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Novel biomarkers and risk factors associated with cardiometabolic dysfunction in heart failure

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Novel biomarkers and risk factors associated with cardiometabolic dysfunction in heart failure

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Knowledge Mapping of the Links Between the Gut Microbiota and Heart Failure: A Scientometric Investigation (2006–2021)

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Background: There is considerable research value and extensive application perspectives to explore the link between gut microbiota and heart failure. The purpose of this study is to provide an overview of overall characteristics, evolutionary pathways, frontier research hotspots, and future trends in this field.

Methods: Research datasets were acquired from the Web of Science Core Collection (WoSCC) between January 1, 2006 and December 31, 2021. Three different analysis tools including one online platform, VOS viewer V1.6.17.0, and CiteSpace V5.8.R2 software were used in order to conduct collaboration network analysis, co-cited analysis, co-occurring analysis, and citation burst detection.

Results: A total of 873 publications in the WoSCC database met the requirement. The overall characteristics analysis showed that a steady growth trend in the number of publications and citations, with the predominant literature type being articles and the most frequent subject category being cardiac cardiovascular systems. The United States was the most prolific country and the center of national collaboration. Cleveland Clinic and Nathalie M. Delzenne provided the leading influence with publications, the cooperation between the institutes and authors were relatively weak. Moreover, gut microbiota, heart failure, risk factor, obesity, and inflammation were the keywords that appeared more frequently in the clustering analysis of reference co-citation and keyword co-occurrence. Burst detection analysis of top keywords showed that trimethylamine *N*-oxide (TMAO), bile acid, blood pressure, hypertension, and fermentation were the new research foci on the association between gut microbiota and heart failure. Strategies to improve gut microbiota hold promise as a new approach to treat heart failure.

Conclusion: The comprehensive bibliometric study indicates that the structured information may be helpful in understanding research trends in the link between gut microbiota and heart failure, and locating research hotspots and gaps in this domain, especially further advances in this field will lead to significant breakthroughs in the development of novel therapeutic tools for metabolic modulation of heart failure.

Keywords: heart failure, gut microbiota, CiteSpace, VOS viewer, scientometric, frontier research hotspots

INTRODUCTION

Heart failure, the terminal stage of many cardiovascular diseases, affects approximately 40 million people in the world (1). It is well known that people can suffer from heart failure for a variety of reasons, but the most common risk factors for heart failure are hypertension, coronary artery disease, obesity, diabetes, smoking, and genetics (1, 2). The "gut hypothesis" in heart failure has been prevalent for many years, arguing that the heart failure is exacerbated by the translocation of gut microbiota and elevated levels of circulating endotoxins caused by intestinal ischemia and congestion, suggesting an inevitable link between the gut microbiota and its metabolites and the pathogenesis of heart failure (3, 4). Of interest is the fact that research in this field is still in a phase of rapid exploratory development.

Despite the fact that the bidirectional communication pathways between the gut microbiota and the cardiovascular system are not completely understood, there appears to be four main pathways (**Figure 1**). Impaired intestinal barrier function and inflammation levels, including high serum endotoxin, lipopolysaccharide, and cytokine levels, have been reported in patients with heart failure (5, 6). Gut microbiota and its metabolites, such as trimethylamine (TMA)/trimethylamine *N*-oxide (TMAO), short-chain fatty acids (SCFAs), and bile acids, also influence the host inflammation and cardiac biofunctions. In addition, studies have shown that environmental factors – diet, medications, and the surrounding milieu – play substantial roles in shaping our gut microbiome. Therefore, a deeper understanding of the gut microbiota and its metabolites would help to tease out the beneficial effects on heart failure in this complex multi-level network.

Knowledge mapping is the scientometric analysis method that provides a visual representation of scientific knowledge, it can be used to explore, analyze, and summarize the process and structure of knowledge development in both spatial and temporal dimensions. This method provides a diverse perspective that is not available in traditional literature reviews and systematic reviews, and has been applied in many fields domestically and internationally (7). Previous bibliometric studies have focused on the gut microbiota in obesity (8), or the microbiome-gut-brain axis (9), or gut microbiota in depression (10), but have not addressed the gut microbiota in heart failure. The purpose of this study is to analyze the overall characteristics, evolutionary pathways, frontier research hotspots, and future research development trends of the link between gut microbiota and heart failure using bibliometric methods, aiming to promote the diversification, deepening, and internationalization of research in this field.

MATERIALS AND METHODS

Data Source

The Web of Science Core Collection (WoSCC) is the most authoritative citation-based database with powerful indexing functions, and is widely used in scientometric analysis (11, 12). Therefore, this study selected to retrieve publications related to gut microbiota and heart failure in the WoSCC of Science Citation Index Expanded (SCIE). Our search terms combined medical subject headings words and keywords such as "heart failure" and "gut microbiota." The full search terms are available in the study protocol (**Supplementary Table 1**). To avoid bias in data updates, the above operations were all executed within 1 day. A total of 986 results were found from January 1, 2006 to December 31, 2021 (retrieved on January 14, 2022). The type of literature studies was restricted to article or review, and literature language was set to English, with the specific inclusion and exclusion results shown in **Figure 2**. The Microsoft Excel 2019 and SPSS 23.0 were used to classify, descriptively analyze, and statistically evaluate the data extracted from the literatures. Moreover, the eligible literature studies were stored in download_txt format and exported for further use.

Data Analysis and Visualization

Conceptual design of the study is presented in **Supplementary Figure 1**. All valid documents retrieved from WoSCC were converted to CiteSpace version 5.8.R2 (developed by Professor Chen Chaomei from Drexel University), VOS viewer version 1.6.17.0 (developed by Professor Eck and Waltman from Leiden University) and online platform[1] for visual analysis (11, 13, 14). The parameters of CiteSpace were as follows: time span (2006–2021), years per slice (2), selection criteria (Top 50). In addition, VOS viewer software was used to conduct the network analysis of the frequent keywords. The parameters in the network analysis of the frequent keywords were as follows: the minimum number of occurrences of a keyword was 5 and resolution was 0.7. Descriptive indicators (number of publications and citations per year, literature types, subject categories, authors, and journals), relational indicators (collaborations of countries/regions, institutions, and authors), and qualitative indicators (bursts, betweenness centralities, and citation scores) were used in the assessment of bibliographic catalogs. We used a variety of scientometric methods, such as typical cluster analysis, co-citation analysis, and co-occurrence analysis, in order to identify intellectual structure, international

[1] http://bibliometric.com/

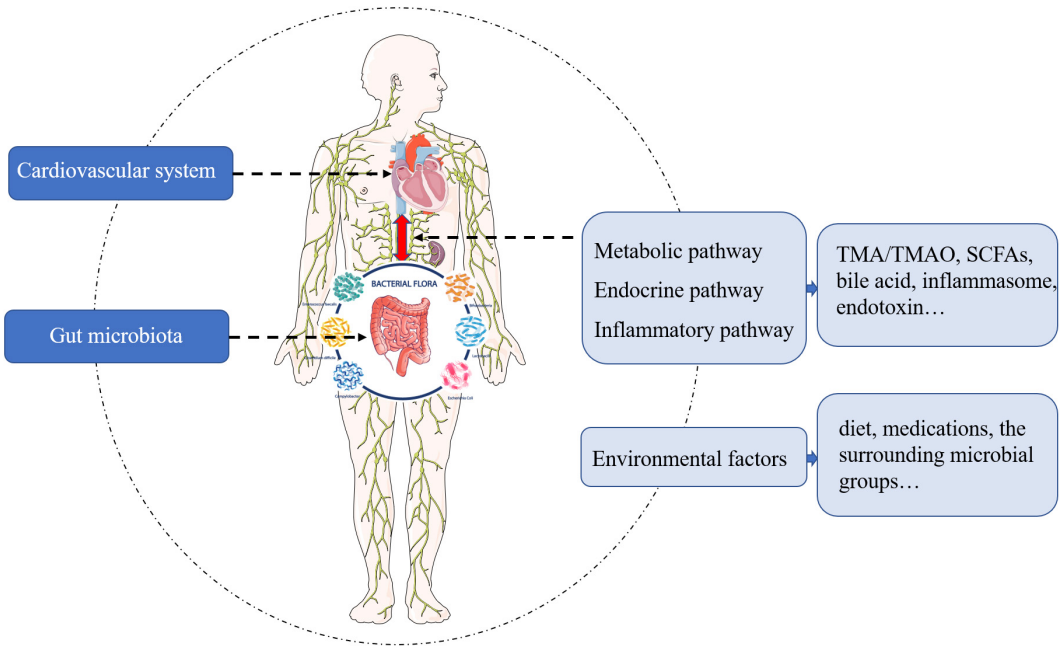

FIGURE 1 | The pathways and mediators of bidirectional communication between cardiovascular system and gut microbiota.

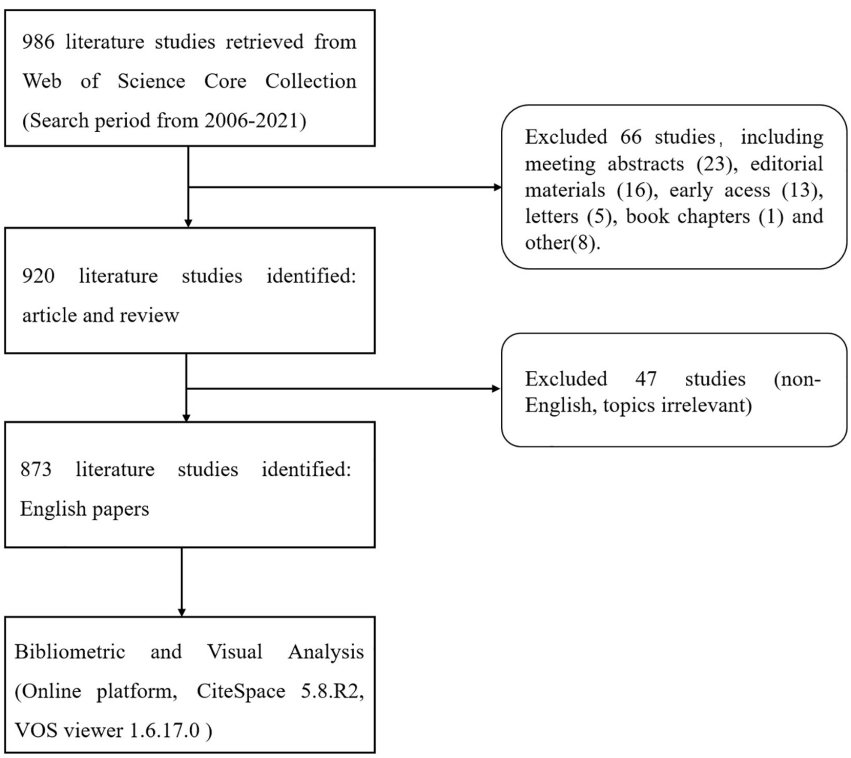

FIGURE 2 | Flowchart of the inclusion and exclusion criteria.

collaborations, evolutionary pathways, as well as frontier research hotspots and future research development trends. In addition, some details of the setting description should be made known, where the ID #0 is assigned to the largest cluster formed in the cluster analysis, the ID #1 is assigned to the second largest, and so on.

RESULTS

Analysis of Publication Outputs and Prediction of Growth Trend

A total of 873 literature studies meeting the inclusion and exclusion criteria were finally retrieved for this study. A total of 714 (81.79%) articles and 159 (18.21%) reviews, respectively, were identified (**Figure 3A**). In addition, analysis of subject categories may provide insight into the subject focus of the current study, as shown in **Figure 3B**, there were top five subject categories in the analyzed publications: cardiac cardiovascular systems (n = 144, 16.49%), nutrition dietetics (n = 90, 10.31%), general internal medicine (n = 74, 8.48%), microbiology (n = 74, 8.48%), and pharmacology/pharmacy (n = 64, 7.33%). Over the past 16 years, the annual article productions had increased from 1 in 2006 to 141 in 2021, and also annual citations had steadily increased from 1 in 2006 to 6166 in 2021, indicating that related topic captures increasingly more attention from researchers. Furthermore, trend predictions were performed using linear, logarithmic, polynomial, power, exponential, and moving average function types. **Figures 3C,D** illustrated that the polynomial function provided the best fit to this prediction model as it has the highest R^2 (0.9811, 0.9944, respectively), while the specific forecasting equations were $y = 0.0503x^3 - 0.5726x^2 + 5.2786x + 5.0742$ and $y = 4.2063x^3 - 66.017x^2 + 393.16x - 497.54$, respectively. Hence, ~176 articles related to gut microbiota and heart failure may be published in 2022.

Analysis of Scientific Collaboration Networks

There were 40 countries, 196 institutions, and 257 authors involved in the publication on the relationship between gut microbiota and heart failure, respectively. **Table 1** summarizes the top 10 high-yield countries, institutions and authors according to publications and centrality. The top five countries, in terms of the number of publications, were the United States, People's Republic of China, Spain, Canada, and United Kingdom. In order of centrality, the United States, Spain, Canada, People's Republic of China and India topped the list. The Pearson's correlation analysis revealed that there was a significant correlation between publications and centrality at the country level (r = 0.859, $p < 0.01$). **Figure 4A** shows a map depicting the collaboration network among countries. Thus, taking a broader view of publications and centrality, the United States (publications: 281, centrality: 0.45) was identified as the most influential country in the field. Furthermore, the cross-country collaborations visualization map was generated by the online bibliometric analysis (**Supplementary Figure 2**), the results showed that the United States remains dominant. **Figure 4B** shows the cluster of institutions that performing gut microbiota

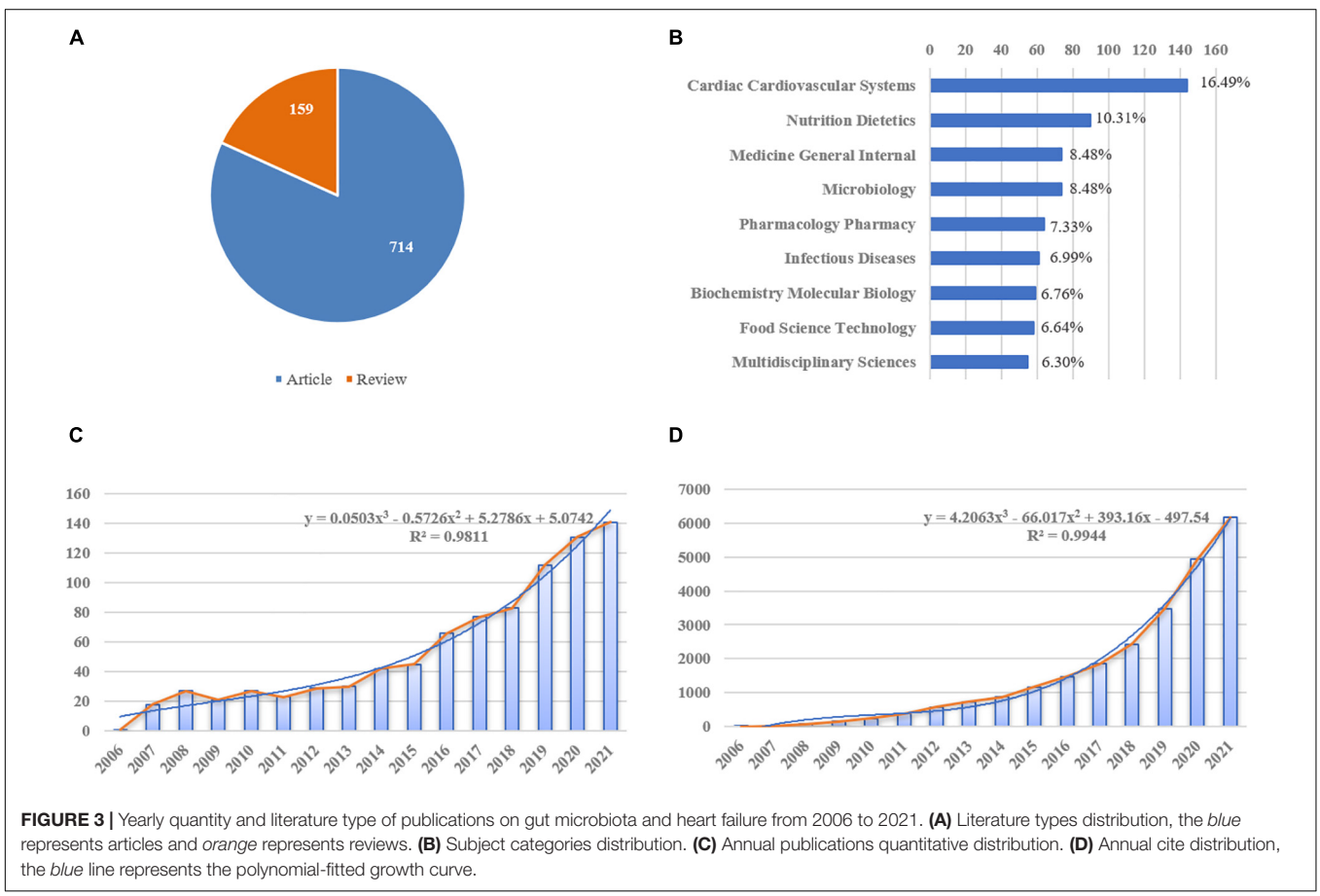

FIGURE 3 | Yearly quantity and literature type of publications on gut microbiota and heart failure from 2006 to 2021. **(A)** Literature types distribution, the *blue* represents articles and *orange* represents reviews. **(B)** Subject categories distribution. **(C)** Annual publications quantitative distribution. **(D)** Annual cite distribution, the *blue* line represents the polynomial-fitted growth curve.

TABLE 1 | Ranking of the top 10 countries, institutions, and authors based on publications and centrality.

Items	Publications			Centrality		
	Ranking	Country	Number	Ranking	Name	Number
Country	1	United States	281	1	United States	0.45
	2	People's Republic of China	167	2	Spain	0.21
	3	Spain	57	3	Canada	0.20
	4	Canada	56	4	People's Republic of China	0.15
	5	United Kingdom	45	5	India	0.14
	6	France	44	6	Italy	0.13
	7	Germany	44	7	Australia	0.13
	8	Italy	42	8	France	0.12
	9	Japan	35	9	Germany	0.11
	10	Australia	32	10	Turkey	0.11
Institution	1	Cleveland Clinic	16	1	Charite-Medical University of Berlin	0.21
	2	University of California Davis	13	2	Cleveland Clinic	0.18
	3	University of Barcelona	11	3	Northwestern University	0.15
	4	University of Calgary	10	4	University of Washington	0.15
	5	Charite-Medical University of Berlin	9	5	University of British Columbia	0.15
	6	Catholic University of Louvain	9	6	University of California Davis	0.1
	7	Northwestern University	8	7	Capital Medical University	0.1
	8	University of California, San Francisco	8	8	McMaster University	0.1
	9	University of Toronto	8	9	University of Pittsburgh	0.09
	10	Chinese Academy of Medical Sciences	8	10	Harvard Medical School	0.09
Author	1	Nathalie M. Delzenne	9	1	Nathalie M. Delzenne	0
	2	Audrey M. Neyrinck	8	2	Audrey M. Neyrinck	0
	3	Raylene A. Reimer	8	3	Raylene A. Reimer	0
	4	Patrice D. Cani	7	4	Patrice D. Cani	0
	5	You-Lin Tain	6	5	You-Lin Tain	0
	6	Stanley L. Hazen	5	6	Stanley L. Hazen	0
	7	Marius Troseid	5	7	Marius Troseid	0
	8	Sunhye Lee	5	8	Sunhye Lee	0
	9	Francine Z. Marques	5	9	Francine Z. Marques	0
	10	W. H. Wilson Tang	5	10	W. H. Wilson Tang	0

research in heart failure. Its most productive institution was Cleveland Clinic with 16 publications, followed by University of California Davis (13), and University of Barcelona (11). Similarly, the Pearson's correlation analysis concluded that there was a significant correlation between publications and centrality at the institutions level ($r = 0.635$, $p < 0.01$). In addition, three authors published more than eight publications in this field. The most productive author with nine publications was identified as Nathalie M. Delzenne, while Audrey M. Neyrinck (eight publications) and Raylene A. Reimer (eight publications) ranked second and third, respectively. There was a relative partial cooperation among Nathalie M. Delzenne, Audrey M. Neyrinck, and Patrice D. Cani (**Figure 4C**). However, the centrality between all of them was zero. Similarly, the VOS viewer software was also used to identify top countries, institutions and authors, and the results showed general agreement with the above results, as presented in **Supplementary Figure 3**.

Analysis of Journals and Co-cited Journals

Journals are an important vehicle for presenting the results of academic research. Literature studies included in the study were published in 489 different journals, many of which were specialized journals. As shown in **Table 2**, the three most influential journals in terms of number of publications were *Plos One*, *Journal of Nutrition*, and *American Journal of Physiology-Gastrointestinal and Liver Physiology*. Most of the productive journals were in Q1, except for *European Journal of Clinical Microbiology and Infectious Disease*. As one of the most important metrics for scientometric investigation, co-citation analysis has been widely used. These co-cited journals were all in Q1 and had highly impact factor (IF), *PloS One* (408, IF = 3.240), *New England Journal of Medicine* (330, IF = 91.245), *Nature* (326, IF = 49.962), and *Circulation* (316, IF = 29.690) were top-ranked by citation counts. Meanwhile, visualization map of journal co-citation network was conducted to explore relationships between different journals from 2006 to 2021 (**Supplementary Figure 4**).

Analysis of Co-cited Authors

Co-cited author analysis can be used to identify research groups at "unknown" institutions or universities and to guide subsequent collaborations among scholars in the field. **Supplementary Figure 5** is the co-citation author map, showing the collaboration between authors. As shown in **Table 3**, the top 10 co-cited authors, their affiliations, and their *h*-indexes were listed. In regard to the number of citations, Patrice D. Cani ranked first, with 145 citations, followed by W. H. Wilson Tang, Peter J.

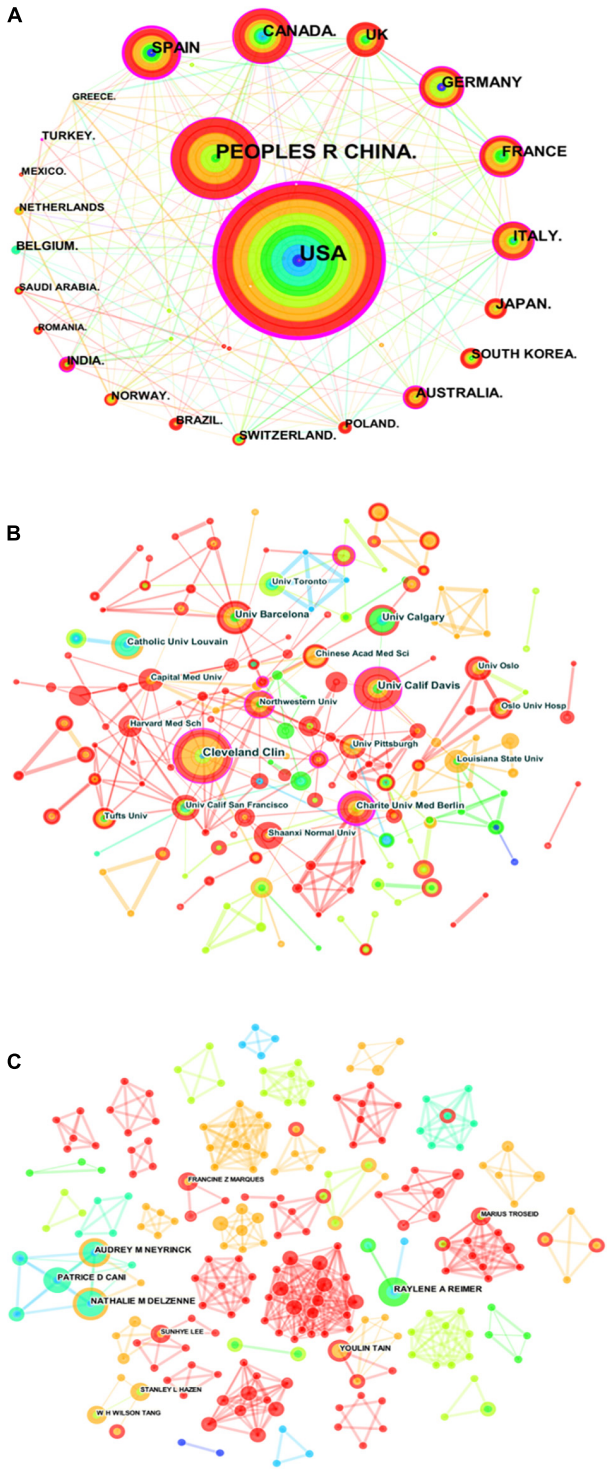

FIGURE 4 | Visualization knowledge maps of the scientific collaboration network for gut microbiota research in the heart failure field between 2006 and 2021. **(A)** Inter-country collaboration network map. **(B)** Inter-institutional collaborative network map. **(C)** Inter-author collaborative network map. *Circle node* on the map represents a country, institute, or author, while *link lines* between nodes indicate collaborative relationships. The more published articles are, the larger their node area is. The outermost *purple ring* denotes the centrality level, and the nodes with high centrality are in the core position.

Turnbaugh, and Ruth E. Ley, whereas the remaining authors had fewer than 100. In terms of *h*-indexes, Patrice D. Cani also ranked first, followed by Fredrik Backhed and W. H. Wilson Tang.

Analysis of Co-cited References

Analysis of co-cited references reveals the authoritative nature of the research in the field and the great contribution of the authors. **Figure 5A** shows the clustering visualization map of co-cited references based on the CiteSpace software, which was divided into 114 clusters based on indexing terms, and finally a log-likelihood ratio algorithm (LLR) was used to extract the largest nine clusters. The figure displays them with different convex hulls, including heart failure (cluster #0), high fat diet (cluster #1), infection (cluster #2), gut (cluster #3), cholesterol (cluster #4), procalcitonin (cluster #5), infective endocarditis (cluster #7), inulin (cluster #10), and light-intensity training (cluster #18). **Table 4** presents details on the largest nine clusters of references in the co-citation network. In addition, the timeline view is a visualization method that combines the clustering analysis and time-slicing techniques. **Figure 5B** then shows the largest nine clusters in a timeline view, illustrating the temporal scientific relevance of co-cited references. A total of 398 nodes and 1,512 links was displayed in the visualization map of co-cited references, and the total *Q*-value was 0.8052, harmonic mean (*Q*, *S*) = 0.886, and the mean silhouette value of each cluster was above 0.9, manifesting that the cluster quality was credible and significant.

As shown in **Table 5**, the characteristics of the top 10 highly co-cited references concerning gut microbiota research in the heart failure field were summarized. Each of these references was found in cluster #0, with the top-ranked reference being published by Tang et al. (64) (15), followed by Koeth et al. (63) (16), and Tang et al. (58) (17).

Analysis of Co-occurring Keywords

Keywords co-occurrence analysis can be used to identify research topics and to analyze research hotspots, as well as to monitor the transition of research frontiers within a certain knowledge domain. VOS viewer was utilized for keyword co-occurrence clusters with a minimum of five occurrences, as shown in **Figure 6**, the size of each node indicates the occurrence of the keyword. Three clusters were shown in different colors, and nodes with common attributes were classified into a color-coded cluster, represented by green, blue, red, which revolved around gut microbiota, heart failure, and risk factor, respectively. The details of each cluster are shown in **Supplementary Figure 6**. In addition, a landscape generated using clusters of keywords based on CiteSpace software presents the following six blocks (**Figure 7A**), the overlapping portions of the image indicate where studies could be classified within more than one cluster. **Supplementary Table 2** shows the details of the largest six clusters in co-occurring keywords. In addition, time-zone view of co-occurring keywords are shown in **Figure 7B**, consisting of 189 nodes and 1,074 links that represent the keywords and their co-occurrence relationships. In terms of co-occurrence frequency, the top 10 ranked keywords are shown in **Table 6**, including "heart failure," "gut microbiota," "obesity,"

TABLE 2 | Ranking of the top 10 journals and co-cited journals for gut microbiota research in the heart failure field.

Items	Ranking	Name	Country	Counts	IF (2020)[#]	JCR (2020)*
Journal	1	Plos One	United States	11	3.240	Q1
	2	Journal of Nutrition	United States	7	4.798	Q1
	3	American Journal of Physiology-Gastrointestinal and Liver Physiology	United States	6	4.052	Q1
	4	Food and Function	United Kingdom	6	5.396	Q1
	5	Journal of Nutritional Biochemistry	United States	6	6.048	Q1
	6	Molecular Nutrition and Food Research	Germany	6	5.914	Q1
	7	Nutrients	Switzerland	6	5.717	Q1
	8	Scientific Reports	United Kingdom	6	4.379	Q1
	9	British Journal of Nutrition	United Kingdom	5	3.718	Q1
	10	European Journal of Clinical Microbiology and Infectious Diseases	Germany	5	3.267	Q2
Co-cited Journal	1	Plos One	United States	408	3.240	Q1
	2	New England Journal of Medicine	United States	330	91.245	Q1
	3	Nature	United Kingdom	326	49.962	Q1
	4	Circulation	United States	316	29.690	Q1
	5	Proceedings of the National Academy of Sciences of the United States of America	United States	287	11.205	Q1
	6	Journal of the American College of Cardiology	Netherlands	278	24.094	Q1
	7	European Heart Journal	United Kingdom	228	29.983	Q1
	8	Lancet	United Kingdom	226	79.321	Q1
	9	Clinical Infectious Diseases	United Kingdom	214	9.079	Q1
	10	Science	United States	205	47.728	Q1

[#]*IF, impact factor. *JCR, journal citation reports. Q, quartile in category.*

TABLE 3 | Ranking of the top 10 co-cited authors with the most citations.

Ranking	Times cited	Author	Affiliation	*h*-Index
1	145	Patrice D. Cani	Universite Catholique Louvain	92
2	119	W. H. Wilson Tang	Cleveland Clinic	81
3	117	Peter J. Turnbaugh	University of California, San Francisco	48
4	104	Ruth E. Ley	Max Planck Institute for Developmental Biology	64
5	98	Zeneng Wang	Cleveland Clinic Foundation	52
6	79	Fredrik Backhed	University of Gothenburg	87
7	70	Anja Sandek	University of Gottingen	24
8	69	Robert A. Koeth	Cleveland Clinic	8
9	67	Junjie Qin	BGI-Shenzhen	12
10	60	J. Gregory Caporaso	Northern Arizona University	63

"risk factor," "inflammation," "TMAO," "metabolism," "antibiotic therapy," "management," and "disease." Of note are the following, the keywords "Obesity" and "TMAO" have become the top research hotspots since 2012 and 2016 (**Figure 7**), appearing in, respectively, 122 and 71 citing publications (**Table 6**).

Analysis of Burst Detection

Burst detection analysis may identify the emerging concepts and future trends that have caught the attention of peer investigators. The strength of the burst and the duration of the burst state are the two attributes of the citation burst. By using CiteSpace, **Figure 8** displays references with the strongest citation bursts during the period of 2006–2021. References with citation bursts first appeared in 2008 (18, 33–36), and the most recent references with citation bursts appeared in 2018 (6, 26, 30–32, 37–49). The strongest burst (strength: 12.85) appeared in 2016 for a 2011 article (27). A total of 13 references had a burst that lasted until 2021 (6, 26, 30–32, 37–41, 49–51). The references with citation bursts between 2010 and 2018 accounted for 90.00%.

Figure 9 shows the top 25 keywords with citation bursts. Their stronger burst rate indicates greater attention to the research topic, which can be a good indicator of the research frontier in this period. Over the past decade, surgery ranked first with the highest burst strength (strength: 6.02), followed by infective endocarditis (strength: 5.51), TMAO (strength: 4.89), antibiotic therapy (strength: strength: 4.64), and blood pressure

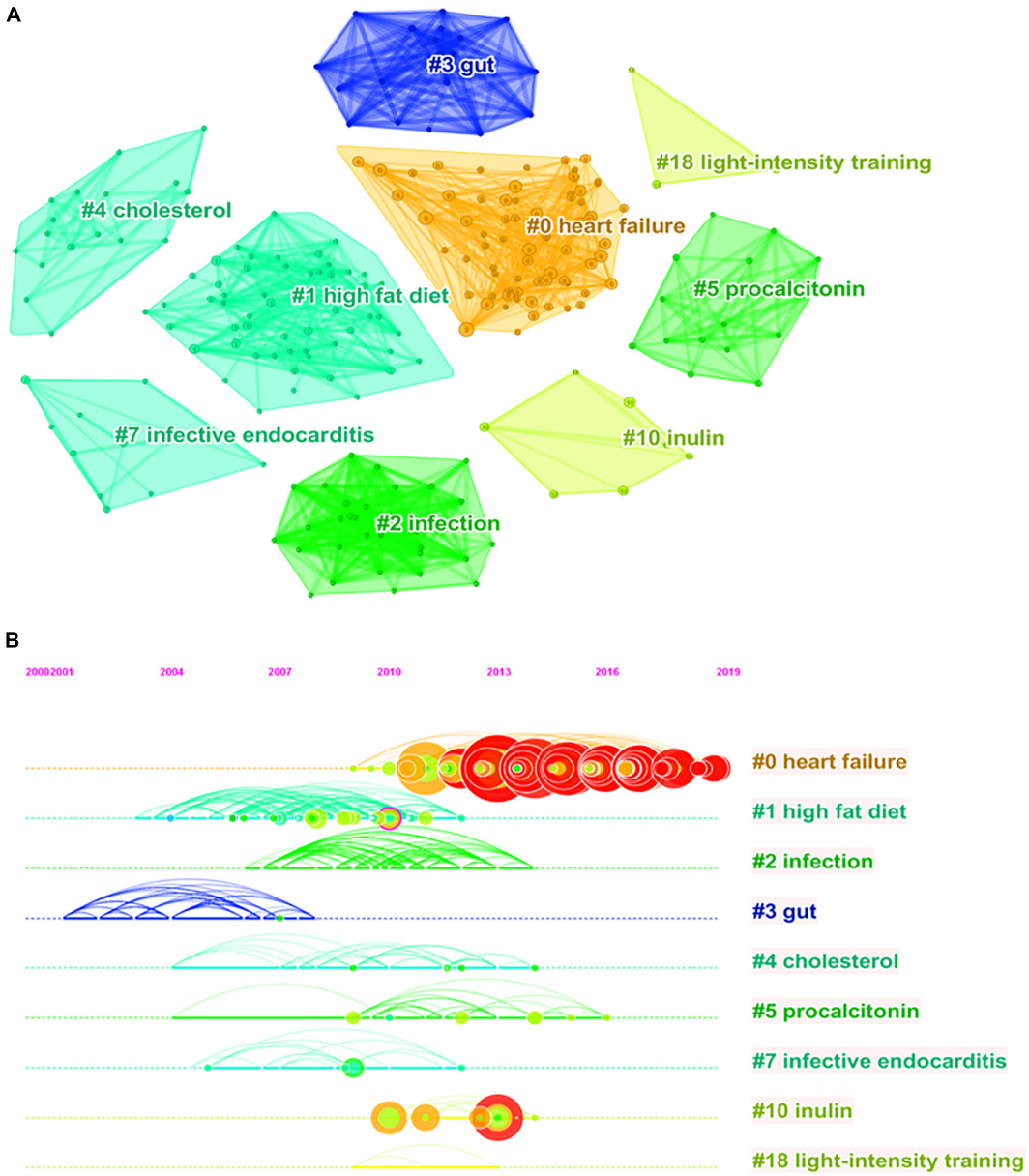

FIGURE 5 | Reference co-citation network knowledge map for research of gut microbiota in the heart failure field from 2006 to 2021. **(A)** Clustering visualization map of the reference co-citation, labeled with the largest nine clusters. **(B)** Timeline visualization map of the reference co-citation.

(strength: 4.63). More importantly, TMAO, blood pressure, hypertension, supplementation, bile acid, and fermentation became the focus from 2018 until now, indicting they were current research hotspots.

DISCUSSION

With the rapid increase in the number of deaths from heart failure each year, the search for possible factors and new therapeutic strategies has become imperative. It has been reported that an imbalance between the gut microbiota alter the microecological environment of a gastrointestinal tract, resulting in numerous diseases. Since then, gut microbiota has attracted the attention of researchers. Is modulating gut microbiota to restore injured heart function a possible therapeutic strategy? Over the past nearly two decades, researchers have made extensive efforts to elucidate the link between them. With the help of scientometric investigation, it is possible to provide the history and current status of research in the subject area, and to predict future research directions. To best of our knowledge, this is the first-ever study to provide systematic information on the link between gut microbiota and heart failure through bibliometric analysis coupled with visualized mapping. Researcher may benefit from

TABLE 4 | The largest nine clusters in the reference co-citation network.

Cluster	Size	Mean silhouette	Mean year	Label (LLR algorithm)	Representative Reference
0	72	0.995	2015	Heart failure	Tang et al. (15)
1	60	0.949	2008	High fat diet	Cani et al. (18)
2	32	1	2010	Infection	Sohail et al. (19)
3	19	0.988	2004	Gut	Sandek et al. (20)
4	18	1	2010	Cholesterol	Parnell et al. (21)
5	16	1	2011	Procalcitonin	Schuetz et al. (22)
7	12	1	2008	Infective endocarditis	Habib et al. (23)
10	7	1	2012	Inulin	Everard et al. (24)
18	3	0.987	2011	Light-intensity training	Turnbaugh et al. (25)

"Size" means the number of references that a cluster contains. "LLR" means log-likelihood ratio.

these results by gaining a basic understanding and identifying areas of interest or trends, encouraging them to conduct further research in this field.

General Information

On the basis of the WoSCC database, we analyzed a total of 873 literature studies that were indexed by SCIE from 2006 to 2021, of which 81.79% were original articles and 18.21% were reviews. As evidenced by a qualitative and quantitative analysis conducted using online platform, VOS viewer and CiteSpace software, the number of scientific research outputs pertaining to the link between gut microbiota and heart failure has increased significantly during the past 16 years. The United States, which lead in the number of publications, also had a maximum centrality of 0.45, and was the country that plays a central role in promoting cooperation among individual countries. Remarkably, China, as a developing country, had shown tremendous progress in this field with 167 relevant articles, Zhu et al. (32, 48) and Qin et al. (50) had published a series of articles around the link between microbial products (e.g., TMAO) and heart failure. As for the authorship, Nathalie M. Delzenne, Audrey M. Neyrinck, and Patrice D. Cani had established a relatively stable collaboration, ranking first, second, and fourth in terms of number of publications, respectively. However, we realized that the collaborative efforts among top researchers seem to be insufficient, and that countries and institutions should encourage scholars to actively increase their collaborations and publish higher quality articles.

Analysis journal and co-cited journals can provide important information for researchers to choose the proper journal in which to submit their manuscripts. Most relevant studies were published in Q1 journals, while those with more co-citations appeared in the journals with world-class influence, such as *New England Journal of Medicine*, *Nature*, *Circulation*, *Lancet*, and *Science*. These results suggested that the link between heart failure and gut microbiota has attracted the attention of numerous

TABLE 5 | Ranking of the top 10 co-cited references for gut microbiota research in the heart failure field.

Ranking	Cited by	References	Title	Source title	Year of publication	Type of document
1	64	Tang et al. (15)	Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis	Journal of the American College of Cardiology	2014	Article
2	63	Koeth et al. (16)	Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis	Nature Medicine	2013	Article
3	58	Tang et al. (17)	Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk	The New England Journal of Medicine	2013	Article
4	49	Marques et al. (26)	High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice	Circulation	2017	Article
5	48	Wang et al. (27)	Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease	Nature	2011	Article
6	47	Yang et al. (28)	Gut dysbiosis is linked to hypertension	Hypertension	2015	Article
7	46	Wang et al. (29)	Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis	Cell	2015	Article
8	45	Pasini et al. (30)	Pathogenic gut flora in patients with chronic heart failure	JACC Heart Failure	2016	Article
9	41	Luedde et al. (31)	Heart failure is associated with depletion of core intestinal microbiota	ESC Heart Failure	2017	Article
10	41	Zhu et al. (32)	Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk	Cell	2016	Article

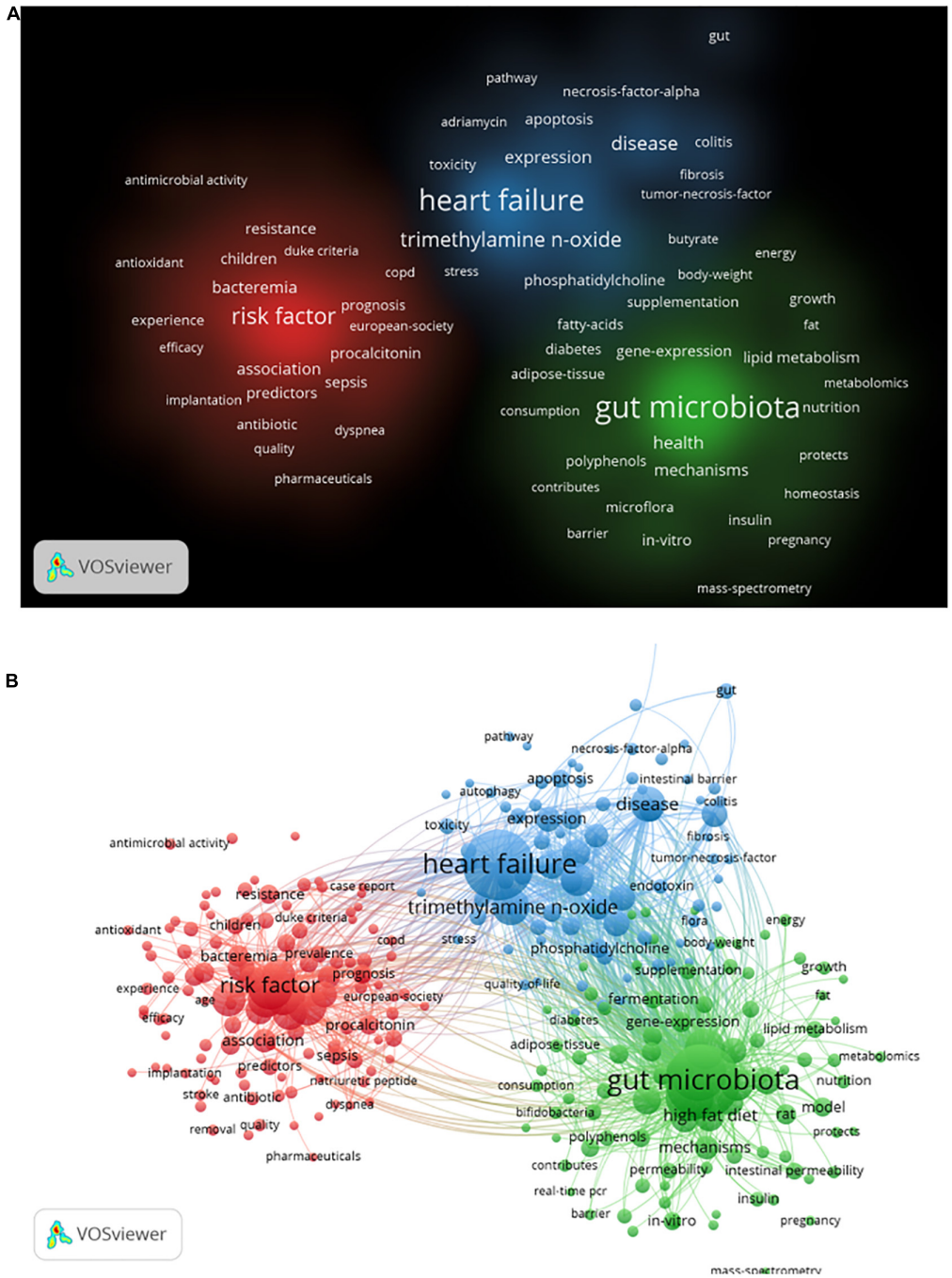

FIGURE 6 | Visualization of keyword co-occurrence analysis from 2006 to 2021 based on the VOS viewer software. **(A)** The density visualization map, and the depth of the color was positively correlated with the occurrences of keywords. **(B)** The network visualization map, all the keywords could be clustered into three categories.

scholars, and its research difficulties and value have also been recognized by scholars. However, only a 10% concordance rate was observed between the top 10 most active journals and the top 10 co-cited journals, indicating a need to improve the quality of research in this field, as well as to strengthened international collaboration among scholars to produce high-quality research.

The published literature studies focused on cardiac cardiovascular systems, microbiology, and pharmacology pharmacy, as well as infectious diseases, biochemistry molecular biology, food science technology, nutrition, reflecting the multidisciplinary intersection that is a characteristic of research in this field. Multidisciplinary intersection will aid in breaking through the technical condition limitation of the research

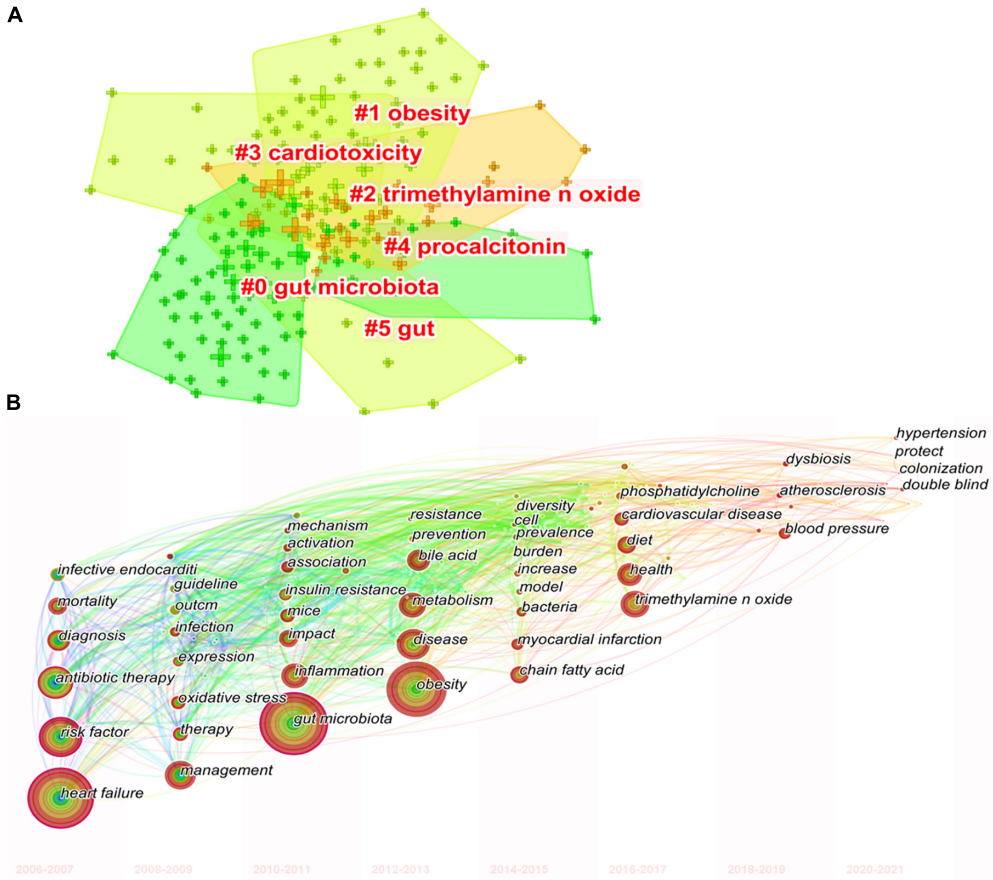

FIGURE 7 | The keywords clustering knowledge map for research of gut microbiota in the heart failure field from 2006 to 2021. **(A)** Clustering visualization map of the co-occurring keywords, labeled with the largest six clusters. **(B)** Time-zone view of co-occurring keywords, with the size of each node proportional to the frequency of keyword occurrences.

between gut microbiota and heart failure, and will provide the impetus for the development of this field.

Knowledge Base

Analysis of reference co-citation networks showed that all the top 10 highly co-cited references fell under the largest theme cluster #0 ("heart failure"). Among the top 10 co-cited references, the first and third were both published by Professor W. H. Wilson Tang group in *JACC* 2013 and *The New England Journal of Medicine* 2014 (15, 17), respectively, where the authors further found that patients with heart failure had elevated TMAO levels compared to patients without heart failure, and elevated TMAO levels were associated with higher long-term mortality risk in spite of traditional risk factors and cardiorenal indexes, with landmark implications. The second most co-cited reference was published by Robert A. Koeth in *Nature Medicine* in 2013 (16), in which it was demonstrated that gut microbiota metabolism of L-carnitine plays an important role in atherosclerosis pathogenesis and suggested new potential therapeutic targets for preventing cardiovascular disease. Moreover, cluster #1 was a collection of studies on high-fat diets, a representative study showing that high-fat feeding alters intestinal flora, which in turn increases intestinal LPS permeability, and several inflammatory markers were analyzed and mRNA expressions of PAI-1, TNF-α, and IL-1 were found to be completely eliminated by antibiotic treatment after high-fat feeding (18). Furthermore, high-fat diets lead to abnormal glucose metabolism and cardiac tissue damage through uric acid-dependent mechanisms, whereas butyric acid (a type of SCFAs) protects against high fat diet-induced cardiometabolic disturbances by inhibiting uric acid and enhancing glutathione antioxidant defenses (52). Cluster #10 focused on elucidating the role of inulin in the gut microbiota, and studies have shown that inulin-type fructans (ITFs) have been implicated as regulators of

TABLE 6 | Ranking of the top 10 keywords for gut microbiota research in the heart failure field in terms of frequency.

Ranking	Keyword	Frequency	Ranking	Keyword	Frequency
1	Gut microbiota	173	6	TMAO	71
2	Heart failure	149	7	Metabolism	70
3	Obesity	122	8	Antibiotic therapy	69
4	Risk factor	93	9	Management	64
5	Inflammation	73	10	Disease	60

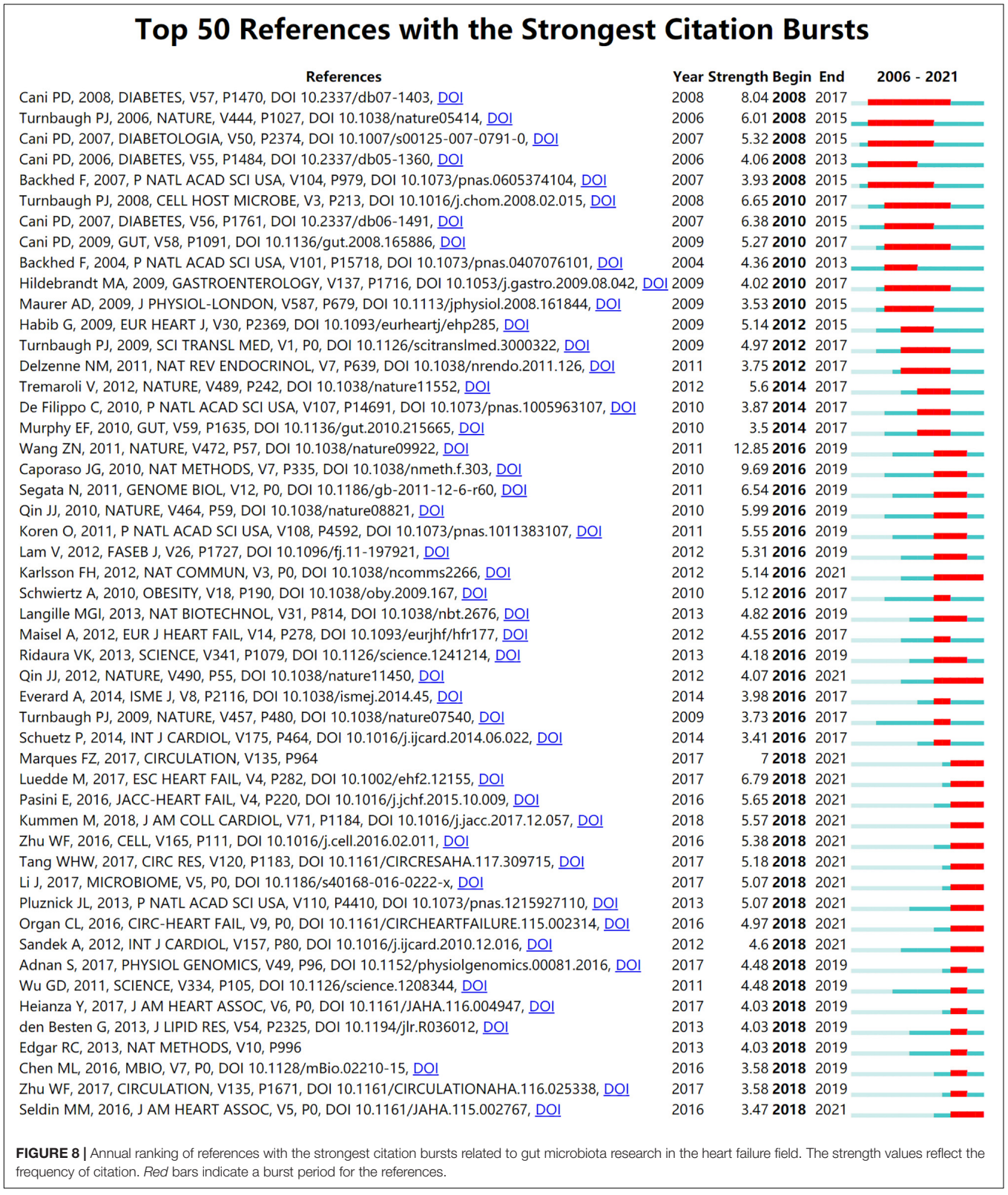

FIGURE 8 | Annual ranking of references with the strongest citation bursts related to gut microbiota research in the heart failure field. The strength values reflect the frequency of citation. *Red* bars indicate a burst period for the references.

microbial ecology and host physiology in animals and humans. Rodent models of genetic and diet-induced obesity also showed ITF to be beneficial to reducing body weight gain and fat mass accumulation, improving glucose tolerance and insulin resistance, improving intestinal barrier function, and reducing inflammation (53). In addition, other highly co-cited references

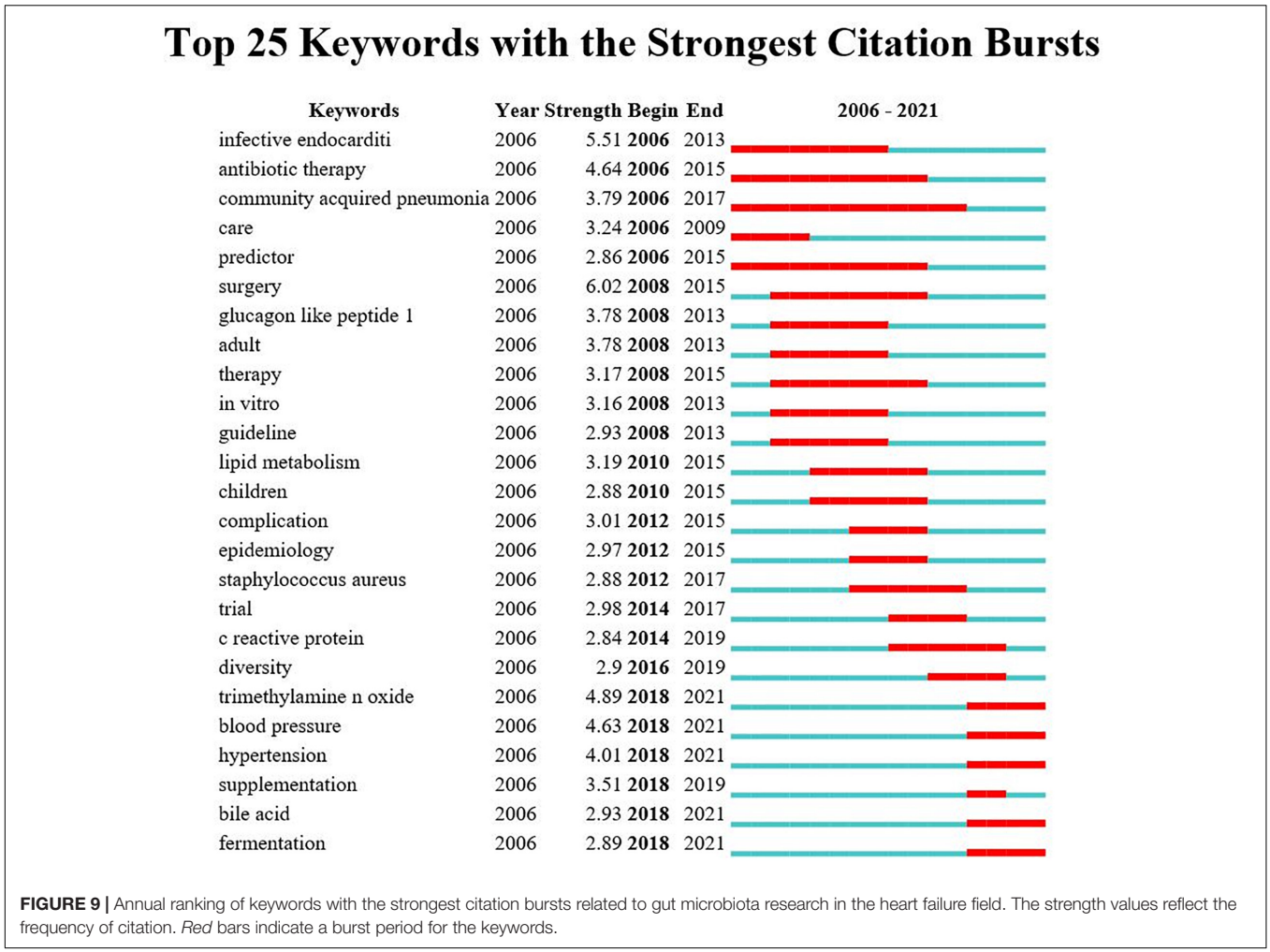

FIGURE 9 | Annual ranking of keywords with the strongest citation bursts related to gut microbiota research in the heart failure field. The strength values reflect the frequency of citation. *Red* bars indicate a burst period for the keywords.

shed light on the possible link between heart failure and gut microbiota around high-fat diet (26), phosphatidylcholine (17, 27), cholesterol, and high-intensity training.

As shown in keywords clustering map in **Figure 6**, it was observed that all the keywords could separate into three clusters, besides the subject terms "heart failure" and "gut microbiota," it is more important to focus on the role of "risk factor," for example, studies have shown comorbidities such as diabetes or chronic kidney disease are a high risk for heart failure patients, and that antibiotic therapy may be a potential treatment by modification of the gut microbiota (**Supplementary Figure 6**). More interestingly, as shown by the clustering visualization and time-zone view of co-occurrence keywords (**Figure 7**), early studies of gut microbiota in patients with heart failure focused on removing gut decontamination with broad-spectrum antibiotics in an attempt to reduce inflammation levels and bacterial translocation. Infective endocarditis is characterized by a high rate of staphylococcus aureus infection, which leads to a relatively high rate of development of moderate-to-severe heart failure (54, 55). Similarly, Sandek et al. also observed bacterial overgrowth in patients with heart failure that consisted of mucosal biofilm with increased bacterial adhesion, which could further lead to chronic inflammation and malnutrition (20, 56). Probiotic supplement studies reported that administration of *Lactobacillus plantarum* 299v decreased circulating leptin levels, reduced myocardial infarcts and improved ventricular function and remodeling after left anterior descending artery ligation (57), and another study showed that saccharomyces boulardii was effective in improving left ventricular ejection fraction and left atrial diameter in patients with chronic heart failure (58). With the in-depth study of gut microbiota, metabolites represented by TMAO and SCFA have played an important role in promoting the progression of heart failure and other cardiovascular disease. In 2017, Marques et al. demonstrated the importance of consuming a high-fiber diet and supplementing with SCFAs to lower blood pressure, minimize cardiac remodeling, and inhibit cardiac hypertrophy and fibrosis through the modification of gut microbial modulation (26). Evidence from a meta-analysis by Zheng et al. suggested that high long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs), obtained in the diet from seafood or in the form of supplements, may have preventive effects on heart failure (59). Therefore, the modulation of dietary composition and intestinal metabolites are expected to be new therapeutic targets of heart failure treatment.

Emerging Topics

The dynamic nature of trends in this field are partially characteristic of the references with citation bursts. Statistics from CiteSpace found that the first representative reference with the strongest currently ongoing citation bursts was an article published by Wang et al., which illustrated that elevated plasma levels of the three dietary lipid phosphatidylcholine metabolites (namely choline, TMAO, and betaine) were associated with increased cardiovascular risk based on metabolomics studies (27). The second highest burst of reference focused on big data mining software in microbiology, introducing quantitative insights into microbial ecology (QIIME), a microecological sequencing data analysis process developed by Caporaso et al. and published in the *Nature Methods* in 2010 (60), which laid the methodological foundation for the link between gut microbiota and heart failure. The software has become the most widely used analysis tool in the microbiota field, with over 10,000 citations, and was named one of the 25 milestone events in human microbiota research in the last 70 years by *Nature*. Furthermore, the publication with the third strongest citation bursts, by Cani et al. (18), was published in 2008, in the *Diabetes* with the burstness strength of 8.04, and the burstness has lasted for 10 years (2008–2017). This new finding implies that intestinal permeability may be increased by changes in gut microbiota, which in turn may control metabolic endotoxemia, inflammation, and associated disorders (18). The citation burstness analysis showed that exploring the link between gut microbiota and heart failure is multi-disciplinary and needs to be explored with the help of emerging research tools, and it is believed that the future development of multi-omics association and artificial intelligence technologies may breathe new life into this field.

These research hotspots have revealed many novel findings that have contributed to a rise in publications. The "burst keywords" can be categorized into three phases based on when they started and when they ended. The first stage included "infective endocarditis," "antibiotic therapy," "community acquired pneumonia," "care," and "predictor," these disease extensions suggest that gut microbiota may be associated with heart failure. The second stage included "glucagon like peptide 1," "lipid metabolism," "staphylococcus aureus," "c reactive protein," etc., elucidating the possible influence of inflammatory indicators and various disorders of glycolipid metabolism on the pathogenesis of heart failure, and paving the way for the study of specific mechanisms of gut microbiota in heart failure. The third stage, that is, the keywords with high burst intensity that started in 2018 and have currently ongoing bursts, specifically contain "TMAO," "blood pressure," "hypertension," "bile acid," and "fermentation." A growing body of literature suggest that modulation of intestinal metabolites (TMAO, bile acid, and SCFAs) holds promise as new therapeutic targets for heart failure patients, which was consistent with the strongest citation keywords burst of this study in 2018. Animal experiments in heart failure from Organ et al. found that either choline supplementation, or direct TMAO feeding, could result in higher levels of systemic TMAO, aggravated myocardial fibrosis, as well as worsened hemodynamic and anatomic parameters in mice after trans-aortic constriction (TAC) (41). Beyond animal model studies showing heightened TMAO is associated with worse adverse ventricular remodeling and function in heart failure models, clinical data also revealed that higher TMAO levels were associated with poorer prognoses with respect to all-cause mortality, hospitalization, and heart transplantation for patients with heart failure (61). And even one study showed that TMAO was a better predictor of prognosis than BNP (62). Coincidentally, Suzuki et al. in patients with heart failure showed that the inclusion of TMAO in the risk model, combined with clinical scores, improved risk stratification for in-hospital mortality, while the combination of TMAO and NT-proBNP would provide additional prognostic predictive information (63). Meanwhile, clinical trial studies have found that the application of antibiotic intervention reduces TMAO synthesis and thus myocardial hypertrophy and myocardial fibrosis (64). Further extension of the findings of the relationship between TMAO levels and cardiovascular disease, some studies had also demonstrated that plasma TMAO levels were associated with the risks of incident thrombotic event and had a degree of elevating effect on blood pressure (32, 48). Bile acids are another important metabolite of gut microbiota, a cross-sectional study showed an increased ratio of secondary to primary bile acids in the serum of chronic heart failure patients, and univariate analysis showed that this ratio was associated with a decrease in overall survival (3). It has been shown that bile acid responsive receptors, namely TGR5 agonists or FXR inhibitors, exert cytoprotective effects on the heart by improve myocardial responses to physiological, inotropic, and hemodynamic stress (65, 66). Meanwhile, an increasing number of emerging molecules have been shown to be independent novel biomarker of heart failure risk stratification, such as secreted frizzled-related protein 2 (SFRP2) and SFRP5 (2, 67), and it is believed that future application with intestinal metabolites will be able to better predict the development of heart failure.

Research conducted in recent years had found that metabolic disorders can have unhealthy effects on cardiovascular health, which include hypertension, atherosclerosis, heart failure, dyslipidemia, diabetes, and chronic kidney disease as well (38). A high prevalence of hypertension is particularly prevalent in heart failure patients, as 91% had underlying hypertension at the time of their diagnosis (68). Therefore, it is necessary to study how gut microbiota affects the regulation of blood pressure. As a result of experimental hypertension, spontaneous hypertension rats were found to have significantly higher plasma levels of TMA than normotensive rats, and enalapril as a hypotensive agent was able to significantly reduce the plasma levels of TMA as well (69). Other studies had demonstrated that SCFAs were correlated with blood pressure levels in the pathogenesis of hypertension, and SCFAs may lower blood pressure by regulating vasodilation, specifically possibly through the G protein-coupled receptor orphan type (Gpr41) and the olfactory receptor 78 (Olfr78), which exert blood pressure modulating effects. Similar studies related to co-morbidities such as diabetes and chronic kidney disease are increasingly being conducted, and although there is the large body of evidence to date pointing to TMAO and SCFAs as the major gut metabolites involved in heart failure

and co-morbidities such as diabetes and chronic kidney disease (63, 70–72), other dietary nutrients and fermentation metabolites should not be ignored (73).

Currently, the supplementation or improvement of gut microbiota has become a frontier topic in the field of heart failure, including dietary interventions, antibiotic interventions, probiotic and prebiotic therapy, faecal microbiota transplantation (FMT), TMA-lyase inhibitors, etc. (74). It is possible to improve the intestinal flora structure of heart failure by transplanting beneficial bacteria, low yielding TMAO intestinal flora, and high yielding SCFAs intestinal flora. However, whether FMT can improve heart failure by restoring the diversity and function of the intestinal flora still needs extensive experimental and clinical studies.

Hence, in the future, to better understand this multi-layered complex gut ecological dysregulation-heart failure relationship, it will be necessary to study larger sample sizes and broader populations, as well as to adjust for various factors and medications that may affect the growth of gut bacterial. Further, to make sense of the vast amount of data generated by these studies, artificial intelligence techniques such as deep learning neural networks or others may be needed to help decipher meaningful patterns.

Strengths and Limitations

To our knowledge, this scientometric investigation is the first of its kind to identify and characterize the association with gut microbiota and heart failure. The application of scientometric provides clearer insights into the evolving focuses and trends in research than traditional narrative reviews. Researchers could utilize these information to identify new research directions and explore potential cooperation opportunities in this field. However, there are several limitations to our study. Firstly, the data collection was only retrieved from the WoSCC database, potentially resulting in bias and incompleteness in the included studies. Additionally, for the purposes of better presentation of the results and guaranteeing the quality of all included literature studies, we only included articles and reviews published in English. Finally, despite our normalization procedures, bias may have still existed because some authors have the same name, and some keywords are expressed differently, the database is constantly updated, etc. These limitations may be addressed in a better way in similar studies in the future. Despite these limitations, the findings are still considered to be an effective representation of the research output of gut microbiota research in the heart failure field on a global scale.

CONCLUSION

This study shows a gradual expansion of worldwide research on gut microbiota and heart failure from 2006 to 2021, indicating a well-developed and promising research field. The most frequent subject category was cardiac cardiovascular systems. The United States constituted the core research forces. Strikingly, the cooperation between the institutes and authors were relatively weak. Further advances in this field will make significant breakthroughs in the development of novel therapeutic tools for metabolic modulation of heart failure. In conclusion, this is the first scientometric analysis that provides a comprehensive overview of research on the link between gut microbiota and heart failure, which could help us realize the main research institutions and authors, core journals, evolutionary pathways, frontier research hotspots, and future trends in this field.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

FM, JW, and HT conceived and designed the study. FM wrote the original draft preparation. YG and RL assisted in literature searching. MT conducted an analysis based on WoS. MT, HZ, and FM conducted the software analysis. MZ, JZ, and SH provided the figures and tables. FM, MT, YG, and RL reviewed and edited the manuscript for content and style. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.882660/full#supplementary-material

Supplementary Figure 1 | Conceptual design of the study.

Supplementary Figure 2 | The cross-country collaborations visualization map was generated by the online bibliometric analysis.

Supplementary Figure 3 | Visualization knowledge maps of the scientific collaboration network based on the VOS viewer software. **(A)** Inter-country collaboration network map. **(B)** Inter-institutional collaborative network map. **(C)** Inter-author collaborative network map.

Supplementary Figure 4 | Visualization collaboration network of co-citation journal from 2006 to 2021 based on the CiteSpace software.

Supplementary Figure 5 | Visualization collaboration network of co-citation author from 2006 to 2021 based on the CiteSpace software.

Supplementary Figure 6 | Specific details of the network visualization map of keyword co-occurrence analysis based on the VOS viewer software.

Supplementary Table 1 | Search strategy in Web of Science Core Collection (January 14, 2022).

Supplementary Table 2 | The details of the largest six clusters in co-occurring keywords.

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B-Lines by Lung Ultrasound Can Predict Worsening Heart Failure in Acute Myocardial Infarction During Hospitalization and Short-Term Follow-Up

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Background: Acute myocardial infarction (AMI) with pulmonary edema shows a worse prognosis. Lung ultrasound (LUS) is a new tool for evaluating subclinical pulmonary congestion. It has been proved to predict prognosis in heart failure; however, whether it can be used as a short-term prognostic marker in AMI and provide incremental value to Killip classification is unknown.

Methods: We performed echocardiography and LUS by the 8-zone method in patients enrolled in Guangdong Provincial People's Hospital undergoing percutaneous coronary intervention for AMI from March to July 2021. The lung water detected by LUS was defined as B-lines, and the sum of the B-line number from 8 chest zones was calculated. Besides, the classification into LUS according to the pulmonary edema severity was as follows: normal (B-line numbers <5), mild (B-line numbers ≥ 5 and <15), moderate (B-line numbers ≥ 15 and <30), and severe (B-line numbers ≥ 30). The NT-proBNP analysis was performed on the same day. All patients were followed up for 30 days after discharge. The adverse events were defined as all-cause death, worsening heart failure in hospitalization, or re-hospitalization for heart failure during the follow-up.

Results: Sixty three patients were enrolled consecutively and followed up for 30 days. The number of B-lines at admission (median 7[3–15]) was correlated with NT-proBNP ($r = 0.37$, $p = 0.003$) and negatively correlated with ejection fraction ($r = -0.43$; $p < 0.001$) separately. In the multivariate analysis, B-line number was an independent predictor of short-term outcomes in AMI patients (in-hospital, adjusted OR 1.13 [95% CI: 1.04–1.23], $P = 0.006$; 30-day follow-up, adjusted OR 1.09 [95% CI: 1.01–1.18], $P = 0.020$). For in-hospital results, the area under the receiver operating characteristic curves (AUCs) were 0.639 ($P = 0.093$), 0.837 ($P < 0.001$), and 0.847 ($P < 0.001$) for Killip, LUS and their combination, respectively. For the diagnosis of 30-day adverse events, the AUCs were 0.665 for the Killip classification ($P = 0.061$), 0.728 for LUS ($P = 0.010$), and 0.778 for their combination ($P = 0.002$).

Conclusion: B-lines by lung ultrasound can be an independent predictor of worsening heart failure in AMI during hospitalization and short-term follow-up and provides significant incremental prognostic value to Killip classification.

Keywords: lung ultrasound, acute myocardial infarction, B-lines, pulmonary edema, heart failure

INTRODUCTION

Myocardial infarction is an acute syndrome caused by the sudden blockage of the coronary arteries. Even though the coronary arteries have been opened in time, the prognosis remains poor in some patients. Acute myocardial infarction (AMI) complicated by heart failure is considered the main cause of increased mortality. Early risk stratification is essential for the postoperative management of AMI (1).

Killip Classification was initially described in 1967 and extensively used in the risk stratification of AMI patients, as it was considered to have a significant prognostic value. However, lung auscultation has shown poor sensitivity and accuracy in detecting mild pulmonary edema, which could decrease the accuracy of Killip classification (2).

Conversely, lung ultrasound (LUS) has been gaining attention over the past decade as a non-invasive tool for the detection and quantification of pulmonary congestion in both ambulatory and hospitalized patients with heart failure (HF) (3). Sonographic assessment of extravascular lung water is based on reverberation artifacts from the pleural line, which are thought to originate from the interlobular septa thickened by fluid. These discrete laser-like vertical hyperechoic reverberations are known as "B-lines" (also "comet tails" or "lung comets") (4). Therefore, LUS is sensitive to the water deposited in the lung, potentially playing an important role in monitoring pulmonary congestion during hospitalization and improving risk assessment.

