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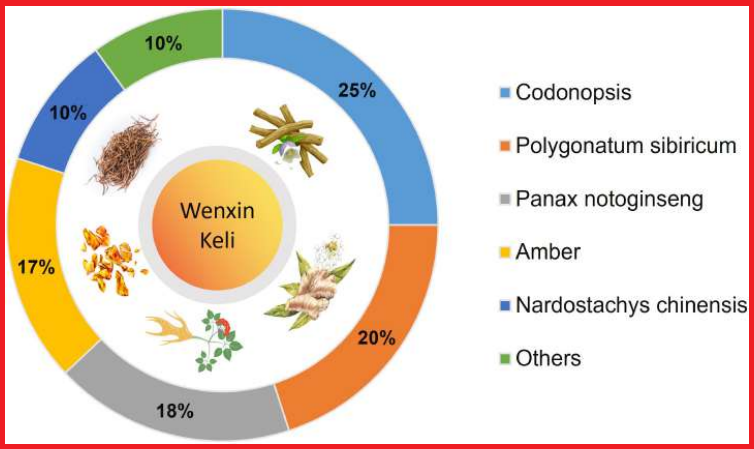
Original Investigations



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THE ANATOLIAN JOURNAL OF CARDIOLOGY

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The Anatolian Journal of Cardiology aims to publish qualified and original clinical, experimental and basic research on cardiology at the international level. The journal's scope also covers editorial comments, reviews of innovations in medical education and practice, case reports, original images, scientific letters, educational articles, letters to the editor, articles on publication ethics, diagnostic puzzles, and issues in social cardiology.

The target readership includes academic members, specialists, residents, and general practitioners working in the fields of adult cardiology, pediatric cardiology, cardiovascular surgery and internal medicine.

The editorial and publication processes of the journal are conducted in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the European Association of Science Editors (EASE), the Committee on Publication Ethics (COPE) and the HEART Group.

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- An ethics committee report prepared in conformity with the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects and the Guide for the Care and Use of Laboratory Animals is required to be submitted with experimental and clinical studies, drug trial studies and some case reports. Authors may be asked by the Editor-in-Chief's Office for an ethics committee report or similar in other circumstances also. Manuscripts reporting the results of experimental studies must explain in detail all procedures which volunteer subjects and patients have undergone and a statement indicating that consent for the study has been obtained from all subjects should be included in the text. Animal studies should clearly specify how pain or discomfort has been relieved.

Artificial Intelligence (AI)–Assisted Technology

At submission, the journal should require authors to disclose whether they used artificial intelligence (AI)– assisted technologies (such as Large Language Models [LLMs], chatbots, or image creators) in the production of submitted work. Authors who use such technology should describe, in both the cover letter and the submitted work, how they used it. Use of AI for writing assistance should be reported in the acknowledgment section. Authors who used AI technology to conduct the study should describe its use in the methods section in sufficient detail to enable replication to the approach, including the tool used, version, and prompts where applicable. Chatbots (such as ChatGPT) should not be listed as authors because they cannot be responsible for the accuracy, integrity, and originality of the work, and these responsibilities are required for authorship. Therefore, humans are responsible for any submitted material that included the use of AI-assisted technologies. Authors should carefully review and edit the result because AI can generate authoritative-sounding output that can be incorrect, incomplete, or biased. Authors should not list AI and AIassisted technologies as an author or co-author, nor cite AI as an author. Authors should be able to assert that there is no plagiarism in their paper, including in text and images produced by the AI. Humans must ensure there is appropriate attribution of all quoted material, including full citations.

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1. Title Page
2. Main Text
3. Tables, Graphs and Figures
4. Copyright Transfer Form
5. Author Contribution Form
6. ICMJE Uniform Disclosure Form for Potential Conflicts of Interest

1. Title Page

Information about the authors and their institutions should not be included in the main text, tables, figures and video documents. Since submitted manuscripts are evaluated by the reviewers through the online system, personal identification is excluded in the interests of unbiased interpretation. Thus, only information about the manuscript as specified below should be included on the title page. For each type of manuscript, it is mandatory to upload a title page as a separate Microsoft Word document through the online submission system. The title page should include the names of the authors with their latest academic degrees, and the name of the department and institution, city and country where the study was conducted. If the study was conducted in several institutions, the affiliation of each author must be specified with symbols. The correspondence address should contain the full name of the corresponding author, postal and e-mail addresses, phone and fax numbers. If the content of the manuscript has been presented before, the name, date and place of the meeting must be noted. Disclosure of conflict of interest, institutional and financial support, author contributions, acknowledgments, and ORCID iDs of the authors should be included on the title page.

2. Main Text

The manuscripts submitted to our journal are classified and evaluated according to the manuscript types stated below. During preliminary evaluation, the editors assess whether a manuscript's format and sub-headings are prepared in accordance with the journal's guidelines. Therefore, it is important that authors check the accuracy of the main text in terms of the following.

A. Manuscript types
- **Original investigation**
- **Editorial comment**
- **Review**
- **Education**
- **Scientific letter**
- **Case report**
- **Original image**
- **Letter to the editor**
- **Publication ethics**
- **Scientific puzzle**
- **Miscellaneous articles**

B. References

C. Special Terms and Conditions

A. Manuscript types

- **Original Research**
- Title
- Highlights: Each submission should be accompanied by 3 to 5 "highlight points" which should emphasize the most striking results of the study and highlight the message that is intended to be conveyed to the readers. It should be limited to 70 words.
- Structured Abstract: It should be structured with Objective, Methods, Results and Conclusion subheadings and should be limited to 250 words.
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- Main Text: It should consist of Introduction, Methods, Results, Discussion, Limitations of the Study and Conclusion sections and should not exceed 5000 words excluding the references.
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Statistical Analysis: Statistical analysis should be conducted in accordance with the guidelines on reporting statistical data in medical journals [Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93 and Lang T, Altman D. Basic statistical reporting for articles published in clinical medical journals: the SAMPL Guidelines. In: Smart P, Maisonneuve H, Polderman A (editors). Science Editors' Handbook, European Association of Science Editors, 2013.]. The software used for statistical analysis must be described. Data must be expressed as mean ± standard deviation when parametric tests are used to compare continuous variables. For nonparametric tests, data must be expressed as median (minimum-maximum) or percentiles (25th and 75th percentiles). In advanced and complex statistical analyses, relative risk (RR), odds ratio (OR) and hazard ratio (HR) must be supported by confidence intervals and p values. The outcomes of statistical analyses and interpretation of the results must be in evidence-based scientific language (Editors of Heart Group journals. Statement on matching language to the type of evidence used in describing outcomes data. Anatolian Journal of Cardiology 2012;12:709).

- **Editorial Comment**

Authors are selected and invited by the Editor-in-Chief. This type of manuscript aims at providing a brief commentary on an article published in the journal by a researcher who is an authority in the relevant field or by the reviewer of the article.

- Title
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Reviews prepared by authors with extensive knowledge on a particular field, which has been reflected in international literature by a high number of publications and citations, are evaluated. The authors may be invited by the Editor-in-Chief. A review should be prepared in the format describing, discussing and evaluating the current level of knowledge or topic that is to be used in the clinical practice and it should guide further studies.

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  Knowledge confirmed by the results of recent research studies, involved in education at academic level and used in clinical practices should be presented in detail in this type of manuscript.
- Title
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  Manuscripts which announce a new scientific invention, are clinically significant, and are in the form of a preliminary report are accepted for publication as scientific letters.
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- **Case Report**
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- Main Text: It should include Introduction, Case Report, Discussion and Conclusion sections and should not exceed 700 words excluding the references.
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NOTE 1: Case reports that include video images have a better chance of publication.

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  Impressive and rare images that reflect significant findings based on clinical science,
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Images obtained directly or on operation, visuals revealed through EKG and/or imaging methods, and macroscopic-microscopic anatomical, pathological findings of attentiongrabbing and rare cases can be published. Unlike original images, the diagnosis and outcome of the case and image are hidden at the beginning in diagnostic puzzles. Four multiple-choice questions are prepared. In the following pages of the journal, the correct answer in terms of precise diagnosis and outcome is given with an explanation and didactic images and then discussed. The Editor-in-Chief will convert suitable images into the format of a diagnostic puzzle with the authors' permission.

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Journal: Muller C, Buttner HJ, Peterson J, Roskomun H. A randomized comparison of clopidogrel and aspirin versus ticlopidine and aspirin after placement of coronary artery stents. *Circulation*. 2000;101(6):590-593.

Book Chapter: Sherry S. Detection of thrombi. In: Strauss HE, Pitt B, James AE, eds. *Cardiovascular Medicine*. St Louis: Mosby; 1974:273-285.

Book with Single Author: Cohn PF. *Silent Myocardial Ischemia and Infarction*. 3rd ed. New York: Marcel Dekker; 1993.

Editor(s) as author: Norman IJ, Redfern SJ, eds. *Mental Health Care for Elderly People*. New York: Churchill Livingstone; 1996.

Conference Paper: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, eds. MEDINFO 92. Paper presented at the 7th World Congress on Medical Informatics; September 6-10 1992; Geneva, Switzerland. Amsterdam: North-Holland; 1992:1561-1565.

Scientific or Technical Report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: October 1994. Report No: HHSIGOE 169200860.

Thesis: Kaplan SI. *Post-hospital Home Health Care: Elderly Access and Utilization*. Dissertation. Washington University; 1995.

Manuscripts accepted for publication but not published yet: Leshner AI. *Molecular Mechanisms of Cocaine Addiction. N Engl J Med*. Forthcoming 1997.

Epub ahead of print articles: Aksu HU, Ertürk M, Gül M, Uslu N. Successful treatment of a patient with pulmonary embolism and biatrial thrombus. *Anatolian J Cardiol*. December 26, 2012. doi: 10.5152/akd.2013.062. [Epub ahead of print].

Manuscript published in electronic format: Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis (serial online)*. l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: http:/ www.cdc.gov/ncidodlEID/cid.htm.

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  This information should be added to the relevant section of the online submission system and included in the Title Page.
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- Tables should be prepared in a Microsoft Office Word document using the command 'Insert Table' and inserted at the end of the references in the main text.
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- All visuals should have a high resolution (minimum 300 dpi).
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Drawings should be performed by professionals. No grey colours should be used.
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- Roman numbers should be avoided in tables and figures within the text and their titles.
- Decimal numbers used in text, tables and figures should be separated by commas in Turkish sections and by dots in English sections.
- Tables should be easily understandable and should not repeat the data in the main text.
- In addition to the pictures included in case reports and original images, video and movie images are published on the journal's website. These images should be prepared in MPEG format with a maximum size of 2 MB. They should be submitted to the journal with the manuscript documents. The names of patients, doctors, institutions and places should be omitted from all documents.

NOTE: All visuals in the manuscripts are printed in black and white without charge. If color printing is required, Turkish authors must pay 300 TL, while foreign authors must pay 300 USD. For payment methods, please contact the Editor-in-Chief's Office of the Anatolian Journal of Cardiology.

EVALUATION AND PUBLICATION

- The main text submitted to the journal must be in English. Manuscripts written in other languages are not accepted. Citation potential is higher for manuscripts in English.
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THE ANATOLIAN JOURNAL OF CARDIOLOGY

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THE ANATOLIAN JOURNAL OF CARDIOLOGY

Official journal of the TURKISH SOCIETY OF CARDIOLOGY

Wenxin Keli, Clopidogrel, Vericiguat and New Impact Factor: 2.1

Cardiac arrhythmias remain a leading cause of cardiovascular morbidity and mortality worldwide. Although conventional antiarrhythmic drugs are effective, their use is constrained by proarrhythmic risks and extracardiac toxicity. Wenxin Keli (WXKL), a traditional Chinese medicine formula, has demonstrated antiarrhythmic properties in preclinical and clinical studies with a reportedly favorable safety profile. Fu et al from China reviewed this topic in detail.

The most commonly used graft material in CABG, the left internal mammary artery (LIMA), is considered the gold standard due to its high long-term patency rates. Clopidogrel, an antithrombotic agent widely used in CABG patients that targets $P2Y_{12}$ receptors on platelets, has also been suggested to influence vascular responses due to the presence of these receptors in vascular smooth muscle cells. However, the direct vascular effects of clopidogrel on LIMA remain unknown. Öz et al from Türkiye tried to solve this topic.

The initiation and maintenance of atrial fibrillation (AF) are predominantly driven by progressive atrial structural remodeling, characterized by fibrosis and electrical conduction abnormalities. While vericiguat has shown efficacy in attenuating cardiac remodeling in heart failure, its impact on AF remains insufficiently characterized. Zhang et al from China investigated the cardioprotective effects of vericiguat in a Sprague-Dawley rat model of AF.

Cardiac resynchronization therapy (CRT) is an established treatment for selected patients with heart failure (HF); however, a substantial proportion of patients do not derive clinical or echocardiographic benefit. Systemic inflammation has been implicated in adverse myocardial remodeling and may influence response to CRT. Şipal et al from Türkiye aimed to evaluate the association between the systemic immune-inflammation index (SII) and CRT response.

Cardiometabolic index (CMI) is closely associated with metabolic disorders related to obesity, which may be implicated in the pathological process of liver disease by inducing oxidative stress. However, previous observational studies have reached inconsistent conclusions on the association between CMI and hepatic fibrosis. Yan et al from China assessed the association of CMI and Hepatic fibrosis with oxidative stress by utilizing a population-based study.

Transradial angiography (TRA) is widely used in contemporary coronary procedures. Although clinically apparent peripheral nerve injury after TRA is uncommon subclinical nerve involvement may go unrecognized. Dandinoğlu et al from Türkiye used neurological examination, standardized neuropathic pain questionnaires and nerve conduction studies (NCS) for objectively evaluate peripheral nerve function after TRA.

And a case report, letters.

I hope this new issue of our journal will be interest of our readers.

Çetin Erol

Editor-in-Chief, Ankara, Türkiye

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THE ANATOLIAN JOURNAL OF CARDIOLOGY

Official journal of the TURKISH SOCIETY OF CARDIOLOGY

Pharmacological Insights and Clinical Challenges of Wenxin Keli in Arrhythmia Treatment

ABSTRACT

Cardiac arrhythmias remain a leading cause of cardiovascular morbidity and mortality worldwide. Although conventional antiarrhythmic drugs are effective, their use is constrained by proarrhythmic risks and extracardiac toxicity. Wenxin Keli (WXKL), a traditional Chinese medicine (TCM) formula, has demonstrated antiarrhythmic properties in preclinical and clinical studies with a reportedly favorable safety profile. This article provides a critical synthesis of WXKL's pharmacological mechanisms, including ion channel modulation (INa, ICaL, Ito), calcium handling, and calcium/calmodulin-dependent protein kinase II signaling, with explicit appraisal of translational limitations. Clinical trial evidence and meta-analyses for atrial fibrillation (AF), premature ventricular contractions, and other indications were systematically evaluated, with detailed discussion of effect sizes, heterogeneity, and methodological quality. Key challenges unique to WXKL as a TCM-derived product—formulation variability, quality control limitations, and regulatory approval pathways—are analyzed in depth. A prioritized research agenda is proposed to address evidence gaps.

Keywords: Anti-arrhythmia, clinical application, pharmacological effects, traditional Chinese medicine, Wenxin Keli

REVIEW

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INTRODUCTION

Cardiac arrhythmias affect millions of individuals worldwide and are associated with increased risks of stroke, heart failure, and sudden cardiac death.[1] Current guideline-based arrhythmia management primarily includes catheter ablation and pharmacological therapy: catheter ablation is recommended as the first-line curative option for drug-refractory arrhythmias,[1,2] while standard antiarrhythmic drugs (e.g., amiodarone, metoprolol) remain essential due to their accessibility and role as adjunctive treatment.[2,3] However, conventional antiarrhythmics are plagued by significant drawbacks: Class I agents may provoke proarrhythmia, Class III agents carry risks of QT prolongation and torsade de pointes, and many drugs exert negative inotropic effects or extracardiac toxicity.[4,5] These limitations have motivated the search for multi-targeted therapies with improved safety profiles. Wenxin Keli (WXKL), therefore, is not intended to replace first-line therapies (standard antiarrhythmics or catheter ablation); instead, it serves as a complementary or alternative option, particularly for patients who are intolerant of or refractory to conventional treatments.

Wenxin Keli is a Chinese medicine formula approved by the China National Medical Products Administration for the treatment of atrial and ventricular arrhythmias.[6] It comprises 5 herbal components: *Codonopsis pilosula*, *Polygonatum sibiricum*, *Panax notoginseng*, Amber (*Succinum*), and *Nardostachys jatamansi*. Over the past decade, more than 100 preclinical studies and several dozen clinical trials have investigated its antiarrhythmic properties. While multiple narrative reviews have summarized this literature, critical appraisal of evidence quality, discussion of conflicting findings, and examination of product-specific challenges remain scarce.

This review has 4 objectives: (1) to critically synthesize current knowledge of WXKL's pharmacological mechanisms, highlighting strengths and gaps in the preclinical evidence; (2) to systematically evaluate the design, quality, and results of clinical trials and meta-analyses involving WXKL; (3) to analyze the unique challenges of formulation consistency, quality control, and regulatory approval that affect its global translation; and (4) to propose a prioritized research agenda to address unresolved questions. By adopting an analytical rather than descriptive approach, a balanced evidence-based foundation for clinicians and researchers was intended to be provided.

LITERATURE SEARCH AND STUDY SELECTION

This review was conducted following the principles of systematic reviews where applicable. The following databases were systematically searched from inception through December 2024: PubMed, EMBASE, Cochrane Library, Web of Science, China National Knowledge Infrastructure, Wanfang Data, and VIP information database. Search terms included combinations of "Wenxin Keli," "Wenxin Granule," "antiarrhythmia," "atrial fibrillation," "premature ventricular contraction," "heart failure," and "arrhythmia."

Inclusion criteria were: (1) studies evaluating WXKL for any arrhythmia indication; (2) randomized controlled trials (RCTs), observational studies, systematic reviews, and meta-analyses; (3) human or preclinical studies with mechanistic relevance; and (4) articles published in English or Chinese. Exclusion criteria were: (1) case reports, commentaries, or editorials without original data; (2) duplicate publications; and (3) studies without accessible full text. Two investigators independently screened titles and abstracts, with disagreements resolved by consensus. The selection process was documented in accordance with PRISMA guidelines where applicable.

PHARMACOLOGICAL MECHANISMS: PRECLINICAL EVIDENCE AND CRITICAL APPRAISAL

Wenxin Keli has been studied extensively in isolated cardiomyocytes, animal models, and ex vivo preparations. These investigations have revealed a multi-target pharmacological profile. However, the translational relevance of many findings remains uncertain due to methodological limitations, the absence of human tissue validation, and the use of drug concentrations that may not be clinically achievable. The principal mechanisms are summarized below, along with a critical appraisal of the supporting evidence.

HIGHLIGHTS

- Provides a critical appraisal of preclinical and clinical evidence for Wenxin Keli (WXKL), identifying key translational gaps and methodological limitations.
- Systematically evaluates meta-analyses with attention to effect sizes, heterogeneity, risk of bias, and publication bias.
- Analyzes formulation variability, quality control deficiencies, and regulatory barriers that impede global acceptance.
- Proposes a prioritized research roadmap to advance WXKL toward evidence-based, internationally approved therapy.

DRUG COMPOSITION AND COMPLEXITY

Wenxin Keli contains over 71 identified compounds, including ginsenosides, steroidal saponins, alkaloids, and organic acids.[7] The proportions of the various herbs in WXKL have been meticulously formulated to optimize its overall therapeutic efficacy. As illustrated in Figure 1, the varying proportions of distinct ingredients within the formulation further augment the specificity and efficacy of WXKL. This formulation, characterized by its scientific rigor and abundant active components, collectively provides a robust basis for WXKL's multi-pathway and multi-target antiarrhythmic effects. Network pharmacology analyses have predicted 68 potentially active ingredients and 83 arrhythmia-related targets, implicating pathways involved in inflammation, oxidative stress, adrenergic signaling, and calcium homeostasis.[8,9]

While *in silico* studies generate useful hypotheses, they rely on databases of predicted interactions and often lack experimental confirmation. The actual contribution of individual compounds to antiarrhythmic effects remains undefined. Synergistic interactions between constituents have not been rigorously deconvoluted, and no studies have systematically compared the potency of the full formula vs. its individual components or combinations thereof.

MODULATION OF CARDIAC ION CHANNELS

The principal mechanism proposed for WXKL's antiarrhythmic effects is modulation of multiple ion channels on the cardiomyocyte membrane. Evidence derives largely from patch-clamp studies in isolated animal cells.

Sodium Channels (INa)

Burashnikov et al[10] demonstrated that WXKL (5-10 mg/mL) concentration-dependently inhibited peak sodium current (INa) in canine atrial and ventricular myocytes, with a greater effect in atrial cells.[10] This atrial-selective inhibition was attributed to differences in resting membrane potential and steady-state inactivation kinetics between atrial and ventricular myocytes at rapid activation rates.[11] Subsequent studies reported suppression of late sodium current (INaL), reducing early and delayed afterdepolarizations in rabbit ventricular myocytes.[12]

These experiments were conducted at supra-therapeutic concentrations (5-10 mg/mL). The peak plasma concentration of WXKL constituents after oral administration in humans has not been definitively established, but available pharmacokinetic data suggest that concentrations achieved in vivo are substantially lower.[13] Atrial selectivity has not been confirmed in human atrial myocytes or in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). No studies have examined state-dependent block or use-dependence, which are critical determinants of clinical safety and efficacy.

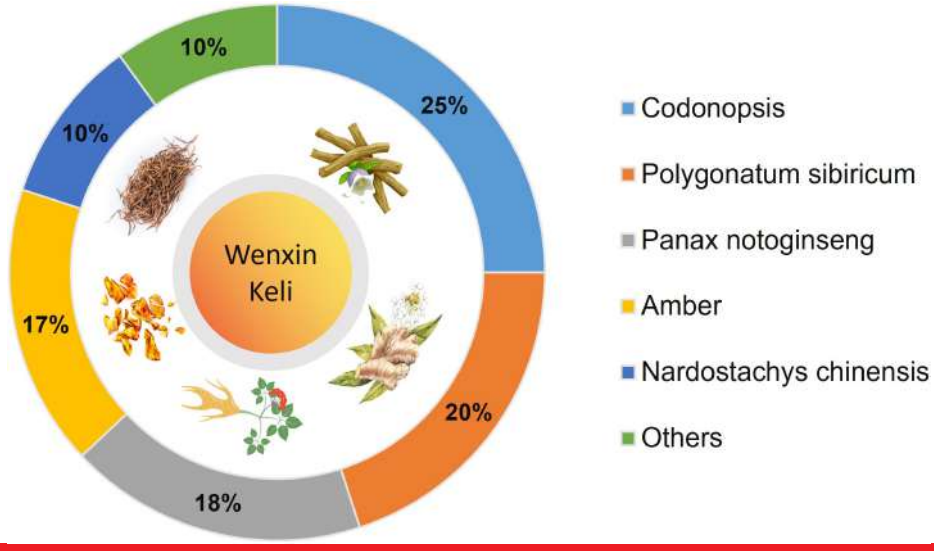

Figure 1. The composition and proportion of WXKL.

Calcium Channels (ICaL)

In post-myocardial infarction rat models, WXKL increased sarcoplasmic reticulum calcium content and reduced the incidence of early and delayed afterdepolarizations.[14] Wenxin Keli accelerated L-type calcium channel inactivation and prolonged recovery from inactivation, potentially mitigating calcium overload.[15]

These studies utilized indirect assessments of calcium handling (fluorescent indicators) rather than direct measurement of ICaL. Experiments were conducted in rodent myocytes, which differ substantially from humans in calcium handling proteins, repolarization reserve, and rate dependence. No studies have examined WXKL effects on human L-type calcium channels expressed in heterologous systems or in hiPSC-CMs.

Potassium Channels (Ito, IKr, IKs)

Wenxin Keli has been shown to reduce the transient outward potassium current (Ito) in rat ventricular myocytes and canine ventricular preparations.[16,17] Ito inhibition underlies the proposed efficacy in Brugada syndrome models.[18] Effects on delayed rectifier currents (IKr, IKs) have not been systematically characterized.

The absence of data on IKr and IKs represents a significant gap, given that inhibition of these currents is a common mechanism of drug-induced QT prolongation and proarrhythmia. Until WXKL's effects on these currents are characterized, its cardiac safety profile remains incompletely defined.

Intracellular Calcium and Signaling Pathways

Several studies have implicated the calcium/calmodulin-dependent protein kinase II (CaMKII) pathway as a key target. In rat models of transverse aortic constriction and myocardial infarction, WXKL reduced CaMKII phosphorylation, normalized calcium transients, and decreased arrhythmia susceptibility.[19,20] Gene expression studies reported downregulation of SCN5A and ADRB2 and upregulation of CHRM2 in infarcted rat hearts.[21]

These studies did not employ genetic or pharmacological CaMKII inhibition as a comparator, making it difficult to attribute antiarrhythmic effects specifically to CaMKII modulation. All experiments were conducted in rodents; confirmation in human tissues is lacking. The gene expression changes, while statistically significant, were modest (typically <2-fold) and of uncertain functional relevance.

Energy Metabolism, Oxidative Stress, and Inflammation

Additional reported effects include enhancement of glucose oxidation and branched-chain amino acid degradation during ischemia-reperfusion,[22] suppression of inflammatory cytokines (interleukin-6, tumor necrosis factor alpha),[23] inhibition of transforming growth factor beta-mediated p38/JNK signaling,[24] and attenuation of mitochondrial oxidative stress via PKC-δ/NOX2/ROS pathway modulation.[25]

These pleiotropic effects are consistent with the multicomponent nature of traditional Chinese medicine (TCM) formulas,[26] but causality is difficult to establish. Most studies are observational and lack mechanistic linkage between the metabolic/inflammatory changes and arrhythmia suppression. The contribution of individual herbal constituents to these effects has not been dissected. All studies were conducted in rodent models; human data are absent.

Regulation of miRNAs and Gap Junctions

Two studies reported that WXKL upregulated connexin 43 (Cx43) expression, increased ventricular fibrillation threshold, and modulated miR-1 and miR-133 expression in infarcted rats.[27,28]

These findings derive from single research groups with small sample sizes and no independent replication. The specificity of miRNA changes and their functional relevance to arrhythmia suppression remain speculative. The Cx43 upregulation was assessed only by Western blot; functional gap junction communication was not directly measured.

Antiplatelet Effects

One study investigated the antiplatelet activity of WXKL. Tao et al[29] demonstrated that linoleic acid, a constituent of WXKL, inhibited platelet aggregation in vitro, reduced adhesion on collagen-coated surfaces under flow conditions, and attenuated thrombus formation in a $FeCl_3$-induced carotid artery injury model. Mechanistically, linoleic acid selectively suppressed P-selectin–mediated platelet activation and disrupted the phosphorylation cascades of protein kinase B (Akt), mitogen-activated protein kinases (MAPKs), and phospholipase C β3 (PLCβ3).

This study provides rigorous mechanistic evidence for an antiplatelet effect. However, its direct relevance to arrhythmia treatment is uncertain. First, the study focused on thrombosis, not arrhythmogenesis; no experimental link was established between platelet inhibition and suppression of atrial or ventricular arrhythmias. Second, the effect was attributed to a single constituent (linoleic acid); whether the full WXKL formula exerts equivalent or synergistic antiplatelet activity, and at what clinically achievable concentrations, remains unknown. Third, the clinical implications are twofold and require caution: if WXKL possesses clinically meaningful antiplatelet effects, it could offer additional vascular protection in atrial fibrillation (AF) patients, but it may also increase bleeding risk when co-administered with anticoagulants or antiplatelet agents. Formal drug–drug interaction and pharmacodynamic studies are urgently needed.

Overall Appraisal of Preclinical Evidence

The existing preclinical literature provides plausible mechanisms by which WXKL might exert antiarrhythmic effects (Figure 2). However, the evidence is predominantly derived from non-human models, employs concentrations of uncertain clinical relevance, and lacks rigorous experimental controls (Table 1). Key limitations of the current body of research include the complete absence of investigations conducted in human cardiomyocytes or hiPSC-CMs, as well as a notable lack of pharmacokinetic-pharmacodynamic correlation studies. Moreover, there has been no systematic characterization of the effects of WXKL on the rapid (IKr) and slow (IKs) components of the delayed rectifier potassium current, and the specific contribution of individual herbal constituents to its observed therapeutic effects remains poorly defined. The reliability of the available evidence is further compromised by limited independent replication of preclinical and clinical findings, together with a substantial risk of publication bias.

Mechanistic claims should therefore be regarded as hypothesis-generating rather than conclusive.

CLINICAL APPLICATION RESEARCH AND EVIDENCE SYNTHESIS

Atrial Fibrillation

Clinical Trial Evidence

Randomized controlled trials of WXKL for paroxysmal AF are limited in both number and methodological quality. An open-label, randomized trial comparing WXKL with sotalol in 120 patients with hyperthyroidism-induced paroxysmal AF reported similar rates of sinus rhythm maintenance at 4 weeks (76.7% vs. 73.3%, $P = .67$); however, the study lacked blinding, did not include a placebo control, and was underpowered for equivalence.[30]

A meta-analysis of 8 small RCTs (n = 624) suggested that WXKL, alone or combined with conventional antiarrhythmic drugs, increased the likelihood of sinus rhythm maintenance (OR 3.21; 95% CI 2.14-4.82).[31] However, all included trials were conducted in China, none were double-blinded, allocation concealment was inadequately reported, and

Figure 2. A condensed overview of the various mechanisms and effects of WXKL.

Table 1. Key Limitations of Preclinical WXKL Studies

Domain	Limitation	Implication
Experimental models	Predominantly rodent, canine; no human cardiomyocyte data	Uncertain translational relevance
Drug concentrations	Supra-therapeutic (5-10 mg/mL); no PK/PD correlation	Clinical achievability unknown
Ion channel studies	IKr, IKs not characterized; no state-dependence assessment	Proarrhythmic risk profile incomplete
Mechanistic specificity	No genetic/pharmacological inhibitors as comparators	Attribution of effects to specific pathways uncertain
Replication	Most findings from single laboratories	Potential publication bias

heterogeneity was moderate ($I^2=52\%$). Publication bias could not be excluded due to the small number of studies. Consequently, the current evidence base is insufficient to support a definitive recommendation for WXKL as monotherapy for rhythm control in AF.

Combination Therapy Trials

Zhang et al[32] randomized 41 patients with recent-onset AF (<48 hours) to intravenous amiodarone alone or amiodarone plus oral WXKL (18 g 3 times daily). Conversion rates at 24 hours did not differ between groups (81.0% vs. 75.0%, $P=.72$). However, conversion time was significantly shorter in the combination group (291 ± 235 minutes vs. 725 ± 475 minutes, $P=.003$). No serious adverse events occurred. Limitations include open-label design, small sample size, and baseline imbalance in diabetes prevalence and QTc interval.

Premature Ventricular Contraction

Pivotal Randomized Controlled Trial

The largest trial to date randomized 1200 patients with frequent premature ventricular contractions (PVCs) (>1000/24h, no structural heart disease) to WXKL (9 g 3 times daily) or placebo for 4 weeks.[33] The primary endpoint was change in 24-hour PVC count. Wenxin Keli significantly reduced PVC burden compared with placebo (median reduction: 61% vs. 22%; $P<.001$). The proportion of patients achieving ≥90% PVC reduction was 31.5% in the WXKL group vs. 8.2% in the placebo group. PVC-related symptom scores also improved significantly.

Methodological considerations: This trial was well-designed with adequate randomization, double-blinding, and central electrocardiogram (ECG) analysis. However, 20% of randomized patients were excluded from the per-protocol analysis, raising concerns about attrition bias. The placebo run-in period was not reported. Comparative effectiveness against guideline-recommended therapy (beta-blockers) was not assessed.

Meta-Analyses

Huang et al conducted a meta-analysis of 11 RCTs (n=1243) comparing WXKL plus metoprolol vs. metoprolol alone for PVCs.[34] The combination therapy significantly improved clinical efficacy (RR 1.32; 95% CI 1.24-1.40; $P<.00001$). However, several critical limitations were identified in the included trials. All eligible studies were conducted exclusively in China. The overall risk of bias was high: only 3 of 11 studies reported appropriate randomization, and none described allocation concealment or adopted a blinded design. Substantial heterogeneity was present ($I^2=67\%$) but was not explored by subgroup or sensitivity analyses. Furthermore, asymmetry in the funnel plot indicated a potential risk of publication bias, although Egger's test was not reported to confirm this.

