Research Article

Dongmei Wei*, Rui Li, Tao Si, Hankang He, Wei Wu

Screening and bioinformatics analysis of key biomarkers in acute myocardial infarction

<https://doi.org/10.1515/pteridines-2020-0031>

received August 03, 2021; accepted September 28, 2021

Abstract: Acute myocardial infarction (AMI) is the most severe manifestation of coronary artery disease. Considerable efforts have been made to elucidate its etiology and pathology, but the genetic factors that play a decisive role in the occurrence of AMI are still unclear. To determine the molecular mechanism of the occurrence and development of AMI, four microarray datasets, namely, GSE29111, GSE48060, GSE66360, and GSE97320, were downloaded from the Gene Expression Omnibus (GEO) database. We analyzed the four GEO datasets to obtain the differential expression genes (DEGs) of patients with AMI and patients with non-AMI and then performed gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, and Protein-protein interaction (PPI) network analysis. A total of 41 DEGs were identified, including 39 upregulated genes and 2 downregulated genes. The enriched functions and pathways of the DEGs included the inflammatory response, neutrophil chemotaxis, immune response, extracellular space, positive regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) transcription factor activity, response to lipopolysaccharide, receptor for advanced glycation end products (RAGE) receptor binding, innate immune response, defense response to bacterium, and receptor activity. The cytoHubba plug-in

in Cytoscape was used to select the most significant hub gene from the PPI network. Ten hub genes were identified, and GO enrichment analysis revealed that these genes were mainly enriched in inflammatory response, neutrophil chemotaxis, immune response, RAGE receptor binding, and extracellular region. In conclusion, this study integrated four datasets and used bioinformatics methods to analyze the gene chips of AMI samples and control samples and identified DEGs that may be involved in the occurrence and development of AMI. The study provides reliable molecular biomarkers for AMI screening, diagnosis, and prognosis.

Keywords: acute myocardial infarction, bioinformatics, GEO database

1 Introduction

Acute myocardial infarction (AMI) is myocardial necrosis due to acute and persistent coronary ischemia. AMI remains a leading cause of morbidity and mortality worldwide, despite dramatic improvements in coronary intervention technology and drugs in the past several decades [1].

Accumulating evidence has demonstrated that atherosclerosis is the pathological basis of coronary heart disease (CHD). Lipid metabolism and inflammatory responses play critical roles in plaque rupture, which leads to the occurrence and development of AMI [2]. However, among many risk factors, only 50–60% of the causes of CHD (including AMI) can be explained, and the remaining 40–50% are attributed to genetic factors [3]. At present, most scholars believe that genetic factors may be the cumulative effect of multiple micro-effect genes in the occurrence of CHDs. The genetic factors that play a decisive role in the occurrence of CHD are still unclear. Therefore, traditional assessments of cardiovascular risk factors (including hypertension, diabetes, and smoking) are essential for the prevention and prognosis prediction of AMI [4], but still cannot sufficiently predict the risk of recurrent events. Some molecular markers, such as brain natriuretic peptide and c-reactive protein, or other serum inflammatory markers in the

* **Corresponding author: Dongmei Wei**, Department of Cardiovasology, Liuzhou Traditional Chinese Medical Hospital, Liuzhou, Guangxi Province, 545001, People's Republic of China, e-mail: wei_dm8815@163.com

Rui Li: Guangzhou University of Chinese Medicine, Guangzhou, Guangdong Province, 510405, People's Republic of China, e-mail: lirui001202009@163.com

Tao Si: Guangzhou University of Chinese Medicine, Guangzhou, Guangdong Province, 510405, People's Republic of China, e-mail: sitao012020@163.com

Hankang He: Department of Cardiovasology, Liuzhou Traditional Chinese Medical Hospital, Liuzhou, Guangxi Province, 545001, People's Republic of China, e-mail: hehankang19650301@163.com