Therefore, indications for LUS are growing in cardiology, especially in patients with heart failure or dyspnea (5, 6). LUS can predict the prognosis of patients with acute and chronic heart failure (7, 8), and LUS-guided treatment can reduce the rehospitalization rate of patients with HF (9). A recent study suggested that LUS added to the Killip classification was more sensitive than physical examination to identify patients with ST-Elevation Myocardial Infarction (STEMI) at risk of in-hospital mortality (10). Furthermore, another study found that B-lines can help predict HF in patients with acute myocardial infarction during hospitalization (11). However, whether B-lines can predict the short-term prognosis of AMI after discharge is unknown. We aimed to evaluate the short-term prognostic ability of LUS in patients with AMI.

MATERIALS AND METHODS

Study Design and Participants

The prospective cohort enrolled 63 consecutive patients admitted with an AMI diagnosis (with or without ST-segment elevation) to the Emergency Department of Guangdong Provincial People's Hospital from March to July 2021. Those who did not meet the inclusion criteria were excluded from the study. Inclusion criteria were: (i) age >18 years; and (ii) suspected AMI diagnosis, based on the presence of typical symptoms associated with ischemic abnormalities in the electrocardiogram, fulfilling the diagnostic criteria for AMI according to current guidelines (1). Exclusion criteria were: (i) pulmonary fibrosis or other severe diseases hampering image acquisition (significant pleural effusion, severe emphysema, pulmonary cancer, and so on), and absence of Killip classification or LUS at admission; (ii) non-obstructive myocardial infarction; or (iii) pregnancy. The research was approved by the Ethics Committee of Guangdong Provincial People's Hospital. Written informed consent was obtained from all patients.

All patients were treated with optimal medical therapy according to current guidelines (12, 13), and appropriate percutaneous coronary intervention (PCI) technical strategies were performed in time.

Echocardiography and Lung Ultrasound

Patients underwent echocardiography and LUS as soon as possible on admission. Bedside transthoracic echocardiography and LUS were performed using Philips 7C ultrasound equipment with a 2.5 MHz phased array transducer. Left ventricle (LV) volumes and ejection fraction (EF) were obtained by two-chamber and four-chamber views using the biplane method of disk summation (modified Simpson's rule), according to the recommendations of the American Society of Echocardiography. The anteroposterior diameter of the left atrium (LA) can be measured in the parasternal long-axis view at the level of the aortic sinuses by using the leading-edge to the leading-edge convention. As recommended, measurements of tricuspid annular plane systolic excursion (TAPSE) were obtained. Diastolic function was assessed from the mitral inflow pattern by pulsed Doppler and tissue Doppler imaging to obtain the E/e' ratio (14).

LUS was performed at the same time as the echocardiography, and patients were placed in the supine position. The LUS examination was performed by the 8-zone method, and the number of B-lines of each zone was counted (15). We summed the total number of B-lines in 8 zones, and the classification into LUS according to the pulmonary edema severity was as follows: normal (B-line numbers <5), mild (B-line numbers ≥5 and <15), moderate (B-line numbers ≥15 and <30), and severe (B-line numbers ≥30) (16). All examinations were performed by one operator, unaware of laboratory data and clinical results.

Killip Classification

Patients were evaluated by an experienced cardiologist at the emergency department to assess the signs and symptoms of

TABLE 1 | Patient characteristics ($N = 63$).

Characteristic	Overall ($n = 63$)
Age (years)	63 ± 12
Men	50 (79%)
STEMI	44 (70%)
Medical history	
Hypertension	30 (48%)
Diabetes	25 (40%)
Atrial fibrillation	4 (6%)
Chronic kidney disease	1 (2%)
Smoking (previous or current)	25 (40%)
Previous PCI	13 (21%)
Admission characteristics	
Systolic blood pressure (mmHg)	124 ± 23
Diastolic blood pressure (mmHg)	77 ± 16
dyspnea	14 (22%)
Ankle edema	1 (2%)
Acute pulmonary inflammation	2 (3%)
Cardiac shock	8 (13%)
Killip classification	
I	38 (60%)
II	12 (19%)
III	6 (10%)
IV	7 (11%)
Number of vessels	
Single vessel	11 (18%)
Multiple vessels	50 (79%)
B-lines	7 (3-15)
Normal	2 (1-4)
Mild	8 (7-13)
Moderate	16 (11-18)
Severe	23 (18-27)
Laboratory results	
NT-proBNP (pg/ml)	4,793 (803–4939)
TnT (ng/ml)	3,103 (719–4767)
sST2 (ng/ml)	40 (26–40)
Creatinine (mg/dl)	97 (66–102)
Albumin (g/dl)	36 (34–38)
Echocardiography	
EF(%)	47 (38–54)
LA diameter (mm)	35 ± 5
E/e' ratio	15 ± 6
TAPSE (mm)	21 ± 3

STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary Intervention; LUS, lung ultrasound; NT-proBNP, N-terminal pro-brain natriuretic peptide; TnT, troponin T; sST2, soluble ST2; EF, ejection fraction; LA, left atrium; TAPSE, Tricuspid Annular Plane Systolic Excursion.

clinical congestion, and the Killip classification was provided blinded to the results of the lung ultrasound. The classification into Killip I–IV was as follows: Killip I, no evidence of heart failure; Killip II, signs indicating a mild to moderate degree of heart failure (S3 gallop, rales half way up the lung fields, or elevated jugular venous pressure); Killip III, acute pulmonary edema (bilateral rales in more than half of both lung fields and dyspnea at rest), and Killip IV, cardiogenic shock (systolic blood pressure <90 mmHg and signs of poor perfusion) (17).

Biochemical Analysis

All peripheral venous blood samples were taken on admission and discharged into sterile tubes containing Ethylene Diamine Tetraacetic Acid (EDTA). N-terminal pro-brain natriuretic peptide (NT-proBNP) analysis was performed using the Abbott Architect assay (Abbott Diagnostics, Abbott Park, IL, USA). In addition, troponin T (TnT) was measured using the electrochemiluminescence method (Roche Diagnostics), and soluble ST2(sST2) was assessed using the Presage ST2 Assay (Waltham, MA).

Follow-Up and Outcomes

All patients were followed up for 30 days after discharge. The adverse events were defined as all-cause death, worsening heart failure in hospitalization (required intravenous diuretic treatment or diuretic increase), or re-hospitalization during the follow-up. The electronic medical records in the hospital were reviewed, and the follow-up data were obtained by telephone interviews and clinic visits from discharge. The coronary angiography data that the number of obstructed coronary vessels observed during the procedure was also recorded.

Statistical Analysis

Continuous variables were expressed as mean (± SD) or median (25th−75th percentiles), as appropriate. Categorical variables were presented as counts and percentages. The B-line number was analyzed as a continuous variable (total B-line numbers) and a categorical variable (LUS classification, the four grades defined above). The correlation between the total B-line numbers, NT-proBNP, and EF was assessed with a non-parametric Spearman correlation coefficient analysis.

Logistic regression models (unadjusted and adjusted) were used to assess the continuous association between B-lines and short-term outcomes. Models were adjusted for potential confounding variables using a forward-conditional selection procedure, including age, Killip classification, log-transformed NT-proBNP concentration, etc. These covariates were chosen based on their clinical importance concerning the outcome, using a limited number of variables to prevent overfitting.

Kendall's tau correlation was used to assess the relationship between Killip and LUS classification. The ROC curve was analyzed to evaluate the efficacy of the different variables, and the areas under the curve (AUCs) were calculated. Two-sided significance levels of 0.05 were used for all analyses. Data were analyzed using SPSS (version 25.0.0; IBM Company).

RESULTS

Patient Characteristics

The main clinical data of the 63 patients included are reported in **Table 1**. 79% of patients were male in the whole cohort, with

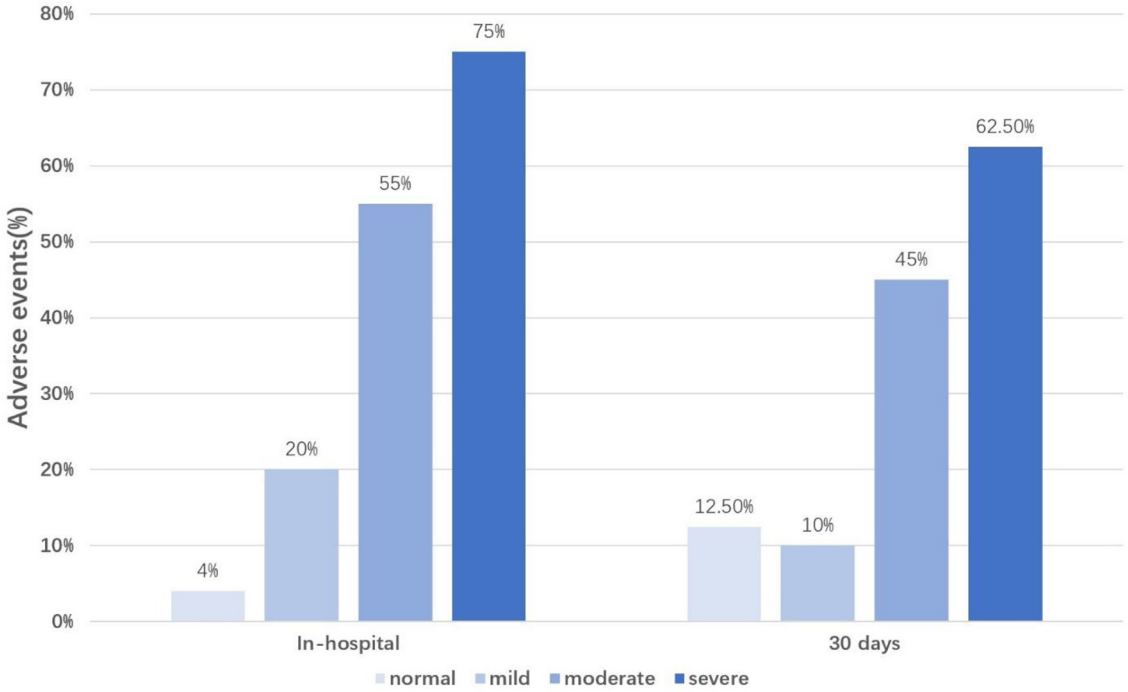

FIGURE 1 | Percentage of adverse events occurrence according to the LUS classification. LUS classification, the total number of B-lines was divided into four groups according to the severity of pulmonary edema (normal, B-line numbers <5; mild, B-line numbers ≥5 and <15; moderate, B-line numbers ≥15 and <30; severe, B-line numbers ≥30); In-hospital, during the hospitalization; 30 days, during 30 days follow-up.

an average age of 63 ± 12 years and a median EF of 47% (38–54%). Furthermore, 70% of patients had STEMI, and 79% had multiple vessel lesions. A few patients had atrial fibrillation (6%) or chronic kidney disease (2%), which may be the confounding factor that affected B-lines or NT-proBNP concentration.

When patients arrived in the emergency room, 13% had a cardiac shock, only 22% had dyspnea, and <5% showed ankle edema. Overall, 60% of patients were classified as Killip I class. However, the median B-lines at admission were 7 (IQR, 3–15), indicating that most of them had mild pulmonary edema at least. Patients also had high levels of NT-proBNP, sST2, and TnT. Echocardiography showed mild dilation in the left atrium, and the E/e' ratio increased (**Table 1**).

B-Lines Acted as an Independent Predictor of Short-Term Adverse Events

The number of B-lines correlated with NT-proBNP values ($r = 0.37$, $p = 0.003$) and negatively correlated with EF measured by echocardiography ($r = -0.43$; $p < 0.001$), which are usually used to predict heart failure and poor prognosis. In our sample, 17 patients (27%) and 15 patients (24%) developed adverse events during hospitalization and 30 days of follow-up, respectively. During hospitalization, 3 (5%) died from all causes, and 14 (22%) patients developed worsening HF, while during the 30 days follow-up, 3 (5%) died, 9 (14%) developed worsening HF, and 3(5%) were readmitted.

Compared to patients with dry lungs or mild pulmonary edema, adverse cardiovascular events were more significantly increased in patients with moderate to severe pulmonary edema (**Figure 1**).

We constructed an unadjusted and adjusted logistic regression model to identify the factors that could predict the short-term outcomes of AMI among the included co-variables; results are shown in **Tables 2**, **3**. For in-hospital events, on univariate analysis, age (OR 1.08 [95% CI: 1.02–1.14], $P = 0.006$), total B-line numbers (OR 1.14 [95% CI:1.05–1.24], $P = 0.002$), creatinine (OR 1.02 [95% CI: 1.01–1.04], $P = 0.040$), Killip II-IV (OR 2.95 [95% CI: 0.94–9.29], $P = 0.041$), Log NT-proBNP (OR 4.14 [95% CI:1.29–13.32], $P = 0.017$), EF (OR 0.94[95% CI:0.89–0.99], $P = 0.027$), and LA diameter (OR 1.16 [95% CI:1.02–1.32], $P = 0.026$) all had statistical significance, but only the first three contributed independent information in a multivariable model.

For the 30-day follow-up, the univariate analysis showed that previous PCI (OR 6.00 [95% CI: 1.39–25.86], $P = 0.016$), total B-line numbers (OR 1.10 [95% CI: 1.02–1.18], $P = 0.020$), Killip II-IV (OR 3.50 [95% CI: 1.02–12.03], $P = 0.047$), Log NT-proBNP (OR 3.20 [95% CI: 1.03–9.94], $P = 0.044$), and EF (OR 0.92 [95% CI: 0.86–0.98], $P = 0.008$) predicted outcome at 30 days. However, only total B-line numbers contributed independent information in a multivariable model. The association between total B-line numbers and the outcome was similar during the in-hospital period (adjusted OR 1.13 [95% CI: 1.04–1.23], $P = 0.006$) and 30 days after discharge (adjusted OR 1.09 [95% CI: 1.01–1.18], $P = 0.020$). Therefore, B-lines can be a stable and independent predictor of short-term adverse events in AMI patients.

TABLE 2 | Univariate analysis for in-hospital and 30 days composite outcome.

Characteristic	In-hospital		30 days	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Age (years)	1.08 (1.02–1.14)	**0.006***	1.05 (0.99–1.11)	0.070
STEMI	4.40 (0.89–21.60)	0.070	1.04 (0.27–3.98)	0.950
Diabetes	2.11 (0.68–6.53)	0.200	1.31 (0.40–4.34)	0.660
Shock	3.23 (0.71–14.76)	0.130	3.08 (0.55–17.35)	0.200
Previous PCI	1.98 (0.54–7.21)	0.300	6.00 (1.39–25.86)	**0.016***
Killip II-IV	2.95 (0.94–9.29)	**0.041***	3.50 (1.02–12.03)	**0.047***
Number of vessels	5.12 (0.66–39.58)	0.120	2.19 (0.46–10.45)	0.330
Total B-line numbers	1.14 (1.05–1.24)	**0.002***	1.10 (1.02–1.18)	**0.020***
Log NT-proBNP	4.14 (1.29–13.32)	**0.017***	3.20 (1.03–9.94)	**0.044***
Log TNT	3.34 (0.99–11.21)	0.050	1.80 (0.62–5.17)	0.280
Creatinine (mg/dl)	1.02 (1.01–1.04)	**0.040***	1.01 (0.99–1.03)	0.107
EF (%)	0.94 (0.89–0.99)	**0.027***	0.92 (0.86–0.98)	**0.008***
LA (mm)	1.16 (1.02–1.32)	**0.026***	1.11 (0.97–1.26)	0.130
E/e' ratio	1.06 (0.97–1.16)	0.220	1.04 (0.94–1.13)	0.470

STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary Intervention; Log NT-proBNP, log-transformed N-terminal pro-brain natriuretic peptide; Log TNT, log-transformed troponin T; EF, ejection fraction; LA, left atrium; OR, odds ratio.
**P < 0.05.*
Statistically significant results are marked in bold.

TABLE 3 | Multivariable analysis for in-hospital and 30 days composite outcome.

Characteristic	In-hospital		30 days	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Age (years)	1.08 (1.01–1.14)	**0.020***	1.04 (0.97–1.12)	0.280
Total B-line numbers	1.13 (1.04–1.23)	**0.006***	1.09 (1.01–1.18)	**0.020***
Killip IV	17.52 (0.89–344.33)	0.060	2.82 (0.27–29.45)	0.390
Number of vessels	3.17 (0.23–42.85)	0.390	0.83 (0.14–5.05)	0.840
Log NT-proBNP	1.17 (0.15–9.23)	0.880	1.15 (0.24–5.58)	0.870
Creatinine (mg/dl)	1.03 (1.01–1.07)	**0.037***	1.01 (0.99–1.03)	0.300

Log NT-proBNP, log-transformed N-terminal pro-brain natriuretic peptide; OR, odds ratio; In-hospital means outcomes during the hospitalization; 30 days means outcomes during 30 days after discharge.
**P < 0.05.*
Statistically significant results are marked in bold.

Correlation Between LUS and Killip Classification

In different Killip grades, the severity of pulmonary edema showed a large variation (**Figure 2**). In patients with Killip I, normal and mild pulmonary edema accounted for up to 87%. However, with the development of heart failure, the proportion of patients with moderate and severe pulmonary congestion increased significantly. Among the patients with Killip II, 58% had at least moderate lung water, while in Killip III, 66% had at least moderate lung water. Unexpectedly, in patients with Killip IV, 43% were normal, while severe edema only accounted for 14% of patients. Kendall's tau correlation analysis showed a correlation between LUS and Killip classification ($\kappa = 0.26$, $P = 0.018$). A similar correlation was also found between the total B-line numbers and Killip classification ($\kappa = 0.27$, $P = 0.008$).

Comparison of LUS and Killip Classification to Predict Adverse Events

The ability of the LUS and Killip classification to diagnose the short-term clinical outcomes of AMI was analyzed using ROC curve analysis. The area under the ROC curve for the prediction of in-hospital adverse events was 0.639 for the Killip classification (95% CI 0.48–0.80, $P = 0.093$), 0.837 for the LUS classification (95% CI 0.73–0.95, $P < 0.001$), and 0.847 for the combination of LUS and Killip classification (95% CI 0.75–0.95, $P < 0.001$). Moreover, for the diagnosis of 30-day cardiovascular adverse events, the AUCs were 0.665 for the Killip classification (95% CI 0.50–0.83, $P = 0.061$), 0.728 for LUS classification (95%CI 0.56–0.89, $P = 0.010$), and 0.778 for the combination of LUS and Killip classification (95%CI 0.63–0.92, $P = 0.002$). These two ROC curves showed that the combination of LUS and Killip classification was superior to Killip classification alone in predicting short-term adverse outcomes in AMI (**Figure 3**).

DISCUSSION

In a prospective cohort of patients with AMI undergoing PCI, we found that B-lines by lung ultrasound can be an independent predictor of adverse cardiovascular events during hospitalization and 30 days of follow-up. AMI patients with more severe pulmonary edema may have a higher risk of adverse events. According to the ROC curves, the combination of LUS and Killip had a superior predictive value for evaluating the short-term outcomes in AMI. It has been proved that LUS can provide significant incremental prognostic value to Killip classification.

Although some studies have evaluated the in-hospital mortality of patients with myocardial infarction by LUS, this is the first study to further evaluate the short-term prognostic value of B-lines.

It is well-known that AMI patients often face complication related to acute left heart failure, which is associated with a higher risk of short-term mortality compared to AMI alone (18). Pulmonary congestion is a prominent element in HF. However, lung auscultation has limited sensitivity and specificity (19). Pulmonary congestion is likely to be ignored, and prompt diuretic treatment cannot be provided. Most patients had at least mild pulmonary edema in this study, but 60% were diagnosed with Killip I. Therefore, edema may have been underestimated due to some false negatives in the Killip grades.

LUS is becoming an effective method for detecting interstitial pulmonary edema (15). Although the LUS protocol has not been unified thus far, we chose the simplified 8-zone method because it was considered effective and less time-consuming. In our study, a positive correlation was found between the number of B-lines and NT-proBNP level, and a negative correlation was found in EF. Our results indicated that B-lines by lung ultrasound could be a promising marker to predict heart failure, such as secreted frizzled-related protein 5(SFRP5) and extracellular volume fraction by cardiovascular magnetic resonance (20, 21). Alberto Palazzuoli et al. also confirmed that B-lines are strongly associated with clinical assessment, biomarkers, or echocardiography. B-lines at discharge add

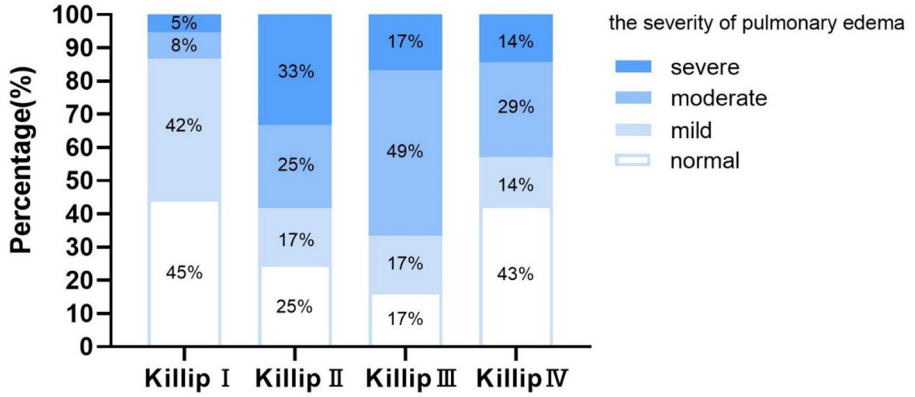

FIGURE 2 | Proportion of the severity of lung water in patients with Killip I–IV. Normal, B-line numbers <5; Mild, B-line numbers ≥5 and <15; Moderate, B-line numbers ≥15 and <30; Severe, B-line numbers ≥30.

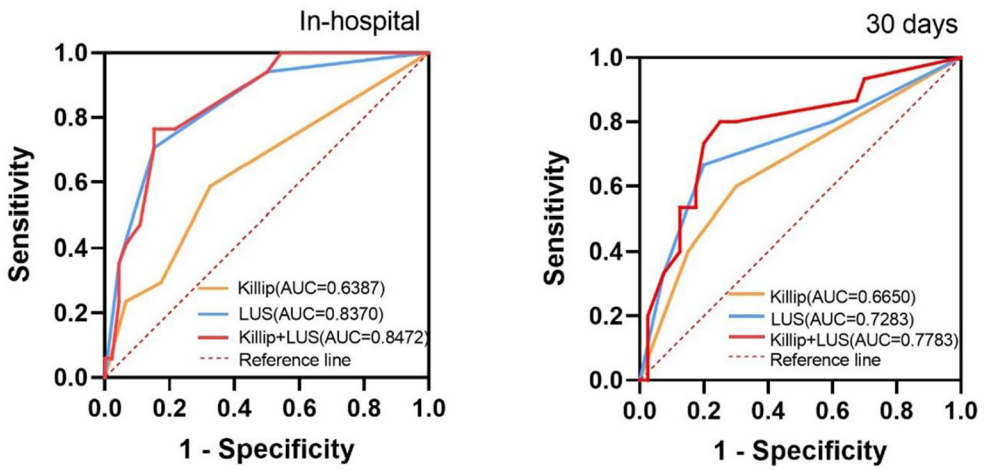

FIGURE 3 | Receiver operating characteristic curves for "Killip", "LUS", and "Killip+LUS" classification to predict the in-hospital and 30 days composite outcome. AUC, the area under the curve. In-hospital, during the hospitalization; 30 days, during 30 days follow-up; Killip, Killip classification; LUS, LUS classification; Killip+LUS, the combination of LUS and Killip classification.

important information regarding risk stratification in acute heart failure (AHF) patients.

In addition to heart failure, recent studies have begun to focus on the prognostic value of B-lines for AMI. AMI is usually complicated with LV afterload and filling pressures increasing, leading to pulmonary congestion. However, In Gustavo N. Araujo et al.'s study, inflammation and vascular permeability were also contributed to extravascular lung water increase in AMI patients. Bedetti et al. found that LUS added additional prognostic value to the Global Registry in Acute Coronary Events score in 470 patients admitted with acute coronary syndromes (22). Jorge et al. also found that LUS performed at admission can help to predict heart failure in patients with AMI (11). In our sample, among patients without lung water, only 4% (during hospitalization) and 12.5% (during 30-day follow-up) had adverse events, indicating that negative results of LUS are more likely to indicate a good prognosis for patients with AMI. However, with the gradual aggravation of pulmonary edema, the proportion of patients with adverse events gradually increased, suggesting that we may need to pay more attention to patients with AMI combined with moderate to severe pulmonary edema.

Our results confirmed that there was a correlation between LUS and Killip classification. Although the severity of pulmonary edema assessed by LUS was not as severe as expected in Killip IV patients, this might have occurred because they were more usually treated with non-invasive or invasive ventilation, which could reduce lung water before LUS examination (23). Killip classification relies mainly on lung rales to determine congestion at admission; however, as mentioned above, auscultation results can be false negatives. Araujo et al. suggested that admission LUS added to the Killip classification was a feasible and more sensitive method of identifying patients with STEMI at risk for in-hospital outcomes than physical examination (10). Moreover, our ROC curves also support this notion and add some new information:

the combination of LUS and Killip was superior to Killip alone for predicting short-term adverse outcomes in AMI, in hospital and during 30 days of short-term follow-up.

Lastly, in univariate and multivariate regression analyses, B-lines can be used as a stable independent predictor of the short-term adverse events of AMI. The correlation between the total B-line numbers and outcomes during hospitalization was slightly higher than during the 30-day follow-up. Platz et al. (3) assessed the prognostic importance of B-lines in acute heart failure (AHF). They found that the relationship between B-lines and outcomes was stronger closer to hospital discharge and diminished over time, consistent with our results. This may be because pulmonary edema caused by AHF is usually transient. It may be feasible to conduct large clinical trials to evaluate whether LUS-guided therapy can improve the prognosis of AMI patients.

Limitation

This was a single-center cohort study with small sample size, limiting our results' generalizability and rendering the estimation of their associations imprecise. In addition, the follow-up period was relatively short. Further extensive independent studies are warranted to confirm the strong association observed between B-line numbers and AMI patients' risk stratification.

In addition, due to the urgent condition of certain AMI patients, they did not have time to perform LUS before PCI. However, they all completed LUS within 24 h of the perioperative period, which may have had a minor effect on our data and results (24). Lastly, since patients are admitted supine, height and weight could not be measured, and BMI cannot be calculated, which may have affected the calibration of some indicators.

CONCLUSION

In a prospective cohort of patients with AMI undergoing PCI, we found that B-lines by lung ultrasound can be an independent predictor of worsening heart failure in AMI during hospitalization and short-term follow-up. Besides, B-line numbers can provide significant incremental prognostic value to Killip classification.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Guangdong Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Material preparation, data collection, and analysis were performed by JH, SY, and ZL. The first draft of the manuscript was written by JH and all authors commented on previous versions of the manuscript. YZ and HD are equally accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the study conception and design and read and approved the final manuscript.

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ORIGINAL RESEARCH
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Association Between Dietary Fiber Intake and Heart Failure Among Adults: National Health and Nutrition Examination Survey 2009–2018

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Objective: To explore the association between dietary fiber and heart failure (HF).

Methods: Data were collected from the 2009–2018 National Health and Nutrition Examination Survey. Dietary fiber intake data were obtained from two 24-h dietary recall interviews. Logistic regression and restricted cubic spline models were used to explore the association of dietary intakes of total, cereal, fruit, and vegetable fiber with HF prevalence.

Results: A total of 21869 adults were included in this study. After adjusting for multiple confounding factors, the odds ratios (OR) and 95% confidence intervals (CI) for HF was 0.49 (0.28 to 0.87, P for trend = 0.016) for the highest tertile versus lowest tertile of total fiber intake. Similar results were observed for cereal but not fruit and vegetable fiber intake. Dose-response analysis indicated that dietary intake of total and cereal fiber were inversely associated with HF in a linear manner.

Conclusion: Intakes of total and cereal fiber were inversely associated with HF in adults.

Keywords: heart failure (HF), dietary fiber intake, dose-response, National Health and Nutrition Examination Survey (NHANES), nutrition

INTRODUCTION

Heart failure (HF) is a growing global public health burden, with more than 37.7 million individuals estimated to be affected worldwide (1), and the prevalence of HF is increasing as populations age, and poor lifestyle determinants rise (2). Although advances in treatments and devices for HF have substantially improved the survival and quality of life in patients, the mortality rates remain high, with the 10-year survival rate at only 25% (3). Therefore, it is of great importance to identify modifiable factors for the prevention or delay of HF and its complications.

Dietary fiber, which comprises many different, mainly plant-based, that are not completely digested in the human gut, has become increasingly popular in recent years due to its health

benefits. Although nutrition guidelines encourage increased consumption of dietary fiber and a consensus statement from the Heart Failure Society of America recommended plant-based diets for patients with HF (4, 5), most individuals in the United States consume less than half of the recommended levels of daily dietary fiber. Notwithstanding the increase in the daily recommended amount to 30 g, just 13% of men and 4% of women adhere to this recommendation (6). The apparent benefits of dietary fiber intake vary substantially from large relative risk reduction to no benefit depending on the outcome or the geographical region (Europe, the United States, Japan, or China). Emerging evidence from randomized trials and observational studies has shown the potential role of dietary fiber intake in reducing coronary heart disease (7, 8), stroke (8), peripheral vascular disease (9), mortality (10), and cancer (11).

However, previous studies were centered just on the association between dietary fiber and many cardiovascular diseases with no scrutiny of its involvement in HF. Several studies have reported that dietary fiber can improve intestinal flora and risk factors for heart failure (e.g., insulin resistance, inflammation, and metabolic disorders) (12–14), suggesting a potential association between dietary fiber and heart failure. We thus examined the relation between HF prevalence and dietary fiber intake.

MATERIALS AND METHODS

Study Population

The National Health and Nutrition Examination Survey (NHANES) is a nationally representative study to assess the health and nutritional status of the non-institutionalized civilian population in the United States. This survey combines several interviews which were initially executed in participants' homes, and subsequent health examinations were completed in a mobile examination center (MEC). This database is global and public with data released every 2 years and accessed from their website. The sampling method and data collection details have been published elsewhere (15).

This study is based on an analysis of data from NHANES cycles between 2009 and 2018. As shown in **Figure 1**, participants who were pregnant or lactating women (n = 576) were excluded. Participants with missing self-reported HF history (n = 20,358) or incomplete dietary recall (n = 6,797), those documenting extreme total energy intakes, i.e., in women: <500 or >5,000 kcal/day and in men: <500 or >8,000 kcal/day (n = 98) were also excluded. Our study included a final total of 21,869 adult participants. As NHANES is a publicly available dataset, this study was exempt from approval by an institutional review board. Informed consent was sourced from the participants prior to the interview and examination steps.

Dietary Fiber Intake

Dietary fiber intake was assessed by two 24-h dietary recall interviews collected by skilled dietitians. While the MEC was the site of the initial dietary interview, the second interview was collected by telephone after 3 to 10 days. The U.S. Department of Agriculture's Dietary Research Food and Nutrition Database for Dietary Studies scrutinized the nutrient intakes. Cereal, vegetable, and fruit dietary fiber are calculated by the sum of the corresponding food code in the Individual Foods files. The average 2-day fiber amount was employed to compute the dietary fiber intakes from the two aforementioned interviews and was adjusted to the body weight. In this study, dietary fiber intake was categorized into tertiles.

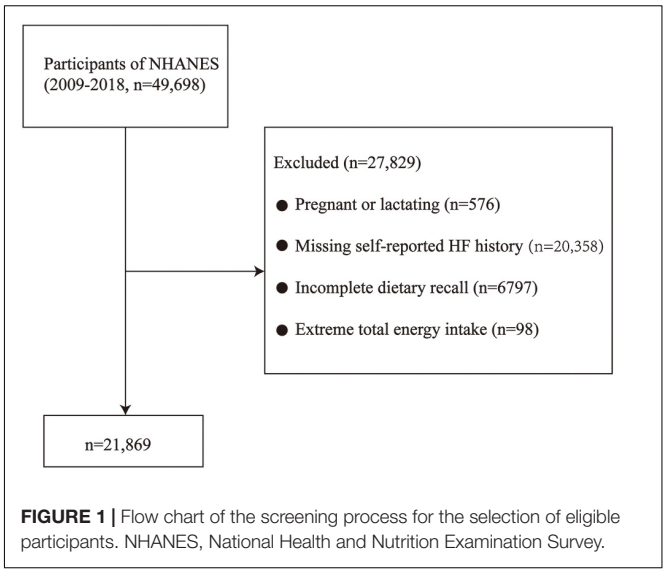

FIGURE 1 | Flow chart of the screening process for the selection of eligible participants. NHANES, National Health and Nutrition Examination Survey.

Heart Failure

Similar to a prior NHANES study (16), HF was defined as participants who have conducted the audiometric assessment and had a self-reported HF history. The latter entailed an affirmative answer to, "Has a doctor ever diagnosed you with heart failure?"

Covariates

Data of age, sex, race, education level, family income, and smoking status were collected from household interviews with standardized questionnaires. Bodyweight, height, and blood pressure were obtained when people participated in the physical examinations at a MEC. Plasma glucose and total cholesterol were measured at baseline when the participants provided their blood samples. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). Race classification was Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other races. Education level was categorized as less than high school, high school or equivalent, or college and above. Family income was classified as <$20,000, $20,000–$55,000, $55,000–$75,000, and ≥$75,000. Smoking status was grouped into "never" (never smoked or less than a hundred cigarettes in life), "current" (more than a hundred cigarettes in life and is also ongoing currently), or "former" (more than a hundred cigarettes in life but currently not smoking). Diabetes was defined as a fasting blood glucose level of ≥7.0 mmol/l, 2-h plasma glucose

TABLE 1 | Characteristics of participants by HF, NHANES 2009–2018.

Characteristics	HF	Non-HF	*P* value
Participants No. (%)	788	21,081	
Male, No. (%)	434 (55.10)	10,039 (47.60)	<0.001
Age, mean (SD),y	67.00 (12.26)	49.42 (17.42)	<0.001
Race, No. (%)			<0.001
Mexican American	68 (8.60)	2,984 (14.20)	
Other Hispanic	70 (8.90)	2,131 (10.10)	
Non-Hispanic White	408 (51.80)	8,741 (41.50)	
Non-Hispanic Black	194 (24.60)	4,517 (21.40)	
Other race	48 (6.10)	2,708 (12.80)	
Income, No. (%)			<0.001
<20,000	76 (45.80)	4,761 (57.90)	
20,000–55,000	46 (27.70)	1,402 (17.10)	
55,000–75,000	25 (15.10)	904 (11.00)	
>75,000	19 (11.40)	1,151 (14.00)	
Education levels, No. (%)			<0.001
Less than high school	256 (32.50)	4,416 (20.90)	
High school or equivalent	210 (26.6)	4,670 (22.20)	
College or above	322 (40.90)	11,995 (56.90)	
Smoking, No. (%)			<0.001
Never	313 (39.70)	12,056 (57.20)	
Former	152 (19.30)	3,974 (18.90)	
Current	323 (41.00)	5,039 (23.90)	
Hypertension No. (%)	715 (90.70)	12,913 (61.30)	<0.001
Diabetes No. (%)	384 (48.70)	3,445 (16.30)	<0.001
TC (SD) (mmol/L)	4.46 (1.12)	4.98 (1.07)	<0.001
Total Fiber intake, mean (SD), (mg/kg/day)	168.42 (101.04)	218.76 (135.36)	<0.001
Cereal fiber intake mean (SD), (mg/kg/day)	91.00 (68.87)	119.17 (88.47)	<0.001
Vegetable fiber intake mean (SD), (mg/kg/day)	43.50 (40.54)	60.62 (64.42)	<0.001
Fruit fiber intake mean (SD), (mg/kg/day)	29.37 (32.91)	39.77 (42.76)	<0.001
BMI, mean (SD) (kg/m2)	32.33 (8.53)	29.37 (7.02)	<0.001
Total energy, mean (SD) (kcal/day)	1783.42 (731.10)	2013.70 (797.37)	<0.001
Total protein, mean (SD) (g/day)	79.75 (34.74)	66.58 (31.06)	<0.001
Total fat, mean (SD) (g/day)	77.45 (37.60)	65.93 (34.94)	<0.001
Total vitamin A, mean (SD) (mg/day)	0.62 (0.53)	0.56 (0.50)	<0.001
Total vitamin B_6, mean (SD) (mg/day)	2.07 (1.34)	1.69 (1.05)	<0.001

BMI, body mass index; TC, total cholesterol. Continuous data were expressed as mean and SD. Categorical was expressed as numbers and percentages.

level of ≥11.1 mmol/l, use of diabetes medications, or self-reported diabetes diagnosis. For hypertension, systolic blood pressure and diastolic blood pressure of ≥130 and ≥80 mmHg, respectively, hypertension medication consumption or self-reported hypertension diagnosis were taken into account. The aggregate daily energy, protein, fat, vitamin A, and vitamin B_6 from the diet and supplement usage was employed to compute the total energy intake, total protein intake, total fat intake, total vitamin A intake, and total vitamin B_6 intake, respectively.

Statistical Analysis

While continuous data were reported as mean and SD, or median with interquartile range, numbers and percentages were employed to express categorical data. Intergroup variation in the former entailed one-way ANOVA and χ^2 tests for the latter. Logistic regression models were employed to assess the association between dietary fiber intake and HF prevalence. While Model 1 was unadjusted, model 2 was inclusive of the adjustment for age, sex, and race and model 3 was inclusive of model 2 variables with total energy intake, total protein intake, total fat intake, total vitamin A intake, total vitamin B_6 intake, BMI, education, annual household income, smoking category, hypertension, diabetes, and total cholesterol (TC) levels. As mentioned above, subsequent to the categorization of the dietary fiber intake as tertiles, the lowest tertile was then employed as the reference group. The odds ratios (ORs) and 95% CIs were calculated. Subgroup analyses ensued for dietary fiber intake and

TABLE 2 | ORs and 95% CIs for HF according to tertiles of dietary fiber intake, NHANES 2009–2018.

Fiber intake level (g/kg/day)	OR (95%CI)		
	Model 1	Model 2	Model 3
Total fiber intake			
T1 (<0.142)	1 (ref)	1 (ref)	1 (ref)
T2 (0.142–0.240)	0.74 (0.63, 0.87)**	0.63 (0.53, 0.75)**	0.58 (0.38, 0.89)*
T3 (>0.240)	0.42 (0.35, 0.51)**	0.39 (0.32, 0.48)**	0.49 (0.28, 0.87)*
P for trend	<0.001	<0.001	0.016
Cereal fiber intake			
T1 (<0.069)	1 (ref)	1 (ref)	1 (ref)
T2 (0.069–0.133)	0.77 (0.66, 0.91)**	0.62 (0.53, 0.74)**	0.72 (0.47, 1.11)
T3 (>0.133)	0.44 (0.37, 0.54)**	0.35 (0.29, 0.43)**	0.59 (0.36, 0.96)*
P for trend	<0.001	<0.001	0.032
Vegetable fiber intake			
T1 (<0.025)	1 (ref)	1 (ref)	1 (ref)
T2 (0.025–0.064)	0.80 (0.67, 0.96)**	0.77 (0.63, 0.92)**	1.14 (0.74, 1.75)
T3 (>0.064)	0.56 (0.45, 0.68)**	0.57 (0.47, 0.70)**	1.19 (0.73, 1.92)
P for trend	<0.001	<0.001	0.439
Fruit fiber intake			
T1 (<0.015)	1 (ref)	1 (ref)	1 (ref)
T2 (0.015–0.043)	0.84 (0.71, 0.99)**	0.65 (0.54, 0.78)**	0.85 (0.55, 1.34)
T3 (>0.043)	0.53 (0.43, 0.65)**	0.36 (0.29, 0.45)**	0.56 (0.33,0.94)*
P for trend	<0.001	<0.001	0.070

*OR, odds ratio; CI, confidence interval; T, tertile. Model 1 is unadjusted. Model 2 adjusted for age and sex. Model 3 adjusted for age, sex, race, total energy intake, total protein intake, total fat intake, total vitamin A intake, total vitamin B_6 intake, BMI, educational level, annual household income, smoking status, hypertension, diabetes and total cholesterol (TC) levels. The lowest tertile of dietary fiber intake was used as the reference group, *P < 0.05; **P < 0.01.*

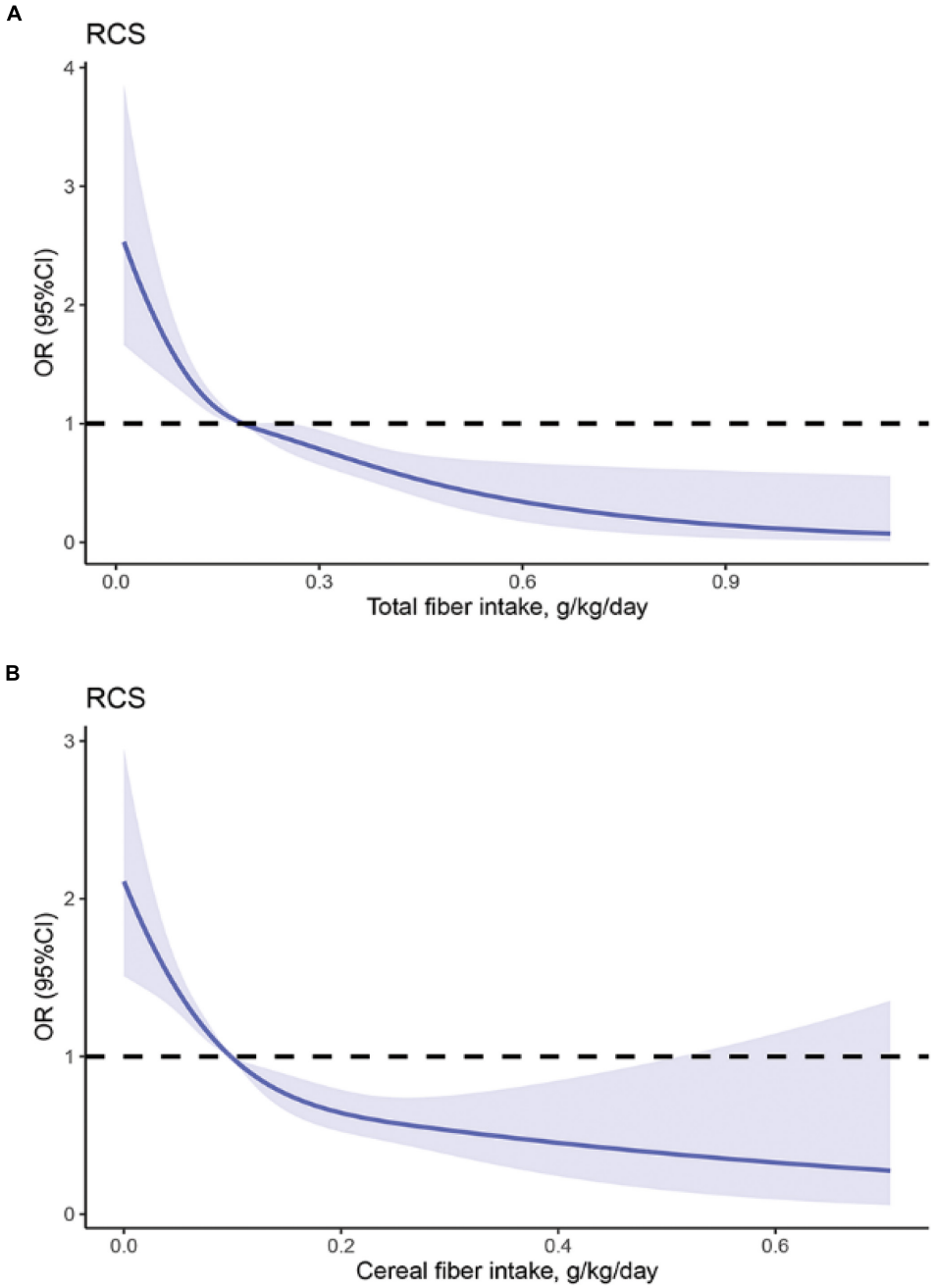

FIGURE 2 | The restricted cubic spline model showed a dose-response relationship between total, cereal fiber intake and HF. **(A)** = Total fiber, P for non-linearity = 0.14; **(B)** Cereal fiber, Pfor non-linearity = 0.27; The restricted cubic splines model adjusted for age, sex, race, total energy intake, total protein intake, total fat intake, total vitamin A intake, total vitamin B_6 intake, BMI, educational level, annual household income, smoking status, hypertension, diabetes and total cholesterol (TC) levels. The solid line and dashed line represent the estimated ORs and the corresponding 95% confidence intervals, respectively. OR, odds ratio.

the prevalence of HF by sex, race, hypertension, diabetes, and obesity status. To assess the dose-response association between dietary fiber intake and the prevalence of HF, we fitted a cubic spline regression using the same covariates adjusted in model 3 and located three knots at the 5th, 50th, and 95th. The probabilities here were all two-sided with significance at $P < 0.05$. All analyses were conducted using R version 3.3.3.

RESULTS

A total of eligible 21,869 participants were included in our study. Mean (SD) age was 50.1 (17.6) years: 10,473 (47.9%) were men, and 9,149 (41.8%) were non-Hispanic White. Population characteristics are presented in **Table 1**. The HF prevalence is 3.6%. As opposed to non-HF participants, patients with HF

TABLE 3 | ORs and 95% CIs for HF according to tertiles of total fiber intake, stratified by sex, race, hypertension, diabetes, and obese status, NHANES 2009–2018.

Subgroup	Fiber intake level	Total fiber intake		Cereal fiber intake	
		OR (95%CI)	P for interaction	OR (95%CI)	P for interaction
Sex			0.43		0.35
Male	T1	1		1	
	T2	0.51 (0.28, 0.95)		0.73 (0.40, 1.33)	
	T3	0.58 (0.28, 1.24)		0.59 (0.30, 1.16)	
Female	T1	1		1	
	T2	0.71 (0.38, 1.34)		0.74 (0.40, 1.39)	
	T3	0.44 (0.18, 1.11)		0.66 (0.32, 1.38)	
Race			0.68		0.63
Mexican American	T1	1		1	
	T2	0.91 (0.18, 4.58)		0.17 (0.03, 1.19)	
	T3	0.55 (0.08, 4.02)		0.43 (0.08, 2.39)	
Other Hispanic	T1	1		1	
	T2	1.26 (0.26, 6.07)		0.19 (0.03, 1.36)	
	T3	1.15 (0.16, 8.59)		1.47 (0.24, 9.09)	
Non-Hispanic White	T1	1		1	
	T2	0.31 (0.17, 0.58)		0.69 (0.39, 1.22)	
	T3	0.40 (0.19, 0.86)		0.41 (0.21, 0.80)	
Non-Hispanic Black	T1	1		1	
	T2	1.77 (0.70, 4.43)		1.08 (0.45, 2.59)	
	T3	0.44 (0.08, 2.35)		1.11 (0.37, 3.30)	
Other race	T1	1		1	
	T2	1.51 (0.72, 6.76)		1.18 (0.61, 4.51)	
	T3	0.42 (0.06, 4.08)		0.95 (0.23, 2.74)	
BMI			0.58		0.63
<25	T1	1		1	
	T2	0.50 (0.18, 1.41)		1.03 (0.24, 4.31)	
	T3	0.27 (0.08, 0.92)		0.90 (0.23, 3.62)	
25–30	T1	1		1	
	T2	1.14 (0.46, 2.85)		0.66 (0.28, 1.59)	
	T3	0.68 (0.22, 2.11)		0.33 (0.13, 0.86)	
>30	T1	1		1	
	T2	0.40 (0.22, 0.75)		0.70 (0.40, 1.21)	
	T3	0.75 (0.33, 1.73)		0.76 (0.39, 1.51)	
Hypertension			0.91		0.70
Yes	T1	1		1	
	T2	0.57 (0.36, 0.88)		0.68 (0.44, 1.06)	
	T3	1.29 (0.09, 19.67)		0.60 (0.36, 0.99)	
No	T1	1		1	
	T2	0.57 (0.05, 6.35)		1.64 (0.25, 10.79)	
	T3	0.38 (0.03, 4.91)		0.13 (0.06, 2.36)	
Diabetes			0.14		0.44
Yes	T1	1		1	
	T2	0.57 (0.29, 1.15)		1.07 (0.54, 2.13)	
	T3	0.83 (0.34, 2.03)		1.10 (0.51, 2.37)	
No	T1	1		1	
	T2	0.63 (0.36, 1.09)		0.58 (0.33, 1.01)	
	T3	0.39 (0.19, 0.83)		0.41 (0.21, 0.78)	

BMI, body mass index; OR, odds ratio; CI, confidence interval; T, tertile. Model is adjusted for age, sex, race, total energy intake, total protein intake, total fat intake, total vitamin A intake, total vitamin B_6 intake, BMI, educational level, annual household income, smoking status, hypertension, diabetes and total cholesterol (TC) levels. The lowest tertile of dietary fiber intake was used as the reference group.

were more likely to be older, male, white, highly educated, a current smoker, and were less likely to earn a substantial income and consume high dietary nutrition (such as fiber, protein, fat, vitamin A, and vitamin B_6) and total energy. They also tended to have lower total cholesterol but higher BMI and higher hypertension and diabetes prevalence.

In univariate logistic regression (**Table 2**), a consistent association was observed between higher fiber intake (total, cereal, vegetable, and fruit) and lower HF prevalence. These associations remained significant following age, sex, and race adjustments (model 2). After further adjustment for total energy intake, total protein intake, total fat intake, total vitamin A intake, total vitamin B_6 intake educational level, annual household income, smoking status, hypertension, diabetes, TC, and body mass index (model 3), the ORs of HF were 0.49 (95% CI 0.28–0.87, $P_{for\ trend} = 0.016$), 0.59 (95% CI 0.36–0.96, $P_{for\ trend} = 0.032$), 1.19 (95% CI 0.73–1.92, $P_{for\ trend} = 0.439$), and 0.56 (95% CI 0.33–0.94, $P_{for\ trend} = 0.070$) for the highest tertile of the total, cereal, vegetable, and fruit fiber, respectively, as opposed to the lowest tertile. An association remained for the highest tertile of total and cereal fiber with a lowered HF prevalence, while the association between vegetable and fruit fiber with HF was no longer significant.

The dose-response relationship analysis between total, cereal fiber intake, and HF is shown in **Figure 2**. A linear relationship was observed between total dietary fiber intake and HF (*P* for non-linearity = 0.14). HF prevalence significantly decreased with the increase in total fiber intake. This linear inverse association was also observed for the cereal fiber intakes and HF prevalence (*P* for non-linearity = 0.27). The dose-response relationship analysis between vegetable and fruit fiber with HF was not performed as no significant association was observed in the multivariable logistic regression analysis (model 3).

The association between dietary fiber intake and HF in subgroup analyses was displayed in **Table 3**. Subsequent to adjustment for the same covariates in model 3, the subgroup analysis shows that the intake of total and cereal fiber has no significant interaction with sex, race, hypertension, diabetes, and obesity status.

DISCUSSION

This study found an association between higher total and cereal fiber intake and lower HF prevalence in adults. Dose-response analysis showed a linear relationship between total and cereal fiber intake with HF prevalence.

Dietary fiber, common nutrition, has a variety of benefits for several diseases (6). A randomized, crossover study documented lowered hyperinsulinemia and plasma lipid concentrations with a high intake of dietary fiber (mainly the soluble type) to improve glycemic control in patients with type 2 diabetes (17). A meta-analysis encompassing 18 studies and 672,408 participants was indicative of an inverse association between dietary fiber consumption and coronary heart disease risk (18). The risk of stroke was lowered by 60% in an 8-year follow-up study with Japanese patients with type 2 diabetes who were in the fourth quartile of total dietary fiber as opposed to those in the first quartile (19). In a large prospective study with a long follow-up period (16.8 years), an inverse association emerged between all-cause mortality and dietary fiber (20). However, the association between HF and dietary fiber intake was not probed in any such work. Our study investigated this association to reveal a lower HF prevalence with higher total and cereal fiber intakes in a dose-responsive manner.

This study found a linear relationship between total fiber intake and HF. HF risk decreased with the increase in total fiber intake, and the rate reduction slightly plateaued at a consumption of 0.3 g/kg/day of total fiber. Similarly, we found a similar dose-response pattern for cereal fiber intake, the rate reduction plateaued at about 0.2 g/kg/day. This observation is consistent with a previous study demonstrating that the risk of hypertension gradually decreased as total dietary fiber intake increased to 0.35 g/kg/day (21). The dose-response relationship in our study suggests the protective role of dietary fiber intake in reducing HF risk, especially for those who do not meet the recommended intake.

The mechanism by which dietary fiber intake might impact HF remains to be elucidated in detail. Several potential pathophysiological mechanisms may contribute to the association between dietary fiber and HF. First, reductions in blood cholesterol and glucose might be involved in the inverse association between dietary fiber intake and HF (22). A study showed that the β-glucans, derived from dietary fiber, lower bile acid reabsorption which in turn reduces the levels of circulating cholesterol (23). The entrapment of sugar by soluble fiber in the small intestine to form a barrier inhibiting amylase and slower glucose absorption is known to diminish blood glucose and improve insulin sensitivity (24). Second, dietary fiber has been shown to improve intestinal flora and increase colonic fermentation (production of SCFA) in the large bowel (25, 26). Animal models have documented that high dietary fiber can improve intestinal flora and reduce blood pressure, cardiac fibrosis, left ventricular hypertrophy, and delay the process of HF in hypertensive mice (26, 27). In addition, other studies reported that high consumption of dietary fiber increased the abundance of SCFA, which might decrease risk factors for HF, such as insulin resistance (28), chronic inflammation, and metabolic disorders (29). The amalgamation of epidemiological and experimental analyses on dietary fiber facilitates us to propose that increasing the dietary fiber intake can cause betterment in HF.

Our study has important clinical implications. Given the increasing prevalence and disease burden of HF along with the lack of approved pharmacological treatment, it is important to scour for modifiable risk factors and to develop preventive strategies. Previous studies have found significant relationships between dietary nutrients and HF (30, 31). Our findings support a potentially adverse association between total, cereal dietary fiber and HF prevalence. The drafting of questionnaires on dietary fiber intake can help to identify individuals at high risk for HF. These observations emerge to reinforce the current recommendations of increasing dietary fiber consumption as part of a healthy diet to prevent HF.

Our study has several advantages. Our study included a large population of different races, social backgrounds, and geographical areas to assess the association between dietary fiber intake and the prevalence of HF. This allowed us to investigate the relationship between dietary fiber and HF for people from different demographics and social backgrounds. However, a few limitations are also put forth here. First, drawing inferences for causal interpretations is challenged towing to the cross-sectional model of our work. Second, despite controlling several potential confounding factors, the possibility of unmeasured confounding factors causing residual confusion remains. Third, the association between specific fiber types (i.e., soluble and insoluble fiber) and HF was not probed which may impact the HF risk.

CONCLUSION

A higher intake of total and cereal dietary fiber was associated with a lower prevalence of heart failure in adults, in a dose-response manner. These findings provide further support for the current recommendations that promote increased consumption of dietary fiber to prevent HF.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was reviewed and approved by the National Center for Health Statistics Research Ethics Review Board, and written informed consent was obtained from all NHANES participants.

AUTHOR CONTRIBUTIONS

HZha and ZL contributed to conception and design of the study. DG, HZho, and ZM acquired the data. XZ, YX, and XW performed the statistical analysis. JC and QZh wrote the first draft of the manuscript. QZe and DX critically revised the manuscript. All authors read and approved the final manuscript.

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Association Between Prognostic Nutritional Index and Prognosis in Patients With Heart Failure: A Meta-Analysis

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Background: The prognostic nutritional index (PNI) has been proposed as a marker of malnutrition and associated with the prognosis of cardiovascular disease. However, whether PNI can serve as a potential biomarker for the prognosis of heart failure (HF) upon those established risk factors were still controversial. This meta-analysis aimed to generate comprehensive evidence on the prognostic value of PNI in patients with HF.

Methods: Multiple databases (PubMed, Embase, the Cochrane Library, and Google Scholar) were searched for related studies up to January 31, 2022. Observational studies accessed associations between PNI levels and the prognosis in patients with HF were included for meta-analysis. The hazard ratios (HRs) and 95% confidence intervals (CI) were calculated.

Results: Fourteen studies, comprising 19,605 patients with HF were included for meta-analysis. The median follow-up duration was 18.5 months. Compared with those with higher PNI (normal nutritional status), patients with HF with lower PNI (malnourished) were associated with a higher risk of all-cause mortality (HR 1.53, 95% CI 1.27–1.85) and composite major adverse cardiac outcomes (MACEs; HR 2.26, 95% CI 1.54–3.31) in the multivariable-adjusted model. Furthermore, when PNI was defined as per 1 increment as a continuous metric, higher PNI was associated with a decrease in all-cause mortality (per 1 increment of PNI: HR 0.94, 95% CI 0.88–0.96) and MACEs (per 1 increment of PNI: HR 0.97, 95% CI 0.95–0.98).

Conclusions: The PNI can serve as an easily calculated bedside "malnutrition-inflammation" biomarker in HF. Lower PNI was associated with a worse prognosis in patients with HF.

Keywords: prognostic nutritional index, heart failure, all-cause mortality, cardiovascular disease, prognosis

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INTRODUCTION

Heart failure (HF) is the end state of various heart diseases. It was estimated that over 37.7 million individuals were affected with HF worldwide (1, 2). During the past decades, there have been significant improvements in the management of HF, especially with the use of new drugs, including angiotensin receptor neprilysin inhibitors and sodium-glucose cotransporter-2 inhibitors, and the

prognosis of HF had been improved (3). However, the risk of mortality and re-hospitalization are still high, which become a growing public health burden worldwide (4–6). Therefore, it was important to further address novel pathogenic mechanisms, prognostic factors, and treatment modalities in HF (7–9).

Malnutrition is an important prognostic factor in HF. It was estimated that up to 50% of patients with HF were malnourished (10). Current guidelines for the management of HF recommend assessment of nutritional status in chronic HF (3, 11); however, there is no consensus on the tools or metrics for measuring malnutrition. Recently, a novel and simple metric, the prognostic nutritional index (PNI), had been proposed to evaluate the nutritional status in multiple clinical settings, including cancers, and post-operative pneumonia (12, 13). Recent studies also showed that lower PNI is associated with an increased risk of mortality or major adverse cardiac events (MACEs) in patients with acute or chronic HF (14–18). However, whether PNI can serve as a potential biomarker for the prognosis of HF upon those established risk factors were still controversial.

Herein, we conducted a systematic review and meta-analysis of observational studies to generate comprehensive evidence on the prognostic value of PNI in patients with HF, and further explore whether such a relationship is modified by other risk factors.

MATERIALS AND METHODS

Search Strategies and Study Selection Criteria

This study was performed under the guideline of the Meta-analysis of Observational Studies in Epidemiology (MOOSE) Group (19). We searched multiple databases (PubMed, Embase, the Cochrane Library, and Google Scholar) from inception until January 31, 2022. The search was developed by combining the MeSH heading and text strategies, with the terms "prognostic nutritional index," "PNI" or "malnutrition" and "heart failure," "cardiac failure," "myocardial failure," "cardiac dysfunction," or "myocardial dysfunction." The search was limited to human studies and writing in English or Chinese. We also manually checked the reference lists of the included studies to identify other potential related articles.

The inclusion criteria for meta-analysis were: (1) observational studies involving adult participants with a diagnosis of HF; (2) the PNI was recorded at enrolment; and (3) the association between PNI score and the prognosis [including all-cause mortality or composite major adverse cardiac outcomes (MACEs)] of HF were reported during follow-up. The PNI value was calculated as: PNI $= 10 \times$ serum albumin concentration (g/d6) $+ 0.005 \times$ total lymphocyte count (per mm^3) (20).

The exclusion criteria were: (1) cross-sectional studies without follow-up evaluation; (2) reported other nutritional indexes but not PNI; and (3) duplicated publications from the same observational studies in such situations, only the most recently published data were included for analysis.

Data Extraction and Study Quality Evaluation

Two researchers (CM and JW) conducted the literature searching and item screening independently. Potentially related articles were reviewed and study information was extracted into a predefined form. We evaluated the quality of the included studies according to NOS (the Newcastle–Ottawa Quality Assessment Scale for cohort studies), which assesses the selection, comparability, and exposure/outcome, respectively (21). Up to a maximum of nine points can be awarded in NOS and we defined studies were graded in quality as poor ($<$4 points), fair (4–6 points), or good ($\geq$7 points), respectively (7, 22).

Statistical Analysis

We assessed whether PNI was associated with the prognosis in patients with HF. The primary outcome was all-cause mortality. The secondary outcome was MACEs, including all-cause mortality, and HF re-hospitalization or all-cause re-hospitalization. The association of the outcomes and PNI value was reported in different ways in the included studies, such as per 1 increment as a continuous metric; or as normal/malnutrition in classified trait. Therefore, we calculated the hazard ratios (HRs) for per 1 score increment in PNI level, and also pooled data as malnutritional vs. normal nutritional status, respectively. To explore the effect of confounders on the estimated risks, unadjusted and multivariable-adjusted relative risks were both calculated. If multiple results were reported from different statistical adjustment models, we extracted the data which had adjusted the maximal number of confounders for analysis.

The HRs and their corresponding standard errors (SEs) were pooled by the inverse variance approach. In case outcomes were presented as odds ratios (ORs) or risk ratios (RRs), they were used as an approximate HR in meta-analysis (7, 22). We use the I^2 statistics to test heterogeneity, and an I^2 value of $>$50% or P for heterogeneity $<$0.1 was considered as statistically significant heterogeneity. A random-effects model was used to combine the pooled estimates if significant heterogeneity was observed. Otherwise, a fix-effects model was used.

Due to the limited number of available studies, we did not perform subgroup analyses for the association between PNI and the prognosis in patients with HF. The sensitivity analyses were conducted by interchanging the statistical models (random-effects models vs. fixed-effects models) or by omitting one study at a time. Publication bias was evaluated by inspecting the funnel plot for the outcomes. Due to the limited number of studies for each analysis (all $n < 10$), we did not further perform Begg's test or Egger's test to explore the publication bias. All the meta-analyses were conducted using RevMan 5.3 (The Cochrane Collaboration, Copenhagen, Denmark). We consider a P-value $<$0.05 as statistically significant.

RESULTS

Studies Retrieved and Characteristics

We retrieved 4,254 related articles from the databases. After deleting the duplicate items, two authors independently screened the titles and abstracts and reviewed the full text of 51

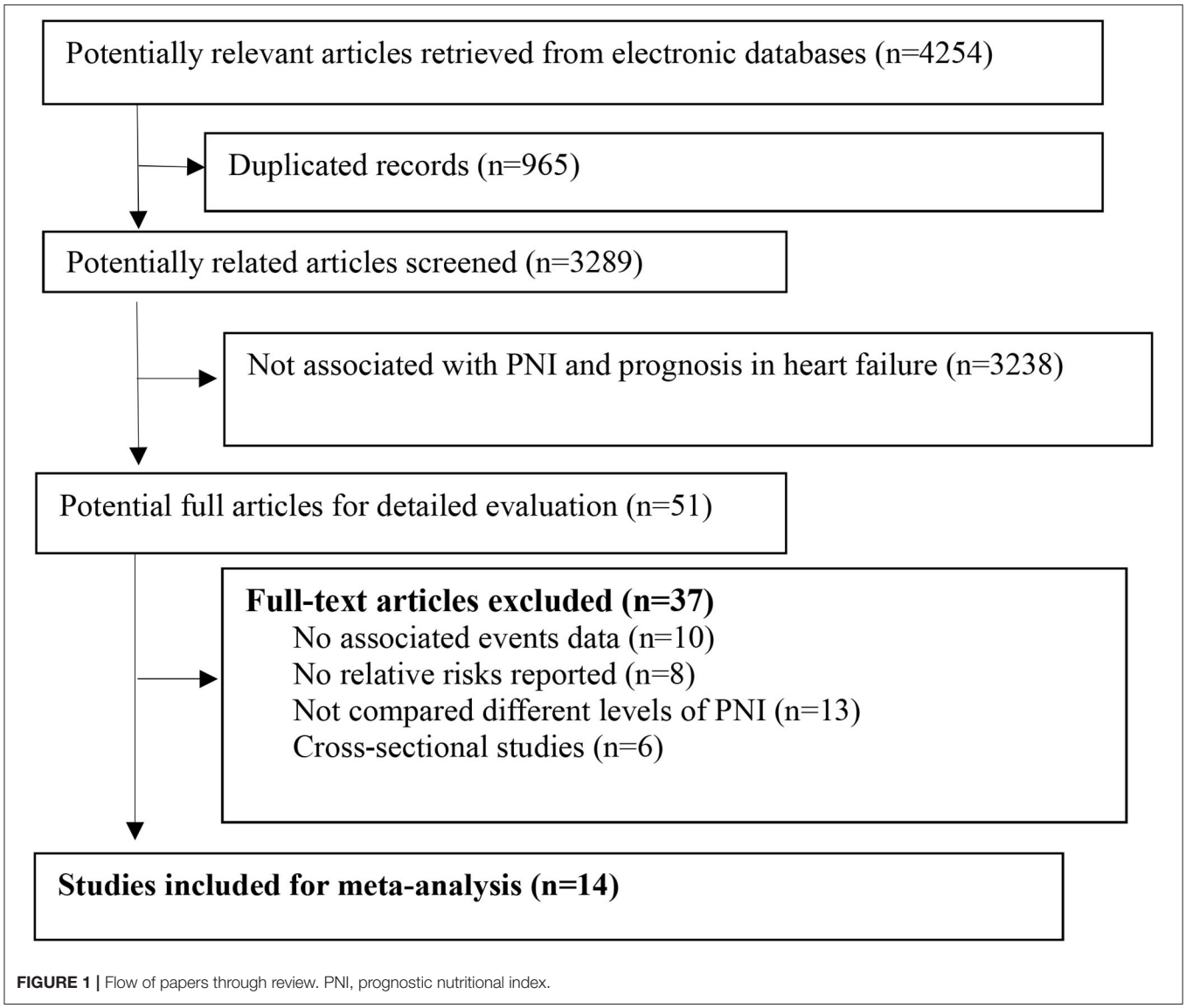

FIGURE 1 | Flow of papers through review. PNI, prognostic nutritional index.

articles (**Figure 1**). Finally, according to the predefined inclusion and exclusion criteria, we included 14 studies comprising 19,605 patients with HF for analysis (14–18, 20, 23–30). The median follow-up duration was 18.5 months. The main baseline characteristics of the included studies are presented in **Table 1**. Most of the included studies adjusted the important confounders for the prognosis of HF (**Supplementary File 1**). According to the predefined NOS assessment, we graded 4 studies with fair study quality and 10 studies with good quality (**Supplementary File 2**).

PNI and Risk of All-Cause Mortality in Patients With HF

In the unadjusted model, patients with lower PNI (malnourished) were associated with a 108% increase in all-cause mortality (HR 2.08, 95% CI 1.63–2.64; $I^2 = 89\%$, P for heterogeneity <0.001), compared with those with higher PNI (normal nutritional status; **Figure 2**). In the multivariable-adjusted model, the association between lower PNI and risk of all-cause mortality was still with statistical significance (HR 1.53, 95% CI 1.27–1.85; $I^2 = 71\%$, P for heterogeneity = 0.004; **Figure 3**).

When PNI was defined as per 1 increment as a continuous metric, we observed that higher PNI was associated with a decrease in all-cause mortality in the unadjusted model (per 1 increment of PNI: HR 0.90, 95% CI 0.87–0.94; $I^2 = 93\%$, P for heterogeneity <0.001; **Figure 4**), as well as in the multivariable-adjusted model (per 1 increment of PNI: HR 0.94, 95% CI 0.88–0.96; $I^2 = 78\%$, P for heterogeneity <0.001; **Figure 5**).

PNI and Risk of MACEs in Patients With HF

Patients with lower PNI were associated with a 154% increase in MACEs (HR 2.54, 95% CI 1.48–4.38; $I^2 = 85\%$, P for heterogeneity <0.001), compared with those with higher PNI in the unadjusted model (**Figure 6**). In the multivariable-adjusted

TABLE 1 | Characteristics of studies evaluated the association between prognostic nutritional index (PNI) and the outcomes in patients with heart failure (HF).

Study	Country/region	Study design	Cohort characteristics	PNI covariate definition	Sample size (% women)	Age (year) (mean or median)	Follow-up (months)	Events for analysis
Kawata et al. (14)	Japan	Retrospective cohort study	AHF	Continuous	141 (46.8%)	84	12.0	MACEs
Ju et al. (15)	China	Retrospective cohort study	AHF	Continuous	8,893 (55%)	81	3.0	All-cause mortality
Çinier et al. (16)	Turkey	Retrospective cohort study	HFrEF	Quartiles	1,100 (20.5%)	60.9	48.0	All-cause mortality
Sze et al. (17)	UK	Prospective cohort study	CHF	Continuous Malnourished (PNI ≤38.0)	467 (33.0%)	76.0	18.5	All-cause mortality MACEs
Candeloro et al. (18)	Italy	Prospective cohort study	AHF	Continuous Malnourished (PNI ≤34)	344 (54.1%)	84.0	5.3	All-cause mortality
Alataş et al. (23)	Turkey	Retrospective cohort study	AHF	Malnourished (PNI ≤41.2)	628 (53.7%)	74.7	NA	All-cause mortality
Zencirkiran and Kahraman (24)	Turkey	Prospective cohort study	HFpEF	Malnourished (PNI < 37.0)	285 (54.4%)	68.0	12.0	MACEs
Chien et al. (25)	China	Retrospective cohort study	HFpEF	Malnourished (PNI < 38.0)	1,120 (60.6%)	77.2	41.8	All-cause mortality MACEs
Takikawa et al. (26)	Japan	Prospective cohort study	AHF	Malnourished (PNI < 38.0)	457 (46.6%)	79.0	12.0	All-cause mortality
Sze et al. (27)	UK	Prospective cohort study	CHF	Malnourished (PNI < 38.0)	3,386 (39.0%)	75.0	52.4	All-cause mortality
Shirakabe et al. (28)	Japan	Retrospective cohort study	AHF	Malnourished (PNI < 38.0)	458 (34.0%)	76.0	12.0	All-cause mortality
Cheng et al. (29)	China	Prospective cohort study	AHF	Continuous Malnourished (PNI ≤44.8)	1,673 (32.0%)	76.0	31.5	All-cause mortality
Sze et al. (30)	UK	Prospective cohort study	AHF	Malnourished (PNI ≤ 38.0)	265 (38.0%)	82.0	19.9	All-cause mortality
Narumi et al. (20)	Japan	Prospective cohort study	CHF	Malnourished (PNI ≤ 38.0)	388 (40.0%)	69.6	28.4	MACEs

AHF, acute heart failure; CHF, chronic heart failure; HF, heart failure; HFrEF, heart failure with preserved ejection fraction; HFpEF, heart failure with preserved ejection fraction; MACEs, major adverse cardiac events; NA, not available; PNI, prognostic nutritional index.

model, lower PNI was still associated with an increased risk of MACEs (HR 2.26, 95% CI 1.54–3.31; I^2 = 64%, P for heterogeneity = 0.04; **Figure 7**).

Only two studies defined PNI as per 1 increment as a continuous metric and evaluated the association between the risk of MACEs. Higher PNI was associated with a decrease of MACEs in the unadjusted model (per 1 increment of PNI: HR 0.95, 95% CI 0.91–1, **Supplementary File 3**), as well as in the multivariable-adjusted model (per 1 increment of PNI: HR 0.97, 95% CI 0.95–0.98, **Supplementary File 4**).

Sensitivity Analyses and Publication Bias Evaluation

Our sensitivity analyses confirmed that the association of PNI with the risks of all-cause mortality and MACEs did not change with the use of fixed-effects models for the random-effects models or recalculation of the relative risks by omitting one study at a time in the meta-analysis. We did not observe significant publication bias for all-cause mortality or MACEs associated with PNI by visual inspection of the funnel plot (**Supplementary Files 5–12**).

DISCUSSION

To the best of our knowledge, this is the first meta-analysis evaluating the prognostic effect of PNI in patients with HF. We found that PNI can serve as an important biomarker in predicting a worse prognosis, including all-cause mortality and/or re-hospitalization in HF, either used as a continuous level or a category metric for defining the nutritional status. Although attenuated, the association between PNI and prognosis of HF was still significant after adjustment for multiple confounders, indicating that the prognostic effect of PNI was independent of other established risk factors.

Malnutrition is common in patients with HF and has been proposed as a modifiable risk factor for improving the prognosis in these patients (31). Nutritional intervention in HF can improve the life quality and reduce the risk of mortality (32).