OTHER INDICATIONS

Cardiac Syndrome X

Only 1 clinical study has investigated WXKL for cardiac syndrome. Xu and Huang[35] reported that WXKL combined with aspirin improved angina symptoms and endothelial function markers (hs−CRP, ET-1, NO) compared with aspirin alone in a small, single-center study. However, the trial was non-randomized, open-label, did not report blinding or allocation concealment, and adverse events were not systematically assessed. The study was published in 2011 with no subsequent confirmatory trials. Therefore, the evidence for WXKL in cardiac syndrome X remains preliminary and insufficient to support routine clinical use.

Brugada Syndrome

Evidence for Brugada syndrome is limited to a single experimental study using arterially perfused canine right ventricular wedge preparations.[18] Wenxin Keli (5 mg/mL) combined with quinidine suppressed arrhythmia triggers by inhibiting Ito. No human data exist. This indication remains exploratory and should not be extrapolated to clinical practice without confirmatory studies.

Safety and Tolerability

Across clinical studies, the most commonly reported adverse events were mild gastrointestinal discomfort (2%-5%) and dizziness (1%-3%), with no severe adverse events definitively attributed to WXKL monotherapy.[33,36] However, critical gaps exist in long-term safety data, which is particularly relevant for combination therapy given that many arrhythmia patients require prolonged treatment with WXKL plus standard antiarrhythmics (e.g., amiodarone, metoprolol) or anticoagulants. To date, no clinical trials have evaluated WXKL's safety beyond 6 months, and post-marketing surveillance data are limited. Given WXKL's complex composition (over 71 identified compounds[7]), long-term administration may pose unrecognized risks, such as cumulative hepatic or renal burden—especially in elderly patients or those with pre-existing organ impairment. For combination therapy, prolonged co-administration with standard antiarrhythmics (e.g., amiodarone, which has inherent hepatic and thyroid toxicity[4]) may increase the risk of additive adverse effects, though this has not been

systematically evaluated. Further long-term, prospective studies are urgently needed to assess the safety of WXKL monotherapy and combination therapy over 12-24 months, with regular monitoring of hepatic (alanine aminotransferase, aspartate aminotransferase), renal (creatinine, estimated glomerular filtration rate (eGFR)), and thyroid function.

In addition to long-term safety concerns, herb-drug interactions represent a key safety consideration, particularly in combination therapy settings. An in vivo study in rats demonstrated that WXKL weakly inhibits the activities of CYP3A4, CYP1A2, CYP2C19, and CYP2E137, which are key enzymes involved in the metabolism of most standard antiarrhythmics (e.g., amiodarone, metoprolol), anticoagulants (e.g., warfarin), and statins.[37] This inhibition may alter plasma concentrations of co-administered drugs, increasing the risk of toxicity (e.g., amiodarone-induced QT prolongation) or reducing therapeutic efficacy, a risk that is amplified when WXKL is frequently used in combination with amiodarone or metoprolol.[32,34] No human pharmacokinetic studies have been conducted to quantify these interactions, and no dose adjustment guidelines exist for co-administered drugs. Additionally, WXKL's antiplatelet potential (attributed to linoleic acid[29]) raises concerns about increased bleeding risk when combined with anticoagulants (e.g., warfarin, novel oral anticoagulants) or antiplatelet agents (e.g., aspirin, clopidogrel)—a common scenario in AF patients requiring stroke prevention. Formal drug–drug interaction studies are critical to clarify these risks and guide clinical practice.

Compounding these safety concerns is the inadequate pharmacovigilance for WXKL, particularly in combination therapy. Current safety data are primarily derived from short-term clinical trials, which are insufficient to detect rare or delayed adverse events (e.g., long-term hepatic injury, drug-drug interaction-related proarrhythmia). For combination therapy, pharmacovigilance efforts should focus on active surveillance of adverse events specific to combination regimens, such as additive cardiac toxicity (QT prolongation) when WXKL is combined with amiodarone or bleeding events when combined with anticoagulants, as well as standardized reporting of adverse events in post-marketing settings with clear documentation of co-administered drugs to identify potential herb–drug interaction signals. The development of a dedicated pharmacovigilance registry for WXKL, particularly for patients on long-term combination therapy, would also help track long-term safety outcomes and identify high-risk subgroups (e.g., elderly patients, those with hepatic/renal impairment, or those on multiple co-medications). Furthermore, healthcare providers should be educated on WXKL's potential herb–drug interactions and the need for close monitoring when used in combination with standard antiarrhythmics, anticoagulants, or antiplatelet agents, while patients should be advised to report any new symptoms (e.g., fatigue, jaundice, bruising) promptly, especially when initiating or adjusting combination therapy.

Heart Failure with Ventricular Arrhythmia

Zheng et al conducted a systematic review and meta-analysis of WXKL plus amiodarone vs. amiodarone alone for heart failure complicated by ventricular arrhythmia.[37] Thirteen trials (n = 1126) were included. The combination therapy significantly improved total effective rate (RR 1.22; 95% CI 1.16-1.29) and reduced heart rate (MD −2.25 bpm; 95% CI −2.61 to −1.88) and PVC frequency (MD −2.03; 95% CI −2.41 to −1.65).

Critical appraisal: Risk of bias was assessed as high in 3 studies and unclear in 10 studies. All trials were conducted in China; none were registered. Heterogeneity for the primary outcome was moderate ($I^2 = 46\%$). Adverse event reporting was inconsistent; pooled analysis showed no significant difference between groups (OR 0.64; 95% CI 0.39-1.07), but CIs were wide. The authors appropriately concluded that "further research is warranted, ideally involving large, prospective, rigorous trials."[38]

Critical Evaluation of Meta-Analyses

Five systematic reviews/meta-analyses evaluating WXKL for arrhythmia indications were identified.[31,34,38-40] According to the AMSTAR-2 assessment criteria, all included reviews were rated as either critically low or low quality, stemming from a series of key methodological shortcomings: all 5 reviews failed to establish a prospective a priori protocol, 4 out of 5 utilized inadequate literature search strategies with no searches of clinical trial registries or gray literature, all 5 provided no list of excluded studies accompanied by justification, 3 out of 5 included no assessment of publication bias, 4 out of 5 failed to consider risk of bias when interpreting overall results, and all 5 conducted no further exploration of heterogeneity beyond the reporting of I^2 statistics.

Detailed study characteristics, effect sizes, heterogeneity estimates, and risk of bias assessments for these meta-analyses (along with key individual RCTs) are summarized in Table 2. These methodological limitations must be considered when interpreting any pooled estimates from the available evidence.

Multiple studies have compared WXKL with conventional antiarrhythmics including metoprolol, propafenone, amiodarone, and mexiletine, generally showing comparable or superior efficacy.[31,34,39,40] However, no studies have compared WXKL with catheter ablation for any arrhythmia indication, representing an important evidence gap given ablation's established role in drug-refractory cases.

Dosing and Administration

An important practical consideration for clinicians is the appropriate dosing of WXKL. The available evidence on dosing regimens, route of administration, and adjustments in special populations or combination therapy is summarized below.

Route of Administration

Wenxin Keli is formulated as a granular preparation for oral administration. In all published clinical studies and approved indications, the granules are dissolved in warm water and taken orally. No intravenous or other parenteral formulation exists or has been studied in humans.

Standard Daily Dosing Regimens

The dosage of WXKL varies according to the condition being treated and whether it is used as monotherapy or

Table 2. Summary of Clinical Evidence for WXKL by Indication (Including RCTs and Meta-Analyses)

Indication	Study	Study Type	Design	Sample Size	Intervention	Key Outcomes	Effect Size/ Heterogeneity	Risk of Bias Summary
Paroxysmal AF	Meng et al 2015[30]	RCT	Open-label	120	WXKL vs. sotalol	Sinus rhythm maintenance: 76.7% vs. 73.3%	$P = .67$ (NS)	High (no blinding, no placebo)
Paroxysmal AF	Chen et al 2013[31]	Meta-analysis	8 RCTs	624	WXKL ± conventional vs. control	Sinus rhythm maintenance	OR 3.21 (2.14-4.82); $I^2 = 52\%$	Moderate heterogeneity; unclear allocation concealment
Recent-onset AF	Zhang et al 2018[32]	RCT	Open-label	41	Amiodarone + WXKL vs. amiodarone alone	Conversion time	291 vs. 725 min ($P = .003$); conversion rates NS	High (open-label, small sample)
Frequent PVCs	Hua et al 2015[33]	RCT	Double-blind, placebo-controlled	1200	WXKL vs. placebo	Median PVC reduction	61% vs. 22% ($P < .001$)	Low for randomization/ blinding; attrition bias concern
Frequent PVCs	Huang et al 2022[34]	Meta-analysis	11 RCTs	1243	WXKL + metoprolol vs. metoprolol alone	Clinical efficacy	RR 1.32 (1.24-1.40); $I^2 = 67\%$	High (poor randomization, no blinding)
HF with VPCs	Li et al 2017[40]	Meta-analysis	8 RCTs	748	WXKL ± conventional vs. control	VPC frequency; LVEF; 6-min walk	MD −427.08 (−526.73 to −327.43); LVEF MD 4.12 (2.97-5.27)	Low methodological quality in included trials
HF with VA	Zheng et al 2018[38]	Meta-analysis	13 RCTs	1126	WXKL + amiodarone vs. amiodarone alone	Total effective rate; AEs	RR 1.22 (1.16-1.29); AEs OR 0.64 (0.39-1.07); $I^2 = 46\%$	High/unclear risk in all 13 studies

AE, adverse event; HF, heart failure; MD, mean difference; NS, not significant; OR, odds ratio; RR, risk ratio; VA, ventricular arrhythmia; VPC, ventricular premature complex.

in combination with conventional antiarrhythmic drugs. Table 3 summarizes the dosing regimens reported in major clinical trials and registration studies.

Dose Variation by Condition and Patient Factors

The available evidence suggests that dosing may differ by indication and by whether WXKL is used alone or adjunctively. For chronic maintenance therapy in outpatient settings (e.g., PVCs, atrial premature beats), doses range from 15 to 30 g/day in divided doses, typically administered for 4 weeks or longer.[33,40] For acute conversion of recent-onset AF when combined with intravenous amiodarone, a higher loading regimen of 18 g 3 times daily (54 g/day) has been used safely for 24 hours.[32] An ongoing dose-ranging study (NCT02319603) is directly comparing 15 g/day vs. 30 g/day for atrial premature beats to establish the optimal dose, but results have not yet been published.

Notably, no studies have investigated whether dose adjustment is required based on age, body weight, renal function, or hepatic impairment. All trials to date have used fixed dosing without stratification by these factors.

Dose Adjustment with Combination Therapy

With respect to the use of WXKL in combination with other antiarrhythmic agents, existing evidence reveals no protocol-mandated dose reductions of WXKL in combination studies. In the trial evaluating WXKL combined with amiodarone, WXKL was administered at 18 g 3 times daily without any dose adjustments, and no serious adverse events were observed.[32] Similarly, in the meta-analysis of WXKL combined with metoprolol, the WXKL dose was consistently 9 g 3 times daily across all included studies, with no dose adjustments reported.[34] However, clinical trial protocols typically prohibit the concomitant use of other Class I-III antiarrhythmic agents during WXKL monotherapy studies to avoid confounding effects on efficacy and safety outcomes,[33] indicating that when WXKL is used as the primary antiarrhythmic agent, co-administration with other antiarrhythmics is generally avoided. Regarding safety, the combination of WXKL with amiodarone was associated with a shorter AF conversion time without an increase in adverse events,[32] while the metoprolol combination meta-analysis suggested a numerical reduction in adverse reactions in the combination group, though this difference did not reach statistical significance.[34]

Important Limitations and Evidence Gaps

Significant evidence gaps persist regarding optimal WXKL dosing. No pharmacokinetic dosing studies have been published to establish concentration–response relationships, leaving uncertainty about whether current fixed doses achieve therapeutic plasma concentrations of active constituents in all patients. Additionally, no dose adjustment guidelines exist for special populations, including patients with renal impairment, hepatic impairment, elderly individuals, or pediatric patients, as all trials have either excluded these groups or failed to report subgroup analyses. Furthermore, no formal drug–drug interaction studies have quantified whether WXKL affects the pharmacokinetics of co-administered antiarrhythmics (e.g., amiodarone, metoprolol) or whether these drugs alter exposure to WXKL constituents. The optimal dose for different arrhythmia subtypes also remains not definitively established; while the ongoing dose-ranging trial may help address this gap, its results are still awaited. Collectively, these evidence gaps underscore the critical need for rigorous pharmacokinetic and pharmacodynamic studies to inform evidence-based dosing recommendations for WXKL.

Critical Challenges in Development and Global Translation

Wenxin Keli, like most TCM formulas, faces inherent challenges that extend beyond those of conventional single-entity drugs. These challenges are not merely academic; they represent substantive barriers to international acceptance, regulatory approval, and evidence-based clinical integration. For WXKL to transition from a domestically approved Chinese medicine to a globally accessible therapeutic option, the following critical issues must be systematically addressed.

Formulation Variability and Quality Control

Sources of Variability

The 5 herbal components of WXKL-*Codonopsis pilosula*, *Polygonatum sibiricum*, *Panax notoginseng*, *Succinum*

Table 3. Summary of WXKL Dosing Regimens in Clinical Studies

Condition	Dosage Regimen	Duration	Key Findings	Source
Frequent premature ventricular contractions (PVCs)	9 g 3 times daily (27 g/day)	4 weeks	61% median PVC reduction vs. 22% placebo; well tolerated	Hua et al[33]
Atrial premature beats	5 g 3 times daily (15 g/day, low dose) OR 10 g 3 times daily (30 g/day, high dose)	4 weeks	Ongoing dose-ranging study; results pending	NCT02319603
Paroxysmal atrial fibrillation (monotherapy)	9 g 3 times daily (27 g/day)	4 weeks	Sinus rhythm maintenance 76.7% vs. 73.3% with sotalol (NS)	Meng et al[30]
Recent-onset AF (adjunct to IV amiodarone)	18 g 3 times daily (54 g/day)	24 hours	Shorter conversion time (291 vs. 725 min); no increased AEs	Zhang et al[32]
Heart failure with ventricular arrhythmia (adjunct to amiodarone)	9 g 3 times daily (27 g/day)	4-24 weeks	Improved total effective rate (RR 1.22); safety similar to control	Zheng et al[38]

All dosing regimens shown are derived from studies that explicitly excluded patients with significant hepatic or renal impairment. No data exist on dose requirements in these populations; extrapolation is not supported by available evidence.

(amber), and *Nardostachys jatamansi* are derived from natural botanical and mineral sources. Their phytochemical compositions and physicochemical properties can vary considerably, influenced by a multitude of interrelated factors:[41] species and chemotype differences including intraspecific genetic variation, misidentification, or adulteration with substitute species; cultivation conditions such as soil composition, climate, altitude, fertilization practices, and pesticide application; harvest time that contributes to seasonal and diurnal fluctuations in secondary metabolite accumulation; and post-harvest processing procedures including drying regimes (sun-drying vs. oven-drying), storage duration and conditions, extraction solvents, and manufacturing parameters.

Despite manufacturer specifications for marker compounds (e.g., ginsenosides Rb1, Rg1; notoginsenoside R1), these markers may not correlate with antiarrhythmic activity. Advanced analytical techniques such as high-performance liquid chromatography (HPLC) fingerprinting and liquid chromatography–tandem mass spectrometry (LC-MS/MS) multi-component quantification have been developed for research purposes, but they are not universally mandated in regulatory submissions, and batch-release specifications vary among manufacturers.

Limitations of Current Quality Control Paradigms

Current quality control for WXKL relies primarily on chemical fingerprinting—ensuring that the chromatographic profile of a production batch matches a reference standard. However, this approach has 3 fundamental limitations:

1. Analytical targets may not be bioactive. The compounds selected as markers are often abundant and chemically stable, but their contribution to antiarrhythmic efficacy is unproven. Conversely, minor, labile, or unidentified constituents may be responsible for therapeutic effects.
2. Chemical equivalence does not guarantee biological equivalence. Two batches with identical HPLC fingerprints may differ in their pharmacological activity due to undetected enantiomeric variation, differential protein binding, or synergistic interactions among unmeasured constituents.
3. No validated bioactivity assays. Unlike conventional drugs, for which potency is defined by a quantifiable biological effect (e.g., enzyme inhibition IC_{50}), no standardized, pharmacopoeial bioassay exists to assess the functional activity of WXKL batches.

Path Forward: Toward International Harmonization

For WXKL to gain acceptance in regulated pharmaceutical markets, the following quality standards must be developed and implemented:

1. Comprehensive characterization: Full phytochemical profiling using ultra-HPLC coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) to establish a definitive chemical library
2. Multi-component quantification: Simultaneous quantification of at least 10-20 representative constituents spanning different chemical classes (ginsenosides, saponins, alkaloids, organic acids)
3. Bioactivity-linked release specifications: Development of cell-based assays [e.g., hiPSC-CM electrophysiology, ion channel Fluorescence Imaging Plate Reader (FLIPR) assays] that correlate with clinical antiarrhythmic potency and can serve as batch-release potency tests
4. Harmonization with ICH guidelines: Alignment with ICH Q3C (residual solvents), Q3D (elemental impurities), Q8 (pharmaceutical development), and Q9 (quality risk management) for eventual regulatory submission in ICH regions

Regulatory Hurdles

In China, WXKL is approved as a prescription drug under the "new drug" category by the National Medical Products Administration. However, its pathway to approval in Western jurisdictions remains ambiguous and fraught with scientific, regulatory, and evidentiary challenges.

US Food and Drug Administration Botanical Drug Pathway

The Food and Drug Administration (FDA) has established a specific guideline for botanical drug development. Key requirements and corresponding gaps for WXKL are summarized in Table 4.

To date, 4 botanical drugs have received FDA approval as prescription products under the Botanical Drug Guidance: Veregen® (sinecatechins, 2006), Mytesi (crofelemer, 2012), Filsuvez® (birch triterpenes), and NexoBrid® (anacaulase-bcdb).[42,43]The first 2 were approved as New Drug Applications, while NexoBrid® was approved as a biologics license application.[42] Neither is a cardiovascular drug. The

Table 4. Food and Drug Administration Botanical Drug Development Requirements and Current Gaps for WXKL

Requirement	Current Status for WXKL	Critical Gap
Phase 1: Chemistry, manufacturing, and controls (CMC)	Partial chemical characterization available; no validated bioassay	No defined active constituent; batch consistency not publicly demonstrated
Phase 1: Preclinical safety assessment	Limited subchronic toxicity studies; no genotoxicity, carcinogenicity, or reproductive toxicology	Non-ICH-compliant; lacks regulatory-grade GLP studies
Phase 2: Well-controlled clinical trials	Multiple RCTs conducted, all in China	Not registered; not GCP-compliant; not acceptable for FDA filing
Phase 3: Confirmatory trials	None	Pivotal trials required for approval

CMC, Chemistry, manufacturing, and controls; GCP, Good clinical practice; GLP, Good laboratory practice; ICH, International Council for Harmonization; IND, Investigational new drug; RCT, Randomized controlled trial.

FDA pathway is therefore feasible but demanding; it requires substantial investment and strategic planning.

European Medicines Agency Herbal Medicinal Products

The European Medicines Agency (EMA) Committee on Herbal Medicinal Products provides 2 registration pathways:

1. Well-established use: Requires bibliographic evidence of safety and efficacy with at least 10 years of recognized medicinal use within the EU. Wenxin Keli does not meet this criterion.
2. Traditional use registration: Requires at least 30 years of medicinal use, including 15 years within the EU. Wenxin Keli also does not meet this criterion.

Thus, WXKL would require a marketing authorization application as a full-fledged medicinal product, supported by a complete dossier of quality, preclinical, and clinical data comparable to that required for any new chemical entity.

Key Regulatory Barriers: An Analytical Summary

Despite the extensive preclinical and clinical literature on WXKL, its path to international regulatory approval is obstructed by 3 interrelated deficits that are fundamental rather than incremental.

First, the chemical complexity of WXKL and the absence of identified active constituent(s) create a cascade of scientific and regulatory ambiguities. Unlike a single-entity drug, for which absorption, distribution, metabolism, and excretion can be tracked unambiguously, WXKL contains hundreds of compounds. It remains unknown whether the antiarrhythmic effect resides in a single potent molecule, a defined combination, or the holistic synergy of the entire mixture. This uncertainty precludes rational dose optimization, prevents pharmacokinetic assessment, makes bioequivalence testing impossible, and blocks the development of mechanism-based quality control assays. Until the active principle(s) are identified—or an acceptable surrogate marker of pharmacological activity is validated—WXKL cannot satisfy the fundamental CMC and nonclinical pharmacology requirements of any ICH-aligned regulatory authority.

Second, the existing nonclinical safety package is incomplete by international standards. Although subchronic toxicity studies have been performed, they were not conducted in compliance with good laboratory practice and do not address genotoxicity, carcinogenicity, or reproductive and developmental toxicity as required by ICH S2, S1A, and S5 guidelines. Safety pharmacology assessments are limited to cardiovascular effects; central nervous system and respiratory system evaluations are absent. These gaps are not merely formalistic: without these studies, the safety of chronic WXKL administration cannot be adequately assessed, and the drug would not be approved for long-term use in any ICH jurisdiction.

Third, the clinical evidence base, while substantial in volume, does not meet the evidentiary standards expected for marketing authorization in the United States or European Union. All published RCTs were conducted in China; none were registered prospectively, and few adhered to ICH E6 Good Clinical Practice (GCP) guidelines. Deficiencies include inadequate documentation of allocation concealment, absence of independent data monitoring, unbundled or poorly described endpoint adjudication, and statistical analysis plans that were not pre-specified. Consequently, even well-conducted domestic trials cannot be used directly to support regulatory filing. A new series of international, multicenter, GCP-compliant trials will be required.

Together, these 3 barriers—unknown active constituents, incomplete regulatory-grade toxicology, and non-compliant clinical development—form a mutually reinforcing obstacle. Progress on any 1 front is contingent on progress on the others. Overcoming them will require a coordinated, strategically phased investment in analytical chemistry, nonclinical safety, and clinical trial infrastructure.

Individual Variability and Personalized Therapy

Traditional Chinese medicine is founded on the principle of bianzheng lunzhi (syndrome differentiation and individualized treatment). In theory, this allows tailoring of herbal formulas to each patient's unique constitutional and pathological state. In practice, however, WXKL is prescribed at a fixed dose (9 g 2 or 3 times daily) regardless of: age, body weight, renal function (creatinine clearance), hepatic function (Child-Pugh class), concomitant medications, and genetic polymorphisms affecting drug metabolism or arrhythmia susceptibility.

Pharmacokinetic Variability

No population pharmacokinetic studies have been published; it is unknown whether plasma concentrations of active constituents differ substantially among individuals, whether renal or hepatic impairment necessitates dose adjustment, or whether significant drug–drug interactions occur with commonly co-prescribed cardiovascular drugs such as statins, beta-blockers, anticoagulants, or antiplatelet agents.

Pharmacodynamic Variability

Response to antiarrhythmic drugs is known to be influenced by genetic polymorphisms in ion channel genes (e.g., SCN5A, KCNQ1, KCNH2), β-adrenergic receptor variants, and disease substrate such as atrial fibrosis, left ventricular hypertrophy, or ischemic burden. No studies have examined whether any of these factors predict differential response to WXKL. Consequently, there are no criteria to guide patient selection, predict therapeutic failure, or identify individuals at increased risk of adverse effects.

Toward Precision Medicine with Wenxin Keli

To realize the promise of personalized therapy, the following research priorities are identified:

1. Population pharmacokinetic modeling: Conduct dense-sampling pharmacokinetic studies in arrhythmia patients to estimate interindividual variability and identify covariates (age, renal function, genotype) that influence drug exposure.
2. Therapeutic drug monitoring: Develop and validate LC-MS/MS assays for quantification of representative

WXKL constituents in human plasma; explore concentration–response relationships.
3. Pharmacogenomic studies: Genotype patients in prospective trials for variants in drug-metabolizing enzymes (CYP2C19, CYP2D6, CYP3A4/5), drug transporters, and arrhythmia-associated genes; correlate with efficacy and safety outcomes.
4. Phenotypic stratification: Evaluate whether WXKL exhibits differential efficacy in specific arrhythmia subtypes (e.g., paroxysmal AF with high vagal tone vs. adrenergic AF, PVCs originating from outflow tract vs. fascicular).
5. Machine learning-based predictive algorithms: Integrate clinical, electrocardiographic, imaging, and -omic data to develop and validate prediction models for individualized response.

Future Research Directions

The preceding critical appraisal reveals substantial evidence gaps across the entire translational spectrum—from basic pharmacology to clinical efficacy, safety, quality control, and regulatory science. To advance WXKL from a promising TCM formula to an evidence-based antiarrhythmic therapy accepted globally, the following prioritized multi-level research agenda is proposed.

MECHANISTIC RESEARCH: FROM DESCRIPTIVE TO MECHANISTIC

Human-Based Experimental Models

A fundamental limitation of existing preclinical research is the near-exclusive reliance on non-human species (rodent, canine, rabbit) and non-cardiac heterologous expression systems. Future studies must prioritize human-relevant models.

Systematic Ion Channel Profiling

Current understanding of the ion channel-mediated effects of WXKL remains fragmentary. To address this gap, a comprehensive and unbiased ion channel profiling screen should be performed using automated patch-clamp platforms (e.g., QPatch, SyncroPatch) to enable high-throughput evaluation of all major cardiac ion channels including NaV1.5, CaV1.2, KV7.1, KV11.1, KV4.3, and HCN4, alongside state-dependent and use-dependent testing protocols to rigorously assess proarrhythmic liability. Concentration–response relationships should also be established across a concentration range relevant to human clinical exposure, with reference values derived from validated clinical pharmacokinetic studies.

Identification of Active Constituent

In the absence of clear evidence identifying the specific compounds responsible for WXKL's antiarrhythmic activity, rational dose optimization, standardized quality control, and formal regulatory approval remain unachievable. A multipronged experimental strategy is therefore required: bioassay-guided fractionation should be applied to sequentially separate WXKL extracts via preparative HPLC, with each fraction tested in electrophysiological assays using hiPSC-CMs to isolate active components and iteratively purify them to single compounds; affinity-based target deconvolution can be performed by immobilizing WXKL constituents on a solid support to pull down interacting proteins from cardiac lysates, with target identification achieved via mass spectrometry; human hepatocyte incubations and in vivo pharmacokinetic studies should be carried out to characterize circulating metabolites, and both parent compounds and metabolites should be evaluated in functional antiarrhythmic assays; finally, the full antiarrhythmic activity of WXKL should be reconstituted using defined mixtures of synthetic or purified constituents to confirm the functional contribution of individual components.

Unbiased Omics Approaches

To move beyond narrow candidate-based hypothesis testing and discover novel mechanisms, unbiased hypothesis-generating omics studies should be implemented, including transcriptomic profiling by RNA-seq in hiPSC-CMs treated with WXKL vs. vehicle, quantitative proteomic mass spectrometry to identify differentially expressed proteins and post-translational modifications, untargeted metabolomics to map metabolic pathway perturbations, and phosphoproteomics for the enrichment and quantification of phosphorylated peptides to identify activated or inhibited signaling nodes. Integration of these multi-omics datasets using systems biology approaches—including network inference, pathway enrichment analysis, and causal reasoning—can reveal previously unanticipated mechanisms of action and prioritize key molecular targets for further experimental validation.

CLINICAL RESEARCH: FROM EFFICACY TO EFFECTIVENESS

High-Quality Confirmatory Trials

Existing trials of WXKL are insufficient for regulatory approval due to methodological limitations and lack of international generalizability. A definitive phase 3 trial should enroll symptomatic paroxysmal AF patients in a double-blind, double-dummy design comparing WXKL, an active comparator, and placebo. The primary endpoint must be AF burden measured by continuous monitoring. The trial requires adequate sample size, 6-12 months treatment duration, international sites, GCP compliance, prospective registration, and independent oversight. Only evidence of this rigor can support global regulatory submission.

Patient–Reported Outcomes and Health Economics

As cardiac arrhythmias substantially diminish patient quality of life, treatment strategies and clinical trial endpoints ought to integrate patient-centered perspectives. Recent studies from Türkiye have contributed to the understanding of AF management and patient-centered outcomes in cardiovascular care.[44,45] Future clinical investigations should therefore incorporate validated disease-specific quality-of-life tools such as the Atrial Fibrillation Effect on Quality of Life (AFEQT) and Arrhythmia-Specific Questionnaire in Tachycardia and Arrhythmia (ASTA) questionnaires, symptom severity grading instruments including the European Heart Rhythm Association score for AF, standardized assessments of healthcare resource utilization including hospital admissions, emergency department visits, and cardioversion procedures, as well as formal cost-effectiveness evaluations based on quality-adjusted life years.

Pragmatic and Comparative Effectiveness Studies

Following initial regulatory approval, pragmatic clinical trials implemented in routine real-world clinical settings will be essential to characterize the comparative effectiveness of WXKL in unselected patient populations, evaluate long-term treatment adherence, and detect rare or delayed adverse events. Suitable research designs may include large-scale simple trials integrated within electronic health record systems, prospective registry-based RCTs (R-RCTs), and target trial emulation approaches using large observational clinical databases.

Special Populations

Targeted clinical studies are urgently needed for patient subgroups that have been systematically excluded from most previous WXKL trials, including elderly patients aged 75 years and older, individuals with chronic kidney disease (eGFR < 60 mL/min/1.73 m^2), those with moderate to severe hepatic impairment, pediatric patients presenting with arrhythmias, and pregnant or lactating women, for whom evidence should be accumulated through post-marketing surveillance studies.

Pharmaceutical Development: Quality by Design

Establishment of a International Reference Standard

A collaborative initiative involving the manufacturer, academic research laboratories, and official pharmacopoeial authorities including the Chinese Pharmacopoeia, United States Pharmacopoeia, and European Pharmacopoeia should be launched to establish a comprehensive international reference standard for WXKL. This standard should comprise a chemically characterized reference extract with a complete LC-MS/MS fingerprint profile, purified reference compounds corresponding to the major ginsenosides, saponins, and alkaloids present in the formulation, as well as a publicly accessible spectral library to support consistent and reliable quality control across laboratories and manufacturing sites.

Development of Bioactivity Assays

To supplement chemical fingerprint-based quality control, a validated and qualified bioactivity assay should be developed for routine batch-release testing of WXKL. Promising candidate assays include high-throughput hiPSC-CM multi-electrode array analysis for the measurement of field potential duration and beat rate, FLIPR membrane potential dye assays for the efficient assessment of ion channel modulation, and Ca^{2+} transient imaging in hiPSC-CMs to quantify drug effects on intracellular calcium handling. Any such assay must be fully validated in accordance with ICH Q2(R1) guidelines, with satisfactory demonstration of accuracy, precision, linearity, specificity, and robustness for regulatory-compliant quality assessment.

Continuous Manufacturing and Process Analytical Technology

The transition from conventional batch manufacturing to continuous manufacturing integrated with real-time release testing represents a promising strategy to minimize batch-to-batch variability in WXKL production. The implementation of process analytical technology, including near-infrared spectroscopy and Raman spectroscopy, would further enable real-time monitoring and control of critical quality attributes throughout the extraction, concentration, and granulation stages of production, thereby enhancing the consistency, stability, and reliability of the final pharmaceutical product.

Regulatory Science and Policy

Food and Drug Administration and European Medicines Agency Engagement

The sponsor should initiate formal and proactive regulatory dialogue with both the FDA and EMA to support the global clinical development of WXKL. For the FDA, a pre-investigational new drug (IND) meeting should be scheduled to present the proposed nonclinical development plan, CMC strategy, and clinical trial design, while seeking official alignment on the acceptability of the botanical IND application. For the EMA, formal scientific advice should be sought through a comparable structured process, with additional discussion regarding the feasibility of adaptive licensing pathways or eligibility for the PRIME (PRIority MEdicines) scheme to expedite development and regulatory assessment.

Development of a Global Clinical Trial Network

To conduct sufficiently well-powered international clinical trials, a dedicated academic clinical trial network focused on cardiovascular TCM research should be established. This network would develop and maintain fully standardized study protocols and case report forms, provide systematic training for participating investigators in ICH GCP, deliver centralized support for randomization, data management, and independent endpoint adjudication, and facilitate robust multi-center collaborative partnerships across Asia, Europe, and North America.

Post-Approval Commitments and Pharmacovigilance

A proactive and comprehensive pharmacovigilance plan should be designed during the pre-approval phase to support long-term safety monitoring of WXKL. This plan will encompass passive surveillance via spontaneous adverse event reporting systems, active surveillance through a prospective dedicated registry enrolling patients receiving WXKL, and systematic signal detection using advanced data mining approaches applied to electronic health records and administrative claims databases.

