sliwa K, Ojji D, Bachelier K, Böhm M, Damasceno A, Stewart S. Hypertension and hypertensive heart disease in african women. *Clin Res Cardiol*. (2014) 103(7):515–23. doi: 10.1007/s00392-014-0660-z

30. Rotschild M, Elias N, Berkowitz D, Pollak S, Shinawi M, Beck R, et al. Autoantibodies against bactericidal/permeability-increasing protein (BPI-ANCA) in cystic fibrosis patients treated with azithromycin. *Clin Exp Med*. (2005) 5(2):80–5. doi: 10.1007/s10238-005-0070-7

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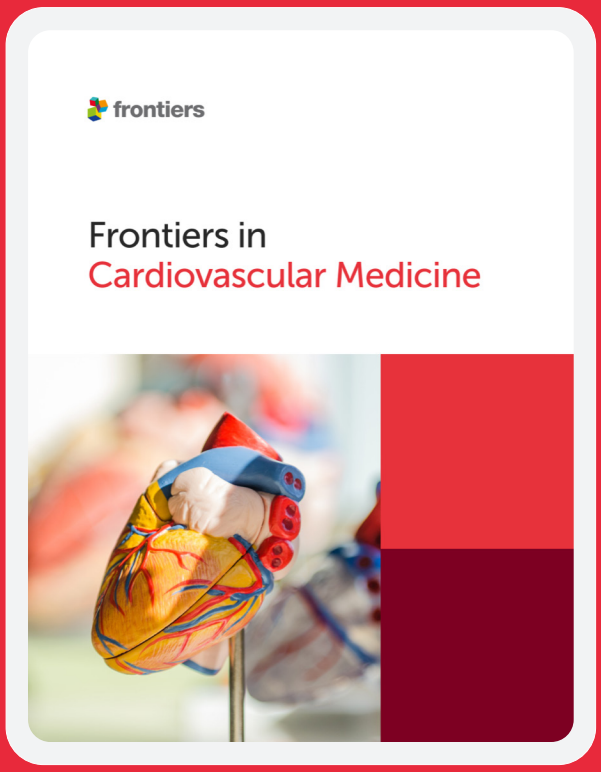

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