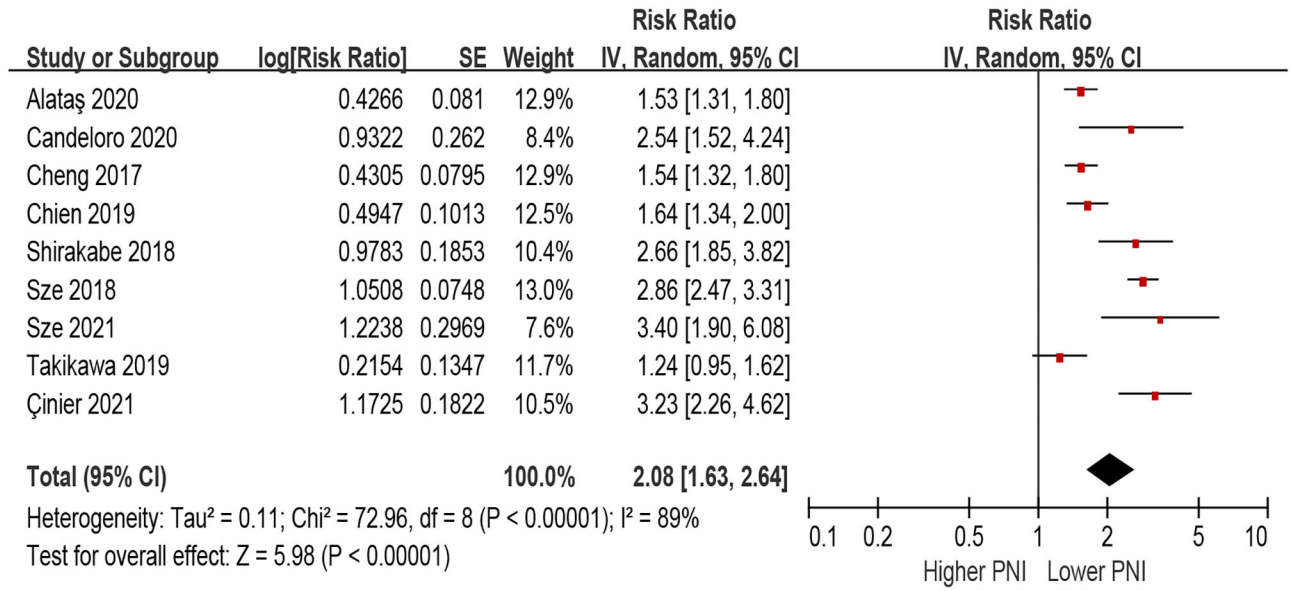

FIGURE 2 | The association between PNI (defined as lower vs. higher) and the risk of all-cause mortality in HF (unadjusted model). CI, confidence interval; HF, heart failure; PNI, prognostic nutritional index.

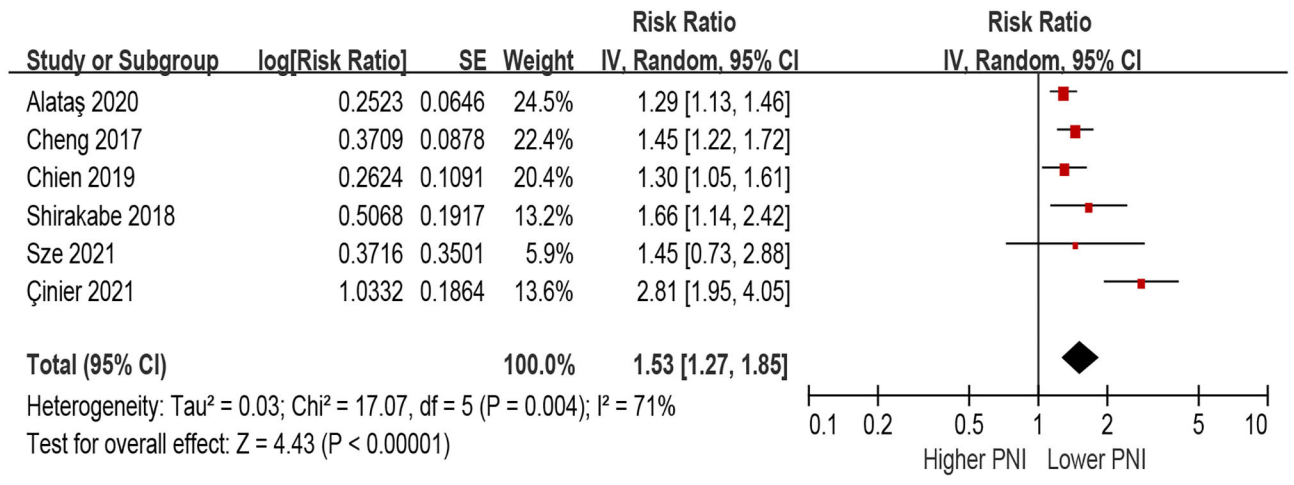

FIGURE 3 | The association between PNI (defined as lower vs. higher) and the risk of all-cause mortality in HF (multivariable-adjusted model). CI, confidence interval; HF, heart failure; PNI, prognostic nutritional index.

Therefore, it is important to evaluate the nutritional status and aid in risk stratification and treatment strategies in the clinical setting. Some multi-dimensional nutritional screening tools, including the Nutritional Risk Screening (NRS-2002), and Mini Nutritional Assessment–Short Form (MNASF) have been developed for evaluating the nutritional risks in patients with HF (33, 34). However, these tools include multiple clinical indexes and dietary factors extracted by questionnaires, which are time-consuming to perform, with limited generalizability in routinely use, and may be confounded by recall bias from the patients.

In contrast, the PNI is a simple metric that can be easily calculated from serum albumin level and lymphocyte count, which are available in most clinical laboratories. Lower PNI (malnutrition) in patients with HF may be contributed to both reduced albumin and lymphocyte count. The components of the PNI, serum albumin, and lymphocyte count, may indicate independent prognostic information in patients with HF. Reduced serum albumin levels may be influenced by malnutritional status or an indication of renal and liver dysfunction. Low lymphocyte count could indicate immune hypo-responsiveness and increased cortisol levels, which could harm the prognosis of HF. Therefore, a lower PNI is not only a metric of malnutrition but also an indication of pro-inflammation status, which provides objective

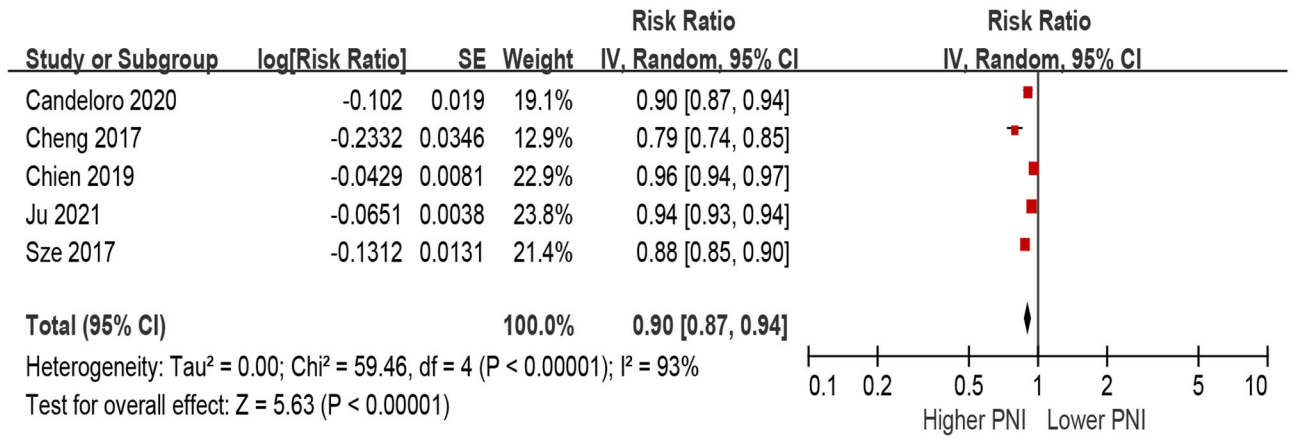

FIGURE 4 | The association between PNI (defined as per 1 increment) and the risk of all-cause mortality in HF (unadjusted model). CI, confidence interval; HF, heart failure; PNI, prognostic nutritional index.

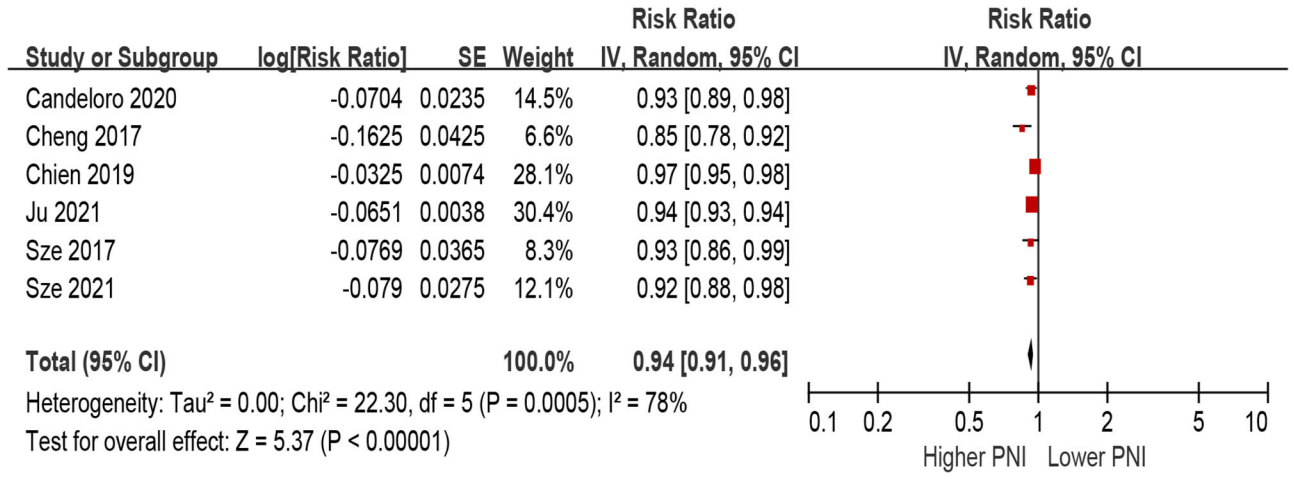

FIGURE 5 | The association between PNI (defined as per 1 increment) and the risk of all-cause mortality in HF (multivariable-adjusted model). CI, confidence interval; HF, heart failure; PNI, prognostic nutritional index.

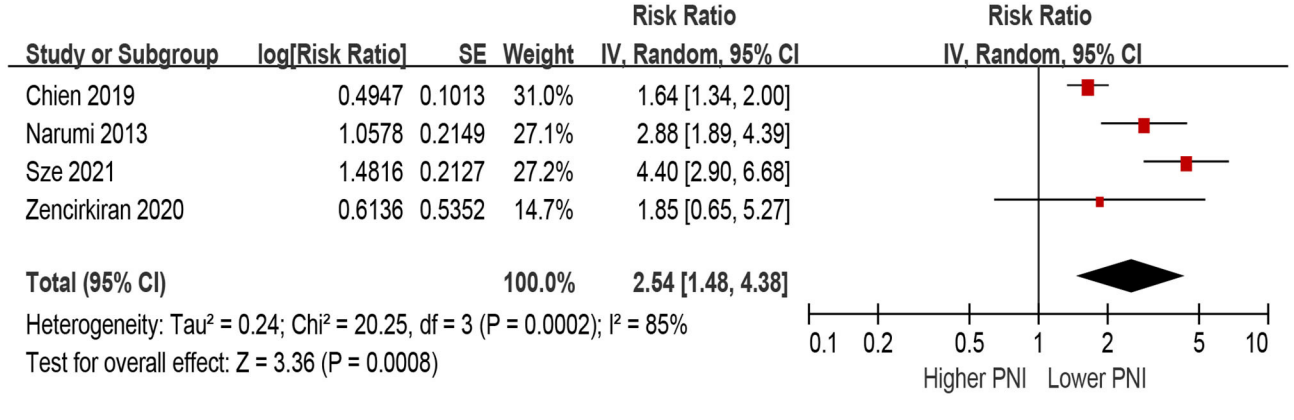

FIGURE 6 | The association between PNI (defined as lower vs. higher) and the risk of major adverse cardiac outcomes (MACEs) in HF (unadjusted model). CI, confidence interval; HF, heart failure; MACEs, major adverse cardiac outcomes; PNI, prognostic nutritional index.

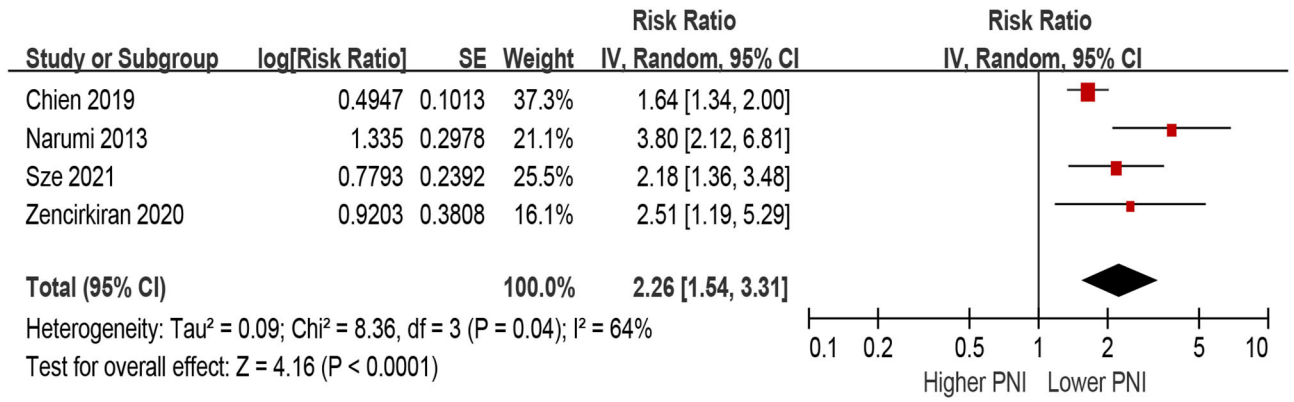

FIGURE 7 | The association between PNI (defined as lower vs. higher) and the risk of MACEs in HF (multivariable-adjusted model). CI, confidence interval; HF, heart failure; MACEs, major adverse cardiac outcomes; PNI, prognostic nutritional index.

information on "malnutrition-inflammation" complex syndrome for determining increased mortality in HF (33, 34). Similar to PNI, some other simple blood-based biomarkers have also been used to assess the nutritional status in HF, such as the controlling nutritional status (CONUT) score (25, 35), and geriatric nutritional risk index (GNRI) (36, 37). However, the CONUT score is calculated from serum albumin, lymphocyte count, and cholesterol, which may be confounded by the broad use of statin in patients with HF. The GNRI requires body weight for calculation, which will be confounded by fluid overload status and dramatic change in HF treatment during hospitalization. Therefore, the PNI may be a more suitable index that can be easily calculated using routine laboratory parameters and offers a practical tool for evaluating the nutritional risk of HF. However, which index is better for predicting the prognosis in HF needed further exploration. Furthermore, the change of PNI during hospitalization would be important to evaluate the association between this metric and the prognosis of HF. Kawata et al. (14) showed that the changes in PNI on admission and at discharge during hospitalization, were associated with 1-year mortality in patients with acute HF. These findings further support the use of PNI to guide the risk stratification in HF.

Some limitations should be noted in the current study. First, the type and definition of HF were different in the included studies, which may contribute to the clinical heterogeneity. Second, the underlying inflammation status was not adjusted in most of the included studies. Therefore, it is difficult to attribute the prognostic effect of PNI to only malnutritional status or a combination of inflammation status. Third, we did not have individual patient data to perform the comprehensive subgroup analysis. Fourth, the cut-point for malnutritional status with PNI was different in the included studies, which would be an important heterogeneity among the studies.

In conclusion, PNI can serve as an easily calculated bedside "malnutrition-inflammation" biomarker in HF. Lower PNI was associated with a worse prognosis in patients with HF. Future studies are needed to explore whether treatment targeting improving the PNI can provide a positive effect on the prognosis of HF.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

J-DW, J-QL, M-YC, and J-XW: research idea and study design. M-YC, J-XW, M-TL, and X-YJ: data acquisition. J-DW, X-LW, Z-WX, and J-QL: data analysis/interpretation. X-LW and Z-WX: statistical analysis. J-DW and J-QL: supervision and mentorship. All authors contributed important intellectual content during manuscript drafting or revision and accept accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.918566/full#supplementary-material

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Triglyceride-Glucose Index and Homeostasis Model Assessment-Insulin Resistance in Young Adulthood and Risk of Incident Congestive Heart Failure in Midlife: The Coronary Artery Risk Development in Young Adults Study

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Objective: This study aimed to assess the association between triglyceride-glucose (TyG) index/homeostasis model assessment-insulin resistance (HOMA-IR) within young adults and congestive heart failure (CHF), and to explore whether TyG index can replace HOMA-IR as a surrogate marker for IR in predicting the risk of CHF.

Methods: A total of 4,992 participants between the ages of 18 and 30 years were enrolled from the Coronary Artery Risk Development in Young Adults (CARDIA) investigation [from 1985 to 1986 (year 0)]. A Cox proportional hazard regression analysis was conducted for assessing correlations between baseline TyG index/HOMA-IR and CHF events, together with the receiver operating characteristic (ROC) curve employed for scrutinizing TyG index/HOMA-IR and the risk of CHF.

Results: During the 31-year follow-up period, 64 (1.3%) of the 4,992 participants developed CHF. In multivariable Cox proportional hazards models, adjusted for confounding factors for CHF, an increased risk of CHF was associated with a per-unit increase in the TyG index [hazard ratio (HR) 2.8; 95% confidence interval (CI), 1.7–4.7] and HOMA-IR (HR 1.2; 95% CI, 1.1–1.3). A Kaplan–Meier curve analysis showed that participants in the TyG index and HOMA-IR index Q4 group had a higher risk of CHF than those in the Q1 group. The area under curve (AUC) for the TyG index and HOMA-IR consisted of 0.67 (95% CI, 0.6–0.742) and 0.675 (95% CI, 0.604–0.746), respectively.

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There were no significant differences between the TyG index and HOMA-IR for AUC (p = 0.986).

Conclusion: The higher TyG index and HOMA-IR are independent risk factors for CHF. The TyG index can replace HOMA-IR in young adulthood as a surrogate marker for IR to predict the risk of CHF.

Keywords: triglyceride-glucose index, HOMA-IR, insulin resistance, congestive heart failure, the CARDIA study

INTRODUCTION

Congestive heart failure (CHF) is a global issue within the public-health scenario and it is also the main causative agent for mortality (1, 2). Many risk parameters, such as diabetes, hypertension, and coronary heart disease, are considered to be intimately related to heart failure (2, 3). Consequently, it is very imperative to identify any risk factors for heart failure early, followed by a timely treatment, to prevent or regulate any progress to heart failure. There is a close interplay between insulin resistance (IR) and the etiology and clinical manifestation of heart failure (4). IR is very common in patients with heart failure (5, 6). The biological effects of exacerbated IR can, in turn, lead to the development or exacerbation of heart failure (7). Multiple investigations demonstrated that IR is an independent risk factor for heart failure (8, 9). Consequently, early diagnosis of IR can predict the manifestation of future heart failure events. Methods for evaluating IR include the euglycemic-hyperinsulinemic clamp test, the quantitative insulin sensitivity check index, HOMA-IR, 1/insulin, and Matusda index (10). Although the euglycemic-hyperinsulinemic clamp test is considered to be an accurate and reliable method for the assessment of IR, such a test is time consuming, complex, and carries an increased financial running cost, rendering it a challenge for such a test to be implemented and promoted within routine clinical practice (11, 12). HOMA-IR has become the most commonly used indicator for the clinical assessment of IR (13). Triglyceride glucose (TyG) index derived from triglyceride and glucose, which was recently proposed as a reliable and inexpensive biomarker for predicting IR (as an alternative to euglycemic-hyperinsulinemic clamp test and HOMA-IR evaluation), has been used within the clinical setting and subject to focus by cardiovascular disease researchers (14). Recent investigations provided further evidence for the clinical manifestation risk of CHF, together with hypertension, ischemic stroke, arteriosclerosis, diabetes, and coronary heart disease being correlated with TyG index (15, 16). However, it is unclear whether the TyG index can predict the occurrence of heart failure. To answer this question, this study focused on the cohort from the Coronary Artery Risk Development in Young Adults (CARDIA) study to longitudinally observe whether there was an association between the TyG index/HOMA-IR and CHF. In addition, this study evaluated whether the TyG index could replace HOMA-IR as the main classifier of IR for predicting CHF event risk.

Abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults; TyG, Triglyceride-glucose; HOMA-IR, Homeostasis model assessment-insulin resistance; CHF, Congestive heart failure; ROC, Receiver operating characteristic; HR, Hazard ratio; CI, Confidence interval; AUC, Area under the curve; IR, Insulin resistance; BMI, Body mass index; eGFR, Estimate the glomerular filtration rate; CKD, Chronic kidney disease; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; TC, Total cholesterol.

MATERIALS AND METHODS

Study Population

From 1985 to 1986 (year 0), the CARDIA multi-center randomized, prospective cohort study was conducted, enrolling 5,115 African-American and Whites aged 18–30 years from the general population or selected census areas from four research centers in the United States. All participants were investigated at years 2, 5, 7, 10, 15, 20, 25, and 30, respectively. The institutional review committee from each research center accepted the research scheme and informed consent from all individual cohort participants was obtained in writing. The baseline data for this study used the 0-year examination data, for a total of 5,114 participants (one patient withdrew consent). Following the exclusion of patients with incomplete clinical data (missing fasting blood glucose, triglyceride, insulin, and missing endpoint records), a total of 4,992 patients formed part of the final analytical queue (**Supplementary Figure 1**). Patients were grouped into four groups, depending on the TyG index quartiles.

Triglyceride-Glucose Index, Homeostasis Model Assessment-Insulin Resistance, and Congestive Heart Failure

Participants at 0 year fasted for at least 8 h, immediately followed by blood collection using an EDTA vacuum vessel. Consequently, plasma was isolated and frozen at −70°C prior to shipping to the laboratory using dry ice. Glucose was determined at baseline using hexokinase UV, calibrated, and followed by the enzymatic analysis of triglyceride levels (17). The TyG index was determined as: Ln [fasting triglycerides (mg/dl) × fasting blood glucose (mg/dl)/2] (18). HOMA-IR was determined as: fasting blood glucose (mmol/L) × fasting serum insulin (μU/ml)/22.5 (19).

Diagnostic validation of CHF necessitated a finalized CHF diagnosis by a physician, together with the implementation of CHF clinical management protocols during the patient hospitalization period (i.e., diuretic/s + digoxin/Glycerin tri-nitrate, hydralazine, ACE-inhibitor/s, or angiotensin receptor blocker/s). All patients were monitored until an endpoint of August 2017.

Covariates

Covariates included in the present analysis were obtained through established protocols/quality assurance processes throughout all centers involved (20). The education level was stratified as high school or less and more high school. Smoking status was stratified as present and present non-smoking (such as past and never smoking). Hypertension was deemed present upon a systolic blood pressure of ≥ 130 mmHg, diastolic blood pressure of ≥ 90 mmHg, or current consumption of anti-hypertension drug/s (21). Obesity was deemed present upon a body mass index (BMI) ≥ 30 kg/m^2 (22). The dietary modification study equation for renal disease diet was implemented in this study to estimate the glomerular filtration rate (eGFR) within serum creatinine: eGFR (ml/min/1.73 m^2) = 175 × standardized $Scr^{-1.154}$ × $age^{-0.203}$ × 1.212 [if African-American] × 0.742 [if female]. Participants with eGFR < 60 ml/min/1.73 m^2 were deemed to have chronic kidney disease (CKD) (23, 24). Detailed descriptions of measurements for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, serum creatinine, and fasting plasma glucose for all participants were previously published.

Statistical Analyses

Normally distributed continuous data were represented by mean and standard deviation (*SD*), while non-normally distributed continuous data were represented by median with interquartile range (IQR). Categorical variables reported percentage frequency. Participants were classified into four groups according to the quartiles of the TyG index. Wilcoxon or Kruskal–Wallis test were employed for analyzing group variations for continuous variables, while the chi-square test was employed for categorical variables. Smooth curve fittings and scatter plots were used to address the relationship between the TyG index and HOMA-IR. The Cox proportional-hazard regression model was employed to determine hazard ratio (HR) and 95% CI for CHF events by quartiles of the TyG index, and HOMA-IR, respectively. The proportional hazard assumption was evaluated by the visualization of Schoenfeld residuals, where such analytical outcomes indicated no evidence of assumption breaches (**Supplementary Table 1**). Multi-collinearity was investigated using variance inflation factors, while TC was removed as a significant variance inflation factor (≥5). Three models were fitted: model 1 was not adjusted; model 2 was adjusted for age, sex, and race; and model 3 was adjusted for variables included in the model 2 and education level, smoking status, hypertension, diabetes mellitus, hypercholesteremia, systolic and diastolic blood pressure, obesity, CKD, HDL-C, and LDL-C. Trend *p*-values were evaluated by a median value within each quartile, as a continuous variable. Kaplan–Meier curve data outcomes were employed for determining the cumulative incidence of CHF events through both the TyG index and HOMA-IR quartiles, with estimation variations being comparatively analyzed through log-rank protocols. The receiver operating characteristic (ROC) curve and area under the curve (AUC) were used to assess both the TyG index-based and HOMA-IR-based capacity for predicting CHF event risk during follow-up. The participants were divided into subgroups according to sex, race, education, obesity, smoking status, hypertension, and CKD status. The results were scrutinized following adjustments for age, sex, race, education, obesity, smoking status, hypertension, diabetes mellitus, hypercholesteremia, CKD, LDL-C, and HDL-C, except for the subgroup variable. All statistical analyses were conducted using R® software (version 4.0.3).[1] The study deemed that *p*-values less than 0.05 (bilateral) conferred statistical significance.

RESULTS

Table 1 shows the baseline characteristics for the total-participating patient cohort, together with the quartile TyG index. During a median (IQR) follow-up of 31 (30.8–31.2) years, 64 out of 4,992 participants (1.3%) developed CHF, with an annual incidence of 41.4/100,000 individuals. With the increase in the quartile of the TyG index of participants, the CHF events increased significantly, from 20.7/100,000 individuals in quartile Q1 to 82.7/100,000 in quartile Q4. The median (IQR) of the TyG index quartiles were 7.3 (7.1–7.4), 7.7 (7.6–7.8), 8.0 (7.9–8.1), and 8.4 (8.3–8.7), respectively. With decreasing quartiles of TyG index, the prevalence of obesity, hypercholesteremia, hypertension, and current smoking were progressively higher (all $p < 0.001$), paralleling the progressive increase of triglycerides, systolic and diastolic blood pressure, HOMA-IR, insulin, HDL-C, LDL-C, TC, and fasting blood glucose (all $p < 0.001$). Conversely, the proportion of women and Whites was progressively lower with the increasing quartiles of the TyG index (all $p < 0.001$).

The scatter plots and smooth curve fittings in **Figure 1** demonstrated the relationship between TyG index and HOMA-IR, where the TyG index was intimately linked to HOMA-IR ($R = 0.339$, $p < 0.001$).

On completing risk analysis, the cumulative CHF incidence among the TyG index and HOMA-IR quartile are illustrated in **Figure 2**. Participants in the quartile of the TyG index Q4 were at an increased risk of CHF events in comparison to the Q1 group throughout the clinical monitoring timeframe (log-rank test, $p < 0.001$; **Figure 2A**). Similar results were observed for HOMA-IR (**Figure 2B**).

The risk of CHF events was increased, with a per-unit increase in the TyG index and HOMA-IR illustrated in **Table 2**. In the non-adjusted model (model 1), per-unit increase in the TyG index correlated to a threefold increase for the risk of CHF (HR 3.0, 95% CI 2.1–4.4). In model 2, adjusted according to race, sex, and age, per-unit increase in the TyG index increased the risk of CHF by a 3.4-fold (HR 3.4, 95% CI 2.3–5.0). In model 3, the risk of CHF event with a per-unit increase in the TyG index was still considerable post-modification for possible confounding factors, with the HR being 2.8-fold (HR 2.8, 95% CI 1.7–4.7). The values of HR for HOMA-IR Models 1, 2, and 3 were 1.2, 1.2, and 1.2, respectively

[1] http://www.R-project.org/

TABLE 1 | Clinical characteristics of the study population according to the TyG index.

Variables	Overall	Quartiles of TyG index				*P*-value
		Q1	Q2	Q3	Q4	
No. of participants	4,992	1,247	1,246	1,251	1,248	
TyG index	7.8 (7.5–8.2)	7.3 (7.1–7.4)	7.7 (7.6–7.8)	8.0 (7.9–8.1)	8.4 (8.3–8.7)	< 0.001
Age, year	25.0 (22.0–28.0)	25.0 (22.0–28.0)	25.0 (22.0–28.0)	25.0 (22.0–28.0)	26.0 (23.0–28.0)	<0.001
Female, *n* (%)	2721 (54.5%)	807 (64.7%)	737 (59.1%)	676 (54.0%)	501 (40.1%)	<0.001
White, *n* (%)	2562 (51.3%)	754 (60.5%)	680 (54.6%)	618 (49.4%)	510 (40.9%)	<0.001
More high school, *n* (%)	2989 (60.0%)	745 (59.9%)	747 (60.0%)	765 (61.2%)	732 (58.8%)	0.681
Obesity, *n* (%)	578 (11.6%)	77 (6.2%)	105 (8.5%)	148 (11.8%)	248 (20.0%)	<0.001
Systolic BP, mmHg	110.0 (103.0–118.0)	107.0 (101.0–115.0)	108.0 (102.0–116.0)	110.0 (104.0–118.0)	113.0 (106.0–121.0)	<0.001
Diastolic BP, mmHg	68.0 (62.0–75.0)	67.0 (62.0–72.0)	67.0 (62.0–74.0)	69.0 (63.0–75.0)	70.0 (64.0–77.0)	<0.001
Current smoking, *n* (%)	1521 (30.5%)	296 (23.8%)	387 (31.1%)	378 (30.2%)	460 (36.9%)	<0.001
Diabetes mellitus, *n* (%)	37 (0.7%)	7 (0.6%)	6 (0.5%)	6 (0.5%)	18 (1.5%)	<0.001
Hypertension, *n* (%)	975 (19.5%)	182 (14.6%)	185 (14.8%)	263 (21.0%)	345 (27.6%)	<0.001
Hypercholesteremia, *n* (%)	102 (2.1%)	16 (1.3%)	15 (1.2%)	24 (2.0%)	47 (3.9%)	<0.001
CKD, *n* (%)	159 (3.2%)	34 (2.7%)	35 (2.8%)	42 (3.4%)	48 (3.8%)	0.351
HDL-C, mg/dL	52.0 (44.0–61.0)	56.0 (50.0–66.0)	54.0 (47.0–63.0)	51.0 (44.0–59.0)	44.0 (37.0–53.0)	<0.001
TC, mg/dL	174.0 (153.0–197.0)	163.0 (144.0–182.0)	169.0 (150.0–189.8)	176.0 (157.0–200.0)	188.0 (167.0–214.0)	<0.001
LDL-C, mg/dL	106.0 (87.0–127.0)	97.0 (80.0–115.0)	102.0 (85.0–121.0)	110.0 (91.0–131.5)	119.0 (97.0–140.0)	<0.001
Triglycerides, mg/dL	62.0 (45.0–84.0)	38.0 (32.5–42.0)	53.0 (49.0–58.0)	71.0 (66.0–78.0)	108.0 (93.0–140.0)	<0.001
Fasting glucose, mg/dL	81.0 (77.0–87.0)	78.0 (74.0–83.0)	81.0 (76.0–85.0)	82.0 (78.0–87.0)	85.0 (80.0–91.0)	<0.001
HOMA-IR	1.8 (1.2–2.7)	1.4 (1.0–2.0)	1.6 (1.1–2.4)	1.9 (1.3–2.7)	2.4 (1.5–3.6)	<0.001
Insulin, μU/mL	8.8 (6.1–13.0)	7.3 (5.1–10.2)	8.1 (5.8–11.6)	9.3 (6.4–13.5)	11.2 (7.4–16.9)	<0.001
Follow-up time, years	31.0 (30.8–31.2)	31.0 (30.8–31.2)	31.0 (30.8–31.2)	31.0 (30.8–31.2)	31.0 (30.8–31.2)	<0.001
CHF	64 (1.3%)	8 (0.6%)	10 (0.8%)	14 (1.1%)	32 (2.6%)	<0.001
Incidence rate per 100,000	41.4	20.7	25.9	36.1	82.7	<0.001

Values are presented as Median (interquartile range, IQR) or number (%). Abbreviations: BMI, body mass index; Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TyG, Triglyceride-glucose; TC, total cholesterol; CKD, chronic kidney diseases; HOMA-IR, homeostasis model assessment-insulin resistance; CHF, congestive heart failure.

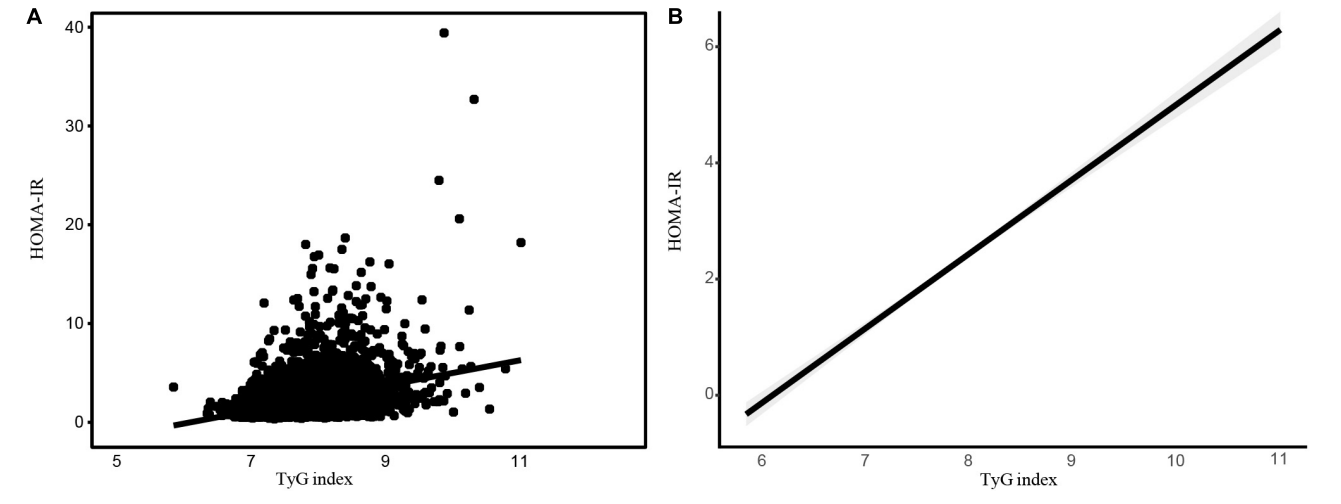

FIGURE 1 | The association between the TyG index and HOMA-IR. The association between the TyG index and HOMA-IR. **(A)** Each black point represents a sample. **(B)** A solid black line represents the smooth curve fit between variables. The gray area represents the 95% of confidence interval (CI) from the fit. Abbreviations: TyG, Triglyceride-glucose; HOMA-IR, homeostasis model assessment-insulin resistance.

($p < 0.001$). The risk of CHF events was still significant, based on the TyG index and the quartile of HOMA-IR (p-trend < 0.001).

Data outcomes from subgroup evaluations are illustrated in **Figure 3**. No significant interactions by sub-groups were observed for the association between sex and race, education

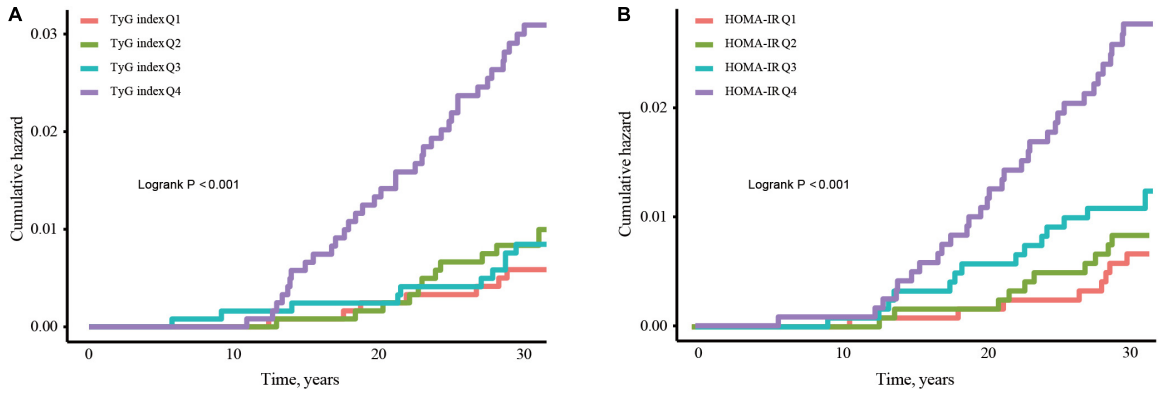

FIGURE 2 | Kaplan–Meier curves for incidence of CHF stratified by quartiles of TyG index **(A)** and HOMA-IR **(B)**. Abbreviations: TyG, Triglyceride-glucose; HOMA-IR, homeostasis model assessment-insulin resistance; CHF, congestive heart failure.

level, obesity, smoking status, hypertension, diabetes mellitus, hypercholesteremia, or CKD status.

The AUCs of the TyG index and HOMA-IR to predict CHF incidence were 0.675 (95% CI, 0.604–0.746) and 0.67 (95% CI, 0.6–0.742), respectively. However, such data outcomes did not exhibit significant variations ($p = 0.986$) (**Figure 4** and **Table 3**).

DISCUSSION

The main results of this prospective observational cohort study of 4,992 young Americans revealed that the TyG index in young adulthood was positively correlated with the incidence of CHF, both pre-and post-adjustments for confounders, and this correlation remained stable even on subgroup analyses, rendering the TyG index to be a potential independent risk factor for CHF. In addition, this investigation validated HOMA-IR as a separate independent risk factor for CHF, in agreement with results previously described in scientific literature. Finally, the study analyzed the AUC of CHF incidence based on the TyG index and HOMA-IR. Through comparative analyses, the TyG index shares the same predicative value as HOMA-IR in predicting CHF incidence. TyG index can be employed as a surrogate marker for IR to predict CHF incidence. The results of this extended, prospective, observational investigation have substantial weight in aiding CHF prophylaxis.

TABLE 2 | Hazard ratio (HR) and 95% confidence intervals (CIs) for CHF according to the TyG index and HOMA-IR.

Variables	HR (95% CI)		
	Model 1	Model 2	Model 3
Quartiles of TyG index			
Q1	1 (reference)	1 (reference)	1 (reference)
Q2	1.3 (0.5, 3.2)	1.3(0.51, 3.3)	1.2 (0.5, 3.1)
Q3	1.8 (0.8, 4.3)	2.0 (0.8, 4.6)	1.7 (0.7, 4.1)
Q4	4.2 (2.0, 9.1)	4.8 (2.2, 10.6)	3.4 (1.4, 8.0)
p-trend	<0.001	<0.001	<0.001
TyG index	3.0 (2.1, 4.4)	3.4 (2.3, 5.0)	2.8 (1.7, 4.7)
Quartiles of HOMA-IR			
Q1	1 (reference)	1 (reference)	1 (reference)
Q2	1.6 (0.6, 4.1)	1.7 (0.6, 4.3)	1.7 (0.6, 4.3)
Q3	1.4 (0.6, 3.8)	1.6 (0.6, 4.0)	1.3 (0.5, 3.5)
Q4	5.3 (2.4, 11.9)	4.4 (2.0, 10.0)	3.2 (1.3, 7.9)
p-trend	<0.001	<0.001	<0.001
HOMA-IR	1.2 (1.1, 1.2)	1.2 (1.1, 1.2)	1.2(1.1, 1.3)

Model 1 does not adjust covariates. Model 2: adjusted for age, sex and race. Model 3: model 2 + adjusted for education, obesity, smoking status, hypertension, diabetes mellitus, hypercholesteremia, CKD, LDL-C and HDL-C. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TyG, Triglyceride-glucose; CKD, chronic kidney diseases; HOMA-IR, homeostasis model assessment-insulin resistance; CHF, chronic heart failure.

It is well acknowledged that IR is intimately linked to the development of heart failure. IR was first identified to be separately linked with the risk of heart failure following the Uppsala longitudinal study of adult men over the age of 70 years (25). In addition, it was reported that IR was capable of predicting the occurrence risk of ventricular systolic and diastolic dysfunction within 20 years among men in their 50s (26, 27). The analyses of IR require complex methods that are challenging to obtain during routine clinical practice (28). HOMA-IR is commonly used for testing IR (13). However, there is no routine measurement of insulin concentration in clinical practice, which leads to HOMA-IR being unsuitable for large-scale clinical implementation. The TgY index is indicative of the metabolic level of triglycerides and glucose, which was first proposed by Simental-Mendía et al. stating that the TyG index can replace the euglycemic-hyper-insulinemic clamp test and HOMA-IR to evaluate IR in healthy participants (18, 29). An increased TyG index is associated with the occurrence of CHF, possibly since IR is recognized as a pivotal player in abnormal glucolipid metabolism (30). Under IR, insulin-mediated glucose uptake in myocytes and adipocytes is impaired, the inhibition against liver glucose production and lipolysis is weakened, while the levels of plasma glucose and triglycerides are increased (31, 32). The increase in blood glucose levels can cause myocardial fibrosis,

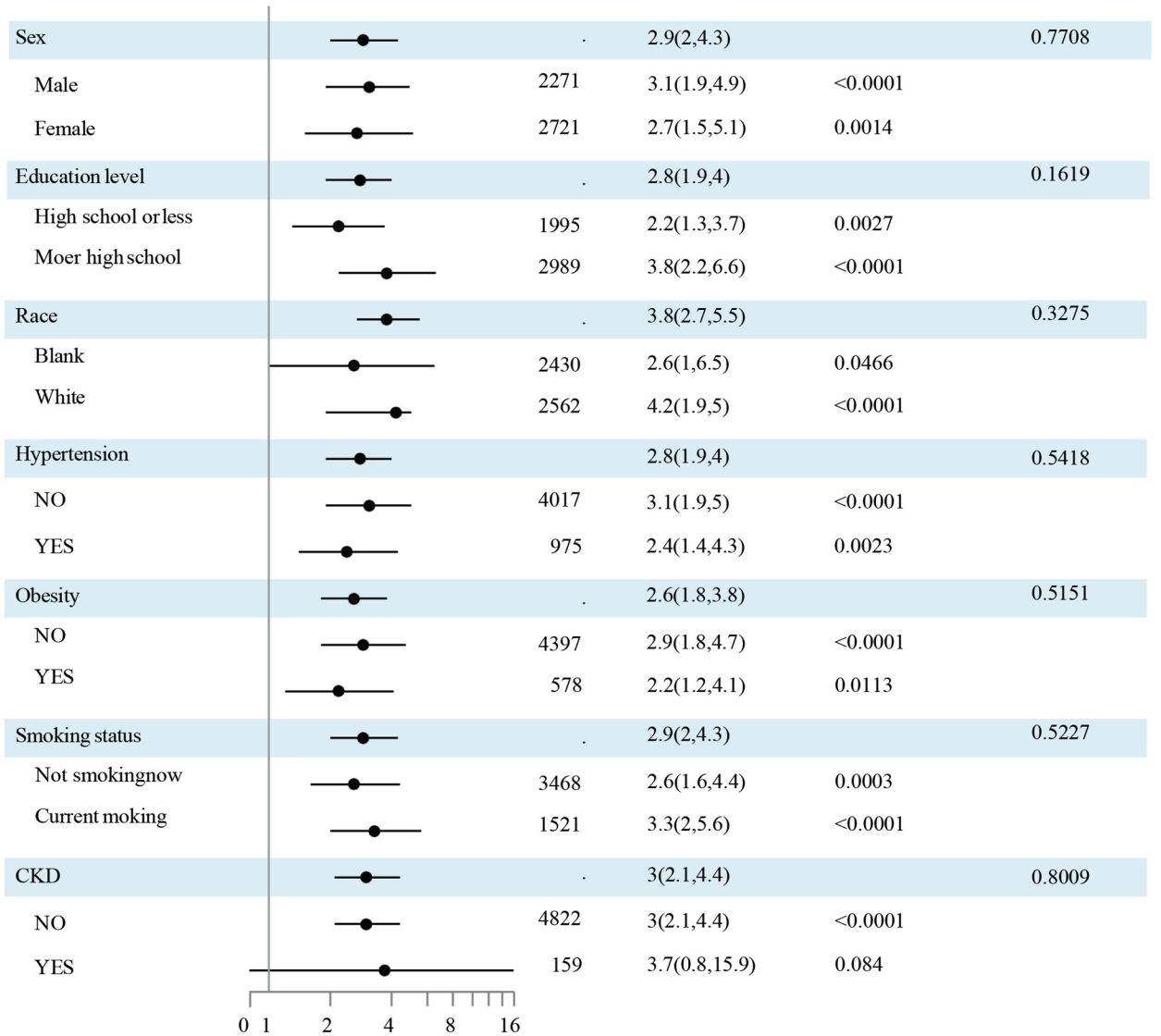

FIGURE 3 | The association of CHF and the TyG index by a subgroup analysis. The association of CHF and the TyG index by a sub-group analysis. Data are hazard ratios (HRs) and 95% confidence limits (95% CLs). The participants were divided into subgroups according to sex, race, education, obesity, smoking status, hypertension, and CKD. The results were evaluated after adjusted for age, sex, race, education, obesity, smoking status, hypertension, diabetes mellitus, hypercholesteremia, CKD, LDL-C, and HDL-C except for the sub-group variable. Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TyG, Triglyceride-glucose; CKD, chronic kidney diseases; HOMA-IR, homeostasis model assessment-insulin resistance; CHF, congestive heart failure.

increased stiffness, and myocardial remodeling, typically leading to the occurrence and development of heart failure (1). Previous studies have also confirmed a positive correlation between the increase in triglycerides and the development of heart failure (33).

Meanwhile, the TyG index is correlated with various risk factors for heart failure. In a 9-year follow-up cohort study on hypertension in China, the TyG index predicted the incidence of hypertension (34). Acute coronary syndrome, hypertension, diabetes mellitus, and other factors can cause cardiac function and structural disorders, leading to heart failure (35, 36). A recent investigation on 546 patients with CHF and type II diabetes mellitus discovered a higher rate of heart failure re-hospitalization and cardiovascular mortality with the TyG index of 9.06, when compared with the TyG index of 8.55 (37). In a study of patients undergoing echocardiography at a hospital in southern Taiwan Province, China, Chiu et al. highlighted a high TyG index to be associated with an increased left atrial diameter and a reduced left ventricular ejection fraction (38). Furthermore, this investigation revealed the predictive value of HOMA-IR on CHF. The increased risk of CHF in the high HOMA-IR

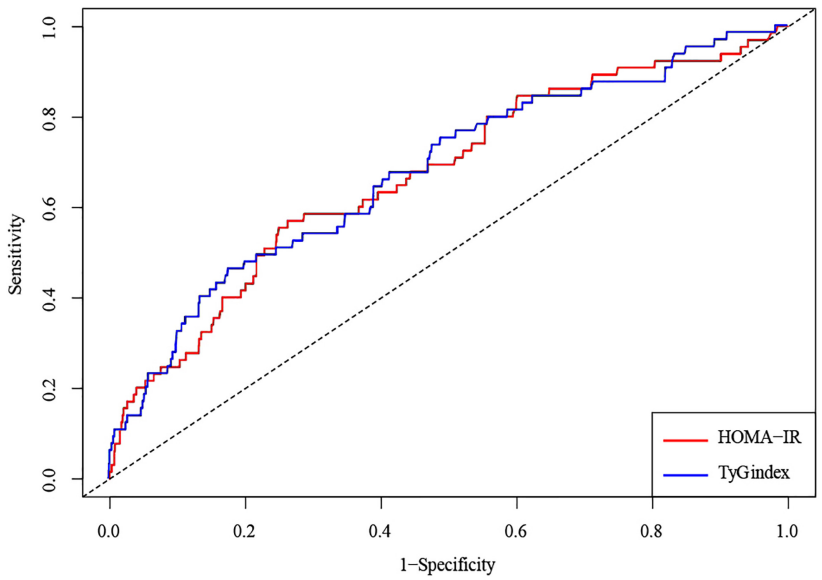

FIGURE 4 | An ROC analysis of the TyG index and HOMA-IR to predict the incident risk of CHF. A solid blue line represents the TyG index; a solid red line represents HOMA-IR. Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; TyG, Triglyceride-glucose; HOMA-IR, homeostasis model assessment-insulin resistance; CHF, congestive heart failure.

population has already been demonstrated in previous studies on patients with diabetes mellitus combined with chronic renal disease, though without coronary heart disease (39). However, this study demonstrated that if individuals are presented with high HOMA-IR despite being young and healthy, their risks of CHF in the future are also increased. The same finding was discovered in the long-term follow-up study of 15,792 cases (aged—45–64 years) by Vardeny et al., stating that HOMA-IR is an independent predictor of heart failure (9). According to Kishi et al., increased IR in young people is an important life-long risk of left ventricular re-modeling and dysfunction in adulthood (40).

This study also demonstrated that the TyG index and HOMA-IR had similar predictive powers for CHF events, with AUC values [0.675 (95% CI, 0.604–0.746) vs. 0.67 (95% CI, 0.6–0.742) $p = 0.986$]. HOMA-IR was employed to assess the relationship between IR and disease (41). However, the TyG index in clinical practice is simpler to perform, rather than HOMA-IR detection, and is cheaper and easier to obtain. Therefore, the TyG index has added advantages in comparison to HOMA-IR regarding the clinical evaluation and prediction of CHF. The TyG index can be used as an alternative index to predict heart failure events.

TABLE 3 | The area under the curve (AUC) of the TyG index and HOMA-IR to predict CHF incidence.

Variables	AUC (95%CI)	*P*-value*
HOMA-IR	0.670 (0.600–0.742)	Reference
TyG index	0.675 (0.604–0.746)	0.986

Asterisk compared with HOMA-IR. Abbreviations: TyG, Triglyceride-glucose; HOMA-IR, homeostasis model assessment-insulin resistance; CHF, congestive heart failure.

We are aware of several limitations in our study. The CARDIA study recruited only young people at the beginning of the research and did not consider people of differing ages and constitutions. Moreover, CARDIA data analysis by ethnicity was limited to African-American and white-American adult individuals, and therefore, such results cannot be cautious to other ethnic groups. Future studies are needed to assess the prevalence of CHF in other ethnicities as well as in children, athletes, and in individuals with specific diseases. Finally, the study did not compare the euglycemic-hyperinsulinemic clamp test (the gold standard for measuring IR) with the TyG index.

CONCLUSION

This study suggests that the TyG index and HOMA-IR in young adulthood are independent risk factors for the development of CHF. However, the TyG index can be easily popularized in clinical practice by low-cost experimental analyses. Heart failure is a major cause of global mortality, resulting in serious economic and social burden. Early identification and intervention of people with an increased TyG index can reduce the incidence of CHF. In view of the increasing prevalence of abnormal glucose and lipid metabolism and high IR, these findings are of great significance to public health.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

XZ and DH performed data analysis and wrote the manuscript. HBZ, XW, and YX contributed to the analysis plan and reviewed and edited the manuscript. QZ, YB, XH, HZ, ZM, and QCZ contributed to the discussion. DX and HR had full access to all of the data in the study, reviewed, edited the manuscript, and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.944258/full#supplementary-material

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ORIGINAL RESEARCH
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Development of Prediction Model to Estimate the Risk of Heart Failure in Diabetes Mellitus

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Background: Heart failure (HF) is a leading cause of mortality and disability in patients with diabetes mellitus (DM). The aim of the study is to predict the risk of HF incidence in patients with DM by developing a risk prediction model.

Methods: We constructed a regression model based on 270 inpatients with DM between February 2018 and January 2019. Binary logistic regression was applied to develop the final model incorporating the predictors selected by least absolute shrinkage and selection operator regression. The nomogram was estimated with an area under the receiver operator characteristic curve and calibration diagram and validated with the bootstrap method.

Results: Risk factors including age, coronary heart disease (CHD), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were incorporated in the final model as predictors. Age ≥ 61 years old, LDL, and CHD were risk factors for DM with HF, with odds ratios (ORs) of 32.84 (95% CI: 6.74, 253.99), 1.33 (95% CI: 1.06, 1.72), and 3.94 (95% CI: 1.43, 13.43), respectively. HDL was a protective factor with an OR of 0.11 (95% CI: 0.04, 0.28). The area under curve of the model was 0.863 (95% confidence interval, 0.812∼0.913). The plot of the calibration showed that there was a good consistency between predicted probability and actual probability. Harrell's C-index of the nomogram was 0.845, and the model showed satisfactory calibration in the internal validation cohort.

Conclusion: The prediction nomogram we developed can estimate the possibility of HF in patients with DM according the predictor items.

Keywords: prediction, nomogram, risk factors, diabetes mellitus, heart failure

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INTRODUCTION

The incidence and mortality rate of diabetes mellitus (DM) are increasing in recent years, especially in developing countries (1, 2). Cardiovascular disease (CVD) is a major complication of blood glucose dysregulation (3). Patients with diabetes have a 2- to 4-fold increased risk of heart failure (HF) compared with those without diabetes (4). There is higher prevalence, incidence, and mortality in diabetic patients with HF compared with those

with diabetes who remain HF-free (5–7). In population-based studies, concomitant DM increases the risk of death in both hospitalized and ambulatory patients with HF (8–11). It is important to note that even in patients with prediabetes, the risk of HF is increased and associated with poor prognosis (12, 13).

DM and HF have considerable morbidity and mortality, when they occur together, which further worsens adverse patient outcomes, quality of life, and costs of care (4). It is important to find the risk factors of diabetes complicated by HF. Therefore, we aimed to develop a simplified prediction model to identify the high-risk of HF early in diabetic patients and conduct early intervention for them.

MATERIALS AND METHODS

Patients

We conducted a trial on a population of resident patients diagnosed with DM in Southern Medical University (The First People's Hospital of Shunde) between February 2018 and January 2019. In total, 270 patients were included. Our outcome was diabetes mellitus with heart failure (DM-HF) disease. We excluded patients who had suffered from DM-HF and received treatment. DM was defined as use of medications for diabetes or fasting blood glucose $\geq$ 7 mmol/L and/or hemoglobin A1c (HbA1c) $\geq$ 6.5% (14). The HF diagnostic criteria were in accordance with Chinese Guidelines for the Diagnosis and Treatment of Heart Failure 2018 (15). Studies involving human participants were reviewed and approved by the Ethics Committee of Southern Medical University (The First People's Hospital of Shunde). The approval number from the Ethics Committee is 20190525. The patients/participants provided their written informed consent to participate in this study.

Data Collection

We collected baseline data from the patients at early hospital admission: (1) patient characteristics including age group ($\leq$ 29 years, 30∼60 years, and $\geq$ 61 years), sex, and history of smoking and drinking; (2) clinical laboratory data included high-density lipoprotein (HDL), low-density lipoprotein (LDL), estimated glomerular filtration rate (eGFR), and aldehyde dehydrogenase 2 (*ALDH2*) gene; (3) cardiovascular conditions including blood pressure and history of coronary heart disease (CHD). We combined the results of published studies with actual clinical examination (16–20), and we used age group, sex, history of smoking and drinking, HDL, LDL, *ALDH2* and cardiovascular conditions as the predictor variables.

Model Development and Validation

Least absolute shrinkage and selection operator (Lasso) logistic regression was conducted to select the optimal predictive factors. A model with excellent performance and the least number of independent variables was given when we adopted the lambda.1se. A multivariate logistic regression analysis was conducted to develop a prediction model by incorporating the variables selected in the Lasso model. The created model was tested in internal validation with the bootstrap resampling technique, in which regression models were fitted in 100 bootstrap replicates. The performance of the model was expressed as the following indicators:

1. A calibration plot was used to estimate the calibration of the final model and bootstrap validation. The difference between the average of observed outcomes and the average of predicted probabilities was reflected with the plot.
2. A R^2 statistic was conducted to evaluate the goodness of fit test of the model. The closer the value to 1, the better the model fits the sample observations.
3. The area under curve (AUC) was used to evaluate the discrimination of the model. If the model can better differentiate among patients who did or did not have HF, the result of AUC was close to 1.
4. Brier score was used to measure the error between the probability of the category predicted by the model and the real value.

All the development and validation of model were analyzed with R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). The R code of the developed model is available in **Supplementary Material 1**. Our study was in accordance with the TRIPOD statement (**Supplementary Material 2**). Continuous data were expressed as median (25th, 75th) and

TABLE 1 | Baseline characteristics of patients included.

Variables	DM-HF	DM	*P*
Patients, *n*	188	82	
Sex			
Male, *n* (%)	111 (59.0)	45 (54.9)	0.524
Age group, *n* (%)			
≤ 29	2 (1.1)	10 (12.2)	
30∼60	40 (21.3)	51 (62.2)	
≥ 61	146 (77.7)	21 (25.6)	**< 0.01**
HDL, mmol/L	1.04 (0.87∼1.25)	1.33 (1.12∼1.72)	**< 0.01**
LDL, mmol/L	2.39 (1.85∼3.15)	1.52 (0.98∼2.45)	**< 0.01**
eGFR, ml/min	102.82 (76.02∼131.39)	62.64 (39.98∼84.24)	**< 0.01**
ALDH2, *n* (%)			
A	19 (10.1)	8 (9.8)	
B	73 (38.8)	28 (34.1)	
C	96 (51.1)	46 (56.1)	0.735
Smoking, *n* (%)	65 (34.6)	24 (29.3)	0.394
CHD, *n* (%)	54 (28.7)	5 (6.1)	**< 0.01**
Systolic pressure, mmHg	131 (117∼150)	127 (120∼135)	1.000
Diastolic pressure, mmHg	79 (70∼93)	81 (75∼92)	0.998
Drinking, *n* (%)	36 (19.1)	17 (20.7)	0.763

DM, diabetes mellitus; HF, heart failure; CHD, coronary heart disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALDH2, aldehyde dehydrogenase 2; A, Lys504Lys; B, Glu504Lys; C, Glu504Glu. The bold P-values mean P < 0.05 and have a significant difference between groups.

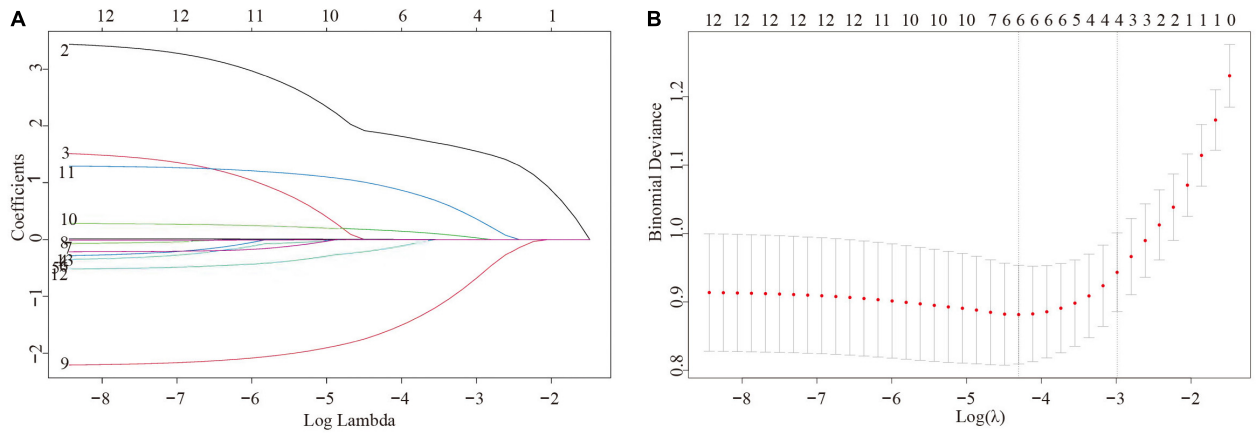

FIGURE 1 | Variable selection using the least absolute shrinkage and selection operator (Lasso) logistic regression model. **(A)** Variable screening process using the control parameter log(λ) **(B)** The 50-fold cross-validation method.

categorical variables as frequencies and percentages. Some data were missing for all the risk factors except for age, sex, eGFR, and the *ALDH2* gene. We filled in missing values with the method of mean/mode completer.

RESULTS

Baseline Characteristics of Patients

A total of 270 patients with DM were included in this analysis. Of the 270 patients, there were 188 patients diagnosed with DM-HF; 77.7% of the patients with DM-HF were ≥ 61 years, and 62.2% of the patients with DM were 30∼60 years. The median of LDL was 2.39 mmol/L (interquartile range 1.85∼3.15) in the DM-HF group and 1.52 mmol/L (interquartile range 0.98∼2.45) in the DM group; 28.7% of the patients with DM-HF had CHD, while only 6.1% of the patients with DM had this disease. There were significant differences in age group, HDL, LDL, eGFR, and CHD between the two groups. The baseline characteristics of patients in the two groups are summarized in **Table 1**.

Development of the Model

Lasso regression was used to screen the four indicators involved in the establishment of the model (**Figures 1A,B**). The selected indicators were age, LDL, HDL, and CHD. The final multivariate regression model is shown in **Table 2**. Age ≥ 61 years old, LDL, and CHD were risk factors for DM-HF, with odds ratios (ORs) of 32.84 (95% CI: 6.74, 253.99), 1.33 (95% CI: 1.06, 1.72), and 3.94 (95% CI: 1.43, 13.43), respectively. HDL was a protective factor for DM-HF with an OR of 0.11 (95% CI: 0.04, 0.28).

Performance of the Model and Internal Validation

We drew calibration curves to assess the degree of calibration of the risk prediction and internal validation of the DM-HF model (**Figures 2A,B**). As shown in **Figure 2A**, the x-axis represents the predicted risk for DM-HF, and the y-axis represents the actual probability of DM-HF. The dotted line represents the realized prediction power, and the solid line represents the prediction of an ideal model. The results showed a good consistency between the nomographic model and the ideal model. The calibration curve of the model also demonstrated a good agreement in the bootstrap validation cohort (**Figure 2B**).

The results in **Table 3** show that the final logistic regression model has good discrimination for DM-HF (AUC = 0.863; 95% CI: 0.812∼0.913; R^2 = 0.477; Brier-score = 0.128). The results of the internal validation indicated that there was negligible model

TABLE 2 | Final multivariate regression model of DM-HF.

Intercept	Multivariate regression		
	β	OR (95% CI)	*P*-value
Intercept	0.15	1.16(0.10∼9.18)	0.89
Age group			
≤ 29	1.00 (referent)		
30∼60	1.56	4.78(0.99∼36.26)	0.07
≥ 61	3.49	32.84(6.74∼253.99)	**< 0.001**
HDL	–2.21	0.11(0.04∼0.28)	**< 0.001**
LDL	0.29	1.33(1.06∼1.72)	**0.02**
CHD			
No	1.00 (referent)		
Yes	1.37	3.94(1.43∼13.43)	0.01

OR, odds ratio; CI, confidence interval. The bold P-values mean P < 0.05 and have a significant difference between groups.

TABLE 3 | Model performances for final regression model and internal validation model.

	Final model	Internal validation
AUC-ROC	0.863(0.812∼0.913)	0.845
R^2	0.477	0.418
Brier-score	0.128	0.137
Slope	1.000	0.869

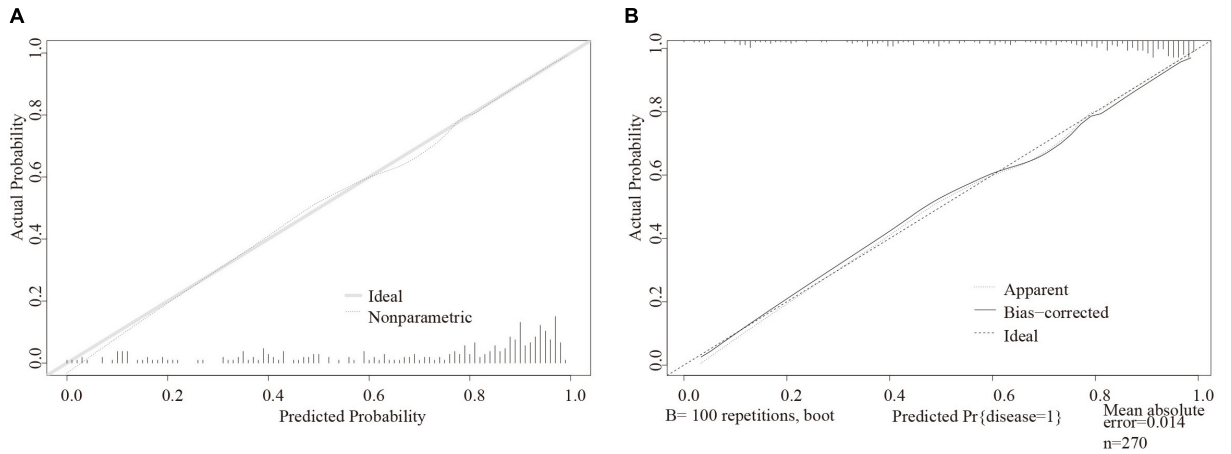

FIGURE 2 | Calibration plot in the final regression nomogram and bootstrap validation. **(A)** Calibration curve for diabetes mellitus with heart failure in the development model. **(B)** Calibration curve for diabetes mellitus with heart failure in the internal validation.

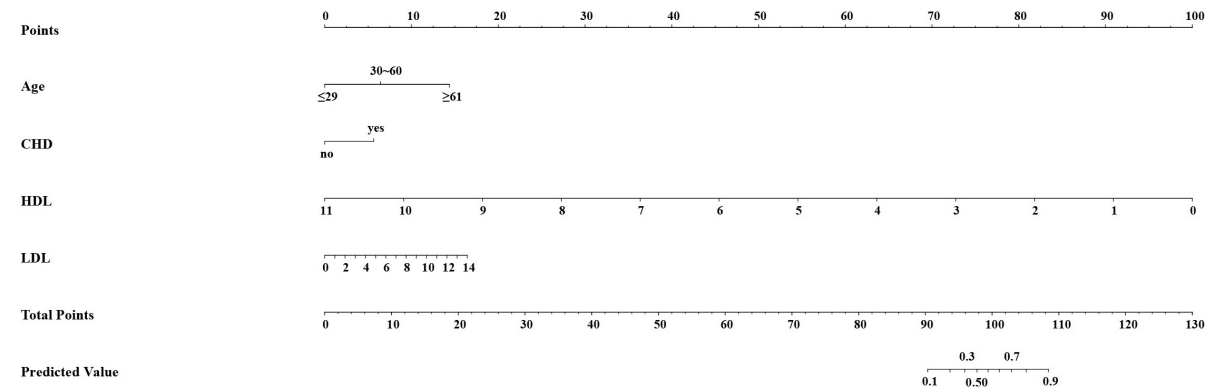

FIGURE 3 | Prediction nomogram of diabetes mellitus with heart failure. The nomogram was developed with age, CHD, HDL, and LDL. CHD, coronary heart disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

optimism in bootstrap resampling (AUC = 0.845; R^2 = 0.418; Brier score = 0.137).

Model Presentation

The final prediction model of DM-HF was displayed as a nomogram (**Figure 3**). We can score according to each variable, and the sum of all the variable scores is the total score. The total score on the DM-HF-predicted value axis represents the probability of DM-HF. The higher the score, the higher the risk that a patient with DM will develop HF. The web calculator can be linked through this URL: Nomogram For Diabetes Mellitus with Heart Failure (shinyapps.io).

DISCUSSION

In our study, a simplified model to predict the risk of DM-HF was constructed and successfully internally validated. The model showed good calibration and discrimination. We also provided the nomogram and web calculator to help one in calculating the risk.

Our study showed that age ≥ 61 years, LDL, and CHD were risk factors for DM-HF, and that high HDL was a protective factor for DM-HF. A multicenter study including 4,447 people concluded that more attention should be paid to elderly people to follow up on their risk of HF (21). In a systematic review and meta-analysis, the association between incident HF and 5-year increase in age (1.47; 1.25–1.73) was reported (19). In many studies, age was a risk factor for DM-HF (22, 23). Diabetic patients with low HDL level [3.62 (2.06–6.36)] have a higher risk of HF (24). Dyslipidemia is a risk factor for DM-HF. Lowering the level of LDL and increasing the level of HDL are engaging goals for reducing the risk of HF in patients with DM (25). The most common cause of HF is ischemic heart disease owing to impaired myocardial perfusion, while there are other common causes including DM and CHD (26). Murtaza et al. believed that long-term diabetes leads to structural and functional changes in the development and progression of HF, independent of myocardial ischemia or microvascular atherosclerotic disease (27).

Despite some published models can predict the risk of HF in patients with diabetes, our Lasso regression model still has its unique advantages. On the one hand, the population we included in our study was different from theirs. The data they used came from the Action to Control Cardiovascular Risk in Diabetes Study Group (ACCORD) trial, which was conducted in 77 centers across the United Stated and Canada (28, 29). All participants had established atherosclerotic coronary vascular disease or were 55–79 years of age with documented atherosclerosis, albuminuria, left ventricular hypertrophy, or two or more other cardiovascular risk factors, while our data came from Southern Medical University (The First People's Hospital of Shunde), which is located in Chinese mainland and covers a catchment area of 3.2 million residents. On the other hand, we used a different statistical tool to construct the prediction model and visualization tools to show the model.

There are some limitations in our study. First, this is a retrospective study, and the results are inevitably biased. Second, the sample size in our research is very limited. In our subsequent research, we will enroll more patients for external verification. Third, some biomarkers had been well established to be associated with the risk of HF, including N-terminal pro B-type natriuretic peptide and soluble suppression of tumorigenesis-2 (30, 31). Furthermore, some other novel biomarkers, such as secreted frizzled-related protein 2 (SFRP2) (32, 33), trimethylamine N-oxide (TMAO) (34), and polyunsaturated fatty acids were also associated with the risk of HF (35). However, we did not include these biomarkers in our prediction model, as we aimed to provide metrics that can be easily extracted from clinical data. In conclusion, we conducted Lasso regression to screen variables and built a risk prediction model for diabetic HF. The model showed good discrimination and calibration in internal validation. A nomogram and a webpage calculator based on the model can make patients or doctors quickly calculate the risk of diabetic HF, which can help patients with diabetes reduce this risk better.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available to uphold patient/participant privacy. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

HQ and WL contributed to the conception and design of the study. CW, PY, and WL organized the database. HQ performed the statistical analysis and wrote the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.900267/full#supplementary-material

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The controlling nutritional status score and clinical outcomes in patients with heart failure: Pool analysis of observational studies

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Background and aims: Malnutrition is very common in patients with heart failure (HF) and is associated with a worse clinical outcome. The Controlling Nutritional Status (CONUT) score is an easily derived index for the evaluation of malnutrition. This study aimed to evaluate the association between the CONUT score and the prognosis in patients with HF.

Methods and results: Electronic databases were searched for potential studies from inception up to February 15, 2022. Observational cohort studies included adult participants with HF, and reported the associations between the CONUT score and the adjusted relative risk (RR) of all-cause mortality, and patients with composite major adverse cardiac outcomes (MACEs) were included. We finally included 18 studies comprising 12,532 participants with HF for analysis. The median age of the patients was 70.5 years old, and 35.4% were women. After a median follow-up duration of 32.5 months, patients with HF with a higher CONUT score were associated with a higher risk of all-cause mortality (per 1 increment of the CONUT score: RR, 1.21, 95% CI, 1.13–1.29, $I^2 = 68\%$, P for heterogeneity = 0.002) and MACEs (per 1 increment of the CONUT score: RR, 1.14, 95% CI, 1.06–1.23, $I^2 = 81\%$, P for heterogeneity <0.0001) after adjusting for other prognostic factors. When the CONUT score was divided into the normal nutritional status and malnourished status, malnourished patients with HF were associated with increased risks of all-cause death (RR, 1.61, 95% CI, 1.40–1.85, $I^2 = 17\%$, P for heterogeneity = 0.29) and MACEs (RR, 2.12, 95% CI, 1.49–3.02, $I^2 = 87\%$, P for heterogeneity <0.0001), compared with those with normal nutritional status.

Conclusions: The CONUT score is associated with the clinical outcomes in patients with HF, and can be used as a screening tool of nutritional status in HF to improve prognosis.

KEYWORDS

heart failure, malnutrition, prognosis, risk, the controlling nutritional status

Introduction

Heart failure (HF) is a complex clinical syndrome that results from any structural or functional impairment of the heart. Accompanied by the aging of society and a decrease in mortality of multiple cardiovascular diseases, the prevalence of HF has increased rapidly, which contributed to a growing health burden worldwide (1, 2). Although guideline-directed medical therapy (GDMT) had made great progress in the management of HF, it was still associated with high morbidity and mortality. It had been reported that, in patients hospitalized due to the exacerbation of HF, the composite outcomes (including 1-year mortality and re-hospitalization) were >20% (3, 4). Therefore, new risk stratification markers and treatment methods are still needed to improve the prognosis of HF.

Malnutrition is very common in patients with HF and is associated with a higher risk of mortality and re-hospitalization (5, 6). Early detection of malnutrition in HF would be useful for identifying patients at high risk of poor clinical outcomes and recommending nutritional interventions to improve prognosis (7). Many tools and indexes had been proposed for screening malnutrition; however, no consensus had been made on which to use in patients with HF (5, 8–10).