CONCLUSION

Wenxin Keli represents one of the most extensively studied TCM formulas for cardiac arrhythmias. Preclinical research has uncovered multiple ion channel and signaling pathway effects that plausibly underlie its antiarrhythmic activity, although the translational relevance of many findings remains uncertain due to the absence of human model validation, undefined active constituents, and incomplete ion channel profiling.

Clinical studies suggest efficacy in reducing AF burden and PVC frequency with a favorable short-term safety profile. However, the evidence base is constrained by methodological

limitations, heterogeneity, potential publication bias, and the absence of internationally conducted, regulatory-grade trials.

Crucially, WXKL's global development is hindered by fundamental challenges in formulation consistency, quality control paradigms that are not aligned with ICH standards, insufficient regulatory toxicology, and unresolved questions about its pharmacokinetics, active constituents, and drug interaction potential.

Rather than asserting therapeutic superiority, the current evidence positions WXKL as a potentially useful adjunct or alternative for selected patients—particularly those intolerant of or refractory to conventional antiarrhythmic drugs. However, this potential can only be realized through a concerted, multi-stakeholder effort involving rigorous mechanistic science, high-quality confirmatory trials, modernization of quality control, and strategic regulatory engagement.

It is hoped that this critical review provides not only a realistic appraisal of where WXKL stands today but also a constructive, actionable roadmap for where it needs to go. The path from traditional remedy to globally accepted therapy is long, but with scientific rigor and international collaboration, it is navigable.

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Modulatory Effects of Clopidogrel on Vascular Response in the Human Internal Mammary Artery: An In Vitro Study

ABSTRACT

Background: Coronary artery disease (CAD) is one of the leading causes of mortality worldwide, and coronary artery bypass grafting (CABG) is frequently preferred as a surgical approach in advanced cases. The most commonly used graft material in CABG, the left internal mammary artery (LIMA), is considered the gold standard due to its high long-term patency rates. Clopidogrel, an antithrombotic agent widely used in CABG patients that targets $P2Y_{12}$ receptors on platelets, has also been suggested to influence vascular responses due to the presence of these receptors in vascular smooth muscle cells. However, the direct vascular effects of clopidogrel on LIMA remain unknown.

Methods: In this study, isolated LIMA segments obtained from 20 patients undergoing CABG were evaluated to compare the contractile responses induced by phenylephrine (PE; 10^{-9}-10^{-4} M) and the endothelium-independent relaxation responses induced by sodium nitroprusside (SNP; 10^{-9}-10^{-4} M), before and after pretreatment with 0.1 µM clopidogrel.

Result: In clopidogrel-treated LIMA segments, all PE-induced contractile responses were significantly attenuated, whereas SNP-induced relaxation was enhanced at low and moderate concentrations; however, this relaxant effect was diminished at the highest dose.

Conclusion: This biphasic effect observed in LIMA segments suggests that clopidogrel may act not only as an antithrombotic agent, but also as a direct modulator of vascular tone. These findings indicate that the local vascular effects of clopidogrel should be considered in developing therapeutic strategies aimed at maintaining graft patency following CABG.

Keywords: Antiplatelet agents, bypass, coronary heart disease, internal mammary artery, organ bath

ORIGINAL INVESTIGATION

INTRODUCTION

Coronary heart disease (CHD), with its increasing prevalence and status as the leading cause of mortality globally, represents a critical public health challenge of worldwide significance.[1-3] Management of CHD involves a multimodal approach.[4-7] In advanced cases, invasive procedures are used to restore arterial patency, while coronary artery bypass grafting (CABG) remains the definitive treatment in severe cases.[8-11] CABG involves bypassing stenotic or occluded coronary arteries using grafts, most commonly the left internal mammary artery (LIMA) or saphenous vein grafts. Among these, LIMA is considered the gold standard, owing to its superior patency rates and long-term outcomes. Studies have demonstrated that LIMA has a 10-year patency rate exceeding 90%, significantly reducing cardiac complications and improving survival compared to other graft options.[12-14]

Despite these advancements, the long-term success of CABG is influenced by multiple factors, including graft patency and the graft's ability to maintain vasodilation and vasoconstriction responses.[15,16] One of the most critical determinants of graft failure is vasoconstriction. Arterial grafts, in particular, are prone to spasm because of their well-developed smooth muscle layer and high α-adrenergic receptor sensitivity. Surgical manipulation and endothelial injury

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further exacerbate this susceptibility, leading to decreased graft flow, thrombosis, and early occlusion.[17] Moreover, the discontinuation of antiplatelet therapy during the preoperative period may increase the risk of postoperative thrombotic events.[18,19] Following CABG, excessive vasoconstriction and thrombosis within the grafted vessels may contribute to early graft failure. Clinical data indicate that up to 20% of venous grafts may occlude within the first postoperative year.[20] Therefore, a comprehensive understanding of how antiplatelet agents (e.g., clopidogrel, ticagrelor) and anticoagulants (e.g., heparin derivatives, direct thrombin, and factor Xa inhibitors) affect vascular reactivity is of great importance in optimizing graft outcomes.[21,22]

Among the pharmacological agents used in this context, clopidogrel—a thienopyridine adenosine diphosphate (ADP) receptor antagonist—has gained widespread acceptance. Clopidogrel has become a cornerstone in preventing thrombotic events in CHD and CABG patients by irreversibly inhibiting platelet aggregation.[23,24] Clopidogrel blocks platelet $P2Y_{12}$ receptors irreversibly and may also affect vascular smooth muscle cells where these receptors are minimally expressed.[25] This suggests that clopidogrel may exert modulatory effects on vascular smooth muscle function, which may vary depending on the specific vascular bed.[26-29]

However, despite its widespread use in patients with CHD and following CABG surgery, the effect of clopidogrel on contractile responses in LIMA has not been investigated. This study aims to investigate the effects of clopidogrel on the contractile and relaxation mechanisms of human LIMA specimens, with the goal of informing the development of novel strategies to optimize postoperative management and enhance long-term graft patency and overall surgical outcomes.

METHODS

Informed consent was obtained from the patients, and the Clinical Research Ethics Committee of Fırat University Faculty of Medicine (Elazığ, Türkiye) approved the use of discarded human LIMA segments in this study (26.10.2017, number 11). This study was conducted in accordance with the principles of the Declaration of Helsinki. No artificial intelligence–assisted technologies, including Large Language Models, chatbots, or image generators, were used in the production of this manuscript.

The sample size of 20 was determined based on an a priori power analysis conducted using G*Power 3.1.9.7 software. For a repeated-measures ANOVA, the analysis was performed assuming a medium effect size ($f = 0.25$), an alpha level of 0.05, and a desired statistical power of 0.80. With 6 repeated measurements and a correlation among repeated measures set at 0.5, the required total sample size was calculated to be approximately 19. Therefore, a sample size of 20 was selected to ensure sufficient power to detect statistically significant effects while also accounting for potential data loss or variability.

Segments of the LIMA were collected from 20 patients undergoing CABG. Demographic and clinical characteristics of these patients are given in Table 1.

In accordance with standard experimental protocols in human vascular research, the distal segments of LIMA—those not utilized during grafting—were selected for evaluation. These segments are routinely obtained during CABG procedures as residual tissue and are commonly used in ex vivo studies due to their accessibility and higher smooth muscle content. While it is acknowledged that different segments of LIMA may exhibit variable anatomical and structural properties, the distal segment remains the most practical and representative choice for investigating vasomotor responses in isolated organ bath experiments.

The IMA were carefully cleaned of loose connective tissue and cut into rings (about 2-3 mm long). The preparations were placed in an isolated tissue bath containing Krebs–Henseleit (KH) solution (composition in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.25, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, EDTA 0.03) at 37°C and pH 7.4, constantly bubbled with a mixture of 95% O_2 and 5% CO_2. Contractile activities were recorded using a physiological force transducer (FDT05, Commat Ltd, Ankara, Türkiye) recorded by MP150WS for Windows (Biopac Systems Inc, CA, USA).[30,31] At the beginning of the experiments, the resting tension of the LIMA vessels was adjusted to 1 g and

Table 1. Some Clinical Features of 20 Patients Undergoing CABG

Clinical features	Mean ± SD, n (%)
Age, years	68.0 ± 4.0
Weight	78.6 ± 8.0
Body mass index	28.2 ± 2.4
Gender	
Male	11 (55)
Female	9 (45)
Smoking	8 (40)
Diseases	
Hypertension	18 (90)
Heart failure	4 (20)
Diabetes	8 (40)
Medication	
Organic nitrates	0 (0)
Aspirin	20 (100)
Beta-blockers	15 (75)
Angiotensin inhibitors	9 (45)
Calcium channel blockers	4 (20)
Hypolipidemics	14 (70)

HIGHLIGHTS

- Clopidogrel reduces PE-induced contraction in the left internal mammary artery (LIMA).
- Clopidogrel modulates sodium nitroprusside(SNP)-induced relaxation in the LIMA.
- The LIMA vascular bed is responsive to clopidogrel.

they were allowed to equilibrate under this resting tension for 120 min. Phenylephrine (PE) and SNP were obtained from Sigma (St. Louis, MO, USA), while clopidogrel was obtained from Sanofi Pharmaceuticals (Türkiye). Phenylephrine (PE) and SNP were dissolved in distilled water immediately before use to ensure stability and reproducibility of concentration–response curves.

Following the equilibration period, PE was administered to the organ bath chamber at 30-second intervals in increasing concentrations from 10^{-9} M to 10^{-4} M. The contraction induced by the highest dose (10^{-4} M) was considered as 100%, and the responses to the lower doses were recorded as percentages relative to this maximum contraction, serving as the control contraction protocol.

To assess the effect of clopidogrel on contractile responses, 0.1 μM clopidogrel was added to the organ bath.[28,32] Following clopidogrel administration, tissues were incubated for 10 minutes. After an incubation period, the PE contraction protocol was repeated with increasing concentrations of PE from 10^{-9} M to 10^{-4} M applied at 30-second intervals. To avoid any systematic influence of the solvent composition or dissolution conditions on the experimental outcomes, Krebs solution was used as the solvent for clopidogrel. This approach was chosen to ensure experimental standardization and to attribute the observed pharmacological effects solely to clopidogrel administration.

Thereafter, to allow the tissue to return to its basal tone, a 90-minute stabilization period was applied, during which the LIMA segments were washed with fresh Krebs solution at 10-minute intervals.

To evaluate the relaxation response, particularly endothelium-independent relaxation, a SNP protocol was applied. Initially, the stable isolated LIMA segment was contracted using 10^{-4} M PE. Subsequently, SNP was administered in increasing concentrations from 10^{-9} M to 10^{-4} M at 30-second intervals. The maximal relaxation observed at 10^{-4} M was accepted as 100%, and relaxation responses at lower concentrations were recorded as percentages of this maximum response.

Subsequently, 0.1 μM clopidogrel was introduced into the organ bath, followed by a 10-minute incubation period, after which the vascular segment was contracted with 10^{-4} M PE. Thereafter, the SNP-induced relaxation protocol was re-applied under identical experimental conditions.[28,30,31]

Statistical analyses were performed using GraphPad Prism version 10.4.1 (GraphPad Software, San Diego, CA, USA). Data are expressed as mean ± standard error of the mean (SEM). Contractile responses to PE were normalized to the maximal contraction. Relaxation responses to cumulative concentrations of SNP (10^{-9} to 10^{-4} M) were calculated as the percentage reduction from PE-induced precontraction. Prior to parametric analyses, the normality of all continuous variables across repeated measurements was assessed using the Kolmogorov–Smirnov test, which confirmed that the data were normally distributed in all phases ($P > .05$). Thus, the assumptions required for parametric testing were satisfied.

Comparisons between the control and clopidogrel groups across drug concentrations were conducted using 2-way analysis of variance (2-way ANOVA). When a significant interaction was detected, Türkiye's multiple comparisons test was used as a post hoc analysis to identify pairwise differences at each concentration. A P-value < .05 was considered statistically significant.

RESULTS

Cumulative concentrations of PE (10^{-9} to 10^{-4} M) induced dose-dependent contractile responses in isolated LIMA vessels. In vessels pretreated with clopidogrel, PE-induced contractions were significantly reduced at all concentrations compared to the control group. According to Türkiye's post hoc multiple comparison test, contractile responses were significantly attenuated in the clopidogrel group at concentrations of 10^{-9} ($P = .023$), 10^{-8} ($P = .022$), 10^{-7} ($P = .010$), 10^{-6} ($P < .001$), 10^{-5} ($P < .001$), and 10^{-4} M ($P < .001$) (Figure 1).

Cumulative concentrations of sodium nitroprusside (SNP) (10^{-9} to 10^{-4} M) induced dose-dependent relaxation in PE-precontracted isolated LIMA rings. In LIMA rings pretreated with clopidogrel, relaxation responses to SNP exhibited concentration-dependent alterations. According to the results of Türkiye's multiple comparison test, relaxation responses were significantly enhanced in the clopidogrel group at 10^{-8} ($P = .017$), 10^{-7} ($P = .007$), and 10^{-6} M ($P = .001$) concentrations, while no significant difference was observed at 10^{-9} ($P = .480$) and 10^{-5} M ($P = .390$) concentrations between the groups. In contrast, at the concentration of 10^{-4} M, pretreatment with clopidogrel significantly inhibited the relaxation response to SNP ($P = .018$) (Figure 2).

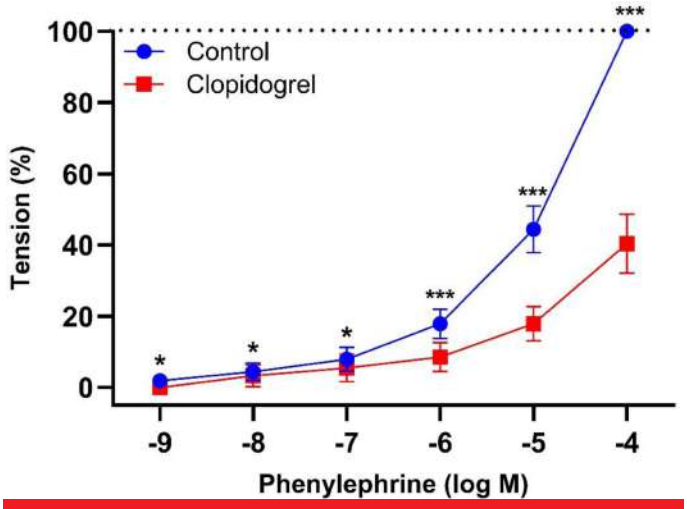

Figure 1. Effect of clopidogrel pretreatment on phenylephrine-induced contractions in isolated human left internal mammary artery (LIMA) segments. Cumulative concentration–response curves to phenylephrine (10^{-9} to 10^{-4} M) were obtained from isolated LIMA segments pretreated with clopidogrel (n = 20). Data are presented as mean ± standard error of the mean (*$P < .05$, **$P < .01$, ***$P < .001$).

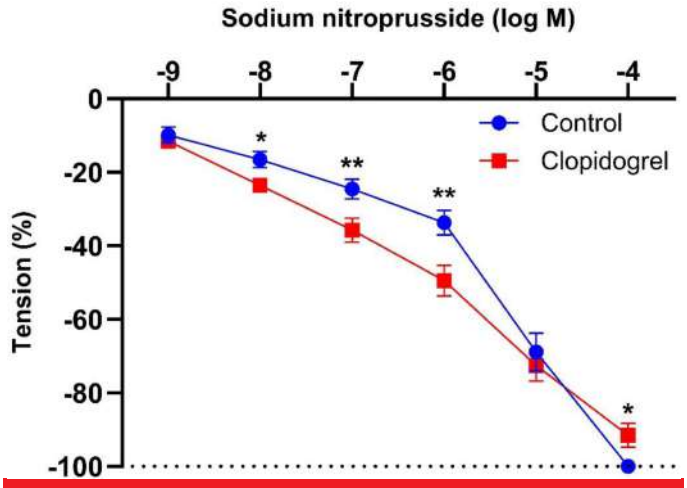

Figure 2. Effects of clopidogrel on sodium nitroprusside-induced relaxation in phenylephrine pre-contracted human left internal mammary artery (LIMA) segments. The magnitude of the relaxation response is expressed as a percentage decrease in vascular tension. Data are presented as mean ± standard error of the mean (n = 20) (*$P < .05$, **$P < .01$, ***$P < .001$).

DISCUSSION

In this study, the effects of clopidogrel on isolated human LIMA rings were investigated. Consistent with existing literature, PE induced dose-dependent contractile responses in LIMA rings. In contrast, in strips pretreated with clopidogrel, PE-induced contractions were markedly attenuated. Notably, the inhibitory effect of clopidogrel on contractile responses became more pronounced with increasing PE concentrations. As expected, SNP elicited vasorelaxation in LIMA rings. In clopidogrel-treated strips, the endothelial-independent relaxation response to low-dose SNP was significantly enhanced. However, at higher SNP doses, the vasodilatory effect was significantly diminished.

It is well established that different segments of the LIMA exhibit anatomical and histological heterogeneity. In the present study, distal segments of the LIMA were selected primarily because the proximal portion is typically used as a graft during CABG surgery, whereas the distal segments, not included in the grafting process, can be ethically and practically obtained as residual tissue. Nevertheless, distal segments are considered the most suitable for evaluating vasomotor responses in isolated organ bath experiments due to their higher smooth muscle content.[33,34]

Due to the limited viability of tissues obtained after surgery and the unavoidable impact of surgical manipulation on endothelial integrity, SNP was selected in the present study to assess endothelium-independent relaxation.[31] The observed enhancement of SNP-induced relaxation in the presence of low-dose clopidogrel suggests an increased sensitivity of vascular smooth muscle cells to nitric oxide (NO). This effect may be mediated through modulation of the NO–cyclic guanosine monophosphate (cGMP) signaling pathway and/or alterations in intracellular calcium handling, including changes in calcium influx or myofilament calcium sensitivity. In this context, previous studies have suggested that antiplatelet agents may enhance NO-mediated vasorelaxation by increasing intracellular cGMP levels and activating downstream cGMP-dependent signaling mechanisms, which can ultimately lead to reduced vascular smooth muscle contractility.[26,35-37]

The biphasic vascular response observed in the present study suggests that clopidogrel may exert its effects not merely through classical vasodilation, but rather via a broader vasomodulatory function. Importantly, this biphasic pattern also points to clinically meaningful implications. Although the in vitro concentration of clopidogrel used in this study (0.1 µM) does not directly replicate systemic plasma levels, the findings indicate that the vascular effects of clopidogrel are neither dose-independent nor unidirectional. Instead, they appear to be concentration-sensitive and bidirectional in nature. This observation underscores the potential importance of dose optimization in the context of preventing graft spasm following CABG. During the perioperative period, fluctuations in hemodynamic conditions and surgical manipulation of the vessel wall may alter local drug distribution, leading to vascular effects that differ from those observed at systemic levels. In this context, it should be considered that local drug exposure may produce variable, dose-dependent effects on vascular tone. Further translational and clinical studies are warranted to elucidate the dose–response relationship and to clarify the potential clinical implications of these findings.

Currently, antiplatelet agents play a pivotal role in preventing ischemia-related atherosclerotic and thrombotic events and are widely used in the post-CABG setting.[38] Among these agents are acetylsalicylic acid, glycoprotein IIb/IIIa receptor antagonists, and thienopyridines such as ticlopidine and clopidogrel, which block platelet ADP receptors.[39,40]

It has been demonstrated that ADP receptors are expressed not only on platelets but also on vascular smooth muscle cells, highlighting their potential role in modulating vascular responses.[27] One study reported that thienopyridines can induce in vitro vasorelaxation, independent of hepatic biotransformation, by directly affecting vascular tissues and smooth muscle cell cultures.[26] Another study suggested that this effect may not be strictly dependent on endothelial NO release, as pretreatment with the NO synthase inhibitor L-NAME did not suppress thienopyridine-induced relaxation, unlike in acetylcholine-mediated pathways.[25] These findings suggest a mechanism potentially linked to intracellular calcium modulation.[26] Several studies have reported that ticagrelor enhances adenosine-mediated vasodilation, while acetylsalicylic acid influences the prostacyclin–thromboxane balance, and that these mechanisms may collectively alter graft hemodynamics following coronary bypass surgery.[41,42]

Clopidogrel is known to undergo hepatic biotransformation to an active thiol metabolite, which irreversibly inhibits the $P2Y_{12}$ receptor. However, several studies have reported that the parent compound of clopidogrel may also exert direct regulatory

effects on various vascular and smooth muscle tissues independent of hepatic metabolism.[26,28,32] Moreover, the presence of certain esterase and CYP2C9/CYP3A4-like enzymatic activities in human vascular tissues suggests the possibility of partial local metabolic conversion.[43,44] Within this framework, the present study was designed not to replicate the in vivo pharmacokinetic process, but rather to explore the potential direct effects of the parent form of clopidogrel on contractile and relaxation mechanisms in human LIMA segments. The short plasma half-life of the active thiol metabolite and the difficulty of stabilizing it under in vitro conditions further justify the methodological rationale of this approach.[45]

Clopidogrel has been shown to reduce inflammation and preserve endothelial NO synthase expression in ischemic coronary arteries of rabbits, supporting a NO-mediated mechanism in its vascular effects.[46-48] Pre-treatment with clopidogrel has also been reported to reduce vasoconstrictive responses to serotonin (5-HT), endothelin-1, and platelet-rich plasma/arachidonic acid mixtures in rat and rabbit aortic rings.[49] Similarly, Jakubowski et al[48] demonstrated a rapid and direct endothelial effect of clopidogrel, independent of its antiplatelet action, in isolated guinea pig hearts. In contrast, other studies have reported no significant inhibition of $P2Y_{12}$ receptor-mediated vasoconstriction in patients treated with clopidogrel,[25] and even high oral doses of clopidogrel failed to inhibit this response meaningfully.[50] Nevertheless, clopidogrel has been shown to improve endothelial function in patients with stable coronary artery disease,[51] and in hypertensive rat mesenteric arteries, it normalized PE-induced vascular contraction and restored impaired acetylcholine-induced relaxation.[47] However, it remains unclear whether these effects are exclusive to the mesenteric vasculature or extend to other vascular beds. Furthermore, it is not yet established whether clopidogrel prevents structural and functional alterations in affected vessels.

Moreover, it should be noted that 90% of the patients included in the present study were hypertensive, 40% were diabetic, and all were receiving acetylsalicylic acid therapy. Each of these comorbid conditions is known to exert substantial effects on vascular reactivity.[52-54] Despite this pronounced clinical heterogeneity and high-risk profile, the observation that clopidogrel significantly attenuated PE-induced contraction may be regarded as one of the major strengths of the study.

In the literature, a study employing a hypothesis similar to that of the present research reported that clopidogrel in combination with aspirin, or clopidogrel monotherapy, may be effective in maintaining high graft patency rates in the early postoperative period following CABG. Furthermore, the same study indicated that the clopidogrel + aspirin combination did not confer a significant advantage over clopidogrel monotherapy in this context.[55] In another study, it was suggested that clopidogrel use after CABG may not improve 1-year graft patency.[56]

In this context, these collective findings underscore the importance of considering and further investigating the potential effects of clopidogrel on vascular resistance. The discrepancies reported in the literature are likely attributable to structural and functional differences among the vascular beds examined. This study serves as a pioneering investigation by demonstrating, for the first time, the potential vascular effects of clopidogrel within the LIMA vascular bed.

Future research incorporating comparative evaluations of different antiplatelet agents and translational approaches supported by clinical vascular function assessments may enable the establishment of a more comprehensive pharmacodynamic profile of these agents.

Limitations of the Study and Conclusion

Our study provides a novel perspective by demonstrating that the LIMA vascular bed is responsive to clopidogrel. In LIMA rings treated with clopidogrel, vasoconstrictor responses to PE were reduced, while vasodilator responses to SNP were enhanced at low doses and suppressed at high doses. These findings support the notion that the effects of clopidogrel are not limited to its antithrombotic properties, but may also involve a modulatory role on vascular function. This is particularly important for maintaining graft patency following CABG and underscores the need to consider the potential local vascular effects of clopidogrel. However, a major limitation of our study is the lack of advanced in vitro analyses that could provide deeper mechanistic insights into the effects of clopidogrel. This shortcoming limits our ability to fully elucidate the cellular and molecular actions of the drug on vascular smooth muscle cells. In addition, due to the limited sample size, a formal subgroup analysis comparing vascular responses between diabetic and non-diabetic patients could not be performed. Nevertheless, at an observational level, clopidogrel appeared to attenuate PE-induced contraction in both groups in a comparable manner, a finding that warrants further investigation in larger cohorts. In future studies, the use of specific inhibitors targeting NO, prostanoid, or calcium signaling pathways may provide valuable insights into the detailed mechanisms underlying the vascular effects of clopidogrel. Moreover, since the vascular tissues in the present study were obtained immediately after CABG surgery, the viability and endothelial integrity of these segments were inherently limited. Therefore, to maintain experimental consistency and minimize confounding factors related to endothelial viability, the study focused on endothelium-independent relaxation responses, as previously described in the literature.[31] Nonetheless, evaluating acetylcholine-mediated endothelium-dependent relaxation could have contributed to a more comprehensive understanding of the underlying mechanisms. Expanding this research to larger populations and exploring different clopidogrel dosing regimens would undoubtedly enhance its scientific value. In this regard, the present study provides a foundational framework for future investigations aiming to elucidate the vascular pharmacodynamics of clopidogrel in greater depth.

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Fırat University (Approval No.: 11; Date: 26.10.2017).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: E.K., I.M.O., and L.U. designed the study. All authors performed the experimental procedures. Z.D.O. performed the statistical analyses and wrote the final version of the manuscript. All authors read and approved the final version of the manuscript.

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Vericiguat Alleviates Atrial Electrical and Structural Remodeling in Rats with Atrial Fibrillation

ABSTRACT

Background: The initiation and maintenance of atrial fibrillation (AF) are predominantly driven by progressive atrial structural remodeling, characterized by fibrosis and electrical conduction abnormalities. While vericiguat has shown efficacy in attenuating cardiac remodeling in heart failure (HF), its impact on AF remains insufficiently characterized.

Methods: This study investigated the cardioprotective effects of vericiguat in a Sprague-Dawley rat model of AF. Twenty-four male SD rats (250-300 g, 8-10 weeks old) were randomly assigned to control, AF, and vericiguat-treated groups. Echocardiography and histological assessments were performed, along with quantification of NT-proBNP, Collagen I, CaMKII, Cx43, ATG7, P62, and LC3II/I in the left atrium.

Results: Compared to controls, AF rats exhibited atrial enlargement, elevated myocardial fibrosis, and significant alterations in molecular markers. Vericiguat treatment effectively reversed these pathological changes. Specifically, NT-proBNP, Collagen I, CaMKII, ATG7, and LC3II/I were upregulated, while Cx43 and P62 were downregulated in the AF group—changes that were mitigated by vericiguat.

Conclusion: Collectively, these findings suggest that vericiguat attenuates atrial remodeling and AF progression by modulating autophagy and suppressing fibrosis.

Keywords: Atrial fibrillation, autophagy, electrical remodeling, structural remodeling, vericiguat

ORIGINAL INVESTIGATION

INTRODUCTION

Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia and is strongly associated with increased risks of ischemic stroke, systemic thromboembolism, and heart failure (HF) progression, thereby contributing substantially to the global cardiovascular disease burden. The 2024 European Society of Cardiology (ESC) guidelines project a twofold rise in AF prevalence over the coming decades, driven primarily by population aging and advances in diagnostic technologies. Atrial fibrillation (AF) has become a notable public health concern.[1,2] Current AF management strategies focus largely on pharmacological rate control and complication prevention, with catheter ablation as the primary interventional option.[3] However, the high recurrence rate and therapeutic resistance of AF post-ablation impose significant economic and societal burdens, underscoring its status as a global health challenge.[4,5] Consequently, elucidating the pathophysiological mechanisms underlying AF and translating these insights into targeted therapies remains a critical unmet need in cardiovascular medicine.

Atrial fibrillation (AF) pathogenesis is fundamentally driven by tightly coupled electrical and structural remodeling processes within the atrial myocardium,[6] although the precise molecular underpinnings remain incompletely defined. Electrical remodeling is characterized by disrupted calcium handling, shortened action potential duration (APD), and impaired conduction. Structural remodeling, in contrast, involves interstitial fibrosis and atrial enlargement—particularly left atrial dilation, which is now recognized as an independent risk factor for AF initiation and maintenance.[7,8] Emerging evidence implicates dysregulated autophagy

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as a contributing factor in both electrical and structural remodeling,[9,10] suggesting that modulation of autophagy may offer a novel therapeutic avenue for AF.

Vericiguat, a soluble guanylate cyclase stimulator approved for HF treatment, mitigates oxidative stress in cardiomyocytes, exerts anti-fibrotic effects, and improves ventricular remodeling.[11] Large-scale clinical trials have demonstrated that vericiguat provides clinical benefit in HF patients with comorbid AF,[12] although the underlying mechanisms remain unclear. Preclinical and clinical data indicate that vericiguat reduces both electrical and structural remodeling in the ventricles.[13-16] Building upon this foundation, the present study investigated whether vericiguat attenuates atrial remodeling in AF-induced Sprague–Dawley rats and explored its potential regulatory role in atrial autophagy.

METHODS

Establishment of Atrial Fibrillation Rat Model

All SD rats were housed under standardized conditions: temperature 23.0 ± 0.5°C, humidity 50%-60%, and a 12-hour light/dark cycle. Atrial fibrillation (AF) was induced using a modified protocol based on references.[17,18] Rats received daily tail vein injections of a mixed solution containing acetylcholine (ACh, 66 μg/mL; A111014, Aladdin) and $CaCl_2$ (10 mg/mL; 499609, Sigma-Aldrich) for 14 consecutive days. Control rats received an equivalent volume of saline (1 mL/kg) via the same route. Fourteen days after the establishment of the AF model, animals were randomly divided into three groups (n = 8 each): Control, saline via oral gavage; AF, AF induction plus saline gavage; and AF + Vericiguat, AF induction followed by vericiguat 0.5 mg/kg/day (Bayer, USA) via oral gavage for four weeks.[19-21]

Electrocardiographic Analysis and Recording

Anesthesia was induced via intraperitoneal injection of sodium pentobarbital (40 mg/kg). Electrocardiographic monitoring (12-lead ECG, ECG-2303B; Guangzhou Sanrui Electronics Co., Ltd., China) was performed by attaching limb leads to the extremities. Continuous intravenous infusion of ACh-$CaCl_2$ was administered for 14 days, with daily assessments of AF duration. Atrial fibrillation (AF) was confirmed by continuous ECG monitoring, characterized by irregular RR intervals, absence of P-waves, and presence of fibrillatory wave (f-wave) patterns.[18]

Echocardiography Analysis

Cardiac structure and function were evaluated after four weeks using transthoracic echocardiography (LOGIQ E11, GE, USA). Parameters measured included left atrial anteroposterior diameter (LAD), left ventricular ejection fraction (LVEF), and fractional shortening (FS).

Biochemical Assessment

Blood samples were collected, centrifuged, and the serum was stored at −80°C. NT-proBNP concentrations were measured using a commercial enzyme-linked immunosorbent assay kit (ZC-36153, Zhuocai Biotechnology Co., Ltd., Shanghai, China). Absorbance was recorded using a microplate reader (SpectraMax iD3, Molecular Devices Co., Ltd., Shanghai, China).

Histological Staining (Hematoxylin and Eosin and Masson's Trichrome)

Left atrial tissues were fixed, embedded, and sectioned for histological analysis. Sections were stained with hematoxylin and eosin (H&E) or Masson's trichrome, followed by sequential dehydration in graded ethanol, xylene clearing, and mounting with resinous medium. Whole-slide scanning was performed using a digital scanner (Pannoramic 250, 3DHISTECH, Hungary). Representative fields at 400× magnification were imaged to assess tissue morphology and fibrosis. Fibrotic area was quantified using ImageJ software (version 1.8.0, NIH), and calculated as:

$$\text{Fibrosis area fraction (\%)} = (\text{collagen fiber area} / \text{total area}) \times 100.$$

Immunohistochemistry

For immunohistochemical analysis, sections were deparaffinized, rehydrated, and cooled. Antigen retrieval was performed with 3% H_2O_2 for 25 min at room temperature in the dark, followed by three PBS washes (5 minutes each). Sections were incubated overnight at 4°C with primary antibodies in blocking buffer: collagen I (1:200, AF7001, Affinity), ATG7 (1:1000, ET1610-53, HuaAn), and LC3B (1 μg/mL, ab192890, Abcam). After phosphate-buffered saline (PBS) rinsing, horseradish peroxidase (HRP)-conjugated secondary antibodies were applied at 37°C for 30 minutes. Diaminobenzidine (DAB) chromogen was added and monitored microscopically for optimal signal development. Hematoxylin counterstaining (3 minutes) was followed by dehydration (ethanol 75%-100%) and xylene clearing. Whole-slide images were analyzed using Halo software to quantify DAB-positive area percentages from three representative fields per section.