Wei Wu: Guangzhou University of Chinese Medicine, Guangzhou, Guangdong Province, 510405, People's Republic of China, e-mail: 20195200020@stu.gzucm.edu.cn

prediction of AMI have gained increasing attention, but the present study shows that the prediction ability of those biomarkers is not so strong; finding more sensitive markers to explain the initial disturbance and the underlying mechanisms of AMI is an urgent need to solve the problem [5]. With the development of bioinformatics, a large amount of big data related to genomics, transcriptomics, proteomics, and metabolomics have been produced. Through the combination of bioinformatics analysis and computer science, these data can be analyzed to study the interrelation between multiple genes. Previous studies have also demonstrated that the application of tissue-based microarray gene expression profiles in a variety of cardiac diseases can help to identify ischemic and nonischemic causes [6]. We can also analyze the relevant gene chips of patients with AMI to identify the different genes and provide ideas for in-depth research on the molecular mechanism of the genetic aspect of myocardial infarction and early diagnosis.

However, the analysis of a single gene chip may yield false-positive results, which may lead to the analysis conclusion not being credible. Therefore, in this study, we analyzed four Gene Expression Omnibus (GEO) datasets to obtain the differential genes of patients with AMI and patients with non-AMI and then performed gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, Protein–protein interaction (PPI) network analysis, and systematic analysis of the relationship between different genes and their regulatory network. We hope to determine the molecular mechanism of the occurrence and development of AMI, thus providing a reliable diagnostic marker for early diagnosis.

2 Materials and methods

2.1 Differential expression analysis

Microarray data: The GEO database is a gene expression database created and maintained by the National Center for Biotechnology Information (NCBI). It contains high-throughput gene expression data submitted by research institutions around the world [7]. We downloaded the four datasets GSE29111 [8], GSE48060 [9], GSE66360

[10], and GSE97320 [11] from the GEO database. The probe annotation information of the gene chips of these four datasets comes from the same platform, GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array). The dataset for GSE29111 includes 36 AMI samples and 16 Unstable Angina samples. GSE48060 incorporates 31 AMI samples and 21 normal samples. GSE66360 consists of 49 AMI samples and 50 normal samples. GSE97320 contains 3 AMI samples and 3 normal samples. A total of 119 AMI and 90 control samples were included. Detailed information about these datasets is listed in Table 1.

Data processing: Based on the same platform, the original data of these four datasets were integrated into one dataset and then the batch processing was standardized using the R package (sva) [12]. The “affy” package in R (version 3.4.2, <http://www.R-project.org/>) [13] was used to preprocess the original data, including background correction, matrix data normalization, missing value supplementation, etc. The probe information matched the latest annotation file downloaded from the NCBI gene database. No probes that matched it were deleted. If a gene corresponded to multiple probes, the probe with the lowest corrected p -value was selected. Quantile standardized preprocessing was performed by the “limma” package [14] before identifying the differentially expressed genes (DEGs).

Identification of DEGs: First, through probe annotation, gene expression data were extracted, and LOG2 logarithmic conversion was performed. Then, the limma package of R was used to analyze the DEGs of AMI samples and control samples in the gene chip data. The screening criteria for DEGs were set to $\log_2(\text{fold change})$ ($\log_2 \text{FC}$) > 1 and adjusted p -value (adj. p -value) < 0.05 . Finally, hierarchical clustering analysis was performed on the DEGs of AMI samples and control samples.

2.2 GO enrichment and KEGG pathway analyses

GO enrichment analysis [15] and KEGG [16] pathway analysis were performed by using the Database for Annotation, Visualization and Integrated Discovery (DAVID) [17] online tool. GO enrichment analyses annotate and clas-

Table 1: Details of the GEO myocardial infarction data

Reference	GEO accession	Platform	AMI total (n)/Male (n, %)	Control total (n)/Male (n, %)	Country
Rothman et al. (2011)	GSE29111	GPL570	36/30.83	16/12.75	United Kingdom
Suresh et al. (2014)	GSE48060	GPL570	31/18.58	21/14.67	USA
Muse et al. (2017)	GSE66360	GPL570	21/16.76	22/9.41	USA
Fan et al. (2018)	GSE97320	GPL570	3/2.67	3/3.100	China

sify genes according to biological process (BP), molecular function (MF), and cellular component (CC) categories.