The Controlling Nutritional Status (CONUT) score, developed by Ignacio et al., (11) had been reported to be one of the most robust markers of nutritional status. It is calculated from a patient's serum albumin, total cholesterol level, and total peripheral lymphocyte count. Therefore, The CONUT score is an immune-nutritional index, which can evaluate the protein reserve, lipid metabolism, and immunocompetence. Recently, studies have shown that malnourished status determined by the CONUT score is associated with worse outcomes in patients with HF (12–16). However, these studies were with small sample size and different patient characteristics, which resulted in inconsistent results in the association between the CONUT score and the clinical outcomes in patients with HF. Based on the inconsistency of previous studies, we conducted a meta-analysis of observational cohort studies to evaluate the association between the CONUT score and the prognosis in HF.

Methods

We performed the systematic review and meta-analysis according to the recommendations of the MOOSE (Meta-analysis of Observational Studies in Epidemiology) Group (17). Electronic databases, including PubMed, Embase, Google Scholar, the Cochrane Library, and Wanfang, were searched for related studies from inception until February 15, 2022. We developed the search strategies using the terms "Controlling Nutritional Status," "CONUT," or "malnutrition" and "heart failure," "cardiac dysfunction," or "myocardial dysfunction" and "prognosis," or "death" or "MACE." We limited our search to human studies and writing in Chinese or English, and further read the reference lists of the included studies or other systematic reviews to identify potential missing related articles.

TABLE 1 Parameters for assessment of the CONUT Score.

Parameter	Score			
Serum albumin (g/ml)	≥3.5	3.0–3.49	2.50–2.99	<2.50
Albumin score	0	2	4	6
Total cholesterol (mg/dl)	≥180	140–179	100–139	<100
Cholesterol score	0	1	2	3
Lymphocytes (count/ml)	≥1,600	1,200–1,599	800–1,199	<800
Lymphocytes score	10	1	2	3

Two researchers (X-WH. and J-JL) independently searched the databases and screened the retrieved items. Potentially related studies were reviewed in full text, and the studies' information was extracted into a pre-defined form. We included studies for meta-analysis if there were: (1) observational cohort studies included adult participants (age ≥18 years old); (2) all the participants were diagnosed with HF; (3) the CONUT score was evaluated at baseline status, which was based on serum albumin, lymphocyte count, and total cholesterol measures (range from 0 to 12); (4) the association between the CONUT score (as a continuous or category metric) and the prognostic outcomes of HF were reported in an adjusted model, which was controlling the other related prognostic factors. We excluded those studies if they were: (1) cross-sectional studies; (2) the follow-up evaluation was <3 months; (3) the relative risk (RR) was not adjusted for other confounders, and (4) duplicated publications from identical cohort studies with the same outcomes.

The CONUT score was calculated based on the patients' serum albumin, total cholesterol, and total peripheral lymphocyte levels (Table 1). The range of the CONUT scores is 0 to 12, and a higher score indicated that the patient was with worse nutritional status (11–16). The quality of the included studies was accessed by the NOS (the Newcastle–Ottawa Quality Assessment Scale for cohort studies), which evaluates the selection (four items with one point in each item), comparability (one item with up to two points), and exposure/outcome (three items with one point in each item), respectively (18, 19). Therefore, up to a highest of 9 points can be awarded in NOS. According to previous reports, the included studies were graded as low quality (<4 points), moderate quality (4–6 points) or high quality (≥7 points), respectively (20, 21).

In this meta-analysis, the primary outcome interested was all-cause mortality in patients with HF. The secondary outcome was composite major adverse cardiac outcomes (MACEs), including all-cause mortality and HF hospitalization. We pooled the association between the exposure (CONUT score) and outcomes in multivariable-adjusted statistical models. If multiple statistical models were reported, we used the data that

adjusted the most comprehensive confounders for analysis. As the associations between the CONUT score and the interested outcomes were reported in different ways in the included studies (e.g., per 1 increment as a continuous metric; or as normal nutritional/malnourished status in the category trait), we pooled the RRs for per 1 increment in the CONUT score, as well as malnourished vs. normal nutritional status, respectively. The RRs (logarithmically transformed) and their corresponding standard errors (SEs) were pooled by the inverse variance approach. In case outcomes were presented as odds ratios (ORs) or hazard ratios (HRs), they were regarded as an approximate RR and used in the meta-analysis (22).

Heterogeneity among studies was evaluated with the I^2 statistic, an I^2 value of <50% or P for heterogeneity <0.1 was considered an indication of no-significant heterogeneity observed among the studies. However, even when no-significant heterogeneity was shown, we combined the results using the DerSimonian and Laird random-effects models over the fixed effects model, considering that, to some extent, both clinically and methodologically were unavoidable (for example, cohort design, the definition of HF, and adjustment of potential confounders) (23). In case of no heterogeneity, the results of fixed and random effects models are the same, while, if there was significant heterogeneity among the included studies, the random-effects model would be more conservative. To further test the stability of the results, we conducted sensitivity analyses by changing the statistical models from random-effects models to fixed-effects models. We also performed sensitivity analyses by deleting one study each time and recalculating the pooled results. The Publication bias was accessed by inspecting the funnel plot for the outcomes. All the statistical analyses were performed with RevMan 5.3 (The Cochrane Collaboration, Copenhagen, Denmark). A P value <0.05 is considered statistically significant.

Results

Baseline characteristics of the included studies

After searching the electronic databases, we retrieved 5,423 potentially related article items. The duplicate items with identical titles, authors, publication journals, and years were deleted. Two investigators (XH and YH) independently screened the titles and abstracts. Then, 62 potentially related full articles were reviewed, and 18 studies were finally included in the pooled analysis according to the pre-defined criteria (Figure 1) (12–16, 24–36). There were 12,532 participants with HF in the included studies, with a median follow-up duration of 32.5 months. The median age of the patients was 70.5 years old, and 35.4% were women. The baseline characteristics of the participants are presented in Table 2. According to the NOS assessment of observational studies, five included studies were graded as fair quality and 13 studies were as good quality (Supplementary File 1). The adjusted confounders in the included studies are summarized in Supplementary File 2.

Association between CONUT score and risk of all-cause death in HF

When the CONUT score was reported as a continuous index, we observed that a higher CONUT score was associated with a higher risk of all-cause mortality in patients with HF after adjusting for multiple prognostic factors (per 1 increment of the CONUT score: RR, 1.21, 95% CI, 1.13–1.29, Figure 2). However, significant heterogeneity was observed in the included studies ($I^2 = 68\%$, $P = 0.002$).

When the CONUT score was divided into the normal nutritional status and malnourished status, the patients with a higher CONUT score (malnourished) were associated with a 61% increased risk of all-cause death in HF (RR, 1.61, 95% CI, 1.40–1.85), compared with those with normal nutritional status (a lower CONUT score) (Figure 3) after being adjusted for other prognostic factors. No significant heterogeneity was observed in the included studies ($I^2 = 17\%$, $P = 0.29$). Furthermore, the increased risk of all-cause mortality was only observed in those with moderate to severe malnutrition (the CONUT score ≥5; RR, 1.79; 95% CI, 1.35–2.37), but not in those with mild malnutrition (the CONUT score, 2–4; RR, 1.20; 95% CI,.85–1.71) (Figure 4).

Association between CONUT score and risk of MACEs in HF

The patients with higher CONUT scores were associated with a higher risk of MACEs in the patients with HF after being adjusted for multiple prognostic factors (per 1 increment of the CONUT score: RR, 1.14; 95% CI, 1.06–1.23). Significant heterogeneity was observed in the included studies ($I^2 = 81\%$, $P < 0.0001$) (Figure 5).

Similarly, when the CONUT score was divided into the normal nutritional status and malnourished status, malnourished patients with HF were associated with a 112% increased risk of MACEs (RR, 2.12; 95% CI, 1.49–3.02), compared with those with normal nutritional status in the multivariable-adjusted model (Figure 6), while significant heterogeneity was observed in the included studies ($I^2 = 87\%$, $P < 0.0001$). Compared with patients with HF, with normal nutritional status, those with mild (RR, 1.63; 95% CI, 1.08–2.46) or moderate to severe malnutrition (RR, 3.96, 95% CI, 1.41–11.13) were associated with a high risk of MACEs (Figure 7).

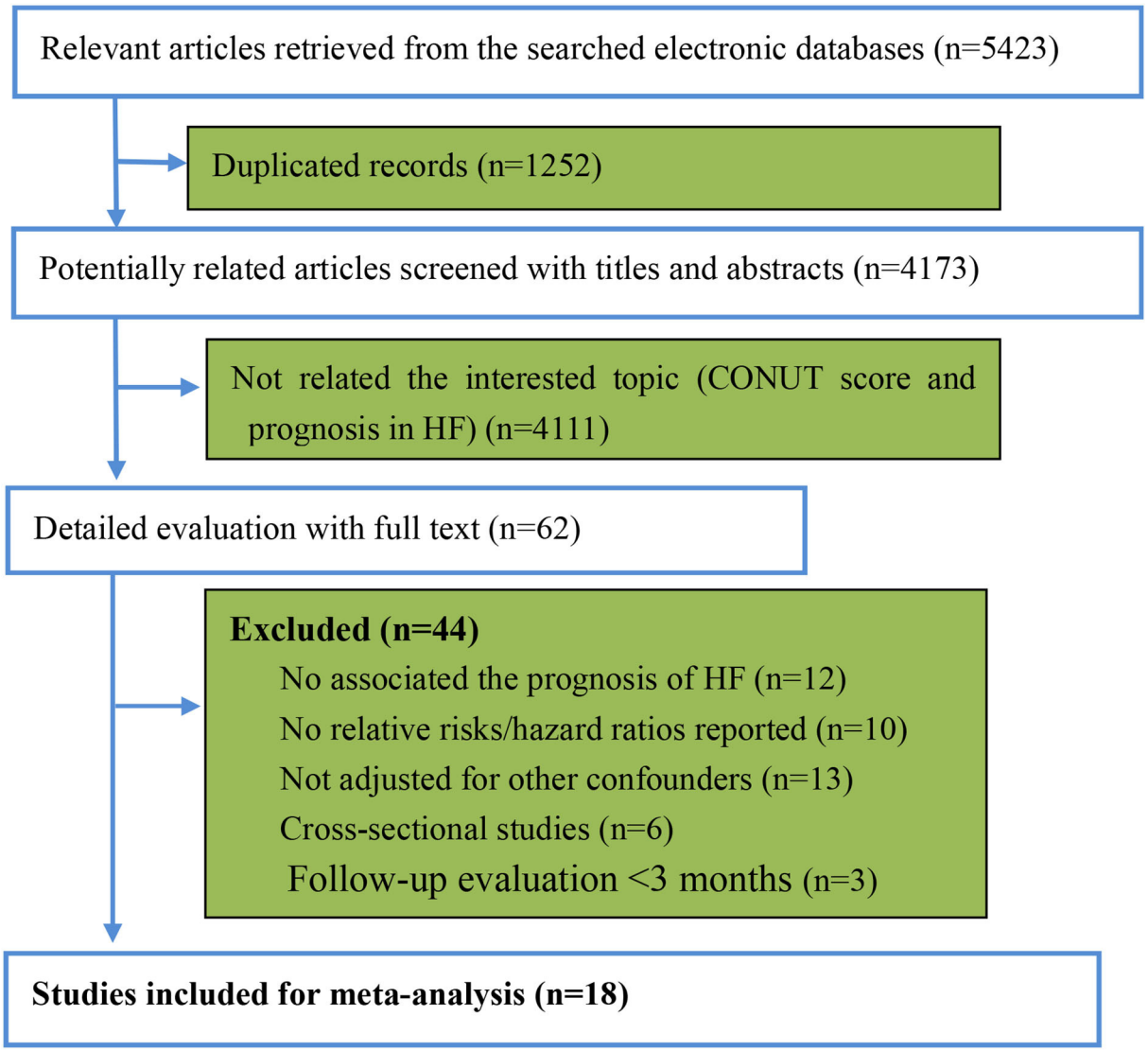

FIGURE 1
The review flow of the retrieved studies. CONUT, controlling nutritional status; HF: heart failure.

Sensitivity analyses and publication bias evaluation

The sensitivity analyses confirmed that the association between the CONUT score and the prognosis in the patients with HF did not change with the use of statistical models (fixed-effects models vs. the random-effects models) or recalculation of the RRs by omitting one study at a time. NO significant publication bias was observed for the analyses of all-cause mortality or MACE associated with the CONUT score as a continuous or as a category index by inspection of the funnel plot (Supplementary Files 3–6).

Discussion

In this meta-analysis, we showed that the CONUT score, which is derived from three commonly detected laboratory biomarkers (e.g., serum albumin, the total cholesterol level, and total peripheral lymphocyte CONUT), is associated with the clinical outcomes in patients with HF. Furthermore, such association was detected when the CONUT score was defined either as a continuous index, or a category divided into the normal nutritional status and malnourished status. These findings support the use of the CONUT score as a screening tool for nutritional status in HF, and guiding the risk stratification, as well as nutritional interventions to improve prognosis in HF.

TABLE 2 Baseline characteristics of the included studies.

Study	Country	Cohort design	HF type	Nutritional status by CONUT score	Sample size (*n*)	Female (%)	Mean age (years)	Follow-up (months)	Outcome
Narumi et al. (25)	Japan	Prospective	CHF	Malnourished (≥5)	388	40.0%	69.6	28.4	MACE
Nochioka et al. (24)	Japan	Prospective	CHF	Continuous variable Malnourished (≥2)	3,421	28.4%	66.9	34.7	All-cause mortality MACE
Nakagomi et al. (26)	Japan	Prospective	CHF	Malnourished (≥3)	114	25.4%	66.0	67.5	MACE
Iwakami et al. (16)	Japan	Prospective	AHF	Continuous variable Malnourished (≥2)	635	38.0%	75.0	27.0	All-cause mortality
La Rovere et al. (28)	Italy	Prospective	AHF	Continuous variable Malnourished (≥2)	466	14.0%	61.0	12.0	All-cause mortality
Nishi et al. (27)	Japan	Retrospective	AHA	Continuous variable Malnourished (≥2)	482	38.2%	71.7	45.1	All-cause mortality
Sze et al. (15)	UK	Prospective	AHF	Continuous variable	265	38.0%	82.0	19.9	All-cause mortality
Shirakabe et al. (14)	Japan	Retrospective	AHF	Malnourished (≥2)	458	34.0%	76.0	12.0	All-cause mortality
Yoshihisa et al. (29)	Japan	Retrospective	AHF	Continuous variable	1,307	39.4%	66.5	38.2	All-cause mortality
Alvarez-Alvarez et al. (30)	Spain	Retrospective	CHF with CRT	Malnourished (≥2)	302	22.5%	70.0	50.4	MACE
Hamada et al. (31)	Japan	Retrospective	CHF	Malnourished (≥5)	67	41.8%	85.0	12	MACE
Chien et al. (13)	China	Retrospective	CHF/HFpEF	Continuous variable Malnourished (>3)	1,120	60.6%	77.2	41.8	All-cause mortality MACE
Uemura et al. (32)	Japan	Retrospective	AHF	Continuous variable Malnourished (≥2)	170	40.6%	67.6	36.5	MACE
Komorita et al. (33)	Japan	Prospective	CHF/HFpEF	Continuous variable	506	45.3%	71.6	50.0	MACE
Sze et al. (10)	UK	Prospective	CHF	Continuous Malnourished (≥2)	467	33.0%	76.0	18.5	All-cause mortality MACE
Ikeya et al. (34)	Japan	Retrospective	CHF with CRT	Continuous Malnourished (≥5)	263	23.2%	69.0	31.0	All-cause mortality
Lu et al. (35)	China	Prospective	AHF	Malnourished (≥2)	396	28.5%	59.8	34.0	All-cause mortality
Takada et al. (36)	Japan	Prospective	AHF	Malnourished (≥2)	1,705	36.0%	71.0	17.5	MACE

AHF, acute heart failure; CONUT, controlling nutritional status; CHF, chronic heart failure; CRT, cardiac resynchronization therapy; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; MACE, major adverse cardiac events.

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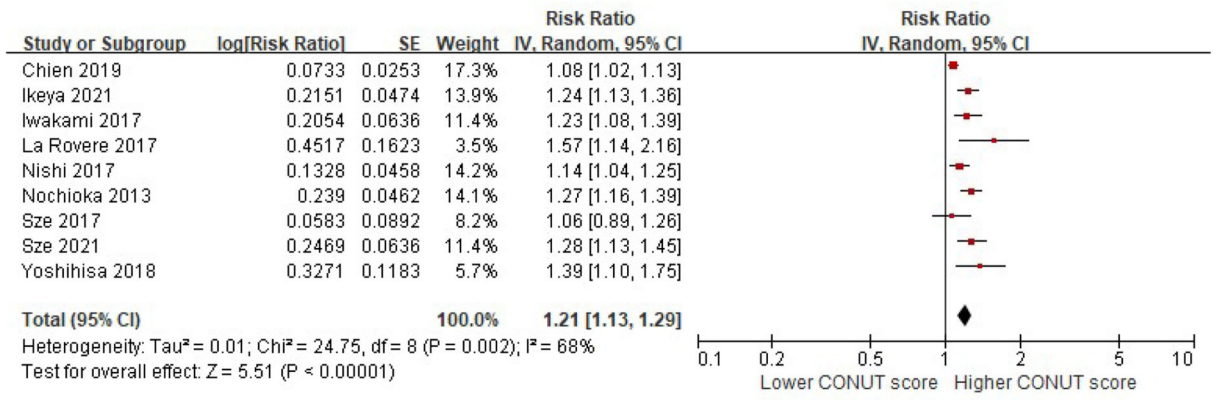

FIGURE 2
A forest plot of comparison: All-cause mortality in patients with HF associated with per-1 increase of the CONUT score. CONUT, controlling nutritional status; HF, heart failure.

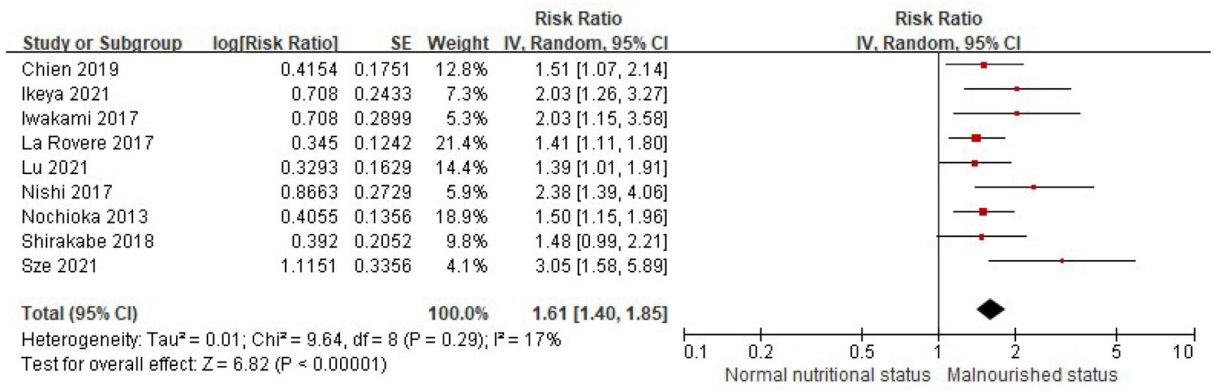

FIGURE 3
A forest plot of comparison: All-cause mortality in patients with HF associated with malnutrition status defined by the CONUT score. CONUT, controlling nutritional status; HF, heart failure.

Similar to our study, a previous meta-analysis by Li et al. (37) included 10 studies involving 5,196 patients with HF, and the results showed that the malnourished patients with HF had an increased risk of follow-up mortality (RR, 2.01; 95% CI, 1.58–2.57). However, the risk of MACEs, including risk of the re-hospitalization, was not evaluated in Li's study. In our meta-analysis, we included a much larger sample size (18 studies with 12,532 participants), which allowed us to perform a much more comprehensive analysis, and our results showed that the risk of MACE in HF was also increased with a higher CONUT score. Furthermore, we found that the worse prognosis (including all-cause mortality and MACEs) was more significant in patients with HF, with moderate to severe malnutrition. Therefore, patients with moderate to severe malnutrition should be emphasized to require more intensive nutritional interventions (e.g., increased protein and energy intake) added to the GDMT, and regular follow-up is needed to improve their prognosis (38).

Several underlying mechanisms may be related to the worse prognosis in HF patients with malnutrition. First, gastrointestinal congestion and gut edema can cause appetite loss and malabsorption (39, 40). Second, the chronic inflammatory state in HF would cause metabolic disturbances, activation of the sympathetic nerve system, and anabolic-catabolic imbalance (41, 42). Third, disturbance of cytokine, adipokines, and metabolites may also play a role in the association between malnutrition and clinical outcomes in HF (43, 44).

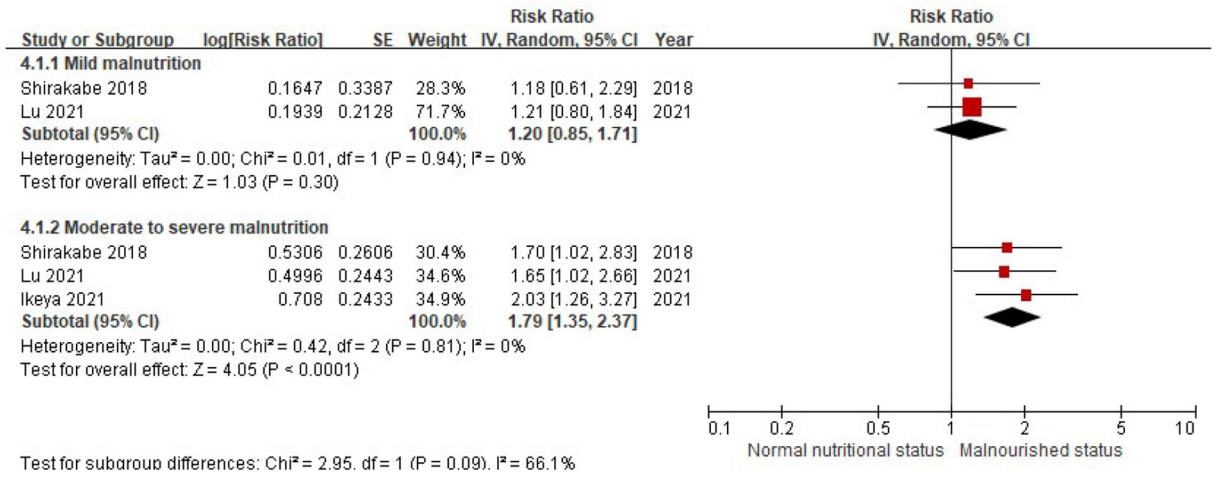

FIGURE 4
A forest plot of comparison: All-cause mortality in patients with HF associated with different levels of malnourished status defined by the CONUT score. CONUT, controlling nutritional status; HF, heart failure.

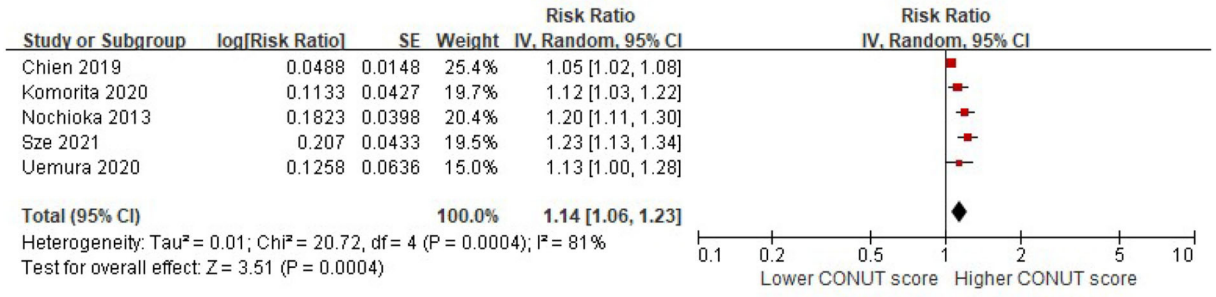

FIGURE 5
A forest plot of comparison: The risk of MACEs in patients with HF associated with per-1 increase of the CONUT score. CONUT, controlling nutritional status; HF, heart failure; MACEs, major adverse cardiac events.

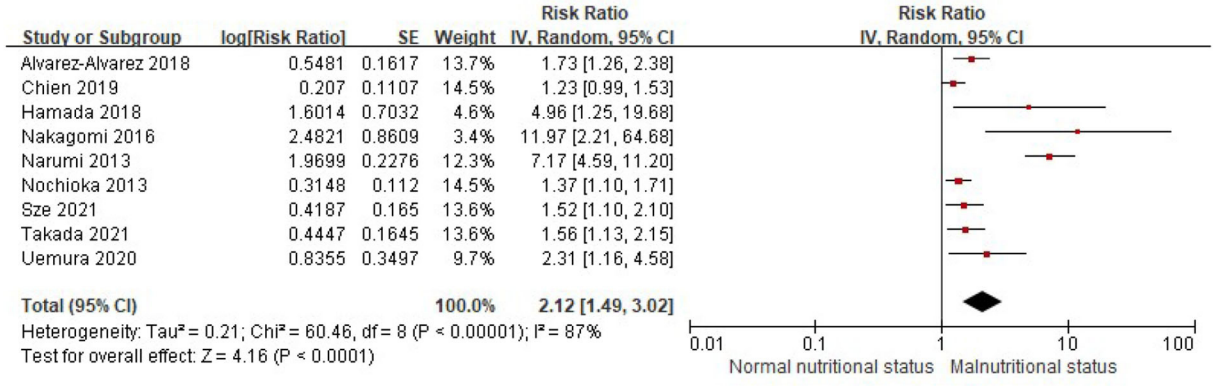

FIGURE 6
A forest plot of comparison: The risk of MACEs in patients with HF associated with malnutrition status defined by the CONUT score. CONUT, controlling nutritional status; HF, heart failure; MACEs, major adverse cardiac events.

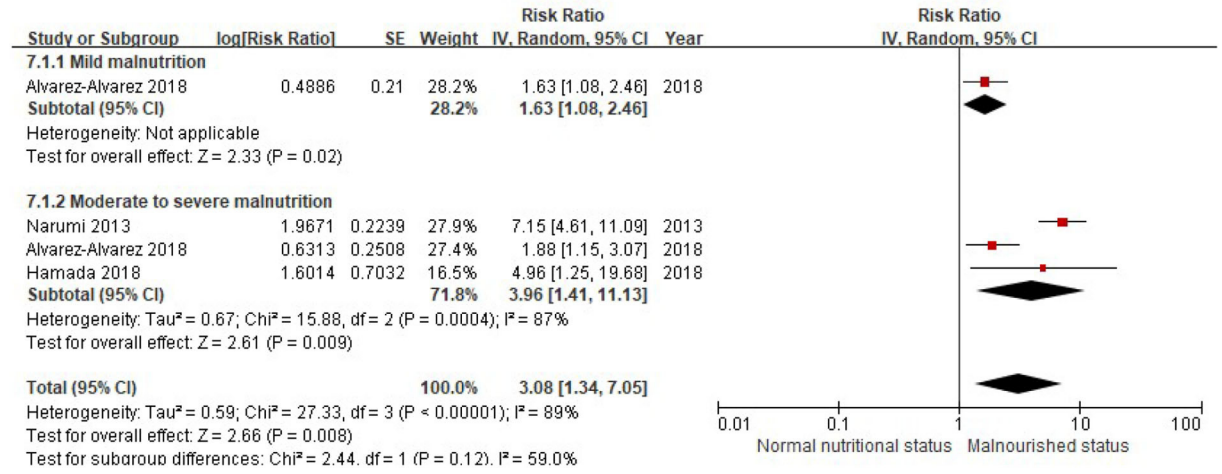

FIGURE 7
A forest plot of comparison: MACEs in patients with HF associated with different levels of malnourished status defined by the CONUT score. CONUT, controlling nutritional status; HF, heart failure; MACEs, major adverse cardiac events.

Except for the CONUT score, some other simple nutritional indexes had also been proposed in patients with HF (8). For example, the prognostic nutritional index (PNI), which was calculated from the serum albumin and total peripheral lymphocyte count, was reported to be associated with a poor prognosis in patients with acute and chronic HF (46, 47). However, the cut-point for malnutrition by the PNI was inconsistent in different studies, which would hamper its wildly clinical use (48). It had been cautious that the total cholesterol level was included as a component in calculating the CONUT score, which would overestimate the prevalence of malnutrition in patients with HF, as most of them may receive statins treatment and resulted in a lower total cholesterol level (5). In the same cohort of patients, it had been shown that the prevalence of malnutrition would be up to 54% when defined by the CONUT, while only 8% when defined by the PNI (45). However, in patients without statins or other lipid-lowing drug treatment, the inclusion of total cholesterol level may be more comprehensive for evaluating the nutritional status, as it also considered the lipid metabolism (9).

Some limitations in our study should be addressed. First, as discussed above, the CONUT score can be significantly affected by the treatment of statins or other lipid-lowering drugs. However, the proportion of statins treatment was unavailable in most of the included studies. Second, most of the included studies only evaluated the nutritional status at enrollment, but not evaluated the change of nutritional status during the follow-up. However, our results support the conclusion that the baseline nutritional status at enrollment is associated with the prognosis in patients with HF. Third, limited studies were available for the analysis of the different levels of malnutrition and the prognosis. Further studies are needed to document whether mild malnutrition was associated with poor clinical outcomes in HF. Fourth, due to the unavailability of individual patients' data, we cannot perform the analysis of risk discrimination (e.g., c-statistic) and reclassification (e.g., net reclassification improvement or an integrated discrimination index).

Conclusion

The CONUT score is an easily available nutritional index associated with the clinical outcomes in patients with HF. Further studies are needed to explore whether the CONUT score can be used as a screening tool for nutritional status in HF and guide the nutritional interventions to improve prognosis in HF.

Data availability statement

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

Author contributions

X-WH and BB: research idea and study design. X-WH and J-JL: data acquisition, data analysis/interpretation, and statistical analysis. BB: supervision and mentorship. All the authors contributed important intellectual content during manuscript drafting or revision and accept accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.961141/full#supplementary-material

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ORIGINAL RESEARCH
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Abnormal Calcium Metabolism Mediated Increased Risk of Cardiovascular Events Estimated by High Ankle-Brachial Index in Patients on Peritoneal Dialysis

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Su X, He W, Zhang M, Zhang Y, Zhu L, Chen J and Huang H (2022) Abnormal Calcium Metabolism Mediated Increased Risk of Cardiovascular Events Estimated by High Ankle-Brachial Index in Patients on Peritoneal Dialysis. Front. Cardiovasc. Med. 9:920431. doi: 10.3389/fcvm.2022.920431

Cardiovascular disease (CVD) is the leading cause of death in peritoneal dialysis (PD) patients. But the relationship between regular PD and the risk of major adverse cardiovascular events (MACE) remains controversial. The possible risk factors are not fully elucidated. This study aims to investigate the possible factors affecting the risk of MACE estimated by high ankle-brachial index (ABI) in PD patients. A total of 243 patients were enrolled and divided into chronic kidney diseases (CKD) stage 1, non-dialyzed CKD stages 2–5, and PD groups. The prevalence of high ABI, indicating increased MACE, was elevated with CKD progression but not further increased in PD patients. Systolic blood pressure was closely correlated with high ABI in non-dialyzed CKD patients ($\beta = 0.059$, $P = 0.001$). But in PD patients, serum calcium had a crucial effect on high ABI ($\beta = -9.853$, $P < 0.001$). Additionally, PD patients with high ABI tended to dialyze inadequately (Kt/V < 1.7) compared to those with normal ABI (29.0 vs. 13.3%, $P = 0.031$). Further mediation analysis revealed that ∼86.2% of the relationship between Kt/V and high ABI was mediated by serum calcium in PD patients (mediation effect = 86.2%, ab = −0.220, 95% CI: −0.381 to −0.059, $P = 0.008$), especially in those starting PD before 55 years of age and with normal body mass index. This present study indicated that improvement of PD adequacy by maintaining calcium balance might be a promising method to reduce the risk of MACE estimated by high ABI for PD patients.

Keywords: ankle-brachial index, peritoneal dialysis, calcium, vascular calcification, Kt/V

INTRODUCTION

Chronic kidney disease (CKD) is a substantial burden threatening global health (1). Among the causes of death, cardiovascular disease (CVD) is still the leading one of CKD death, which is increased with the development of CKD stages and is even up to 10–20 times higher in dialysis patients than in the general population (2, 3). Therefore, CVD prevention is important for

CKD, especially for patients on dialysis. Since traditional risk factors such as hypertension and hypercholesterolemia cannot fully explain the high mortality of CVD in the CKD population, untraditional risk factors, especially vascular calcification (VC), have caused great concern recently (4). Defined as the deposition of extraosseous calcium (Ca) in the vasculature, VC is a common complication in CKD patients (5). Studies have demonstrated that VC is strongly associated with CVD mortality in patients with CKD (6–8). Ankle-brachial index (ABI) is a simple and non-invasive method widely used in clinical practice to identify peripheral artery disease. High ABI (>1.3) indicates artery stiffness and has a close relationship with VC. It is reported that high ABI is independently associated with major adverse cardiac events (MACE) (9, 10), but remain unclear in dialysis patients. Adragao et al. reported that CKD patients with high ABI exhibited nearly 7-fold higher risk of cardiovascular mortality (11). Therefore, ABI is a promising indicator for VC and provides a better portable CVD risk assessment in CKD patients.

The state of uremic milieu is shown to be involved in the development of VC (5). However, patients with different CKD stages have different risk factors for VC development (12). Especially for dialysis patients, besides the common risk factors similar to non-dialyzed patients, other factors like hemodynamic stability and mineral metabolism also play important roles in VC as well (11, 13). Peritoneal dialysis (PD) is considered a relatively better choice for patients with end-stage renal disease than hemodialysis (HD) as its preference for hemodynamic stability and volume regulation (13). Regular PD is demonstrated to reduce mortality, but its effect on cardiovascular risks remains controversial (14). Moreover, the possible risk factors are not fully elucidated.

Therefore, we conducted a cross-sectional study to explore the risk factors affecting the risk of MACE evaluated by ABI among patients with different CKD stages or PD treatment. Then, we further investigated the possible mediators for high ABI in PD patients and sought possible strategies.

METHODS

Study Population

This was a single-center observational study of consecutive CKD patients who were referred to the Tungwah Hospital of Sun Yat-sen University between November 2015 and November 2016. A total of 243 patients were enrolled in this study. All patients must undergo the ABI test.

The e-GFR of all included patients were calculated based on CKD-EPI equation (15). According to the CKD diagnostic criteria, patients were divided into three groups based on their e-GFR levels (1). Patients with the estimated-glomerular filtration rate (e-GFR) < 15 mL/min per 1.73 m^2 and receiving the regular continuous ambulatory peritoneal dialysis (CAPD) treatment for more than 3 months were defined as the PD group. Initiation of dialysis usually depended on the patient's preferences and medical necessity. It will be considered when one or more of the following are present: symptoms or signs attributable to kidney failure (e.g., neurological signs and symptoms attributable to uremia, pericarditis, anorexia, medically resistant acid-base or electrolyte abnormalities, reduced energy level, weight loss with no other potential explanation, intractable pruritus, or bleeding); inability to control volume status or blood pressure; and a progressive deterioration in nutritional status refractory to interventions (16, 17). Non-dialyzed patients with e-GFR < 90 mL/min per 1.73 m^2 were defined as non-dialyzed CKD stages 2–5 group, and the other patients who had the evidence of renal structural or functional abnormalities but e-GFR ≥ 90 mL/min per 1.73 m^2 were defined as CKD stage 1 group.

Clinical data were extracted from the hospital database. Individuals with age < 20 years, familial hyperlipidemia, severe hepatic dysfunction, carcinoma, potential infectious or inflammatory diseases, autoimmune diseases, peripheral artery disease, corticosteroid therapy, either one leg with ABI ≤ 0.9 were excluded from this study. In the PD group, patients who had peritonitis in the past 3 months and cannot receive CAPD should be excluded.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committee of the Tungwah Hospital of Sun Yat-sen University. Informed consent was obtained from each participant.

Laboratory Parameters

Laboratory parameters were all measured by using blood samples. Each patient should fast overnight for at least 10 h before venipuncture. Biochemical parameters, Ca, serum phosphorus (P), creatinine, alkaline phosphatase (ALP), fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), cystatin C (CysC), etc. were analyzed by a standardized and certified TBA-120 auto-analyzer (Toshiba Medical Systems, Japan) in the institutional central laboratory.

Dialysis Adequacy

To estimate dialysis adequacy, weekly Kt/V was calculated based on 24-h urine performed prior to the scheduled visit to the PD unit. Weekly Kt/V values were calculated according to the standard method recommended in the kidney disease outcomes quality initiative guidelines (18). The cut-off value of Kt/V was 1.7, as recommended by K/DOQI guidelines (19). Kt/V ≥ 1.7 indicated adequate PD while Kt/V < 1.7 indicated insufficient PD.

Measurement of ABI

Ankle-brachial index was measured by a non-invasive vascular screening device (VP-1000, OMRON, Japan) that was reported before (20, 21).

Definition of Adverse Cardiovascular Events

History of adverse cardiovascular events was collected including cardiac death, coronary heart disease (CHD), congestive heart failure (CHF), acute myocardial infarction (AMI), and acute cerebral infarction (ACI). CHD was defined as ≥50% diameter

TABLE 1 | Clinical characteristics of non-dialyzed CKD stages 1–5 and PD patients.

	CKD stage 1	CKD stage 2–5 (Non-dialyzed)	PD	*P*-value
N	52	77	114	
Age (y)	43 ± 7	47 ± 13	44 ± 14	0.122
Male (%)	51.9	50.6	64.0	0.128
BMI (kg/m^2)	22.7 ± 3.0	23.0 ± 5.0	22.1 ± 3.7	0.329
SBP (mmHg)	126 ± 23	135 ± 20	152 ± 21	<0.001*
DBP (mmHg)	82 ± 15	82 ± 15	89 ± 13	0.001*
Smoking (%)	7.7	9.1	39.5	<0.001*
Hypertension (%)	48.1	64.9	87.7	<0.001*
DM (%)	11.5	16.9	14.0	0.690
FPG (mmol/L)	5.20 ± 0.90	5.36 ± 1.16	5.22 ± 1.76	0.762
ALP (U/L)	76 ± 25	78 ± 25	73 ± 16	0.309
Ca (mmol/L)	2.30 ± 0.14	2.24 ± 0.22	2.16 ± 0.24	<0.001*
P (mmol/L)	1.10 ± 0.19	1.11 ± 0.23	1.73 ± 0.54	<0.001*
K (mmol/L)	4.08 ± 0.37	4.24 ± 0.52	4.04 ± 0.75	0.087
TC (mmol/L)	4.60 ± 0.81	4.88 ± 1.02	4.77 ± 1.25	0.366
LDL-C (mmol/L)	2.63 ± 0.78	2.93 ± 0.94	2.72 ± 1.01	0.170
HDL-C (mmol/L)	1.25 ± 0.38	1.28 ± 0.42	1.25 ± 0.39	0.874
TG (mmol/L)	1.73 ± 1.38	1.70 ± 0.90	1.72 ± 1.34	0.994
Cr (mmol/L)	70.2 ± 18.1	143.4 ± 113.1	979.7 ± 322.3	<0.001*
CysC (mg/L)	0.91 ± 0.21	1.53 ± 0.83	5.80 ± 1.54	<0.001*
e-GFR [ml/(min·1.73m^2)]	111.7 ± 20.0	58.1 ± 22.7	–	<0.001*
Kt/V	–	–	2.04 ± 0.36	–
PD period (m)	–	–	38.8 ± 27.5	–
Residual renal function (KRU > min)			7.26 ± 3.18	
High ABI (%)	9.8	23.1	27.2	0.045*

*ABI, ankle-brachial index; ALP, alkaline phosphatase; BMI, body mass index; Ca, calcium; CKD, chronic kidney disease; Cr, creatinine; CysC, cystatin C; DBP, diastolic blood pressure; DM, diabetes mellitus; e-GFR, estimated-glomerular filtration rate; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; K, serum potassium; LDL-C, low-density lipoprotein cholesterol; P, phosphorus; PD, peritoneal dialysis; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; *P < 0.05.*

stenosis of coronary arteries by either coronary angiography or CT angiography (22). CHF was diagnosed according to 2016 ESC Guidelines for the diagnosis and treatment of chronic heart failure (23). AMI was diagnosed according to 2015 ESC Guidelines for the management of acute coronary syndromes (24). ACI was defined as an acute neurological event lasting more than 24 h associated with the evidence of ischemic focus of the brain in computer tomography or magnetic resonance imaging. Cardiac death was defined as death caused by AMI, arrhythmias, or CHF. The combination of CHD, CHF, AMI, ACI, and cardiac death was defined as MACE in this study.

Statistical Analysis

Data were presented as frequencies for categorical variables, mean values with standard deviation (SD) for normally distributed continuous variables, and median values with 25 and 75% percentiles for ordinal variables. The group differences among different CKD-stage groups were assessed by the analysis of variance (ANOVA) or Pearson chi-square according to the data types. Independent risk factors for high ABI were identified by binary logistic regression with the forward conditional method.

Mediation analysis was conducted to explore the mediator of the relationship between age, e-GFR or Kt/V, and high ABI. Mediation existed when four conditions were met: first, the predictor (in this case age, e-GFR or Kt/V) must have a significant relationship with the outcome variable (high ABI) in pathway c; second, the predictor must also have a significant relationship with the potential mediators in pathway a; third, the mediator must have a significant relationship with the outcome when the effect of the predictor on the outcome was controlled for pathway b; fourth, the relationship between predictor and outcome must be decreased (lower than in pathway c) when controlling for the mediators in pathway c'. If the predictor remained significant when the mediator was controlled for, the mediation was considered partial. When controlling for the mediator rendered the predictor non-significant, mediation was considered complete. In this article, both ABI (ABI $\geq$ 1.3 as 1 and 0.9 < ABI < 1.3 as 0) and Kt/V (Kt/V $\geq$ 1.70 as 1 and <1.70 as 0) were dichotomous variances while age, e-GFR, and all mediators were continuous. Ordinary least squares regression or logistic regression might be chosen according to the types of variances. Therefore, the parameter estimates a, b, c, c' and their standard

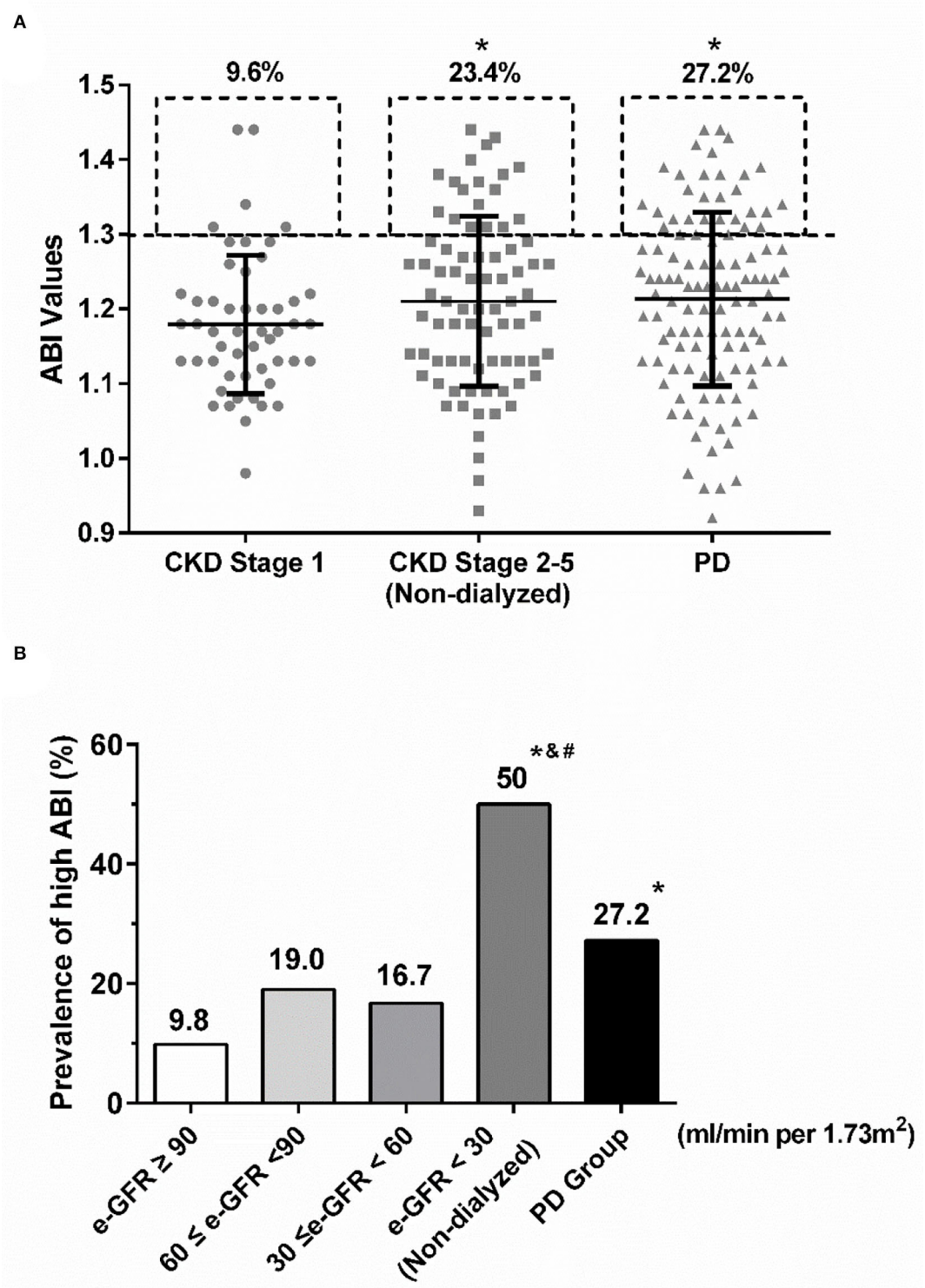

FIGURE 1 | The prevalence of high ABI in various CKD stages. **(A)** The rates of high ABI were similarly increased in non-dialyzed CDK stage 2–5 and PD groups compared with CKD stage 1. *$P < 0.05$ vs. CKD stage 1 group. **(B)** Similar upgraded trend of high ABI was shown in the decline of e-GFR. However, the prevalence of high ABI in PD patients was much lower than that in non-dialyzed CKD patients with e-GFR < 30 ml/min per 1.73m^2. *$P < 0.05$ vs. the group with e-GFR ≥ 90 ml/min per 1.73 m^2; # $P < 0.05$ *vs.* the group with 60 ≤ e-GFR < 90 ml/min per 1.73 m^2; and & $P < 0.05$ vs. the group with 30 ≤ e-GFR < 60 ml/min per 1.73 m^2. ABI, ankle-brachial index; CKD, chronic kidney disease; e-GFR, estimated-glomerular filtration rate; PD, peritoneal dialysis.

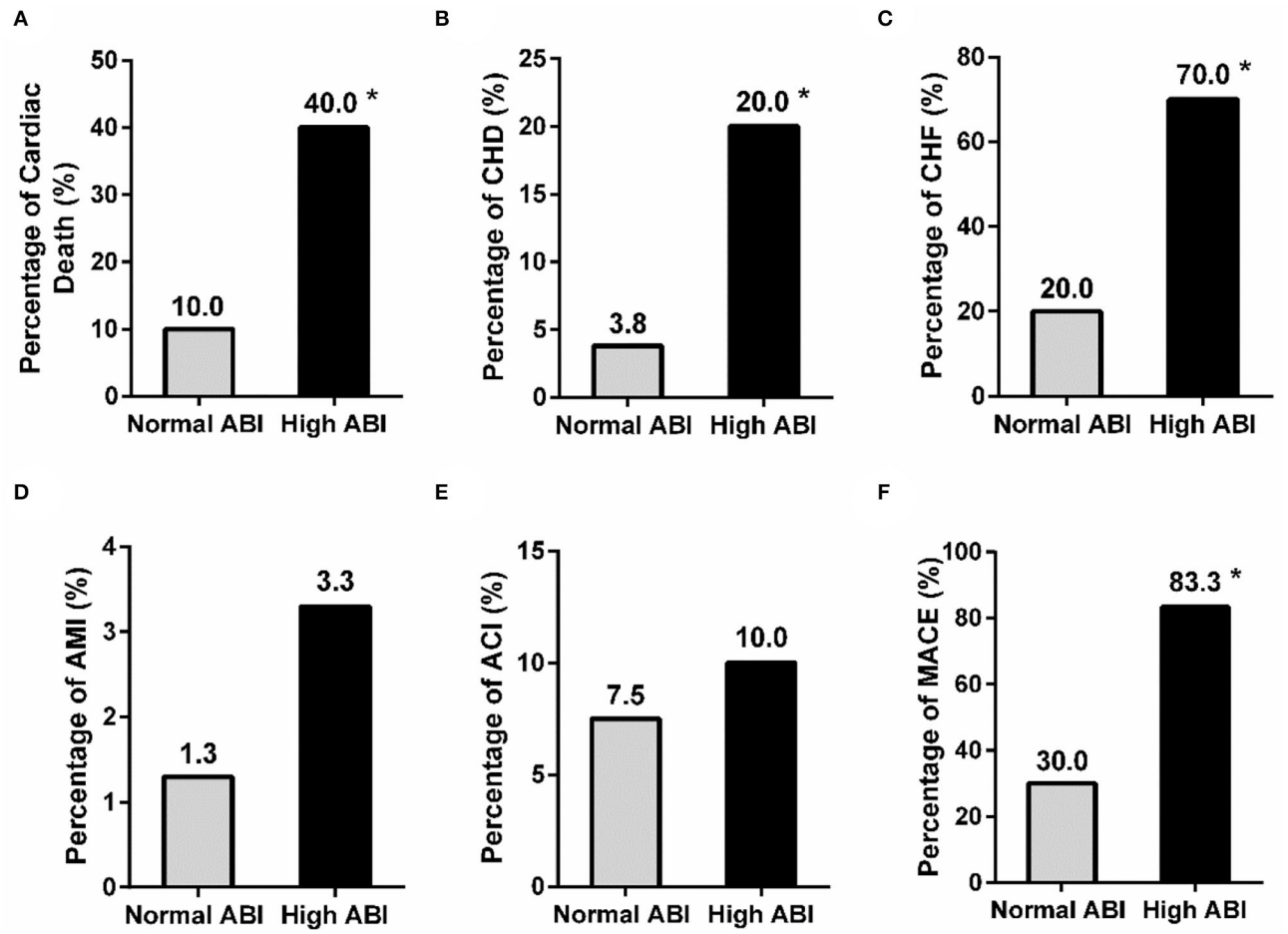

FIGURE 2 | Comparison of the history of adverse cardiovascular events between the normal and high ABI groups in PD patients. The prevalence of cardiac death, CHD, and CHF **(A–C)**, but not AMI and ACT **(D,E)**, was significantly different between patients with normal and high ABI. A significant difference was also observed in the rate of MACE **(F)** between the two groups. *$P < 0.05$ vs. normal ABI group. ABI, ankle-brachial index; ACI, acute cerebral infarction; AMI, acute myocardial infarction; CHD, coronary heart disease; CHF, congestive heart failure; MACE, major adverse cardiac events.

errors calculated above must be standardized according to the methods suggested by MacKinnon. Then the indirect effect was calculated by standardized parameters and tested for significance by the Sober test. The mediated effect size was also evaluated by a formula ab/(ab + c′), where a, b, and c' were all standardized (25).

All statistical analyses were performed using the software SPSS version 22.0 (SPSS, Inc, Chicago, IL), and 2-sided *P*-values < 0.05 were considered statistically significant.

TABLE 2 | The binary logistic regression analysis of the independent risk factors for high ABI.

Groups		*β*	SE	*P*-value
CKD stage 1	TC	4.305	1.747	0.014*
CKD stage 2–5 (non-dialyzed)	SBP	0.058	0.018	0.010*
	DM	1.542	0.689	0.025*
PD	Age	0.074	0.023	0.001*
	Ca	−9.853	2.020	<0.001*

*Binary logistic regression analysis with forward conditional method was used to assess the independent risk factors of high ABI in the CKD stage 1, non-dialyzed CKD stage 2–5 and PD groups. The factors used for analysis were age, sex, BMI, SBP, hypertension, DM, smoking, TC, LDL-C, Ca, Pi P, e-GFR. *P < 0.05. ABI, ankle-brachial index; BMI, body mass index; Ca, calcium; CKD, chronic kidney disease; DM, diabetes mellitus; e-GFR, estimated-glomerular filtration rate; PD, peritoneal dialysis; Pi P, phosphorus; SBP, systolic blood pressure; SE, standard error; TC, total cholesterol.*

RESULTS

Comparison of Clinical Characteristics Among CKD Stage 1, Non-dialyzed CKD Stages 2–5, and PD Groups

The clinical characteristics of CKD stage 1, non-dialyzed CKD stages 2–5, and PD groups are shown in **Table 1**. The PD patients tended to be smokers and exhibited higher rate of hypertension with higher blood pressure than the other groups (smoking: 39.5 vs. 9.1 vs. 7.7%, $P < 0.001$; hypertension: 87.7 vs. 48.1 vs. 64.9%, $P < 0.001$; SBP: 152 ± 21 mmHg vs. 135 ± 20 mmHg vs. 126

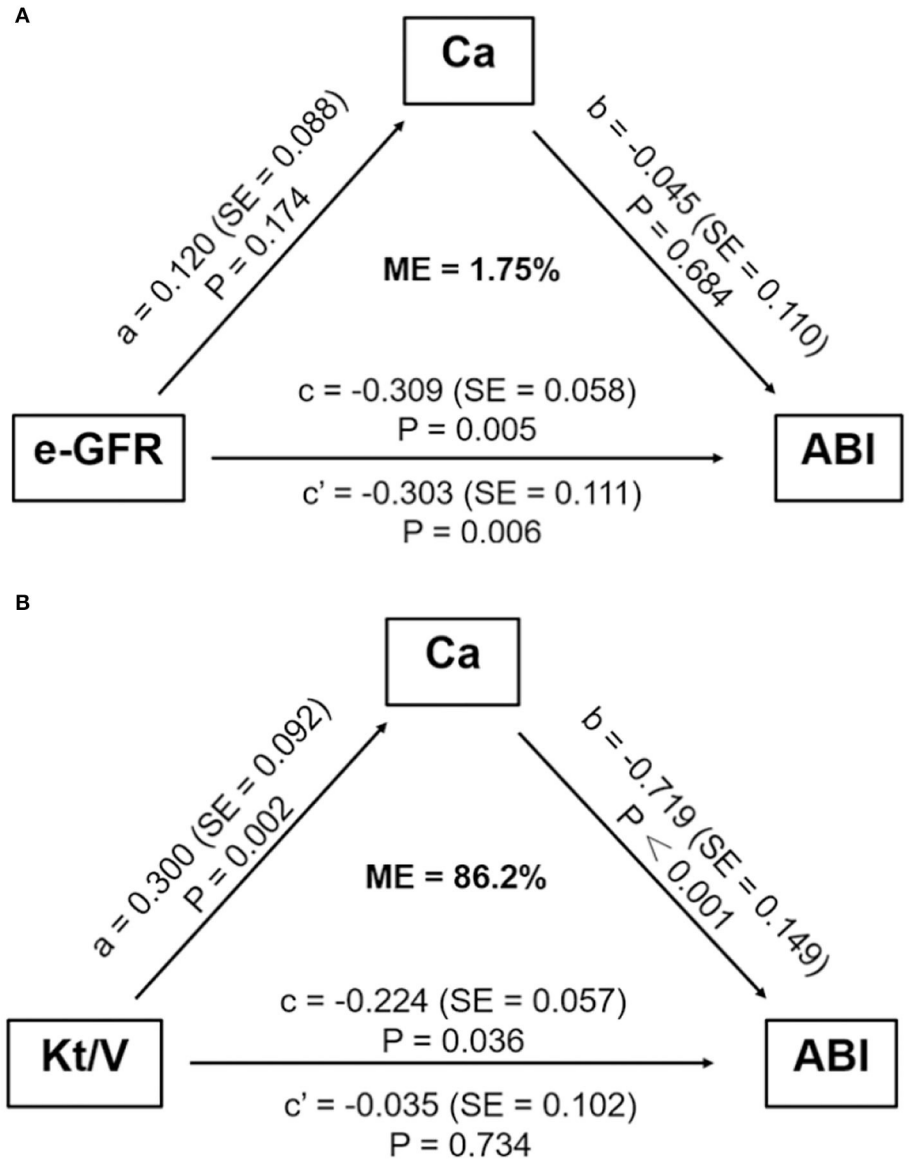

FIGURE 3 | Mediation analysis on the association between e-GFR or Kt/V and ABI. **(A)** The mediation effect of serum calcium did not exist in the association between e-GFR and high ABI in non-dialyzed CKD patients. **(B)** Serum calcium mediated the association between Kt/V and high ABI in PD patients. ABI, ankle-brachial index; Ca, calcium; e-GFR, estimated-glomerular filtration rate; ME, mediation effect; SE, standard error.

± 23 mmHg, $P < 0.01$; and DBP: 89 ± 13 mmHg vs. 82 ± 15 mmHg vs. 82 ± 15 mmHg, $P < 0.001$). Moreover, the levels of P and CysC were increased with the elevation of CKD stages and reached the highest in PD patients (P: 1.10 ± 0.19 mmol/L vs. 1.11 ± 0.23 mmol/L vs. 1.73 ± 0.54 mmol/L, $P < 0.001$; CysC: 0.91 ± 0.21 mg/L vs. 1.53 ± 0.83 mg/L vs. 5.80 ± 1.54 mg/L, $P < 0.001$), while Ca showed the opposite trend (2.30 ± 0.14 mmol/L vs. 2.24 ± 0.22 mmol/L vs. 2.16 ± 0.24 mmol/L, $P < 0.001$). However, no significant differences were found in age, sex ratios, body mass index (BMI), prevalence of diabetes mellitus (DM), the levels of FPG, TC, HDL-C, LDL-C, and TG ($P > 0.05$) (**Table 1**).

The Prevalence of High ABI Was Increased With CKD Progression

Prevalence of high ABI was significantly different among CKD stage 1, non-dialyzed CKD stages 2–5, and PD groups (9.6 vs. 23.4 vs. 27.2%, $P = 0.045$) (**Figure 1A**). Non-dialyzed CKD stages 2–5 and PD patients shared similar prevalence of high ABI and both were higher than that of CKD stage 1 patients (non-dialyzed CKD stage 2–5 vs. CKD stage 1: $P = 0.054$; PD vs. CKD stage 1: $P = 0.012$; non-dialyzed CKD stage 2–5 vs. PD: $P = 0.521$; **Figure 1A**). Furthermore, about 50% of non-dialyzed patients with e-GFR < 30 ml/min per 1.73 m^2 had high ABI

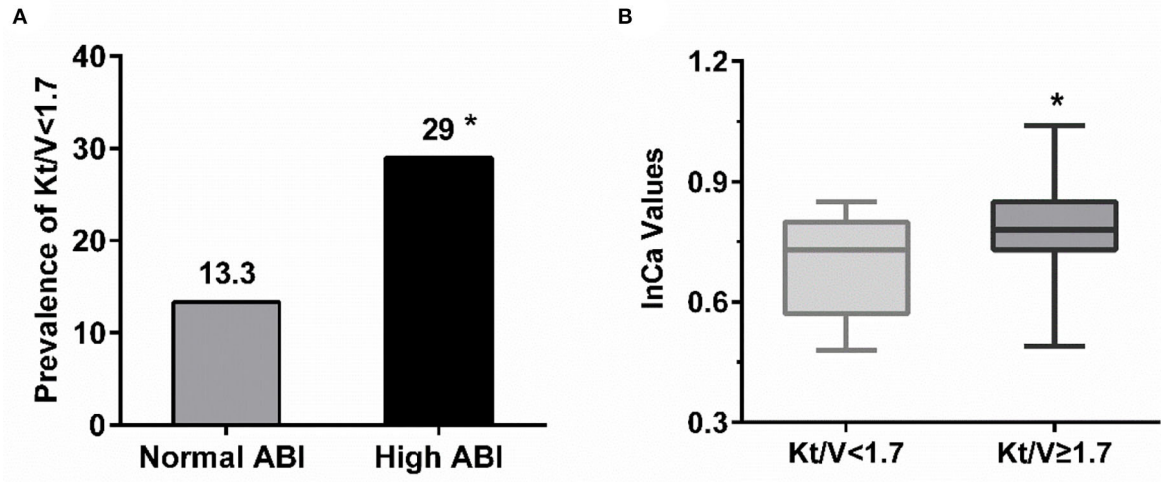

FIGURE 4 | The relationship among adequacy dialysis and high ABI. **(A)** PD patient with high ABI tended to have Kt/V lower than 1.7 compared with those with normal value. *$P < 0.05$ vs. normal ABI group. **(B)** The level of serum calcium in PD patients with Kt/V $\geq$ 1.7 were relatively higher but within normal range. *$P < 0.05$ vs. Kt/V < 1.7. ABI, ankle-brachial index; Ca, calcium.

(**Figure 1B**). Interestingly, the prevalence tended to decrease in PD patients compared with those with similar e-GFR but without dialysis (e-GFR < 30 ml/min per 1.73 m^2: 50.0% vs. PD: 27.2%, $P = 0.477$) (**Figure 1B**). However, no significant difference was observed in ABI values among various groups or e-GFR levels. The results showed that high ABI occurred more frequently with the decrease of e-GFR but the prevalence was not further increased in PD patients.

Different Risk Factors Contributed to ABI Between PD and Non-dialyzed Patients

As known, high ABI has been reported to be associated with a high risk of adverse CVD in non-dialyzed patients (10). Our results showed that PD patients with high ABI also suffered more cardiac death, CHD, CHF, and total MACE than those with normal ABI (cardiac death: 40.0 vs. 10.0%, $P < 0.001$; CHD: 20.0 vs. 3.8%, $P = 0.012$; CHF: 70.0 vs. 20.0%, $P < 0.001$; MACE: 83.3 vs. 30.0%, $P < 0.001$) (**Figure 2**). The prevalence of AMI and ACI tended to be increased in the high ABI group than those in the normal ABI group, although the difference was not significant (AMI: 3.3% vs. 1.3%, $P = 0.473$; ACI: 10.0 vs. 7.5%, $P = 0.702$) (**Figure 2**). This indicated that high ABI was closely associated with a high prevalence of CVD in PD.

To explore the possible risk factors affecting high ABI between PD and non-dialyzed CKD patients, binary logistic regression analysis was used. The results showed that TC was a risk factor for high ABI in CKD stage 1 group while SBP and DM were in the non-dialyzed CKD stages 2–5 group (TC: $\beta = 4.305$, SE = 1.747, $P = 0.014$; SBP: $\beta = 0.058$, SE = 0.018, $P = 0.010$; DM: $\beta = 1.542$, SE = 0.689, $P = 0.025$) (**Table 2**). In the PD group, both age and Ca were the risk factors for high ABI (age: $\beta = 0.074$, SE = 0.023, $P = 0.001$; Ca: $\beta = -9.853$, SE = 2.020, $P < 0.001$) (**Table 2**). Taken together, the results suggested that traditional cardiovascular risk factors contributed to high ABI in non-dialyzed CKD patients while the serum level of Ca played an important role in PD ones.

The Mediation Effects of Serum Calcium on High ABI in CKD Patients

We further studied whether any mediators functioned in the association between e-GFR and high ABI in non-dialyzed CKD patients. As shown in **Figure 3A**, indirect effect of Ca was not significant in the relationship between e-GFR and ABI for non-dialyzed CKD patients (ab = −0.005, 95% CI: −0.390 to 0.022, $P = 0.696$). This result indicated that Ca did not act as a mediator in the relationship between e-GFR and high ABI in non-dialyzed CKD patients.

For PD patients, e-GFR was no longer suitable for evaluating kidney function. Instead, Kt/V was usually used to estimate dialytic adequacy. Kt/V $\geq$ 1.7 indicated dialytic adequacy while Kt/V < 1.7 suggested inadequacy. About 29% of PD patients with high ABI had Kt/V lower than 1.7, which was significantly higher than those with normal ABI (29.0 vs. 13.3%, $P = 0.031$) (**Figure 4A**). Moreover, PD patients with Kt/V < 1.7 tended to have lower Ca level compared to those with Kt/V $\geq$ 1.7 (0.69 $\pm$ 0.122 vs. 0.78 $\pm$ 0.11, $P = 0.001$) (**Figure 4B**). As Ca was the risk factor for high ABI in PD patients, we explored whether Ca mediated the association between Kt/V and ABI in PD patients. In PD patients, especially over 30 years old, the indirect effect of Ca with Kt/V became significant (ab = −0.215, 95% CI: −0.371 to −0.059, $P = 0.006$). Moreover, the total effect of Kt/V on ABI became non-significant after including Ca in the model (total effect c = −0.224, $P = 0.036$; direct effect c' = −0.034, $P = 0.734$). This indicated that ∼86.2% of the relationship between Kt/V and ABI was mediated by Ca. The finding showed that Ca acted as a mediator in the relationship between Kt/V and ABI in PD patients (**Figure 3B**). Further, we conducted a subgroup analysis to identify the PD subgroups with high ABI mediated

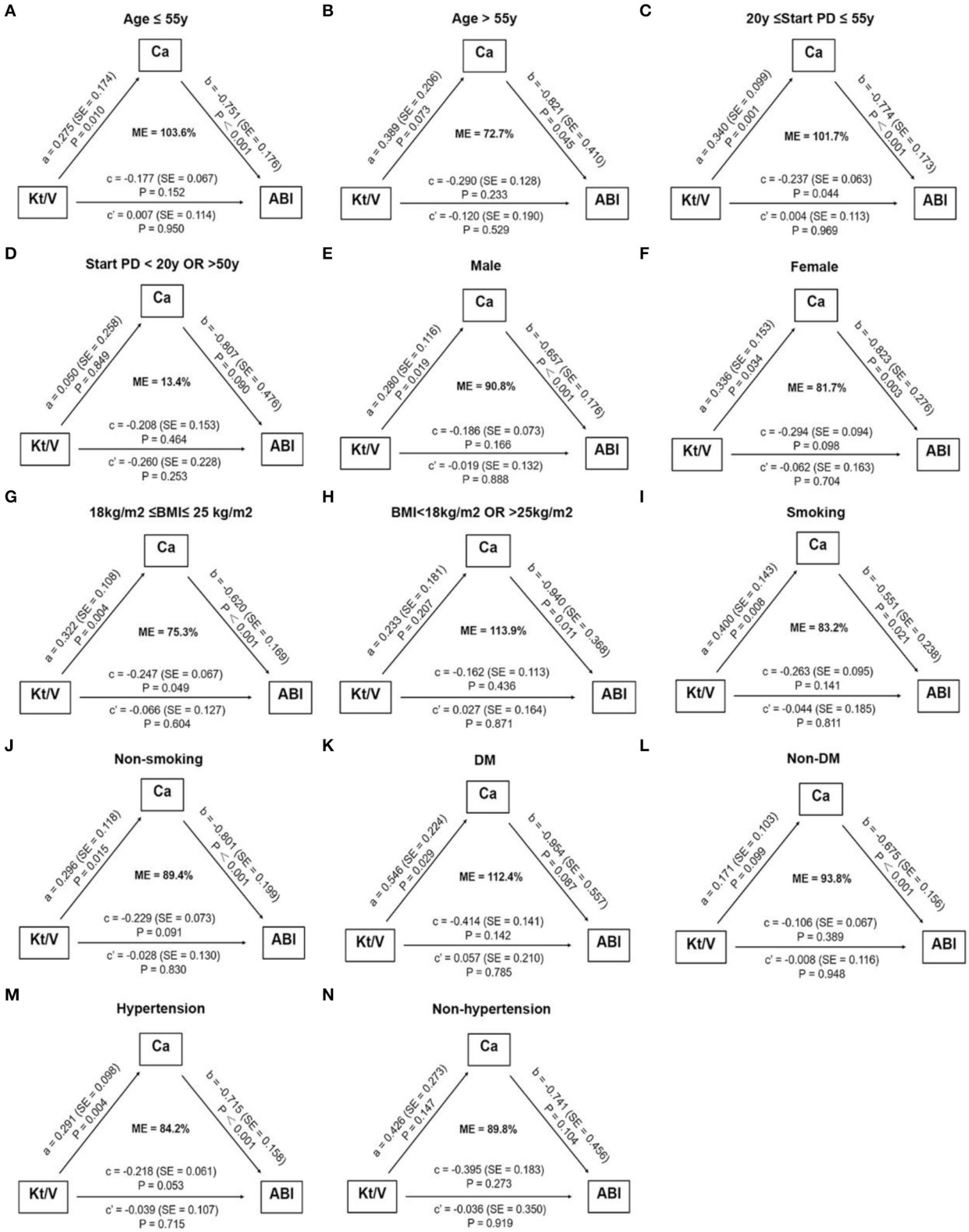

FIGURE 5 | Subgroup analysis of assessing the mediation effect of serum calcium. The mediation effect of serum calcium on the association between Kt/V and high ABI only existed in PD patients starting dialysis before 55 years of age **(C)** and with normal BMI **(G)**. No mediation effect of serum calcium was observed in other subgroups **(A,B,D–F,H–N)**. The number of patients in subgroups: age ≤ 55 y: $n = 88$, Age > 55 y: $n = 26$; 20 y ≤ start PD ≤ 55 y: $n = 93$, start PD > 20 y OR < 50 *(Continued)*

FIGURE 5 | y: $n = 17$; male: $n = 73$, female: $n = 41$; 18 kg/m^2 ≤ BMI ≤ 25 kg/m^2: $n = 79$, BMI < 18 kg/m^2 OR > 25 kg/m^2: $n = 31$; smoking: $n = 45$, non-smoking: $n = 69$; DM: $n = 16$, non-DM: $n = 98$; hypertension: $n = 100$, non-hypertension: $n = 14$. ABI, ankle-brachial index; BMI, body mass index; Ca, calcium; DM, diabetes mellitus; e-GFR, estimated-glomerular filtration rate; ME, mediation effect; PD, peritoneal dialysis; SE, standard error.

by Ca. As shown in **Figure 5**, the mediated effect of Ca became significant in PD patients starting PD before 55 years of age (ME = 101.7%, ab = −0.263, 95% CI: −0.452 to −0.074, $P = 0.006$) (**Figure 5C**) and with normal BMI (ME = 75.3%, ab = −0.200, 95% CI: −0.369 to −0.031, $P = 0.020$) (**Figure 5G**). However, the mediating effect did not exist in other subgroups (all $P > 0.05$).

DISCUSSION

Our study mainly found that the prevalence of high ABI, a strong MACE indictor, was increased with CKD progression. Interestingly, the prevalence of high ABI in PD patients was not further increased compared with non-dialyzed ones, which was possibly related to PD adequacy. Further mediation analysis revealed the important mediated effect of Ca on the relationship between PD adequacy and high ABI, especially in patients starting dialysis before 55 years of age and with normal BMI.

Although the development of effective dialysis and medication treatment for dialysis patients have been improved, CVD mortality is still predicted to be further increased by 2030, which causes great concern worldwide (1). In the late stages of CKD, many medications lack efficacy. For example, statins are supposed to be beneficial for the reduction of CVD mortality in non-dialyzed patients while similar protective effects cannot be seen in dialysis patients (26, 27). It indicates that other nontraditional risk factors may also contribute to the development of CVD in the dialysis population. As an important nontraditional risk factor, VC is supposed to be associated with incremental CVD mortality (6, 7). Many studies have demonstrated that the prevalence of VC is higher in CKD patients and elevates with CKD progression (28, 29). Likewise, our results also reveal that the prevalence of high ABI, predicting VC, increases with the decline in e-GFR levels. But intriguingly, the prevalence of high ABI is not further increased among PD patients. It seems that major risk factors influencing VC in PD patients are different from non-dialyzed ones. As PD patients with high ABI tend to have increased risk of MACE, the possible contributors of VC in PD patients warrant further exploration.

Numerous factors have been reported to contribute to VC in CKD patients, among which abnormal mineral metabolism is the most important contributor (30). As known, the kidney is a major organ regulating mineral balance. Thus, impaired kidney function frequently develops a deficiency of mineral metabolism, and the condition becomes the worst at the end-stage of CKD. Mineral disorder is commonly seen in dialysis patients, who are also more prevalent with VC (31). Importantly, abnormal mineral metabolism has been demonstrated to contribute to the large burden of CVD in dialysis patients (32). The additional health risks imposed by abnormal mineral metabolism are greatly challenging the management of patients with CKD although the traditional risk factors have well been controlled, especially for dialysis patients (8, 31). In this study, we observe that serum Ca, rather than traditional risk factors, plays a critical role in high ABI among PD patients. It seems that Ca balance might be a major contributor to VC after patients started PD (33). In fact, the essential role of Ca in the development of VC has been well elucidated (34). Growing evidence from randomized controlled trials demonstrates that more progression of VC and higher mortality are observed in CKD patients receiving calcium-based phosphate binders than those receiving non-calcium-containing phosphate binders because of the high risk of Ca overload (35, 36). Therefore, well-controlled Ca balance is critical for CKD patients, especially for dialysis patients with markedly reduced urinary Ca excretion.