RT-qPCR Assay

Left atrial tissues were homogenized in TRIzol using a high-throughput cryogenic grinder (MB-LD48S, Zhejiang Mibei Instruments Co., Ltd., China). Total RNA was extracted using the RNAeasy Mini Kit (Accurate Biotechnology, Hunan, China), and purity was assessed with a Nano-500 microspectrophotometer (Hangzhou Aosheng Instruments Co., Ltd., China). First-strand cDNA was synthesized using a reverse transcription kit (Accurate Biotechnology), followed by RT-qPCR using SYBR Green Master Mix (Accurate Biotechnology) on a CFX Connect RT-qPCR system (Bio-Rad, USA; 788R06671). Primer sequences are listed in Table 1. Reactions were conducted in 20 μL volumes under the following cycling conditions: 95°F for 30 seconds, then 40 cycles

HIGHLIGHTS

- Vericiguat ameliorates structural remodeling in atrial fibrillation (AF) rats, as shown by attenuated atrial dilation and reduced collagen I deposition.
- Vericiguat demonstrates potent anti-fibrotic and anti-remodeling effects by attenuating atrial dilation and collagen I deposition in a rat model of AF.
- We identify the normalization of dysregulated autophagy as a novel mechanism underlying vericiguat's cardioprotective action.

Table 1. Primer sequences used for quantitative real-time PCR analysis.

Gene	Forward (5'-3')	Reverse (5'-3')
ATG7	AATGATGTGGTGGCTCCAGG	CCTCAGGATGCTGCAAGACA
Collagen I	CACTGCAAGAACAGCGTAGC	AAGTTCCGGTGTGACTCGTG
GAPDH	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA

Gene-specific primers were designed using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

of 95°F for 5 seconds, and 60°F for 30 seconds. Each analysis included three independent biological replicates with technical triplicates. Gene expression was normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Primer design and validation.

The coding sequence of the rat GAPDH, ATG7, collagen I gene was downloaded from the NCBI Nucleotide database. Gene-specific primers were designed using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Design parameters were configured as follows: product size range of 70-150 bp, optimal primer length of 20 nucleotides (18-22 range), optimal melting temperature (Tm) of 60°C (58-62°C range), and optimal GC content of 50% (40-60% range). To ensure specificity, the "Primer specificity check" parameter was set to query the Norway rat (taxid:10116) RefSeq mRNA database. From the output, a primer pair spanning an exon-exon junction was selected to preclude genomic DNA amplification and was further evaluated for minimal secondary structure using OligoAnalyzer Tool.

Western Blotting

Total protein was extracted from rat left atrial tissue, and concentrations were quantified using the bicinchoninic acid assay (Beyotime, China). Equal amounts of protein (30 μg/lane) were denatured in 5× sodium dodecyl sulfate (SDS) loading buffer (Solarbio) and separated via 10%-15% gradient SDS-PAGE (80 V for 30 minutes followed by 120 V for 60 minutes). Proteins were then transferred to PVDF membranes (Millipore, USA) at 200 mA for 90 minutes. Membranes were blocked with a protein-free rapid sealing solution (PS108, Epizyme) and incubated overnight at 4°C with primary antibodies against: Collagen I (1:500, AF7001, Affinity), CaMKII (1:4000, 12666-2-AP, Proteintech), Cx43 (1:4000, 26980-1-AP, Proteintech), ATG7 (1:1000, ET1610-53, HuaAn), P62 (1:1000, A7785, ABclonal), LC3B (1:2000, ab192890, Abcam), GAPDH (1:30,000, AC001, ABclonal), and Tubulin (1:3000, 11224-1-AP; Proteintech). After washing with Tris-buffered saline with Tween 20, membranes were incubated with horseradish peroxidase–conjugated goat anti-rabbit IgG (1:10,000, bs-0295G-HRP, Bioss) for 1 hour at room temperature. Signal detection was performed using ECL substrate (Bio-Rad), and images were acquired with a ChemiDoc XR system (Bio-Rad, USA). Band intensities were quantified using Image Lab software (version 6.1). Each analysis included three independent biological replicates

Statistical Analysis

Data analysis was conducted using GraphPad Prism (version 8.01; GraphPad Software, San Diego, CA, USA). Normality of the data was assessed using the Shapiro–Wilk test. Results are presented as mean ± standard deviation (SD). For comparisons between two groups, the Student's *t*-test was used (e.g., for the frequency and duration of AF episodes between the AF and Ver + AF groups), while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A *P*-value of less than .05 was considered statistically significant.

RESULTS

Electrocardiogram Analysis

Daily electrocardiographic monitoring was performed on the rats. A total of 16 rats developed paroxysmal AF starting on the seventh day following continuous injection of the ACh-$CaCl_2$ mixture. Atrial fibrillation (AF) induction was characterized by the absence of P waves, the appearance of fibrillatory (f) waves, and irregular RR intervals. Over the subsequent seven days, the frequency and duration of AF episodes following each drug injection were recorded (Table 2). According to the 2024 ESC guidelines on the definition of AF,[1] modeling was successfully established in all 16 rats. These rats were then randomly divided into the AF group and the Ver + AF group, while the control group, which received an equal volume of saline, exhibited no abnormalities in the electrocardiogram (Figure 1C).

Echocardiographic Analysis

After four weeks of continuous vericiguat administration, echocardiographic assessment revealed no significant differences in LVEF or FS between the AF and control groups. However, the LAD was significantly increased in the AF group and was notably reduced following vericiguat treatment (Figure 1A and B; Table 3).

Vericiguat Significantly Attenuated Atrial Fibrillation–Induced Morphological Alterations

Hematoxylin and eosin (HE) staining showed that cardiomyocytes in the control group were well-organized with aligned nuclei, whereas the AF group exhibited disorganized myocardial fibers, necrosis, and inflammatory infiltration. These pathological changes were markedly attenuated by vericiguat (Figure 2A). Masson's trichrome staining revealed significantly elevated collagen deposition and collagen volume fraction (CVF) in the AF group, both of which were significantly reduced by vericiguat treatment (Figure 2B and C).

Table 2. Serum NT-proBNP levels in control, AF, and Ver+AF groups.

	Control	AF	Ver + AF
NT-proBNP (pg/mL)	361 ± 23.75***	790 ± 66.54	560.3 ± 55.12*

*P<.05,**P < .01,***P < .001 vs. atrial fibrillation (AF) group.

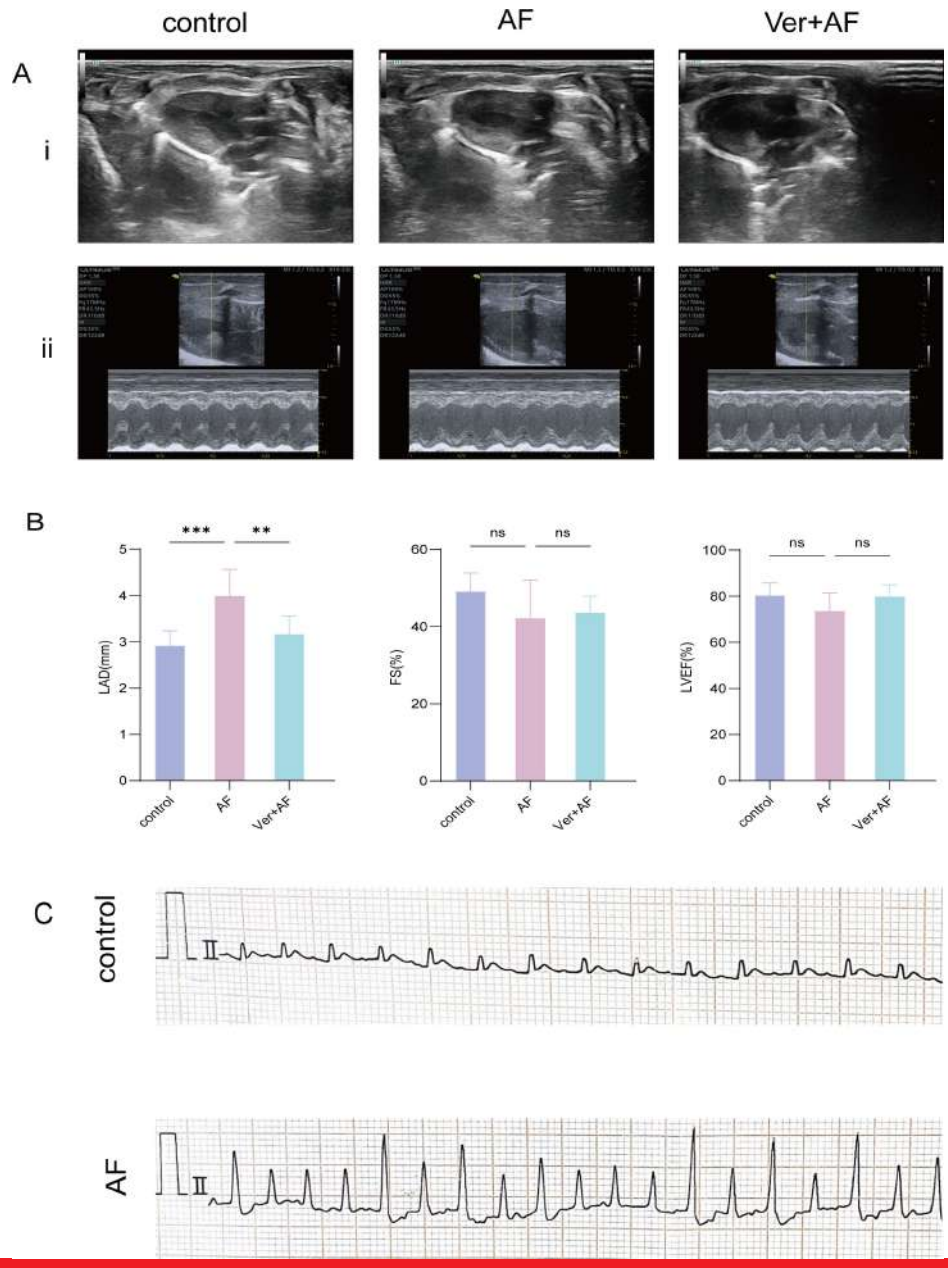

Figure 1. (A) Echocardiographic alterations in experimental groups. i: long axis/B-ultra; ii: short axis/M-ultra. (B) left atrial diameter (LAD mm), left ventricular shortening fraction (FS%), and left ventricular ejection fraction (LVEF%) of rats in each group. Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA. *$P < .01$,*$P < .001$ vs AF group, n = 8 per group. (C) ECG analysis in the atrial fibrillation (AF) group and the control group.**

ELISA Analysis of Serum NT-proBNP in Each Group of Rats

Serum NT-proBNP levels were significantly elevated in the AF group and were substantially reduced following vericiguat treatment (Figure 2D; Table 4).

Vericiguat Attenuated the Upregulation of Collagen I and CaMKII, as well as the Downregulation of Cx43, in the AF Model

Western blot, immunohistochemistry (IHC), and RT-qPCR analyses demonstrated that the AF group exhibited upregulation of Collagen I and CaMKII, along with downregulation

Table 3. Echocardiographic parameters in control, AF, and Ver+AF groups

	Control	AF	Ver + AF
Left atrial diameter (mm)	2.886 ± 0.345***	4.389 ± 0.917	3.475 ± 0.377*
FS(%)	48.64 ± 4.399	42.4 ± 9.505	43.97 ± 4.215
EF(%)	79.02 ± 3.742	74.33 ± 7.681	78.12 ± 3.854

*P<0.05,**P < 0.01,***P < 0.001 vs atrial fibrillation (AF) group.
AF, atrial fibrillation; EF, ejection fraction (%); FS, fractional shortening (%); LAD, left atrial diameter (mm).

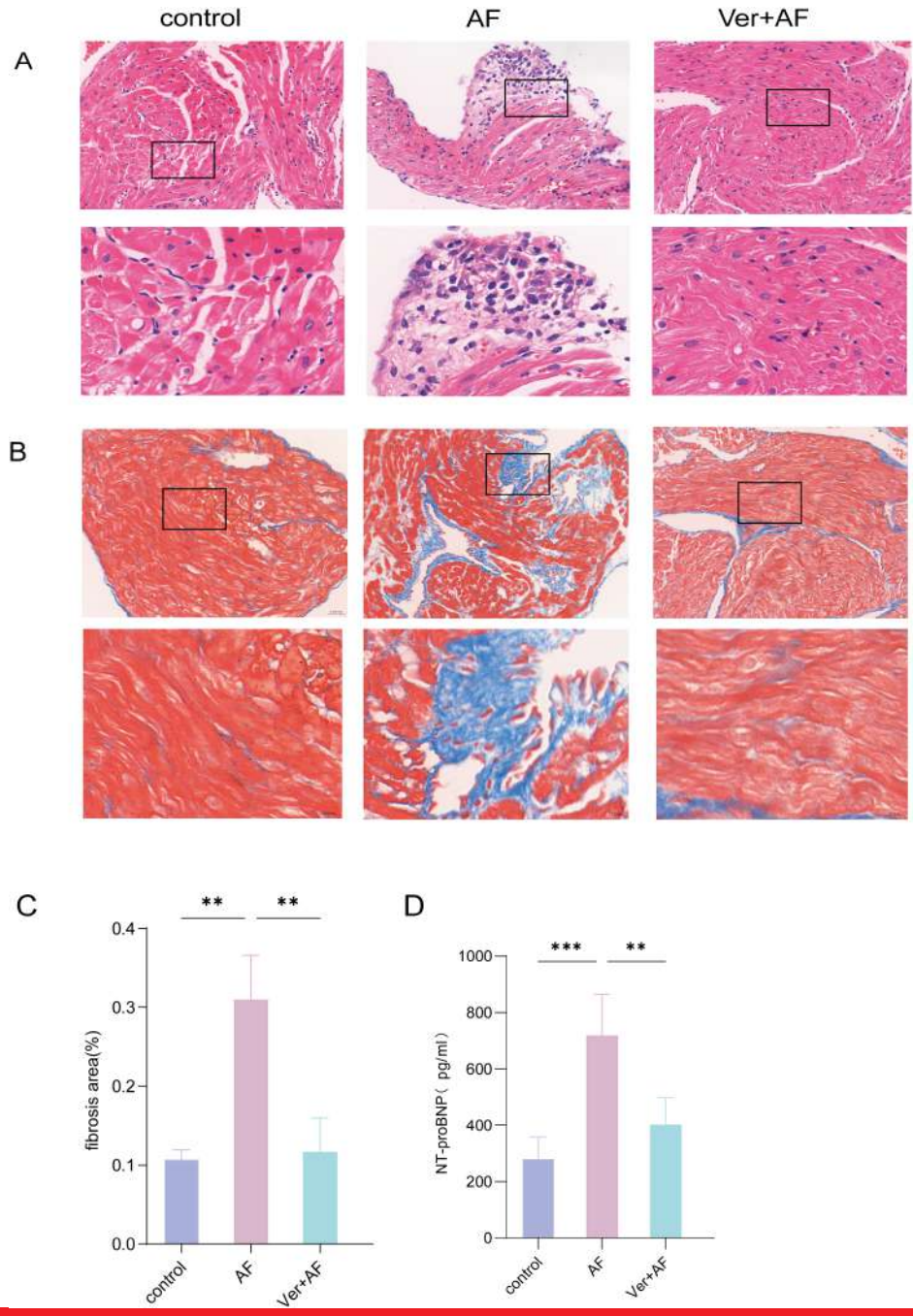

Figure 2. Histological and serological changes in rat myocardial tissues: (A) Representative images of HE-stained left atrial tissues from each group. (B) Masson-stained left atrial tissues across experimental groups. (C) Collagen volume fraction (CVF%) in the left atrium (magnification: 40× and 100×). (D) Serum NT-proBNP levels in different groups. Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA. *P<.05, **P < .01, ***P < .001 vs atrial fibrillation (AF) group. n = 3 per group for panels A-C, n = 5 for panel D.

of Cx43. Vericiguat effectively reversed these changes (P < .05 vs. AF group; Figure 3A-G), indicating its role in modulating both electrical and structural remodeling in AF.

Vericiguat Suppressed the Upregulation of ATG7 and LC3II/I, as well as the Downregulation of P62

Consistent with this, expression of autophagy-related proteins was altered in the AF group, with significant upregulation of ATG7 and LC3II/I and downregulation of P62. Vericiguat treatment reversed these trends (Figure 4A-G), suggesting that its therapeutic mechanism may involve suppression of pathological autophagy activation in AF.

DISCUSSION

Key findings of this study are as follows: (1) AF rats exhibited significantly increased collagen I expression and left

Table 4. Comparison of atrial fibrillation frequency and duration between AF and Ver+AF groups.

	Average Frequency (Time)/Seven Days	Average Duration (s)/ Seven Days
AF	7.339 ± 0.481	348.6 ± 5.721
Ver + AF	7.268 ± 0.524	350.4 ± 8.029
P value	.783 (ns)	.614 (ns)

Data are expressed as mean ± standard deviation. Comparisons between two groups were performed using Student's *t*-test; ns indicates no statistically significant difference (*P*> .05).
AF, atrial fibrillation.

atrial diameter, both of which were attenuated by vericiguat treatment. (2) Elevated CaMKII expression and decreased connexin 43 (Cx43) levels in AF rats were reversed with vericiguat administration. (3) Dysregulation of autophagy-related proteins—upregulation of LC3II/I and ATG7, and downregulation of P62—was normalized by vericiguat, implicating autophagy modulation as a potential mechanism of action.

Calcium/calmodulin-dependent protein kinase II (CaMKII) plays a central role in atrial electrical remodeling by phosphorylating ryanodine receptor 2 (RyR2), thereby enhancing sarcoplasmic reticulum calcium release and promoting intracellular calcium overload. This leads to shortened APD and aberrant electrical activity that facilitates AF initiation and perpetuation.[22,23] Connexin 43 (Cx43), the principal gap junction protein in atrial myocytes, ensures coordinated impulse propagation.[24] Its downregulation or disorganization disrupts electrical conduction, creating a substrate for microreentry and arrhythmia maintenance.[25] Notably, excessive autophagy has been shown to suppress Cx43 expression, linking proteostatic imbalance to arrhythmogenesis.[26] Atrial fibrosis, characterized by extracellular matrix expansion and disorganized collagen I deposition,[27,28] impairs conduction homogeneity and promotes delayed depolarization, increasing vulnerability to reentrant arrhythmias. This fibrotic remodeling not only initiates AF but also sustains its recurrence by establishing a persistent arrhythmogenic substrate.

Autophagy is a regulated catabolic process that facilitates the degradation and recycling of intracellular components via lysosomal pathways. It is activated by stressors such as oxidative stress, hypoxia, and nutrient deprivation. Upon activation, the ULK1 complex initiates autophagosome formation, and ATG7 promotes the lipidation of LC3-I to

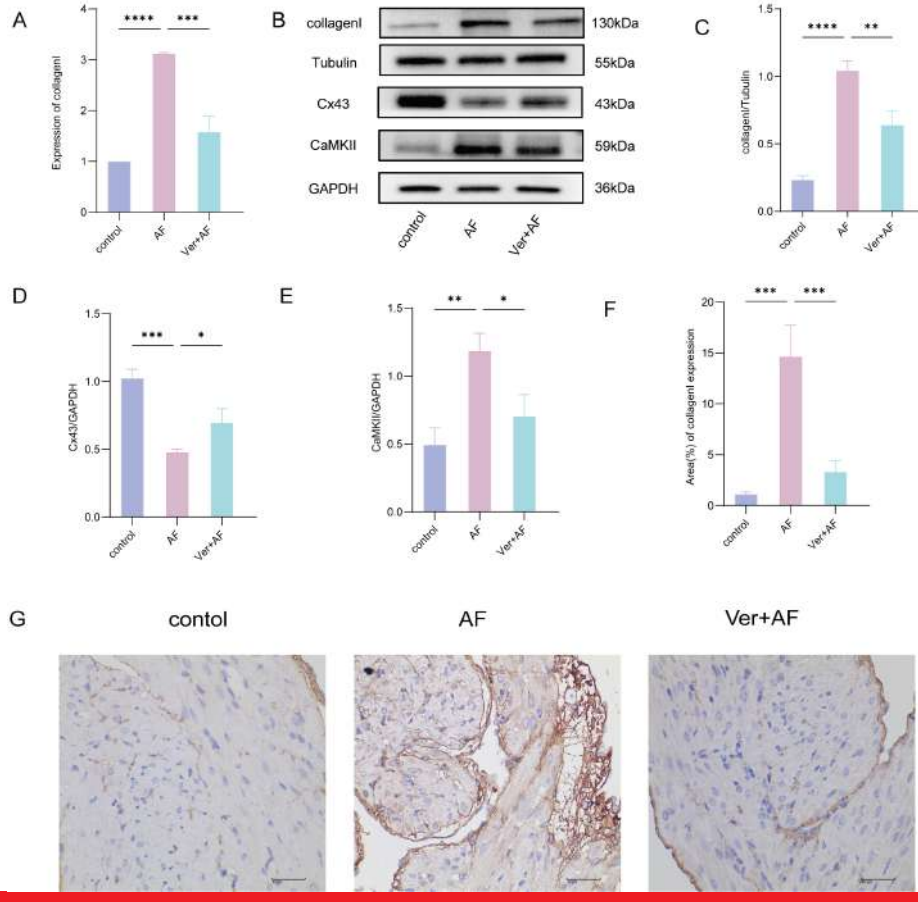

Figure 3. (A) RT-qPCR analysis of Collagen I mRNA expression levels across experimental groups. (B-E) Western blot quantification of Collagen I, Cx43, and CaMKII protein levels. (F, G) Representative immunohistochemical (IHC) images showing Collagen I deposition (magnification: 40×; n = 3 per group). Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA. *P<.05, **P < 0.05, **P < .01, ***P < .001, ****P < .0001 vs. atrial fibrillation (AF) group.

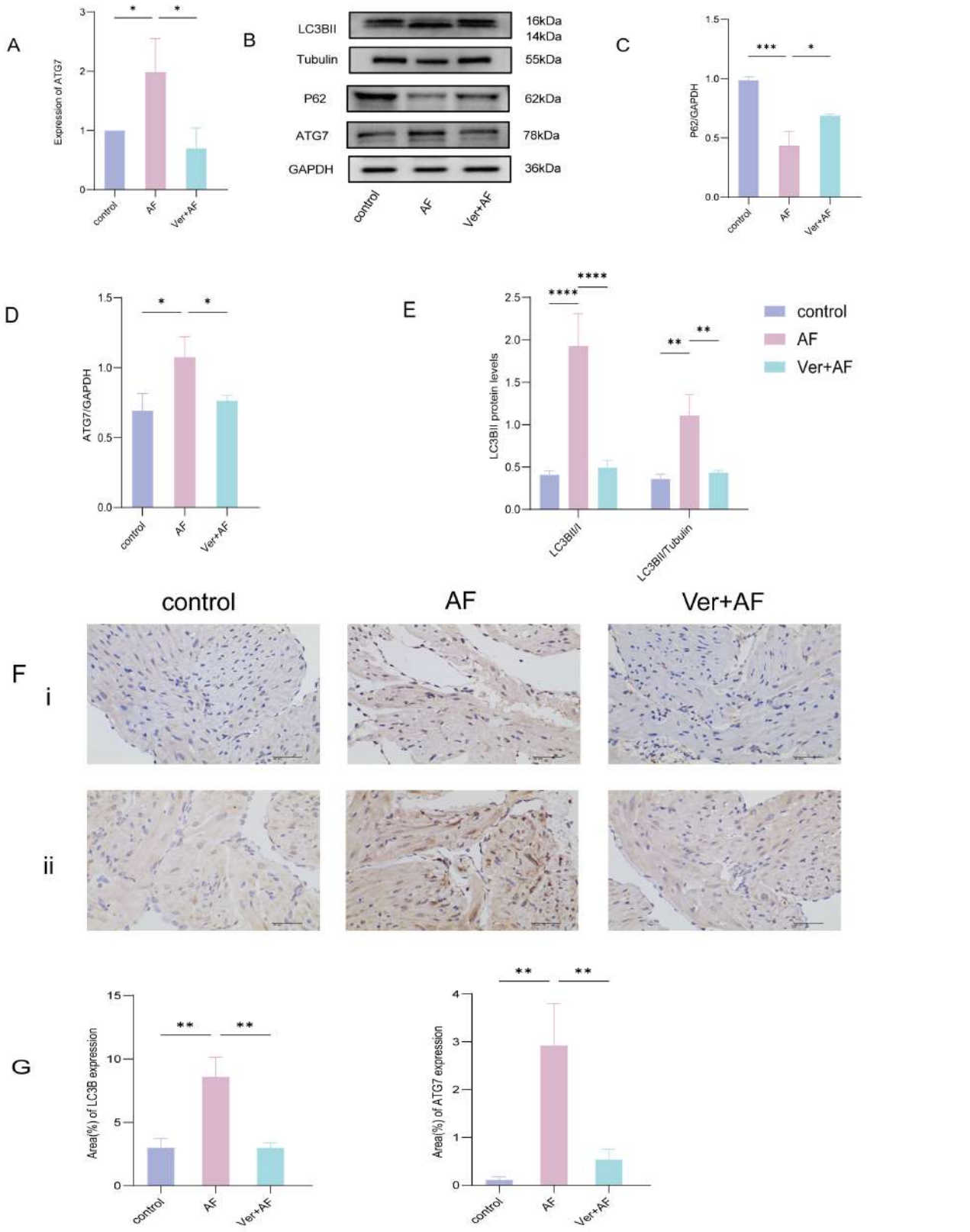

Figure 4. (A) RT-qPCR analysis of ATG7 mRNA expression levels across experimental groups. (B–E) Western blot quantification of ATG7, P62, and LC3II/I protein expression levels. (F,G) Representative immunohistochemical (IHC) images showing tissue-specific expression of (i) ATG7 and (ii) LC3II/I (magnification: 40×; n = 3 per group). Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA. *P<.05, **P < 0.01, ***P < .001 vs. atrial fibrillation (AF) group.

form LC3-II. LC3-II then incorporates into autophagosomal membranes, aiding in the sequestration of cytoplasmic substrates. Concurrently, p62/SQSTM1 binds to ubiquitinated cargo and anchors it to autophagosomes. Mature autophagosomes fuse with lysosomes for the degradation and recycling of metabolic byproducts.[29,30] Excessive autophagy promotes atrial fibrosis through mechanisms like increased extracellular matrix deposition and collagen I overproduction, which facilitate AF persistence and recurrence.[10,31-33]

To characterize fibrotic remodeling, we comprehensively assessed atrial collagen I deposition across experimental groups using multiple modalities. The AF group exhibited significantly elevated collagen I expression and increased CVF relative to controls, corroborating previous reports linking fibrosis to AF progression.[16,34,35] Echocardiographic analysis further revealed marked left atrial enlargement in AF rats, while LVEF and FS remained unaltered. In parallel, serum NT-proBNP levels—a biomarker of myocardial stress—were significantly elevated in the AF cohort. Notably, vericiguat treatment attenuated these pathological changes, consistent with its documented benefits in mitigating ventricular remodeling in HF models.[36] Given the contributions of CaMKII upregulation and connexin 43 (Cx43) downregulation to atrial electrical remodeling, we quantified their expression profiles. Atrial fibrillation (AF) was associated with pronounced increases in CaMKII expression and concomitant reductions in Cx43 levels, indicative of impaired calcium handling and disrupted gap junctional communication. Vericiguat administration reversed these molecular derangements, suggesting its capacity to restore electrophysiological stability by simultaneously modulating calcium signaling and intercellular connectivity.

Furthermore, we observed distinct alterations in autophagy-related markers in the AF group: a significantly elevated LC3II/I ratio, increased mRNA and protein expression of *ATG7*, and reduced levels of p62. These changes suggest a shift in autophagic activity, indicating that paroxysmal AF may lead to changes in autophagic activity in atrial cardiomyocytes, in line with previous findings.[9,37,38] These observations suggest that restoring autophagic balance might help reduce AF susceptibility. Our study demonstrated that vericiguat reversed the alterations in the above proteins.

In summary, our study provides evidence that vericiguat may alleviate paroxysmal AF, potentially through modulation of atrial autophagy.

Limitations

However, our study has several limitations. The relatively small sample size, particularly in histological and molecular analyses, may limit the generalizability of our findings. Additionally, we focused solely on the effects of vericiguat in a paroxysmal AF model, and future studies should evaluate its mechanisms in other AF subtypes, such as persistent AF.

AI Disclosure: The authors declare that no generative AI tools (such as large language models, chatbots, or image creators) were used in any stage of the preparation or writing of this manuscript.

Ethics Committee Approval: Animal Ethics Statement: Twenty-four male Sprague Dawley rats (8 weeks old, 250–300 g) were obtained from the Animal Experiment Center of North Sichuan Medical College and approved by the Ethics Committee of North Sichuan Medical College (License No.: SYXK[Sichuan]2023-0076, Nanchong, China). All procedures adhered to the Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of North Sichuan Medical College (Approval No. [2024]005). Efforts were made to minimize animal suffering.

Peer-review: Externally peer-reviewed.

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Association Between Systemic Immune-Inflammation Index and Cardiac Resynchronization Therapy Response in Patients with Heart Failure

ABSTRACT

Background: Cardiac resynchronization therapy (CRT) is an established treatment for selected patients with heart failure (HF); however, a substantial proportion of patients do not derive clinical or echocardiographic benefit. Systemic inflammation has been implicated in adverse myocardial remodeling and may influence response to CRT. This study aimed to evaluate the association between the systemic immune-inflammation index (SII) and CRT response.

Methods: This retrospective, single-center study included 110 patients with HF who underwent CRT implantation. Clinical, echocardiographic, and laboratory parameters, including SII, were assessed at baseline and at 6-month follow-up. Cardiac resynchronization therapy response was defined using combined echocardiographic and clinical criteria. Associations between inflammatory markers and CRT response were analyzed.

Results: Nonresponders demonstrated significantly higher baseline SII levels compared with responders. During follow-up, SII values increased significantly in nonresponders, whereas no significant change was observed in responders. Baseline C-reactive protein (CRP) levels were similar between groups. Chronic ischemic heart disease and elevated baseline SII were independently associated with CRT nonresponse. The discriminatory ability of baseline SII for CRT nonresponse was moderate.

Conclusion: Elevated SII was associated with an increased likelihood of CRT nonresponse and persistent inflammatory activity during follow-up. These findings suggest that SII reflects an unfavorable inflammatory and biological milieu related to impaired CRT response, rather than a direct causal mechanism. Systemic immune-inflammation index may serve as a complementary biomarker for risk stratification in patients undergoing CRT.

Keywords: Cardiac resynchronization therapy, heart failure, inflammation, nonresponder, systemic immune-inflammation index

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INTRODUCTION

Heart failure (HF) is a progressive clinical syndrome characterized by impaired cardiac function and substantial morbidity and mortality. Despite advances in pharmacological therapy, many patients remain symptomatic. Cardiac resynchronization therapy (CRT) improves symptoms, ventricular function, and survival in appropriately selected patients[1]; however, approximately 30% of patients fail to demonstrate meaningful benefit despite meeting guideline-based indications.[2]

The variability in CRT response has prompted extensive investigation into clinical, electrocardiographic, imaging, and laboratory predictors.[3-8] In recent years, systemic inflammation has emerged as an important contributor to HF progression and adverse remodeling. Elevated inflammatory markers, including C-reactive protein, interleukins, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio, have been associated with poor cardiovascular outcomes.[9-14]

The systemic immune-inflammation index (SII), derived from neutrophil, lymphocyte, and platelet counts, integrates inflammatory, immune, and prothrombotic

pathways. Although SII has been evaluated in several cardiovascular conditions, its association with CRT response has not been adequately explored. Therefore, this study aimed to investigate the relationship between SII and CRT response, with particular emphasis on distinguishing association from causation.

METHODS

Patients

The study population consisted of 110 HF patients who underwent CRT implantation in the center between January 2019 and January 2024, in accordance with the current guideline recommendations. The study included 112 HF patients who met the following criteria:

- Persistent symptoms despite optimal medical therapy [New York Heart Association (NYHA) class II-IV].
- Severe left ventricular systolic dysfunction (left ventricular ejection fraction [LVEF] ≤ 35%).
- Presence of left bundle branch block (LBBB) on a 12-lead electrocardiogram (ECG) with prolonged QRS duration (≥130 milliseconds) in sinus rhythm.
- Successful implantation of the left ventricular lead in recommended coronary sinus side branches.
- Achieved over 90% biventricular pacing during follow-up.