2.3 PPI network construction

A PPI network functional enrichment analysis of the identified DEGs was constructed by using the STRING online database [18]. The cutoff point was set to a credibility score higher than 0.4. The PPI network was visualized using Cytoscape software [19].

2.4 Recognition of hub genes

To select hub genes from these DEGs, we used the cytoHubba [20] plug-in in Cytoscape software, which can explore important hubs in an interactome network by several topological algorithms. Three of those algorithms, namely, the density of maximum neighborhood component (DMNC), maximal clique centrality (MCC), and maximum neighborhood component (MNC), were used to identify the hub genes. We used Venn diagrams to find mutual genes from the results of the three algorithms.

3 Results

3.1 Controlling the quality and identification of DEGs

R software was used to analyze all the original data. First, quality was evaluated. The residual, grayscale, weight, and residual symbol maps were made to show the uniform color distribution. The relative logarithmic expression (RLE) and normalized unscaled standard error (NUSE) boxplot showed that the chip expression values were close to each other; RLE did not deviate significantly from 0, and NUSE did not deviate significantly from 1, indicating reliable quality between chipsets (Figures S1 and S2). The RNA degradation diagram showed that RNA degradation started from the 5-prime end, and no abnormal RNA degradation occurred (Figure S3). The slope of the curve was between 0° and 45° , indicating a moderate degradation rate. This indicates that the dataset met the requirements of data analysis. The results before the score normalization process are shown in Figure 1a, and the results after the process are shown in Figure 1b. It can be seen from Figure 1b that the processed chip data value distribution is better. Finally, after comparing the two sets of data of AMI samples and control samples, we found 41 DEGs, including 39 upregulated genes and 2 downregulated genes. These DEGs are

Figure 1: (a) Before quantile standardization. (b) After quantile standardization. The X coordinate represents the samples, and the Y coordinate represents gene expression values.

Figure 2: Volcano plot of the DEGs. The Y axis is log FC, and the X axis is $-\log_{10}(\text{adj. } p\text{-value})$. Each dot represents a gene. Red dots represent DEGs that are upregulated, green dots represent DEGs that are downregulated, and black dots represent genes that are not significantly different.

represented by volcano graphs (Figure 2). In the volcano graphs, $p < 0.05$ is used as the criterion for defining significant differences. Red dots indicate upregulated DEGs, while green dots indicate downregulated DEGs. The heatmap (Figure 3) shows the hierarchical clustering of DEGs. The clusters of differential genes are displayed on the left side of the heatmap, and the clusters of the samples are displayed above the heatmap.

3.2 Functional enrichment analysis of the DEGs

We used DAVID to perform functional analysis of these DEGs. Figure 4 shows the top ten significant GO terms. As shown in Figure 5 and Table 2 we found that DEGs between AMI and control samples were mainly enriched in inflammatory response, neutrophil chemotaxis, immune response, extracellular space, positive regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) transcription factor activity, response to lipopolysaccharide, receptor for advanced glycation end products (RAGE) receptor binding, innate immune response, defense response to bacterium, and receptor activity.

3.3 Pathway analyses

According to the p -value, the top ten KEGG pathways for the DEGs are shown in Figure 6 and Table 3 including the hematopoietic cell lineage pathway ($p = 0.002882095$), cytokine-cytokine receptor interaction pathway ($p = 0.007920517$), malaria pathway ($p = 0.011431864$), legionellosis ($p = 0.013770733$), nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway ($p = 0.014759858$), tuberculosis ($p = 0.020316804$), complement and coagulation cascades ($p = 0.021902284$), rheumatoid arthritis ($p = 0.034396554$), amoebiasis ($p = 0.048229511$), and tumor necrosis factor (TNF) signaling pathway ($p = 0.049049747$). All these pathways may be related to the development of AMI. Among all the pathways, the cytokine-cytokine receptor interaction pathway involves the largest number of five DEGs. The hematopoietic cell lineage signaling pathway was the most significant pathway according to the p -value.