Besides using drugs such as phosphate binders and vitamin D, adequate dialysis is a specific and effective way to maintain mineral balance for dialysis patients. As known, inadequate dialysis contributes greatly to increased risk of all-cause and CVD mortality in dialysis patients (37). Dialysis adequacy also plays a role in the development of VC. Estimated by low Kt/V level, inadequate dialysis is reported to be an independent risk factor for VC in dialysis patients (38, 39). Likewise, we find that the Kt/V level of PD patients with high ABI tends to be <1.7 and PD adequacy is associated with a lower prevalence of high ABI. This might be because insufficient PD could lead to internal environmental disorders and mineral metabolism imbalance, which then initiates vascular lesions and VC (5, 31). Indeed, further mediation analysis estimates that ∼84.4% of the relationship between Kt/V and ABI might be mediated by Ca in PD patients. It suggests that maintaining neutral Ca balance after adequate dialysis is important for reducing the prevalence of high ABI for PD patients. It might be another reason to explain why recent studies have reported that PD patients seem to face additional CVD risk (14). Therefore, dialysis treatment for PD patients should be individualized after comprehensive assessments, which include both the adequacy of dialysis and the level of post-dialysis Ca. Nevertheless, due to the limited data, further investigation is needed to verify our results.

Another interesting result we discovered is that the mediation effect of Ca only exists in PD patients starting dialysis before 55 years of age and with normal BMI. It seems that only in such relatively low-risk PD patients, adequate dialysis could retard the development of VC by maintaining a neutral Ca balance. This might explain why some PD patients with adequate dialysis also suffer from high ABI, as both age and abnormal nutritional status are important contributors to VC in CKD patients (30, 40). With a series of traditional cardiovascular risks, PD patients tend to suffer from advanced VC, and even adequate dialysis could not bring further benefits to its regression. Therefore, initiating PD

earlier than 55 years of age and keeping BMI in the normal range may help patients benefit more from dialysis adequacy.

There are several limitations in this study. First, this is a cross-sectional, observational study. It might be impossible to fully confirm the relationship among dialysis adequacy, Ca, and high ABI. But we conduct the medication analysis which is used to analyze the statistically causal relationship. Second, the number of PD patients enrolled is relatively small and selection bias exists as patients without the contraindications of PD are included in this study. Therefore, further prospective studies with a larger sample size are needed to verify our findings. Third, we used ABI to estimate VC instead of computed tomography (CT) scanning. Although CT is the most common method used to assess VC, it may be not suitable for CKD patients because of the renal damage of the contrast agent. As a simple and noninvasive method, ABI has no renal damage and is more convenient and economical for CKD patients in systematic VC detection.

In conclusion, we provide clinical evidence that PD inadequacy correlated with increased high ABI occurrence, which predicts incremental risk of MACE, and that Ca might be an important mediator, especially for those starting PD before 55 years of age and with normal BMI. Improvement of PD adequacy by controlling neutral Ca balance seems to be promising to reduce the risk of MACE evaluated by high ABI for patients on PD.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Tungwah Hospital of Sun Yat-sen University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HH contributed to conception and design of the study and the analyses. XS, WH, MZ, and YZ participated in data collection. WH contributed to the statistical analysis and the draft manuscript. LZ, JC, and HH reviewed the manuscript and made final changes. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.920431/full#supplementary-material

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D-Dimer Is Associated With Coronary Microvascular Dysfunction in Patients With Non-obstructive Coronary Artery Disease and Preserved Ejection Fraction

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Background: Coronary microvascular dysfunction (CMVD), an important etiology of ischemic heart disease, has been widely studied. D-dimer is a simple indicator of microthrombosis and inflammation. However, whether an increase in D-dimer is related to CMVD is still unclear.

Materials and Methods: This retrospective study consecutively enrolled patients with myocardial ischemia and excluded those with obstructive coronary artery. D-dimer was measured at admission and the TIMI myocardial perfusion grade (TMPG) was used to distinguish CMVD. Patients were divided into the two groups according to whether the D-dimer was elevated (>500 ng/ml). Logistic models and restricted cubic splines were used to explore the relationship between elevated D-dimer and CMVD.

Results: A total of 377 patients were eventually enrolled in this study. Of these, 94 (24.9%) patients with CMVD had older age and higher D-dimer levels than those without CMVD. After full adjustment for other potential clinical risk factors, patients with high D-dimer levels (>500 ng/ml) had a 1.89-times (95% CI: 1.09–3.27) higher risk of CMVD than patients with low D-dimer levels. A non-linear relationship was found between concentrations of D-dimer and CMVD. With increased D-dimer level, the incidence of CMVD increased and then remained at a high level. Stratified analysis was performed and showed similar results.

Conclusion: Elevated D-dimer level is associated with the incidence of CMVD and potentially serves as a simple biomarker to facilitate the diagnosis of CMVD for patients with angina.

Keywords: non-obstructive coronary artery disease, preserved ejection fraction, coronary microvascular dysfunction, TIMI myocardial perfusion grade, D-dimer

INTRODUCTION

Although clinical practice strategies have optimized the prevention and treatment of ischemic heart disease over the past few years, ischemic heart disease has a complex pathophysiology that goes beyond the traditional role of obstructive coronary artery disease (CAD). Coronary microvascular dysfunction (CMVD), a phenotype of ischemic heart disease, is defined as the clinical syndrome of angina without obstructive CAD (1). CMVD may contribute to angina by reducing coronary blood flow, which is prevalent and associated with an increased risk of future adverse cardiovascular outcomes (2, 3). Studies have shown that CMVD is mediated by risk factors traditionally recognized as related to cardiovascular disease, although these factors account for a small sample, leaving a large proportion that cannot be explained (4, 5). Potential pathophysiological mechanisms of ischemia involved in CMVD with non-obstructive CAD have been previously proposed such as microvascular thrombosis, inflammation, and edema (6), indicating a changed myocardial microcirculation environment. However, there is currently a lack of simple tools to identify CMVD and more predictors are still desperately needed.

D-dimer, a degradation product of cross-linked fibrin, is widely recognized as a marker of thrombosis (7). Elevated D-dimer has a prognostic value for adverse cardiovascular events in healthy people and patients with CAD (8, 9). Zhang et al. found that D-dimer in admission could be used to identify the no-reflow phenomenon in patients with ST-segment elevation myocardial infarction, highlighting the advantage of D-dimer in identifying microvascular embolism (10). Previous studies have shown that elevated D-dimer levels are related to microvascular thrombosis (11), inflammation (12), and endothelial injury (13), which contribute to CMVD. The TIMI myocardial perfusion grade (TMPG) is a simple indicator derived from coronary angiography to evaluate coronary microcirculation and identify CMVD (14–16). Although D-dimer is a simple indicator of the microcirculatory environment, the relationship between D-dimer level and CMVD has only rarely been studied.

In this study, we hypothesized that D-dimer levels might be associated with CMVD evaluated by the TMPG and could be used as an available biomarker for early screening for CMVD.

MATERIALS AND METHODS

Study Population

This retrospective study included 1,654 consecutive patients who were admitted for suspected CAD from September 2014 to September 2015 at the Guangdong Provincial People's Hospital. Suspected CAD is clinically based on symptoms of ischemia and/or electrocardiographic ischemic changes. Patients with obstructive coronary artery stenosis (defined as $\geq$70% luminal diameter narrowing of an epicardial stenosis or $\geq$50% luminal diameter narrowing of the left main artery, n = 1,218), with impaired left ventricular ejection fraction (LVEF) ($<$40%, n = 35) and without coronary angiography data (n = 24) were excluded (**Figure 1**). Demographic data, risk factors, and coronary angiography results were collected based on the electronic medical records.

This study was approved by the Ethics Committee of Guangdong Provincial People's Hospital and informed verbal consent was obtained from all the patients. This research was conducted in accordance with the Declaration of Helsinki.

Coronary Angiography

All the patients underwent coronary angiography using the Judkins technique. Coronary angiography was performed with a radial approach and the femoral artery was used in a minority of patients, as clinically necessary. We used 5-Fr or 6-Fr Judkins left and right diagnostic catheters for left and right coronary angiography, respectively. The degree of coronary artery stenosis was judged and recorded by two interventional cardiologists. Evaluation of the TMPG flow was performed by two experienced cardiologists who were blinded to patient's demographic and clinical information. In the case of disagreement, a third cardiologist was consulted and the majority opinion was adopted.

The TMPG was classified into four grades (17) as follows: (1) TMPG 0: failure of dye to enter the microvasculature, indicating a lack of tissue-level perfusion; (2) TMPG 1: dye enters slowly but fails to exit the microvasculature. There is a ground-glass appearance or opacification of the myocardium in the distribution of the vessel that fails to clear from the microvasculature and dye staining is present on the next injection (30 s); (3) TMPG 2: delayed entry and exit of dye from the microvasculature. Dye strongly persists after three cardiac cycles of the washout phase and either does not or only minimally diminishes in intensity during washout; and (4) TMPG 3: normal entry and exit of dye from the microvasculature. Dye is gone or is mildly/moderately persistent after three cardiac cycles of the washout phase and noticeably diminishes in intensity during the washout phase. The blush that is of only mild intensity throughout the washout phase but fades minimally is also classified as grade 3.

Definitions and Laboratory Examination

The TMPG flow was used to assess coronary microvascular function and CMVD was distinguished with the TMPG flow $<$3. Hypertension, diabetes, chronic kidney disease (CKD), smoking, and alcohol consumption were diagnosed according to self-report and discharge diagnosis. A blood routine was detected using the Sysmex XE-5000 machine. High-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglyceride, lipoprotein(a), albumin, uric acid, and creatinine were detected using the Beckman AU5800 spectrophotometer *via* colorimetry or immunoturbidimetry. D-dimer was detected using the Sysmex CA-1500 *via* immunoturbidimetry.

Echocardiographic Analysis

Transthoracic echocardiography examination was performed by experienced sonographers using the GE Vivid E95 (GE Healthcare, Milwaukee, WI, United States) interfaced with a 2.5–3.5-MHz phased array probe. LVEF was measured using Simpson's method.

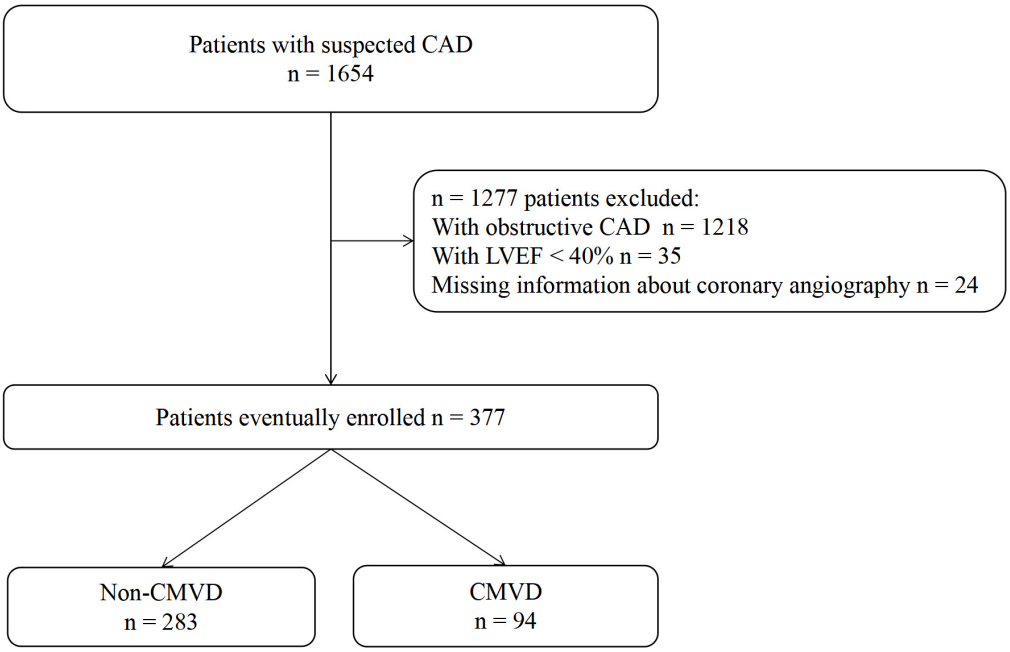

FIGURE 1 | Study flowchart.

Statistical Analysis

The total procedure for statistical analysis was divided into four steps. First, we used the *t*-test for normally distributed data, the Mann–Whitney *U* test for non-normally distributed data, and the Chi-squared test or Fisher's exact test for categorical variables to identify significant differences between the two groups. Second, we used the logistic regression models simultaneously for unadjusted, minimally adjusted, and fully adjusted analyses to evaluate the associations between D-dimer and CMVD. Considering the potential influence of age on D-dimer concentration, we added the sensitivity analyses to assess the relationship using an age-adjusted cut-off (500 ng/ml, if age is <50 years or age in years ×10 in patients ≥50 years) (18, 19). Third, because D-dimer is a continuous variable, to visually assess the non-linear relationship between D-dimer level and risk of CMVD, a restricted cubic spline curve was used. Fourth, subgroup analyses were performed using stratified Chi-square models; interactions among subgroups were examined using likelihood ratio tests. Comparisons with $P < 0.05$ (two-sided) were considered to be statistically significant. All of the analyses were performed with Stata 15.0 (StataCorp LLC, College Station, TX, United States), R version 3.4.3 (The R Project for Statistical Computing, Vienna, Austria), and EmpowerStats (X&Y Solutions Incorporation, Boston, MA, United States).

RESULTS

Patient Characteristics

A total of 377 patients who presented with symptoms of ischemia changes and without obstructive CAD were finally included. Of those, 94 patients had evidence of CMVD assessed using the TMPG flow. The clinical characteristics of patients with CMVD and non-CMVD are shown in **Table 1**. Patients with CMVD were more likely to be older, have more frequent smoking behavior, and have more hypertension than those without patients without CMVD. D-dimer levels were significantly higher in patients with CMVD than in controls [360.00 (270.00–747.50) vs. 330.00 (270.00–470.00)], while sex, alcohol consumption, diabetes, renal function, blood lipids, and previous medication use presented no difference between the two groups.

Association Between D-Dimer and Coronary Microvascular Dysfunction

Results for the association between D-dimer and CMVD are shown in **Table 2**. In the unadjusted model, the odds ratio (OR) for CMVD of D-dimer was 2.12 (95% CI: 1.29–3.50). After fully adjusting for other potential clinical risk factors, including age, sex, hypertension, diabetes, smoking, alcohol consumption, and platelets, the risk of CMVD among those with high D-dimer levels (>500 ng/ml) was 1.86-times (95% CI: 1.09–3.19) higher than the risk among patients in low D-dimer levels ($P < 0.05$). The association between the age-adjusted D-dimer and CMVD remained remarkably significant.

The relationship between D-dimer and the risk of CMVD is given in **Figure 2**. The restricted cubic spline curve showed a non-linear relationship between the D-dimer level and the prevalence of CMVD (P for linearity <0.05). An elevated D-dimer level was significantly associated with an increased risk of CMVD ($P = 0.0241$). When the D-dimer was at a margin level (500 ng/ml), the OR for CMVD was 1.30.

TABLE 1 | Demographic and clinical characteristics of patients included in the study.

	Non-CMVD $n = 283$	CMVD $n = 94$	*p*-value
Age, years	61 ± 11	64 ± 9	0.043
Male sex	150 (53.00%)	56 (59.57%)	0.268
Current smoking	64 (22.61%)	31 (32.98%)	0.045
Alcohol consumption	16 (5.65%)	7 (7.45%)	0.529
Hypertension	134 (47.35%)	65 (69.15%)	< 0.001
Diabetes	50 (17.67%)	19 (20.21%)	0.580
CKD	30 (10.60%)	11 (11.70%)	0.766
WBC, 10^9/L	7.20 ± 2.04	7.33 ± 1.88	0.269
Platelets, 10^9/L	223.42 ± 61.03	216.52 ± 66.36	0.354
Hemoglobin, g/L	132.50 ± 15.33	134.86 ± 16.28	0.203
Glucose, mmol/L	5.61 ± 1.58	5.86 ± 2.37	0.248
TG, mmol/L	1.58 ± 1.25	1.66 ± 1.09	0.601
TC, mmol/L	4.62 ± 1.24	4.55 ± 1.01	0.615
LDL-C, mmol/L	2.68 ± 1.08	2.61 ± 0.87	0.587
HDL-C, mmol/L	1.16 ± 0.29	1.15 ± 0.27	0.783
Creatine, μmol/L	78.20 ± 27.60	77.72 ± 22.31	0.877
D-dimer, ng/mL	330.00 (270.00–470.00)	360.00 (270.00–747.50)	0.028
CRP, mg/L	1.52 (0.55–4.35)	1.64 (0.57–3.58)	0.669
LVEF, %	66 (62–69)	65 (61–70)	0.383
Previous drug treatment			
ACE inhibitor or ARB, %	44 (15.55%)	14 (14.89%)	0.879
Beta-blocker, %	42 (14.84%)	10 (10.64%)	0.306
Calcium channel blocker, %	30 (10.60%)	15 (15.96%)	0.165
Diuretics, %	7 (2.47%)	1 (1.06%)	0.685
Statin, %	50 (17.67%)	19 (20.21%)	0.580

CMVD, coronary microvascular dysfunction; CKD, chronic kidney disease; WBC, white blood cells; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; CRP, C-reactive protein; LVEF, left ventricular ejection fraction; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker.

TABLE 2 | Association of CMVD and D-dimer in study participants.

Variable	Model 1†	Model 2†	Model 3†	Model 4†
D-dimer was converted into a binary variable according to a cutoff of >500 ng/mL				
OR	2.12	1.91	1.88	1.86
95% CI	1.29–3.50	1.13–3.22	1.10–3.23	1.09–3.19
Chi-square (DF)	8.49 (1)	11.91 (3)	25.11 (7)	26.31 (8)
P-value	0.003	0.016	0.021	0.024
D-dimer was converted into a binary variable according to age-related cutoff value				
OR	2.25	2.06	1.95	1.92
95% CI	1.29–3.91	1.17–3.62	1.10–3.48	1.08–3.43
Chi-square (DF)	7.95 (1)	12.40 (3)	24.87 (7)	26.00 (8)
P-value	0.004	0.012	0.023	0.027

†*Model 1: Unadjusted. Model 2: Adjusted for age and sex. Model 3: Adjusted as in Model 2 and hypertension, diabetes, smoking, and alcohol consumption. Model 4: Adjusted as in Model 3 and platelets.*
CMVD, coronary microvascular dysfunction. OR, odds ratio; CI, confidence interval; DF, degrees of freedom.

Subgroup Analyses

In subgroup analysis, patients with high D-dimer levels (>500 ng/ml) have CMVD more frequently than those without in most of the strata (**Figure 3**). No significant interactions for the association between D-dimer and CMVD were found among individuals stratified by age, sex, hypertension, diabetes, smoking, CKD, and LDL-C levels.

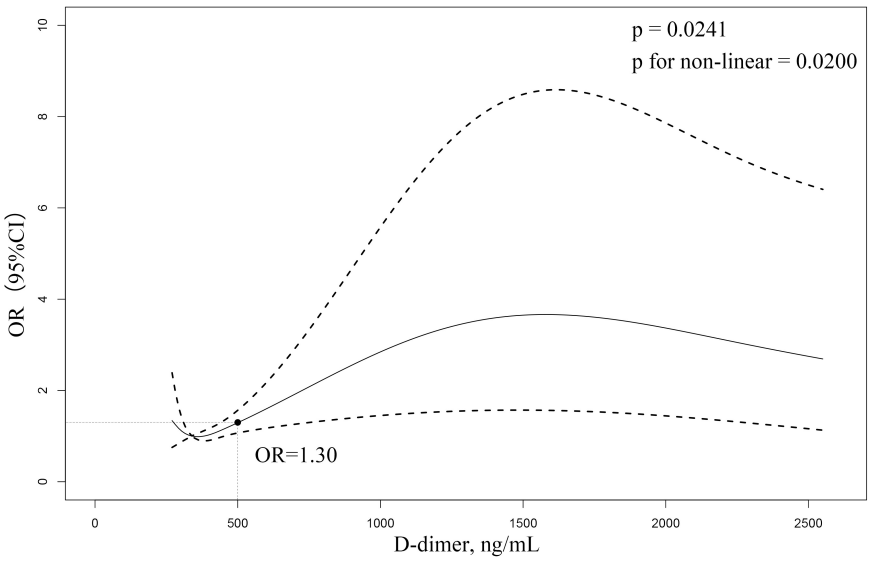

FIGURE 2 | Restricted cubic spline curve to fit the relationship between D-dimer level and CMVD. The model adjusted for age, sex, hypertension, diabetes, smoking, alcohol consumption, and platelets. The middle area of the dash represents 95% confidence interval (CI), and the reference line represents D-dimer margin level. The relationship between D-dimer level and CMVD is not shown for those D-dimer >2,500 ng/mL due to the large (95%) CI.

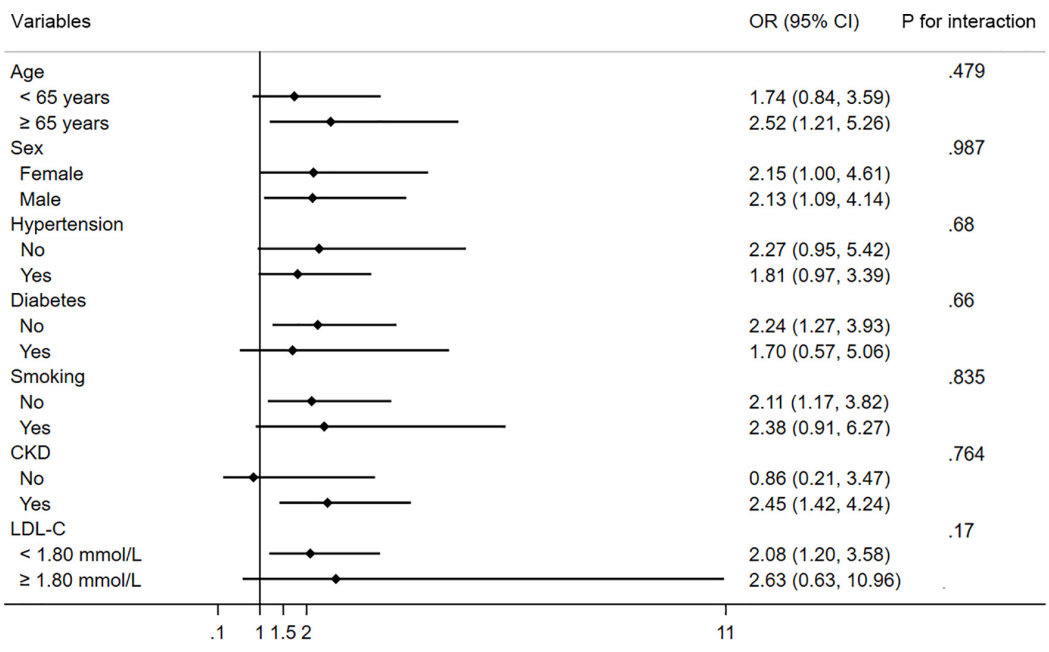

FIGURE 3 | Subgroup analysis of the association between CMVD and D-dimer in prespecified and exploratory subgroups. CI, confidence interval.

DISCUSSION

In the present study, we found that elevated D-dimer level was strongly associated with the risk of CMVD and the association was independent of traditional risk factors. There was a dose–response relationship between D-dimer concentration and the incidence of CMVD. Elevated D-dimer levels could be used as a biomarker to identify CMVD.

D-dimer, a biomarker of microthrombosis, has been previously reported to reflect microcirculation (11). Previous studies have shown that elevated D-dimer levels are associated with cerebral circulation and microvascular complications in patients with diabetes mellitus, indicating the role of D-dimer in evaluating microvascular function (20, 21). However, there has been little study on the correlation between D-dimer and coronary microcirculation. In our study, the concentration

of D-dimer was higher in patients with CMVD than in those without CMVD. Elevated D-dimer levels had a significant relationship with the occurrence of CMVD and the non-linear relationship between them also supports it. In the general population and patients with CAD, elevated baseline D-dimer levels were associated with poor cardiovascular outcomes (8, 9, 22), which may be attributed to the presence of CMVD. In addition, there is also an individual susceptibility to the occurrence of CMVD (23). Acquired risk factors such as older age, hypertension, diabetes, and hyperlipidemia may increase the occurrence of CMVD. In our study, patients with high D-dimer levels were older, had a higher incidence of hypertension, and were more likely to smoke. These comorbidities might contribute to CMVD.

The mechanisms by which D-dimer reflects microcirculation mainly include microvascular thrombosis, endothelial dysfunction, and inflammation. Previous studies on CMVD after ST-segment elevation myocardial infarction have shown that elevated D-dimer levels were largely influenced by thrombus burden and distal microvascular thrombosis (10). In addition to distal microvascular embolization, Erkol et al. found that *in situ* thrombosis may also contribute to poor myocardial perfusion (24). D-dimer, a biomarker that can reflect the severity of hypercoagulability, can be increased by microvascular embolism. Research involving patients with angina, non-obstructive CAD, and normal left ventricular function has shown that endomyocardial biopsy-proven endothelial cell activation occurred more frequently in patients with CMVD (25). Normal coronary blood flow and myocardial perfusion rely on the normally functioning endothelium, which regulates smooth muscle function through the release of vasodilators, such as nitric oxide (26). Inversely, damage to endothelial cells would lead to platelet activation and a coagulation cascade (27). The correlation between elevated D-dimer and endothelial cell dysfunction has been widely reported in previous studies (28–30). The significantly independent association between D-dimer levels and endothelial function determined *via* flow-mediated dilatation of the brachial artery supports the role of D-dimer as a biomarker for endothelial dysfunction (31), suggesting that an increased D-dimer level indicates the presence of endothelial dysfunction. Another potential mechanism contributing to CMVD is vascular inflammation. Prior studies have suggested that traditional cardiovascular risk factors may be important and they fail to fully account for the increased risk of the development of CMVD (32). In this regard, Klein et al. reported that patients with biopsy-proven myocardial inflammation infiltrate had an apparently reduced coronary flow reserve, indicating an association between inflammation and the occurrence of microvascular dysfunction (33). What is more, high-sensitivity C-reactive protein (CRP) is inversely related to coronary flow reserve in patients with angina and without obstructive CAD, showing a direct relationship between inflammation and CMVD (34). D-dimer, as an acute phase reactant, is increasingly recognized as a marker of inflammatory reaction (27). D-dimer concentrations have been reported to be related to vascular inflammation and microvascular complications in patients with metabolic and infectious diseases, such as diabetes mellitus, chronic obstructive pulmonary disease, and HIV infection (10, 35). Our results indicated that inflammation markers, such as white blood cell count and CRP, were slightly higher in patients with CMVD, although not significantly. In all, the microenvironment of inflammation and endothelial damage reflected by D-dimer plays an important role in the development of CMVD among patients with ischemic heart disease.

This study has several limitations. First, the present study had a retrospective cross-sectional design, so no causal relationship can be inferred between D-dimer levels and CMVD. Second, the research was conducted in a single center and the sample size is relatively small, so the conclusions drawn here cannot be generally extrapolated. Third, other factors that influence the level of D-dimer were not considered in this study, including infectious diseases, pulmonary embolism, and subclinical deep vein thrombosis. However, the effect of these factors can be ruled out because individuals with highly abnormal D-dimer levels were excluded and the vast majority of the study population had D-dimer concentrations of <2,500 ng/ml. Fourth, it is widely recognized that the TMPG is a clinically accessible and practical method to assess CMVD. However, other more accurate quantitative methods could be considered as options for further studies, such as cardiac MR, index of microcirculatory resistance, and fractional flow reserve.

CONCLUSION

In summary, D-dimer levels are significantly associated with the incidence of CMVD. This biomarker may be useful in identifying patients with CMVD for ischemic heart disease.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Guangdong Provincial People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XH, YL, and HD conceived and designed study, and drafted and refined the manuscript. WW, BY, LZ, and XH collected and complied, and analyzed the data. YZ, GL, and HD revised the manuscript critically. All authors have read and agreed to the published version of the manuscript.

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Long-term prognostic value of macrophage migration inhibitory factor in ST-segment elevation myocardial infarction patients with metabolic syndrome after percutaneous coronary intervention

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Metabolic syndrome (MetS) is a major risk factor for cardiovascular disease and negatively affecting the prognosis of patients with ST elevation myocardial infarction (STEMI). Macrophage migration inhibitory factor (MIF) is a multipotent cytokine involved in various cardiovascular and inflammatory diseases. In this prospective study, we investigate the value of MIF in the long-term prognosis of STEMI combined with MetS after emergency PCI. Circulating MIF levels were measured at admission, and major adverse cardiovascular and cerebrovascular events (MACCE) were monitored during the follow-up period of 4.9 (3.9–5.8) years. MACCE occurred in 92 patients (22.9%), which was significantly higher in MetS (69/255, 27.1%) than in the non-MS subgroup (23/146, 15.8%, $P < 0.05$). Patients with MetS developed MACCE had the highest admission MIF level. Kaplan-Meier survival analysis using the cutoff value of admission MIF (143 ng/ml) showed that patients with a higher MIF level had a greater incidence of MACCE than those with lower MIF levels in both the MetS ($P < 0.0001$) and non-MetS groups ($P = 0.016$). After adjustment for clinical variables, the value of MIF $\geq$ 143 ng/ml still had the predictive power for the MetS group [HR 9.56, 95% CI (5.397–16.944),$P < 0.001$]; nevertheless,

it was not the case in the non-MetS group. Our findings indicated that MetS is a critical risk factor for adverse clinical outcomes in patients with STEMI, and a high admission MIF level has predictive power for the long-term MACCE, which is superior in STEMI patients with MetS and better than other traditional predictors.

KEYWORDS

metabolic syndrome, macrophage migration inhibitory factor, MACCE, ST- segment elevation myocardial infarction, coronary artery disease

Introduction

Metabolic syndrome (MetS) is characterized by a constellation of metabolic disorders including impaired glucose tolerance, central obesity, dyslipidemia, and hypertension (1). As a result of economic growth and medical advancement, changes in lifestyle and dietary intake and aging have become the major risks for the development of MetS (2–4). The prevalence of MetS has increased significantly worldwide, not only in developed countries but also in developing countries over the past decades. In the United States, the prevalence of MetS was 23.7% (age-adjusted) during 1988–1994 (5), which sharply increased to 32.5–36.9% during 2011–2016 (6). Using the revised National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria, the International Collaborative Study of Cardiovascular Disease in ASIA (InterASIA) showed that the prevalence of MetS was 13.7% among adults aged 35–74 years in China in 2001 (3), which increased to 33.9% among adults aged 18 years and older in 2010 based on the China Non-communicable Disease Surveillance data (7). Recently, the China Health and Recruitment Longitudinal Study (CHARLS) revealed a further elevation of MetS prevalence up to 39.7% in middle-aged and elderly Chinese during 2011–2015 (8).

On a global scale, approximately 16.7 million patients die from cardiovascular disease (CVD) every year, representing the leading cause of mortality in the world (9). Growing evidence has strongly indicated that MetS is the major risk factor for CVD (10–14). A meta-analysis including 87 studies involving 951,083 participants reported that MetS was associated with a twofold increased risk of CVD (relative risk, RR: 2.35; 95% CI: 2.02–2.73) and CVD-related mortality (RR: 2.40; 95% CI: 1.87–3.08), and a 1.5-fold elevation in all-cause mortality (RR: 1.58; 95% CI: 1.39–1.78) (15). Thus, patients with MetS were at higher risk for cardiovascular outcomes. Furthermore, MetS was also associated with a higher risk for myocardial infarction (MI) and stroke (15, 16). Patients classified as MetS suffered from acute MI had worse outcomes at follow-up (11). Therefore, early prediction of major adverse cardiovascular and cerebrovascular events (MACCE) in MI patients with MetS bears an important clinical value.

Macrophage migration inhibition factor (MIF) acts as a pro-inflammatory factor and is widely expressed in different cell types and involved in many inflammatory-related disorders (17, 18). Association of MIF in myocardial ischemia and infarction has been reported in clinical and experimental settings (19). Notably, early elevation of plasma MIF levels in patients following ST-segment elevation MI (STEMI) correlated with acute and chronic infarct size and the degree of cardiac remodeling (20). Our previous study demonstrated that admission MIF levels could predict long-term MACCE in patients with STEMI (21). MetS, as a major risk factor for CVD, has drawn great attention in clinical settings. However, it is not known whether admission MIF levels carry the same prognostic importance for development of MACCE in patients with MetS subjected to an acute MI. Furthermore, it is unclear if the same MIF value can be used to predict long-term outcome in both patients with or without MetS after acute MI. The aim of this study is to address these key questions in patients with STEMI after percutaneous coronary intervention (PCI).

Materials and methods

Study design and participants

We consecutively recruited patients with STEMI aged >18 years admitted to our hospital from January 2014 to October 2018 who underwent emergency PCI after

Abbreviations: MIF, macrophage migration inhibitory factor; STEMI, ST-elevation myocardial infarction; MetS, metabolism syndrome; PCI, percutaneous coronary intervention; CVD, cardiovascular disease; CHD, coronary heart disease; hs-TnT, high sensitive-troponin T; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MACCE, major adverse cardio and cerebrovascular events; WBC, white blood cells; hs-CRP, high sensitive C-reactive protein; Grace score, Global Registry of Acute Coronary Events.

the onset of chest pain. All patients/participants provided written information. This project is in line with the Declaration of Helsinki, and the research protocol was approved by the Human Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Approval ID: K201301-09).

Inclusion criteria

The diagnosis of STEMI was defined as a plasma level of cardiac high sensitive-troponin T (hs-TnT) >0.1 μg/ml after symptom onset together with at least one of the following: (1) chest pain lasting for >20 min; (2) Electrocardiograph (ECG) exhibiting elevation of ST segment >1 mm or a new pathological Q wave (22). MetS was defined based on the modified NCEP ATPIII criteria (1) and included three or more of the following components: (1) abdominal obesity (body mass index, BMI ≥ 30 for men and ≥ 25 kg/m^2 for women) (23); (2) elevated triglycerides (TG ≥ 1.69 mmol/L); (3) reduced high-density lipoprotein-cholesterol (HDL-C, < 1.03 mmol/L in men and < 1.29 mmol/L in women); (4) systolic blood pressure (SBP, >130 mmHg) or diastolic blood pressure (DBP >85 mmHg) or use of antihypertensive medications; and (5) fasting plasma glucose >5.6 mmol/L or use of antidiabetic medications. In this study, we used BMI as a surrogate parameter for central obesity, which had been adopted and verified in previous studies (24–27).

Exclusion criteria

Patients with one or more of the following conditions were excluded: malignancy, thrombolysis, cardiomyopathy, previous history of PCI or coronary artery bypass grafting (CABG), recurrent MI, infectious disease, active inflammatory disease, renal failure, severe liver disease, peripheral arterial disease, or hematologic disease.

Definition of cardiovascular risk factors

Body mass index was calculated by dividing body weight (kilograms) by the height in meter squares. Overweight/obesity was classified as a BMI ≥ 30 kg/m^2 for men and ≥ 25 kg/m^2 for women (27). Persons who reported regular tobacco use in the previous 6 months were considered as current smokers. Hypertension was defined as history of hypertension and/or repeated systemic BP measurements exceeding 140/90 mmHg or use of antihypertensive medications. Diabetes was defined as a history or presence of diabetes and/or a fasting plasma glucose level of >7.0 mmol/L on two separate occasions or a random glucose value >11.1 mmol/L on at least one occasion before the present admission or use of antidiabetic medications. Concentrations of TC >6.2 mmol/L, TG >2.3 mmol/L, LDL-C >4.1 mmol/L, and HDL-C < 1.0 mmol/L were defined as hypercholesterolemia, hypertriglyceridemia, high LDL-C, or low HDL-C, respectively, according to Chinese dyslipidemia guidelines (28). Dyslipidemia was defined as any of the four lipid abnormalities mentioned above. The Global Registry of Acute Coronary Events (Grace) risk score is recognized as a validated predictor of adverse cardiovascular events in patients with AMI (29, 30). It is calculated based on age, heart rate, systolic BP, creatinine level, history of congestive heart failure, PCI and MI, ST-segment changes on admission ECG, and elevated levels of cardiac enzymes or markers.

Sample collection and laboratory test

Intravenous blood samples were collected at admission for the MIF level test, and the median symptom-to-sampling time was 5.8 h (25th–75th percentile, 3.5–8.0 h). Plasma MIF levels were measured using the Quantikine MIF ELISA kit (R&D Systems, United States) according to the manufacturer's specifications. The next day after PCI, intravenous blood samples were collected at the Coronary Care Unit and routine whole blood tests and biochemical tests, including high-sensitive C-reactive protein (hs-CRP), were performed in the laboratory of the First Affiliated Hospital Center of Xinjiang Medical University using a commercial automated platform. High sensitive-cardiac troponin T (hs-TnT) was tested at admission and every 4 h after admission to determine the peak.

Coronary angiography and percutaneous coronary intervention

All patients with STEMI were admitted with aspirin 300 mg, load dose of clopidogrel 300 mg, and standard intravenous heparin 70 U/kg and then underwent emergency coronary angiography followed by PCI. PCI procedures were performed by experienced interventional cardiologists. Multivessel lesions were defined as >50% stenosis in more than one major coronary artery. According to the Gensini score (31, 32), the severity of the injury was 1 (0–25%), 2 (25–50%), 4 (50–75%), 8 (75–90%), 16 (90–99%), and 32 (completely occluded vessels), respectively, and this ratio was multiplied by the segment location weighting factor to obtain the Gensini score for each patient. PCI was considered successful if the patient had a grade 3 blood flow rating for MI thrombolysis (TIMI) in the coronary artery associated with the area of MI and postoperative residual stenosis < 10% (33). After PCI, all patients received dual antiplatelet therapy: 100 mg aspirin, 75 mg clopidogrel daily for at least 1 year, and other cardiovascular-related medications at the discretion of the treating physician.

Echocardiography

All patients were assessed by transthoracic echocardiography within 48 h after primary PCI using the Vivid 7 Ultrasound System (GE Medical Systems, United States). Standard echocardiography was conducted for the assessment of left ventricular (LV) dysfunction.

Study endpoints

Study endpoints included MACCE during the follow-up period, including all-cause mortality, target lesion reconstruction, recurrent angina or AMI, readmission due to heart failure, arrhythmia, or/and stroke. All clinical events were defined according to standardized definitions. If patients presented with multiple events, only the first event was considered for event-free survival analysis.

Follow-up visits

Follow-up visits included telephone interviews, outpatient visits, and inpatient clinical records of readmitted patients. Information on the deceased patient was obtained from hospital records or telephone contacts with relatives of the patient. The follow-up period ended in October 2020. MACCE were recorded at 1, 3, and 6 months after discharge and every 6 months thereafter.

Quality control

Professionally trained investigators used a uniformly designed questionnaire to collect general patient information, laboratory results, coronary angiography results, and MACCE events through our electronic medical records and paper cases. The database was created using the Epidata 3.0 software, and the data entry was performed by two investigators independently. The data were verified in a batch of 10 again to ensure the accuracy of data entry.

Statistical analysis

Based on a prospective cohort study design and according to our previous study, the incidence of long-term MACCE in ACS was 38% in the high MIF group, and the hazard ratio was 2.8 (21). We set $\alpha = 0.05$ and power = 0.9 to calculate the sample size. We also assumed a 10% loss during the follow-up period. Therefore, a sample of 110 patients per group was required. Data were collected using Epidata3.1 (Odense, Denmark) and double checked. Continuous variables with a Gaussian distribution are presented as mean ± standard deviation (SD), and those with a non-Gaussian distribution are presented as median values with corresponding 25th–75th percentiles. The differences between groups were evaluated using Student's unpaired *t*-test with Welch's correction or the Mann-Whitney rank test. Categorical variables were expressed as numbers and frequencies, and the difference between groups was detected using the Pearson chi-square test or Fisher exact chi-square test. The cutoff values of admission levels of MIF for predicting MACCE were determined by using the receiver operating characteristic (ROC) plot with the maximal corresponding values of Youden's index (sensitivity + specificity-1). To visualize the relationship between the cutoff and MACCE during the follow-up, Kaplan-Meier plots were generated, and the log-rank test was used to compare the resulting curves. Potentially influential variables of MACCE among traditional risk factors and variables in univariate cox regression with a value of $P < 0.05$ were tested by collinearity diagnosis in advance, and those variables with interaction between each other and with the variance inflation factor (VIF) ≥ 5 were excluded. The variables that did not show interaction were finally included for a multivariable Cox proportional hazard regression analysis to assess whether a high admission MIF level is an independent predictor of a long-term adverse clinical outcome. Results of univariate and multivariate Cox proportional hazard regression models are presented as a hazard ratio (HR) and 95% confidential interval (CI). $P < 0.05$ was considered statistically significant. Analyses were performed using SPSS Statistics 26 (IBM) and GraphPad Prism 6 (United States).

Results

Baseline characteristics of participants

The flowchart of the study design is shown in **Figure 1**. We consecutively recruited 476 patients with STEMI into the study during January 2014–October 2018. Of them, 51 patients were excluded due to thrombolysis therapy, active inflammatory diseases, cancer, renal failure, previous history of MI, and percutaneous transluminal coronary angioplasty (PTCA), and 425 patients with STEMI who received PCI were included. After PCI, patients were divided into non-MetS and MetS groups according to the criteria of MetS diagnosis (1). There were 13 patients who died during hospitalization, and in-hospital mortality was similar between the two groups (4 in non-MetS vs. 9 in MetS, $P > 0.05$). After discharge, 412 patients were eligible to enter the follow-up period. We lost contact with 11 (2.6%) patients during the follow-up period. Finally, 401 patients with STEMI were followed up during the 4.9-year period for assessment of long-term clinical outcomes. Baseline characteristics of study participants are presented in **Table 1**. Although age and gender distribution were similar between the non-MetS and MetS groups, the number of patients in MetS was 1.75-fold more than those in the non-MetS group.

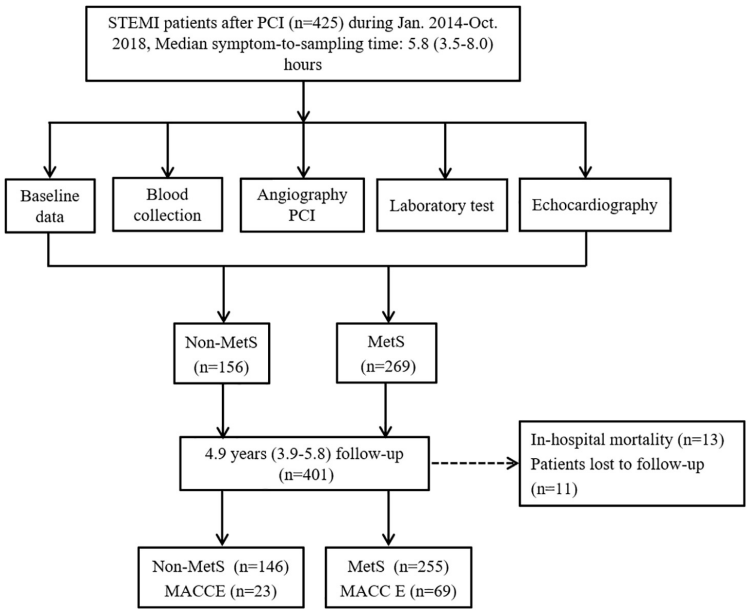

FIGURE 1
The flowchart of study design with inclusion and exclusion procedures. MACCE, major adverse cardiovascular and cerebrovascular events; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction; MS, metabolic syndrome.

It was expected that all variables related to MetS, including percentage of diabetes mellitus and hypertension, and BMI, fasting glucose, total cholesterol (TC), triglycerides (TG), and higher density lipid-cholesterol (HDL-C) were greater in the MetS group (n = 255) than in the non-MetS (n = 146) group (all $P < 0.001$). White blood cell counting (WBC), hs-CRP, and peak hs-TnT were higher in the MetS group when compared to the non-MetS group (both $P < 0.05$), indicating more severe systemic inflammation and cardiac injury in the MetS group. Angiographic analysis showed that the MetS group had a higher Gensini score and a prevalence of multivessel disease vs. the non-MetS group (both $P < 0.05$). While the admission MIF level, N-terminal pro-brain natriuretic peptide precursors (NT-proBNP), Grace score, and symptom onset-to-reperfusion time were comparable between the two groups. There was no statistical difference in medication between the non-MetS and MetS groups except for ACEI or ARB drugs ($P < 0.001$) (**Table 1**).

ST-segment elevation MI patients with metabolic syndrome had a higher incidence of major adverse cardiovascular and cerebrovascular events

During the 4.9 (interquartile range 3.9–5.8) years of follow-up, 92 (22.9%) cases of MACCE were recorded, and the MetS group had a greater occurrence of total MACCE than the non-MetS group (27.1% vs. 15.8%, P = 0.010). The category of MACCE is displayed in **Table 2**. Compared to the non-MetS group, the incidences of all-cause mortality and target lesion revascularization were significantly higher in patients with MetS. The prevalence of other adverse events was comparable between the two groups.

Major adverse cardiovascular and cerebrovascular events and metabolic syndrome associated with higher migration inhibitory factor levels

We further compared admission MIF levels in STEMI patients with or without MACCE, non-MetS STEMI patients with or without MACCE, and MetS STEMI patients with or without MACCE, respectively (**Figure 2**). The admission MIF level was higher in overall STEMI patients with MACCE vs. non-MACCE patients (165 ± 69 vs. 107 ± 51 ng/ml, $P < 0.0001$, **Figure 2A**). In STEMI patients with MetS, those who developed MACCE had significantly greater MIF levels than those without MACCE (180 ± 65 vs. 104 ± 50 ng/ml, $P < 0.0001$, **Figure 2B**). However, in non-MetS STEMI patients, MIF levels were slightly elevated in those with developed MACCE vs. MACCE-free patients but did not reach statistical significance (141 ± 71 vs.

TABLE 1 Baseline clinical characteristics of all participants.

Variables	STEMI (n = 401)		P-value
	Non-MetS (n = 146)	MetS (n = 255)	
Age (years)	58.7 ± 11.9	57.2 ± 12.1	*0.222*
Male, n (%)	124 (84.9)	204 (80.0)	*0.218*
Current smoker, n (%)	82 (56.2)	15 1 (59.2)	*0.551*
Diabetes mellitus, n (%)	18 (12.3)	100 (39.2)	*<0.001*
Hypertension, n (%)	45 (30.8)	154 (60.4)	*<0.001*
BMI (kg/m^2)	24.2 ± 3.2	27.5 ± 3.8	*<0.001*
WBC (×10^9/L)	10.7 ± 3.4	11.4 ± 3.5	*0.033*
Fasting glucose (mmol/L)	8.36 ± 3.59	10.19 ± 4.01	*<0.001*
TC (mmol/L)	5.03 ± 1.12	5.54 ± 1.31	*<0.001*
TG (mmol/L)	1.09 (0.78∼1.59)	2.08 (1.51∼2.91)	*<0.001*
HDL-C (mmol/L)	1.08 ± 0.26	0.88 ± 0.17	*<0.001*
LDL-C (mmol/L)	3.07 ± 0.87	3.04 ± 0.93	*0.759*
NT-proBNP (pg/mL)	342 (80∼994)	422 (116∼1,234)	*0.153*
LVEF (%)	59.2 ± 5.3	59.0 ± 6.2	*0.689*
Peak hs-TnT (ng/mL)	2.02 (0.85∼4.06)	2.50 (1.12∼5.47)	*0.035*
Adm. MIF (ng/ml)	116 ± 55	121 ± 63	*0.368*
hs-CRP (mg/L)	12.2 (4.0∼18.6)	14.1 (6.9∼21.4)	*0.015*
Grace score	154 ± 21	151 ± 24	*0.365*
Gensini score	52 (39∼82)	63 (42∼88)	*0.010*
Multi-vessel disease, n (%)	71 (48.6)	155 (60.8)	*0.018*
Symptom onset to reperfusion (h)	5.9 (3.9∼8.4)	6.4 (3.9∼8.6)	*0.612*
Medication at discharge			
Anti-platelet therapy (%)	139 (95.2)	245 (96.1)	*0.676*
ACEIs/ARBs (%)	58 (39.1)	153 (60.0)	*<0.001*
β-blockers (%)	102 (69.9)	166 (65.1)	*0.329*
Statin (%)	142 (97.3)	249 (97.6)	*0.811*

Date are expressed as mean ± SD or median (25th-75th percentiles), or exact number and percentage.
STEMI, ST-segment elevation myocardial infarction; MetS, metabolic syndrome; Adm, admission; MIF, macrophage migration inhibitory factor; hs-CRP, high sensitive C-reactive protein; BMI, body mass index; WBC, white blood cell; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; NT-proBNP, N-terminal precursor brain natriuretic peptide; LVEF, left ventricular ejection fraction; hs-TnT, high sensitive-troponin T; Grace, Global Registry of Acute Coronary Events; ACEIs/ARBs, angiotensin converting enzyme inhibitors/angiotensin receptor blocker.

111 ± 51 ng/ml, P = 0.061, Figure 2C). As hs-CRP is an inflammatory marker, we also compared its levels in the same way as MIF, and there were no statistical differences between STEMI patients with or without MACCE in overall STEMI patients and STEMI patients with or without MetS (Supplementary Figure 1).

TABLE 2 Category of MACCE occurred during the follow-up period.

	Non-MetS (n = 146)	MetS (n = 255)	P-value
Total MACCE, n (%)	23 (15.8)	69 (27.1)	*0.010*
All-cause mortality, n (%)	1 (0.7)	13 (5.1)	*0.022*
Target lesion revascularization, n (%)	2 (1.4)	17 (6.7)	*0.015*
Rehospitalization owing to recurrent angina, n (%)	9 (6.2)	18 (7.1)	*0.731*
Rehospitalization owing to AMI, n (%)	3 (2.1)	7 (2.8)	*0.753*
Rehospitalization owing to heart failure, n (%)	4 (2.7)	8 (3.1)	*1.000*
Rehospitalization owing to Arrhythmia, n (%)	3 (2.1)	4 (1.6)	*0.709*
Stroke, n (%)	1 (0.7)	2 (0.8)	*1.000*

Date are expressed as exact number and percentage.
MACCE, major adverse cardio- and/or cerebro-vascular events, MetS, metabolic syndrome; AMI, acute myocardial infarction.

A higher admission migration inhibitory factor level predicted long-term clinical outcomes and it was superior than other prognostic indicators

The ROC plots using admission MIF values for all patients with STEMI were generated. The area under the ROC curve for MIF predicting MACCE in patients with STEMI was 0.78 (Figure 3A). The optimal cutoff value for MIF based on the maximum of Youden's index on the ROC curve was 143 ng/ml with 63.0% sensitivity and 83.2% specificity in predicting long-term clinical outcomes. Using the same method, the cutoff values for hs-TnT, NT-proBNP, and Grace score (Figures 3B–D) and inflammatory indicators (hs-CRP, Supplementary Figure 2A) were calculated, respectively, and the AUCs for those indexes were inferior to the admission MIF.

Based on the cutoff value of admission MIF (143 ng/ml), STEMI patients with or without MetS were further divided into the high- and low-MIF level groups. As shown in Table 3, in the non-MetS group, patients with the high-MIF level (≥ 143 ng/ml) had a greater incidence of diabetes and MACCE than those with the low-MIF level (< 143 ng/ml, both P < 0.05). In the MetS group, patients with the high-MIF level had a greater peak level of hs-TnT, Gensini score, and higher incidence of MACCE. Other clinical characteristics, including medications, were similar between the two groups.

Patients with STEMI discharged from the hospital were separated into two groups using the MIF cutoff value of 143 ng/ml. Cumulative incidences of MACCE using Kaplan-Meier curves are shown in Figure 4. In the STEMI without MetS group, high-MIF level patients had a greater incidence of MACCE (42.4%) than those with low-MIF levels (18.0%,

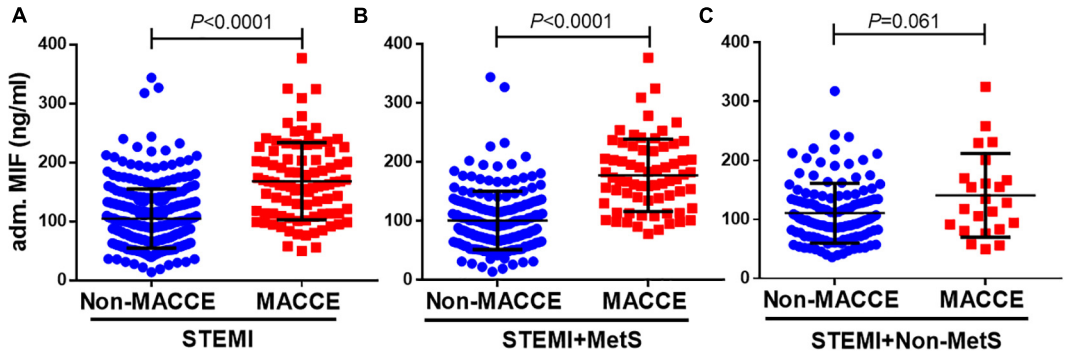

FIGURE 2
Admission MIF levels between patients with MACCE and non-MACCE. Overall patients with STEMI [**(A)**, non-MACCE, *n* = 309; MACCE, *n* = 92], STEMI + MetS [**(B)**, non-MACCE, *n* = 186; MACCE, *n* = 69], and STEMI + non-MetS [**(C)**, non-MACCE, *n* = 123; MACCE, *n* = 23]. MIF, macrophage migration inhibitory factor.

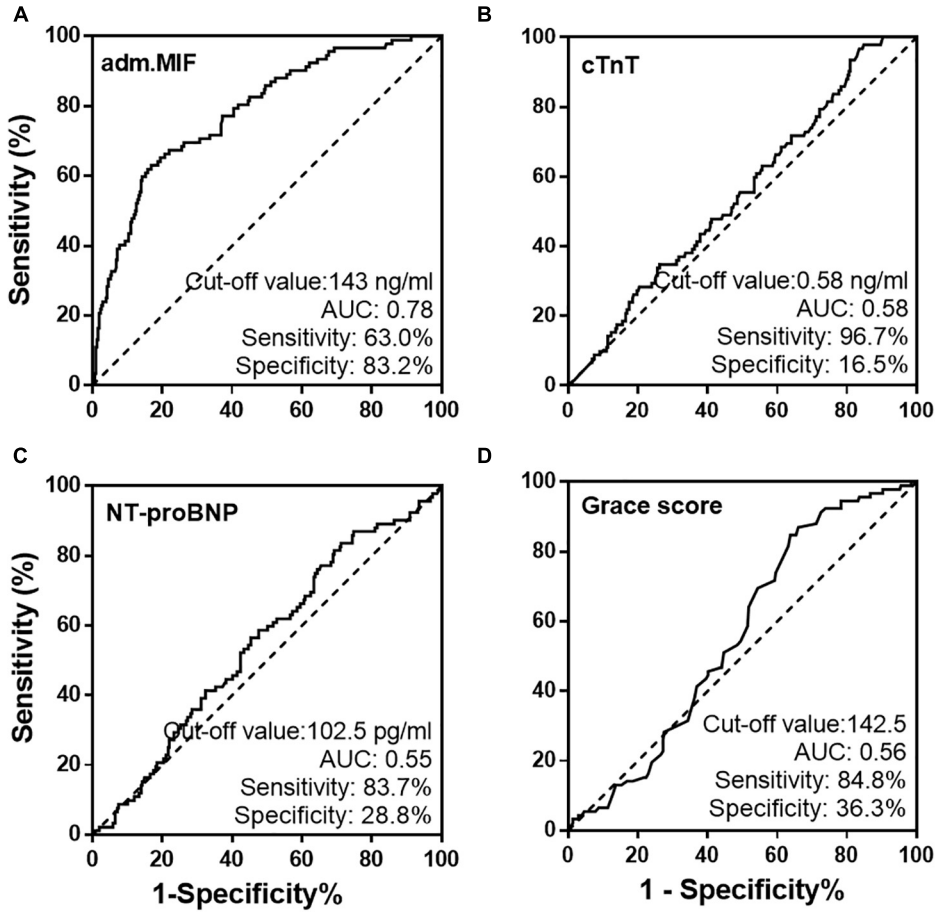

FIGURE 3
Receiver operating characteristic curves of admission MIF **(A)** and traditional prognostic indicators, cTnT **(B)**, NT-proBNP **(C)**, and Grace score **(D)** from all STEMI patients.

Figure 4A, $P = 0.016$) at the end of the follow-up period. In the STEMI with MetS group, the prevalence of MACCE was markedly higher in patients with high-MIF levels (76.9%) than those with low-MIF levels (18.7%) at the end of a 4.9-year follow-up period (Figure 4B, $P < 0.001$). The predictive power of MIF was much stronger compared to the non-MetS group.

The cutoff value (0.58 ng/ml) of hs-TnT had predictive value for MACCE in both the non-MetS and MetS groups (Figures 4C,D, $P < 0.05$). Nevertheless, NT-proBNP with a cutoff value of 102.5 ng/ml predicted MACCE only for the non-MetS group (Figure 4E, $P = 0.035$) but not for the MetS group (Figure 4F, $P = 0.060$). The cutoff value (142.5) of the Grace score had strong predict power for the MetS group (Figure 4H, $P < 0.001$) but not for the non-MetS group (Figure 4G, $P = 0.237$). We also assessed the power of hs-CRP using the cutoff value (17.5 mg/L); it lost the power to predict MACCE in both the non-MetS and MetS groups (Supplementary Figures 2B,C). These results indicate that a higher admission MIF level was associated with a greater incidence of MACCE, which was superior in the MetS group and better than other traditional indicators.

Elevated admission migration inhibitory factor level is an independent predictor for long-term adverse clinical outcomes

To estimate whether the cutoff value of admission MIF level is an independent factor to predict long-term clinical outcomes, the following statistical methods were applied. First, univariate Cox regression analysis was performed on all participants to screen variables for the next step of multivariate Cox regression analysis, and the results are displayed in Supplementary Table 1. *Second,* a collinearity diagnostic approach was used to exclude potentially interactive variables with VIF ≥ 5, the VIF of all variables presented in Table 3 was less than 5, and therefore,

TABLE 3 Baseline clinical characteristics of STEMI patients with or without MetS grouped by the cut-off value of adm. MIF (143 ng/ml).

Variables	STEMI without MetS ($n = 146$)		*P*-value	STEMI with MetS ($n = 255$)		*P*-value
	Adm. MIF < 143 ng/ml ($n = 110$)	Adm. MIF ≥ 143 ng/ml ($n = 36$)		Adm. MIF < 143 ng/ml ($n = 179$)	Adm. MIF ≥ 143 ng/ml ($n = 76$)	
Age (years)	58.3 ± 11.6	60.1 ± 12.7	*0.445*	57.8 ± 12.1	55.9 ± 12.3	*0.284*
Male	93 (84.5)	31 (86.1)	*0.820*	141 (78.8)	63 (82.9)	*0.451*
Current smoker	64 (58.2)	18 (50.0)	*0.390*	99 (55.3)	52 (68.4)	*0.051*
Diabetes mellitus, *n* (%)	10 (9.1)	8 (22.2)	*0.038*	77 (43.0)	23 (30.3)	*0.056*
Hypertension, n (%)	32 (29.1)	13 (36.1)	*0.428*	106 (59.2)	48 (63.2)	*0.556*
BMI (kg/m^2)	24.23 ± 3.39	24.09 ± 2.62	*0.814*	27.49 ± 3.82	27.65 ± 3.78	*0.750*
WBC ($\times 10^9$/L)	10.77 ± 3.37	10.35 ± 3.42	*0.524*	11.39 ± 3.53	11.52 ± 3.56	*0.797*
Fasting glucose (mmol/L)	8.05 ± 2.63	9.31 ± 5.56	*0.199*	10.31 ± 3.91	9.92 ± 4.24	*0.476*
TC (mmol/L)	5.10 ± 1.15	4.83 ± 0.97	*0.205*	5.51 ± 1.33	5.60 ± 1.29	*0.605*
TG (mmol/L)	1.10 (0.78∼1.62)	1.03 (0.78∼1.48)	*0.616*	2.01 (1.45∼2.84)	2.18 (1.74∼3.05)	*0.245*
HDL-C (mmol/L)	1.07 ± 0.28	1.12 ± 0.21	*0.301*	0.88 ± 0.18	0.89 ± 0.17	*0.496*
LDL-C (mmol/L)	3.04 ± 0.87	3.21 ± 0.87	*0.532*	3.05 ± 0.89	3.01 ± 1.02	*0.761*
NT-pro BNP (pg/mL)	342 (79∼894)	383 (80∼1,420)	*0.352*	418 (97∼1,433)	489 (141∼1,157)	*0.906*
LVEF (%)	58.8 ± 5.2	60.3 ± 5.4	*0.132*	58.7 ± 6.6	59.4 ± 4.8	*0.398*
CK-MB max (U/L)	220 (117∼364)	281 (115∼338)	*0.653*	246 (133∼388)	269 (158∼428)	*0.148*
Peak hs-TnT (ng/mL)	1.86 (0.74∼3.95)	2.21 (1.53∼4.20)	*0.150*	2.23 (1.01∼5.31)	3.14 (1.65∼6.90)	*0.013*
hs-CRP (mg/L)	11.3 (3.2∼18.6)	13.4 (8.5∼19.5)	*0.109*	14.1 (6.4∼21.3)	13.4 (7.6∼21.9)	*0.917*
MACCE, *n* (%)	13 (11.8)	10 (27.8)	*0.023*	21 (11.7)	48 (63.2)	*<0.001*
Grace score	154 ± 21	153 ± 23	*0.797*	150 ± 24	153 ± 23	*0.349*
Gensini score	54 (38∼82)	47 (39∼80)	*0.661*	57 (42∼85)	80 (50∼100)	*0.006*
Multi vessel disease, *n* (%)	52 (47.3)	19 (52.8)	*0.566*	106 (59.2)	49 (64.5)	*0.432*
Symptom onset to reperfusion (h)	5.9 (3.9∼8.5)	6.0 (3.6∼8.2)	*0.550*	6.4 (3.9∼9.1)	6.1 (4.1∼8.3)	*0.591*
Medication at discharge						
Anti-platelet therapy (%)	105 (94.6)	34 (97.1)	*1.000*	174 (97.2)	71 (93.4)	*0.169*
ACEIs/ARBs (%)	43 (38.7)	15 (42.9)	*0.664*	102 (57.0)	51 (67.1)	*0.131*
β-blockers (%)	76 (68.5)	26 (74.3)	*0.513*	113 (63.1)	53 (69.7)	*0.311*
Statin (%)	109 (98.2)	33 (94.3)	*0.243*	173 (96.6)	76 (100.0)	*0.183*

Date are expressed as mean ± SD or median (25th–75th percentiles), or exact number and percentage.
STEMI, ST-segment elevation myocardial infarction; MetS, metabolic syndrome; Adm, admission; MIF, macrophage migration inhibitory factor; BMI, body mass index; WBC, white blood cell; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; NT-proBNP, N-terminal precursor brain natriuretic peptide; LVEF, left ventricular ejection fraction; hs-TnT, high sensitive-troponin T; hs-CRP, hypersensitive C-reactive protein; Grace, Global Registry of Acute Coronary Events. ACEIs/ARBs, Angiotensin converting enzyme inhibitors/Angiotensin receptor blocker.

they were eligible for multivariate Cox regression analysis (data not shown). *Third*, based on the nature of square-transformed admission MIF and the cutoff value (≥ 143 ng/ml) of admission MIF generated by the ROC curve, multivariate Cox regression analysis was conducted. **Table 4** shows the results of multivariate Cox proportional hazard models used for assessing the independent predictive value of MIF for long-term MACCE. In the crude model, two MIF values had the same predictive capacity for MACCE in both the MetS subgroup and the non-MetS group ($P < 0.05$). However, in Model 1, after adjusting for age, men, history of hypertension and diabetes, and BMI, the predictive power of MIF ≥ 143 ng/ml remained for the MetS group with HR 9.10, 95% CI (5.323–15.568, $P < 0.001$), whereas it was not observed in the non-MetS subgroup. In Model 2, except for those confounding factors used in Model 1, peak TnT, LDL-C, NT-proBNP, LVEF, and Gensini and Grace scores were also included for the adjustment. The value of MIF ≥ 143 ng/ml still had predictive power for the MetS group [HR 9.56, 95% CI (5.397–16.944), $P < 0.001$]. Nevertheless, it was not the case in the non-MetS group. These results demonstrate that a higher admission MIF level (≥ 143 ng/ml) is an independent predictive factor for long-term MACCE in STEMI patients with MetS.

Discussion

A number of studies have observed that plasma MIF levels in patients with STEMI were elevated in the early stages after the onset of chest pain (20, 21, 34). Most importantly, the admission MIF level in patients with STEMI was found to be correlated to the size of the myocardial infarction (20), and further, our previous study has shown that admission MIF levels can predict both in-hospital mortality and long-term MACCE (21). These findings demonstrate the potential of admission MIF as a novel biomarker to predict the clinical adverse outcome in the setting of acute MI. MetS is a well-known cardiovascular risk factor, and

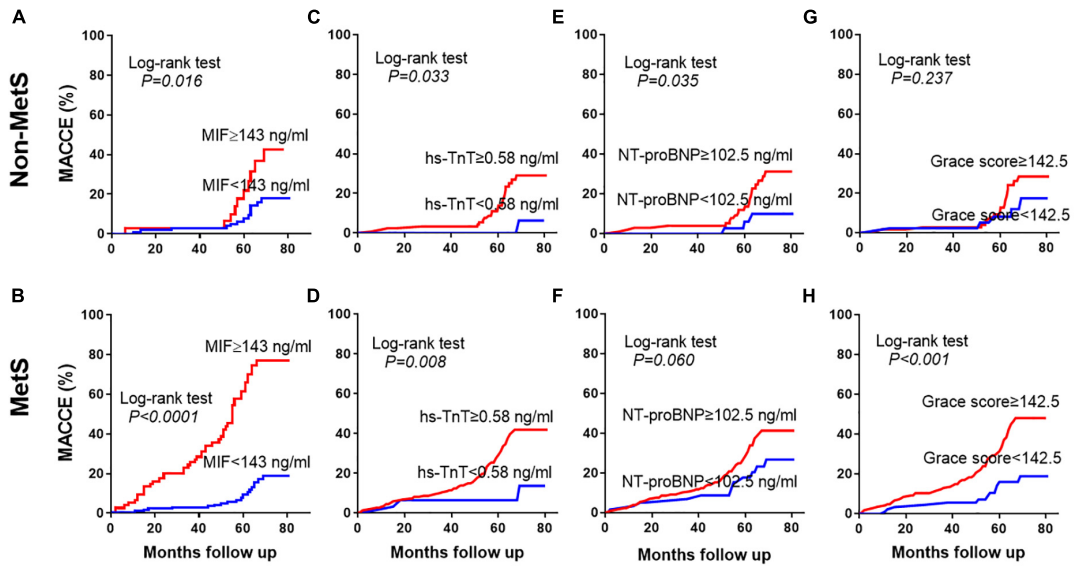

FIGURE 4
Prediction of MACCE using different prognostic indicators. Kaplan-Meier curves showing the incidence of MACCEs in the non-MetS and MetS groups during the 4.9-year follow-up period using the cutoff values of admission MIF **(A,B)**, hs-TnT **(C,D)**, NT-proBNP **(E,F)**, and Grace score **(G,H)**.

TABLE 4 Multivariate cox proportional hazards models for MACCE in both MetS and non-MetS groups.

Group	Variable	Crude model		Model 1		Model 2	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
MetS	MIF level square	1.51 (1.385~1.655)	<0.001	1.54 (1.407~1.702)	<0.001	1.60 (1.441~1.785)	<0.001
	MIF level ≥ 143 ng/ml*	8.17 (4.874~13.719)	<0.001	9.10 (5.323~15.568)	<0.001	9.56 (5.397~16.944)	<0.001
Non-MetS	MIF level square	1.22 (1.034~1.449)	0.019	1.17 (0.984~1.397)	0.075	1.23 (1.019~1.493)	0.031
	MIF level ≥ 143 ng/ml*	2.68 (1.174~6.115)	0.019	2.41 (0.958~5.165)	0.101	2.04 (0.805~6.101)	0.061

Model 1: adjusted for age, male, history of hypertension/diabetes, BMI and admission MIF.
Model 2: adjusted for model 1 + peak hs-TnT, LDL-C, NT-proBNP, LVEF, Gensini score and Grace score.
MACCE, major adverse cardio- and/or cerebro-vascular events; HR, hazard ratio; CI, confidence interval.
*The cut-off value of 143 ng/ml was generated from the receiver operating characteristics (ROC) curve analysis in all participants.

it has a great negative impact on CVD, especially coronary artery disease (CAD). However, whether admission MIF levels (the earliest available sampling time) also bear a predictive power for the prognosis of patients with STEMI complicated with MetS is unknown.

Our study has made several findings. *First*, the overall MACCE during the 4.9-year (3.9–5.8) follow-up period was 3-fold higher in STEMI patients with MetS compared to those without MetS. This is in line with the negative impact of MetS on CVD (11, 15, 16, 35). The sharply increasing prevalence of MetS, nowadays, has become a global health challenge affecting all nations and races. A similar trend of change in the prevalence of MetS was reported among US adults from 1988 to 2016 (5, 6, 36) and among Chinese adults from 2000 to 2015 (3, 7, 8). Two early studies investigated the relationship between MetS and coronary heart disease (CHD), MI, and stroke, and reported that in men, the MetS age-adjusted relative risk (RR) was 2.54 (95% CI 1.62–3.98) for CHD (10), and the MetS was significantly related in multivariate analysis of MI/stroke with an OR of 2.05 (95% CI 1.64–2.57) (16). A meta-analysis that involved 951,083 participants found that the MetS is associated with a twofold increase in CVD, CVD mortality, MI, and stroke and a 1.5-fold increase in all-cause mortality (15). In an American study, 69% of MetS were detected in 1,129 hospitalized patients due to acute MI, and the worse clinical outcomes (i.e., mortality and rehospitalization) were 38% in patients with MetS vs. 27% in patients with non-MetS during the 12 month follow-up period (11). In the current small-scale study, diagnostic criteria for MetS were met by 63.6% of patients with STEMI during hospitalization and 27.1% of patients with MetS developed MACCE vs. 15.7% in patients with non-MetS during a long-term follow-up period, which is consistent with the results of the American study. These results further support the consensus that MetS is the major cardiovascular risk and clearly raise the alarm that ischemic heart disease concomitant with MetS is more likely to develop adverse clinical outcomes even after blood flow reconstruction. Our findings extend the predictive power of a high admission MIF level from overall patients with STEMI, as we previously reported (21), into the specific high-risk cohort.

Second, although the overall admission MIF levels were comparable between non-MetS and MetS groups, they were significantly higher in patients complicated with MetS-developed MACCE than in those MACCE-free counterparts. While the MIF levels in patients without MetS who developed MACCE were also elevated, they did not reach statistical significance when compared MACCE-free counterparts. Furthermore, using the cutoff value of MIF generated by the ROC curve to predict the long-term adverse outcomes, we found that patients in the higher MIF level ($\geq$ 143 ng/ml) subgroup had a greater incidence of MACCE in both the non-MetS and MetS groups during the 4.8-year follow-up period. The predictive power was much higher in patients with MetS. These results demonstrate that a higher admission MIF level was associated with a greater incidence of MACCE, which was superior in the MetS subgroup and better than other traditional prognostic indicators such as hs-TnT, NT-proBNP, and Grace score. Although admission MIF could not differentiate non-MetS and MetS in the very early phase of MI, adverse effects emerged during the follow-up period. This may highlight the negative influence of Mets in this clinical setting.

As demonstrated in our previous study, admission MIF levels from the first available blood samples (as early as 3.5 h after symptom onset) were correlated with myocardial infarct size detected by the golden standard method, magnetic resonance imaging (MRI), at day 3 (the acute phase) and at 3 months (the chronic phase) after acute MI (20). This indicates that the early surge of MIF level, which actually reflects the extent of acute myocardial ischemia/necrosis, likely masks its difference as an inflammatory biomarker between the non-MetS and MetS groups. Supportive evidence of a higher hs-TnT level and greater Gensini score in the high MIF level ($\geq$ 143 ng/ml) subgroup of this cohort signifies more severe myocardial damage. Interestingly, a study using integrated backscatter intravascular ultrasound (IB-IVUS) analyzed coronary plaques and found that the percentage of lipid area and volume was significantly increased, while the percentage of fibrous volume was decreased in stable CAD patients with MetS vs. patients with non-MetS (37). This finding established a direct evidence of MetS is associated with lipid-rich plaque, which contributes to the increasing risk of plaque vulnerability. Moreover, MetS was also found to be associated with a worse no-reflow during emergency PCI for STEMI, and, consequently, a worse prognosis (38). The underlying mechanism is more severe damage to microcirculation (39).