Patients were excluded from the study if they had the following:

- Mechanical heart valves.
- A history of myocardial infarction or coronary artery bypass grafting within the last 6 months.
- Malignancies, chronic inflammatory diseases, or autoimmune diseases.
- Hematological diseases, moderate-to-severe renal, or liver dysfunction.

HIGHLIGHTS

- Elevated baseline systemic immune-inflammation index (SII) levels were associated with nonresponse to cardiac resynchronization therapy (CRT).
- During follow-up, SII values increased significantly in nonresponders, whereas responders demonstrated relative inflammatory stability rather than a significant reduction in SII.
- High baseline SII levels were independently associated with a significantly increased likelihood of being a CRT nonresponder and with poorer clinical and echocardiographic outcomes.
- Persistent elevation of inflammatory biomarkers—including SII, C-reactive protein, B-type natriuretic peptide (BNP), and troponin—in nonresponders suggests that ongoing systemic inflammation may limit CRT efficacy.
- These findings indicate that SII may serve as a complementary, easily accessible biomarker for patient characterization and risk stratification before CRT implantation.

- Non-LBBB morphology on ECG.
- Patients with pacemaker to CRT upgrades.

Additionally, patients with a life expectancy of less than 12 months or a follow-up period shorter than 6 months were not included. This study is a retrospective analysis of prospectively collected data, and institutional ethics approval was obtained in accordance with the principles of the Helsinki Declaration.

Blood Samples

Venous blood samples were collected before the procedure and at the sixth-month follow-up. Laboratory parameters such as hemoglobin, creatinine, leukocytes, platelets, neutrophils, lymphocytes, C-reactive protein (CRP), and low-density lipoprotein (LDL) levels were recorded. Based on these results, the SII was calculated using the formula: SII = Platelet count × Neutrophil count / Lymphocyte count.

Clinical Evaluation

All patients underwent a clinical evaluation, including NYHA classification, QRS duration, and comorbidity assessment. This was performed by an independent physician blinded to other data. QRS duration was measured on surface ECG, taking the widest QRS complex from leads II, V1, and V6. Patients were evaluated for age, sex, history of coronary artery disease, diabetes, hyperlipidemia, hypertension, and other comorbidities.

Patients with a history of coronary artery disease and/or prior myocardial infarction were classified as ischemic. Those without a history of myocardial infarction or revascularization and with no more than 50% atherosclerotic lesions in 2 or more epicardial vessels, the left main coronary artery, or the proximal left anterior descending artery were classified as non-ischemic.

Echocardiography

Standard echocardiographic imaging was performed both before CRT implantation and at the sixth-month follow-up. Echocardiographic imaging was conducted in a resting state with the patient in the left lateral decubitus position, using the equipment available in the clinic (Vivid 7 Ultrasound System; GE, Horten, Norway). Measurements were made according to the recommendations of the American Society of Echocardiography. The LVEF was calculated using the modified Simpson method.

Device Implantation and Optimization Protocol

All patients received a CRT device with a defibrillator (CRT-D). The CRT-D implantation was performed transvenously via the left subclavian route. Before the placement of the left ventricular (LV) lead, routine coronary sinus venography was performed. The LV lead was preferably positioned in the lateral or posterolateral branches of the coronary sinus. The right atrial lead was implanted in the atrial appendage, and the right ventricular lead was positioned in the apex region. Atrioventricular (AV) and interventricular interval optimization was performed by an experienced cardiologist using Doppler echocardiography, with transmitral flow and velocity-time integral measurements used to achieve the shortest possible QRS duration and maximize ventricular synchrony.

To evaluate the potential impact of LV lead positioning on CRT response, pacing sites (posterior, posterolateral, and lateral coronary sinus branches) between responders and nonresponders were compared, with lead positioning determined using fluoroscopic guidance during implantation. To further assess its influence on CRT outcomes, a secondary analysis was conducted at the 6-month follow-up, evaluating the relationship between lead position and key echocardiographic and clinical parameters, including LVEF improvement, NYHA class reduction, and hospitalization rates.

Definition of Cardiac Resynchronization Therapy Response

Left ventricular reverse remodeling was assessed quantitatively based on the improvement in LVEF following CRT, with an increase of ≥5% at the 6-month follow-up considered indicative of a positive echocardiographic response. Clinical response was defined as an improvement of at least 1 NYHA functional class level without any HF-related hospitalizations during the follow-up period. Patients meeting both echocardiographic and clinical response criteria were classified as responders, while those who did not fulfill these criteria were classified as nonresponders.

Statistical Analysis

The statistical analyses were conducted using IBM SPSS Statistics v26 software. The normality of the variables was assessed using the Kolmogorov–Smirnov test. The chi-squared test and Fisher's exact test were utilized to compare categorical variables. Fisher's exact test was applied when more than 20% of the cells had expected frequencies less than 5, while the chi-squared test was used when this condition was not met. *P*-values were two-tailed. For continuous variables, the Mann–Whitney *U*-test was conducted to compare the medians (median: 25th-75th percentiles) of 2 independent groups, and the Student's *t*-test was performed to compare the means (mean ± SD) of 2 independent groups. A *P*-value below .05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory ability of variables in predicting CRT response. The area under the curve (AUC) was used as a measure of predictive power, with a *P*-value below .05 indicating statistical significance. Confidence intervals for the AUC were calculated to determine the reliability of the estimates. Cox regression analysis was employed to identify independent predictors of CRT response. Hazard ratios with 95% CIs were calculated to estimate the strength and direction of associations between predictors and outcomes. Variables with *P*-values below .05 in the univariate analysis were included in the multivariate Cox regression model. Statistical significance was defined as a *P*-value below .05.

Ethical Approval

This study was approved by an independent Institutional Ethics Committee (Approval Date: November 13, 2025; Decision Number: 2025-128) and conducted in accordance with the principles of the Declaration of Helsinki. Because this research involved a retrospective analysis of previously recorded clinical data, the requirement for informed consent was waived by the ethics committee.

RESULTS

A total of 110 patients who met the inclusion criteria and experienced no complications were included in the study. Of these, 35.5% (n = 39) were female, and 64.5% (n = 71) were male. Among the participants, 43.6% (n = 48) had chronic ischemic heart disease, 21.1% (n = 23) had diabetes, and 30.9% (n = 34) had hypertension. Pacing was performed in the posterior branch in 72.7% (n = 80), the posterolateral branch in 20.9% (n = 23), and the lateral branch in 6.4% (n = 7). Symptomatically, 45.5% (n = 50) of the patients were classified as NYHA Class II, and 54.5% (n = 60) as Class III prior to the procedure. Echocardiographic evaluations conducted before the procedure showed mild mitral regurgitation in 62.7% (n = 69), moderate mitral regurgitation in 29.1% (n = 32), and severe mitral regurgitation in 8.2% (n = 9).

Patients were divided into 2 groups based on their response to CRT: responders (n = 81) and nonresponders (n = 29). There were no significant differences between the groups in terms of age ($P = .927$) or gender distribution ($P = .930$). Similarly, no significant differences were observed in the prevalence of diabetes ($P = .126$) or hypertension ($P = .627$) between groups. However, chronic ischemic heart disease was significantly more prevalent in the nonresponder group (65.5%) compared to the responder group (35.8%) ($P = .006$).

Baseline LVEF was similar between groups, with median values of 28% (nterquartile range [IQR]: 25-32) in responders and 28% (IQR: 25-31) in nonresponders ($P = .762$). The nonresponder group had significantly higher baseline platelet counts ($P < .001$), SII ($P = .007$), and troponin levels ($P = .020$). Other hematological and biochemical parameters, baseline QRS duration, and mitral regurgitation severity showed no significant differences between groups (Table 1).

The distribution of LV lead placement sites was comparable between CRT responders and nonresponders. Posterior, posterolateral, and lateral pacing sites showed no significant differences between groups ($P = .991$) (Table 1). Given the lack of statistical significance, LV lead positioning did not appear to influence CRT response in this study.

Analysis of changes in SII between baseline and follow-up showed a statistically significant increase in SII values in the nonresponder group ($Z = -2.865$, $P = .004$), indicating elevated systemic inflammation in nonresponders over time. In contrast, in the responder group, no significant difference was found in SII values between baseline and follow-up ($Z = -0.951$, $P = .342$).

At the 6-month follow-up, significant differences were observed between the groups in hematological, biochemical, and echocardiographic parameters (Table 2). The nonresponder group had significantly higher neutrophil counts ($P = .037$) and platelet counts ($P < .001$), while no significant difference was observed in lymphocyte levels ($P = .242$). Inflammatory and biochemical markers, including SII ($P < .001$), CRP ($P < .001$), BNP ($P < .001$), and troponin ($P < .001$), were significantly higher in nonresponders. Left ventricular ejection fraction was significantly lower in the nonresponder group ($P = .004$), whereas mitral regurgitation severity did

Table 1. Baseline Characteristics of Responders and Nonresponders

	Total n = 110	CRT Responder n = 81	CRT Nonresponder n = 29	*P*
Age (years)	70 (63-76)	70 (62-77)	70 (66-75)	.927
Gender (F/M) %	39/71 (34.8/63.4)	29/52 (35.8/64.2)	10/19 (34.5/65.5)	
Chronic ischemic heart disease, n (%)	48 (42.9)	29 (35.8)	19 (65.5)	.006
Diabetes, n (%)	23 (20.5)	14 (17.3)	9 (31)	.126
Hypertension, n (%)	34 (30.4)	24 (29.6)	10 (34.5)	.627
Pacing site, n (%)				
Posterolateral	80 (71.4)	59 (72.8)	21 (72.4)	.991
Lateral	23 (20.5)	17 (21.0)	6 (20.7)	
Posterior	7 (6.3)	5 (6.2)	2 (6.9)	
Glomerular filtration rate, median (IQR)	62.00 (54.75-69.00)	62.00 (56.00-69.00)	65.00 (52.5-69.00)	.881
Basal neutrophil, median (IQR)	4.60 (3.68-6.00)	4.60 (3.60-6.05)	4.60 (3.70-5.65)	.984
Basal platelet, median (IQR)	239 000 (186 750-272 500)	222 000 (177 500-258 000)	272 000 (255 000-298 000)	<.001
Basal lymphocyte, median (IQR)	1.90 (1.55-2.20)	1.90 (1.60-2.25)	1.70 (1.50-2.15)	.237
Basal SII, median (IQR)	581.57 (407.78-806.66)	551.28 (363.71-749.34)	714.00 (507.05-1066.02)	.007
Basal CRP, median (IQR)	2.40 (1.58-3.13)	2.50 (1.45-3.20)	2.40 (1.65-3.00)	.701
Basal BNP, median (IQR)	904.50 (573.25-1367.00)	950.00 (628.50-1529.50)	756.00 (480.00-1159.50)	.173
Basal troponin, median (IQR)	0.010 (0.010-0.020)	0.010 (0.010-0.020)	0.010 (0.010-0.010)	.020
Basal QRS, median (IQR)	163 (158-168)	162 (158-167)	163 (159-169)	.333
Basal mitral regurgitation, n (%)				
No regurgitation	0 (0)	0 (0)	0 (0)	.943
Mild regurgitation	69 (61.6)	51 (63.0)	18 (62.1)	
Moderate regurgitation	32 (28.6)	23 (28.4)	9 (31.0)	
Severe regurgitation	9 (8)	7 (8.6)	2 (6.9)	
Basal Ejection fraction, median (IQR)	28 (25-32)	28 (25-31)	28 (25-32)	.762
Basal NYHA, n (%)				.900
II	50 (45.5)	37 (45.7)	13 (44.8)	
III	60 (54.5)	44 (54.3)	16 (55.2)	
ACEI/ARB, n (%)	110 (100)	81 (100)	29 (100)	1.000
Beta bloker, n (%)	110 (100)	81 (100)	29 (100)	1.000
SGLT-2 inh., n (%)	87 (79.09)	65 (80.2)	22 (75.9)	.720

ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; CRT, cardiac resynchronization therapy; e-GFR, estimated glomerular filtration rate; F, female; IQR, interquartile range; M, male; MRA, mineralocorticoid receptor antagonist; SGLT-2 inh., sodium-glucose cotransporter-2 inhibitor, SII, systemic immune-inflammation index.

not differ significantly ($P = .062$). The QRS duration was significantly longer in nonresponders ($P < .001$).

Regarding clinical outcomes, hospitalization rates were significantly higher in the nonresponder group ($P = .001$). Although the incidence of implantable cardioverter-defibrillator (ICD) shocks was higher in nonresponders, the difference was not statistically significant ($P > .05$).

Logistic regression analysis identified chronic ischemic heart disease and high baseline SII levels as significant predictors of CRT nonresponse. The model achieved an overall accuracy of 74.5%, correctly classifying 44.8% of nonresponders and 85.2% of responders. Chronic ischemic heart disease was associated with a higher likelihood of nonresponse ($P = .007$), with affected patients being 3.5 times more likely to be nonresponders (odds ratio [Exp(B)] = 3.524, 95% CI: 1.405-8.836). High baseline SII levels were also significantly associated with CRT nonresponse ($P = .020$), with an approximately 3-fold increased likelihood of nonresponse (odds ratio [Exp(B)] = 3.030, 95% CI: 1.187-7.736). Gender was not a significant predictor of CRT response ($P = .828$), with an odds ratio of 1.112 (95% CI: 0.427-2.901).

The AUC for the ROC analysis of the baseline SII index was 0.669 (Figure 1), with a standard error of 0.059 and a *P*-value of .007. The 95% CI for the AUC ranged from 0.553 to 0.784, demonstrating moderate predictive power.

At 6 months, no significant association was observed between LV lead positioning and clinical or echocardiographic response to CRT. Patients with posterior, posterolateral, and lateral lead placements exhibited similar rates of LVEF improvement and NYHA class reduction (Table 2). These findings suggest that LV lead position alone was not a determinant of CRT response in this cohort.

Baseline characteristics were comparable between responders and nonresponders with respect to age, sex, and major comorbidities, except for a higher prevalence of chronic

Table 2. Six-Month Follow-Up Values of Both Groups

	Total n = 110	CRT Responder n = 81	CRT Nonresponder n = 29	*P*
Control neutrophil, median (IQR)	5.40 (4.10-6.33)	5.10 (4.10-6.20)	5.90 (4.50-7.40)	.037
Control platelet, median (IQR)	214.000 (183.000-266.000)	202 000 (160 000-256 000)	266 000 (232 500-300 500)	<.001
Control lymphocyte, median (IQR)	1.90 (1.38-2.20)	1.90 (1.40-2.30)	1.80 (1.25-2.10)	.242
Control SII, median (IQR)	633.96 (453.37- 945.54)	579.57 (370.50-798.06)	924.59 (704.84-1155.19)	<.001
Control CRP, median (IQR)	2.10 (1.30-2.53)	1.60 (1.20-2.10)	3.10 (2.30-3.70)	<.001
Control BNP, median (IQR)	599.00 (263.00-947.00)	365.00 (217.00-745.00)	947.00 (722.00-1634.50)	<.001
Control troponin, median (IQR)	0.020 (0.010-0.040)	0.010 (0.010-0.025)	0.060 (0.030-0.125)	<.001
Pacing QRS, median (IQR)	128 (124-134)	126 (122-131)	135 (132-140)	<.001
Control mitral regurgitation, n (%)				
No regurgitation	3 (2.7)	3 (3.7)	0 (0)	.062
Mild regurgitation	83 (74.1)	64 (79.0)	19 (65.5)	
Moderate regurgitation	22 (19.6)	14 (17.3)	8 (27.6)	
Severe regurgitation	2 (1.8)	0 (0)	2 (6.9)	
Control EF, median (IQR)	31 (28-35)	32 (29-36)	29 (26-32)	.004
Hospitalization, n (%)	5 (4.5)	0 (0)	5 (17.2)	.001
ICD shock, n (%)	2 (1.8)	0 (0)	2 (6.9)	.068

CRT, cardiac resynchronization therapy; IQR, interquartile range.

ischemic heart disease among nonresponders. Baseline SII levels were significantly higher in nonresponders, whereas baseline CRP levels did not differ between groups.

During follow-up, SII values increased significantly in the nonresponder group, indicating persistent or worsening systemic inflammation. In contrast, no significant difference was observed between baseline and follow-up SII values in the responder group, suggesting relative inflammatory stability.

QRS duration at follow-up was longer in nonresponders. Given the standardized implantation and optimization protocol, this finding was interpreted as reflecting differences in electrical remodeling and myocardial substrate rather than unequal CRT optimization.

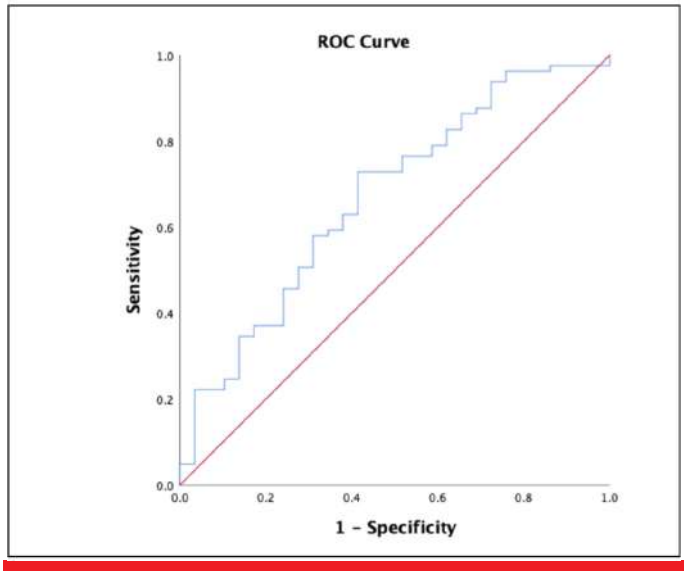

Figure 1. Receiver operating characteristic curve for the predictive performance of the systemic immune-inflammation index in identifying nonresponders to cardiac resynchronization therapy.

In the nonresponder group, 5 patients (17%) experienced HF-related hospitalization during the follow-up period. Importantly, all baseline blood samples were obtained during clinically stable conditions and at least 4 weeks after the last episode of hospitalization or acute decompensation. Patients with blood sampling performed during acute decompensated HF were not included in the analysis.

DISCUSSION

The principal finding of this study is that elevated SII is associated with CRT nonresponse and with persistent systemic inflammation during follow-up. Importantly, these findings should be interpreted as associative rather than causal. At baseline, higher SII levels in nonresponders ($P = .007$) indicate that pre-existing systemic inflammation is associated with a reduced likelihood of CRT success, reinforcing the hypothesis that inflammatory burden may impair ventricular remodeling and response to resynchronization therapy. Over time, nonresponders exhibited a further increase in SII levels ($P < .001$), whereas responders maintained relatively stable values, suggesting that persistent inflammation may contribute to the lack of functional and structural improvement despite CRT implantation. These findings suggest that SII not only serves as a pre-procedural risk stratification tool but also reflects ongoing inflammatory processes that may impair CRT efficacy, making it a valuable biomarker for patient selection and long-term monitoring.

Cardiac resynchronization therapy is an effective treatment modality for patients with HF and prolonged QRS duration, aimed at improving symptoms, enhancing quality of life, and reducing mortality risk.[15] Given the progressive nature of HF, the potential of CRT to improve cardiac function has

established it as a pivotal therapeutic option.[16] However, identifying patients who will respond favorably to CRT remains a clinical challenge, underscoring the need for novel biomarkers to predict therapeutic response. Differences in follow-up QRS duration likely reflect heterogeneous myocardial substrate and electrical remodeling rather than disparities in CRT optimization. Inflammation-related myocardial fibrosis and microvascular dysfunction may contribute to impaired resynchronization and limited QRS narrowing.

Systemic immune-inflammation index has emerged in recent years as a valuable biomarker that reflects a combination of inflammatory and thrombotic processes in various clinical contexts. Calculated using neutrophil, platelet, and lymphocyte counts, SII serves as a comprehensive measure of inflammatory burden and immune response.[17]

In this study, the aim was to investigate the role of the SII as a prognostic marker in HF patients undergoing CRT. Given the potential impact of systemic inflammation on CRT outcomes, previous research has explored its influence on patient response rates and clinical prognoses. Stavrakis et al[17] previously reported that elevated inflammatory markers negatively impact response rates and are associated with worse clinical outcomes in CRT patients. Similarly, high SII values have been shown to reflect increased inflammatory burden and thrombotic activity, which may limit the effectiveness of CRT.[18] In alignment with these findings, these results demonstrated that responders exhibited a notable reduction in inflammation, suggesting that alleviating inflammatory processes may have a beneficial impact on myocardial remodeling. These observations reinforce the potential role of SII as a predictive biomarker and emphasize the importance of addressing inflammation to optimize outcomes in CRT recipients.

In the present study, responders did not demonstrate a significant change in SII values between baseline and follow-up, indicating relative inflammatory stability over time. In contrast, nonresponders exhibited a significant increase in SII levels, reflecting persistent or worsening systemic inflammation. This divergence suggests that the absence of inflammatory progression, rather than a reduction in inflammatory burden, may characterize patients who respond favorably to CRT. Within this context, previous studies have suggested that favorable ventricular remodeling after CRT may be accompanied by attenuation of systemic inflammatory activity, particularly in selected patient populations. Karaüzüm et al[19] demonstrated that a reduction in inflammation following CRT is associated with improved LVEF levels. This suggests that CRT not only provides mechanical hemodynamic benefits but also supports myocardial recovery by reducing inflammation. This effect was particularly pronounced in patients with low baseline SII values.[20]

The NYHA classification is a standard tool for assessing the severity of HF symptoms. In this study, higher baseline SII values were associated with more severe NYHA classes (III-IV), and the responder group exhibited improvements in NYHA classification alongside reductions in SII following CRT. Specifically, patients in NYHA III-IV who experienced symptomatic improvement after CRT demonstrated significant decreases in SII, underscoring the role of inflammation in CRT success. Cleland et al[15] previously reported that CRT supports symptomatic improvement, particularly in patients with NYHA III-IV classification. Given that SII reflects both inflammatory and thrombotic processes, it may serve as a more comprehensive predictor of NYHA class and CRT response.

Mitral regurgitation (MR) is a common complication in HF patients and has the potential to improve through the hemodynamic benefits of CRT. In this study, higher baseline SII values were associated with more severe MR, and significant improvement in MR severity was observed in the responder group alongside reductions in SII following CRT. Di Biase et al[21] reported similar findings, demonstrating significant MR improvement in patients who exhibited reduced inflammatory markers post-CRT. This suggests that CRT mitigates the burden on mitral valve dysfunction by modulating inflammation, thereby supporting MR improvement.

The relationship between baseline and 6-month SII values and QRS durations provides valuable insights into the electrical effects of CRT. Elevated pre-CRT SII values were generally associated with prolonged QRS durations, and patients with higher baseline SII values showed limited QRS shortening following CRT. In contrast, the responder group exhibited significant QRS shortening, accompanied by reductions in SII. The potential pathophysiological mechanisms underlying these findings, as noted in previous studies, include the role of heightened inflammation in promoting myocardial fibrosis, which impairs ventricular synchronization and prolongs QRS duration.[22] Additionally, increased platelet levels may contribute to microcirculatory dysfunction, adversely affecting electrical conduction, which further explains these observations.[23] These findings highlight the influence of inflammation on electrical synchronization and underscore the importance of SII as a marker in understanding CRT outcomes.

To comprehensively evaluate inflammation's impact on CRT response, this study assessed hematological and biochemical markers such as CRP, neutrophils, platelets, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR). Elevated levels of these parameters in nonresponders indicate persistent inflammation and associated thrombotic activity, potentially impairing ventricular remodeling and limiting CRT efficacy. Conversely, responders demonstrated significantly lower and decreasing levels of these inflammatory markers post-CRT, highlighting the role of inflammation control in enhancing CRT's mechanical and clinical benefits.

Previous investigations have shown that long-term CRT can enhance intrinsic ventricular activation, with responders exhibiting marked reductions in native QRS duration

and improvements in dyssynchrony indices such as interventricular mechanical delay (IVMD) and opposing wall delay (OWD).[24] This pattern of intrinsic electromechanical recovery underscores the importance of myocardial substrate in determining CRT efficacy. Consistent with this framework, these findings suggest that elevated SII levels may hinder the heart's ability to achieve such intrinsic improvements, potentially contributing to the variability in remodeling and clinical response observed among CRT recipients.

These findings underscore the critical role of inflammation and related biological processes in determining CRT response. Elevated SII, along with increased levels of CRP, NLR, and PLR in nonresponders, emphasizes the predictive value of these markers. Incorporating SII and related inflammatory biomarkers into pre-implantation assessments may help identify optimal CRT candidates, facilitating more targeted management strategies and potentially improving patient outcomes. Although baseline CRP levels were similar between responders and nonresponders, SII demonstrated significant baseline differences and dynamic changes over time. This discrepancy likely reflects fundamental differences between these biomarkers. Cardiac resynchronization therapy represents a single acute-phase reactant influenced by transient inflammatory stimuli, whereas SII integrates neutrophil activation, lymphocyte suppression, and platelet-related prothrombotic activity. Consequently, SII may better capture chronic, low-grade inflammatory and immune dysregulation that characterizes advanced HF.

These findings provide valuable insights into the impact of inflammation on CRT outcomes and emphasize the need for further research. Future large-scale studies could explore innovative approaches to managing inflammation in this patient population, potentially enhancing CRT success rates and refining therapeutic strategies.

Study Limitations

Our study has several limitations. First, being a retrospective and single-center study limits the generalizability of the findings. Second, not all inflammation-related biomarkers were analyzed, and the potential effects of other inflammatory markers could not be evaluated. Third, the study considered only the increase in LVEF as an echocardiographic response to CRT, due to the absence of other important parameters. Additionally, the relatively short follow-up period may be insufficient to assess long-term outcomes.

Despite these limitations, this study suggests that the use of the SII may be valuable in predicting the response to CRT therapy and provides a meaningful contribution to the literature.

CONCLUSION

Elevated SII is associated with CRT nonresponse and persistent inflammatory activity during follow-up. Systemic immune-inflammation index appears to reflect underlying inflammatory and immune dysregulation linked to impaired CRT benefit rather than exerting a causal effect. Incorporation of SII into pre-implant evaluation may aid in patient characterization and risk stratification.

Data Availability: The data supporting this study are available from the corresponding author upon reasonable request.

Ethics Committee Approval: This study was approved by the Kocaeli City Hospital Scientific Research Ethics Committee (Approval Date: 13 November 2025; Protocol Number: 2025-128).

Informed Consent: As this is a retrospective study, the requirement for informed consent was waived by the ethics committee.

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Association Between Cardiometabolic Index and Liver Fibrosis: Mediation Analysis of Oxidative Stress Factors

ORIGINAL INVESTIGATION

ABSTRACT

Background: Liver fibrosis (LF) is a serious complication of chronic liver disease that may progress to cirrhosis and liver cancer, posing a daunting threat to human health. Cardiometabolic Index (CMI) is closely associated with metabolic disorders related to obesity, which may be implicated in the pathological process of liver disease by inducing oxidative stress. However, previous observational studies have reached inconsistent conclusions on the association between CMI and hepatic fibrosis. This study aimed to assess the association of CMI and LF with oxidative stress by utilizing a population-based study.

Methods: A total of 3170 participants from the National Health and Nutrition Examination Survey (2017-2020) were included. A weighted logistics regression model was generated to analyze the correlation between CMI and LF, followed by the construction of the restricted cubic spline (RCS) model to explore the potential nonlinear relationship between the 2. In addition, the potential mediating role of oxidative stress factors (serum albumin, uric acid, γ-glutamyl transferase [GGT]) in the association of CMI with hepatic fibrosis was further investigated by regression analysis.

Results: With confounding factors adjusted, CMI was found to be associated with an increased risk of LF (odds ratio [OR] = 2.27, $P < .001$, 95% CI: 1.60-3.23). The stratification results showed that compared with the first quartile range, the LF risk of the second and third quartile ranges increased by 3.08 times (OR = 3.08, $P < .001$, 95% CI: 1.90-4.98) and 6.43 times (OR = 6.43, $P < .001$, 95% CI: 3.84-10.75), respectively. Further RCS analysis suggested that this association had nonlinear characteristics (P-nonlinear $< .0001$). Mediation analysis demonstrated that the intermediate proportions of serum albumin, uric acid, and GGT in the impact of CMI on LF were approximately 8.04%, 8.15%, and 5.60%, respectively.

Conclusion: This study demonstrated a significant positive linkage between CMI and LF, highlighting the mediating role of oxidative stress factors (serum albumin, uric acid, and GGT) in this linkage.

Keywords: Cardiometabolic Index, liver fibrosis, mediation analysis, National Health and Nutrition Examination Survey, oxidative stress

INTRODUCTION

Liver fibrosis (LF) is fibrous scarring induced by the accumulation of extracellular matrix proteins (mainly type 1 and type 3 cross-linked collagen).[1] Fibrosis is a common feature of chronic inflammatory disease progression.[2] If the process of LF continues to progress, it can later develop into cirrhosis or even liver cancer,[3] which seriously endangers human life and health. Liver fibrosis usually results from chronic liver injury, and risk factors include chronic hepatitis virus infection, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) induced by metabolic syndrome (which is renamed as metabolic dysfunction-associated steatotic liver disease [MASLD]), and cholestasis.[4]

With the prevalence of obesity, MASLD has developed into one of the most common global causes of chronic liver disease in the world.[5] Metabolic dysfunction–associated steatotic liver disease is closely related to metabolic syndrome and its related hypertension, type 2 diabetes, dyslipidemia, and obesity.[6,7] Currently, commonly used scoring systems for clinical assessment of LF in MASLD, such as

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Fibrosis-4 index (FIB-4) and Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS), have included metabolic indicators such as body mass index (BMI) and blood glucose. However, these indicators often focus on a single dimension and are difficult to fully reflect the synergistic effect of central obesity and lipid metabolism disorders.[8-10]

The Cardiometabolic Index (CMI), as an integrated indicator, is calculated by multiplying the waist-to-height ratio (WHtR, reflecting central obesity) and the triglyceride/high-density lipoprotein cholesterol ratio (TG/HDL-C, reflecting lipid metabolism). It combines simplicity and multidimensional evaluation advantages.[11] Although the existing fibrosis score already covers some metabolic parameters, CMI may more accurately capture the damaging effects of metabolic disorders on the liver by coupling body fat distribution with lipid metabolism abnormalities. For example, WHtR is more indicative of visceral fat accumulation than BMI,[12,13] while the TG/HDL-C ratio is closely related to insulin resistance and hepatic lipid deposition.[14] Therefore, CMI may provide additional predictive value beyond traditional single indicators. In recent years, the clinical value of CMI has been gradually revealed in metabolic diseases, including atherosclerosis,[15] ischemic stroke,[16] and hypertension.[17] A recent study found a strong positive correlation between higher CMI and the risk of NAFLD/MASLD in the general population.[18] However, there is still controversy regarding the association between CMI and LF.[19,20] For example, based on the same National Health and Nutrition Examination (NHANES) dataset, Yan et al[20] discovered that the higher CMI is indicated the higher incidence of LF (adjusted odds ratio [OR]: 1.84, 95% CI: 1.84-1.85), but Cheng et al[19] did not find an association between the 2 (OR: −0.009, 95% CI: −0.47 to 0.29). This contradiction may stem from differences in the definition of LF in research, incomplete control of confounding factors, or insufficient exploration of the potential mechanisms of CMI (such as oxidative stress).

Oxidative stress is one of the key mechanisms driving liver injury and inducing LF. Ethanol intake, chronic viral infection, and iron deposition can exacerbate the production of reactive oxygen species (ROS),[21] thereby inducing hepatic stellate cell (HSC) activation and extracellular matrix deposition.[22] Dysregulation of lipid metabolism can also exacerbate liver damage by increasing oxidative stress levels.[23] Serum albumin, uric acid, and γ-glutamyl transferase (GGT) are mediating variables of oxidative stress and are associated with the regulation of oxidative damage and the progression of LF.[24-26] Serum albumin, as an endogenous antioxidant, reduces oxidative damage by clearing ROS and chelating transition metals such as iron and copper. The decrease in its level is correlated with the severity of LF.[27,28] Uric acid has an antioxidant function at physiological concentrations, but at high concentrations, it can promote ROS generation by activating Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase, forming a "dual effect" and is associated with advanced LF.[29,30] Gamma-glutamyl transferase reflects oxidative stress status by participating in glutathione metabolism, and its elevation suggests glutathione depletion and progression of LF.[24,31,32] However, there is still a lack of population-level evidence on whether CMI affects LF by regulating oxidative stress pathways.