3.4 Construction of the PPI network

We used the STRING database to investigate PPI networks, including 39 upregulated genes and two downregulated genes. Subsequently, a PPI network of those DEGs was drawn and is shown in Figure 7.

3.5 Selection of hub genes from the PPI network

We used the cytoHubba plug-in in Cytoscape to select the most significant hub gene from the PPI network. Among the algorithms in the cytoHubba plug-in, we chose the three most commonly used algorithms, namely, DMNC, MNC, and MCC. Based on these three algorithms, we selected the top fifteen hub genes of each algorithm (Figure 8a–c). Finally, we used the Venn diagram to intersect the top fifteen hub genes of each selected algorithm to obtain the final ten mutual hub genes (Figure 8d). A total of ten genes were identified as hub genes. The names, abbreviations, and MFs of these ten hub genes are shown in Table 4. The functional analyses of these hub genes were analyzed with DAVID. The results of the hub genes are shown in Table 5.

4 Discussion

AMI is the most severe manifestation of coronary artery disease [1]. Numerous previous studies have confirmed

Figure 3: Heatmap of the DEGs. The clusters of differential genes are displayed on the left side of the heat map, and the clusters of the samples are displayed above the heat map. The gene symbols of these differential genes are shown on the right side of the heat map. The samples from these four datasets are shown at the bottom of the heatmap.

that atherosclerosis is the primary etiology factor of AMI and that the inflammatory response plays a critical role in the development of AMI [21]. Although traditional AMI risk factors such as smoking and diabetes have some reference value for disease prevention and prognosis judgment, the specificity of this risk factor has certain limitations [22]. Identifying new biomarkers at the current stage is conducive to early screening and diagnosis of AMI.

In this study, the bioinformatics method was used to screen the data of GEO chips to analyze the DEGs in the peripheral blood samples of patients with AMI. Compared with the healthy control group, 41 genes in peripheral blood samples of patients with AMI had differential expression, of which 2 were downregulated and 39 were upregulated. Further analysis of GO functional enrichment of DEGs and construction of a PPI network were conducted to explore their differential expression. The

DEGs were mainly enriched in inflammatory response, neutrophil chemotaxis, immune response, extracellular space, and positive regulation of NF- κ B transcription factor activity. Previous studies have reported that the inflammatory response plays a critical role in the occurrence and development of AMI [21,23]. Elevated neutrophils are important markers of the inflammatory response. At the same time, neutrophils are an essential part of innate immunity. Neutrophils chemoattract to coronary plaques and infarcted myocardium and mediate tissue damage by releasing matrix-degrading enzymes and reactive oxygen species (ROS) [24]. The extracellular space is an irregular and interconnected narrow space between cells. As the microenvironment where brain cells and neural networks live and work directly, the extracellular space affects the activity of brain cells. It is involved in the occurrence and development of various neurological diseases [25]. Reports of connections between the

Figure 4: Distribution of DEGs in AMI for different GO-enriched functions.

extracellular space and heart disease are rare. Nevertheless, extracellular vesicles, which originate from diverse subcellular compartments, are released into the extracellular space and act as regulators of the transfer of biological information [26]. Circulating extracellular vesicles can be diagnostic and prognostic biomarkers for primary and secondary prevention of cardiovascular diseases [27]. In addition, recent studies have suggested that NF- κ B is a

crucial nuclear transcription factor that mediates the release of inflammatory factors and plays a vital role in the pathophysiological process of AMI [28]. Overall, the above research results are consistent with our analysis. From the above 41 DEGs, we selected 10 DEGs as hub genes. GO and KEGG pathway enrichment analysis revealed that the ten hub genes were mainly enriched in inflammatory response, neutrophil chemotaxis, immune response,

Figure 5: GO analysis of the DEGs. Different colors differentiate the three categories. BP: biological process, CC: cellular component, and MF: molecular function.