Third, multivariate Cox regression analysis identified that a high admission MIF level is an independent predictor of the long-term MACCE in both the non-MetS and MetS subgroups, which was superior in the MetS subgroup in our study cohort. Therefore, a higher admission MIF level can identify this specific subgroup patients with a higher risk in the setting of acute MI, and it is valuable for cardiologists making a better management in advance. As most risk factors for MetS are modifiable, identification of a high-risk cohort of patients that may benefit from more aggressive risk factor modification (11).

Study limitations

This current study had several limitations. *First,* the relatively small sample size of this single-center prospective study limited the power of our findings, which requires large-scale multicenter studies to reinforce. *Second*, in our study, only patients with STEMI who received primary PCI were included. The predictive value of admission MIF levels in MetS patients who suffered from acute coronary artery syndrome,

especially including STEMI and non-STEMI, and received different interventions such as thrombolysis or CABG should be included in a future study. *Third*, as MetS is systemic inflammatory state (1), if dynamic changes in inflammatory parameters were included in our study, which may better characterize the relationship of the admission MIF level and the prognosis. *Fourth,* infarct size, which is known to influence the final outcomes, was not evaluated. However, we included factors such as peak hs-TnT, Gensini score, percentage of multivessel disease, and the Grace score for further analysis, which may help to minimize this weak point in this current study and validate our findings and conclusion.

Conclusion

Our study found that STEMI patients with MetS who developed MACCE had significantly higher admission MIF levels than those in MACCE-free patients. STEMI patients with MetS who had an elevated admission MIF level (i.e., $\geq$ 143 ng/ml) were more likely to develop an adverse clinical outcome after discharge. A higher admission MIF level can be taken as an independent predictive factor to stratify STEMI patients with MetS for a more precise therapy.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the First Affiliated Hospital of Xinjiang Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

X-LY and QZ conducted the study and drafted the manuscript. FL and Y-JY contributed to the statistical analysis. B-BF, W-LL, X-HZ, and G-LD contributed to the data collection and quality control. X-ML, X-MG, and Y-NY were responsible for the funding support and contributed to the study design and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.947395/full#supplementary-material

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Redistribution of adipose tissue is associated with left atrial remodeling and dysfunction in patients with atrial fibrillation

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Objective: Adipose tissue is recognized as a crucial regulator of atrial fibrillation (AF). However, the effect of epicardial adipose tissue (EAT) on the pathophysiology of AF might be different from that of other adipose tissues. The purpose of this study was to explore the distribution features of different adipose tissues in AF patients and their relationships with left atrial (LA) remodeling and function.

Methods: A total of 205 participants (including 112 AF and 93 non-AF patients) were recruited. Color doppler ultrasound was used to measure the thickness of subcutaneous, extraperitoneal, and intra-abdominal adipose tissue. Cardiac CT scan was performed to measure the mean thickness of EAT surrounding the whole heart (total-EAT) and specific regions, including left atrium (LA-EAT), left ventricle, right ventricle, interventricular groove, and atrioventricular groove. LA anatomical remodeling and function were measured by echocardiography, while electrical remodeling was evaluated by P-wave duration and dispersion using Electrocardiography (obtained after cardioversion or ablation in AF patients). Relationship between the thickness of different adipose tissues and LA remodeling and function was analyzed.

Results: The thickness of subcutaneous, extraperitoneal, and intra-abdominal adipose tissue was similar between AF and non-AF patients, and had no or only weak association with LA remodeling and dysfunction. However, compared to non-AF participants, total-EAT thickness significantly increased in both paroxysmal and persistent AF patients (non-AF vs. paroxysmal AF vs. persistent AF: 6.31 ± 0.63 mm vs. 6.76 ± 0.79 mm vs. 7.01 ± 1.18 mm, $P < 0.001$), which was positively correlated with the LA size and P-wave duration and dispersion, and negatively correlated with LA ejection fraction and peak strain rate. More interestingly, EAT thickness in AF patients did not increase uniformly in different regions of the heart. Compared to EAT surrounding the other regions, LA-EAT was found to accumulate more greatly, and had a closer relationship

to LA remodeling and dysfunction. Multivariate logistic regression analysis also showed that LA-EAT was significantly correlated with the presence of AF (OR = 4.781; 95% CI 2.589–8.831, $P < 0.001$).

Conclusion: Rather than other adipose tissues, accumulation and redistribution of EAT, especially surrounding the LA, is associated with LA remodeling and dysfunction in AF patients.

KEYWORDS

atrial fibrillation, epicardial adipose tissue, cardiac remodeling, left atrial remodeling, dysfunction

Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinic that significantly increases the risk of stroke, heart failure, and all-cause mortality (1, 2). Although significant progress has been made in preventing and treating AF, the incidence rate continues to increase over time. Previous studies have confirmed that left atrial remodeling and dysfunction is the essential change of pathophysiology for AF (3). As the body's largest endocrine organ, adipose tissue is considered as a crucial regulator of AF, which may affect the occurrence, progression, and prognosis of AF (4). However, due to the broad dispersion of adipose tissue throughout the body and intra-organ location, the visceral fat with high secretory activity around the heart usually has a more practical effect than fat in other parts, promoting atrial remodeling and increasing the susceptibility to AF (5). As a kind of organ-specific adipose tissue in direct contact with the surface of the heart, epicardial adipose tissue (EAT) can secrete paracrine or endocrine inflammatory cytokines, adipokines, or other factors that influence the health or diseases of the heart (6). These facts give rise to the hypothesis that adipose tissue might be redistributed in AF patients and play a role in left atrial (LA) remodeling and dysfunction. Therefore, this study aimed to explore the distribution features of different adipose tissues in AF patients and their relationships with LA remodeling and dysfunction.

Materials and methods

Patient characteristics

This is a single-center prospective cross-sectional study conducted in the Department of Cardiovascular Medicine, the Third Affiliated Hospital, Sun Yat-sen University. A total of 205 inpatients were enrolled between September 2020 and August 2021, including 112 patients with non-valvular AF (61 paroxysmal AF and 51 persistent AF) diagnosed according to the ESC guideline (1), and 93 patients with sinus rhythm as control group. Patients younger than 18 years old, pregnant or lactating women, patients with organic heart diseases (including valvular heart disease, hyperthyroid cardiomyopathy, dilated cardiomyopathy, and ischemic cardiomyopathy), and patients with any other systemic disorders (including hepatic disorders, renal dysfunction, neoplastic diseases, connective tissue disease, and thyroid disorders), were excluded. The research protocol has been reviewed and approved by the medical ethics committee of the Third Affiliated Hospital of Sun Yat-sen University, which is in line with the ethical guidelines of the Declaration of Helsinki in 1975 (7). All participants voluntarily participated in the study and signed informed consent. Study participants strictly followed the study protocol to reduce information bias.

Data collection

Baseline data were collected from all subjects, including age, gender, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, lifestyle (smoking and drinking), and comorbidity (hypertension, hyperlipidemia, diabetes, coronary heart disease, and stroke). Blood samples were collected after 8-h fasting and biochemical parameters were examined by Hitachi 7180 chemical analyzer, including triglyceride, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting plasma glucose, glycosylated hemoglobin, uric acid, blood urea nitrogen, creatinine, serum cystatin C, and estimated glomerular filtration rate (eGFR). Besides, medications that may affect the structure or function of the heart were also collected.

Measurement of adipose tissues

Abdominal Color Doppler ultrasound was used to measure the thickness of subcutaneous, extraperitoneal, and intra-abdominal adipose tissue. Subcutaneous adipose tissue was

evaluated by measuring the abdominal wall fat thickness at 1 cm above the umbilicus (longitudinal section of the line between the xiphoid process and umbilicus). Extraperitoneal adipose tissue was evaluated by measuring the distance between the left extrahepatic peritoneum and the abdominal white line under the xiphoid process. Intra-abdominal adipose tissue was evaluated by measuring the distance between the posterior wall of the abdominal aorta and peritoneum at 1 cm above the umbilicus (cross-cut between the xiphoid process and umbilicus). All adipose tissue measurements were independently performed by two experienced echocardiographers who were blinded to the clinical data of the patients. The average values were used for the statistical analysis.

Cardiac CT scan was performed to measure the thickness of EAT by using a 320 × 0.5 mm detector configuration scanner (Toshiba Aquilion One Dynamic Volume CT, Japan) with the gantry rotation time of 350 ms. Tube voltage and current were adapted to body mass index (BMI) and thoracic anatomy. Tube voltage was 100–135 kV and maximal tube current was 380–480 mA (depending on BMI and thoracic anatomy). All patients received 50–70 ml (depending on BMI) non-ionic contrast medium (Isovue-370; Bracco Diagnostics, Guangzhou, China) at a flow rate of 6.0 ml/s followed by 20 ml normal saline injected with a dual injector (Mallinckrodt Empower CTA DUAL Injector, Mallinckrodt Inc., Cincinnati, OH, United States). Bolus tracking was set in the descending aorta using a 180 Hounsfield unit threshold. We scanned with retrospective ECG-triggered dose modulation, and reconstructed at the end-diastole phase of left ventricle. The initial data set with 0.50 mm slice thickness and 0.25 mm reconstruction interval was used for further analysis. The dose-length product (DLP) was obtained from the patient protocol of the scanner. The effective dose (measured in millisieverts) was defined as the product of the DLP and a conversion coefficient for the chest. The axial source images were transferred to an image post-processing workstation (Vitrea 2.0; Vital Images, Minnetonka, MN, United States) for quantitative analysis of EAT surrounding the heart. The maximal EAT thickness was determined from the myocardial surface to the pericardium (perpendicular to the pericardium) and having threshold attenuation values of −30 to −250 Hounsfield units. The ventricular short axis view (at the basal, mid ventricular and apical levels) horizontal long axis view and short-axis view of LA with a slice thickness of 2 mm were obtained by multiplanar reconstructions of the raw data. The mean measurements of the ventricular short axis view (basal, mid, apical) were used for analyses. The short axis view of LA was reconstructed as a plane perpendicular to the long axis of standard 2- and 4-chamber views of LA at the level of the mid LA. In the ventricular short-axis view, seven segments of EAT thickness were measured, including right ventricular (RV) anterior free wall superior, RV anterior free wall inferior, RV superior wall, RV diaphragmatic wall, superior interventricular (IV) groove, inferior IV groove, and left ventricular (LV) lateral wall. In the horizontal long-axis view, four segments of EAT thickness were measured, including LV apex, RV apex, left atrioventricular (AV) groove, and right AV groove. In the short-axis view of LA, the EAT thickness was measured as the shortest distance between the mid-LA wall and three anatomical markers, including esophagus (LA-Eso), pulmonary artery (LA-PA) and descending aorta (LA-Desc Ao). Mean EAT thickness surrounding the whole heart (total-EAT) was calculated as the average value of all segments. Mean EAT thickness surrounding LA (LA-EAT) was calculated as the average value of LA-Eso, LA-PA, and LA-Desc Ao. Mean EAT thickness surrounding LV (LV-EAT) was calculated as the average value of LV apex and lateral walls. Mean EAT thickness surrounding RV (RV-EAT) was calculated as the average value of 5 segments (RV anterior free wall superior, RV anterior free wall inferior, RV superior wall, RV diaphragmatic wall, and RV apex). Mean value of EAT thickness in IV grooves (IVG-EAT) was calculated as the average value of superior and inferior IV grooves. And mean value of EAT thickness in AV groove (AVG-EAT) was calculated as the average value of the left and right AV grooves. We measured the epicardial fat by a single investigator (with 15 years' experience in cardiac CT diagnosis and was blinded to clinical information) with digital calipers. Repeat measurements were made at intervals of more than 1 month. The intraobserver reproducibility was 0.893 [95% confidence interval (CI) 0.837–0.926]. Another investigator (with 11 years' experience in cardiac CT diagnosis and was blinded to clinical information) selected 25 patients randomly to measure the epicardial fat. The interobserver reliability was 0.847 (95% CI 0.804–0.891).

Evaluation of left atrial remodeling and function

Left atrial anatomical remodeling and function were evaluated by experienced registered cardiologists using Philips ie33 color Doppler ultrasound diagnostic instrument, followed the guidelines of the American Association of echocardiography (ASE) for evaluating the changes of cardiac structure and function through two-dimensional speckle tracking imaging and real-time three-dimensional imaging. The LA anteroposterior diameter (LAAPD) was measured according to the parasternal left ventricular long axis section. The left and right atrial diameter (LALRD) and LA superior and inferior diameter (LASID) were measured according to the apical four-chamber view. The mean value of LA minimum volume (LAVmin) and LA maximum volume (LAVmax) were measured according to the images of the four-chamber heart. At the same time, the LA total ejection fraction [LAtEF = (LAVmax – LAVmin)/LAVmax] and active ejection fraction [LAaEF = (LAVp – LAVmin)/LAVp] was calculated. Furthermore, the LA peak strain rate (SRs) in

left ventricular systole and the peak strain rate (SRe) in early left ventricular diastole were measured by a two-dimensional speckle tracking technique.

Left atrial electrical remodeling was evaluated by measuring the P-wave duration and dispersion using 12 lead Electrocardiography during sinus rhythm. For patients with AF, sinus rhythm was achieved by cardioversion or ablation. The paper speed of ECG was set to 50 mm/s, with an amplitude of 20 mm/mv. The P-waves of select leads II, III, a VF, and V1 were identified. P-wave duration was retrieved by measuring the distance between the beginning and ending point of the P wave intersected with the equipotential line. Five continuous measurements of P-wave duration were obtained and the average value was calculated. The maximum and minimum P-wave durations were used to calculate the P-wave dispersion (Pd = maximum P-wave duration - minimum P-wave duration). All ECG recordings were analyzed independently by two investigators.

Statistical analysis

Categorical variables were expressed as numbers and percentages, while continuous variables were expressed as mean ± standard deviation. Kolmogorov-Smirnov test was used to study the normal distribution of data. Student's *t*-test and Mann-Whitney *U* test were used to compare continuous variables between two groups, and analysis of variance (ANOVA) or Wilcoxon signed-rank test was used to compare continuous variables among three groups. Chi-square analysis, Fisher's Exact test, or McNemar's test were used for proportion comparison. Pearson correlation coefficient and significance test were used to determine the association between adipose tissue and LA remodeling and dysfunction. The univariate and multivariate logistic regression models were used to analyze the correlation between EAT and the presence of AF. Model 1 was adjusted for heart rate, uric acid, and serum cystatin C, which got $P < 0.05$ in univariate analysis. Model 2 was additionally adjusted for the traditional risk factors that may affect AF, including age, gender, BMI, hypertension, hyperlipidemia, diabetes, coronary artery disease, stroke, smoking status, alcohol abuse, eGFR, and medications. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software version 20.0 (SPSS, Chicago, IL, United States).

Results

Patients' characteristics

The baseline data characteristics of the study population were summarized in **Table 1**, including 93 patients with sinus rhythm (mean age 64.46 ± 10.92 years; 58.06% male), 61 patients with paroxysmal AF (mean age 67.34 ± 11.38 years; 65.57% male), and 51 patients with persistent AF (mean age 68.25 ± 10.40 years; 64.71% male). The mean values of heart rate ($P < 0.001$), serum uric acid ($P = 0.042$), and cystatin C ($P = 0.001$) were significantly increased in both paroxysmal and persistent AF patients when compared to those with sinus rhythm. Other baseline data did not show significant differences among groups ($P > 0.05$).

Left atrial remodeling and dysfunction in atrial fibrillation patients

The changes in indexes of LA remodeling and dysfunction in AF patients were listed in **Table 2**. When compared to those in patients with sinus rhythm, variables representing LA size, including LAAPD, LALRD, LASID, LAVmax, and LAVmin, were significantly increased in patients with AF, irrespective of paroxysmal or persistent ($P < 0.001$). However, variables representing LA function, including LAtEF, LAaEF, SRs and SRe, were significantly decreased in patients with AF ($P < 0.001$). When referred to LA electrical remodeling, P-wave duration and dispersion were also significantly increased in AF patients than those in patients with sinus rhythm ($P < 0.001$). In addition, all variables of LA remodeling and dysfunction seemed to further worsen in patients with persistent AF than those with paroxysmal AF ($P < 0.001$).

Distribution features of adipose tissues in atrial fibrillation patients

As shown in **Figure 1**, the thicknesses of subcutaneous and intra-abdominal adipose tissues were similar between AF and non-AF patients ($P = 0.716$ and 0.382, respectively). The thickness of extraperitoneal adipose tissue tended to decrease in AF patients, although without statistical significance ($P = 0.054$). In contrast, when compared to that in non-AF patients, the thickness of total-EAT significantly increased in both paroxysmal and persistent AF patients (6.31 ± 0.63 vs. 6.76 ± 0.79 vs. 7.01 ± 1.18 mm, $P < 0.001$), indicating that accumulation of total EAT surrounding the whole heart was occurred in AF patients. Multivariate logistic regression analysis showed that total-EAT (OR = 2.615, $P = 0.001$ in Model 1; OR = 2.448, $P = 0.005$ in Model 2) was independently associated with the presence of AF (**Table 3**).

Interestingly, it was found that EAT in AF patients did not accumulate uniformly in different regions of the heart, and redistribution of EAT was observed. As shown in **Figure 2** and **Table 4**, the thickness of LA-EAT ($P < 0.001$), LV-EAT ($P = 0.007$), and RV-EAT ($P = 0.013$) progressively elevated from control groups, paroxysmal AF group, to persistent AF group.

TABLE 1 Baseline characteristics of participants.

Variables	Sinus rhythm (n = 93)	Paroxysmal AF (n = 61)	Persistent AF (n = 51)	P-value
Age, years	64.46 ± 10.92	67.34 ± 11.38	68.25 ± 10.40	0.093
Male, n (%)	54 (58.06)	40 (65.57)	33 (64.71)	0.577
BMI, kg/m^2	23.73 ± 2.89	24.59 ± 4.14	24.89 ± 3.37	0.106
Hypertension, n (%)	50 (53.76)	31 (50.82)	26 (50.98)	0.919
Hyperlipidemia, n (%)	36 (38.71)	20 (32.79)	19 (37.25)	0.752
DM, n (%)	24 (25.81)	20 (32.79)	20 (39.22)	0.240
CAD, n (%)	18 (19.35)	12 (20.00)	10 (19.61)	0.995
Stroke/TIA, n (%)	6 (6.45)	8 (13.11)	9 (17.65)	0.108
Current smoker, n (%)	33 (35.48)	24 (39.34)	22 (43.14)	0.658
Alcohol abuse, n (%)	13 (13.98)	8 (13.11)	6 (11.76)	0.932
SBP, mmHg	136.72 ± 19.65	135.69 ± 21.29	137.57 ± 26.28	0.902
DBP, mmHg	80.84 ± 10.81	80.13 ± 10.38	81.43 ± 13.24	0.831
HR, bpm	75.65 ± 11.61	77.75 ± 20.39	88.10 ± 15.55	<0.001
TC, mmol/L	4.43 ± 1.23	4.42 ± 1.21	4.50 ± 1.15	0.930
TG, mmol/L	1.48 ± 0.70	1.24 ± 0.63	1.44 ± 0.75	0.107
LDL-C, mmol/L	2.75 ± 1.03	2.85 ± 1.03	2.88 ± 1.01	0.716
HDL-C, mmol/L	1.05 ± 0.24	1.05 ± 0.23	1.06 ± 0.28	0.943
FBG, mmol/L	6.08 ± 1.77	6.20 ± 1.61	6.32 ± 1.53	0.701
HbA1c, %	6.47 ± 1.47	6.63 ± 1.32	6.70 ± 1.40	0.621
UA, μmol/L	394.04 ± 115.12	426.70 ± 102.12	439.08 ± 112.68	0.042
BUN, μmol/L	5.87 ± 2.49	6.01 ± 1.61	6.55 ± 2.94	0.270
SCr, μmol/L	76.11 ± 22.41	77.74 ± 17.16	84.68 ± 30.04	0.101
CysC, mg/L	0.96 ± 0.26	1.14 ± 0.31	1.15 ± 0.36	0.001
eGFR, mL/min/1.73 m^2	81.64 ± 21.41	82.06 ± 16.62	74.36 ± 20.26	0.101
ACEI/ARB, n (%)	31 (33.33)	18 (29.51)	10 (19.61)	0.218
β-blocker, n (%)	42 (45.16)	24 (39.34)	20 (39.22)	0.698
Anticoagulant, n (%)	50 (53.76)	26 (42.62)	28 (54.90)	0.316
Statin, n (%)	69 (74.19)	36 (59.02)	32 (62.75)	0.114

The data were shown as the mean ± SD, or n (%). BMI, body mass index; DM, diabetes mellitus; CAD, coronary artery disease; TIA, transient ischemic attack; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; FBG, fasting blood glucose; UA, uric acid; BUN, blood urea nitrogen; SCr, serum creatinine; CysC, cystatin C; eGFR, estimated glomerular filtration rate; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers.

TABLE 2 Indexes of LA remodeling and function in participants.

Variables	Sinus rhythm (n = 93)	Paroxysmal AF (n = 61)	Persistent AF (n = 51)	P-value
LAAPD, mm	29.08 ± 6.05	33.69 ± 5.60	39.14 ± 5.22	<0.001
LALRD, mm	31.29 ± 5.82	34.69 ± 6.02	37.29 ± 6.39	<0.001
LASID, mm	39.48 ± 5.78	47.67 ± 6.13	51.33 ± 6.02	<0.001
LAVmax, ml	33.15 ± 5.51	42.89 ± 9.36	49.18 ± 9.76	<0.001
LAVmin, ml	10.77 ± 3.58	18.23 ± 7.84	30.27 ± 6.96	<0.001
LAtEF,%	65.22 ± 5.59	55.81 ± 7.02	40.63 ± 6.34	<0.001
LAaEF,%	45.91 ± 5.78	41.85 ± 8.98	37.64 ± 7.57	<0.001
SRs,%	32.69 ± 9.31	19.88 ± 6.84	11.25 ± 4.82	<0.001
SRe,%	10.54 ± 2.73	7.87 ± 4.23	3.06 ± 3.38	<0.001
P-wave duration, ms	99.40 ± 6.54	107.64 ± 9.51	123.07 ± 16.09	<0.001
P-wave dispersion, ms	19.75 ± 6.28	26.87 ± 11.79	31.40 ± 15.88	<0.001

The data are shown as the mean ± SD. AF, atrial fibrillation; LAAPD, LA anteroposterior diameter; LALRD, LA left and right diameter; LASID, superior and inferior diameter of left atrium; LAVmax, LA maximum volume; LAVmin, LA minimum volume; LAtEF, LA total emptying fraction; LAaEF, LA active ejection fraction; SRs, strain rate during ventricular systole; SRe, strain rate during early ventricular diastole.

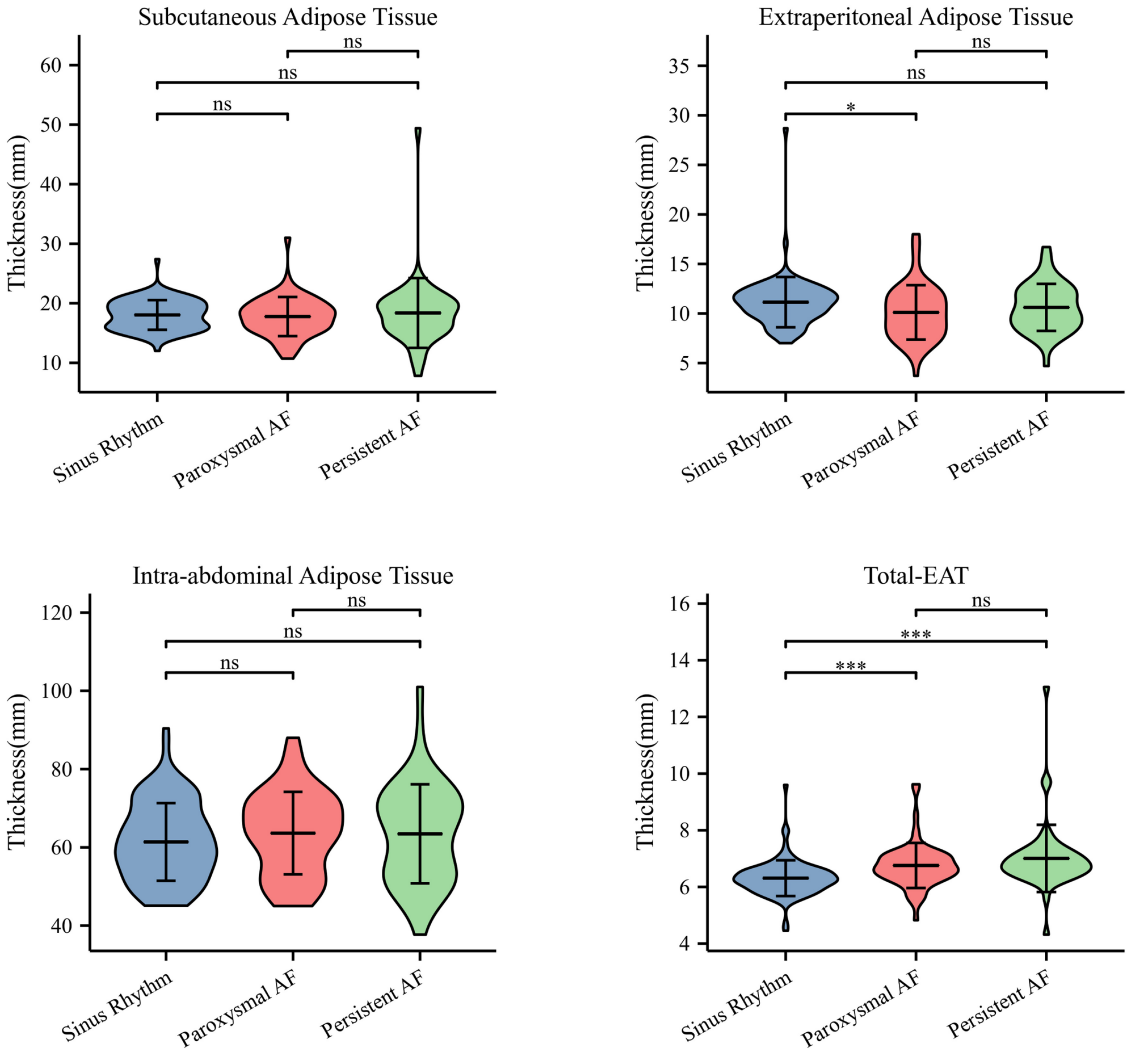

FIGURE 1
Distribution characteristics of different adipose tissues in patients with AF. Only thickness of total-EAT was significantly increased in AF patients compared to that in patients with sinus rhythm. *$P < 0.05$ and ***$P < 0.001$. AF, atrial fibrillation; EAT, epicardial adipose tissue; Total-EAT, mean EAT thickness surrounding the whole heart.

TABLE 3 Logistic regression analysis to evaluate the impact of EAT on the presence of AF.

Variables	Univariate			Model 1[a]			Model 2[b]		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Total-EAT	3.321	1.920–5.746	<0.001	2.615	1.498–4.564	0.001	2.448	1.302–4.604	0.005
LA-EAT	4.737	3.126–7.180	<0.001	3.938	2.566–6.045	<0.001	4.781	2.589–8.831	<0.001
LV-EAT	1.502	1.064–2.122	0.021	1.534	0.976–2.411	0.063	1.245	0.815–1.903	0.310
RV-EAT	1.580	1.108–2.253	0.012	1.453	0.955–2.213	0.081	1.274	0.838–1.936	0.258
IVG-EAT	0.888	0.692–1.140	0.353	1.147	0.872–1.509	0.326	0.824	0.586–1.160	0.267
AVG-EAT	0.864	0.772–0.967	0.011	0.915	0.654–1.280	0.604	0.888	0.762–1.036	0.131

EAT, epicardial adipose tissue; AF, atrial fibrillation; Total-EAT, mean EAT thickness surrounding the whole heart; LA-EAT, mean EAT thickness surrounding left atrium; LV-EAT, mean EAT thickness surrounding left ventricle; RV-EAT, mean EAT thickness surrounding right ventricle; IVG-EAT, mean EAT thickness in interventricular grooves; AVG-EAT, mean EAT thickness in atrioventricular groove. [a] Model 1 was adjusted for HR, UA, and CysC, which have $P < 0.05$ in univariate regression analysis. [b] Model 2 was additionally adjusted for the traditional risk factors, including age, gender, BMI, hypertension, hyperlipidemia, diabetes, coronary artery disease, stroke, smoking status, alcohol abuse, eGFR, ACEI/ARB, β-blocker, anticoagulant, and statin.

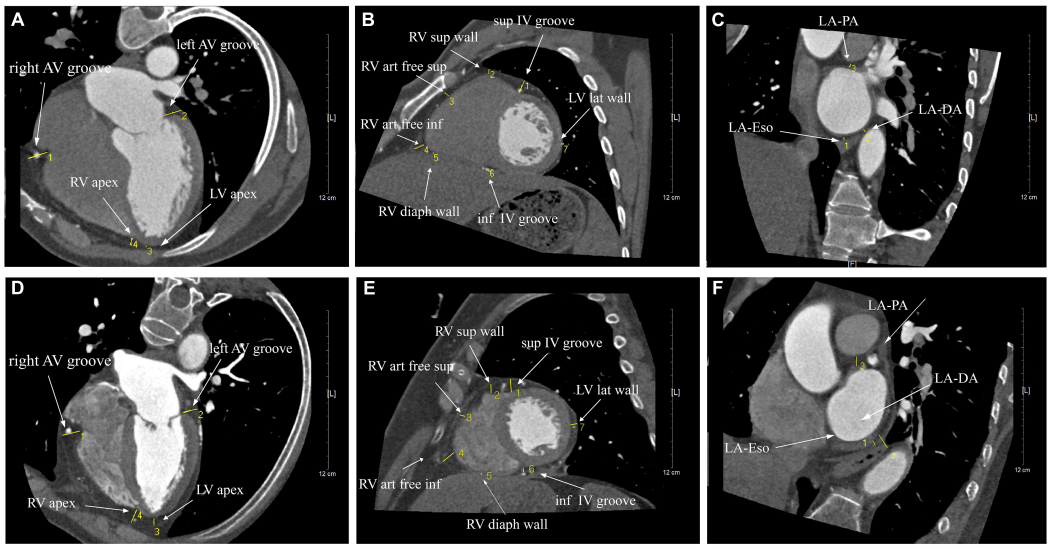

FIGURE 2
The illustration of the thickness of EAT surrounding the heart in patients with sinus rhythm **(A–C)** and AF **(E,F)**. Cardiac CT images were measured in three different views, including the parasternal short-axis view **(A,D)**, the horizontal long-axis view **(B,E)**, and the short-axis view **(C,F)**.

TABLE 4 Redistribution of EAT in different regions of the heart in patients with AF.

Variables	Sinus rhythm (n = 93)	Paroxysmal AF (n = 61)	Persistent AF (n = 51)	P-value
LA-EAT, mm	5.06 ± 0.80	7.36 ± 1.44	7.67 ± 2.00	0.000
LV-EAT, mm	3.12 ± 0.59	3.30 ± 1.17	3.72 ± 1.56	0.007
RV-EAT, mm	5.20 ± 0.64	5.50 ± 1.08	5.77 ± 1.72	0.013
IVG-EAT, mm	13.06 ± 2.36	12.04 ± 2.55	12.19 ± 2.94	0.031
AVG-EAT, mm	7.34 ± 0.93	7.18 ± 1.19	7.20 ± 1.35	0.648

The data are shown as the mean ± SD. AF, atrial fibrillation; EAT, epicardial adipose tissue; LA-EAT, mean EAT thickness surrounding left atrium; LV-EAT, mean EAT thickness surrounding left ventricle; RV-EAT, mean EAT thickness surrounding right ventricle; IVG-EAT, mean EAT thickness in interventricular grooves; AVG-EAT, mean EAT thickness in atrioventricular groove.

However, the thickness of IVG-EAT reduced significantly in both paroxysmal and persistent AF patients (P = 0.031), while the thickness of AVG-EAT had no significant difference among three groups (P = 0.648). Furthermore, the ratio of change in EAT relative to the control group in different regions of the heart was presented in Figure 3, which indicated that the ratio of change in LA-EAT in AF patients, irrespective of paroxysmal or persistent, was much higher than those in other regions of the heart. Multivariate logistic regression analysis showed that LA-EAT (OR = 3.938; $P < 0.001$ in Model 1; OR = 4.781; $P < 0.001$ in Model 2), rather than EAT in other regions, was independently associated with the presence of AF after adjusting for other risk factors of AF (Table 3).

Redistribution of adipose tissues and left atrial remodeling and dysfunction

As shown in Figure 4, total-EAT was significantly associated with almost all indexes of LA remodeling and dysfunction in AF patients. It seemed that total-EAT was positively correlated with LA size (including LAAPD, LALRD, LASID, LAVmax and LAVmin) and P-wave duration and dispersion, and negatively correlated with LA function (LAtEF, SRs, and SRe). Subcutaneous adipose tissue was associated with partial indexes of LA remodeling and dysfunction, including LAAPD, LALRD, LAVmax, LAVmin, LAaEF, SRs, and SRe, while extraperitoneal and intra-abdominal adipose tissues had no or only weak association with those indexes.

When referred to EAT in different regions of the heart, as shown in Figure 5, LA-EAT was found to be significantly associated with all indexes of LA remodeling and dysfunction in AF patients (all $P < 0.05$). However, EAT in other regions were only associated with partial indexes of LA remodeling and dysfunction. Further linear trend analysis showed that LA-EAT was positively correlated with LAAPD, LALRD, LASID, LAVmax, LAVmin, and P-wave duration and dispersion (all $P < 0.05$), and negatively correlated with LAtEF, LAaEF, SRs and SRe (all $P < 0.05$) (Figure 6).

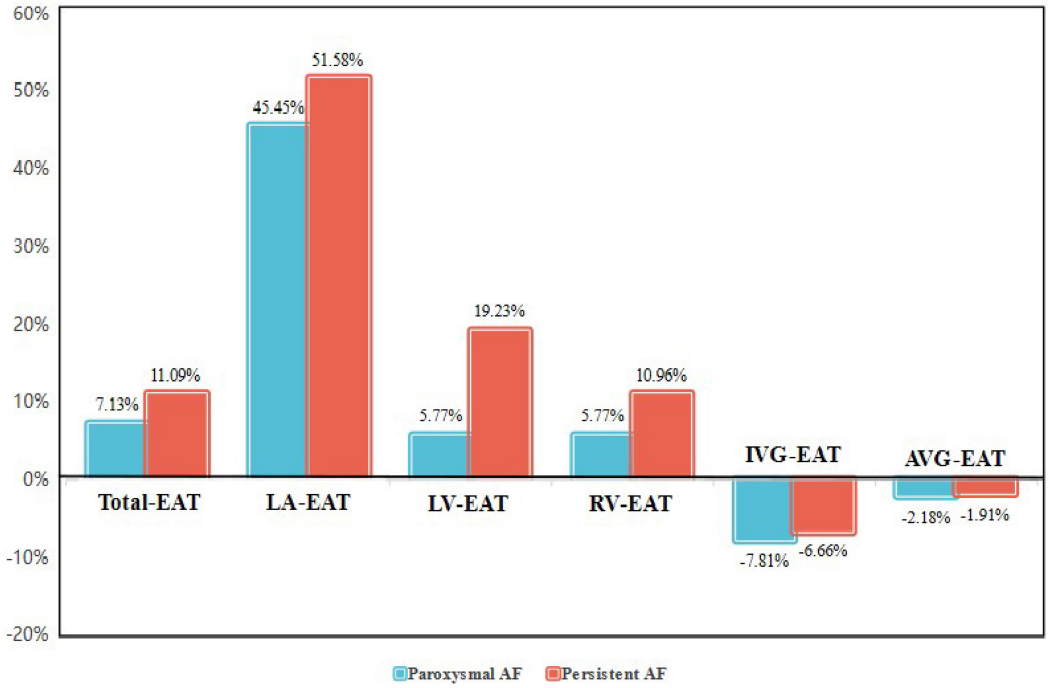

FIGURE 3
The ratio of change in EAT relative to the control group in different regions of the heart in paroxysmal and persistent AF patients. AF, atrial fibrillation; EAT, epicardial adipose tissue; Total-EAT, mean EAT thickness surrounding the whole heart; LA-EAT, mean EAT thickness surrounding left atrium; LV-EAT, mean EAT thickness surrounding left ventricle; RV-EAT, mean EAT thickness surrounding right ventricle; IVG-EAT, mean EAT thickness in interventricular grooves; AVG-EAT, mean EAT thickness in atrioventricular groove.

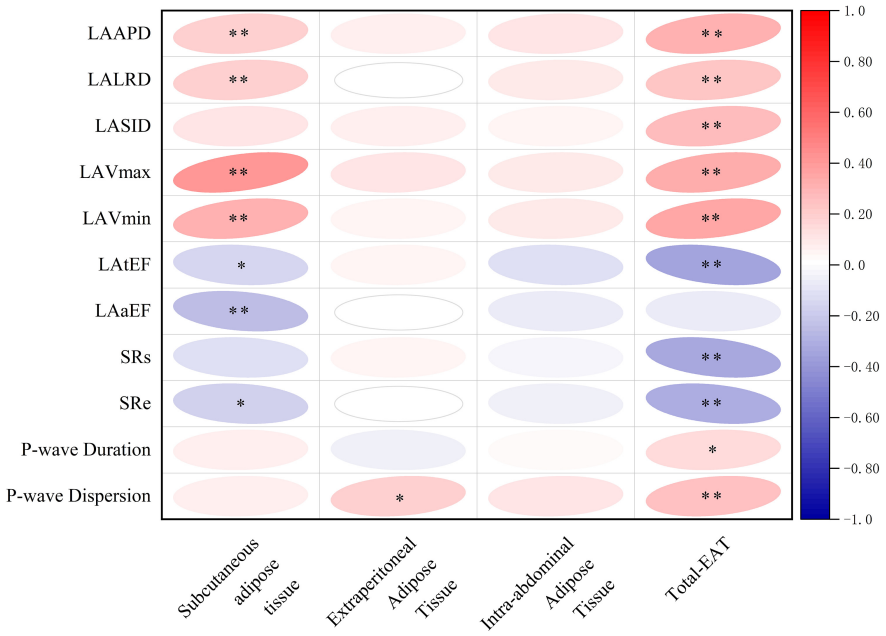

FIGURE 4
Correlations of different adipose tissues with LA remodeling and dysfunction in AF patients. $^{*}P < 0.05$, $^{**}P < 0.01$. EAT, epicardial adipose tissue; Total-EAT, mean EAT thickness surrounding the whole heart; LAAPD, LA anteroposterior diameter; LALRD, LA left and right diameter; LASID, superior and inferior diameter of left atrium; LAVmax, LA maximum volume; LAVmin, LA minimum volume; LAtEF, LA total emptying fraction; LAaEF, LA active ejection fraction; SRs, strain rate during ventricular systole; SRe, strain rate during early ventricular diastole.

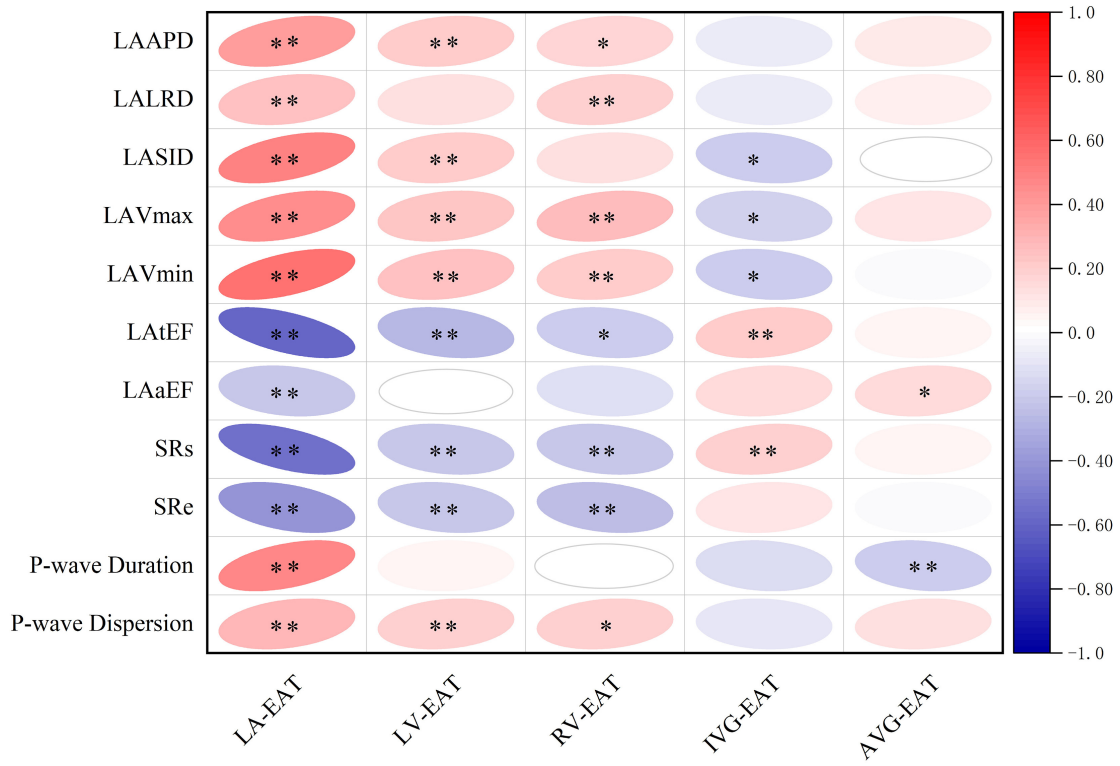

FIGURE 5
Correlation of EAT in different regions of the heart with LA remodeling and dysfunction in AF patients. *$P < 0.05$, **$P < 0.01$. EAT, epicardial adipose tissue; LA-EAT, mean EAT thickness surrounding left atrium; LV-EAT, mean EAT thickness surrounding left ventricle; RV-EAT, mean EAT thickness surrounding right ventricle; IVG-EAT, mean EAT thickness in interventricular grooves; AVG-EAT, mean EAT thickness in atrioventricular groove; LAAPD, LA anteroposterior diameter; LALRD, LA left and right diameter; LASID, superior and inferior diameter of left atrium; LAVmax, LA maximum volume; LAVmin, LA minimum volume; LAtEF, LA total emptying fraction; LAaEF, LA active ejection fraction; SRs, strain rate during ventricular systole; SRe, strain rate during early ventricular diastole.

Discussion

The issue that adipose tissue distribution affects cardiovascular disease has been widely discussed (8–10). The present study found that systemic redistribution of body adipose tissue occurred in AF patients, where the accumulation of total EAT surrounding the heart had a closer correlation with the LA remodeling and dysfunction in AF patients than the adipose tissue in other parts of the body, including subcutaneous, extraperitoneal, and intra-abdominal ones. More interestingly, when we further studied the epicardial distribution feature of adipose tissue surrounding specific regions of the heart, we found that the change in LA-EAT was particularly significant, and had a closer relationship to LA remodeling and dysfunction compared to EAT surrounding the other regions of the heart. These results suggest that systemic and epicardial distribution of adipose tissue might have an impact on the pathophysiology of AF.

Over the past decades, a growing data from epidemiological, clinical and translational studies have demonstrated that EAT is associated with the presence, severity, and recurrence of AF (8–10). Poggi et al. suggested that a tight paracrine cross-talk existed between EAT and myocardium due to their anatomical and functional features, and the dysfunctional EAT can determine a pro-inflammatory environment in the surrounding myocardial tissue (10). Batal et al. found that increased posterior LA fat thickness appears to be associated with AF burden independent of age, body mass index, or LA area (8). Despite these advances, however, significant uncertainty exists and many questions remain unanswered. The differences of the present study to previous ones are as follows: (1) Most previous studies only focused on the impact of EAT on AF, but rarely simultaneously assessed the other body fat as control. The assessment of systemic and epicardial distribution features of adipose tissues in AF patients in the present study was more detailed and comprehensive. Therefore, we make it clear that compared with the overall fat environment, changes in the local fat microenvironment of the heart have more critical value for the pathophysiology of AF. (2) For evaluating the change of LA in AF patients, traditional index of LA size or area has been used in most previous studies (11–13). Nakamori et al. confirmed the correlation between

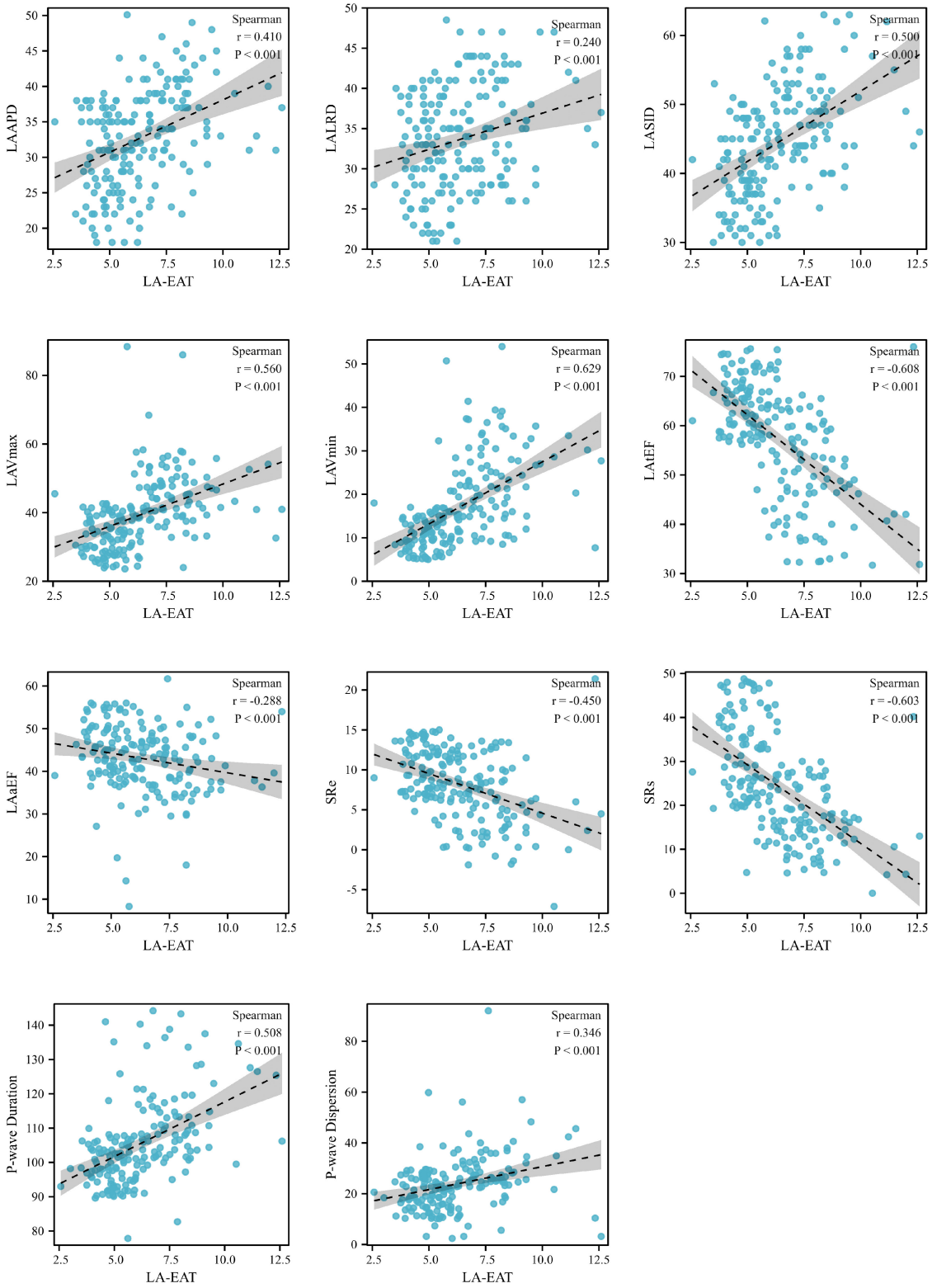

FIGURE 6
Linear trends between LA-EAT and LA indexes in AF patients. AF, atrial fibrillation; LV-EAT, mean Epicardial adipose tissue thickness surrounding left ventricle; AF, atrial fibrillation; LAAPD, LA anteroposterior diameter; LALRD, LA left and right diameter; LASID, superior and inferior diameter of left atrium; LAVmax, LA maximum volume; LAVmin, LA minimum volume; LAtEF, LA total emptying fraction; LAaEF, LA active ejection fraction; SRs, strain rate during ventricular systole; SRe, strain rate during early ventricular diastole.

EAT and LA remodeling by measuring LA diameter and volume (13). In Yorguns' study, EAT was associated with the presence of AF and the diameter of the LA (12). However, on the basis of traditional anatomical parameters, the present study added new functional indicators for assessing the full picture of LA remodeling and dysfunction in AF patients, including LAtEF, LAaEF, SRs, and SRe. These parameters can provide more sample and convincing evidence for our conclusion. (3) The quantitative indicators of EAT by cardiac CT scan include thickness and volume (14–16). Although EAT volume can better reflect the overall situation of adipose tissue surrounding the heart, special workstation is required, with complex measurement method and long analysis time (15). In comparison, measuring the thickness of EAT is simpler and more convenient. A recent meta-analysis found that EAT was associated with AF, measured either as volume or thickness (14). In the study, the thickness was used as the evaluation method for adipose tissue to clarify the correlation between local fat microenvironment and AF, which facilitated the follow-up clinical practice.

The latest view considers that AF is the last stage of atrial remodeling, and the main pathological mechanisms include cardiac structural and electrophysiological disorders (10). The present study found that EAT, especially LA-EAT, may contribute to cardiac structural and electrical remodeling and dysfunction, leading to the occurrence of AF. Previous studies showed that EAT can change the microenvironment and myocardial function around the LA by secreting pro-fibrosis and pro-inflammatory factors through direct contact with the LA myocardium, resulting in cardiac structural and electrical remodeling, such as myocardial fibrosis, slow conduction of electrical activities in the atrium and increased heterogeneity (17, 18). Proliferative adipose tissue can extend into the myocardial cells along with the stroma, induce atrial fibrosis through fat infiltration, and changes the connection mode between myocardial cells, resulting in voltage reduction and abnormal conduction in the LA (19–21). In addition, EAT may affect the atrial electrical activity and participate in cardiac electrophysiological remodeling by affecting the function and number of autonomic plexus around the heart, especially the function of ganglion plexus in fat pad EAT (22).

After observing that EAT is related to the occurrence, maintenance, and severity of AF, studies found that EAT can help prevent and treat AF (23–26). The association between EAT and AF is independent of systemic obesity and more substantial than that of fat in other parts of the body, suggesting the feasibility and effectiveness of EAT as a biomarker of AF in clinical settings. The relationship between LA-EAT and AF is more significant than the fat in other regions of the heart, which can provide more accurate guidance for future local research and intervention of epicardial fat. CT, MRI, and other imaging methods can accurately quantify EAT in different regions to identify the high-risk group of patients prone to AF, guide risk stratification and predict AF recurrence (27). In addition, considering the possible mechanism of EAT affecting AF, as a potential and emerging therapeutic target, EAT can be managed by reducing weight, glucose and lipid, and regulating local cardiac metabolism to improve the adverse effects of EAT on the heart (23–26). EAT can also guide radiofrequency ablation to reduce the difficulty of the operation and improve the efficiency of AF treatment by constructing the EAT model (28).

The findings of this study have to be seen in the light of some limitations. First, the sample size was relatively small. More case data is needed to improve clinical trial design, implementation, and interpretation of results. Second, this is a single-center cross-sectional study assessed the relationship between EAT and AF. Whether accumulation and redistribution of EAT is associated with the prognosis and recurrence of AF was not dynamically and regularly tracked. Therefore, a follow-up database with a broader temporal and spatial dimension must be established. Third, this study only established the relationship between EAT and LA remodeling of AF in clinical practice but did not involve mechanism research. In the near future, further basic researches to explore the pathological molecular mechanism of EAT affecting the local cardiac environment and remodeling are warranted.

To sum up, our study confirmed that EAT had a stronger correlation with AF compared with the other body adipose tissue. Accumulation and redistribution of EAT, especially surrounding the LA, is associated with LA remodeling and dysfunction in AF patients, which may be the critical link to mediating the occurrence of AF.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SL, JMZ, and XT contributed to the study design, formal analysis, and writing – original draft. QC, JW, BW, and ZZ

contributed to the data acquisition and curation. JLZ and HZ made the results visualization. QC and JW contributed to the literature research. QC, XT, and SL contributed to the writing – review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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Incidence and effect of secondary cardiac amyloidosis on outcomes of patients with t(11;14) multiple myeloma

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Background: The t(11;14)(q13;32) is a common chromosome translocation in multiple myeloma (MM), but its prognostic value remains controversial. Immunoglobulin light chain amyloidosis is commonly secondary to multiple myeloma, which can rapidly cause heart failure and high mortality. We aimed to investigate the prevalence of secondary cardiac amyloidosis in MM patients with t(11;14) and to evaluate its impact on survival outcomes.

Methods: We retrospectively identified 52 MM patients with t(11;14) in our center between October 2015 and April 2022. The associations between cardiac amyloidosis and clinical and biological parameters were statistically analyzed, and the impacts of concomitant of cardiac amyloidosis on survival and prognosis of MM patients with t(11;14) were also assessed.

Results: Concomitant presence of cardiac amyloidosis was observed in 15 (28.8%) of all cases. Patients with cardiac amyloidosis had significantly higher NT-proBNP ($p = 0.002$) and higher hs-cTnT ($p < 0.001$), while the patients without cardiac amyloidosis had higher percentage of bone marrow plasma cells ($p = 0.027$), higher incidence of hemoglobin <80 g/L ($p = 0.021$) and bone destruction ($p < 0.001$). The median overall survival (OS) for all patients was 33.4 months after a median follow-up of 23.8 months. The amyloidosis group showed a significantly shorter OS than the non-amyloidosis group (15.3 vs. 41.8 months, $p < 0.001$). Besides, patients harboring NT-proBNP >1,800 pg/ml ($p < 0.001$) or hs-cTnT ≧40 pg/ml ($p = 0.001$) or light chain (LC) only isotype ($p = 0.033$) had a significantly shorter mean OS compared with patients with lower NT-proBNP or hs-cTnT or other M-protein isotype. Univariate analyses showed that NT-proBNP >1,800 pg/ml, hs-cTnT ≧40 pg/ml, LC only isotype, and concomitant presence of cardiac amyloidosis were independently associated with shorter OS, while NT-proBNP >1,800 pg/ml still retained the prognostic value for OS in multivariate analyses.

Conclusion: The t(11;14) MM patients with coexisting cardiac amyloidosis may represent a distinct clinical entity that confers a poor outcome. These findings may have important clinical and biological implications.

KEYWORDS

multiple myeloma, cardiac amyloidosis, heart failure, survival, risk factor

Introduction

Multiple myeloma (MM) is a malignant neoplasm characterized by clonal proliferation of malignant plasma cells in bone marrow (1). It accounts for about 10% of all hematologic malignancies and is the second most common hematologic malignancy after non-Hodgkin lymphoma (2). In MM, detection of cytogenetic abnormality is essential for predicting patient prognosis and disease management. The t(11;14)(q13;q32) is a frequently occurring chromosome translocation in MM and presents in 15–24% of newly diagnosed cases (3–6). This translocation cause IGH/CCND1 fusion and transcriptional activation of CCND1 oncogene by the IGH promoter/enhancer sequences, which contribute to multiple myeloma biology (6). The influence of t(11;14) on outcome remains controversial. Historically, the presence of t(11;14) was associated with relatively favorable outcomes, and patients with t(11;14) have been classified as standard risk MM up to now (3, 7–10). However, recent studies have challenged this paradigm with the conclusion that the prognosis of patients with t(11;14) is less favorable (11–13). These debatable results suggest that additional vital factors may affect the outcomes of t(11;14) MM, which should be managed accordingly.

Immunoglobulin light chain amyloidosis (AL) has long been recognized as a complication of MM. This complication results from the extracellular deposition of β-pleated sheet amyloid proteins which are consist of abnormal monoclonal immunoglobulin light chains (LC) secreted by abnormal plasma cells. These amyloid proteins are resistant to degradation and can infiltrate multiple organs, causing progressive organ dysfunction and death (14). Researches have suggested that up to 38% of MM patients may have subclinical AL, and 10–15% of patients may develop clinically overt AL during the course of the disease (15–19). Despite impressive therapeutic advances in the field of MM, MM-associated AL was also considered to be an independent high-risk prognostic factor for MM patients even in the absence of symptoms at diagnosis (18). The heart is the most commonly involved organ and cardiomyopathy appears in ∼70% of AL patients (20). Moreover, asymptomatic patients with echocardiographic evidence of cardiac involvement usually develop cardiac symptoms later (21). Studies have shown that the survival time of patients with heart failure caused by cardiac amyloid deposition is much shorter than that of patients without heart failure (22). Therefore, the existence and extent of cardiac involvement is the key determinant of the prognosis of AL patients. However, there have been few studies regarding the concomitant presence of cardiac amyloidosis and t(11;14) MM, and it is unclear about the incidence rate of secondary cardiac amyloidosis in t(11;14) patients and its impact on the prognosis.

To address these issues, we examined the prevalence and clinical implications of secondary cardiac amyloidosis in a cohort of 52 MM patients with t(11;14), and assessed the impact of concomitant cardiac amyloidosis on patient survival outcomes. These findings may provide valuable information for the prognosis and optimal treatment of MM patients with t(11;14).

Materials and methods

Patients

This was a retrospective, cross-sectional, medical record review. A total of 52 consecutive MM patients with t(11;14) detected on fluorescence *in situ* hybridization (FISH) between October 2015 and April 2022 at Guangdong Provincial People's Hospital were included in this study. The diagnosis was made according to the 2014 International Myeloma Working Group criteria (23). The symptomatic MM and smoldering MM were both included in this study. The staging was made according to the International Staging System (ISS) and revised ISS (R-ISS), where the required laboratory parameters were available (24, 25). Their medical information regarding demographic characteristics, laboratory findings, treatment, response to induction, survival status, etc were collected in accordance with the Declaration of Helsinki. X-ray, computed tomography, magnetic resonance imaging (MRI), or positron emission tomography/computed tomography were used to determine bone destruction. This study was approved by the Ethics Committee of the Guangdong Provincial People's Hospital.

Fluorescence *in situ* hybridization

All the patients had complete interphase FISH (iFISH) studies available. iFISH analysis was performed on CD138-purified plasma cells using probe sets for the detection of deletions, numerical aberrations and translocations. A total of 200 interphase nuclei were analyzed for each probe set, and the cut-offs recommended by the European Myeloma Network were used: for deletions and numerical aberrations, the cut-off level was set at 20%; for translocations in the IgH locus as well as other translocations, the cut-off level was set at 10% (10).

Diagnosis of amyloidosis

Cardiac amyloidosis is diagnosed when the below criteria are met: a. tissue or organ biopsy proven amyloidosis and b. typical cardiac imaging features or/and abnormal cardiac biomarkers: abnormal age-adjusted NT-proBNP or abnormal hs-Troponin with all other causes for these changes excluded. The echocardiographic evidence indicates cardiac amyloidosis, usually manifested as ventricular wall thickening, poor diastolic filling, and abnormal longitudinal strain. The cardiac MRI evidence indicates cardiac amyloidosis, usually manifested

as ventricular wall thickening, diffuse late gadolinium enhancement, and increased global extracelullar volume.

Amyloid deposits were established by histologic examination with Congo red staining in bone marrow, abdominal fat, or organ biopsy. If the stain was positive, and apple-green birefringence was observed under polarized microscopy, the patient was considered to have amyloid. Immunohistochemistry or immunofluorescence of the monoclonal LCs or proteomic analysis with mass spectrometry was performed in selected cases. The organ involvement for amyloidosis was assessed as follows: kidney (24-h urine protein >0.5 g/day, predominantly albumin), liver (total liver span >15 cm in the absence of heart failure or alkaline phosphatase >1.5 times institutional upper limit of normal), nerve (symmetric lower extremity sensorimotor peripheral neuropathy, gastric-emptying disorder, pseudo-obstruction, voiding dysfunction not related to direct organ infiltration), gastrointestinal tract (direct biopsy verification with symptoms), soft tissue (direct biopsy verification) (26).

Response and outcome

Patients were treated with different induction therapy regimens, and some patients received autologous stem cell transplantation (ASCT). The induction regimens include bortezomib and dexamethasone, or bortezomib and dexamethasone plus cyclophosphamide, or bortezomib and dexamethasone plus thalidomide, or bortezomib and dexamethasone plus lenalidomide, etc. Response to therapy was assessed using the International Myeloma Working Group uniform response criteria (27). Overall survival (OS) was defined as the time from diagnosis of MM to the time of death or last follow-up. The cut-off date for follow-up was April 2022.

Statistical analysis

Statistical analysis was conducted using SPSS software version 19. We summarized categorical variables as proportions and continuous variables as median (range). Categorical variables were compared using Chi-squared test or Fisher's exact, and continuous variables were compared using Student's *t*-test or Mann-Whitney *U*-test. Survival was analyzed using the Kaplan-Meier method and compared between groups using the log-rank test. Patients who were alive at the time of analysis were censored on the date of the last follow-up. Univariate and multivariate analyses of independent factors for OS were performed using the Cox proportional hazard model. A two-sided p-value <0.05 was considered significant for all statistical tests.

Results

Patient characteristics

Table 1 shows the clinical characteristics and treatments of 52 patients with MM carrying t(11;14) included in our study. Briefly, the median age was 65 years and 63% were males. Most of the patients were LC only isotype, followed by IgG isotype, three were non-secretory, and one was smoldering MM. The LC of λ and κ was in 52% of patients and 40% of patients, respectively. The median bone marrow plasma cells were 32%. According to ISS and R-ISS stages, most had favorable features, such as low ISS and R-ISS stage (69% ISS I–II, 82% R-ISS I–II). About the cytogenetic abnormalities, del(17p) and 1q21 gain were detected in 5.8% and 28.8% of patients, respectively.

Prevalence of cardiac amyloidosis in t(11;14) MM

For the cohort as a whole, the concomitant presence of cardiac amyloidosis was observed in 15 (28.8%) of all t(11;14) cases. The diagnosis of cardiac amyloidosis was established by demonstrating amyloid in various target organs or tissues including heart (one), liver (one), soft tissue (eight), gastrointestinal (two), and bone marrow (three). The involvement of other organs outside the heart was as follows: soft tissue (nine), kidney (seven), gastrointestinal tract (two), peripheral nerves (two), and liver (one). Overall, involvement in 1, 2, and 3 or more organs occurred in 2 (13.3%), 6 (40.0%), and 7 (46.7%) patients, respectively. Among the patients with cardiac amyloidosis, cardiac MRI evidence of amyloidosis and echocardiographic evidence of amyloidosis were detected in 10 and 11 patients, separately. The median interventricular septal thickness was 13 mm, and the median ejection fraction was 55% on echocardiography. Additionally, abnormal electrocardiogram were detected in 11 patients, including low voltage in the limb, bundle branch block, atrial arrhythmia, pseudo-infarct Q-waves, etc.

Correlation of cardiac amyloidosis with other clinicobiological characteristics

Statistical analysis was performed to evaluate possible associations between cardiac amyloidosis and the clinical and biological parameters. Patients were separated into two groups based on the presence or absence of cardiac amyloidosis, and the results are summarized in Table 2. Compared with the patients without cardiac amyloidosis, patients with cardiac amyloidosis had significantly higher NT-proBNP ($p = 0.002$) and higher hs-cTnT ($p < 0.001$). Hemoglobin <80 g/L ($p = 0.021$) and bone destruction ($p < 0.001$) were significantly more common in

TABLE 1 Patient characteristics.

Characteristics	Number of patients, *n* (%) or median (range)
Age, years, median (range)	65 (39–83)
Male gender, *n* (%)	33 (63.5)
Hemoglobin, g/L, median (range)	96 (54–151)
Serum albumin, g/L, median (range)	32 (17–43)
β_2-microglobulin, mmol/L, median (range)	6.78 (1.25–27.78)
LDH, U/L, median (range)	224 (15–593)
Calcium, mmol/L, median (range)	2.36 (82–3.20)
Ccr, ml/min/1.73 m^2, median (range)	55 (5–139)
Abnormal FLC ratio (<0.26 or >1.65), *n* (%)	47 (90.4)
BMPCs, %, median (range)	32 (3–94)
M-protein isotype, *n* (%)	
IgG	20 (38.5)
IgA	3 (5.8)
IgD	1 (1.9)
LC only	24 (46.2)
Others	4 (7.7)
LC isotype, *n* (%)	
κ	21 (40.4)
λ	27 (51.9)
ISS stage, *n* (%)	
I	12 (23.1)
II	24 (46.2)
III	16 (30.8)
R-ISS stage, *n* (%)	
I	9 (17.3)
II	34 (65.4)
III	9 (17.3)
Presence of bone destruction, *n* (%)	24 (46.2)
Cytogenetic abnormalities, *n* (%)	
del(17p)	3 (5.8)
1q21 gain	15 (28.8)
t(4;14)	1 (1.9)
Concomitant of cardiac amyloidosis, *n* (%)	15 (28.8)
Treatment regimen, *n* (%)	
VD	9 (17.3)
VCD	19 (36.5)
VTD or VRD	9 (17.3)
Others	15 (28.8)
Underwent ASCT, *n* (%)	2 (3.8)

LDH, lactate dehydrogenase; Ccr, creatinine clearance rate; FLC, free light chain; LC, free light chain; BMPCs, bone marrow plasma cells; LC, light chain; ISS, International Staging System; R-ISS, revised International Staging System; VD, bortezomib, dexamethasone; VCD, bortezomib, cyclophosphamide, and dexamethasone; VTD, bortezomib, thalidomide, and dexamethasone; VRD, bortezomib, lenalidomide, and dexamethasone; ASCT, autologous stem cell transplantation.

the patients without cardiac amyloidosis, and higher percentage of bone marrow plasma cells ($p = 0.027$) was also observed in this group. The incidence of IgG isotype was higher in the amyloidosis negative group ($p = 0.027$), while the incidence of LC only isotype was higher in the amyloidosis positive group ($p = 0.002$). On the other hand, the patients with amyloidosis seemed to have preferential λ LC isotype, but there was no significant difference between the two groups. There were trends that the patients with cardiac amyloidosis had lower albumin level, higher lactate dehydrogenase and alkaline phosphatase levels, but the results were not statistically significant. The other characteristics including age, gender, β2-microglobulin, hypercalcemia, renal dysfunction, difference between involved and uninvolved free light chain (dFLC), and stages were similar between the two groups. In addition to high-risk IgH translocations, del(17p) and 1q21 gain are another high-risk cytogenetic abnormalities. However, there was no significant correlation between cardiac amyloidosis and the presence of del(17p) or 1q21 gain.

Treatment and response

Among all patients, most patients were treated with bortezomib-based regimens (75.0%), and 25.0% were treated with immunomodulator-based regimens. Only two patients (3.8%) received ASCT. There were no data available for three patients. A total of 17 out of 52 patients with available data achieved ≥ very good partial response.

Overall survival

After a median follow-up of 23.8 months (range 0.5–67 months), the median OS for all patients was 33.4 months (95% CI 25.8–41.1 months). Among the 15 patients with concomitant presence of cardiac amyloidosis, 14 (93.3%) patients died during the follow-up period, and 8 (57.1%) patients died within 1 month after diagnosis. All these patients died of complications of amyloidosis. When patients were stratified based on the presence or absence of cardiac amyloidosis, the result showed that patients with cardiac amyloidosis had a significantly shorter mean OS compared with patients without cardiac amyloidosis (15.3 vs. 41.8 months, $p < 0.001$; Figure 1A). Besides, we found that the mean OS of patients with LC only isotype was shorter than that of patients with other M-protein isotypes (24.6 vs. 41.0 months, $p = 0.033$; Figure 1B).

As the current staging systems for systemic AL amyloidosis are based on the levels of circulating markers of cardiac and dFLC, we further analyzed the impact of cardiac biomarkers

TABLE 2 Baseline characteristics according to the presence or absence of cardiac amyloidosis.

Characteristics	Amyloidosis positive ($n = 15$)	Amyloidosis negative ($n = 37$)	p-Value
Age, years, median (range)	64.7 (46–82)	65.2 (39.0–83.0)	0.864
Gender-male, *n* (%)	12 (80.0)	21 (56.8)	0.203
Hemoglobin <80 g/L, *n* (%)	1 (6.7)	15 (40.5)	0.021*
Serum albumin <25 g/L, *n* (%)	4 (26.7)	5 (13.5)	0.419
β_2-microglobulin >5.5 mmol/L, *n* (%)	4 (26.7)	12 (32.4)	0.752
LDH >250 U/L, *n* (%)	6 (40.0)	9 (24.3)	0.318
Calcium >2.75 mmol/L, *n* (%)	0 (0)	4 (10.8)	0.311
Ccr <40 ml/min/1.73 m^2, *n* (%)	6 (40.0)	13 (35.1)	0.760
NT-proBNP >332 pg/ml, *n* (%)	15 (100.0)	19 (51.4)	0.002*
hs-cTnT ≧40 pg/ml, *n* (%)	12 (80.0)	5 (13.5)	<0.001*
ALP >187.5 U/L, *n* (%)	2 (13.3)	1 (2.7)	0.196
BMPCs, %, median (range)	20.9 (4.0–80.0)	36.6 (3.0–94.0)	0.027*
dFLC ≥180 mg/L, *n* (%)	10 (66.7)	22 (59.5)	1.000
M-protein isotype, *n* (%)			
IgG	2 (13.3)	18 (48.6)	0.027*
LC only	12 (80.0)	12 (32.4)	0.002*
LC isotype, *n* (%)			
κ	4 (26.7)	17 (45.9)	0.230
λ	11 (73.3)	16 (43.2)	0.068
ISS stage, *n* (%)			
I	4 (26.7)	8 (21.6)	0.926
II	7 (46.7)	17 (45.9)	
III	4 (26.7)	12 (32.4)	
R-ISS stage, *n* (%)			
I	2 (13.3)	7 (18.9)	0.823
II	11 (73.3)	23 (62.2)	
III	2 (13.3)	7 (18.9)	
Presence of bone destruction, *n* (%)	2 (13.3)	26 (70.3)	<0.001*
Cytogenetic abnormalities, *n* (%)			
del(17p)	0 (0)	3 (8.1)	0.546
1q21 gain	3 (20.0)	12 (32.4)	0.503

LDH, lactate dehydrogenase; Ccr, creatinine clearance rate; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac Troponin T; ALP, alkaline phosphatase; BMPCs, bone marrow plasma cells; dFLC, difference between involved and uninvolved free light chain; LC, light chain; ISS, international staging system; R-ISS, revised international staging system.
*$p < 0.05$.

levels on the survival of all MM patients with t(11;14). The results showed that patients harboring NT-proBNP >1,800 pg/ml or hs-cTnT ≧40 pg/ml had a significantly shorter mean OS compared with patients with lower NT-proBNP or hs-cTnT (NT-proBNP >1,800 pg/ml vs. NT-proBNP ≤1,800 pg/ml: 12.3 vs. 41.3 months, $p < 0.001$; hs-cTnT ≧40 pg/ml vs. hs-cTnT <40 pg/ml: 15.6 vs. 39.1 months, $p = 0.001$; Figures 1C,D). Nevertheless, no significant difference was observed in OS between the patients with dFLC >180 mg/L and the patients with dFLC ≤180 mg/L (32.2 vs. 37.1 months, $p = 0.463$), probably due to the small sample size.