Therefore, this study conducted a cross-sectional study using NHANES data to investigate whether the association between CMI and LF is independent of traditional metabolic indicators. In addition, considering the crucial role of oxidative stress in the progression of liver disease, this study also investigated whether oxidative stress factors (serum albumin, uric acid, and GGT) play a mediating role in the relationship between CMI and LF. This study can provide new evidence for the clinical application of CMI in risk stratification of LF, but it does not reveal the potential mechanism of the metabolic oxidative stress axis in LF.

HIGHLIGHTS

- There is a positive correlation between Cardiometabolic Index (CMI) and liver fibrosis (LF).
- Oxidative stress factors (serum albumin, uric acid, and γ-glutamyl transferase [GGT]) play a mediating role in this process.
- This project uncovered a significant positive linkage between CMI and LF, highlighting the mediating role of oxidative stress factors (serum albumin, uric acid, and GGT) in this linkage.

METHODS

Study Population

The data in this work came from the NHANES (https://www.cdc.gov/nchs/nhanes/index.html), which is a comprehensive study launched by the Centers for Disease Control and Prevention and the National Center for Health Statistics. The survey is aimed at assessing the nutritional and health status of the deinstitutionalized US population through stratified and multi-stage sampling. The relationship between CMI and LF was examined using survey data from 2017 to 2020 (n = 15 560). After excluding missing and irrelevant data on LF (n = 5860), CMI data (n = 5668), and data from subjects with other missing covariate data (n = 697), a total of 3170 individuals were included at last (non-LF samples: 2849; LF samples: 321). Figure 1 is a flowchart for screening subjects.

Dependent Variable: Liver Fibrosis

By using the FibroScan 502 V2 Touch system, NHANES staff performed liver stiffness measurement (LSM) on subjects using vibration-controlled transient elastography technology. This study used LSM ≥ 8.2 kPa as the criterion for determining LF based on the study of Eddowes et al.[33] According to liver biopsy, the study is a large cohort of NAFLD and reveals that 8.2 kPa is the optimal cutoff value for maximizing the Youden index when diagnosing significant fibrosis ($F \geq F2$).[33]

Independent Variable: Cardiometabolic Index

CMI = WHtR × [TG (mmol/L)/HDL-C (mmol/L)], where WHtR = waist circumference (cm)/height (cm).[11] Waist circumference and height data were obtained through physical

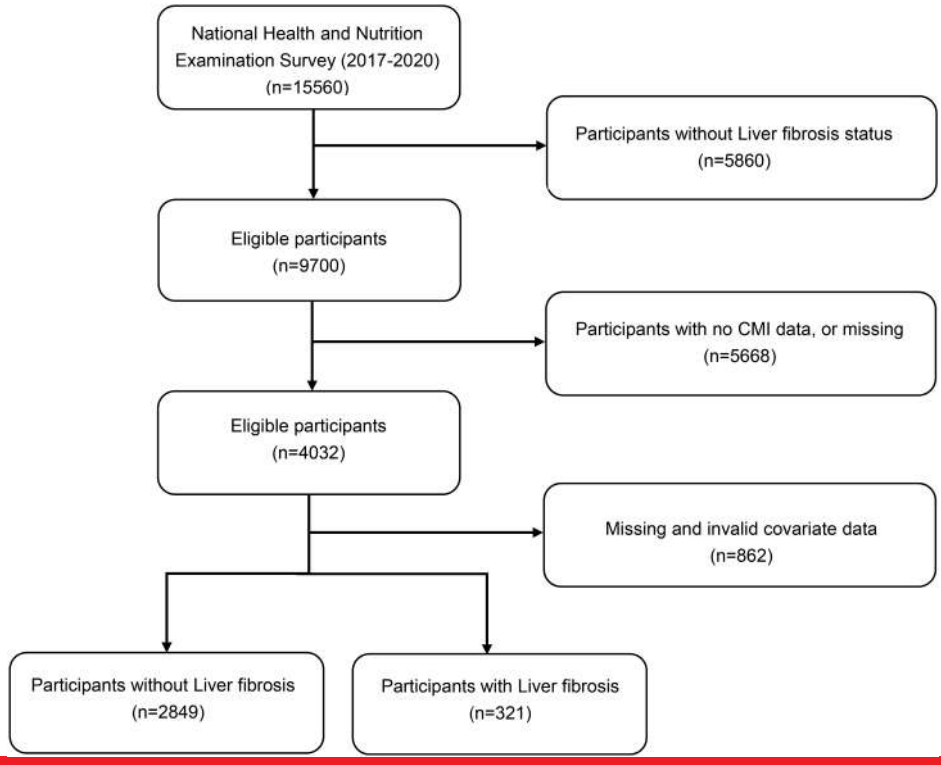

Figure 1. Flowchart of participant screening in NHANES.

examination. Triglycerides and HDL-C levels were detected by a Roche/Hitachi Cobas 6000 chemical analyzer.

Mediating Variables: Oxidative Stress Factors

Serum albumin, uric acid, and GGT were selected as the oxidative stress factors for mediation analysis in this project. In NHANES, serum albumin was obtained by the bromocresol purple staining method, and uric acid and GGT were determined by Roche Cobas 6000 (c501 module) chemistry analyzer.

Covariates

The covariates in this work included sex, race, age, BMI, serum albumin, smoking status, alcohol consumption, uric acid, blood bilirubin, alanine aminotransferase (ALT), blood urea nitrogen (BUN), aspartate transaminase (AST), GGT, history of liver disease, hepatic steatosis, MASLD, hepatitis C virus (HCV), total cholesterol (TC), TGs, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, total sugar intake, and total energy intake. Non-HDL-C was calculated by subtracting HDL-C levels from TC.[34] Body mass index was calculated when body weight (kg) was divided by the square of height (m) and analyzed by the following categories: underweight/healthy weight (BMI < 25 kg/m^2) and overweight or obese (BMI ≥ 25 kg/m^2).[35] Smoking status was determined based on the concentration of serum cotinine, with a concentration $\geq$10 ng/mL indicating smokers and a concentration <10 ng/mL indicating non-smokers.[36]

The measurement or questionnaire for all variables can be queried on the NHANES website (https://www.cdc.gov/nchs/nhanes/index.html).

Statistical Analysis

All analyses were processed using R (V4.3.3) software. The baseline table was plotted by utilizing the tableone package. The categorical variables are expressed as sample size and proportion [n (%)], and the continuous variables are expressed as mean and SD. A weighted logistics regression model was constructed using the survey package to analyze the association of CMI and its tertiles with hepatic fibrosis and to disclose its relevance in different populations through further subgroup analyses. The selection of confounding variables in this study was based on a theoretical framework supported by previous literature and the interrelationships between variables. Variance inflation factor was used to evaluate multicollinearity. To control for confounding factors that may affect the results, models were established to gradually evaluate confounding effects: in the Crude model, no adjustment for confounding factors was made. In Model 1, adjustments were made for age, sex, and race. In Model 2, age, sex, race, BMI, smoking, drinking, total bilirubin, ALT, BUN, AST, non-HDL-C, total sugar intake, and total energy intake were adjusted. A restricted cubic spline (RCS) was constructed using the rms package in a regression model after adjusting for all confounders to dissect the nonlinear relationship between CMI and hepatic fibrosis. In addition, regression analysis was employed to dig out the potential mediating role of serum albumin, uric acid, and GGT in the association of CMI with hepatic fibrosis. Mediation analysis was performed by utilizing the mediation package. Thousand non-parametric iterations were employed to estimate the 95% CI of the mediation effect and evaluate the stability of the results. Different researchers have used

weighted or unweighted methods when analyzing data. Although the complex sampling of NHANES can enhance the representativeness and applicability of research results, in some cases, weighted and unweighted analyses may yield different conclusions. Therefore, this study conducted non-weighted regression analysis as a sensitivity analysis to verify the robustness of the results of this study. $P < .05$ indicated a statistically significant difference.

RESULTS

Baseline Characteristics

A total of 3170 subjects were enrolled in this project, with an average age of 46.88 ± 17.33 years. Grouping was based on whether LF was present. The group with LF had a higher average age (52.59 vs. 46.33, $P < .001$), a significantly higher proportion of BMI ≥ 25 kg/m^2 (87.1% vs. 70.2%, $P < .001$), and a significantly higher CMI compared to the non-diseased group (0.87 vs. 0.58, $P = .001$). Biochemistry indices showed that the levels of uric acid, ALT, BUN, AST, and GGT in the population with LF were significantly higher than those in the non-diseased population. In terms of blood lipid indicators, the TG of patients with LF was significantly higher than those without LF (121.39 vs. 102.54 mg/dL, $P < .001$), while the levels of TC and HDL cholesterol were significantly lower than those without LF ($P < .05$). In addition, from the perspective of disease, a higher proportion of the LF population had a history of liver disease (9.2% vs. 4.2%, $P = .002$), steatosis (73.8% vs. 38.9%, $P < .001$), MASLD (82.2% vs. 40.1%, $P < .001$), and HCV (6.2% vs. 1.8%, $P = .035$) (Table 1).

Association Between Cardiometabolic Index and Liver Fibrosis

Table 2 displays the results of the weighted logistics regression model between CMI and LF. In the Crude model, CMI was associated with increased risk of LF (OR = 2.16, $P < .001$, 95% CI: 1.71-2.74). After preliminary adjustments for age, sex, and race (Model 1), the trend remained unchanged (OR = 2.09, $P < .001$, 95% CI: 1.64-2.65). After further adjustment of BMI, smoking, drinking, total bilirubin, ALT, BUN, AST, non-HDL-C, total sugar intake, and total energy intake (Model 2), the association between CMI and LF remains statistically significant (OR = 2.27, $P < .001$, 95% CI: 1.60-3.23). Additionally, the CMI was stratified by tertiles. The trend test uncovered that the risk of LF increased significantly with the increase of the CMI tertile interval (P for trend < .001). In Model 2, the risk of LF increased by 3.08 (OR = 3.08, $P < .001$, 95% CI: 1.90-4.98) and 6.43 (OR = 6.43, $P < .001$, 95% CI: 3.84-10.75) times for the second and third quartile intervals, respectively, compared to the first quartile interval.

Further subgroup analysis identified the association between CMI and LF in different subgroups (Table 3). After adjusting for all confounding factors, in both male (OR = 2.04, $P < .001$, 95% CI: 1.49-2.81) and female populations (OR = 2.62, $P < .01$, 95% CI: 1.48-4.65), CMI was associated with an increased risk of LF. Additionally, participants who were overweight or obese (BMI ≥ 25 kg/m^2) (OR = 2.30, $P < .001$, 95% CI: 1.63-3.25), who were drinkers (OR = 2.34, $P < .001$, 95% CI: 1.57-3.48), and with hepatic steatosis (OR = 1.58, $P < .05$, 95% CI: 1.08-2.32) were also significantly connected with an elevated risk of LF.

Nonlinear Relationship Between Cardiometabolic Index and Liver Fibrosis

After adjusting for all confounding factors, the nonlinear relationship between CMI and LF was explored using the RCS in a weighted logistic regression model. The results revealed that the overall trend between CMI and LF was significant (P-overall < .0001). With the increase in CMI, the risk of LF was significantly elevated. When CMI was greater than 0.451, the risk of LF was further increased. In addition, a significant nonlinear relationship between CMI and LF (P-nonlinear < .0001) was also detected (Figure 2).

Mediation Analysis

In the mediation analysis, the potential mediating roles of oxidative stress factors (serum albumin, uric acid, and GGT) in the association between CMI and LF were assessed. The results demonstrated that in the overall effect of CMI on LF, about 8.04% (95% CI: 3.61, 14.00) of the effect was attributed to the mediation of serum albumin (Figure 3A). In addition, uric acid and GGT accounted for approximately 8.15% (95% CI: 2.33, 14.00) and 5.60% (95% CI: 1.98, 10.00) of the mediating effect on LF in CMI, respectively (Figure 3B and C). These findings suggested that oxidative stress factors play an instrumental mediating role between CMI and LF.

Further analysis was conducted on the potential mediating roles of oxidative stress factors such as serum albumin, uric acid, and GGT in the association between CMI and LF in sex subgroups. The results showed that in males, GGT accounted for approximately 4.65% (95% CI: 0.03, 11.00) of the mediating proportion in the process of CMI affecting LF. In females, approximately 9.18% (95% CI: 1.96, 19.00) of the total effect of CMI on LF was attributed to the mediating role of serum albumin. Uric acid and GGT accounted for 13.55% (95% CI: 2.53, 27.00) and 5.21% (95% CI: 0.78, 10.00) of the mediating proportion in the process of CMI affecting LF, respectively (Table 4).

Sensitivity Analysis

Sensitivity analysis of non-weighted logistic analysis showed that in Model 2, the risk of LF increased by 2.95 (OR = 2.95, $P < .001$, 95% CI: 1.93-4.61) and 6.87 (OR = 6.87, $P < .001$, 95% CI: 4.46-10.86) times for the second and third quartiles, respectively, compared to the first quartile. These results indicate a strong correlation between CMI and the increased risk of LF (Table 5).

DISCUSSION

Based on the data from the NHANES, this investigation probed into the association between CMI and LF as well as the mediating role of oxidative stress factors (serum albumin, uric acid, GGT) in this association. The results indicated that CMI was associated with an increased risk of LF. Furthermore, the mediation analysis further revealed that serum albumin, uric acid, and GGT played mediating roles in the association between CMI and LF, with mediation proportions of 8.04%, 8.15%, and 5.60%, respectively.

Since its introduction in 2015, CMI has become a novel anthropometric measure for assessing an individual's metabolic health. CMI was originally used for the recognition

Table 1. Baseline Characteristics of Study Participants

Characters	Total, n (%)	Normal, n (%)	Liver Fibrosis, n (%)	*P*
Overall	3170	2849 (91.1)	321 (8.9)	
Demographic information				
Age (years)	46.88 (17.33)	46.33 (17.37)	52.59 (15.89)	<.001
Race				.507
Mexican American	427 (9.3)	376 (9.2)	51 (10.4)	
Other Hispanic	316 (6.7)	287 (6.7)	29 (6.4)	
Non-Hispanic White	1123 (63.7)	998 (63.4)	125 (66.5)	
Non-Hispanic Black	790 (10.9)	710 (11.0)	80 (9.9)	
Other race	514 (9.4)	478 (9.6)	36 (6.8)	
Sex				.138
Male	1564 (49.7)	1377 (48.9)	187 (58.2)	
Female	1606 (50.3)	1472 (51.1)	134 (41.8)	
BMI (kg/m^2)				.001
<25	844 (28.3)	809 (29.8)	35 (12.9)	
≥25	2326 (71.7)	2040 (70.2)	286 (87.1)	
Alcohol				.049
No	290 (6.7)	259 (6.2)	31 (10.9)	
Yes	2880 (93.3)	2590 (93.8)	290 (89.1)	
Smoke				.995
Non-smoker	2423 (77.5)	2180 (77.5)	243 (77.5)	
Active smoker	747 (22.5)	669 (22.5)	78 (22.5)	
Biochemical parameters				
CMI	0.61 (0.52)	0.58 (0.51)	0.87 (0.61)	<.001
Serum albumin (g/L)	40.77 (3.24)	40.84 (3.17)	40.04 (3.83)	.026
Uric acid (mg/dL)	5.40 (1.38)	5.35 (1.37)	5.97 (1.40)	<.001
Total bilirubin (mg/dL)	0.51 (0.32)	0.51 (0.32)	0.55 (0.29)	.110
ALT (U/L)	22.43 (17.40)	21.50 (16.44)	32.00 (23.20)	<.001
BUN (mmol/L)	5.20 (1.82)	5.13 (1.70)	5.90 (2.66)	<.001
AST (U/L)	21.56 (11.30)	20.90 (10.09)	28.36 (18.57)	.003
GGT (IU/L)	27.87 (30.16)	25.66 (24.24)	50.56 (60.55)	<.001
Total cholesterol (mg/dL)	184.19 (39.48)	184.90 (39.01)	176.91 (43.43)	.009
Triglycerise (mg/dL)	104.21 (61.57)	102.54 (61.20)	121.39 (62.82)	<.001
LDL-C (mg/dL)	109.37 (34.70)	109.89 (34.33)	103.99 (38.01)	.056
HDL-C (mg/dL)	54.00 (15.64)	54.52 (15.76)	48.67 (13.28)	<.001
Non-HDL-C (mg/dL)	130.19 (39.15)	130.38 (38.73)	128.23 (43.25)	.481
Total sugars intake (gm)	106.99 (78.73)	107.25 (78.78)	104.31 (78.30)	.499
Total energy intake (kcal)	2178.05 (964.84)	2174.09 (956.85)	2218.78 (1044.29)	.399
Comorbidity information				
Liver disease history				.002
No	3015 (95.3)	2733 (95.8)	282 (90.8)	
Yes	155 (4.7)	116 (4.2)	39 (9.2)	
Steatosis				<.001
No	1801 (58.0)	1718 (61.4)	83 (26.2)	
Yes	1369 (42.0)	1131 (38.9)	238 (73.8)	
MASLD				<.001
No	1735 (56.1)	1684 (59.9)	51 (17.8)	
Yes	1435 (43.9)	1165 (40.1)	270 (82.2)	
HCV				.035
No	3101 (97.8)	2805 (98.2)	296 (93.8)	
Yes	69 (2.2)	44 (1.8)	25 (6.2)	

n (%) represented the categorical variable and mean (SD) represented the continuous variable. n was unweighted. n (%), mean, and SD were weighted.
ALT, alanine aminotransferase; AST, aspartate transferase; BMI, body mass index; BUN, blood urea nitrogen; CMI, Cardiometabolic Index; GGT, γ-glutamyl transferase; HCV, hepatitis C; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MASLD, metabolic dysfunction–associated steatotic liver disease.

Table 2. The Associations Between Cardiometabolic Index and Liver Fibrosis

Participants	Crude Model	Model 1	Model 2
CMI (continuous)	OR = 2.16, *P* < .001, 95% CI: 1.71-2.74	OR = 2.09, *P* < .001, 95% CI: 1.64-2.65	OR = 2.27, *P* < .001, 95% CI: 1.60-3.23
CMI (categorical)			
T1 (<0.296)	Ref.	Ref.	Ref.
T2 (0.296-0.646)	OR = 2.78, *P* < .001, 95% CI: 1.63-4.72	OR = 2.45, *P* = .006, 95% CI: 1.33-4.49	OR = 3.08, *P* < .001, 95% CI: 1.90-4.98
T3 (≥0.646)	OR = 5.17, *P* < .001, 95% CI: 3.32-8.05	OR = 4.50, *P* < .001, 95% CI: 2.74-7.39	OR = 6.43, *P* < .001, 95% CI: 3.84-10.75
P_{trend}	<.001	<.001	<.001

Crude model: no adjustment for confounding factors. Model 1: adjustments for age, sex, and race. Model 2: adjustments for age, sex, race BMI, smoking, alcohol consumption, total bilirubin ALT, BUN, AST, Non-HDL-C, total sugar intake, total energy intake.
CMI, Cardiometabolic Index; OR, odds ratio.

Table 3. Relationship Between Cardiometabolic Index and Liver Fibrosis by Subgroup Analysis

Participants	Crude	Model 1	Model 2
Sex			
Male	OR = 1.85, *P* < .001, 95% CI: 1.36-2.51	OR = 1.83, *P* < .001, 95% CI: 1.35-2.48	OR = 2.04, *P* < .001, 95% CI: 1.49-2.81
Female	OR = 2.62, *P* < .001, 95% CI: 1.89-3.63	OR = 2.58, *P* < .001, 95% CI: 1.85-3.60	OR = 2.62, *P* = .004, 95% CI: 1.48-4.65
BMI			
BMI < 25	OR = 0.69, *P* = .589, 95% CI: 0.17-2.77	OR = 0.29, *P* = .153, 95% CI: 0.05-1.66	OR = 0.52, *P* = .493, 95% CI: 0.06-4.18
BMI ≥ 25	OR = 1.95, *P* < .001, 95% CI: 1.49-2.55	OR = 1.97, *P* < .001, 95% CI: 1.51-2.56	OR = 2.30, *P* < .001, 95% CI: 1.63-3.25
Alcohol			
No	OR = 2.62, *P* = .050, 95% CI: 1.00-6.83	OR = 2.56, *P* = .077, 95% CI: 0.89-7.32	OR = 2.66, *P* = .084, 95% CI: 0.85-8.30
Yes	OR = 2.13, *P* < .001, 95% CI: 1.61-2.80	OR = 2.04, *P* < .001, 95% CI: 1.54-2.69	OR = 2.34, *P* < .001, 95% CI: 1.57-3.48
Steatosis			
No	OR = 1.49, *P* = .420, 95% CI: 0.55, 4.02	OR = 1.17, *P* = .803, 95% CI: 0.31, 4.39	OR = 2.88, *P* = .057, 95% CI: 0.96-8.64
Yes	OR = 1.51, *P* = .005, 95% CI: 1.14, 1.98	OR = 1.55, *P* = .003, 95% CI: 1.19, 2.03	OR = 1.58, *P* = .025, 95% CI: 1.08-2.32

Crude model: no adjustment for confounding factors. Model 1: adjustments for age, sex, and race. Model 2: adjustments for age, sex, race BMI, smoking, alcohol consumption, total bilirubin ALT, BUN, AST, Non-HDL-C, total sugar intake, total energy intake.
BMI, body mass index; OR, odds ratio.

of diabetes. A study has confirmed its significant association with hyperglycemia,[11] which can reflect obesity and lipid metabolism. Obesity and dyslipidemia are strongly linked with the onset of MASLD.[37,38] Cardiometabolic Index is positively linked with the risk of MASLD in the general population.[18] This linkage has also been confirmed in the Chinese adult population, where CMI is a convenient indicator for MASLD screening and quantification.[39] Metabolic dysfunction–associated steatotic liver disease activates liver regeneration and fibrosis processes in the context of pathological damage such as liver fat accumulation, oxidative stress, inflammation, and apoptosis and facilitates the development of LF.[40] These findings indicated a significant positive correlation between CMI and LF, which is in line with previous studies.[20]

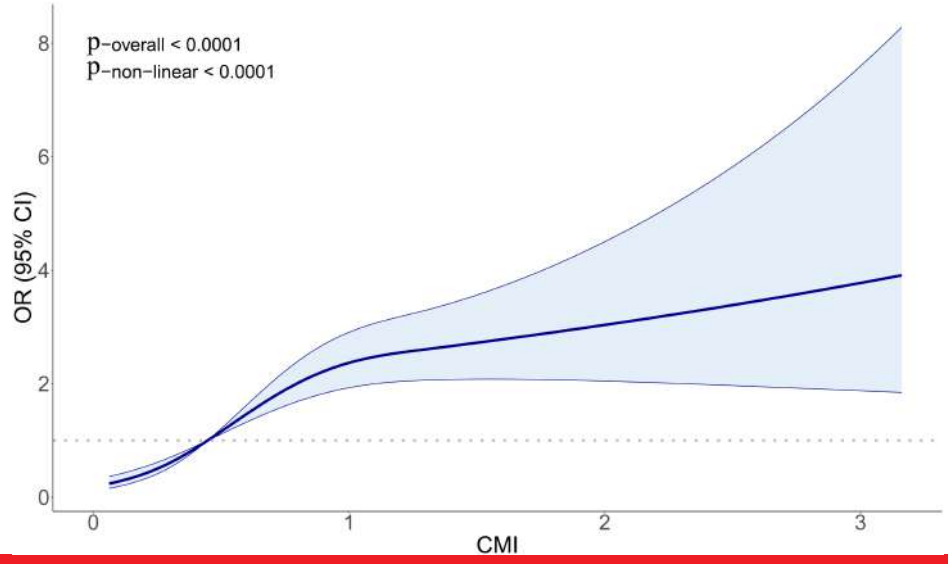

Figure 2. The nonlinear associations between CMI and LF. Adjustments were made based on age, sex, race, BMI, smoking, alcohol consumption, total bilirubin, ALT, BUN, AST, non-HDL-C, total sugar intake, and total energy intake. The solid line and the blue area represent the estimated value and its corresponding 95% CI, respectively.

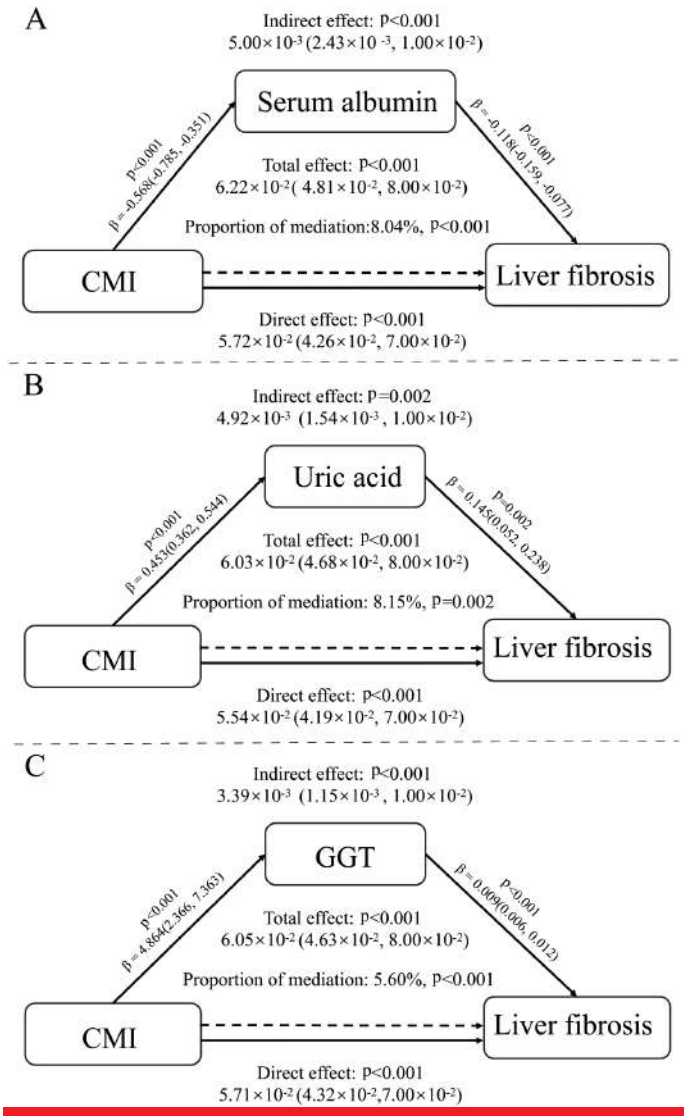

Figure 3. Mediation analysis of oxidative stress factors on the relationship between CMI and LF. In the mediation analysis, the following variables were adjusted: age, sex, race, BMI, smoking, alcohol consumption, total bilirubin, ALT, BUN, AST, non-HDL-C, total sugar intake, and total energy intake.

In addition, this study also included non-HDL-C, a key lipid indicator, in the analysis. Numerous studies have shown that non-HDL-C is not only closely associated with cardiovascular metabolic abnormalities but also with the occurrence and progression of hepatic steatosis and fibrosis.[41,42] A previous study has confirmed that this indicator is an independent predictor of NAFLD.[43] In a high-quality meta-analysis, the good predictive efficacy of non-HDL-C and its ratio (non-HDL-C/HDL-C) is confirmed in the occurrence and diagnosis of MASLD.[42] A large sample study based on NHANES indicated that elevated levels of non-HDL-C/HDL-C were independently associated with an increased risk of LF.[44] In the baseline characteristics of this study, there was no significant difference in non-HDL-C levels between the LF group and the non-LF group. However, after incorporating multiple confounding factors (Model 2), the association between CMI and LF remained robust (OR = 2.27). This result suggests that although non-HDL-C itself did not show differences between different LF states, incorporating it into the model still helps to more comprehensively control potential metabolic confounding factors, and CMI still exhibits predictive ability independent of non-HDL-C. Therefore, non-HDL-C may have additional or complementary value in the risk assessment of liver metabolic injury, while CMI provides a convenient indicator for integrating obesity and lipid metabolism abnormalities. The 2 may have the potential for synergistic application in identifying the risk of LF.

There is a positive linkage between CMI and LF, especially in overweight or obese individuals, alcohol drinkers, and people with a history of liver steatosis. Obesity is a key risk factor for the progression of MASLD to LF.[45] Obesity may develop insulin resistance by activating pro-inflammatory M1-type macrophages in adipose tissue and releasing pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α.[46] Obesity and insulin resistance may upregulate the expression of pro-inflammatory cytokines[47] and activate HSCs, thereby reinforcing the development of hepatic fibrosis. Furthermore, long-term alcohol consumption is a known cause of alcoholic liver disease and LF.[48] The main metabolite of ethanol, acetaldehyde, is highly toxic to hepatocytes and can lead to hepatocyte death and apoptosis. Meanwhile, ROS generated during ethanol catabolism causes oxidative stress and further induces hepatocyte death.[49] This persistent damage to hepatocytes causes mesenchymal cells to proliferate, thus resulting in fibrosis. Hepatic steatosis is also closely related to insulin resistance.[50,51] Fatty liver further exacerbates the development of insulin resistance by hindering the elimination of insulin from the portal vein, forming a vicious cycle. The imbalance of pro-inflammatory cytokines, free fatty acids, and adiponectin resulting from the vicious cycle can exacerbate the inflammatory response and is considered the main pathological mechanism of LF in patients with fatty liver.[52] Therefore, lifestyle management is particularly essential for these high-risk groups, including dietary adjustments, increasing physical activity, controlling alcohol consumption, and improving metabolic health. These measures may help reduce the risk of LF and refine the prognosis of liver disease.

The mediation analysis highlighted the mediating roles of oxidative stress factors, serum albumin, uric acid, and GGT in the association between CMI and LF. Serum albumin acts as an essential antioxidant in the human body.[27] The level of serum albumin reflects the body's oxidative stress state. A negative linkage between albumin levels and stages of LF has been revealed,[28] which is in line with the results showing that elevated levels of serum albumin can reduce the risk of LF ($\beta = -0.118$). Uric acid has dual properties of an antioxidant and a pro-oxidant.[53,54] Under physiological conditions, uric acid may elevate oxidative stress levels by increasing the production of ROS.[29] Hyperuricemia is connected with advanced LF in patients with MASLD.[30] Gamma-glutamyl transferase is not only a marker of liver damage, but also

Table 4. Mediation Analysis of Oxidative Factors on the Relationship Between Cardiometabolic Index and Liver Fibrosis by Sex Subgroup Analysis

Oxidative Stress	Direct Effect	Indirect Effect	Mediated Proportion % (95% CI)
Male			
Serum albumin	OR = 0.057, *P*<.001, 95% CI: 0.034, 0.080	OR = 0.002, *P* = .140, 95% CI: −0.001, 0.010	4.13 (−1.18, 12.00)
Uric acid	OR = 0.056, *P*<.001, 95% CI: 0.033, 0.080	OR = 0.002, *P* = .210, 95% CI: −0.001, 0.010	3.30 (−1.99, 11.00)
GGT	OR = 0.055, *P*<.001, 95% CI: 0.033, 0.070	OR = 0.003, *P* = .050, 95% CI: 0, 0.010	4.65 (0.03, 11.00)*
Female			
Serum albumin	OR = 0.062, *P*<.001, 95% CI: 0.039, 0.080	OR = 0.006, *P* = .012, 95% CI: 0.002, 0.010	9.18 (1.96, 19.00)*
Uric acid	OR = 0.058, *P*<.001, 95% CI: 0.035, 0.080	OR = 0.009, *P* = .018, 95% CI: 0.002, 0.020	13.55 (2.53, 27.00)*
GGT	OR = 0.065, *P*<.001, 95% CI: 0.042, 0.090	OR = 0.004, *P* = .010, 95% CI: 0.001, 0.010	5.21 (0.78, 10.00)*

Adjustments for age, sex, race BMI, smoking, alcohol consumption, total bilirubin, ALT, BUN, AST, non-HDL-C, total sugar intake, and total energy intake.
GGT, gamma-glutamyl transferase; OR, odds ratio.
*$P < .05$.
**$P < .01$.
***$P < .001$.

plays a pivotal role in the metabolism of glutathione, with its elevation usually indicating oxidative damage to liver cells.[55] In a cross-sectional study, elevated GGT can affect metabolic syndrome and progressive LF in patients with MASLD.[32]

It is worth noting that interesting differences in sex stratification analysis were observed. In females, the mediating ratio of serum albumin and uric acid is higher than that in males, while the mediating effect of GGT exists in both males and females, but in a lower proportion. The sex dimorphism of this association strength suggests potential biological mechanism differences. Estrogen has been found to exhibit potential protective effects in preventing or delaying the progression of LF. Estrogen can alleviate liver lipid deposition,[56] reduce oxidative stress induced by lipotoxicity in liver mitochondria, alleviate inflammation, and protect liver cells from damage.[57,58] Therefore, in women, pathways related to antioxidant capacity, such as albumin and uric acid, may play a more prominent role in liver damage associated with metabolic disorders. On the contrary, the association between CMI observed in males and LF may be related to the involvement of Y chromosome genes (especially *SRY* genes) in regulating fibrosis response. According to previous animal and molecular studies, the *SRY* gene can transcriptionally regulate platelet-derived growth factor receptor α (*Pdgfr*α) expression and promote HMGB1 release and subsequent activation of HSCs. By upregulating the expression of profibrotic genes, males are more likely to experience liver tissue repair imbalance and fibrosis exacerbation when subjected to oxidative stress or increased metabolic load.[59] It should be emphasized that these explanations are still speculative at present, and further clarification of the role of sex in metabolism-related LF is needed at the genetic and molecular levels in the future.