Table 2: Top ten GO terms of DEGs

Term	Count	p-value	Genes
Inflammatory response	14	7.02×10^{-13}	C5AR1, S100A8, CXCL2, S100A9, CHI3L1, TLR2, NLRP3, S100A12, SLC11A1, CCL20, VNN1, IL1B, BCL6, CLEC7A
Neutrophil chemotaxis	8	9.80×10^{-11}	C5AR1, S100A8, CCL20, S100A9, IL1B, CSF3R, TREM1, S100A12
Immune response	12	1.03×10^{-9}	IL1R2, SLC11A1, LILRB2, CD36, C5AR1, AQP9, CCL20, CXCL2, IL1RN, TLR2, IL1B, NFIL3
Extracellular space	15	2.43×10^{-7}	NAMPT, S100A8, IL1RN, CXCL2, S100A9, PLBD1, CHI3L1, LILRB2, THBD, CD36, CCL20, IL1B, VCAN, SERPINA1, CSTA
Positive regulation of NF-κB transcription factor activity	7	4.19×10^{-7}	IRAK3, S100A8, S100A9, TLR2, IL1B, NLRP3, S100A12
Response to lipopolysaccharide	7	1.43×10^{-6}	SLC11A1, IRAK3, THBD, C5AR1, S100A8, CXCL2, TLR2
RAGE receptor binding	4	1.58×10^{-6}	S100P, S100A8, S100A9, S100A12
Innate immune response	9	3.48×10^{-6}	S100A8, CLEC4E, S100A9, TLR2, VNN1, CLEC7A, TREM1, NLRP3, S100A12
Defense response to bacterium	6	1.56×10^{-5}	SLC11A1, S100A8, CLEC4E, CCL20, S100A9, S100A12
Receptor activity	6	1.05×10^{-4}	LILRB2, THBD, CLEC4E, TLR2, CSF3R, TREM1

Figure 6: Top ten KEGG pathway analyses of the DEGs.

RAGE receptor binding, extracellular region, and positive regulation of NF- κ B transcription factor activity. In contrast, changes in KEGG were mainly enriched in the NOD-like receptor signaling pathway.

Among the ten hub genes, S100A8 and S100A9 showed the highest coexpression and combined score. S100A8 and S100A9, which are members of the S100 calcium-binding family of proteins and typically exist as S100A8/S100A9 heterodimers, are associated with inflammation [29] and atherosclerosis [30]. As the most important of all the damage-associated molecular pattern proteins in sterile inflammatory conditions, in recent years, the role of S100A8/S100A9 in myocardial infarction has attracted an increasing amount of interest [31]. S100A8/S100A9 is released locally after myocardial infarction and enhances the inflammatory response associated with myocardial ischemia-reperfusion injury [32,33]. Elevated plasma S100A8/A9 levels are related to the risk of

future coronary events [34]. Thus, it is regarded as a new biomarker and therapeutic target for AMI [31,35]. S100A12, also a calgranulin family protein released by white blood cells, is associated with inflammatory cardiovascular diseases. S100A12 levels in plasma can be used to predict the prognosis of patients with chronic coronary artery disease [36].

Interleukin-1 (IL-1) plays an essential role in the signaling, activation, and regulation of immune cells, and mediates T and B cell activation, proliferation and differentiation, and the inflammatory response [37,38]. There are two subtypes of IL-1, namely, IL-1 α and IL-1 β ; two receptor forms, that is, interleukin-1 receptor type 1 (IL1R1) and interleukin-1 receptor type 2 (IL1R2); an interleukin-1 receptor accessory protein (IL1RAP); and an interleukin-1 receptor antagonist (IL1RN). With the help of IL1RAP, IL-1 can form a membrane-penetrating complex with IL1R1, and IL-1/IL1R1/IL1RAP recruit IL-1 receptor-associated kinase

Table 3: Partial results of pathway analysis of DEGs

Term	Count	p-value	Genes
Hematopoietic cell lineage	4	0.002882095	IL1R2, CD36, IL1B, CSF3R
Cytokine-cytokine receptor interaction	5	0.007920517	IL1R2, CCL20, CXCL2, IL1B, CSF3R
Malaria	3	0.011431864	CD36, TLR2, IL1B
Legionellosis	3	0.013770733	CXCL2, TLR2, IL1B
NOD-like receptor signaling pathway	3	0.014759858	CXCL2, IL1B, NLRP3
Tuberculosis	4	0.020316804	CLEC4E, TLR2, IL1B, CLEC7A
Complement and coagulation cascades	3	0.021902284	THBD, C5AR1, SERPINA1
Rheumatoid arthritis	3	0.034396554	CCL20, TLR2, IL1B
Amoebiasis	3	0.048229511	IL1R2, TLR2, IL1B
TNF signaling pathway	3	0.049049747	CCL20, CXCL2, IL1B

Figure 7: PPI network of DEGs. The balls represent the gene nodes, and the connecting lines represent the interactions.