Cox regression analysis

Univariate and multivariate analyses of potential prognostic factors for OS are summarized in Table 3. In the univariate analyses, NT-proBNP >1,800 pg/ml (HR = 5.510, $p < 0.001$), hs-cTnT ≧40 pg/ml (HR = 3.385, $p = 0.001$), LC only isotype (HR = 2.152, $p = 0.040$), and concomitant of cardiac amyloidosis (HR = 3.591, $p = 0.001$) were associated with significantly shorter OS (Table 2). In the multivariate analysis, all the parameters with a $p < 0.05$ in the univariate analysis for OS were included in the Cox model. The result showed that only

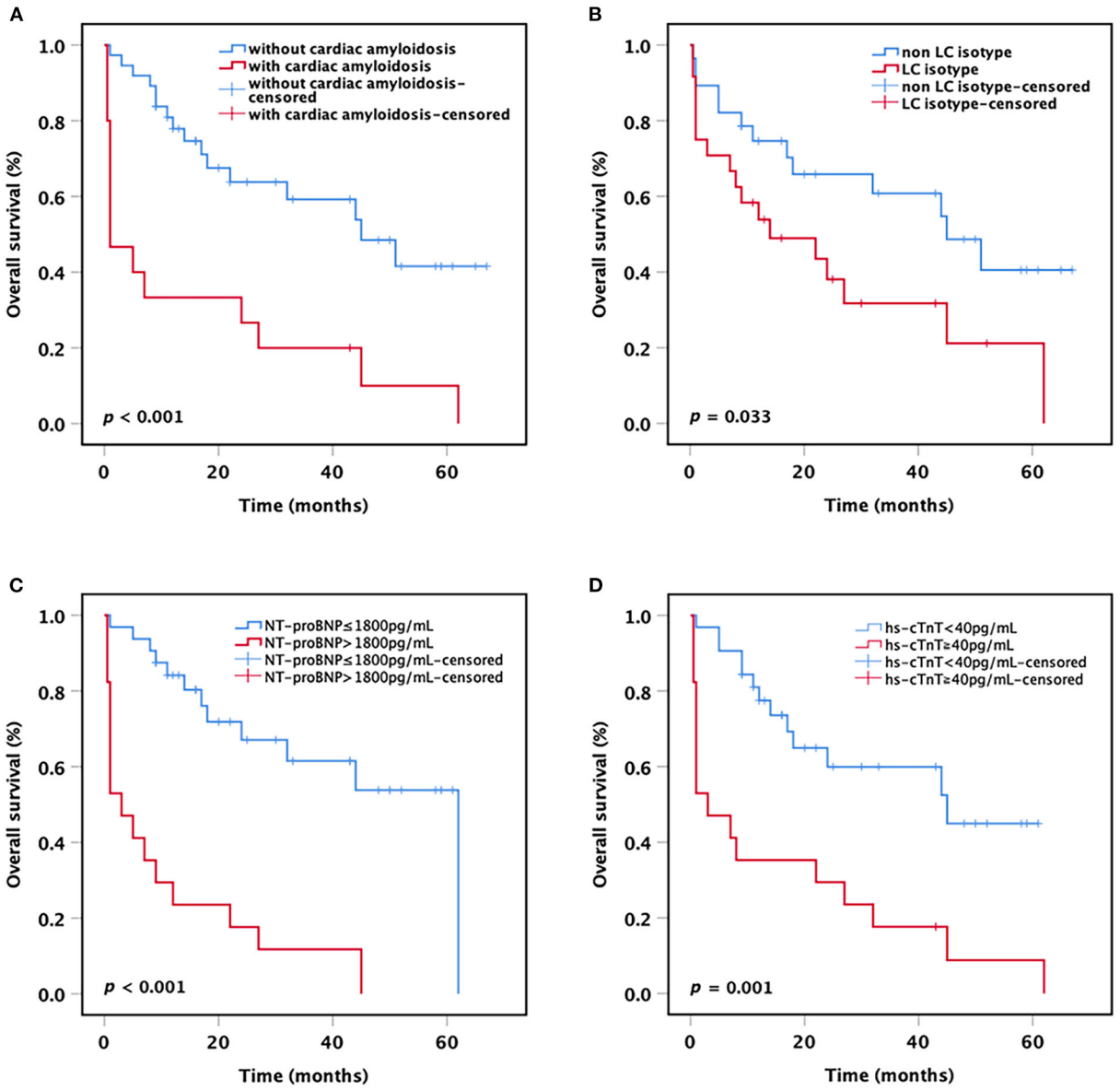

FIGURE 1
Kaplan-Meier curves. OS of t(11;14) MM patients **(A)** with or without cardiac amyloidosis, **(B)** with or without LC only, **(C)** with or without NT-proBNP >1,800 pg/ml, and **(D)** with or without hs-cTnT ≧40 pg/ml. OS, overall survival; LC, light chain; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac Troponin T.

NT-proBNP >1,800 pg/ml was significantly independent factors for shorter OS (HR = 3.965, $p = 0.003$).

Discussion

Multiple myeloma is a heterogeneous disease both clinically and genetically. The t(11;14) is a frequently occurring chromosome translocation in MM, while the prognostic value of t(11;14) in MM remains controversial in the era of novel agents. This discrepancy suggests that it is essential to identify other clinical or biological factors affecting the outcomes of t(11;14) MM. In this study, a high incidence of secondary cardiac amyloidosis was observed in t(11;14) MM, and the concomitant presence of cardiac amyloidosis was demonstrated to adversely affect the prognosis of patients with t(11;14).

Both MM and AL are malignant plasma cell proliferative diseases with unique characteristics. Previous studies have shown that the t(11;14) is detected in 15–24% of MM, while in 40–60% of AL that is higher than MM (3–6, 28), then it atrracted

TABLE 3 Univariate and multivariate survival analysis.

Variable	Univariate		Multivariate	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Serum albumin <35 g/L	0.814 (0.379–1.747)	0.597	–	–
β_2-microglobulin >5.5 mmol/L	1.576 (0.758–3.275)	0.223	–	–
LDH >250 U/L	1.186 (0.554–2.540)	0.660	–	–
NT-proBNP >1,800 pg/ml	5.510 (2.551–11.899)	<0.001*	3.965 (1.614–9.740)	0.003*
hs-cTnT ≧40 pg/ml	3.385 (1.598–7.170)	0.001*	1.506 (0.632–3.589)	0.355
dFLC ≥180 mg/L	1.380 (0.574–3.316)	0.472	–	–
LC only isotype	2.152 (1.035–4.474)	0.040*	0.761 (0.282–2.056)	0.590
ISS III (vs. ISS I/II)	1.576 (0.758–3.275)	0.223	–	–
R-ISS III (vs. R-ISS I/II)	1.019 (0.415–2.502)	0.968	–	–
With 1q21 gain	0.649 (0.276–1.530)	0.323	–	–
Concomitant of cardiac amyloidosis	3.591 (1.735–7.433)	0.001*	1.844 (0.658–5.171)	0.245

LDH, lactate dehydrogenase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac Troponin T; dFLC, difference between the involved and uninvolved free light chain; LC, light chain; ISS, international staging system; R-ISS, revised international staging system; HR, hazard ratio; CI, confidence interval.
*p < 0.05.

our attention to conduct this study to investigate whether the MM patients with t(11;14) are more prone to AL amyloidosis. It is known that the heart is the most common involved organ in AL amyloidosis, and the presence of cardiac involvement is the most significant prognostic factor for AL (14). Therefore, we concentrated on the cardiac amyloidosis in the present study.

In this single-center retrospective study, we investigated 52 patients with t(11;14) MM, and found that the prevalence of secondary cardiac amyloidosis was 28.8%. It should be noted that all the patients with cardiac amyloidosis in this study were clinically overt, and the prevalence of subclinical cardiac amyloidosis was perhaps higher. According to previous reports, clinically overt AL involving various organs accounted for up to 15% of all MM patients (15–19). This indicates to us that the incidence of amyloidosis is increased in the t(11;14) group, or the patients with t(11;14) MM are more prone to amyloidosis. The result also indicates that clinicians should have a high index of suspicion for cardiac amyloidosis in t(11;14) patients, especially those presenting with abnormal clinical or laboratory cardiac indicators.

Cardiac amyloidosis can present as restrictive cardiomyopathy, arrhythmia and heart failure comprise of systolic and diastolic heart failure (29). When a patient exhibits signs of palpitations, dyspnea on exertion, orthopnea, or syncope, and the laboratory finding shows abnormal increase of NT-pro BNP or hs-Troponin, or the cardiac imaging indicates amyloidosis, the presence of cardiac amyloidosis should be suspected. The next is to search for evidence of amyloidosis through biopsy specimens, and the biopsy site can include subcutaneous tissue, labial salivary gland, rectal mucosal, kidney and so on, which are common deposition sites of amyloid protein. In the present study, the most frequently selected site is soft tissue (53.3%) including subcutaneous tissue and labial salivary gland, which are minimally invasive. Endocardial biopsy is the gold standard method for the diagnosis of cardiac amyloidosis, but it is invasive and requires much experience (30). There was only one patient who received an endocardial biopsy in this study. Besides, the kidney was the most frequently affected organ besides the heart, which was consistent with previous reports (14).

When the patients were separated into two groups based on the presence or absence of cardiac amyloidosis, the results showed that the patients with cardiac amyloidosis had significantly higher NT-proBNP ($p = 0.002$) and higher hs-cTnT ($p < 0.001$) than the patients without amyloidosis, indicating increased incidence of heart failure in cardiac amyloidosis. Additionally, we found the patients with cardiac amyloidosis showed relatively lower tumor burden compared with the patients without amyloidosis, which was reflected in the lower percentage of bone marrow plasma cells, lower incidence of severe anemia, and bone destruction. This characterastics is similar to systemic AL manifests as lower tumor burden but systemic organ damage. According to previous reports, the patients with t(11;14) had a significantly higher proportion of LC only isotype (35%) in comparison to other cytogenetic abnormalities (4, 31). In our study, LC only isotype was also demonstrated to be the most common M-protein isotype for the whole t(11;14) cohort (46.2%), meanwhile, we found that the LC only isotype showed closely associated with cardiac amyloidosis (cardiac amyloidosis vs. no cardiac amyloidosis: 80.0% vs. 32.4%, $p = 0.002$). These results suggest that MM patients with amyloidosis may produce more pathogenic free light chain (FLC) causing symptoms of systemic AL, even before the development of symptomatic MM. Futhermore, it

is reported that the λ LC isotype accounts for the majority of systemic AL (32), and λ predominance was also observed in patients with amyloidosis in our study, but not statistically significant, probably due to the small sample size.

We know that the most important prognostic factor in patients with systemic AL is whether there is cardiac involvement, and sudden cardiac death is an important cause of high early mortality in AL patients (20). Nevertheless, there was limited data about the impact of cardiac amyloidosis on the survival of MM patients. In this study with t(11;14) MM, we found that the patients with cardiac amyloidosis had a significantly shorter mean OS compared with patients without cardiac amyloidosis (15.3 vs. 41.8 months, $p < 0.001$). We also found that there were 8 (57.1%) patients who died of complications of amyloidosis within 1 month after diagnosis. Moreover, the concomitant presence of cardiac amyloidosis was demonstrated to be an independent factor for shorter OS in univariate analysis (HR = 3.591, $p = 0.001$). These results matched our priors that the t(11;14) myeloma represents a heterogeneous disease rather than a unique clinical and biological entity. Not all patients with t(11;14) belong to "standard risk disease," which need comprehensive evaluation. Once the patients are complicated with cardiac amyloidosis, the prognosis will be very poor, and the presence of secondary cardiac amyloidosis may be a good risk-stratification factor needed to be validated further.

Staging of AL amyloidosis is based on NT-proBNP >1,800 pg/ml, hs-cTnT ≧40 pg/ml, and dFLC ≥180 mg/L (14), we also evaluated the value of these three indicators in the prognosis of t(11;14) MM. The results showed that NT-proBNP >1,800 pg/ml (HR = 5.510, $p < 0.001$) and hs-cTnT ≧40 pg/ml (HR = 3.385, $p = 0.001$) were independent factors for shorter OS in univariate analyses, and NT-proBNP >1,800 pg/ml retained the prognostic value for OS in multivariate analyses (HR = 3.965, $p = 0.003$). Our study did not find a significant correlation between the level of dFLC with OS in t(11;14) MM.

Additionally, we found that the patients with LC only isotype had shorter mean OS compared with patients harbor other M-protein isotype ($p = 0.033$), and LC only isotype was an independent factor for shorter OS in univariate analysis (HR = 3.591, $p = 0.040$). These results indicate that the LC only isotype may represent a poorer prognosis of the disease. Of note, ISS, R-ISS, and other high-risk cytogenetic abnormalities like 1q21 lost their prognostic values in our study, indicating that the t(11;14) MM requires a unique staging system that takes into account cardiac function indicators.

The outcomes for patients with MM have improved significantly in the era of novel agents (33), most of the patients also received novel agent therapies including bortezomib and immunomodulators in our study, nevertheless, the patients with secondary cardiac amyloidosis were unlikely to benefit from therapy and had a high early death. Therefore, we need to explore the optimal management for this complication in order to improve the prognosis. Rapid and profound elimination of pathogenic FLC is essential to ameliorate cardiac function, so it will be valuable to assess the potential benefits of various induction protocols and ASCT in this disease. BCL2 inhibition venetoclax has demonstrated robust single-agent activity in t(11;14) MM that harbor high expression of antiapoptotic protein BCL-2, which can be evaluated in t(11;14) with cardiac amyloidosis as well in the future (31). Moreover, immunotherapies targeting the amyloid deposits are undergoing clinical trials, which are expected to reverse organ dysfunction (20).

To the best of our knowledge, this is the first study to find an association between the concomitant of cardiac amyloidosis in t(11;14) MM and shorter OS. Limitations of this study include the small sample size of patients, large data missing, lack of patients treated with other novel agents such as daratumumab, and lack of analysis with other coexisting high-risk factors. Additional studies with larger cohorts of patients are warranted to evaluate the clinical entity of secondary cardiac amyloidosis in MM patients with t(11;14).

Conclusion

In summary, we observed a high prevalence of secondary cardiac amyloidosis in MM patients with t(11;14), and the presence of cardiac amyloidosis was a key determinant of patient survival that in need of optimal management.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Guangdong Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JWa and SY acquired the data, performed the analysis, and wrote the manuscript. PL participated in data analysis. LZe, LW, and WL were responsible for data curation. LZh and JWe were involved in study design, supervision, and acquiring funding. All

authors contributed to the study conception, design, read, and approved the final manuscript.

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Conflict of interest

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

Publisher's note

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Elevated α-hydroxybutyrate dehydrogenase is associated with in-hospital mortality in non-ischemic dilated cardiomyopathy

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Background: Previous Study Found That Implantation of a Cardioverter-Defibrillator Likely Caused a Worse Prognosis in Older Patients With non-Ischemic Systolic Heart Failure. This Suggests That More Precise Risk Stratification Is Needed in Elderly Patients. We Conducted a Retrospective Study to Evaluate the Association of α-Hydroxybutyrate Dehydrogenase (α-HBDH) With Mortality During Hospitalization in Elderly Patients With non-Ischemic Dilated Cardiomyopathy (NIDCM).

Methods: 1,019 Elderly Patients (age ≥60 Years) Diagnosed With NIDCM Were Retrospectively Enrolled From January 2010 to December 2019. Univariate and Multivariate Analyses Were Showed to Explore the Relationship Between α-HBDH and in- Hospital Death.

Results: Patients in elevated α-HBDH group (>182 U/L) had a longer hospital stays and higher in-hospital mortality. Univariate logistics regression analysis showed that elevated α-HBDH was significantly related to mortality (OR: 7.004, 95% CI: 3.583–13.693, $p < 0.001$). Receiver operator characteristic (ROC) curve analysis reflected that α-HBDH levels had excellent predictive power for in-hospital death (AUC = 0.810, 95% CI: 0.745–0.876, $p < 0.001$). After adjustment of age, serum creatine, albumin and LVEF, multivariate regression analysis validated the association of elevated α-HBDH with increased risk of in-hospital death ($p < 0.05$).

Conclusions: Elevated α-HBDH level is significantly related to in-hospital mortality in older patients with NIDCM.

KEYWORDS

NIDCM, elderly, α-HBDH, in-hospital death, prognosis

Introduction

Non-Ischemic Dilated Cardiomyopathy (NIDCM) Is Defined as Left Ventricle (LV) Enlargement and Global Systolic Function Impairment (LVEF <45%) in the Absence of Coronary Artery Disease or Increased Loading Condition (1). Currently, There Are no Effective Treatments can Prevent the Progression of NIDCM to HF (2). These Patients Often Occur Refractory Heart Failure (HF) and Sudden Cardiac Death (SCD) and Need Benefit From Cardiac Transplantation (3–5). This Condition Brings Great Pain to Patients and Places a Heavy Financial Burden on Global Health Care Systems (6, 7). The DANISH Study Found That Older Patients With non-Ischemic Systolic HF may not Benefit From Implantation of a Cardioverter-Defibrillator (8). This Suggests That More Precise Risk Stratification Is Needed in Elderly Patients.

α-hydroxybutyrate dehydrogenase (α-HBDH) is an auxiliary marker of myocardial injury, which was reported to have an increased specificity for detecting myocardial injury, starting to increase 8–12 h after damage, reaching peak serum concentrations after 48–72 h and returning to baseline after 7–14 days (9). Myocardial injury was associated with progression and poor outcomes of DCM (10). Therefore, we aimed to explore the connection between α-HBDH and in-hospital mortality in elderly patients with NIDCM.

Materials and methods

Study population

We retrospectively enrolled 1,019 patients (age ≥60 years) admitted for NIDCM in Guangdong Provincial People's Hospital (Guangzhou, China) from January 2010 to December 2019. All patients met the diagnostic criteria of NIDCM according to the scientific statement established by the American Heart Association (11). This study received approval of the Ethics Committee of Guangdong Provincial People's Hospital with a waiver of written informed consent due to the retrospective study design. Oral informed consent from patients or their relatives by telephone was recorded by trained nurses during follow-up.

Data source

Clinical information and laboratory results were collected from the electronic medical records by one researcher and randomly confirmed by another researcher. Basal α-HBDH samples were collected on the following morning after admission and measured by colorimetric method. The LV ejection fraction (LVEF) was measured by Simpson's biplane method. Linear internal measurements of the left ventricle and its walls were completed in the parasternal view.

Definition and endpoints

Elevated α-HBDH on admission was defined as a α-HBDH level of more than 182 U/L according to the standards established by our laboratory. There were no patients had reduced α-HBDH (<72 U/L). The primary endpoint was in-hospital death, followed by malignant arrhythmia and acute HF during hospitalization. Malignant arrhythmia included ventricular tachycardia, ventricular fibrillation, and sudden cardiac arrest.

Statistical analysis

Continuous data are expressed as the mean ± SD, compared by One-way ANOVA tests. Categorical variables are presented as numbers, *n* (proportions, %) and compared through Pearson's chi-square tests. Missing values were excluded from the analysis. Univariate and multivariate analyses were performed to evaluate the association of elevated α-HBDH level with in-hospital mortality. Receiver operator characteristic (ROC) curves were drawn to assess the predictive power of α-HBDH. All statistical analyses were performed using SPSS software version 26.0 (IBM Corp., Armonk, New York, USA). All *P*-values were two sided with a significance level of 0.05.

Results

Baseline characteristics

There are 1,019 patients (667 males, 352 females) met the inclusion criteria and were divided into two groups according to the α-HBDH levels on admission: normal α-HBDH (≤182 U/L) and elevated α-HBDH (>182 U/L). In the two groups, the range of age was 60–91 and 60–89 years, respectively, and there were 151 patients older than 75 years old. The mean age and sex composition were similar.

First, the medical history of smoking, diabetes, and hypertension were similar in the two groups. Second, patients with elevated α-HBDH had a higher lactate dehydrogenase (LDH) and lower albumin level and LVEF on admission. Third, there was no statistic difference in the prevalence of malignant arrhythmia and acute HF during hospitalization. Patients in elevated α-HBDH group performed a longer mean hospital stays (11 vs. 8 days) and higher mortality during hospitalization (10.9 vs. 1.7%) (Table 1).

TABLE 1 Baseline demographics and clinical characteristics.

Variables	Patients with NIDCM ($N = 1,019$)		*P*-value
	Normal α-HBDH ($n = 754$)	Elevated α-HBDH ($n = 265$)	
Demographic			
Age, y	68.1 ± 6.1	68.8 ± 6.6	0.124
Male, *n* (%)	503 (66.7)	164 (61.9)	0.155
Medical history			
Smoking history, *n* (%)	211 (28)	61 (23)	0.116
Hypertension, *n* (%)	258 (34.2)	82 (30.9)	0.331
Diabetes, *n* (%)	175 (23.2)	73 (27.5)	0.157
Parameters on admission			
WBC count, 109/L	7.6 ± 3.0	7.3 ± 2.3	0.087
Neutrophil count, 109/L	5.1 ± 2.7	4.8 ± 2.1	0.060
Lymphocyte count, 109/L	1.7 ± 1.3	1.6 ± 0.7	0.939
Hemoglobin, g/L	131.8 ± 18.7	130.4 ± 18.9	0.318
Platelet count, 109/L	198.0 ± 67.3	197.2 ± 69.8	0.878
Albumin, g/L	36.0 ± 4.5	33.5 ± 4.8	<0.001
CREA, umol/L	103.8 ± 68.3	136.9 ± 86.9	<0.001
ALT, U/L	27.3 ± 27.6	158.6 ± 460.2	<0.001
AST, U/L	29.1 ± 28.1	214.8 ± 1,196.5	<0.001
LDH, U/L	189 ± 38	430 ± 621	<0.001
α-HBDH, U/L	137 ± 26	255 ± 88	<0.001
Total bilirubin, umol/L	18.8 ± 10.4	34.3 ± 40.3	<0.001
PAP, mmHg	40.4 ± 14.4	45.7 ± 14.3	<0.001
LVEF, %	33.8 ± 11.2	31.2 ± 10.6	0.003
Hospital stays, days	8 ± 6	11 ± 9	<0.001
Clinical symptoms and outcomes			
Malignant arrhythmia, *n* (%)	51 (6.8)	25 (9.4)	0.155
Acute heart failure, *n* (%)	10 (1.3)	4 (1.5)	0.826
In-hospital mortality, *n* (%)	13 (1.7)	29 (10.9)	<0.001

NIDCM, non-ischemic dilated cardiomyopathy; WBC, white blood cell; CREA, creatinine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PAP, pulmonary artery pressure; LVEF, left ventricle ejection fraction.

Regression analyses and ROC curve

Univariate logistics regression analysis showed that elevated α-HBDH was not related to malignant arrhythmia and acute HF in hospital (both $p > 0.05$), but it was significantly associated with mortality (OR: 7.004, 95% CI: 3.583–13.693, $p < 0.001$). Multivariate logistics regression for in-hospital death and α-HBDH displayed statistic difference in the two groups (OR: 4.217, 95% CI: 1.958–9.082, $p < 0.001$) (Table 2). Meanwhile, multivariate Cox regression analysis confirmed the relationship between elevated α-HBDH and increased risk of in-hospital death (HR: 2.489, 95% CI: 1.217–5.089, $p = 0.012$) (Table 3). The adjustment variables enrolled in the multivariate regression model were age, serum creatine, albumin and LVEF, whose *p*-value was <0.05 in univariate regression analysis.

ROC curve analysis showed that α-HBDH levels had great sensitivity and specificity in predicting mortality (AUC = 0.810, 95% CI: 0.745–0.876, $p < 0.001$) but not malignant arrhythmia and acute HF (both $p > 0.05$) during hospitalization. The area under the curve (AUC) is a measure of the overall predictive validity of the risk and prognosis in the disease where AUC >0.80 signals good validity. The optimal cutoff value was 168 U/L, with a sensitivity of 83.3% and specificity of 66.0% (Figure 1).

Discussion

At present, this is the largest and first study to investigate the connection between α-HBDH levels and in-hospital mortality

TABLE 2 The risk of elevated α-hydroxybutyrate dehydrogenase for in-hospital death during hospitalization.

	Group	Logistic regression analysis		
		OR	95% CI	*P*-value
Univariate analysis	Normal α-HBDH	Ref	/	/
	Elevated α-HBDH	7.004	3.583–13.693	<0.001
Multivariate analysis[a]	Normal α-HBDH	Ref	/	/
	Elevated α-HBDH	4.217	1.958–9.082	<0.001

OR, odds ratio; CI, confidence interval. [a]Adjust for age, serum creatine, albumin, and left ventricle ejection fraction.

TABLE 3 The risk of elevated α-hydroxybutyrate dehydrogenase for in-hospital death during hospitalization.

	Group	Cox regression analysis		
		HR	95% CI	*P*-value
Univariate analysis	Normal α-HBDH	Ref	/	/
	Elevated α-HBDH	4.052	2.069–7.934	<0.001
Multivariate analysis[a]	Normal α-HBDH	Ref	/	/
	Elevated α-HBDH	2.489	1.217–5.089	0.012

HR, hazard ratio; CI, confidence interval. [a]Adjust for age, serum creatine, albumin, and left ventricle ejection fraction.

in patients with NIDCM. The results showed that elevated α-HBDH was an independent risk factor for in-hospital death in elderly patients with NIDCM, which may serve as a potential therapeutic target for reducing mortality during hospitalization.

Previous study has reported that α-HBDH have an increased specificity for detecting myocardial injury (9). NIDCM is characterized by LV enlargement and global systolic function impairment, and often accompanied by myocardial injury (1). Older age is always accompanied by deceleration of cell metabolism, and more extensive damage to organ. α-HBDH is significantly associated with age as a marker of cell death (12). In the present study, we selected a cohort of elderly people (age ≥60 years) and divided them into two groups. There was no statistic difference in age and sex composition between the two groups. However, patients with elevated α-HBDH had a higher rate of in-hospital mortality than those with normal α-HBDH.

The present study showed that patients in elevated group had elevated LDH, and reduced albumin level and LVEF on admission. Changes in α-HBDH levels and these markers may affect mutually, such as reduced albumin level indicates malnutrition (13) and lower LVEF is associated with damage of systolic function of myocardium (14). All these changes are related to poor outcome in patients. It also indicates that the relationship between elevated α-HBDH and poor prognosis is associated with the abnormal situations of several systems. However, future studies are required to elucidate the specific mechanism.

The molecular basis of the irregular cardiac architecture in NIDCM involves changes in the structure and composition of cardiomyocytes, which lead to remodeling of the myocardium (15). Myocardial remodeling may cause the phenotype of a patchwork LV, where myocardial cells are interspersed with necrotic and fibrotic patches and intermittent calcifications (4, 16). These mechanisms increase dilatation of the ventricle and are responsible for the reduction of LVEF, and, consecutively, clinical symptoms (17). Patients with NIDCM are often admitted to the hospital because of HF. Current guidelines suggest that treatment for these patients is mostly supportive general HF treatment (11). Patients often receive the therapy with β-blockers and angiotensin-converting enzyme inhibitors or angiotensin II-receptor blockers (ACEIs/ARBs), which has been proved to the survival of patients in clinical trials (18, 19). However, such patients remain at substantial risk for sudden death from cardiac causes. It is essential to early identify high-risk patients during hospitalization.

α-HBDH is a marker of apoptosis, which can reflect myocardial and renal damage (9). Patients in elevated α-HBDH group had higher serum creatine and lower LVEF. It was reported that α-HBDH was related to the occurrence of atherothrombotic events (20). In the present study, we had excluded patients with ischemic heart disease. The result showed that α-HBDH remained significantly associated with mortality during hospitalization in non-ischemic patients, but not malignant arrhythmia and acute HF. In addition, patients with elevated α-HBDH had longer hospital stays and higher in-hospital mortality (10.9 vs. 1.7%). The underlying mechanism is not clear and future trials are warranted.

The α-HBDH/LDH ratio between 0.63 and 0.81 is normal, while more than 0.9 suggests myocardial lesion, <0.6 suggests liver damage (12). The ratio α-HBDH/LDH in elevated α-HBDH group is 0.59 (255/430, Table 1), but normal in another group (137/189). In addition, the AST and ALT levels were abnormally higher and AST/ALT ratio was more than 1 (214/158, Table 1) in elevated group. This condition indicates that those patients are likely to have severe liver damage or even liver cancer. This suggests that the relationship between α-HBDH and mortality during hospitalization may be related to multiple organ damage, including heart, liver, kidney, and others.

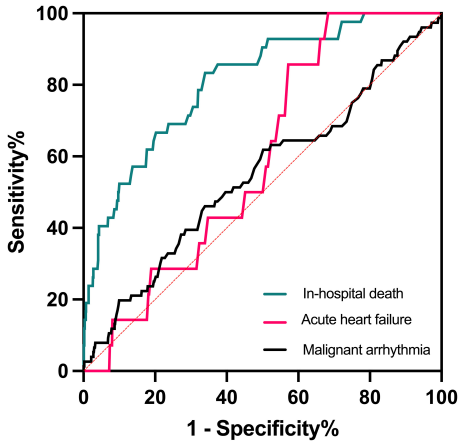

FIGURE 1
ROC curves of α-HBDH in predicting in-hospital endpoints.

Patients with NIDCM are usually admitted because of severe HF symptoms. At present, ACEIs/ARBs and β-blockers are the optimal medical therapy for HF in DCM (4). However, in acute and severe patients, pharmacological therapy may not be sufficient to maintain adequate cardiac function, which will increase the risk of in-hospital death. Those patients may need to benefit from cardiac resynchronization therapy (CRT) or even surgery. In a meta-analysis, CRT was proved to reduce mortality in non-ischemic cardiomyopathy (21). Surgical treatment mainly involves heart transplantation and implantation of long-term mechanical circulatory support (19). The results of this study suggest that elevated α-HBDH on admission was related to higher risk of death, which may help clinicians select the optimal treatment for patients and reduce their in-hospital mortality.

Some limitations should be declared in this research project. First, potential confounding factors may affect the results due to the retrospective study design. Second, α-HBDH levels are measured on admission and the influence of continuous monitoring cannot be ignored. Third, we did not correct for the effect of diuretics and other drugs on outcome.

In a word, we discovered that α-HBDH levels may be an independent risk factor for in-hospital death in elderly patients with NIDCM. Early monitoring of α-HBDH may help identify high risk patients and conduct beneficial intervention.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The datasets generated and/or analyzed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to jianglei@smu.edu.cn.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Guangdong Provincial People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

LJ contributed to the design and conception of the study. XL, WH, XZ, FS, and YL were responsible for the acquisition, analysis, and interpretation of data. XL and WH drafted the manuscript. LJ and NT critically reviewed the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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Conflict of interest

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

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Development and validation of a clinical predictive model for 1-year prognosis in coronary heart disease patients combine with acute heart failure

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Background: The risk factors for acute heart failure (AHF) vary, reducing the accuracy and convenience of AHF prediction. The most common causes of AHF are coronary heart disease (CHD). A short-term clinical predictive model is needed to predict the outcome of AHF, which can help guide early therapeutic intervention. This study aimed to develop a clinical predictive model for 1-year prognosis in CHD patients combined with AHF.

Materials and methods: A retrospective analysis was performed on data of 692 patients CHD combined with AHF admitted between January 2020 and December 2020 at a single center. After systemic treatment, patients were discharged and followed up for 1-year for major adverse cardiovascular events (MACE). The clinical characteristics of all patients were collected. Patients were randomly divided into the training (n = 484) and validation cohort (n = 208). Step-wise regression using the Akaike information criterion was performed to select predictors associated with 1-year MACE prognosis. A clinical predictive model was constructed based on the selected predictors. The predictive performance and discriminative ability of the predictive model were determined using the area under the curve, calibration curve, and clinical usefulness.

Results: On step-wise regression analysis of the training cohort, predictors for MACE of CHD patients combined with AHF were diabetes, NYHA $\geq$ 3, HF history, Hcy, Lp-PLA2, and NT-proBNP, which were incorporated into the predictive model. The AUC of the predictive model was 0.847 [95% confidence interval (CI): 0.811–0.882] in the training cohort and 0.839 (95% CI: 0.780–0.893) in the validation cohort. The calibration curve indicated

good agreement between prediction by nomogram and actual observation. Decision curve analysis showed that the nomogram was clinically useful.

Conclusion: The proposed clinical prediction model we have established is effective, which can accurately predict the occurrence of early MACE in CHD patients combined with AHF.

KEYWORDS

acute heart failure, major adverse cardiac events, prognosis, clinical predictive model, coronary heart disease

Introduction

Acute heart failure (AHF) is a life-threatening condition characterized by acute dyspnea and Systemic congestion caused by abnormal cardiac structure or function, including acute decompensated heart failure (ADHF) or new onset of AHF (1). AHF is the leading cause of cardiogenic shock and cardiac arrest in patients, and the case fatality rate in hospitals is as high as 3–13%, seriously threatening their life safety, and their prognosis is extremely poor (2). The most common causes of AHF are coronary ischemic disease, cardiomyopathy, valvular disease, infective endocarditis, hypertensive heart disease, pulmonary heart disease, renal failure, and metabolic disorders. A significant proportion of coronary heart disease (CHD) patients develop AHF, and CHD is the primary cause of heart failure(HF). Despite recent advances in AHF management, such as advances in pharmacological treatment, cardiac devices, and specific heart failure programs, mortality remains high. The in-hospital fatality rate and 5-year mortality rate of AHF were 3 and 60%, respectively, and the rate of 1-year emergency department visits and emergency re-hospitalization was 50% (3). Uncontrolled AHF complicates clinical treatment and threatens patient safety (4). Thus, an effective and simple prediction model for AHF is urgently needed.

Acute heart failure is associated with a higher risk of death and re-hospitalization, and many researchers have attempted to develop different tools to predict adverse events in patients with AHF. Several researchers have developed risk score models to stratify HF patients, such as the Seattle Heart Failure Model (SHFM), the European Society of Cardiology (ESC) Model, and the American College of Cardiology/American Heart Association (ACC/AHA) Model (5–7). Although the predictive value of these models is widely recognized, some researchers report that these models do not necessarily predict the mortality of individual patients with HF (8). In addition to the high mortality rate, the high rate of re-hospitalization was due to major adverse cardiac events (MACE), which contributed to the poor prognostic outcomes of AHF (9, 10). According to the report (11), each AHF hospitalization resulted in cardiac dysfunction and a gradual decline in the patient's clinical course, increasing the risk of re-hospitalization. Notably, its risk could be markedly increased in the short-term period after an AHF event (12–15). A significant proportion of AHF patients appear to be at an even higher risk of rapid HF progression and death following an acute event. Early, post-discharge follow-up and risk-tailored, intensive HF therapy may reduce AHF re-hospitalization and improve survival in these patients (16). At present, AHF prediction models are mostly used to predict mortality or long-term prognosis, while models for AHF after short-term are very rare. Evidence in the short-term prognosis prediction model of AHF demonstrates that early, coordinated, aggressive treatment reduces inpatient mortality (17). Recent several studies have shown that a number of risk factors are related to short-term mortality after discharge, including age, sex, ventricular function, management, and so on (18–21). Although some established algorithms are currently available, these studies have not been validated in Chinese populations. Therefore, an individualized prediction model is imperative for more accurate MACE prediction in AHF patients.

Nomogram is a new prognosis evaluation tool based on Cox proportional hazards regression model or logistic regression model that predicts individual disease risk graphically and is easily applied clinically. It mainly simplifies the prediction model by calculating a single estimated value of the probability of an event occurrence and provides a personalized prognosis assessment for individual patients to assist clinical decision-making (22). Compared with the traditional risk scoring system, a nomogram can integrate more risk factors, calculate the numerical probability of the target event, quantify the risk more accurately, and apply it more flexibly.

This study aimed to develop a clinical predictive model to predict the risk of short-term (1-year) adverse outcomes in CHD patients combined with AHF based on potential risk factors.

Materials and methods

Patients

A total of 692 CHD patients combined with AHF admitted to Shunde Hospital of Southern Medical University between January 2020 and December 2020 were selected for this study. Inclusion criteria: (1)Patients with CHD who were diagnosed with coronary artery stenosis > 70%; (2) Patients with AHF met the Chinese Guidelines for the Diagnosis and Treatment of Heart Failure 2018 (23); (3) Patients aged ≥ 18 years; and (4) Patients were classified as having grade II–IV cardiac function, by the New York Heart Association (NYHA) classification (24). Exclusion criteria: (1) Patients with congenital heart disease, cardiomyopathy, or valvular disease; (2) Patients complicated with malignant tumors; (3) Patients with hematological system diseases or autoimmune diseases; (4) Patients with prior history of cerebrovascular accident or mental illness; and (5) Patients with clinical data that were incomplete or lost to follow-up.

Data collection

Based on previous studies (25, 26), we selected 40 risk factors that may predict MACE in CHD patients combined with AHF 1 year after discharge from the hospital, including age, sex, hypertension, diabetes, smoking, HF history, chronic kidney disease history, atrial fibrillation history, NYHA grades, systolic blood pressure, diastolic blood pressure, lipoprotein-associated phospholipase A2 (Lp-PLA2), homocysteine (Hcy), serum creatine kinase isoenzyme MB (CK-MB), troponin T, N-terminal pro B-type natriuretic peptide (NT-proBNP), D-dimer, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, Triglycerides, fibrinogen degradation product, serum creatinine, uric acid, fasting plasma glucose, C-reactive protein, white blood cell count, neutrophil count, lymphocyte count, neutrophil to lymphocyte ratio, hemoglobin levels, platelet count, and medication status (such as diuretics, beta-blockers, Statins, Mineralocorticoid receptor antagonists, Angiotensin receptor enkephalinase inhibitors, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers).

Follow-up and grouping

Follow-up data were collected from our hospital's electronic medical record system, patients' outpatient records, and telephone conversations with patients or family members.

The 1-year adverse outcomes were defined as adverse cardiovascular events associated with stroke, Non-fatal myocardial infarction, cardiac death and re-hospitalization due to HF during the first 12 months of follow-up after discharge from the hospital.

Patients were divided into MACE and no MACE groups based on whether MACE occurred 1-year after discharge from the hospital.

Model development and statistical analysis

All statistical calculations were computed using R software. The data were randomly divided into a training cohort ($n = 484$) and a validation cohort ($n = 208$) at about 7:3. Predictors of 1-year outcomes were analyzed using logistic regression based on the training cohort. Step-wise regression based on the Akaike information criterion was used to further select significant variables. The receiver operating characteristic curve (ROC) and its area under the curve (AUC) were used to evaluate the step-wise regression on both the training and validation cohorts. A nomogram was formulated based on the results of logistic regression. The nomogram is based on proportionally transforming the regression coefficient into a 0–100 point scale. The sum of all variables' points could be interpreted as a probability of belonging to a class. The predictive performance of the nomogram was measured using AUC and resampling model calibration, accompanied by the Hosmer-Lemeshow test (a significant test statistic implies that the model does not calibrate perfectly). Decision curve analysis (DCA) was used to determine the clinical usefulness of the models by calculating the net benefits at different threshold probabilities in the combined training and validation datasets. The "rms" package was used for nomogram formulation and calibration. The DCA was performed by using the "rmda" package. P-value < 0.05 was considered to be statistically significant.

Results

Baseline characteristics

The mean age of the 692 patients was 67.13 ± 15.55 years, with 400 (57.8%) males and 292 (42.2%) females. Three hundred seven-nine (54.8%) patients were diagnosed with ADHF, and 313 (45.2%) patients were diagnosed with new onset of AHF. Two hundred fifty-nine (37.4%) patients were followed up with MACE, including 23 (3.3%) patients with cardiac death and 236 (34.1%) patients with re-hospitalization cause by stroke, Non-fatal myocardial infarction, or HF. Finally, 484 patients were assigned to the training cohort, which included 181 (37.4%) patients with MACE, and 208 patients were assigned to the validation cohort, which included 78 (37.5%) patients with MACE.

Clinical data from the training and validation cohorts revealed no statistically significant differences in age, sex, disease history, laboratory tests, NYHA grades, medication status, or MACE incidence ($P > 0.05$) (**Table 1**).

Table 2 compares patients and clinical characteristics in the training cohort between the MACE and No MACE groups.

Prediction model development and validation

Six predictors, including diabetes, HF history, NYHA ≥ 3, NT-proBNP, Lp-PLA2, and Hcy, were selected by step-wise regression based on the Akaike information criterion (**Table 3**). The above independent predictors were incorporated into the nomogram (**Figure 1**). The nomogram for 1-year MACE prognosis prediction indicated an AUC of 0.847 [95% confidence interval (CI): 0.811–0.882], a sensitivity of 0.740, a specificity of 0.815, and an accuracy of 0.787 in the training cohort (**Figure 2A** and **Table 4**). Relatively, in the validation cohort, the nomogram showed an AUC of 0.839 (95% CI: 0.780–0.893), a sensitivity of 0.705, a specificity of 0.877, and an accuracy of 0.812 (**Figure 2B** and **Table 4**). **Figures 3A,B** depicts the nomogram calibration curve, demonstrating good agreement between prediction by nomogram and actual observation in the training and validation cohorts. The Hosmer-Lemeshow test produced a non-significant statistic ($\chi^2 = 6.522$, $P = 0.589$ in the training cohort and $\chi^2 = 5.648$, $P = 0.687$ in the validation cohort, respectively), indicating no perfect fit deviation.

Clinical usefulness of the nomogram

In the training and validation cohort DCAs, the nomogram offered a net benefit over the "happen-all" or "happen-none" strategies at a threshold of 8–88 and 8–92%, respectively (**Figures 3C,D**), demonstrating that our nomogram was clinically useful. For example, in a training cohort with a threshold probability of 40%, using the clinical nomogram could provide an additional net benefit of 0.2 over the "happen-all" or "happen-none" strategy.

Discussion

We included six independent factors associated with 1–year prognosis in CHD patients combined with AHF, including diabetes, HF history, NYHA ≥ 3, NT-proBNP, Lp-PLA2, and Hcy. The developed clinical nomogram model achieved good predictive performance in the training cohort (AUC = 0.847, 95% CI: 0.811–0.882) and validation cohort (AUC = 0.839, 95% CI: 0.780–0.893). The clinical predictive model has been clinically validated.

HF is one of the most common acute and severe diseases in internal medicine, as well as the final stage of the progression of various cardiovascular disorders. It has been reported that a significant number of patients with AHF are re-hospitalized after discharge due to recurrent symptoms or die within months of being discharged from the hospital (27). Our study showed that during 1-year post-discharge follow-up, 259 patients (37.4%) were followed up with MACE, including 23 (3.3%) patients with cardiac death and 236 (34.1%) patients with re-hospitalization cause by stroke, Non-fatal myocardial infarction, or HF. This result is consistent with the findings of the European Heart Organization and the American Heart Association (28–30). They concluded that AHF represented high-risk patients with higher mortality and likelihood of re-hospitalization during the same follow-up period than chronic stable HF. The treatment goal of HF is not only to improve symptoms and quality of life but also to prevent and delay the development of cardiac remodeling by targeting the mechanism of cardiac remodeling to reduce the mortality and hospitalization rate of patients with HF (31). Delayed diagnosis of AHF worsens prognosis by increasing the time to initiate initial treatment, and this delay may be associated with increased morbidity and mortality (32). That is why the individualized prediction of AHF is critical.

According to the 2018 Guidelines for the Diagnosis and Treatment of Heart Failure in China, the associated factors for poor prognosis of patients with HF are decreased left ventricular ejection fraction, continuously increased natriuretic peptide levels, deterioration of NYHA cardiac function grading, hyponatremia, decreased hematocrit value, chronic hypotension, resting tachycardia, and renal insufficiency (33). However, studies on identifying short-term (1-year) prognosis predictors of CHD patients combined with AHF are scarce. Inspired by previous studies, our nomogram model is based on the available clinical high performance in predicting the 1-year prognosis of CHD patients combined with AHF, including diabetes, HF history, NYHA ≥ 3, NT-proBNP, Lp-PLA2, and Hcy. Diabetes can increase the mortality rate in patients with HF by 50–100% (34, 35). Mebazaa et al. have shown that hyperglycemia has a poor short-term prognosis for AHF and can exacerbate its progression. Lassus et al. suggested that both all-cause mortality and cardiovascular mortality are lower in patients with new-onset AHF than in patients with acutely decompensated chronic heart failure (ADCHF) (36, 37). Studies have shown that patients with ADCHF have a longer course of CHD and more significant cardiac remodeling. ADCHF will further aggravate the damage to myocardial cells and extracellular matrix damage, resulting in aggravated cardiac remodeling

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TABLE 1 Baseline characteristics between patients in the training and validation cohorts.

Variables	Total (n = 692)	Training group (n = 484)	Validation group (n = 208)	P-value
Age(yrs)	67.13 ± 15.55	67.54 ± 15.96	66.17 ± 14.55	0.290
Sex (n,%)				0.897
Male	400(57.8%)	279(57.6%)	121(58.2%)	
Female	292(42.2%)	205(42.4%)	87(41.8%)	
Hypertension(n,%)				0.353
YES	447(64.6%)	318(65.7%)	129(62.0%)	
NO	245(35.4%)	166(34.3%)	79(38.0%)	
Diabetes (n,%)				0.818
YES	408(59.0%)	284(58.7%)	124(55.8%)	
NO	284(41.0%)	200(41.3%)	84(41.3%)	
Smoking (n,%)				0.703
YES	345(49.9%)	239(49.4%)	106(51.0%)	
NO	347(50.1%)	245(50.6%)	102(49.0%)	
HF (n,%)				0.878
First	313(45.2%)	218(45.0%)	95(43.7%)	
Former	379(54.8%)	266(55.0%)	113(56.3%)	
CKD (n,%)				0.140
YES	428(61.8%)	308(63.6%)	120(57.7%)	
NO	264(38.2%)	176(36.4%)	88(42.3%)	
AF (n,%)				0.341
YES	506(73.1%)	359(74.2%)	147(70.7%)	
NO	186(26.9%)	125(25.8%)	61(29.3%)	
NYHA (n,%)				0.476
≥3 Level	345(49.9%)	237(49.0%)	108(51.9%)	
<3 Level	347(50.1%)	247(51.0%)	100(48.1%)	
MACE (n,%)				0.979
YES	259(37.4%)	181(37.4%)	78(37.5%)	
NO	433(62.6%)	301(62.6%)	130(62.5%)	
Medication care				
Diuretic (n,%)				0.659
YES	520(75.1%)	365(75.6%)	154(74.0%)	
NO	172(24.9%)	118(24.4%)	54(26.0%)	
Beta-blocker (n,%)				0.899
YES	194(28.0%)	135(27.9%)	59(28.4%)	
NO	498(72.0%)	349(72.1%)	149(71.6%)	
Statin (n,%)				0.343
YES	212(30.6%)	143(29.5%)	69(33.2%)	
NO	480(69.4%)	341(70.5%)	139(66.8%)	
MRA (n,%)				0.650
YES	181(26.2%)	129(26.7%)	52(25.0%)	
NO	511(73.8%)	355(73.3%)	156(75.0%)	
ARNI (n,%)				0.133
YES	215(31.1%)	142(29.3%)	73(35.1%)	
NO	477(68.9%)	342(70.7%)	135(64.9%)	
CCB (n,%)				0.162
YES	233(33.7%)	155(32.0%)	78(37.5%)	
NO	459(66.3%)	329(68.0%)	130(62.5%)	
ACEI (n,%)				0.706

(Continued)

TABLE 1 (Continued)

Variables	Total (n = 692)	Training group (n = 484)	Validation group (n = 208)	P-value
YES	206(29.8%)	142(29.3%)	64(30.8%)	
NO	486(70.2%)	342(70.7%)	144(69.2%)	
ARB (n,%)				0.771
YES	168(24.3%)	116(24.0%)	52(25.0%)	
NO	524(75.7%)	368(76.0%)	156(75.0%)	
Clinical findings				
SBP (mm Hg)	143.41 ± 17.97	143.93 ± 17.85	142.20 ± 18.21	0.244
DBP (mm Hg)	82.98 ± 12.61	83.13 ± 12.81	82.62 ± 12.81	0.623
Lp-PLA2 (ng/L)	183.01 ± 33.67	184.19 ± 35.31	180.27 ± 29.37	0.132
Hcy (umol/L)	14.93 ± 6.11	14.66 ± 5.57	15.57 ± 7.20	0.106
CK-MB (ug/L)	45.74 ± 69.70	45.81 ± 73.94	45.56 ± 59.88	0.996
TnT (ug/L)	0.32 ± 1.00	0.32 ± 1.00	0.33 ± 1.01	0.776
NT-proBNP (ng/L)	1083.99 ± 1352.04	1096.50 ± 1364.31	1054.86 ± 1325.83	0.711
D-Dimer (ug/ml)	1.80 ± 2.44	1.66 ± 1.80	2.13 ± 3.49	0.063
Total cholesterol (mg/dl)	195.23 ± 42.52	194.46 ± 42.14	197.17 ± 44.46	0.430
LDL-C (mg/dl)	112.11 ± 32.86	111.34 ± 32.09	113.66 ± 34.79	0.339
HDL-C(mg/dl)	54.51 ± 14.69	54.51 ± 14.30	55.28 ± 15.46	0.696
Triglycerides(mg/dl)	138.21 ± 98.34	139.10 ± 102.78	139.10 ± 90.37	0.985
FDP(ug/L)	13.23 ± 13.48	12.94 ± 13.41	13.94 ± 13.66	0.370
Scr(umol/L)	220.29 ± 268.13	211.21 ± 253.42	241.40 ± 299.18	0.204
Uric Acid(umol/L)	467.01 ± 191.74	471.31 ± 194.77	457.01 ± 184.58	0.369
FPG(mmol/L)	8.56 ± 5.82	8.51 ± 6.09	8.67 ± 5.17	0.727
CRP(mg/L)	43.36 ± 53.10	43.85 ± 53.44	42.20 ± 52.65	0.709
WBC(103/μl)	9.27 ± 4.87	9.09 ± 4.47	9.71 ± 5.68	0.161
NEUT(103/μl)	3.68 ± 2.14	3.67 ± 2.04	3.72 ± 2.37	0.795
LYM(103/μl)	1.29 ± 1.31	1.26 ± 0.93	1.38 ± 1.93	0.307
NLR(%)	4.29 ± 3.96	4.36 ± 4.01	4.13 ± 4.87	0.479
HGB(g/L)	106.77 ± 31.12	106.41 ± 31.49	107.61 ± 30.28	0.642
PLT(103/μl)	204.74 ± 97.41	206.39 ± 89.96	200.90 ± 113.03	0.497

HF, heart failure history; CKD, chronic kidney disease history; AF, atrial fibrillation history; NYHA, New York heart association; MACE, major adverse cardiovascular events; MRA, mineralocorticoid receptor antagonists; ARNI, angiotensin receptor enkephalinase inhibitors; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; SBP, systolic blood pressure; DBP, diastolic blood pressure; Lp-PLA2, lipoprotein associated phospholipase A2; Hcy, homocysteine; CK-MB, creatine kinase isoenzyme-MB; TnT, troponin T; NT-proBNP, N terminal pro B type natriuretic peptide; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FDP, fibrinogen degradation product; Scr, serum creatinine; FPG, fasting plasma glucose; CRP, C-reactive protein; WBC, white blood count; NEUT, neutrophil count; LYM, lymphocyte count; NLR, neutrophil to lymphocyte ratio; HGB, hemoglobin; PLT, platelet count.

and deterioration of cardiac function (36, 38). NT-proBNP is a bioactive lysis product of brain natriuretic peptide (BNP), produced and released by ventricular myocytes under ventricular wall stress (39). Causes of BNP release during AHF include myocardial cell extension, tissue ischemia, and myocardial remodeling (40, 41). Many experiments confirmed that NT-probNP is a predictor of the prognosis of HF. And our studies showed that NT-probNP is an independent risk factor for short-term prognosis. Moreover, the higher the NT-proBNP index of AHF patients, the more severe the symptoms of HF, and the worse the short-term and long-term prognosis (42). Belkin et al. suggested that NYHA ≥ III is a risk factor for AHF (43). BNP levels and NT-proBNP concentrations of AHF patients increased gradually as their NYHA cardiac classification increased, and the differences between the III/IV and II groups were statistically significant. Sheng et al. have suggested that LP-PLA2 is an independent predictor of vascular endothelial injury, which is associated with the occurrence and prognosis of both ischemic and non-ischemic heart failure (44). High levels of Hcy are independent risk factors for HF induced by CHD (45). Studies showed that the 5- or 3-year mortality rate of HF patients with high levels of Hcy is significantly higher than that of patients with normal levels of Hcy, and Hcy levels may be an independent predictor of the long-term prognosis of patients with HF (46, 47). Our study suggests that LP-PLA2 and Hcy were independent factors for 1-year prognosis of AHF. However, the effect of Hcy and LP-PLA2 levels on the

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TABLE 2 Univariate analyses of variables associated with MACE in the training cohorts.

Variables	Training group (n = 484)	MACE (n = 181)	No MACE (n = 303)	P-value
Age(yrs)	67.54 ± 15.96	66.68 ± 15.67	68.05 ± 16.13	0.361
Sex(n,%)				0.799
Male	279(57.6%)	103(56.9%)	176(58.1%)	
Female	205(42.4%)	78(43.1%)	127(41.9%)	
Hypertension(n,%)				0.542
YES	318(65.7%)	122(67.4%)	196(64.7%)	
NO	166(34.3%)	59(32.6%)	107(35.3%)	
Diabetes(n,%)				<0.001
YES	284(58.7%)	130(71.8%)	154(50.8%)	
NO	200(41.3%)	51(28.2%)	149(49.2%)	
Smoking(n,%)				0.002
YES	239(49.4%)	106(58.6%)	133(43.9%)	
NO	245(50.6%)	75(41.4%)	170(56.1%)	
HF(n,%)				<0.001
First	218(45.0%)	102(56.4%)	116(38.3%)	
Former	266(55.0%)	79(43.6%)	187(61.7%)	
CKD(n,%)				0.723
YES	308(63.6%)	117(64.6%)	191(63.0%)	
NO	176(36.4%)	64(35.6%)	112(37.0%)	
AF(n,%)				0.788
YES	359(74.2%)	133(73.5%)	226(74.6%)	
NO	125(25.8%)	48(26.5%)	77(25.4%)	
NYHA(n,%)				<0.001
≥3 Level	237(49.0%)	117(64.6%)	120(39.6%)	
<3 Level	247(51.0%)	64(35.4%)	183(60.4%)	
Medication care				
Diuretic(n,%)				0.805
YES	365(75.6%)	138(76.2%)	228(75.2%)	
NO	118(24.4%)	43(23.8%)	75(24.8%)	
Beta-blocker(n,%)				0.919
YES	135(27.9%)	50(27.6%)	85(28.1%)	
NO	349(72.1%)	131(72.4%)	218(71.9%)	
Statin(n,%)				0.754
YES	143(29.5%)	55(30.4%)	88(29.0%)	
NO	341(70.5%)	126(69.6%)	215(71.0%)	
MRA(n,%)				0.312
YES	129(26.7%)	53(29.3%)	76(25.1%)	
NO	355(73.3%)	128(70.7%)	227(74.9%)	
ARNI(n,%)				0.522
YES	142(29.3%)	50(27.5%)	92(30.4%)	
NO	342(70.7%)	131(72.4%)	211(69.6%)	
CCB(n,%)				0.551
YES	155(32.0%)	55(30.4%)	100(33.0%)	
NO	329(68.0%)	126(69.6%)	203(67.0%)	
ACEI(n,%)				0.853
YES	142(29.3%)	54(29.8%)	88(29.0%)	
NO	342(70.7%)	127(70.2%)	215(71.0%)	

(Continued)

TABLE 2 (Continued)

Variables	Training group (*n* = 484)	MACE (*n* = 181)	No MACE (*n* = 303)	*P*-value
ARB(n,%)				0.309
YES	116(24.0%)	48(26.5%)	68(22.4%)	
NO	368(76.0%)	133(73.5%)	235(77.6%)	
Clinical findings				
SBP(mm Hg)	143.93 ± 17.85	144.37 ± 15.20	143.67 ± 19.29	0.660
DBP(mm Hg)	83.13 ± 12.81	84.04 ± 11.41	82.59 ± 13.57	0.208
Lp-PLA2(ng/L)	184.19 ± 35.31	206.34 ± 35.51	170.96 ± 27.79	<0.001
Hcy(umol/L)	14.66 ± 5.57	16.81 ± 5.59	13.38 ± 5.16	<0.001
CK-MB(ug/L)	45.81 ± 73.94	52.11 ± 105.95	42.06 ± 44.90	0.148
TnT(ug/L)	0.32 ± 1.00	0.27 ± 0.34	0.34 ± 1.23	0.477
NT-proBNP(ng/L)	1096.50 ± 1364.31	1481.60 ± 1613.94	866.46 ± 1132.45	<0.001
D-Dimer(ug/ml)	1.66 ± 1.80	1.89 ± 1.2.08	1.53 ± 1.59	0.063
Total cholesterol(mg/dl)	194.46 ± 42.14	193.30 ± 40.59	195.23 ± 42.91	0.633
LDL-C(mg/dl)	111.34 ± 32.09	110.95 ± 28.99	111.34 ± 34.02	0.844
HDL-C(mg/dl)	54.51 ± 14.30	52.96 ± 13.53	55.67 ± 15.08	0.034
Triglycerides(mg/dl)	139.10 ± 102.78	148.84 ± 118.72	132.90 ± 90.37	0.109
FDP(ug/L)	12.94 ± 13.41	11.60 ± 11.31	13.73 ± 14.91	0.065
Scr(umol/L)	211.21 ± 253.42	206.89 ± 229.67	213.79 ± 266.93	0.772
Uric Acid(umol/L)	471.31 ± 194.77	493.12 ± 172.47	458.28 ± 206.13	0.047
FPG(mmol/L)	8.51 ± 6.09	9.02 ± 8.56	8.20 ± 3.92	0.154
CRP(mg/L)	43.85 ± 53.44	38.00 ± 51.82	47.34 ± 54.02	0.060
WBC(103/μl)	9.09 ± 4.47	8.40 ± 4.11	9.50 ± 4.63	0.009
NEUT(103/μl)	3.67 ± 2.04	3.67 ± 2.09	3.67 ± 2.01	0.795
LYM(103/μl)	1.26 ± 0.93	1.29 ± 0.77	1.25 ± 1.01	0.983
NLR(%)	4.36 ± 4.01	4.08 ± 3.64	4.53 ± 4.20	0.588
HGB(g/L)	106.41 ± 31.49	109.50 ± 31.30	104.57 ± 31.52	0.095
PLT(103/μl)	206.39 ± 89.96	207.43 ± 83.69	205.77 ± 93.64	0.845

HF, heart failure history; CKD, chronic kidney disease history; AF, atrial fibrillation history; NYHA, New York heart association; MRA, mineralocorticoid receptor antagonists; ARNI, angiotensin receptor enkephalinase inhibitors; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; SBP, systolic blood pressure; DBP, diastolic blood pressure; Lp-PLA2, lipoprotein associated phospholipase A2; Hcy, homocysteine; CK-MB, creatine kinase isoenzyme-MB; TnT, troponin T; NT-proBNP, N terminal pro B type natriuretic peptide; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FDP, fibrinogen degradation product; Scr, serum creatinine; FPG, fasting plasma glucose; CRP, C-reactive protein; WBC, white blood count; NEUT, neutrophil count; LYM, Lymphocyte count; NLR, neutrophil to lymphocyte ratio; HGB, hemoglobin; PLT, platelet count; *P*-value means difference between MACE group and No MACE group.

TABLE 3 Multivariate analysis of independent risk factors associated with MACE.

	Multivariable analysis					
Variables	**B**	**S.E**	**Wals**	**OR**	**95% CI**	***P*-value**
Diabetes	0.627	0.25	6.32	1.873	1.148-3.055	0.012
Heart failure history	0.634	0.24	6.984	1.885	1.178-3.016	0.008
NYHA ≥ 3Level	0.990	0.239	17.107	2.692	1.684-4.303	<0.001
NT-proBNP	0.003	0.001	8.136	1.003	1.001-1.004	0.004
Lp-PLA2	0.031	0.004	60.765	1.031	1.023-1.039	<0.001
Hcy	0.088	0.024	13.246	1.092	1.042-1.145	<0.001

NT-proBNP, N terminal pro B type natriuretic peptide; Lp-PLA2, lipoprotein associated phospholipase A2; Hcy, homocysteine; OR, odds ratio; CI, confidence interval.

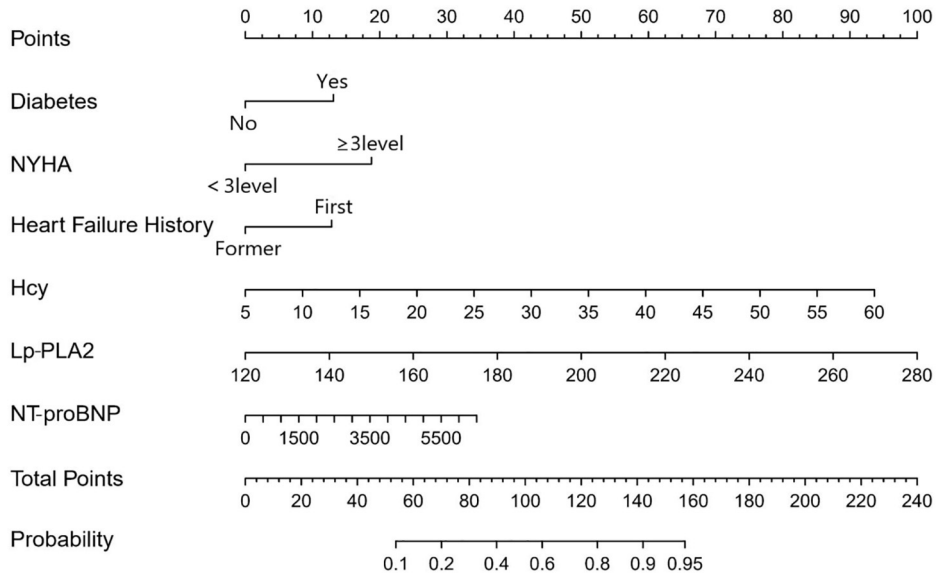

FIGURE 1
Nomogram for 1-year MACE prognosis in coronary heart disease (CHD) patients combined with AHF. The nomogram was developed in the primary cohort, with the Diabetes, HF history, NYHA ≥ 3, NT-proBNP, Lp-PLA2, and Hcy incorporated.

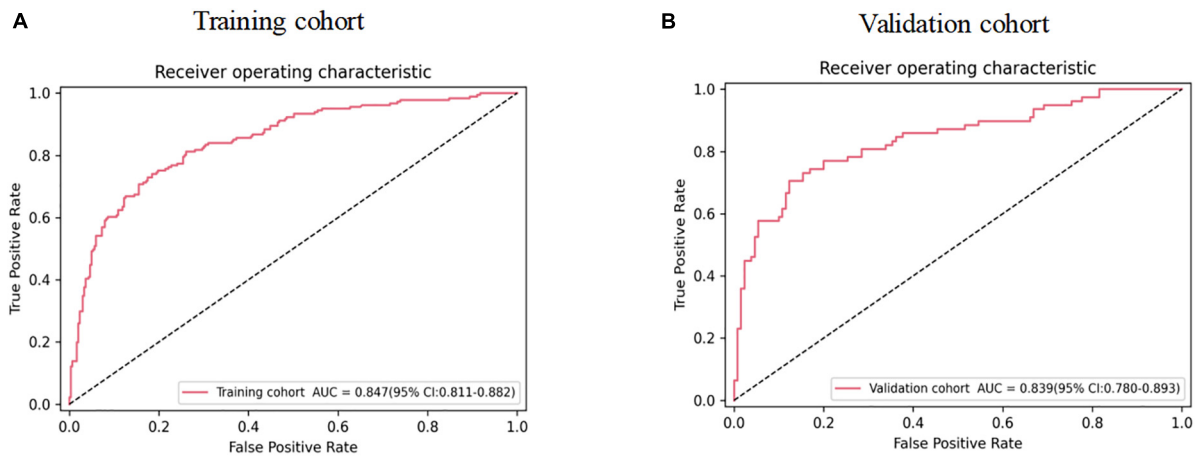

FIGURE 2
The ROC of nomogram in panels **(A,B)** training and validation cohorts. ROC, receiver operating characteristic curve.

TABLE 4 **Predictive value of nomogram in training group and validation group.**

Model	Accuracy	Sensitivity	Specificity	F1_score	AUC (95% CI)
MACE					
Training group (n = 484)	0.787	0.740	0.815	0.722	0.847 (0.811–0.882)
Validation group (n = 208)	0.812	0.705	0.877	0.738	0.839 (0.780–0.893)

AUC, area under the curve; CI, confidence interval.

short-term prognosis of HF has not been widely reported. Thus, larger sample size studies are required to confirm the findings of this study.

As an intuitive expression of the analysis results of a statistical model, a nomogram is more concise and effective in quantifying risks. Studies have confirmed that

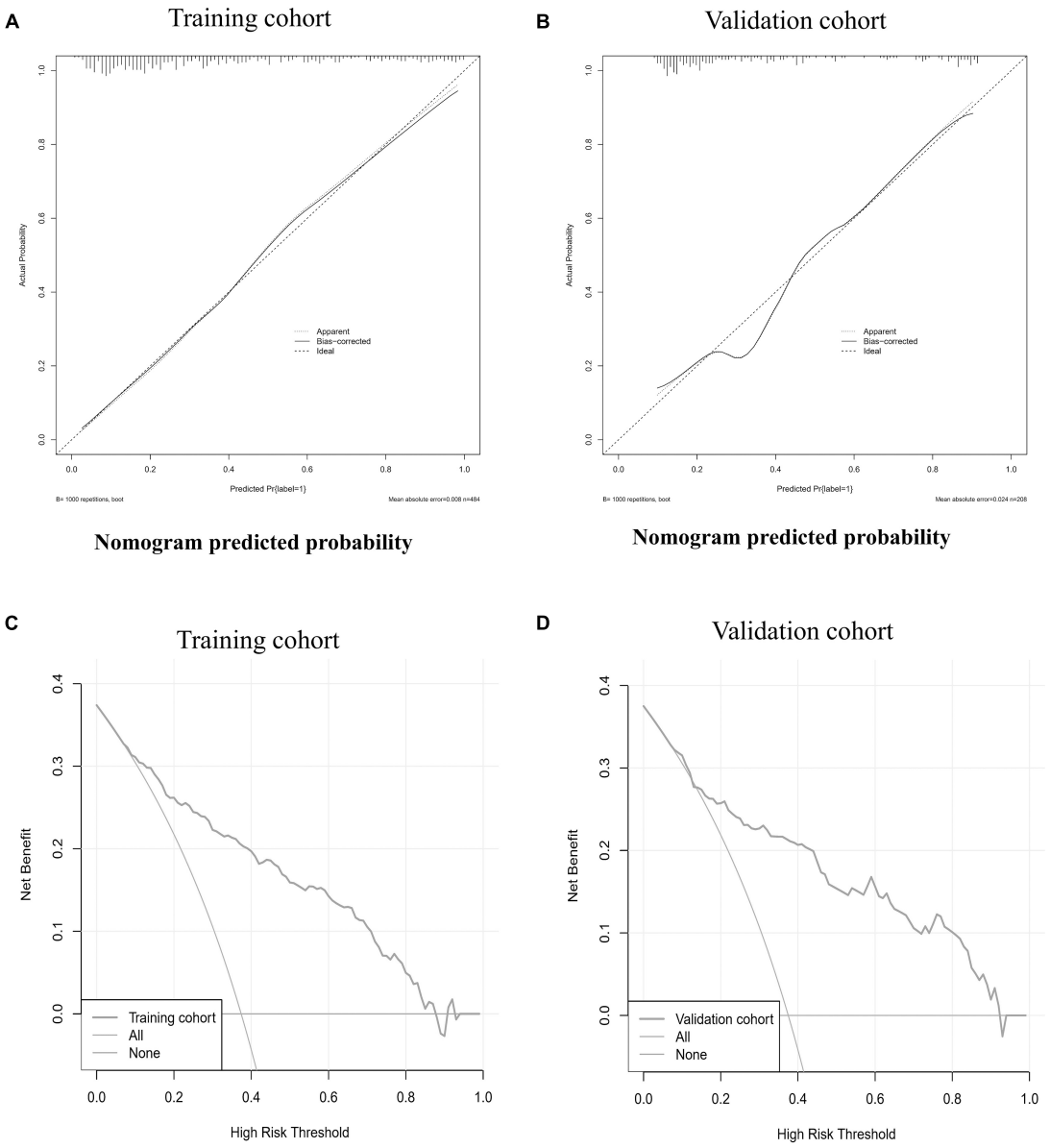

FIGURE 3
The calibration curve and decision curve of the nomogram in training and validation cohorts. The calibration curves of the nomogram in the training cohort **(A)** and validation cohort **(B)** are reported. The x-axis is the nomogram predicted probability and the y-axis is the actual probability. The prediction performance can be measured by the difference of the fitted curve and slope 1 line (diagonal 45-degree line). The diagonal dotted line represents a perfect prediction by an ideal model. The solid line represents the performance of the nomogram, of which a closer fit to the diagonal dotted line represents a better prediction. Decision curve analysis for the nomogram in the training cohort **(C)** and validation cohort **(D)** are showed. The y-axis measures the net benefit. The red line represents the nomogram. The blue line represents all patients had MACE. The orange line represents the assumption that no patients had MACE. The decision curve showed that if the threshold probability of a patient uses the nomogram offered a net benefit over the "happen-all" or "happen-none" strategy at a threshold range form 8–88 to 8–92%, respectively in the training cohort and validation cohort.

the nomogram has a good application effect in predicting the risk of acute kidney injury in patients with acute myocardial infarction after percutaneous coronary intervention (48) and in identifying the risk of HF in patients with CHD (49). However, no studies on the development of 1-year prognosis risk in CHD patients combined with AHF have been published. In our study, 6 independent risk factors affecting the occurrence of MACE in CHD patients

combined with AHF after discharge from the hospital were screened out by step-wise regression, and a personalized nomogram prediction model was constructed. The AUC values for the training and validation cohorts were 0.847 (95% CI: 0.811–0.882) and 0.839 (95% CI: 0.780–0.893), with sensitivity and specificity of 0.740 and 0.705 and 0.815 and 0.877, respectively, suggesting that the nomogram had good prediction ability. It is better than the prediction model reported by Kadoglou et al., which 1-year prediction model has an AUC value of 0.698 (25). The Hosmer-Lemeshow test confirmed that the deviation between the risk prediction value of the nomogram and the actual observed value was not statistically significant (χ^2 = 6.522, P = 0.589 in the training cohort and χ^2 = 5.648, P = 0.687 in the validation cohort, respectively). Moreover, the calibration curve shows the mean absolute error of the internal verification of the nomogram, which is 0.008 and 0.024, respectively, indicating that the nomogram has good calibration and pretest uniformity. DCA curve analysis showed that the nomogram had good clinical applicability. Meanwhile, the prediction indexes required for constructing the nomogram are all derived from the clinical data of patients during hospitalization, which is easy to obtain and does not require complex calculation transformation. In conclusion, the nomogram for predicting the risk of 1-year MACE in CHD patients combined with AHF after discharge from the hospital has a high predictive value and clinical application value, and targeted preventive measures can be formulated for patients to reduce the occurrence of MACE in CHD patients combined with AHF.

This study has several limitations. Firstly, this was a single-center and retrospective study with a small sample size. The training and validation cohorts' data in this study were based on the researcher's location. Therefore, the 1-year MACE clinical predictive model must be verified in additional regional databases. Secondly, we did not analyze the occurrence of MACE in patients in the two subgroups of newly developed AHF and ADCHF due to the smaller sample size and heterogeneity of baseline data, which would lead to decreased accuracy of results. Finally, the clinical relevance or applicability of the nomogram we constructed should be validated in a prospective cohort of patients.

Conclusion

In conclusion, this study identified 6 predictors of 1-year prognosis of MACE in CHD patients combined with AHF. A clinical predictive model is established based on the predictors to identify who will experience short-term MACE, to enhance more effective clinical intervention for CHD patients combined with AHF. DCA confirmed the clinical usefulness of the nomogram.

Data availability statement

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

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XH, SY, JL, BG, FO, and QH: conception and design. XH, QZ, XC, JP, JC, SL, and FO: acquisition of data. XH, SY, BG, FO, LD, YD, JC, and QH: analysis and interpretation of data. XH, SY, BG, and JL: drafting or revising the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Identification of energy metabolism-related biomarkers for risk prediction of heart failure patients using random forest algorithm

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Objective: Energy metabolism plays a crucial role in the improvement of heart dysfunction as well as the development of heart failure (HF). The current study is designed to identify energy metabolism-related diagnostic biomarkers for predicting the risk of HF due to myocardial infarction.

Methods: Transcriptome sequencing data of HF patients and non-heart failure (NF) people (GSE66360 and GSE59867) were obtained from gene expression omnibus (GEO) database. Energy metabolism-related differentially expressed genes (DEGs) were screened between HF and NF samples. The subtyping consistency analysis was performed to enable the samples to be grouped. The immune infiltration level among subtypes was assessed by single sample gene set enrichment analysis (ssGSEA). Random forest algorithm (RF) and support vector machine (SVM) were applied to identify diagnostic biomarkers, and the receiver operating characteristic curves (ROC) was plotted to validate the accuracy. Predictive nomogram was constructed and validated based on the result of the RF. Drug screening and gene-miRNA network were analyzed to predict the energy metabolism-related drugs and potential molecular mechanism.

Results: A total of 22 energy metabolism-related DEGs were identified between HF and NF patients. The clustering analysis showed that HF patients could be classified into two subtypes based on the energy metabolism-related genes, and functional analyses demonstrated that the identified DEGs among two clusters were mainly involved in immune response regulating signaling pathway and lipid and atherosclerosis. ssGSEA analysis revealed that there were significant differences in the infiltration levels of immune cells between two subtypes of HF patients. Random-forest and support vector machine algorithm eventually identified ten diagnostic markers (MEF2D, RXRA,

PPARA, FOXO1, PPARD, PPP3CB, MAPK14, CREB1, MEF2A, PRMT1) for risk prediction of HF patients, and the proposed nomogram resulted in good predictive performance (GSE66360, AUC = 0.91; GSE59867, AUC = 0.84) and the clinical usefulness in HF patients. More importantly, 10 drugs and 15 miRNA were predicted as drug target and hub miRNA that associated with energy metabolism-related genes, providing further information on clinical HF treatment.