Table 5. Unweighted Logistic Regression Analysis on the Associations Between Cardiometabolic Index and Liver Fibrosis in Sensitive Analysis

Participants	Crude Model	Model 1	Model 2
CMI (continuous)	OR = 2.16, *P*<.001, 95% CI: 1.82-2.57	OR = 2.14, *P*<.001, 95% CI: 1.78-2.56	OR = 2.24, *P*<.001, 95% CI: 1.81-2.77
CMI (categorical)			
T1 (<0.296)	Ref.	Ref.	Ref.
T2 (0.296-0.641)	OR = 2.82, *P*<.001, 95% CI: 1.92-4.24	OR = 2.56, *P*<.001, 95% CI: 1.73-3.86	OR = 2.95, *P*<.001, 95% CI: 1.93-4.61
T3 (≥0.641)	OR = 5.77, *P*<.001, 95% CI: 4.03-8.50	OR = 5.34, *P*<.001, 95% CI: 3.69-7.95	OR = 6.87, *P*<.001, 95% CI: 4.46-10.86
P_{trend}	<.001	<.001	<.001

Crude model: no adjustment for confounding factors. Model 1: adjustments for age, sex, and race. Model 2: adjustments for age, sex, race BMI, smoking, alcohol consumption, total bilirubin ALT, BUN, AST, non-HDL-C, total sugar intake, total energy intake.
CMI, Cardiometabolic Index; OR, odds ratio.

This study confirmed that CMI, as a simple tool based on conventional metabolic indicators such as WHtR and TG/HDL-C, can integrate the dual risks of metabolic abnormalities and oxidative stress, providing a new strategy for early screening of LF. By combining oxidative stress markers such as serum albumin, uric acid, and GGT, CMI can not only identify high-risk populations (such as obesity and metabolic syndrome patients) but also suggest potential oxidative damage mechanisms, thereby optimizing primary stratified management. For example, prioritizing FIB-4 scores or imaging examinations for individuals with elevated CMI can achieve efficient resource allocation. In addition, this study revealed for the first time at the population level the specific pathway through which CMI mediates LF through oxidative stress (mediation ratio ranging from 5.60% to 8.15%), providing direct evidence for the pathological mechanism of the "metabolism-oxidative stress-fibrosis" axis. This discovery suggests that combined interventions targeting metabolic and oxidative pathways, such as lipid-lowering therapy combined with antioxidants, may be an effective strategy for delaying fibrosis progression. Future research needs to further validate the universality of CMI in non-obese MASLD, different ethnic and regional populations, or verify the predictive value of CMI dynamic changes on intervention effectiveness through longitudinal cohort studies.

It should be pointed out that although this study focuses on the mediating role of oxidative stress, the influence of other unmeasured mediating pathways cannot be ruled out. Chronic low-grade inflammatory response remains an important biological mechanism between metabolic disorders and LF, and this study did not include inflammatory biomarkers such as C-reactive protein (CRP) and IL-6 for evaluation. In addition, dietary structure and physical activity level can independently affect metabolic status, liver lipid deposition, and fibrosis risk. These unmeasured behaviors or inflammation-related factors may interact with the oxidative stress pathway, thereby participating in or amplifying the association between CMI and LF to some extent. Future research needs to combine more comprehensive inflammatory biomarkers, dietary survey data, and physical activity data, and further use multi-omics methods to systematically analyze the interactions of different pathways in order to more accurately characterize the complex mechanism network of CMI acting on LF.

Although this investigation provides evidence of a positive association between oxidative stress-mediated CMI and hepatic fibrosis, certain limitations persist. Firstly, this study is a cross-sectional study that limits causal inference. The time sequence of exposure mediation outcome cannot be determined, and mediation effects may be influenced by reverse causality and residual confounding. The observed mediation effects should be considered as preliminary evidence, and their robustness needs to be further validated through longitudinal cohorts or intervention studies. Secondly, the extrapolation of research needs to be carefully evaluated. The NHANES data are mainly based on the American population, whose racial structure, dietary habits, and prevalence of liver disease differ from regions such as Asia, the Middle East, and Europe. Therefore, the applicability of the research results to other populations is limited. Thirdly, confounding factors cannot be completely ruled out. Although this study controlled for bias through complex sampling weighting and statistical analysis, this cross-sectional study may still contain unknown or unmeasured confounding factors that affect the observed associations. Finally, the sample for this study was from the general population, and the incidence of LF (especially advanced fibrosis) is relatively low, which may have an impact on the positive predictive value of imaging tools such as FibroScan. However, through stratified analysis and a tiered screening strategy, the limitations of low prevalence rates were partially alleviated. In the future, it is necessary to verify the synergistic application value of CMI and imaging tools in higher-risk populations (such as MASLD outpatient patients) and develop composite models to optimize diagnostic efficiency. Taken together, these findings revealed a positive correlation between CMI and LF and highlighted the key role of oxidative stress factors in it. These findings can proffer a new perspective on the complex relationship between CMI and liver diseases, offering scientific evidence for the prevention and clinical management of LF in the future.

CONCLUSION

Based on the NHANES data, this work systematically revealed a significant positive correlation between CMI and LF and emphasized the mediating role of oxidative stress factors (serum albumin, uric acid, and GGT) in this association. These findings not only deepen the interpretation of the relationship between metabolic abnormalities and LF but also provide a scientific basis for the early prevention of LF. Future studies should further validate these findings in different populations to provide stronger evidence and guidance for the prevention and clinical management of LF.

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Clinical and Electrophysiological Assessment of Peripheral Nerve Function After Transradial Angiography: A Prospective Study

ABSTRACT

Background: Transradial angiography (TRA) is widely used in contemporary coronary procedures. Although clinically apparent peripheral nerve injury after TRA is uncommon, subclinical nerve involvement may go unrecognized. This study aimed to objectively assess peripheral nerve function after TRA using neurological examination, standardized neuropathic pain questionnaires, and nerve conduction studies (NCS).

Methods: This prospective, single-center observational study included consecutive patients undergoing transradial coronary angiography. A total of 107 patients were analyzed. Neurological examination was performed within 24-48 hours after the procedure. Neuropathic symptoms were evaluated using the Douleur Neuropathique en 4 (DN4) and Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) questionnaires. Bilateral nerve conduction studies of the median, ulnar, and radial nerves were performed 1 month after TRA, and side-to-side comparisons were conducted.

Results: Side-to-side differences were observed in selected nerve conduction parameters. These electrophysiological changes predominantly involved the radial nerve on the procedure side, characterized by lower sensory amplitude, reduced conduction velocity, and decreased motor amplitude ($P < .05$). Median and ulnar nerve conduction findings were largely comparable between sides. Douleur Neuropathique en 4 and LANSS scores were in normal ranges and not associated with nerve conduction parameters. No clinically evident local or neurological complications were detected during follow-up.

Conclusion: Transradial angiography may be associated with mild, procedure-side predominant radial nerve conduction changes detectable by NCS, without clinically evident neuropathy. Objective electrophysiological assessment may therefore help identify underrecognized subclinical nerve involvement following TRA.

Keywords; Nerve conduction studies, peripheral nerve injuries, radial nerve, transradial angiography

ORIGINAL INVESTIGATION

INTRODUCTION

In recent years, the transradial approach (TRA) has become the preferred access route for coronary angiography. Its use is associated with fewer access site bleeding events and vascular complications than the transfemoral approach, resulting in better clinical outcomes, particularly in patients with acute coronary syndrome. As a result, international guidelines now recommend TRA as the default strategy for invasive coronary procedures.[1-5]

Transradial access may be associated with complications including radial artery spasm, catheter kinking, arterial dissection or perforation, radial artery occlusion, hematoma, pseudoaneurysm, arteriovenous fistula, and, less commonly, peripheral nerve injury. Although most TRA-related complications are mild and self-limited, some may lead to patient discomfort, limb dysfunction, prolonged hospitalization, and in rare cases, significant morbidity.[6-10]

Peripheral nerve injury after transradial access is rare and its true incidence is likely underestimated, as mild neurological symptoms are frequently overlooked. Proposed mechanisms include local hematoma, contrast extravasation,

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compression-related ischemia, compartment syndrome, and complex regional pain syndrome, all of which may affect adjacent neural structures. In this setting, electrophysiological abnormalities may still be identified even in patients without clinically apparent neuropathic symptoms.[11-13]

Nerve conduction studies (NCS) provide an objective method for assessing peripheral nerve function and are capable of identifying subclinical or transient nerve involvement that may not correlate with patient-reported symptoms or neurological examination.[14,15] However, data regarding systematic electrophysiological evaluation of peripheral nerves following TRA remain limited, and existing studies have reported inconsistent findings.

This prospective study objectively evaluated the presence and characteristics of peripheral nerve involvement following TRA through comprehensive neurological examination, standardized neuropathic pain assessment, and nerve conduction studies.

METHODS

Study Design and Population

This prospective, single-center, observational cohort study was conducted at a tertiary referral hospital. Eligibility screening was performed consecutively in patients undergoing transradial coronary angiography. A total of 132 patients were initially assessed to reflect routine clinical practice and to allow a comprehensive evaluation of potential electrophysiological changes related to intervention.

At baseline, all patients underwent a detailed medical history review and comprehensive neurological examination. One month after the TRA procedure, peripheral NCS were performed on both the procedure side and the contralateral upper extremity.

Inclusion and Exclusion Criteria

Patients undergoing transradial coronary angiography were evaluated for eligibility. Individuals with peripheral polyneuropathy (PNP), a history of nerve injury or upper-extremity surgery/injury involving the peripheral nerves, and those with other neurological conditions known to affect nerve functions were not included. Patients unable to adequately cooperate with electrophysiological testing were also excluded. Carpal Tunnel syndrome (CTS) cases were retained in the primary analysis to reflect routine practice and to avoid unnecessary loss of sample size for non-median nerve comparisons. A prespecified secondary analysis was subsequently performed excluding patients with CTS to reduce potential confounding.

HIGHLIGHTS

- Peripheral nerve involvement after transradial angiography may remain clinically unrecognized.
- Nerve conduction studies revealed mild, procedure-side–predominant radial nerve changes.
- Electrophysiological findings were not associated with neuropathic pain scores.
- Objective assessment may help identify subclinical nerve involvement after transradial access.

Transradial Angiography Procedure

All angiographic procedures were performed by an experienced interventional cardiologist using a standardized transradial approach. Only patients who had undergone diagnostic coronary angiography for standard clinical indications, without additional interventional procedures, were included in the study. A standardized 6-French radial sheath was used in all cases. The average procedural duration was approximately 30 minutes, depending on anatomical complexity. The radial sheath was removed immediately after completion of angiography, and hemostasis was achieved using a dedicated radial compression device according to institutional protocol.

Clinical and Neurological Assessment

The neurological examination included assessment of muscle strength, superficial sensation, deep tendon reflexes, and neuropathic pain–related signs such as allodynia, hyperalgesia, and hyperpathia. This examination was carried out within 24-48 hours after the transradial procedure. Nerve conduction studies were performed separately at the 1-month follow-up visit.

Neuropathic complaints were evaluated using the Douleur Neuropathique en 4 (DN4) and Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) questionnaires as screening tools for neuropathic pain. A DN4 score of 4 or higher and a LANSS score of 12 or higher were considered indicative of neuropathic pain.[16,17]

Procedure-related local complications were systematically assessed during follow-up, and medication histories were reviewed for agents that could affect neuropathic pain assessment.

Electrophysiological Evaluation

Nerve conduction studies were performed 1 month after transradial angiography, as electrophysiological manifestations of peripheral nerve injury often require several weeks to become detectable and may be underestimated if assessed earlier. All recordings were performed in a dedicated electromyography laboratory using a commercially available system. Motor nerve studies used supramaximal stimulation with compound muscle action potentials recorded from standard target muscles. Sensory nerve studies were performed using routine antidromic techniques. Standard filter settings were used throughout the study (motor studies: 2 Hz-10 kHz; sensory studies: 20 Hz-2 kHz). Skin temperature was maintained at ≥32°C and monitored before each examination to minimize temperature-related variability. All recordings were performed in accordance with established electrodiagnostic guidelines. All electrophysiological studies were performed and interpreted by an experienced physiatrist.

Nerve conduction studies parameters were obtained from both the procedure side and the contralateral side, and side-to-side comparisons within the same patient were used to reduce inter-individual variability. All studies were

performed and interpreted by the same physician to ensure methodological consistency.

Laboratory Evaluation

Laboratory parameters known to influence peripheral nerve function were recorded, including vitamin B12, ferritin, thyroid function tests (TSH and free T4), glycemic status (HbA1c), and hematological indices (hemoglobin and mean corpuscular volume). These parameters were evaluated to identify potential metabolic or systemic confounders.

Statistical Analysis

Statistical analyses were performed using standard statistical software. The normality of continuous variables was assessed prior to analysis. Variables with a normal distribution are presented as mean ± standard deviation, whereas non-normally distributed variables are reported as median (interquartile range). Categorical variables are expressed as number and percentage. Side-to-side comparisons within the same patient were performed using paired *t*-tests for normally distributed data and Wilcoxon signed-rank tests for non-normally distributed data. Associations between neuropathic pain scores (DN4 and LANSS) and nerve conduction parameters were evaluated using Spearman's rank correlation coefficient. A *P* value <.05 was considered statistically significant. Given the exploratory nature of the analyses, no formal correction for multiple comparisons was applied.

Ethics

The study was approved by the Local Ethics Committee (15.10.2025-2025-KAEK-47) and was conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

A total of 132 patients who underwent TRA were initially referred for post-procedural neurological and NCS. Among these, 25 patients were excluded based on predefined criteria, including poorly controlled diabetes (HbA1c > 7%; n=7), vitamin B12 deficiency (< 250 pg/mL; n=5), and use of medications that could influence neuropathic pain assessment (n=5). In addition, electrophysiological findings consistent with PNP were identified during NCS in 8 patients. After these exclusions, 107 patients constituted the final study population for the primary analysis. This analysis was performed to observe all potential electrophysiological changes associated with the procedure before excluding conditions that could independently affect peripheral nerve function. Subsequently, patients with CTS (n=16) were excluded, and a predefined secondary analysis was conducted in the remaining 91 patients.

Table 1 summarizes the demographic, laboratory and clinical characteristics of the final study population following exclusion of patients with CTS (n=91). Douleur Neuropathique en 4 and LANSS neuropathic pain scale scores were within normal ranges in the 91 patients included in the secondary analysis.

Side-to-side nerve conduction findings observed in the primary analysis of the entire cohort (n=107) are summarized in Tables 2 and 3. In this analysis, significant side-to-side differences were identified in selected nerve conduction parameters, including median motor nerve latency, amplitude, and conduction velocity, as well as median sensory nerve conduction velocity. Significant differences were also observed in radial nerve motor amplitude and in radial sensory nerve amplitude and conduction velocity. In contrast, ulnar motor and sensory nerve conduction measures were comparable between sides.

After exclusion of patients with CTS (n=91), side-related differences were limited to the radial nerve (Tables 4 and

Table 1. Demographic and Clinical Characteristics of the Study Population After Exclusion of CTS Cases (n=91)

Characteristics	Values
Age, years	56 ± 11
Sex (female/male) (%)	41 (45)/50 (55)
Vitamin B12 (pg/mL)	412 (318-546)
Ferritin (ng/mL)	74 (38-146)
TSH (μIU/mL)	1.92 (1.21-2.68)
Free T4 (ng/dL)	1.12 (0.98-1.26)
HbA1c (%)	5.8 ± 0.8
Hemoglobin (g/dL)	13.7 ± 1.4
MCV (fL)	89.6 ± 4.8
DN4 score	2 (0-3)
LANSS score	5 (2-8)

Values are presented as mean ± standard deviation, median (interquartile range), or number (percentage), as appropriate. DN4, Douleur Neuropathique en 4 Questions; HbA1c, glycated hemoglobin; LANSS, Leeds Assessment of Neuropathic Symptoms and Signs; MCV, mean corpuscular volume; TSH, thyroid-stimulating hormone.

Table 2. Primary Analysis (Overall Cohort, n=107): Side-to-Side Motor Nerve Conduction Comparisons

	Right	Left	*P*
Median motor nerve latency	3.33 (2.45-7.24)	3.31 (2.31-7.4)	**.035***
Median motor nerve amplitude	8.4 (3.3-14.9)	7.45 (1.4-16.5)	**<.001****
Median motor nerve velocity	50 (33-74)	51 (46-71)	**.036***
Ulnar motor nerve latency	2.59 ± 0.36	2.41 ± 0.42	.1
Ulnar motor nerve amplitude	7.65 (5.4-12.7)	7.8 (4.7-14)	.57
Ulnar motor nerve velocity	57.1 ± 7.6	57.9 ± 7.3	.23
Radial motor nerve latency	2.29 (1.25-3.28)	2.38 (1.25-3.75)	.19
Radial motor nerve amplitude	3.1 (0.6-7.4)	5.3 (3.2-7.9)	**.001****
Radial motor nerve velocity	63 (37-76)	64 (41-76)	.95

Values are presented as mean ± standard deviation or median (interquartile range), as appropriate. Right and left sides correspond to the procedure side and contralateral side, respectively. *P* values were obtained using paired statistical tests. Statistically significant *P* values are indicated with an asterisk (*$P < .05$, **$P < .001$).

Table 3. Primary Analysis (Overall Cohort, n = 107): Side-to-Side Sensory Nerve Conduction Comparisons

	Right	Left	*P*
Median sensory nerve latency	2.71 (2.15-5.05)	2.74 (2.08-4.1)	.12
Median sensory nerve amplitude	17.7 (2.86-48.60)	21.1 (1.96-52)	.42
Median sensory nerve velocity	49.78 ± 7.43	51.30 ± 6.98	**.009****
Ulnar sensory nerve latency	2.14 (1.77-3.75)	2.23 (1.18-4.27)	.44
Ulnar sensory nerve amplitude	20 (16.40-56.90)	21 (15.5-59)	.47
Ulnar sensory nerve velocity	54 (42-64)	56.5 (46-68)	.062
Radial sensory nerve latency	1.51 (0.73-3.96)	1.46 (0.94-3.78)	.26
Radial sensory nerve amplitude	20.15 (14.5-66)	23.5 (14.45-64)	**.004****
Radial sensory nerve velocity	55.18 ± 7.21	64.82 ± 7.24	**.027***

Values are presented as mean ± standard deviation or median (interquartile range), as appropriate. Right and left sides correspond to the procedure side and contralateral side, respectively. *P* values were obtained using paired statistical tests. Statistically significant *P* values are indicated with an asterisk (*$P < .05$, **$P < .001$).

5). Lower sensory amplitude and conduction velocity were observed on the procedure side, along with reduced radial motor amplitude ($P = .03$, $P = .042$, and $P = .04$). Radial latencies and median and ulnar NCS findings were similar on both sides.

Despite these procedure-side differences in radial nerve conduction parameters, correlation analyses demonstrated no significant association between DN4 or LANSS scores and radial sensory or motor nerve conduction findings (Table 6).

Table 4. Predefined Secondary Analysis Excluding CTS (n = 91): Side-to-Side Motor Nerve Conduction Comparisons

	Right	Left	*P*
Median motor nerve latency	3.10 (2.45-4.24)	3.05 (2.31-4)	.055
Median motor nerve amplitude	9.7 (5.3-14.9)	8.80 (5.4-16.5)	.061
Median motor nerve velocity	58 (49-74)	58 (52-71)	.054
Ulnar motor nerve latency	2.45 ± 0.38	2.35 ± 0.43	.81
Ulnar motor nerve amplitude	8.6 (5.6-12.7)	8.2 (5.8-14)	.66
Ulnar motor nerve velocity	55.3 ± 5.6	56.7 ± 6.3	.35
Radial motor nerve latency	2.20 (1.25-2.98)	2.30 (1.25-3.60)	.24
Radial motor nerve amplitude	3.2 (1.5-7.4)	5.4 (3.69-7.9)	**.04***
Radial motor nerve velocity	66 (44-76)	62 (41-70)	.65

Table 5. Predefined Secondary Analysis Excluding CTS (n = 91): Side-to-Side Sensory Nerve Conduction Comparisons

	Right	Left	*P*
Median sensory nerve latency	2.6 (2.15-4.15)	2.51 (2.08-3.1)	.26
Median sensory nerve amplitude	27 (20-48.60)	29 (22-52)	.56
Median sensory nerve velocity	54.58 ± 5.23	53.5 ± 5.54	.068
Ulnar sensory nerve latency	2.2 (2.23-3.75)	2.05 (1.18-3.70)	.56
Ulnar sensory nerve amplitude	23 (20.15-54)	23.45 (20-59)	.68
Ulnar sensory nerve velocity	54 (42-63)	55 (46-63)	.07
Radial sensory nerve latency	1.65 (1.63-3.96)	1.60 (1.74-3.78)	.32
Radial sensory nerve amplitude	22 (19.5-66)	24.1 (16.5-62)	**.03***
Radial sensory nerve velocity	54.28 ± 7	62.56 ± 6.84	**.042***

Values are presented as mean ± standard deviation or median (interquartile range), as appropriate. Right and left sides correspond to the procedure side and contralateral side, respectively. *P* values were obtained using paired statistical tests. Statistically significant *P* values are indicated with an asterisk.

No clinically evident procedure-related local complications, including hematoma, extravasation, compartment syndrome, or complex regional pain syndrome, were observed during postprocedural follow-up.

DISCUSSION

This prospective clinical study provides objective evidence of peripheral nerve conduction changes following transradial angiography. By combining detailed neurological examination, standardized neuropathic pain assessment scales, and comprehensive NCS, procedure-side–predominant radial nerve electrophysiological alterations were demonstrated, despite the absence of motor or sensory deficits on clinical examination. This study represents one of the few prospective investigations systematically evaluating potential peripheral nerve involvement after transradial angiography using objective electrophysiological measures.

Table 6. Correlation Between Neuropathic Pain Scores and Radial Nerve Conduction Parameters (n = 91)

	DN4 (*r*, *P*)	LANSS (*r*, *P*)
Radial sensory amplitude	$r = -0.14$, $P = .18$	$r = -0.11$, $P = .27$
Radial sensory velocity	$r = -0.09$, $P = .36$	$r = -0.13$, $P = .21$
Radial motor amplitude	$r = -0.17$, $P = .10$	$r = -0.15$, $P = .14$

Correlation analyses were performed using Spearman's rank correlation.
DN4, Douleur Neuropathique en 4 Questions; LANSS, Leeds Assessment of Neuropathic Symptoms and Signs.

In this study, patients with CTS (n=16) were not excluded from the primary analysis in order to preserve the statistical power of the ulnar and radial nerve conduction assessments. As these conditions predominantly affect the median nerve or reflect generalized neuropathy, their inclusion in the primary analysis enabled a more comprehensive evaluation of non-median nerve conduction findings. To minimize potential confounding effects, a predefined secondary analysis excluding these patients was subsequently performed. After the second analysis, side-to-side differences in non-radial nerves were no longer observed, whereas radial nerve abnormalities persisted, supporting a radial nerve–specific effect rather than a generalized or systemic neuropathic process.

The lack of correlation between DN4/LANSS scores and nerve conduction parameters is consistent with clinical experience. In carpal tunnel syndrome and other entrapment neuropathies, symptoms may be prominent despite normal or only mildly abnormal nerve conduction findings, whereas electrophysiological abnormalities can also be detected in patients with minimal or no symptoms.[18] Another possible explanation for the lack of correlation between neuropathic symptoms and electrophysiological findings relates to patient reporting behavior. Mild dysesthetic symptoms such as numbness or tingling may be underrecognized or underreported unless accompanied by more prominent complaints like weakness or functional impairment. This tendency may contribute to a dissociation between subjective symptom reporting and objective neurophysiological findings. Consistent with this observation, a systematic review reported a pooled incidence of clinically documented nerve damage after transradial access of 0.16%, while the pooled incidence of sensory symptoms such as numbness or tingling was 1.61%, with only 1 study identifying radial nerve damage (1/488; 0.25%).[19,20]

In a paresthesia-focused cohort, most patients had no pathological NCS findings. However, including patients with diabetic polyneuropathy in the primary analysis may have reduced sensitivity for subtle side-to-side differences because polyneuropathy tends to be bilateral and symmetric.[21]

A systematic review evaluating hand dysfunction after transradial artery catheterization reported a very low incidence of clinically documented nerve damage and sensory symptoms such as numbness or tingling. Analyzed studies mostly relied on symptom-based assessments or functional questionnaires, with limited use of objective neurophysiological testing. Findings of this review suggest that while clinically evident hand dysfunction after transradial access is uncommon, mild or subclinical nerve conduction changes may remain underrecognized without systematic electrophysiological evaluation, as demonstrated in the present study.[11]

The pattern of electrophysiological changes observed in this study—predominantly involving radial sensory nerve amplitude and conduction velocity, as well as radial motor nerve amplitude, with preserved latencies—may provide important clues regarding the underlying mechanism. Latency prolongation is typically associated with focal demyelination or conduction block, whereas reductions in amplitude and conduction velocity are more consistent with axonal dysfunction, impaired axonal recruitment, or transient ischemic effects. In the context of transradial access, prolonged or excessive local compression, edema, or microvascular compromise may preferentially affect axonal function without causing apparent demyelination. Such mechanisms could explain why amplitude- and velocity-based parameters were more sensitive to procedure-related changes than latency measures. This pattern is also in keeping with a mild and potentially reversible form of nerve involvement rather than a fixed structural nerve injury.[12,14,22]

The procedure-side–predominant radial nerve conduction changes observed in this study may be related to local factors inherent to transradial access rather than direct nerve injury. Hemostasis after transradial angiography is commonly achieved using prolonged manual compression, elastic bandaging, or dedicated radial compression devices.[23] While these methods are effective in preventing bleeding and radial artery occlusion, excessive pressure, prolonged compression duration, or suboptimal positioning may lead to transient local nerve compression, microvascular compromise, or perineural ischemia. In addition, access-site–related complications such as local edema, minor hematoma formation, or contrast extravasation may contribute to subclinical radial nerve involvement. Given the superficial course of the radial nerve and its close anatomical relationship to the radial artery at the wrist and forearm, the radial nerve may be particularly vulnerable to these local mechanical and ischemic factors. Radial artery occlusion (RAO) is another recognized vascular complication after transradial access and has been reported most frequently in the early post-procedural period, although delayed cases have also been described. While RAO is often clinically silent due to collateral circulation, it may occasionally present with hand numbness or vague pain, which may overlap clinically with symptoms attributed to peripheral nerve involvement.[23] Sensory complaints of vascular origin generally show a broader and more diffuse distribution, often involving the entire hand and sometimes extending into the forearm. In such cases, numbness or vague pain is typically poorly localized and may be accompanied by additional findings such as color changes or temperature asymmetry of the affected limb. In contrast, sensory symptoms related to peripheral nerve involvement tend to follow the anatomical distribution of the affected nerve and are usually more clearly demarcated distally. Color change or temperature difference is not commonly observed in isolated nerve dysfunction. The predominantly amplitude- and velocity-based changes observed in the study, without corresponding latency prolongation or clinical deficits, are consistent with mild, potentially reversible conduction alterations rather than apparent structural nerve injury.[12,14,23,24,25]

During follow-up, some patients continued to apply tight wrapping or excessive protection to the procedure side beyond the immediate post-procedural period. Insufficient

discharge guidance regarding limb use, together with overly protective patient behavior, may contribute to prolonged local compression and transient radial nerve stress.

Study Limitations

There were several limitations to this study. This was a single-center study, which may limit the generalizability of the findings. Although electrophysiological changes were observed on the procedure side, there was no clinically apparent motor or sensory neuropathy, which makes it difficult to draw conclusions about their direct clinical relevance. Because pre-procedural baseline nerve conduction studies were not performed, direct intra-individual temporal comparison of the procedure side remains limited. In addition, the findings observed may represent an early and potentially reversible axonal response related to transient mechanical stress, edema, or inflammatory changes following transradial access. However, since long-term follow-up electrophysiological assessments were not performed, it remains unclear whether these changes persist or resolve over time.

Clinical Implications

Although no clinically evident neuropathy was detected, procedure-side–predominant electrophysiological changes suggest that transradial angiography may be associated with mild radial nerve stress. These findings emphasize the importance of careful post-procedural limb care, particularly appropriate hemostasis and avoidance of unnecessarily prolonged or tight compression, and may help clinicians interpret post-procedural upper-extremity symptoms in the absence of objective neurological deficits.

Ethics Committee Approval: The study was approved by the Bursa City Hospital Clinical Research Ethics Committee (15.10.2025-2025-KAEK-47) and was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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Mixed Reality–Assisted Planning Enables Successful Coil Embolization of Anatomically Complex Peridevice Leak After LAAC

CASE REPORT

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INTRODUCTION

Percutaneous left atrial appendage closure (LAAC) is an established stroke prevention strategy in patients with atrial fibrillation contraindicated for long-term anticoagulation.[1,2] However, peridevice leak (PDL) remains a significant limitation, occurring in 25.8% of patients at 45-day follow-up.[3] Persistent PDL is associated with a 4.25-fold increased risk of transient ischemic attack, ischemic stroke, or systemic embolism (adjusted subdistribution hazard ratio, 4.25; 95% confidence interval (CI): 1.91-9.44; $P < .001$).[4] Posterosuperior PDL locations present particular technical challenges for percutaneous closure. Mixed reality (MR) shows promise for preprocedural planning in complex structural interventions.[5] The first application of MR-guided planning for PDL closure after LAAC is reported.

CASE REPORT

A 69-year-old male with paroxysmal atrial fibrillation (CHA_2DS_2-VASc score 4), hypertension, insulin-dependent type 2 diabetes mellitus, dyslipidaemia, and hypothyroidism underwent LAAC. Anticoagulation was contraindicated due to spontaneous intracerebral haemorrhage in 2015.

In October 2023, a Watchman Flex 31 mm device (Boston Scientific, Marlborough, MA, USA) was implanted under general anesthesia with transoesophageal echocardiographic (TEE) guidance. Two redeployments were required due to initial shallow positioning. Dual antiplatelet therapy (aspirin 100 mg, clopidogrel 75 mg) was initiated following anticoagulation discontinuation at 6 weeks (patient timeline detailed in Supplementary Table 1).

Six-month TEE surveillance revealed a posterosuperior PDL (3 mm jet, 12 × 4 mm cavity) with color Doppler demonstrating bidirectional flow and systolic predominance, communicating with residual appendage tissue (Figure 1A and B). Cardiac computed tomography confirmed complex cavity morphology (12 × 6 × 9 mm) with asymmetric flow characteristics: wide communication between LAA and peridevice cavity (4.5-7.8 mm) (multimodality imaging analysis in Supplementary Table 2).

Preprocedural planning utilized the HJPHub MR (distinct from magnetic resonance imaging) system on Microsoft HoloLens 2 (Microsoft Corporation, Redmond, WA, USA)[6] (specifications in Supplementary Table 3). Three-dimensional holographic visualization enabled detailed assessment of complex left atrial anatomy (Supplementary Video 1), identification of optimal posteroinferior transseptal puncture site, and predicted the need for telescoping catheter technique (Figure 2).

In October 2024, PDL closure was performed under general anesthesia with TEE guidance. Following transseptal puncture, an Agilis 8.5 F steerable sheath and Vista IMA 6 F guiding catheter enabled cannulation of the posterosuperior

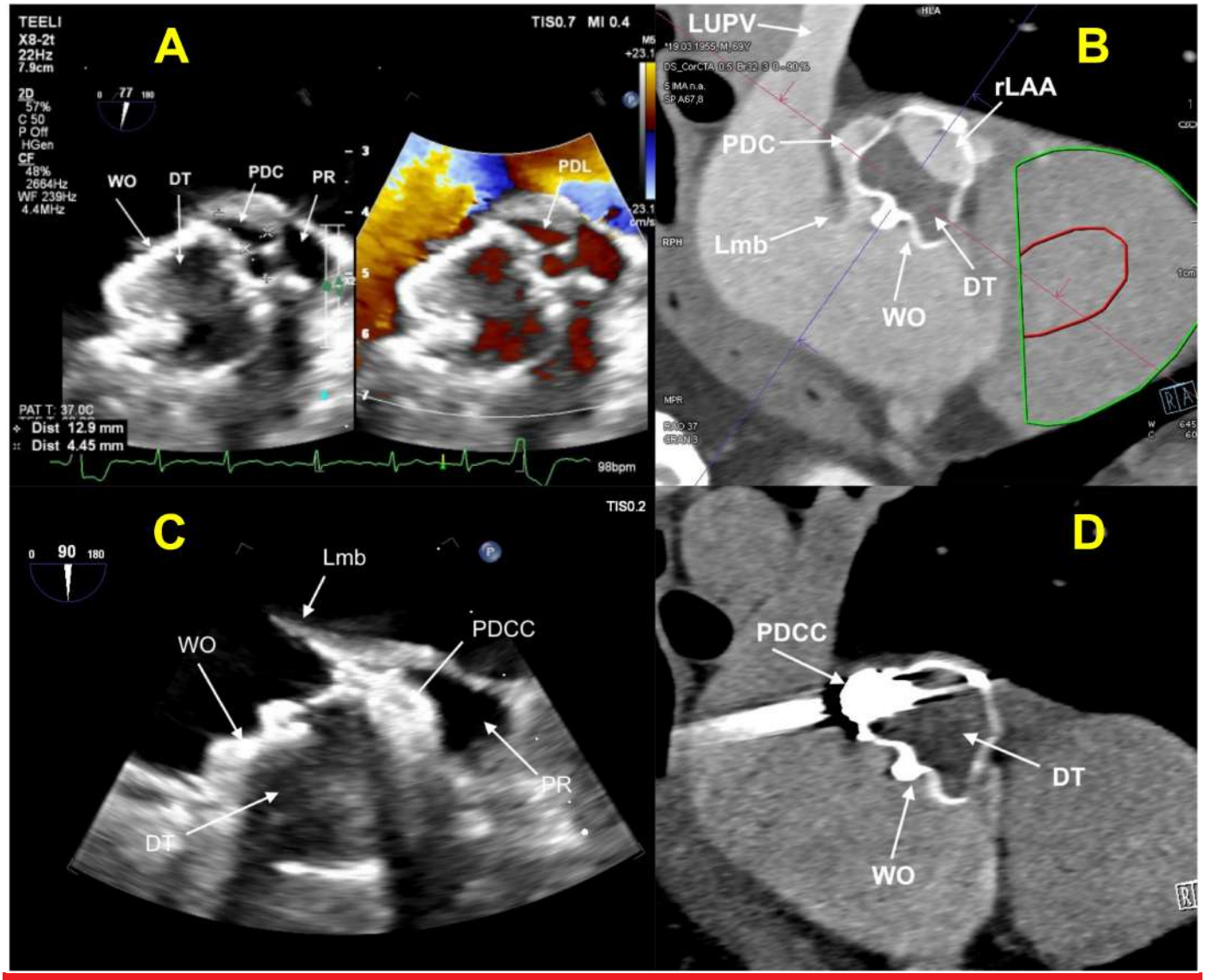

Figure 1. Posterosuperior peridevice leak and device-related thrombus: multimodality. Imaging before and after coil embolization. (A) Two-dimensional TEE with color Doppler demonstrates the WO with an echogenic mass representing DT located under the skirt of the occluder. A posterosuperior PDC (12.9 × 4.45 mm) is visualized adjacent to the occluder. Color Doppler reveals the PDL with flow communication between the rLAA and left atrium. The PR is identified as a distinct structure separate from the pathologic cavity. (B) Contrast-enhanced cardiac CT with multiplanar reconstruction demonstrates the complex anatomy of the PDC and its relationship to the LUPV, Lmb, and residual LAA. Device-related thrombus appears as a hypoattenuating mass within the WO. (C) Follow-up TEE 3 months after coil embolization demonstrates the PDCC with densely echogenic coil material. The Lmb and PR remain visualized as distinct anatomic landmarks. Complete PDL closure is achieved, with planned thrombosis evident. The DT now fills the entire residual LAA cavity—a desirable outcome confirming flow elimination. (D) Follow-up contrast-enhanced CT confirms complete occlusion with the PDCC showing no contrast opacification despite left atrial enhancement, indicating elimination of the peridevice leak. The WO and persistent DT are visualized. CT, computed tomography; DT, device thrombus; LAA, left atrial appendage; Lmb, limbus; LUPV, left upper pulmonary vein; PDC, peridevice cavity; PDCC, peridevice cavity closure (coil-filled); PDL, peridevice leak; PR, pericardial recess; rLAA, residual left atrial appendage; TEE, transesophageal echocardiography; WO, Watchman occluder.

cavity. Wire access was achieved with a Sion Black guidewire through a Lantern 130 cm microcatheter. Closure was accomplished using 1 RUBY POD 12 coil (Penumbra, Alameda, CA, USA) deployed within the cavity, followed by a 15 cm Packing Coil at the neck, achieving complete occlusion confirmed by TEE and angiography (procedural details in Supplementary Table 4 and Supplementary Video 2).

Three-month follow-up imaging demonstrated complete resolution with no contrast penetration behind the occluder on cardiac computed tomography (Supplementary Video 3) and no residual leak on TEE (Figure 1C and D). Given an absolute oral anticoagulation contraindication, the patient continues on aspirin monotherapy with planned annual imaging surveillance, without adverse events.

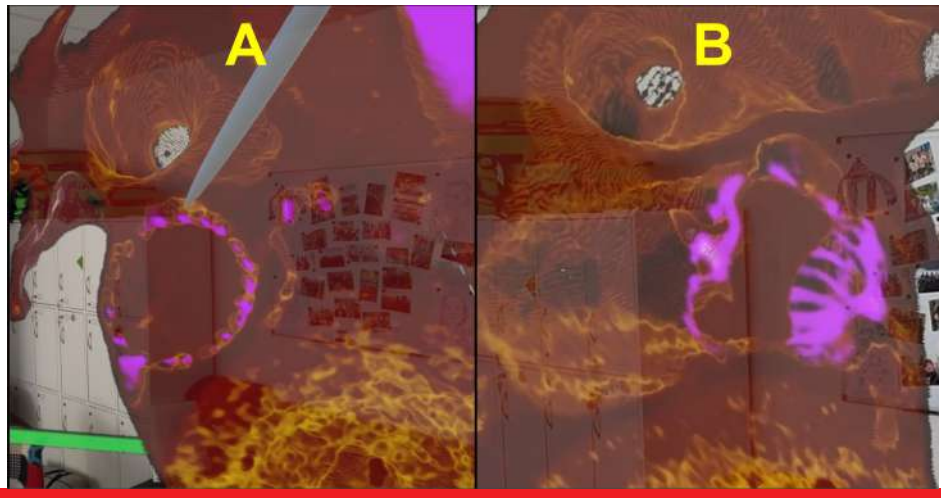

Figure 2. Mixed reality preprocedural planning for peridevice leak closure. First-person operator views through Microsoft HoloLens 2 displaying the HJPHub mixed reality system used for preprocedural planning. The three-dimensional holographic reconstruction visualizes the left atrial anatomy (orange volume rendering) and the Watchman Flex occluder with posterosuperior paradevice leak cavity (purple highlighted structures) superimposed onto the real-world clinical environment. (A) Anterior viewing angle demonstrates the planned transseptal catheter trajectory (gray line) targeting the posterosuperior peridevice cavity. The mitral valve annulus orientation and spatial relationship between the left atrial appendage occluder (purple mesh structure) and the peridevice cavity are clearly delineated, enabling identification of the optimal posteroinferior transseptal puncture site. (B) Rotated viewing angle provides enhanced visualization of the Watchman occluder (purple striped structure) and its relationship to the residual appendage tissue. This perspective demonstrates the complex three-dimensional geometry that predicted the need for telescoping catheter technique to navigate the challenging posterosuperior approach. Interactive manipulation between viewing angles during preprocedural planning facilitated comprehensive spatial understanding unattainable with conventional two-dimensional imaging. Technical note: The real-world clinical environment visible in the background (office furniture, wall-mounted displays) demonstrates the mixed reality augmented visualization capabilities of the HoloLens 2 system, where holographic reconstructions are seamlessly overlaid onto the operator's natural field of view.

DISCUSSION

This case demonstrates several clinically important findings. First, a significant PDL with bidirectional flow was identified communicating with residual appendage tissue containing organized intracavitary thrombus, distinct from device-related thrombus on the occluder surface. The PDL constituted the primary indication for intervention, as persistent flow around the occluder represents ongoing stroke risk. This reinforces the importance of systematic PDL surveillance regardless of antithrombotic strategy, given the established association between PDL and thromboembolism (pooled odds ratio 2.04).[7]

Second, this represents the first application of mixed reality-guided planning for PDL closure. The HJPHub system enabled three-dimensional assessment of complex anatomy, facilitating optimal transseptal puncture site selection. Critically, despite real-time TEE navigation, the cavity entry could not be cannulated—even with live 3D TEE. Only by returning to MR planning during the intervention—to regain three-dimensional spatial orientation after TEE navigation had been exhausted—was successful navigation achieved; real-time catheter guidance was thereafter performed under fluoroscopy. The exceptional procedural complexity—fluoroscopy time 177.5 minutes (~11× published series), air kerma 4415.5 mGy (~10× typical LAAC values), and 6 catheter exchanges—underscores the technical challenge where TEE visualization may be suboptimal. Mixed reality planning proved indispensable, enabling success unattainable with conventional imaging alone. Notably, this indispensable contribution was qualitative in nature—three-dimensional spatial re-orientation rather than quantitative measurements or catheter simulation, neither of which is yet available in the current system; these capabilities represent clear directions for future development.

Third, coil embolization was selected for this irregular, noncircular cavity morphology. Detachable coils represent the most commonly utilized PDL closure device (42.3%) in multicenter registries, offering conformability advantages over plugs or occluders for complex geometries.[8,9] Residual leak represents a recognized complication across transcatheter structural interventions.[10]

Limitations include single-case observation, precluding generalizability, and the need for longer-term follow-up to assess durability. Randomized data evaluating MR-guided planning for structural interventions remain unavailable.

CONCLUSION

Peridevice leak closure procedures are inherently complex, requiring extensive operator expertise and specialized equipment. Mixed reality guidance using the validated HJPHub system facilitated successful coil embolization of a challenging posterosuperior PDL following LAAC.

AI Disclosure: AI-assisted technologies were utilized for language refinement. All clinical data, scientific interpretation, and conclusions represent the independent work of the authors.

Informed Consent: Written informed consent was obtained from the patient.

Declaration of Interests: The authors have no conflicts of interest to declare.

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Supplementary Video 1. Mixed Reality-Guided Preprocedural Planning for Complex Peridevice Leak Closure. This recording from the operator's perspective (via Microsoft HoloLens 2) demonstrates the application of the HJPHub system for a 69-year-old patient with a posterosuperior peridevice leak (PDL) following Watchman Flex implantation. The 3D holographic reconstruction visualizes the left atrial anatomy (orange wireframe) and the complex, non-circular

Supplementary Table 1. Complete Patient Timeline with Antithrombotic Management

Date	Clinical Event	Antithrombotic Regimen
2007	Radiofrequency catheter ablation for atrial flutter	—
2015	Spontaneous intracerebral hemorrhage (temporo-parietal); managed conservatively; established contraindication to long-term OAC	—
August 2023	First documented paroxysmal AF; CHA_2DS_2-VASc score 4 (age 65-74: 1; HTN: 1; DM: 1; prior stroke/TIA: 2)	—
Aug 28-29, 2023	Pre-LAAO workup: coronary angiography (non-significant CAD); TEE (no LA thrombus, MR 2-3/4, LAA 21-26mm)	—
Oct 18, 2023	LAAO: Watchman Flex 31mm implanted; 2 redeployments due to shallow positioning	Apixaban 2.5mg BID + Clopidogrel 75mg
Nov 14, 2023	1-month TEE: Excellent occluder effect; no PDL; device well-seated	Apixaban 2.5mg BID + Clopidogrel 75mg
Nov 30, 2023	Protocol transition: apixaban discontinued	Aspirin 100mg + Clopidogrel 75mg (DAPT)
Apr 16, 2024	6-month TEE: Posterosuperior PDL (~3mm jet; 12×4mm cavity)	Aspirin 100mg + Clopidogrel 75mg (DAPT)
May-Sep 2024	Cardiac CT for 3D characterization; MR preprocedural planning with HJPHub	DAPT continued
Oct 16, 2024	PDL closure: RUBY POD 12 coils + 15cm Packing Coil; complete occlusion	DAPT + enhanced gastroprotection
Dec 2, 2024	6-week TEE: Coils stable; trace residual leak laterally; no thrombus	DAPT continued
Jan 13, 2025	3-month CT + TEE: Complete occlusion; no contrast behind occluder; no residual leak	Aspirin 100mg monotherapy

This timeline integrates clinical events with antithrombotic therapy, highlighting the critical finding that device-related thrombus developed while on dual antiplatelet therapy (DAPT).
AF, atrial fibrillation; BID, twice daily; CAD, coronary artery disease; DAPT, dual antiplatelet therapy; DM, diabetes mellitus; DRT, device-related thrombus; HTN, hypertension; LA, left atrium; LAA, left atrial appendage; LAAO, left atrial appendage occlusion; MR, mixed reality; OAC, oral anticoagulation; PDL, paradevice leak; TEE, transesophageal echocardiography; TIA, transient ischemic attack.

Supplementary Table 2. Multimodality Imaging Analysis

A. Preprocedural Dimensional Measurements: TEE vs Cardiac CT

Parameter	TEE Measurement	Cardiac CT Measurement
PDL orifice	~3mm jet width; 2.99mm drainage	4.5-7.8mm (wide communication)
Peri-device cavity	12×4mm (2D measurement)	12×6×9mm (volumetric)
LAA dimensions (pre-LAAO)	21-26mm	22×27mm
Flow pattern	Posterosuperior jet; systolic predominant	Asymmetric inflow-outflow identified

B. Follow-up Imaging Findings

Timepoint	Modality	Key Findings	Clinical Implication
Immediate post-procedure	TEE + Angiography	Complete PDL occlusion; no residual flow; coils well-positioned	Technical success confirmed
6 weeks (Dec 2, 2024)	TEE	Good procedural effect; coils stable; trace residual leak laterally; no thrombus	Continue DAPT; plan 3-month follow-up
3 months (Jan 13, 2025)	Cardiac CT	Occluder internally thrombosed; NO contrast penetration behind occluder; coils stable	Complete seal achieved; durable occlusion
3 months (Jan 13, 2025)	TEE	Complete occlusion; no residual leak; no thrombus visible	Transition to aspirin monotherapy

Comparative imaging findings demonstrating complementary value of TEE and CT, with follow-up results.
The 3-month CT finding of "no contrast penetration behind occluder" represents the key endpoint confirming complete LAA exclusion from systemic circulation, indicating that the combined Watchman device plus coil embolization achieved the intended goal of LAA isolation.
CT, computed tomography; DAPT, dual antiplatelet therapy; LAA, left atrial appendage; LAAO, left atrial appendage occlusion; PDC, peri-device cavity; PDL,paradevice leak; rLAA, residual left atrial appendage; TEE, transesophageal echocardiography.

peri-device cavity (solid purple structure). This immersive view allowed for the precise identification of the cavity's spatial orientation, facilitating the selection of an optimal posteroinferior transseptal puncture site and confirming the necessity for a telescoping catheter technique to navigate the challenging anatomy.

Supplementary Video 2. Fluoroscopic Deployment and Angiographic Confirmation. This intraprocedural fluoroscopic recording captures the selective cannulation and deployment of a detachable coil (RUBY POD 12) into the posterosuperior peri-device cavity using a telescoping catheter technique (Agilis sheath and Vista IMA guide). The final angiographic sequence demonstrates a dense, stable coil mass within the defect with no residual contrast extravasation, confirming the immediate and complete occlusion of the paradevice leak.

Supplementary Video 3. Holographic Verification of Complete Peridevice Leak Closure. This three-dimensional reconstruction, derived from three-month follow-up cardiac computed tomography, visualizes the spatial relationship between the implanted Watchman Flex device (purple mesh) and the coil-embolized residual shunt space (green volume). The transition to solid blood volume rendering (red) demonstrates a smooth endocardial contour with the absence of contrast penetration into the green occluded cavity, confirming the complete resolution of the paradevice leak.

Supplementary Table 3. HJPHub Mixed Reality System Specifications

Specification	Values
Hardware platform	Microsoft HoloLens 2 (Microsoft Corporation, Redmond, WA, USA)
Software platform	Unity-based HJPHub application
Frame rate	59.6 ± 0.7 fps
Local latency	14.3 ± 0.5 ms
Registration accuracy (TRE)	1.61 ± 0.33 mm
Multi-user latency	26.9 ms (P95: 52.4 ms)
System Usability Scale (SUS) score	77.5 ± 3.8 ("good usability")
Calibration time	38 seconds
Validated applications	LAAO, structural heart interventions

The HJPHub system utilized for preprocedural planning has been previously validated for structural cardiac procedures.
Reference: Hecko J, Precek D, Januska J, et al. Design and validation of a mixed reality workflow for structural cardiac procedures in interventional cardiology. Front Virtual Real. 2025;6:1690439.
Abbreviations: fps = frames per second; LAAO = left atrial appendage occlusion; ms = milliseconds; P95 = 95th percentile; SUS = System Usability Scale; TRE = target registration error.

Supplementary Table 4. Procedural Details

A. Procedural Parameters (October 16, 2024)

Parameters	Values
Total procedure duration	5 hours 10 minutes (13:30-18:40)
Fluoroscopy time	177.5 minutes
Contrast volume	260 mL
Cumulative air kerma	4415.5 mGy
Dose area product	352 Gy·cm^2
Vascular access	Right femoral vein
Anesthesia	General anesthesia with TEE guidance
Transseptal puncture location	Posteroinferior (MR-guided planning)
Number of catheter exchanges	6 (see Part B below)
Complications	None

B. Catheter Exchange Sequence

Step	Catheter/Equipment	Outcome	Rationale for Change
1	Mullins 8.5F → Agilis 8.5F steerable sheath	Base sheath established	Required steerability for posterosuperior approach
2	Flexor 5F 110cm	Insufficient support	PDL orifice reached but inadequate backup for wire advancement
3	MPA 4F 125cm	Better angle but lacked stability	Improved coaxial alignment but catheter tip deflection persisted
4	JR4 125cm diagnostic catheter	Initial leak probing; inadequate backup	Successfully entered PDL cavity but insufficient support for coil delivery
5	MPA GC 7.5F Sheathless (Asahi)	System tension; position loss	Excessive friction between guide catheter and steerable sheath; abandoned
6*	Vista IMA 6F guide catheter	FINAL - Adequate support achieved	Optimal deliverability, backup support, and lumen for microcatheter

C. Closure Device Specifications

Device	Specifications	Deployment Location
RUBY POD Coil (×2)	12mm diameter; detachable; platinum/tungsten (Penumbra, Alameda, CA, USA)	Peri-device cavity (bulk fill)
Packing Coil	15cm length; platinum/tungsten (Penumbra, Alameda, CA, USA)	PDL neck/drainage channel

Table A: Comprehensive procedural parameters including catheter exchange sequence and closure device specifications.
Table B: The posterosuperior PDL location necessitated multiple catheter exchanges. MR planning predicted the need for telescoping technique. Device Selection Rationale: Detachable coils were selected based on: (1) irregular geometry of peri-device cavity (12×6×9mm), not amenable to plug-type devices; (2) narrow drainage channel (1.8-3.2mm), suitable for coil packing; (3) published evidence supporting coil use in 42.3% of PDL closures in multicenter registries; (4) 100% acute procedural success and 85.9% one-year success rates in the LAA Leak Study.
*Final configuration: Agilis 8.5F steerable sheath + Vista IMA 6F guide catheter + Sion Black guidewire + Lantern 130cm 45° microcatheter

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Stiffness or Reflection? A Critical Appraisal of Energy Drink–Induced Vascular Changes

LETTER TO THE EDITOR

To the Editor,

I read with great interest the article by Yaşar et al,[1] entitled "Evaluating the Acute Effects of Energy Drink Consumption on Arterial Stiffness in Healthy Young Adults," recently published in the *Anatolian Journal of Cardiology*.[1] While the study addresses a clinically relevant topic, I would like to highlight some methodological and interpretive concerns.

First, the distinction between the Augmentation Index (AIx) and arterial stiffness is critical. Augmentation Index is a composite parameter influenced by peripheral vascular resistance, wave reflection, and heart rate, rather than a direct measure of wall stiffness.[2] The fact that the gold-standard measure, pulse wave velocity (PWV), remained unchanged throughout the study strongly suggests that the observed changes in AIx reflect transient hemodynamic modulations in wave reflection and vascular tone rather than an actual increase in arterial stiffness.[3]

Second, the absence of a control group is a significant limitation. Without a placebo or a water-intake arm, it is difficult to determine whether the observed effects were specifically caused by the energy drink ingredients or were merely a physiological response to fluid ingestion, caffeine's known acute effects, or even a "white-coat" effect related to the measurement environment.

Third, the 2-hour post-consumption data present a physiological contradiction. The authors reported a significant decrease in total vascular resistance and diastolic blood pressure at the 2-hour mark. This suggests a transient adrenergic surge followed by a compensatory vasodilator rebound, which is inconsistent with a sustained increase in arterial stiffness.[4,5]

In conclusion, given the stable PWV and the complex nature of AIx, I believe that characterizing the findings as an increase in "arterial stiffness" may be misleading. I suggest that the results would be more accurately described as acute modulations of wave reflection dynamics. Therefore, I believe that the results should be reinterpreted as acute hemodynamic modulations of wave reflection dynamics.

Declaration of Interests: The author has no conflicts of interest to declare.

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Reply to the Letter to the Editor: "Stiffness or Reflection? A Critical Appraisal of Energy Drink Induced Vascular Changes"

LETTER TO THE EDITOR
REPLY

To the Editor,

We sincerely thank the reader[1] for their careful evaluation of our article[2] and for their constructive comments.

We agree that the distinction between augmentation index (AIx) and direct measures of arterial stiffness, particularly pulse wave velocity (PWV), is important. AIx is influenced by wave reflection, vascular tone, and heart rate, and therefore, should not be interpreted as a pure measure of intrinsic arterial wall stiffness.[3,4] In contrast, PWV is considered a more direct marker of arterial stiffness.[5] In our study, we did not intend to suggest that a transient increase in AIx in the absence of a change in PWV proves fixed or structural arterial stiffening. Rather, our findings suggest an acute alteration in vascular function and wave-reflection-related indices following energy drink consumption.

As correctly noted, PWV did not change significantly, whereas AIx and AIx@75 increased at the 30th minute. We agree that these findings are more consistent with a short-term hemodynamic or functional vascular response than with persistent structural changes in the arterial wall. We appreciate the opportunity to clarify this point.

We also acknowledge the absence of a placebo or control beverage arm as an important limitation. Our study was designed as a single-arm, repeated-measures pilot study to assess the acute vascular response to energy drink consumption under standardized conditions in healthy young adults. Therefore, our findings should be interpreted with caution, and future placebo-controlled studies are needed to better distinguish the specific effects of energy drinks from non-specific time-dependent or volume-related influences.[6]

Regarding the decrease in total vascular resistance and diastolic blood pressure at the second hour, we believe this does not necessarily contradict the earlier increase in AIx. Rather, these findings may reflect the dynamic and time-dependent nature of the vascular and autonomic response after energy drink intake. Previous literature suggests that energy drinks and their constituents may produce complex and temporally variable cardiovascular responses rather than a single uniform physiological effect.[7] The early increase in wave-reflection-related parameters and the later peripheral hemodynamic changes may represent different phases of the same transient physiological response.

In summary, we agree that our findings should not be interpreted as evidence of acute fixed arterial stiffening in the structural sense. However, the observed transient increase in AIx and AIx@75 still supports the presence of an acute vascular functional response following energy drink consumption.[3-5] We believe these findings are physiologically meaningful and merit further investigation in larger, controlled studies.

We thank the authors once again for their valuable comments and for the opportunity to clarify the interpretation of our results.

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Methodological Considerations Regarding the Combined Use of Erectile Dysfunction and Frontal QRS-T Angle for Predicting Coronary Artery Disease Severity

LETTER TO THE EDITOR

To the Editor,

I read with great interest the article by Özmen Yıldız and Özbilen,[1] which proposes combining erectile dysfunction (ED) and frontal QRS-T angle (fQRSTa) as a composite predictor of coronary artery disease severity. The hypothesis is thought-provoking; however, several methodological concerns may affect the interpretation of the findings.

First, the fQRSTa cutoff of 52.5° was derived via receiver operating characteristic analysis from the same cohort subsequently used for group stratification and regression modeling. This circular approach can inflate the threshold's apparent discriminative performance and bias group comparisons. Without validation in an independent cohort or through internal resampling methods such as bootstrapping, the generalizability of this cutoff remains uncertain.

Second, the regression models have notable gaps in covariate adjustment. Fasting glucose differed significantly between groups ($P < .001$, Table 3) yet was excluded from the models; only hemoglobin A1c (HbA1c) was entered. Since HbA1c may underestimate glycemic burden when hemoglobin is low—as observed in group 4 (median 13.6 g/dL)[2]—this omission is clinically relevant. Moreover, the authors cite "unmeasured factors such as… electrolyte disturbances" among study limitations, yet serum calcium was measured, showed significant between-group differences ($P = .009$), and was nonetheless excluded from the models. This internal contradiction undermines the claim of "full adjustment." Additionally, variance inflation factors were not reported, leaving multicollinearity—particularly between diabetes status and HbA1c (diabetes $\beta = 0.869$, $P = .881$)—unquantified.

Third, diabetic autonomic neuropathy (DAN) represents a critical unmeasured confounder. Both ED and ventricular repolarization heterogeneity reflected by fQRSTa are recognized manifestations of autonomic dysfunction.[3] With approximately 34% of the cohort having diabetes mellitus and group 4 showing the highest prevalence (40.7%), DAN could account for the observed ED–fQRSTa association without requiring a synergistic mechanism. Without autonomic function testing or subgroup analysis stratifying by diabetes status, this alternative explanation cannot be excluded.

Fourth, dichotomizing IIEF-5 scores and fQRSTa—2 continuous variables—into binary categories results in information loss and may obscure dose–response relationships.[4] The wide confidence interval of the interaction term for the Gensini score ($\beta = 17.233$, 95% CI: 1.906-32.560) illustrates the imprecision of this estimate and raises concerns about whether sufficient statistical power existed to detect interaction effects in 236 patients.

Finally, the term "synergistic" warrants caution. A significant interaction term indicates departure from additivity on a given scale but does not establish

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biological synergy.[5] Given that ED and fQRSTa share pathophysiological underpinnings—particularly through the autonomic pathway discussed above—the observed interaction may reflect confounding by a common upstream process rather than a true multiplicative biological effect. Future multicenter studies incorporating external threshold validation, comprehensive autonomic assessments, and continuous variable modeling would substantially strengthen the evidence for this promising dual-marker approach.

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Reply to the Letter to the Editor: "Methodological Considerations Regarding the Combined Use of Erectile Dysfunction and Frontal QRS-T Angle for Predicting Coronary Artery Disease Severity"

LETTER TO THE EDITOR
REPLY

To the Editor,

We thank the author[1] for the interest shown in our study entitled "The synergistic relationship between erectile dysfunction and frontal QRS-T angle in predicting coronary artery disease severity."[2] We appreciate the thoughtful methodological comments and the opportunity to further clarify several aspects of our analysis.

First, the author raised concerns regarding the derivation of the frontal QRS-T angle (fQRSTa) cut off value using receiver operating characteristic analysis within the same study cohort. We agree that external validation may strengthen the generalizability of threshold values. However, there is currently no universally accepted cut off value for the fQRSTa, and previous studies have highlighted the challenges of applying fixed thresholds across different populations.[3] Our aim was not to define a universal diagnostic threshold but to identify a population-specific value suitable for exploratory risk stratification within the study cohort. To address the concern that our findings might be an artifact of this specific cut off, we performed a sensitivity analysis incorporating fQRSTa as a continuous variable in our regression models. The interaction term between erectile dysfunction (ED) and fQRSTa remained statistically significant for both the Gensini score ($\beta = 0.192$, $P = .023$) and the SYNTAX score ($\beta = 0.069$, $P = .009$). This demonstrates that the predictive value of ED and fQRSTa is robust and independent of the variable operationalization method.

Second, the author commented on covariate selection, specifically the exclusion of fasting glucose and serum calcium. In our dataset, fasting glucose and HbA1c were strongly correlated ($r = 0.780$, $P < .001$). Including both would significantly increase the risk of multicollinearity. HbA1c was selected as it reflects long-term glycemic burden and is a more stable predictor in cardiovascular risk modeling.[4] When fasting glucose was added in a sensitivity analysis, it was not an independent predictor (Gensini: $P = .454$; SYNTAX: $P = .184$), and the variance inflation factor (VIF) approached the collinearity threshold (VIF = 4.458). Similarly, adding serum calcium did not yield significance (Gensini: $P = .242$; SYNTAX: $P = .114$). Multicollinearity was rigorously assessed, and all VIF values in our final models remained below 5. Crucially, even when all these additional covariates were included, the interaction term maintained its significance (Gensini: $\beta = 18.319$, $P = .020$; SYNTAX: $\beta = 6.372$, $P = .008$), confirming the stability of our primary findings.

Third, the potential role of diabetic autonomic neuropathy (DAN) as an unmeasured confounder was suggested. While this is an insightful hypothesis, as autonomic dysfunction is indeed linked to both ED and ventricular repolarization abnormalities,[5] our data do not support it as the primary explanation for our findings. Diabetes mellitus was included as a covariate in our multivariable models, partially controlling for its effects. More importantly, we conducted a subgroup analysis stratified by diabetes status. The correlation between ED and

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fQRSTa remained significant in both non-diabetic ($r=0.309$, $P<.001$, n=156) and diabetic patients ($r=0.326$, $P=0.003$, n=80), with remarkably similar coefficients. The relationships between ED and angiographic scores also remained consistent across both groups. Furthermore, the association between ED and coronary artery disease is well-established across diverse populations, independent of diabetes.[6] Given that the prevalence of autonomic neuropathy is exceedingly low in non-diabetic patients, these combined results strongly suggest that the observed associations are robust and independent of DAN.

Fourth, the author noted that dichotomizing continuous variables (IIEF-5 and fQRSTa) may result in information loss and questioned the statistical power given the wide CIs. While categorization was employed to enhance clinical interpretability and align with established thresholds (IIEF-5 ≤21),[7] we acknowledge this methodological concern. To address it, we re-tested the interaction term (IIEF-5 × fQRSTa) using their continuous forms. After adjusting for age, body mass index, diabetes, hypertension, hyperlipidemia, smoking, HbA1c, eGFR, and hemoglobin, the interaction remained significant for both the Gensini ($\beta=-0.015$, $P=.008$) and SYNTAX scores ($\beta=-0.005$, $P=.003$). It should be noted that the negative sign of the interaction coefficient in the continuous model reflects the mathematical direction of the product term, as higher IIEF-5 scores indicate less severe ED; this is fully consistent with the positive direction observed in the dichotomized model, where the presence of ED (lower IIEF-5) combined with a widened fQRSTa was associated with greater coronary disease severity. Furthermore, to address concerns regarding statistical power and precision, we performed a bootstrap analysis with 5000 resamples. The BCa 95% CIs did not cross zero for either the Gensini (−0.025 to −0.002) or SYNTAX models (−0.009 to −0.002), thereby confirming the statistical robustness of the interaction effect.

Finally, regarding the term "synergistic," we agree that a statistically significant interaction does not necessarily establish a definitive biological synergy. We used this term to describe the observed statistical and predictive interaction, where the coexistence of both factors is associated with greater disease severity than either alone. To address the author's argument that this interaction merely reflects a common upstream pathophysiological process (such as DAN), our subgroup stratified by diabetes status provides crucial counter-evidence. We found that the ED−fQRSTa association remained consistently significant in both non-diabetic ($r=0.309$, $P<.001$) and diabetic patients ($r=0.326$, $P=.003$). If the interaction were solely driven by a diabetes-related upstream process, we would not observe such robust and parallel relationships in the non-diabetic cohort. Therefore, we believe that our findings provide valuable additive predictive value, and we welcome future mechanistic studies to further elucidate these links.

We appreciate the constructive comments and believe that continued investigation in larger, multicenter cohorts will further clarify the clinical utility of combining ED and electrocardiographic markers in cardiovascular risk assessment.

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