Conclusion: This study identified ten energy metabolism-related diagnostic markers using random forest algorithm, which may help optimize risk stratification and clinical treatment in HF patients.

KEYWORDS

heart failure, energy metabolism, random forest, nomogram, biomarker

Introduction

Heart Failure (HF) is a complex ailment that characterized by multidimensional nature and primarily results from myocardial infarction, cardiomyopathy, abnormal cardiac load, and arrhythmias. The morbidity and mortality of HF have increased rapidly in recent years, particularly among the elderly (1), and a majority of hospitalized patients die within 5 years of admission (2), affecting over 64 million patients' quality of life (3, 4). The first step in improving the clinical management and survival rate of patients with HF is the rapid and accurate diagnosis of the disease. The current clinical diagnosis of HF relies on a spectrum of biochemical markers, including BNP and NT-proBNP (5, 6). However, several researches demonstrated that BNP lacks sensitivity and specificity as they could increase in various non-HF diseases such as pulmonary arterial hypertension and renal failure (7). Furthermore, BNP and echocardiography is operator-dependent, which limits its diagnostic precision to some degree (5, 8). The emergence of gene testing raised hope for early and diagnosis of HF, which helps better understand the mechanisms underlying the development of HF and identify the potential diagnostic markers. Several biomarkers have been identified to serve as diagnostic and prognostic markers for HF patients (9), whereas the ability of individual markers to differentiate between disease and healthy controls is usually not very powerful (10). Therefore, searching for novel multi-biomarker diagnostic profile was urgently needed to more accurate diagnosis and develop new therapeutic targets in HF patients.

Perturbations of cardiac energy metabolism is an important characteristic of heart failure in the early stages (11–13). During aerobic conditions, the healthy heart derives its contractile energy from fatty acids and glucose, whereas this balance is disrupted under cardiac stress condition, which can have a profound impact on the function of the heart (14). The current study has indicated that energy metabolism is promoted in early and compensated HF states and that a decrease in metabolic capacity may result in the progressive defects seen in more severe cases of HF (10). It is likely that targeted improvements in energy metabolic efficacy will improve the symptomatic status of HF patients. For example, some energy metabolism-related genes such as UCP2 and PRMT1 were found to modulate the energy metabolism in cardiomyocytes after HF, which involved in remodeling of the ventricular wall and the maintenance of cardiac function (15, 16). More importantly, some energy metabolism-related genes like the myocyte enhancer factor 2 (MEF2) family, including MEF2A, MEF2D, has been considered as core transcription factors in cardiac development and reprogramming (17), which may serve as a potential candidate gene for the cardiac abnormalities (18). These studies suggest that further understanding of the value

Abbreviations: HF, Heart failure; DEGs, differentially expressed genes; RF, random forest algorithm; SVM, support vector machine; ROC, Receiver operating characteristic; NF, non-heart failure people; GEO, Gene Expression Omnibus; CM, consensus matrix; PCA, Principal component analysis; ssGSEA, Single-sample gene set enrichment analysis; CDF, cumulative distribution function; MEF2A, Myocyte enhancer factor 2A; MEF2D, myocyte enhancer factor 2D; PPARA, Peroxisome proliferator-activated receptor alpha; PPARD, peroxisome proliferator-activated receptor delta; FOXO1, forkhead box O1; MAPK14, mitogen-activated protein kinase 14; CREB1, cAMP responsive element binding protein 1; PRMT1, protein arginine methyltransferase 1; CaMKII, cardiomyocytes causes multifunctional Ca2+/calmodulin-dependent kinase II; RXRA, Retinoid X receptor alpha; PPP3CB, Protein phosphatase 3 catalytic subunit beta; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CTD, The Comparative Toxicogenomics Database; TTD, The Therapeutic Target Database; UCP2, uncoupling protein 2; PRMT1, protein arginine methyltransferase 1.

of the energy metabolism in HF patients will be crucial to clarifying the process of HF and developing new therapeutic options (19).

Given the huge burden of HF and the important role of energy metabolism, we obtained energy metabolism-relate DEGs between HF and non-heart failure (NF) samples in the GEO database. By the help of these DEGs data, we used random forest algorithm (RF) and support vector machine (SVM) to identify the diagnostic biomarkers in HF and constructed and validated an energy metabolism-related genetic diagnostic nomogram to predict the risk of HF. Moreover, we predicted key genes related drugs and miRNA. In a word, this study could provide theoretical support for early warning signs of HF and assist in improving risk stratification and guiding clinical decision-making.

Materials and methods

Data collection

The two profiling datasets, GSE66360 ($n = 99$) (20) and GSE59867 ($n = 436$) (21) were obtained from gene expression omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/). The training group (GSE66360) includes HF patients induced by acute myocardial infarction ($n = 49$) and non-HF cohort ($n = 50$), and the test group (GSE59867) have HF ($n = 34$) and non-HF ($n = 30$). Normalization of the microarray data was performed using the normalize quantiles function of the preprocessCore package in R software (version 3.4.1). The probes were transformed into gene symbols based on the annotation information provided in the platform. These datasets were stripped of probes corresponding to multiple genes, and then we calculated the average expression value of each gene measured by multiple probes as the final expression value. The energy metabolism-related genes were obtained through the Molecular Signatures Database (http://www.gsea-msigdb.org/gsea/msigdb/) that is one of the most widely used and comprehensive databases of gene sets for performing gene set enrichment analysis. In total, 48 genes related to energy metabolism were collected from Wiki Pathways.

Differential expression analysis of energy metabolism-related genes

The gene expression profiling was annotated through the corresponding annotation packages of the R software. The "limma" R software package was applied to analyze the differential expressed energy metabolism-related genes. Those genes which met the criteria ($P < 0.05$ and $|\log2(\text{fold change})| > 1$) were considered as DEGs. The heatmap and boxplot were constructed to show the differential expressed genes which were highlighted. Finally, the chromosomal locations of the DEGs were demonstrated using Circos and the protein-protein interaction (PPI) networks of the DEGs were predicted by the search tool for the retrieval of interacting genes (STRING, version 11.5; https://cn.string-db.org) with minimum required interaction score ≥ 0.7 (22).

Consensus clustering for heart failure samples

According to energy metabolism-related genes, HF samples were grouped into different classifications using "Consensus Cluster Plus" package in R software (23). Based on the consensus matrix (CM) and cumulative distribution function (CDF) curves of the consensus score, we determined the optimal cluster number (24). Meanwhile, we performed principal component analysis (PCA) between clusters. The "limma" package was then applied to screen for the energy metabolism-related genes between clusters ($|\log_2(\text{fold change})| > 2$ and $P < 0.05$).

Functional and pathway enrichment analysis

To assess the functional enrichment of DEGs identified between clusters, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analyses using "ClusterProfiler" R package (25). GO database describes our understanding of biology from three GO domains, including biological process (BP), molecular function (MF), and cellular component (CC). The KEGG database provides information about high-level functions in the biological system.

Immune landscape analysis

ssGSEA is an extension method of the Gene set enrichment analysis analysis (GSEA), using for quantifying infiltrating immune cells. This tool allows the definition of an enrichment score that represents the absolute enrichment level of the gene sets in each sample within a given dataset. To investigate the association between immune infiltration level and two subtypes, ssGSEA was performed by the R package "GSVA" to investigate the differences in immune cell infiltration in two clusters (26). Person method was used to calculate the correlation between immune cells and genes related to energy metabolism.

Identification of energy metabolism-related diagnostic biomarkers

To screen significant diagnostic biomarkers in HF, two machine learning algorithms including RF and SVM were performed on the identified energy metabolism-related DEGs. These two models were analyzed using the explanatory feature of the R package "DALEX." Optimal models were chosen by plotting residual distributions. The receiver operating characteristic (ROC) curve was used to assess the diagnostic performance of two models (6). The area under the curve (AUC) was used to measure the overall predictive validity of the risk in HF where AUC = 0.50 signals random prediction, $0.60 < \text{AUC} \leq 0.70$ signals poor, $0.70 < \text{AUC} \leq 0.80$ signals fair, $0.80 < \text{AUC} \leq 0.90$ signals good and AUC > 0.90 signals excellent validity. Based on out-of-band data, we calculated the average modeling error rate for all genes using the R package "random forest." A random forest model was then constructed, and the Gini coefficient method was used to calculate dimensional importance value (7).

Construction and validation of the nomogram

Based on the identified energy metabolism-related diagnostic biomarkers by random forest algorithm, we further established a nomogram to predict the occurrence of HF using R package "rms." The "Points" column indicates the score for each gene below, and the "Total Points" column represents the sum of all the scores. Specifically, we obtained the "Points" for each gene by drawing a line straight upward from each gene to the point scale in the nomogram. The "Points" were then added together and positioned on the scale of "Total points" to further convert them into risk probability of HF. The calibration curve was used to assess the nomogram's predictive accuracy, while the ROC curve was used to examine the predictive power of the model.

Prediction of biomarker-related miRNA and drugs

"Enrichr" database is a comprehensive online tool for gene enrichment analysis, including a large number of genomic annotation libraries that can be used for analysis and download, such as transcription, pathways, ontology (GO), diseases/drugs, cell types, which may accumulate biological knowledge for further biological discoveries. In this manuscript, we predicted the regulatory correlation between miRNA with 10 diagnostic biomarkers in "Enrichr" database (https://maayanlab.cloud/Enrichr/) and visualized it using Cytoscape 3.9.1 software. Meanwhile, the related drugs were also evaluated in Enrichr.

Statistical analysis

Statistical analysis was performed by R (version 4.1.1) software. Perl and "limma" package were used to analyze the data. Using "Consensus Cluster Plus" package to classify the samples. *T*-test or Wilcoxon rank-sum test were applied to analyze the continuous variables according to the normality. Pearson chi-square test was applied to examine the differences of categorical variables. All significant thresholds were set at a two-sided $P < 0.05$.

Results

Identification of differentially expressed energy metabolism-related genes

A total of 22 energy metabolism-related DEGs, including PRKAG2, PPARD, MEF2D, ESRRA, RXRA, PPARA, TFB1M, PPARGC1B, PPP3CB, PRMT1, MED1, MAPK14, FOXO3, NCOA1, FOXO1, MEF2A, PPP3R1, HDAC1, PRKAB2, CREB1, CAMK2G, UCP2, were identified as different expression genes (Figure 1A). Among them, 11 genes were significantly downregulated and 11 genes were significantly upregulated (Figure 1B). A gene's chromosomal location can provide information about its evolutionary history, including gene duplication patterns, and gene duplication events (27). Herein, chromosomal location information of these energy metabolism-related genes was performed by the genome visualization tool named CIRCOS, providing insights into the evolution of these gene family. We can find that a total of 44 energy metabolism-related genes were distributed throughout the 18 chromosomes and the highest numbers of these genes ($n = 6$) were located on chromosome 2 (Figure 1C). PPI analysis was conducted to explore the interactions of these energy metabolism-related DEGs at the protein level. As shown in Figure 1D, the PPI network showed that MEF2A, MEF2D, CREB1, HDAC1, MED1 and ESRRA may have higher numbers of interacted proteins.

Molecular subtype of heart failure based on energy metabolism-related genes

Consensus clustering is a method that provides quantitative analysis results to determine possible subtypes based on gene expression profiling data, which can be used to discover new molecular subtypes and thus redefine disease classification. In this study, consensus clustering was performed to identify

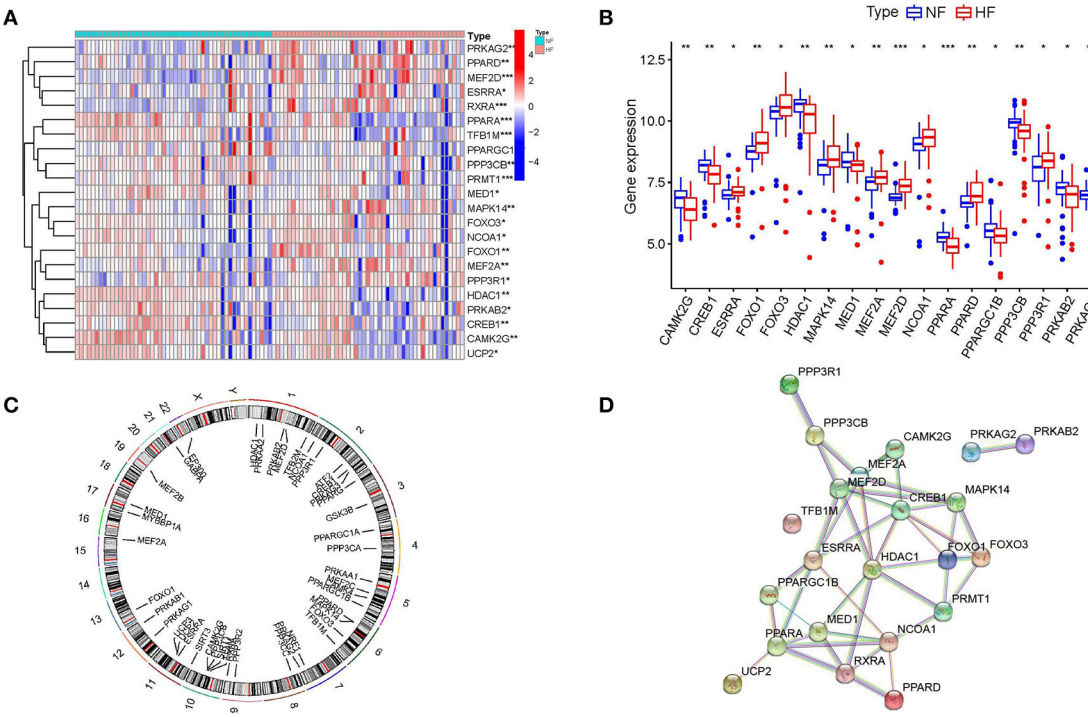

FIGURE 1
Identification of differentially expressed energy metabolism-related genes. **(A,B)** Expressions of differentially expressed energy metabolism-related genes. **(C)** Circos plot showing the location of genes in 22 chromosomes. **(D)** Protein-protein interaction networks.

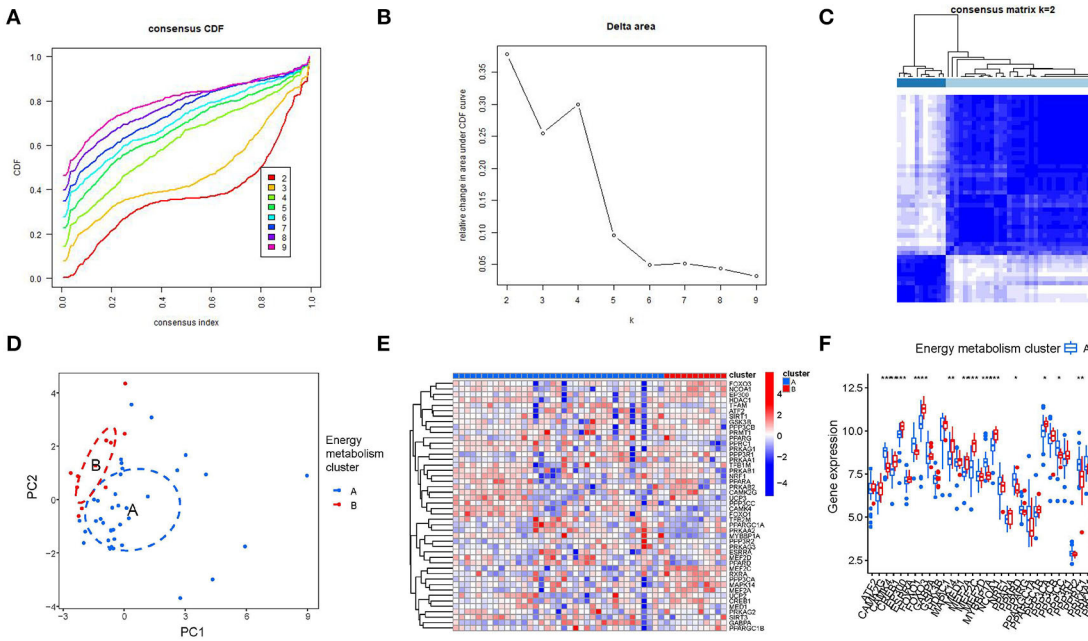

FIGURE 2
Consensus clustering analysis of energy metabolism-related genes. **(A)** The cumulative distribution function (CDF) curve of samples in the HF cohort. **(B)** The relative change in area under the CDF curve for k = 2–9. **(C)** Sample clustering heatmap when consumption k = 2. **(D)** PCA analysis for cluster A and cluster B. **(E,F)** The different expression of 48 genes between two clusters.

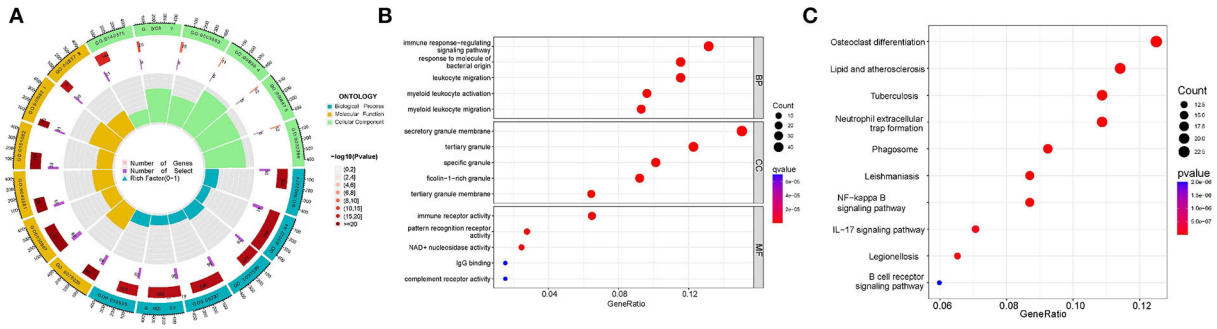

FIGURE 3
Functional analyses of DEGs identified between clusters. **(A,B)** Gene Ontology (GO) functional analysis. **(C)** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

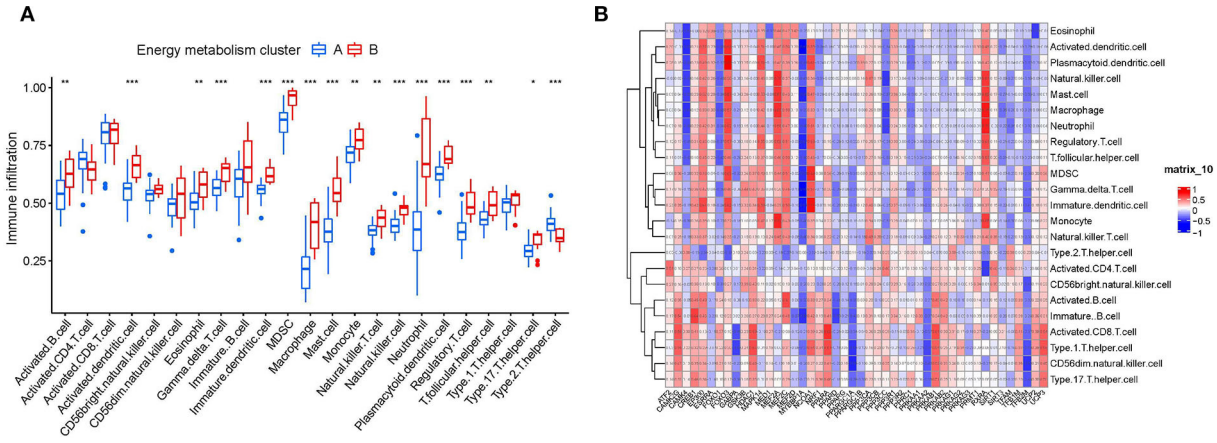

FIGURE 4
Correlation analysis between energy metabolism-related genes and immune microenvironment. **(A)** The relationship between two clusters and the level of immune cell infiltration. *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001; ****p-value < 0.0001. **(B)** The relationship between 46 energy metabolism-related genes and immune cell infiltration.

HF subtypes based on energy metabolism-related genes. According to the CDF curve and the CDF Delta area curve, clustering results are relatively stable when the number of clusters was set to 2 (Figures 2A,B). Figure 2C presented a heatmap of clustering results (k = 2) and the PCA result showed a clear distribution between cluster A and cluster B (Figure 2D), suggesting that energy metabolism-related genes have potential diagnostic value for HF patients. Figure 2E presented a heatmap of 48 genes expression level between two clusters. The result of the boxplot showed that FOXO1, RXRA, CREB1, MAPK14, MEF2A,PPARD, FOXO3, EP300, CAMK3, MEF2C, MYBBP1A, NCOA1, PPP3CA, PPP3CC, PPRC1, TFAM, TFB2M were significant differential expressed between cluster A and cluster B (P < 0.05, Figure 2F).

Functional analyses of different clusters of HF patients

To examining the differences in gene functions and pathways between the subgroups grouped by energy metabolism-related genes, we extracted DEGs using the "limma" R package with a threshold of FDR < 0.05 and $|\log_2 FC| \geq 2$. A total of 378 DEGs were identified between cluster A and cluster B, and then GO and KEGG enrichment analysis were conducted on these DEGs. The result demonstrated that the DEGs were mainly correlated with immune response regulating signaling pathway (BP), secretory granule membrane (CC), immune receptor activity (MF) (Figures 3A,B, Supplementary Figure 1), osteoclast differentiation, and lipid and atherosclerosis (Figure 3C).

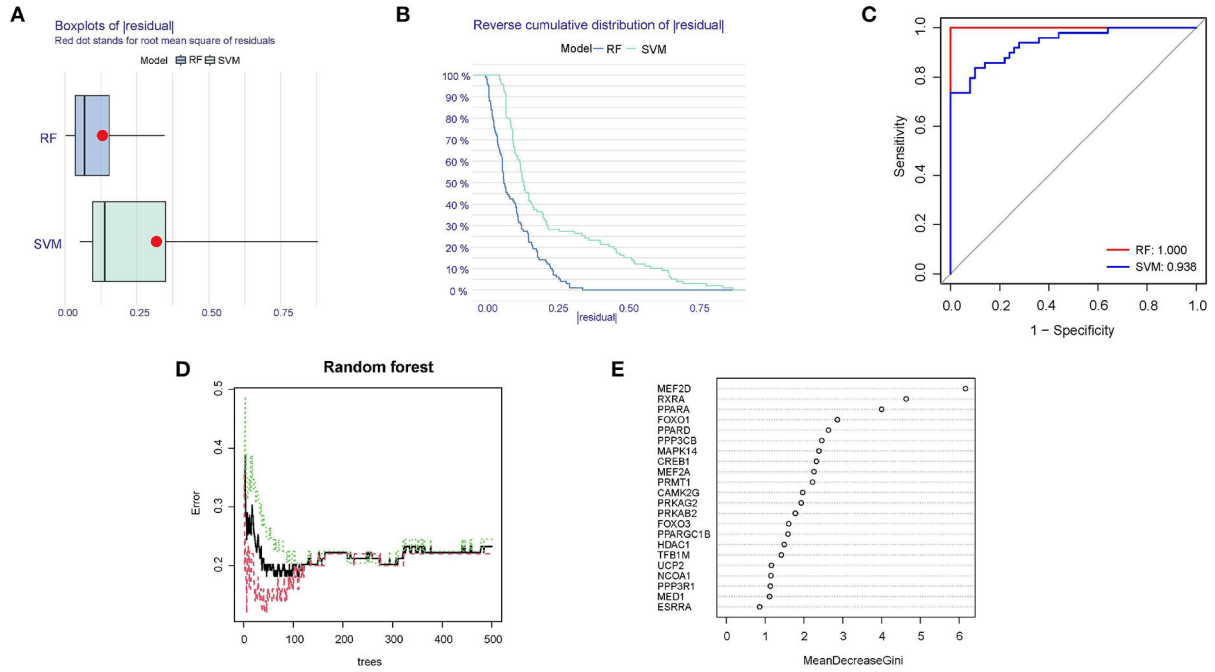

FIGURE 5
Identification of diagnostic biomarkers using RF and SVM model. **(A)** Boxplots of the residuals of the sample. Red dot stands for root mean square of residuals. **(B)** Cumulative residual distribution map of the sample. **(C)** AUC verification results of the two models on the training dataset. **(D)** The influence of the number of decision trees on the error rate. **(E)** Results of the Gini coefficient method in the random forest classifier.

Assessment of the immune infiltration level between clusters

Based on the results of functional analyses, we further explore the correlation of immune cell infiltration level between two clusters. First, we confirmed the role of immune cell infiltration in HF development. As the Supplementary Figure 1 shown, nearly half of immune cell levels (12/23) were found more abundant in HF samples vs. the healthy control ($P < 0.001$), suggesting that patients with HF already show signs of systemic-immune activation, and may contribute to the progression to HF. More importantly, we found that most of immune cells (17/23) have also different infiltration levels in two clusters (Figure 4A). Compared to the cluster B, cluster A generally had lower levels of immune cell infiltration, especially of activated dendritic cell, gamma delta T cell, immature dendritic cell, MDSC, macrophage, mast cell, natural killer cell, neutrophil, plasmacytoid dendritic cell, regulatory T cell, and Type 2 T helper cell ($P < 0.001$). Furthermore, we assessed the correlation between 48 energy metabolism-related genes and 24 immune cells (Figure 4B). There was a strong correlation between the expressions of most of the energy metabolism-related genes and the infiltration of immune cells, especially MAPK14, FOXO1, and RXRA.

Establishment and evaluation of RF and SVM model

Compared RF and SVM, according to the training dataset (GSE66360), we found that RF had the less sample residual (Figures 5A,B). Similarly, the AUC of the random forests model (AUC = 1.000) and SVM model (0.938) showed that RF model had a higher degree of differentiation (Figure 5C). As shown in Figure 5D, 400 decision trees were selected as the final model parameter based on the relationship plot between the model error and the number of trees. Figure 5E presented the variable importance of the output results in the process of the construction of random forest model based on the Gini coefficient method. For further analysis, 10 genes with a significance > 2 were identified as candidate genes. Among ten variables, MEF2D, RXRA, and PPARA were the most important, followed by FOXO1, PPARD, PPP3CB, MAPK14, CREB1, MEF2A, PRMT1.

Establishment of the clinical nomogram

Based on the ten diagnostic biomarkers from the RF model, we developed a clinical predictive nomogram (Figure 6A) and people can use the nomogram score to predict the risk of HF.

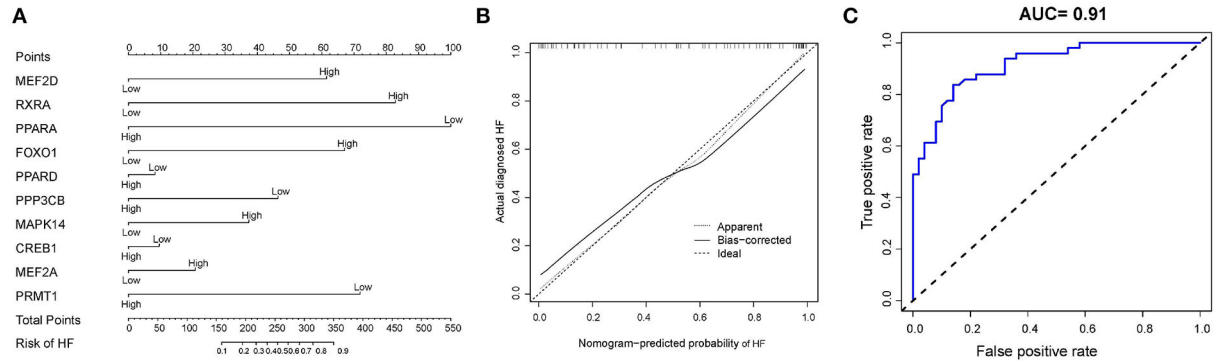

FIGURE 6
Construction of a nomogram model for HF diagnosis in training cohort (GSE66360). **(A)** The nomogram was used to predict the occurrence of HF. **(B)** Calibration curve to assess the predictive power of the nomogram model. **(C)** The receiver operating characteristic (ROC) analysis of nomogram.

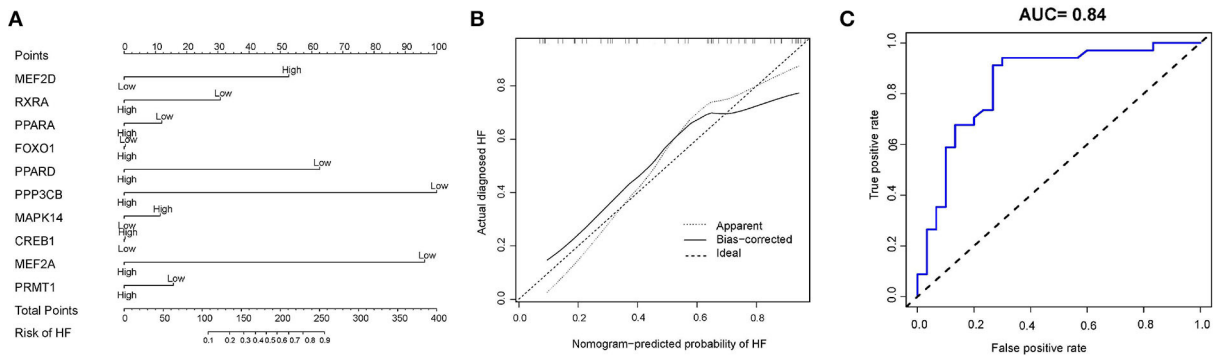

FIGURE 7
Construction of a nomogram model for HF diagnosis in test cohort (GSE59867). **(A)** The nomogram was used to predict the occurrence of HF. **(B)** Calibration curve to assess the predictive power of the nomogram model. **(C)** The receiver operating characteristic (ROC) analysis of nomogram.

Using calibration curve, we evaluated the predictive accuracy of the nomogram and the result indicated the nomogram has high accuracy for risk prediction of HF (Figure 6B). The corresponding ROC analysis revealed that the AUC value of the constructed diagnostic model was 0.91, which proved the predictive performance of this clinical nomogram (Figure 6C). We next verified the stable of the nomogram using the test cohort (GSE59867) (Figure 7A). The calibration curve of this nomogram was close to diagonal line (Figure 7B) and the AUC value was 0.84 (Figure 7C), further validating the accuracy and robustness of our nomogram.

Prediction of related drugs and miRNA

Ten drugs were identified the energy metabolism-related drugs, which may be the potential therapies for heart failure target (Table 1). RXRA, PPARA and PPARD were the top three genes most relevant to these drugs, indicating that these biomarkers have great potential to serve as a drug target for HF treatment. Furthermore, we constructed a miRNA–gene association network and displayed 15 potential miRNA targets of 10 energy metabolism-related biomarkers, which may play a regulatory role in the development of HF (Figure 8). Among them, miR-3177-5p and miR-1284 contributed to the regulation of the highest number of target genes ($n = 6$), followed by miR-4532, miR-4640-3p, miR-4445, miR-515-3p, miR-519e, and miR-3659 ($n = 5$).

Discussion

Heart failure is a deadly chronic disease that owns a high symptom burden and a poor health status. Patients with HF often fail to benefit from treatment as a result of a lack of an early diagnosis, resulting in poor prognosis. It has been demonstrated in numerous studies that HF is caused by severe

TABLE 1 The prediction of energy metabolism-related drugs.

Index	Name	*P*-value	Adjusted *P*-value	Odds ratio	Combined score	Gene
1	phthalic acid CTD 00001559	5.02E-08	2.15E-05	658.58	11068.58	RXRA;PPARA;PPARD
2	DIETHYL PHTHALATE CTD 00000348	1.02E-07	2.91E-05	503.52	8105.11	RXRA;PPARA;PPARD
3	Pirinixic acid TTD 00010254	1.23E-05	3.30E-04	555.03	6273.06	RXRA;PPARA
4	Difenoconazole CTD 00003609	1.48E-05	3.84E-04	499.5	5554.53	RXRA;PPARD
5	15(R)-Prostaglandin D2 CTD 00007048	1.75E-05	4.16E-04	454.07	4973.59	PPARA;PPARD
6	Triphenyltin hydroxide CTD 00000355	1.75E-05	4.16E-04	454.07	4973.59	RXRA;PPARD
7	4602-84-0 CTD 00005951	2.04E-05	4.57E-04	416.21	4494.85	RXRA;PPARA
8	gemfibrozil CTD 00007055	4.87E-07	6.91E-05	285.14	4144.43	RXRA;PPARA;PPARD
9	7614-21-3 CTD 00000893	2.35E-05	4.57E-04	384.17	4094.01	RXRA;PPARA
10	gemfibrozil TTD 00008191	2.35E-05	4.57E-04	384.17	4094.01	RXRA;PPARA

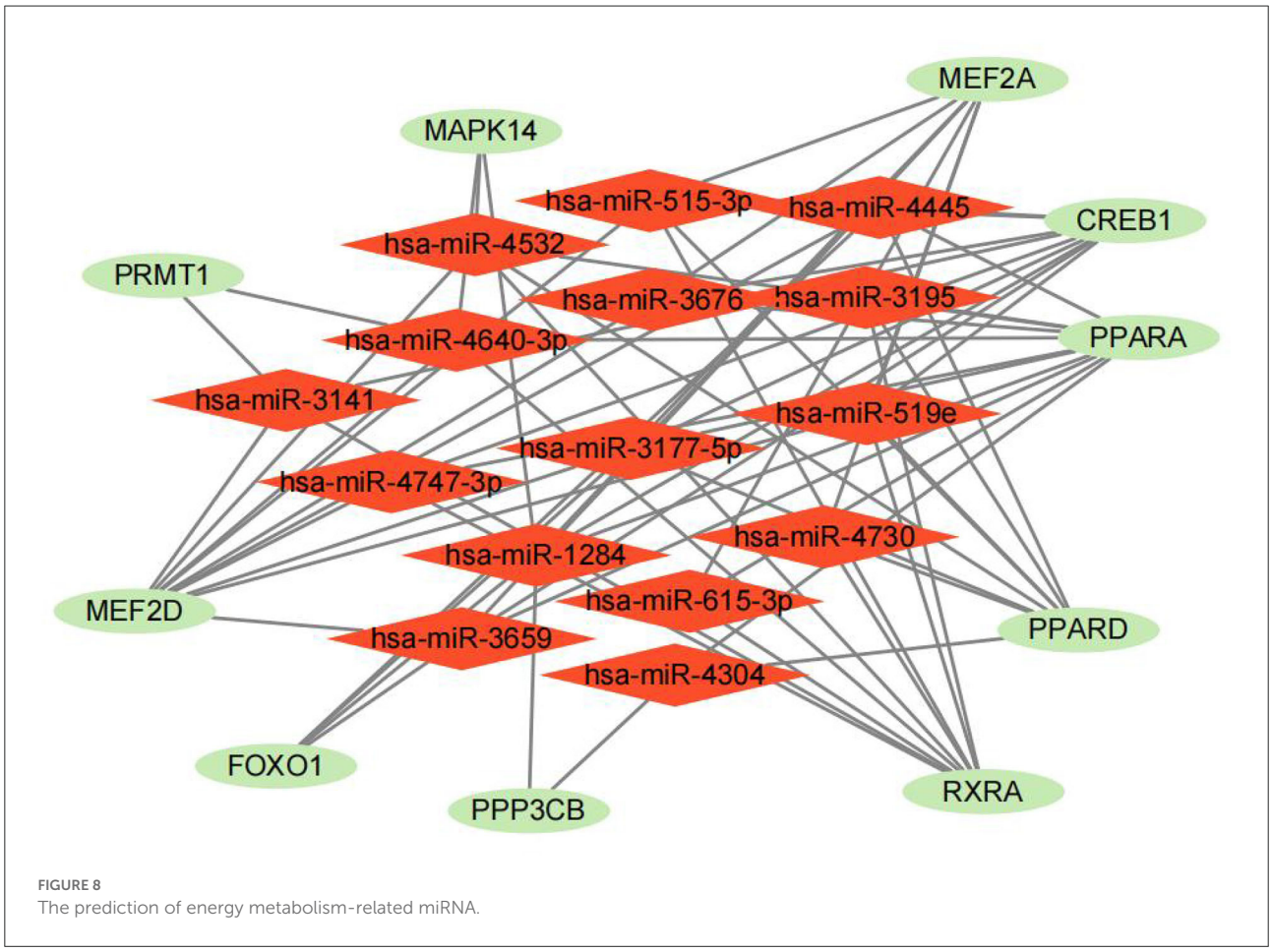

FIGURE 8
The prediction of energy metabolism-related miRNA.

energy metabolism disorders, resulting in insufficient energy supply to the heart (14). As a consequence, researchers are looking for novel diagnostic biomarkers and investigating the molecular level of energy metabolism in HF, which could lead to a number of positive effects on the clinical outcome of HF. Growing evidence demonstrated that microRNAs and mRNAs may be promising biomarkers of cardiovascular disease and HF in particular (9). However, few studies have examined the aberrantly expressed genes associated with energy metabolism in HF vs. normal tissues. Thus, this study sought to identify

candidate biomarkers for detecting HF and explore how energy metabolism may contribute to it.

As far as our knowledge goes, this is a novel study that analyzed GEO datasets to identify diagnostic biomarkers associated with energy metabolism in patients with HF. A total of 22 energy metabolism-related DEGs were identified between HF and normal tissues, including 11 upregulated genes and 11 downregulated genes. The result of consensus clustering analysis indicated that HF patients could be classified into two clusters based on 48 energy metabolism-related genes. Generally speaking, proposing new subtypes through the clustering could contribute to provide more precise treatment options (28) and thus our finding is the first time to prove potential diagnostic and therapeutic utility of energy metabolism gene set in HF. GO and KEGG enrichment analyses indicated that DEGs between the two clusters are mainly associated with the immune response regulating signaling pathway. Based on the result of these functional analyses, we further explored the association of immune infiltration between two clusters and found that HF patients have different immunity status across different cluster. These results generally agree with the previous finding that immune activation plays an essential role in the progression of HF (29). In fact, various forms of HF may be affected by the immune system, according to more recent evidence (30, 31). For example, regulatory T cell may be crucial in suppressing cellular immune responses, controlling both inflammation and infection during the development of HF (32). Some studies also indicated that inflammation-related effector cytokines, such as IL-17 family members and IL-22 are associated with Th17 cells (33), and these effector cytokines were demonstrated to regulate the MMP/TIMP system to influence myocardial fibrosis (34, 35). Excessive numbers of monocytes, macrophages, dendritic cells, and lymphocytes have been found to increase myocyte apoptosis, hypertrophy, and interstitial fibrosis during chronic heart failure (36). These findings are consistent with our own, demonstrating the validity of the results in the present study as well as the crucial role played by the immune response in HF (37). Thus, it is crucial to precisely control various types of immune cells to ensure a safe and effective treatment for HF patients. Furthermore, more research is required to better understand the role of immune cells in the heart in homeostasis and energy metabolism, aiming to identify the therapeutic methods targeting immune in patients with diverse kinds of HF.

Lacking sensitivity and specificity is the main limitation for the early diagnosis in HF (38–40), whereas novel multi-gene diagnostic biomarker may resolve this dilemma. In our study, 10 energy metabolism-related diagnostic markers were identified using random forest algorithm, which allows diagnosis of HF with high stability and accuracy. Among them, MEF2A and MEF2D, are both essential regulator of cardiac morphogenesis and myogenesis, which can bind specifically to the MEF2 element in the regulatory region of many muscle-specific genes (41). PPARA and PPARD, which mainly regulate fatty acids and lipid metabolism, function as transcription activator for cardiac fatty acid oxidation (42). Similarly, FOXO1 (31–34) and MAPK14 (35, 43) are highly expressed during the progress of cardiac hypertrophy which contributes to HF development. Our immune cell association analysis also noted that MAPK14, FOXO1 have a strong association with immune cells, indicated that these two genes may play immune-inhibiting roles in HF. In the contrast, activated CREB1 may reduce the excessive burden on the heart and heart hypertrophy (44, 45). Loss of PRMT1 in cardiomyocytes causes multifunctional CaMKII dysregulation, resulting in dilated cardiomyopathy and heart failure (15, 46). RXRA, receptor for retinoic acid, is demonstrated to be involved in the adipogenic/lipogenic regulation (47, 48), which has significant correlation with cardiovascular aging process, which contributes to the development of HF phenotype and outcome (49). PPP3CB, belonging to α-catalytic subunit gene family members (50), has been reported to be significantly up-regulated in the atrial myocyte hypertrophy of mitral regurgitation patients (51). Based on above 10 biomarkers, we constructed and validated a novel diagnose nomogram to risk prediction of HF patients. Compared with other predicted nomogram (AUC = 0.655∼0.720) (52), the AUC value of our nomogram, which was 0.91 in the training cohort (GSE66360) and 0.84 in the test cohort (GSE59867), indicated that it may have exceptional potential for making an early diagnosis of HF from patient blood samples.

In addition to the construction of clinical nomogram, anticipating gene-miRNA and gene-drug interactions is also an important task that helps understand the potential miRNA targets of energy metabolism-related genes and better guide clinical medication in HF. In this study, we predicted 10 related drugs using Enrichr based on 10 energy metabolism-related biomarkers. Most of these drugs are still in its infancy and remains exploratory. Among them, pirinixic acid, a potent PPARA receptor activator, exhibits anti-inflammatory properties in human neutrophils and may be useful as therapeutic agents (53). In fact, upregulating PPARA has been reported to promote mitochondrial energy metabolism and prevents HF (50) and the activated PPARA could increase high-density lipoprotein and reduce plasma lipids (54). Another predictive drug, diethyl phthalate was found to induce the antioxidant and immune responses in zebrafish embryos under DBP/DEP exposure (48). In a Helsinki Heart Study of primary prevention, gemfibrozil treatment has been found to reduce coronary events by 34% (55), which can also be considered for HF management. However, most predicted drugs lacked clinical outcomes and these components still need further research for clinical targeted therapy. As for miRNA, it is not only a diagnostic biomarker, but also a

therapeutic target for HF. In our prediction, we constructed a miRNA-gene network and these targets and miRNA may be served as potential biomarkers in HF. For example, regulating miR-615-3p/HMGB3 axis have been reported to promote glycolysis under hypoxic conditions at least partly. Notably miR-519e had similar mechanism (56), which helps us better understanding of the molecular mechanism of energy metabolism in HF.

Limitation also exists in this study. First, the important clinical information could not be obtained since it was retrospective in our study. Moreover, the functions and molecular mechanisms of these ten biomarkers in HF need to be further studied *in vitro* and *in vivo* experiments. Furthermore, it will be necessary to conduct large-scale prospective studies with strict follow-up protocols in the future to confirm the clinical feasibility of the proposed biomarkers.

Conclusions

In summary, we applied random forest-based feature selection to identify the 10 high-performance biomarkers for HF classification, and a clinical nomogram was constructed to visualizes the 10 identified biomarkers, which could better guide the clinical decisions. Further prediction potential miRNA and drugs of these 10 biomarkers provided further application on clinical HF treatment. We proved potential diagnostic utility of energy metabolism gene set in HF, and hope to assist in improving risk stratification and provide the potential treatment targets in HF.

Data availability statement

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

Author contributions

H-FT, WS, and ZZ designed, revised, and supervised the study. HC, RJ, RZ, and HW analyzed and organized the data. WH, KC, QY, KG, JL, RW, and SL generated the figure and tables. HC, RJ, WH, and ZZ wrote this manuscript. All authors reviewed and approved the final manuscript.

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The original data in our study were retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo) and we gratefully acknowledge the contributions from public available databases.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.993142/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Boxplot of immune cell subtypes level between HF samples and normal samples. *represents $P < 0.05$, **represents $P < 0.01$, ***represents $P < 0.001$.

SUPPLEMENTARY TABLE 1
Results of GO analysis of DEGs.

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Early carbohydrate antigen 125 as a mortality predictor in hospitalized patients with coronavirus disease 2019

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Background: Carbohydrate antigen 125 (CA125) is an indicator of inflammation, immune response, and impaired cardiac function. The aim was to investigate whether CA125 behaves as a biomarker of severity and poor clinical outcomes in hospitalized patients with coronavirus disease 2019 (COVID-19).

Methods: Serum CA125 [Elecsys CA125 II assay-(Roche Diagnostics GmbH)] was measured in stored biobank samples from COVID-19 hospitalized patients between 01 March 2020 and 17 October 2021. Multiple logistic regression models were built to explore the association between CA125 and clinical outcomes [in-hospital all-cause mortality, need for invasive mechanical ventilation (IMV), or non-invasive respiratory support (non-IRS)], estimating odds ratios (ORs; 95% CI). The gradient of risk of CA125 was evaluated by fractional polynomials.

Results: A total of 691 patients were included, median age of 63 years (50–76), men (57.2%), with high comorbidity. At admission, 85.8% had pneumonia. Median CA125 was 10.33 U/ml (7.48–15.50). The in-hospital mortality rate was 7.2%. After adjusting for confounding factors, CA125 $\geq$ 15.5 U/ml (75th percentile) showed an increased risk of death [OR 2.85(1.21–6.71)], as

age ≥ 65 years, diabetes, and immunosuppression. Furthermore, CA125 as a continuous variable was positive and significantly associated with the risk of death after multivariate adjustment. The mean hospital stay of the patients with CA125 ≥ 15.5 U/ml was longer than the rest of the study population.

Conclusion: CA125 in the first 72 h of hospital admission seems a useful biomarker of mortality in hospitalized patients with moderate–severe COVID-19. If our findings are confirmed, the wide availability of this biomarker would make easy its widespread implementation in clinical practice.

KEYWORDS

CA125, COVID-19, hospitalized, mortality, risk factors

"Early CA125 measurement, widely available in routine clinical practice, seems a useful biomarker of disease severity and mortality risk in hospitalized patients with moderate – severe #COVID-19 #CVD @isabial_iis"

Introduction

Coronavirus disease 2019 (COVID-19) can trigger an inflammatory process with a complex pathophysiology and affect the cardiovascular system directly or indirectly (1), with an impact on the course of the disease (2, 3). The mechanisms of cardiac injury are poorly understood (1). A disordered renin–angiotensin system (RAS) activity (4, 5), mediated by binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2) receptors present in the pulmonary alveoli, vascular and myocardial endothelial cells, could cause a direct cytotoxic effect on these cells (1, 6), besides triggering a severe systemic inflammation and cytokine storm (3, 7) that leads to respiratory dysfunction, myocardial and microvascular lesions (1), and the exacerbation of preexisting heart disease (8).

Early diagnosis and timely intervention in critical cases are crucial, highlighting the unmet need for novel biomarkers to improve diagnostic accuracy, risk stratification, monitoring, and therapy guidance.

The increases in cardiac and inflammatory biomarkers in COVID-19 have been associated with poor prognosis and mortality (9). Carbohydrate antigen 125 (CA125) has emerged as a useful and widely available marker in patients with decompensated heart failure (HF) (10). HF condition is closely related to systemic inflammatory activity and congestion (hydrostatic pressure and serosal effusions). Congestion is a hemodynamic parameter and causes the disease to progress by integrating into the circle of the inflammatory process defined in HF (11). Activation of mesothelial cells in response to increased hydrostatic pressures, mechanical stress, and cytokine activation has been suggested to be the crucial mechanism being the synthesis of CA125 by mesothelial cells (12). Although CA125 biological role is not well understood, it appears to be involved in multiple pathways, including immune innate and adaptive responses (13, 14).

Given that CA125 is not a specific cardiac biomarker, coupled with the fact that systemic inflammation has emerged as an important factor in increased CA125 concentrations (15), it is biologically plausible that this biomarker is useful in diseases in which inflammation is an important mechanism in the pathogenesis. In this regard, a correlation between CA125 and certain proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10, has been identified in HF (16). Also, in cultured human mesothelial cells, the secretion of CA125 can be enhanced by the inflammatory cytokine interleukin-1 beta (IL-1 beta), tumor necrosis factor-alpha (TNF-alpha), or lipopolysaccharide from *Escherichia coli* (17).

As a marker of inflammation, immune response, and cardiac function impairment, we postulated that CA125 may be useful for predicting unfavorable outcomes in patients with COVID-19. The availability of CA125 in most clinical laboratories, together with its standardized measurement and reduced cost, makes this marker attractive for routine use (18).

To provide insights into this issue, the impact of CA125 levels on major outcomes was examined in patients hospitalized with COVID-19.

Methods

Patients and study design

Since the beginning of the pandemic, every adult patient admitted to Hospital General Universitario Dr. Balmis de Alicante – a tertiary center – was

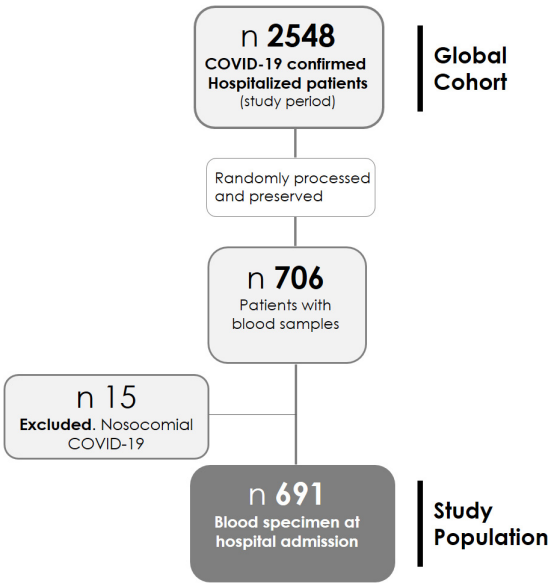

FIGURE 1
Flowchart of coronavirus disease 2019 (COVID-19) hospitalized in the study period.

asked for informed consent to be included in a database and to obtain a blood sample for biobank storage.

Patients hospitalized between 01 March 2020 and 17 October 2021 are studied. Blood samples were collected in EDTA tubes; plasma was separated from whole blood by centrifugation at 3,000 × *g* for 15 min at 4°C, then aliquoted and frozen at −80°C until use, by the BioBank ISABIAL, and integrated with the Spanish National Biobank Network and with the Valencian Biobanking Network. From 2,548 patients admitted during the study period, samples from 706 patients were randomly processed and preserved (**Figure 1**). Those with nosocomial COVID-19 were excluded from this analysis (n = 15), leaving the study sample in 691 patients.

Inclusion criteria were as follows: age ≥ 18 years, not nosocomial confirmed SARS-CoV-2 infection by the RT-PCR-COBAS 6800 System (Roche Molecular Systems, Branchburg, NJ, USA), informed consent signature, and availability of biobank blood sample with extraction in the first 72 h after hospital admission.

Variables and data collection

The clinical features, comorbidity, laboratory and radiological tests, prescribed therapies, and outcomes during the acute phase of the infection by SARS-CoV-2 were extracted from the digital medical records. The laboratory variables have been dichotomized, according to clinically relevant cutoff points or, failing that, according to the upper limit of the reference values of the center (9, 19–23). For the following variables, standard categorizations were followed: age ≥ 65 years, Charlson comorbidity index ≥ 3, estimated glomerular filtration rate < 60 ml/min/1.73 m^2 (by CKD-EPI formula), and hypoxemia (oximetry < 94% and PaO2:FiO2 < 300 mmHg) (24). The Charlson index assigns weights for specific diseases and includes myocardial infarction, congestive HF, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, ulcer disease, mild liver disease, diabetes with or without end-organ damage, any tumor, leukemia, lymphoma, moderate or severe liver disease, metastatic solid tumor, and AIDS.

Measurements and definitions

Serum CA125 was measured from biobank samples, following standardized and reproducible methods of their processing, by electrochemiluminescence immunoassay [Elecsys CA125 II assay-(Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim)] and was dichotomized by its 75th percentile.

Outcomes

The endpoints of this analysis were in-hospital all-cause mortality (main), need for invasive mechanical ventilation (IMV) or non-invasive respiratory support (secondaries), and associated factors.

Statistical analysis

Categorical and continuous variables are given as frequencies (percentages) and as median (interquartile range), respectively. Mann–Whitney *U* and Chi-square tests were used for group comparisons. The correlation between explanatory variables was analyzed by Spearman's Rho. Cumulative incidences of outcomes [95% confidence intervals (95% CI)] were registered.

Multiple logistic regression models were built to explore the association between CA125 and clinical outcomes, estimating odds ratios (ORs; 95% CI) in the global cohort and the subgroups. The variables were included as covariates if shown significant associations in simple models. The gradient of risk of CA125, as a continuous variable, in univariate and multivariate settings, was evaluated by fractional polynomials. The

final covariates included in the multivariate model were as follows: ≥ 65 years of age, Charlson comorbidity > 3, sex, nursing home, confusion, diabetes, hypertension, immunosuppression, eGFR ≤ 60 ml/min/m^2, oximetry at room air < 94%, ferritin > 500 mg/L, troponin T > 14 ng/L, B-type natriuretic peptide > 125 pg/ml, procalcitonin > 0.15 ng/ml, lactate dehydrogenase (LDH) > 250 U/L, C-reactive protein > 10 mg/dl, lymphopenia (< 1,000/mm^3), and the exposure (CA125). The number of patients included in the multivariate analysis was 583 (84.4% of the initial sample). No multiple imputations were performed. Covariates with more than 15% missing were not included in the multivariate analysis. The discriminative ability of the models was assessed by ROC analysis. A specific model was built to study the association between CA125 and mortality in the oldest subpopulation (age ≥ 85 years).

All tests were two-tailed, and a *p*-value of less than 0.05 was used. IBM SPSS Statistics 25 and STATA 15.1 statistical packages were used for the analyses.

This project was performed in the Clinical and Biomedical Research Institute of Alicante (ISABIAL), under the written approval of the local Ethics Committee of Clinical Research (Reference 200379).

Results

Baseline characteristics

Of the 2,548 patients hospitalized in the study period, blood samples from 706 patients were available. Fifteen patients with nosocomial COVID-19 were excluded. Finally, 691 patients were included in this study (refer to the flowchart in **Figure 1**). The basal demographic characteristics, comorbidities, clinical presentation, and outcomes are shown in **Table 1**. For more detailed information, refer to **Supplementary Table 1**.

The population was composed mainly of men (57.2%), with a median age of 63 years (50–76) and high comorbidity (Charlson index ≥ 3 46.6%, hypertension 47.5, obesity 39.7%, and diabetes 22.4%). Notably, 5% had received a complete vaccination (at least 14 days before the onset of clinical symptoms). After a mean of 1 week of symptoms, they were admitted to hospital, with hypoxemia in 32.3% and pneumonia in 85.8% of cases (bilateral pneumonia 34.6%; opacities > 50% of lung surface 22.4%). At admission, 33.6 and 47.3% of patients had T-Troponin > 14 ng/L and pro-BNP > 125 pg/ml, respectively.

Endotracheal intubation was required in 7.5% (52/691) of the patients. The in-hospital mortality rate was 7.2% (50/691). Biobank samples were obtained in 62.4 and 86.1%, in the first 24 and 48 h of hospital admission, respectively. Median CA125 was 10.33 U/ml (7.48–15.50).

TABLE 1 Demographic characteristics, comorbidities, clinical presentation, and clinical outcomes.

	Total (*n* = 691)
Demographics	
Age (years), median (IQR)	62 (50–76)
Age > 65, %	320/691 (46.3)
Males, %	395/691 (57.2)
Vaccine status[a] Complete Partial	35/691 (5.1%)13/691 (1.9%)
Comorbidities	
Diabetes, %	155/691 (22.4)
Hypertension, %	328/691 (47.5)
Chronic respiratory disease	123/690 (17.8)
Smoker (current or former), %	53/538 (9.9)
Charlson comorbidity index ≥ 3, %	341/688 (49.6)
Obesity (BMI ≥ 30), %	190/479 (39.7)
Initial assessment	
Oximetry at room air < 94%, %	211/654 (32.3)
Lymphopenia (< 1000/mm^3), %	332/691 (48.0)
Troponine T > 14 ng/L	216/643 (33.6)
Brain natriuretic peptide > 125 pg/ml, %	303/640 (47.3)
Clinical presentation	
Days of symptoms before admission, median (IQR)	6.8 (4–10)
Dyspnea, %	406/689 (58.9)
Radiological characteristics Bilateral pneumonia, % Unilateral pneumonia, %	236/683 (34.6)350/683 (51.2)
Opacities > 50% of lung surface on X-Rays, %	155/691 (22.4)
Clinical outcomes	
Length hospital stay (days), median (IQR)	8 (5–12)
Non-invasive respiratory support, %	192/691 (27.8)
ICU admission, %	79/691 (11.4)
Length ICU stay (days), median (IQR)	17.2 (6–17)
Invasive mechanical ventilation, %	52/691 (7.5)
Deaths, %	
Global, %	50/691 (7.2)
Group with ≥ 85 years old, %	14/72 (19.4)
Group with < 85 years old, %	36/619 (5.8)
Group with IMV, %	15/52 (28.8)

ICU, intensive care unit; IMV, invasive mechanical ventilation; IQR, interquartile rate. [a]We defined complete vaccination (CV) as symptom onset after 14 days of the second dose of vaccines (a single Janssen dose) and partial vaccination (PV) as administration of only the first dose, or symptom onset within 13 days after the second dose (single dose in Janssen).

Carbohydrate antigen 125 and severity of the disease

Patients in the upper quartiles showed a worse baseline risk profile (**Supplementary Table 2**). They were older, more frequently women, and had more comorbidities, higher cardiac

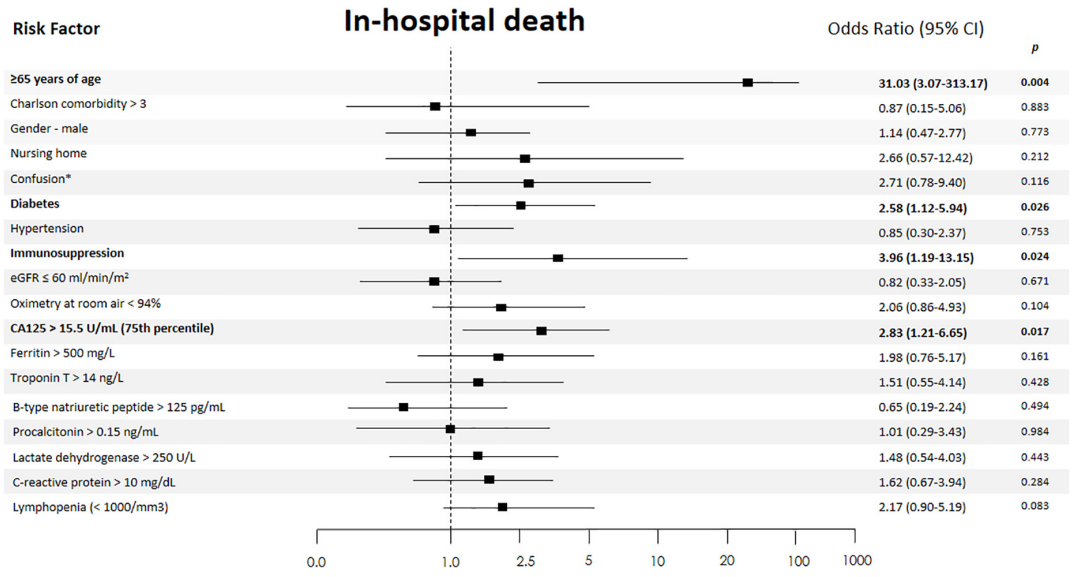

FIGURE 2
Independent risk factors of in-hospital death. The 95% confidence intervals (CIs) of the odds ratios have been adjusted for multiple testing. In bold, independent predictors associated with the outcomes. eGFR, estimated glomerular filtration rate (by CKD-EPI formula); *On admission.

biomarker (troponin T and NT-proBNP) levels, and higher procalcitonin and ferritin, as shown in **Supplementary Table 2**.

There was a weak correlation between the levels of the natural logarithm (ln) CA125 with age (rho 0.21) amino terminal brain natriuretic peptide (NTpro-BNP) (rho 0.22) and T-troponin (rho 0.21), $p < 0.001$. At the same time, a correlation with IL-6 levels on admission was not observed.

Carbohydrate antigen 125 and risk of death

Supplementary Table 3 shows the baseline characteristics across the death status. During hospitalization, 50 patients died (7.2%). Plasma CA125 was higher in patients with fatal outcome [14.35 U/ml (8.30–27.81) vs. 10.24 U/ml (7.44–14.98), $p = 0.008$]. The rates of in-hospital death were significantly higher in the upper CA125 quartile [Q1 ($\leq$ 7.47 U/ml): 5.2%, Q2 (7.48–10.3 U/ml): 5.8%, Q3 (10.3–15.48): 4.1%, and Q4 ($\geq$ 15.5 U/ml): 13.9%; $p < 0.001$].

When CA125 was categorized in quartiles, those in the upper quartile showed a significantly increased risk [OR 2.94 (1.32–6.52)], compared with the lower quartile. In the multivariate regression model, after adjusting for confounding factors, when compared with the three lower quartiles ($<$ 15.5 vs. $\geq$ 15.5 U/ml), those in the upper category remained to show an increased risk of death [OR 2.85 (1.21–6.71)], along with age $\geq$ 65 years [OR 30.4 (3.02–305.02)], diabetes [OR 2.54 (1.10–5.91)], and immunosuppression [OR 4.08 (1.20–13.85)] (**Figure 2**). Lymphopenia was close to statistical significance. Final multivariate risk estimates for all covariates included in the models are presented in **Figure 2**. After multivariate adjustment, CA125 as a continuous was positive and significantly associated with the risk of death (**Figure 3**).

Subgroup analysis revealed that those in the upper quartile vs. the three lower quartiles remained to show a homogenous increased risk of in-hospital death across age ($<$ 65 vs. $\geq$ 65 years), sex (men vs. women), and Charlson index ($<$ 2 vs. $\geq$ 3). The adjusted p-value for the interactions for those belonging to the upper quartile vs. three lower quartiles was 0.483, 0.189, and 0.586 for age, sex, and Charlson status, respectively. **Table 2** shows the risk estimates for each subgroup.

After excluding patients aged $\geq$ 85 years, age $\geq$ 65 years, diabetes, and CA125 $>$ 15.5 U/ml persist as independently associated factors of mortality, whereas confusion and hypoxemia at admission were close to statistical significance.

Carbohydrate antigen 125 and other clinical outcomes

Carbohydrate antigen 125 levels were not associated with the need of IMV [CA125 $>$ 50th percentile OR 0.87 (0.49–1.55), CA125 $>$ 75th OR 0.72 (0.35–1.47)] or non-invasive respiratory support [CA125 $>$ 50th percentile OR 0.88 (0.63–1.23), and CA125 $>$ 75th OR 1.08 (0.73–1.28)].

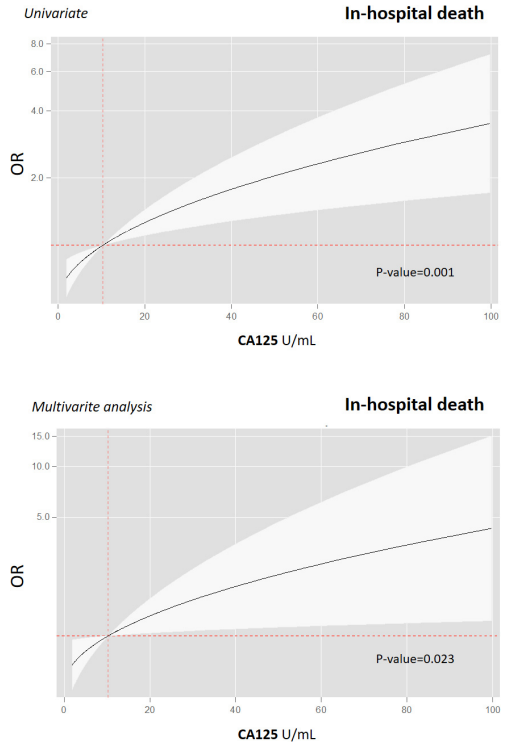

FIGURE 3
Gradient of risk of CA125 for predicting in-hospital death in patients with COVID-19. Prognostic effect of CA125 on in-hospital all-cause mortality and in univariate (upper graph) and multivariate analyses (lower graph). CA125, antigen carbohydrate 125; OR, odds ratios.

TABLE 2 CA125 and adjusted risk of in-hospital death.

	OR (CI 95%)*	*p*-value for interaction
Upper quartiles vs. three lower quartiles (< 15.5 vs. ≥ 15.5 U/ml)		
Whole sample		
Whole sample	2.85 (1.21–6.71)	
Age		
< 65 years	2.21 (1.17–11.91)	0.483
≥ 65 years	3.12 (1.56–5.67)	
Sex		
Men	2.01 (0.95–4.18)	0.189
Women	4.35 (1.51–14.51)	
Charlson index		
< 2	1.95 (0.83–13.65)	0.586
≥ 3	3.21 (1.57–5.32)	

Subgroup analysis. *Adjusted estimates. CA125, antigen carbohydrate 125; OR, odds ratio.

The mean hospital stay of the patients with CA125 higher than the 75 h percentile was longer than the rest of the study population [9.0 (6.0–15.0) vs. 7.0 (5.0–11.0) days, $p = 0.014$].

Discussion

This is the first study analyzing the role of CA125 in the first 72 h of admission as a biomarker of disease severity in hospitalized patients with moderate–severe COVID-19. CA125 was higher in patients with fatal outcomes, whereas did not entail a greater requirement of IMV. Even though pro-BNP, T-troponin, and CA125 correlated positively, these associations were weak. Our findings establish CA125 levels as a sensitive biomarker of severity and poor clinical evolution in hospitalized COVID-19.

Carbohydrate antigen 125, also called cancer antigen 125, carcinoma antigen 125, or mucin 16 (MUC16), is a complex glycoprotein encoded by the MUC16 gene in humans (13, 25). CA125 is mainly synthesized by mesothelial cells in the pericardium, pleura, or peritoneum (25, 26). In recent years, increasing evidence supported the use of CA125 in cardiovascular diseases, particularly in decompensated HF and in the transition to clinical stability (10, 27). Interestingly, in patients with acute HF, this glycoprotein provides additional prognostic information to those provided by well-known prognosticators, including natriuretic peptides (28).

Pathophysiology of the association between CA125 and death in COVID-19

In different CV scenarios, especially in acute HF, plasma levels of CA125 have emerged as proxies of two crucial and interrelated pathophysiological processes, namely, inflammation and congestion (18). Thus, several works have found a positive and significant association between CA125 and surrogate parameters of fluid overload and right-sided HF dysfunction (29). Additionally, higher glycoprotein levels also identified patients with a greater immunoinflammatory milieu (18). For instance, Miñana et al. reported in a cohort of 132 patients admitted with acute HF that CA125 levels above the median (> 60 U/ml) were associated with higher levels of TNF-α, IL-6, and interleukin-1β and lower relative lymphocyte count (30). Also, Kosar et al. showed that the increase in serum CA 125 levels in 35 patients with HF correlates with TNF-α ($r = 0.624$, $p < 0.001$), IL-6 ($r = 0.671$, $p < 0.001$), and IL-10 ($r = 0.545$, $p < 0.001$) (16). These findings contrast with the lack of correlation between the levels of nlCA125 and IL-6 in our series. However, the patients in the upper quartiles of CA125 levels showed higher inflammatory markers such as procalcitonin and ferritin, reflecting the degree of underlying systemic inflammation. The short half-life of IL-6 (2–5 h) (31) and the analysis of only one sample at admission, in our study, and not sequential measurements could explain these discrepancies. In this regard, in patients without COVID-19 with systemic inflammatory response syndrome/sepsis

admitted to ICU, Oda et al. demonstrated that there was no significant difference in the blood IL-6 level on admission between survivors and non-survivors, whereas the mean blood IL-6 level during ICU stay was significantly higher in the non-survivors (31).

We postulate that the mechanisms endorsing the relationship between CA125 and the risk of mortality in COVID-19 are due to at least two main pathophysiological mechanisms that partially overlap. First, we think that CA125 may capture the intensity of the inflammatory response more accurately than other inflammatory markers. Our findings positioned CA125 as an independent biomarker of fatal outcomes in patients hospitalized for COVID-19, above the classical inflammation markers described in the literature (ferritin, procalcitonin, LDH, and C-reactive protein) (32, 33) or biomarkers of myocardial damage (T troponin and pro-BNP) (9). CA125, in contrast to other biomarkers, is a stable biomarker that may capture the severity of immunoinflammatory response in a period of several days (the half-life of CA125 ranges from 5 to 12 days) (18). Second, CA125 may also capture information about the onset of clinical complications such as HF, pulmonary thromboembolism with right-sided dysfunction, or pleural effusion. This greater elevation in the most seriously ill patients could respond to a greater pulmonary involvement because CA125 concentrations are correlated with hemodynamic parameters, right atrial pressure, and pulmonary capillary wedge pressure (34).

Limitations

Some important limitations need to be addressed. First, this is a one-center observational analysis of patients hospitalized with COVID-19. Although the effort to control for relevant confounders was performed, the risk of residual confounding, as a selection bias for available biobank samples, cannot be ruled out in this type of study. Sample size limitations prevented analysis by the SARS-CoV-2 variant. In this study, we only measured this glycoprotein at a one-time point. Thus, we could not explore the kinetic of this biomarker and its influence on risk stratification.

Several gaps are worth mentioning, the pathophysiology of CA125 upregulation in COVID-19 is not well known, and whether CA125 is a marker or plays a role in disease progression remains speculative. The optimal cutoff for defining severity should be corroborated in future research. Finally, we did not register a prior history of HF or cancer in the evaluated sample. CA125 is a well-established marker of different neoplasms. Therefore, we cannot assess its role as a confounding factor in the current findings.

Future directions

These findings require to be validated in larger studies, and more research is needed to define its biological role in patients with COVID-19. The larger sample size may be helpful for confirming current findings and unraveling the clinical utility of the results presented in this study. Additionally, formal prognostic comparison among different inflammatory markers is still required. Further studies are warranted to determine the optimal set of widely available circulating biomarkers useful in patients with COVID-19. Whether CA125 will be among them remains to be confirmed. In the meantime, the usefulness of this biomarker in guiding the intensity of medical therapy seems a reasonable hypothesis that deserves further evaluation.

Logistic advantages

Carbohydrate antigen 125 has potential logistic advantages that deserve to be highlighted. First, the wide availability of CA125 in most clinical laboratories, its measurement following standardized and reproducible methods, and low cost (< 2.5 € per determination) make this marker attractive for routine use in decompensated HF and other diseases. Second, CA125 levels are not substantially modified by age, sex, body mass index, or renal dysfunction. Furthermore, in all of the prior subgroups, plasmatic CA125 retained its prognostic value (18, 35). All of the prior items are crucial aspects that yield us to speculate an easy transition of these findings to the actual clinical practice of patients hospitalized with COVID-19.

Conclusion

Carbohydrate antigen 125 measured in the first 72 h of hospital admission seems a useful biomarker of disease severity in hospitalized patients with moderate-severe COVID-19. Besides, this sensitive biomarker, as a surrogate of congestion and inflammation, may reflect the progression of COVID-19 and is independently associated with in-hospital mortality after adjusting by confounders. If our findings are confirmed, the wide availability of this biomarker will make easy its widespread implementation in clinical practice.

Further research is required to understand better its biological role and its promising utility as a prognostic marker in COVID-19.

Data availability statement

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

Ethics statement

This project was performed in the Clinical and Biomedical Research Institute of Alicante (ISABIAL), under the written approval of the Local Ethics Committee of Clinical Research (Reference 200379). The patients/participants provided their written informed consent to participate in this study.

Author contributions

OM-P, JN, MS-R, VA, and EM: writing – original draft. OM-P, JN, MS-R, VA, VB, SR, HP-C, IR, RA, ML-S, MM-G, and EM: writing – review and editing, and investigation. OM-P, JN, and EM: methodology. OM-P and JN: formal analysis. EM: project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.941512/full#supplementary-material

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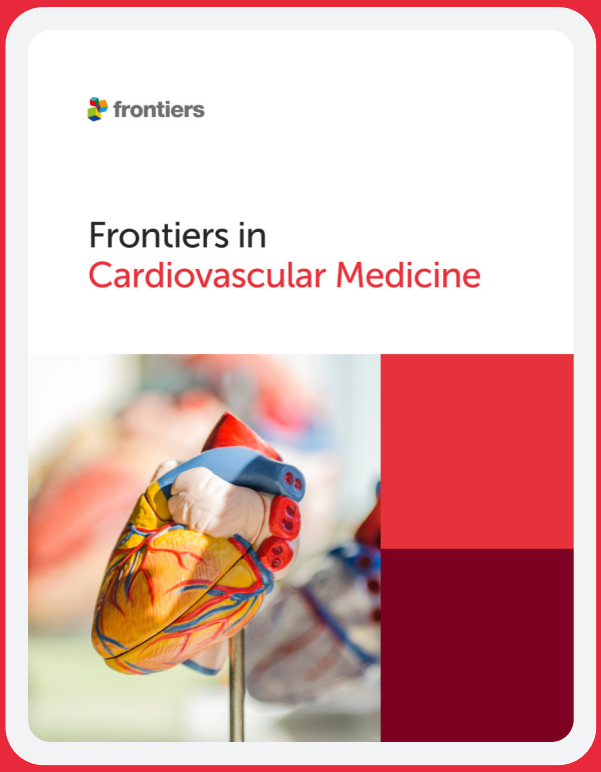

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