(IRAK1) and activate transcription regulators. This further activates NF- κ B, mitogen-activated protein kinase, mammalian target of rapamycin, and other signal transduction pathways, ultimately initiating the IL-1-mediated cellular response. Unlike IL1R1, IL1R2 acts as an endogenous inhibitor

that prevents IL-1 from binding to IL1R1 and inhibits IL-1 signal transduction [39,40]. IL1R2 is highly expressed in several cancer species and is regarded as a novel biomarker for the early diagnosis and therapeutic target of tumors [41–44]. However, IL1R2 has been reported to have a controversial role in coronary disease, which needs further investigation. The elevated IL1R2 prevents homocysteine-induced apoptosis and inflammation injury in coronary atherosclerosis through the inhibition of inflammasome signaling pathway activation [45]. In another study, the expression level of IL1R2 on monocytes/macrophages decreased during atherosclerosis and vascular injury. The reason may include acetylated low-density lipoprotein inhibiting the expression of IL1R2 in macrophages and uncontrolled IL-1-dependent inflammation in this case [46]. However, published bioinformatics analyses have shown that IL1R2 is highly expressed in patients with AMI [47,48]. IL1RN inhibits the activity of IL-1 by binding to the receptor IL1R1 and prevents its signal transduction with the coreceptor IL1RAP. Under advanced pathological conditions, IL1RN inhibits cells by noncompetitively inhibiting the activities of caspase-8 and caspase-9 apoptosis and/or necrosis [49,50,51]. According to reports, IL1RN can reduce the myocardial infarct size and inhibit myocardial cell apoptosis by blocking the inflammatory response during myocardial ischemia-reperfusion injury and plays a potential

Figure 8: (a) The top fifteen hub genes based on the DMNC method. (b) The top fifteen hub genes based on the MNC method. (c) The top fifteen hub genes based on the MCC method. (d) Venn diagram of the mutual hub genes based on the above three algorithms.

Table 4: Functional roles of the ten hub genes

No.	Gene symbol	Full name	Molecular function
1	S100A8	S100 calcium binding protein A8	A calcium- and zinc-binding protein which plays a prominent role in the regulation of inflammatory processes and immune response
2	S100A9	S100 calcium binding protein A9	
3	S100A12	S100 calcium binding protein A12	
4	NLRP3	NLR family, pyrin domain containing protein 3	May function as an inducer of apoptosis
5	CXCL2	Chemokine (C-X-C motif) ligand 2	Produced by activated monocytes and neutrophils and expressed at sites of inflammation
6	IL1RN	Interleukin 1 receptor antagonist	Inhibits the activity of interleukin-1 by binding to receptor IL1R1 and prevents its association with the coreceptor IL1RAP for signaling
7	CLEC7A	C-type lectin domain family 7, member A	Lectin that functions as pattern receptor specific for beta-1,3-linked and beta-1,6-linked glucans
8	C5AR1	Complement component 5a receptor 1	Receptor for the chemotactic and inflammatory peptide anaphylatoxin C5a
9	IL1R2	Interleukin 1 receptor, type II	Non-signaling receptor for IL1 α , IL1 β , and IL1RN. Reduces IL1 β activities
10	TREM1	Triggering receptor expressed on myeloid cells 1	Stimulates neutrophil and monocyte-mediated inflammatory responses

benefit in myocardial remodeling in AMI [52]. Anakinra, a specific IL1RN, demonstrates potent suppression of markers of inflammation in non-ST elevation acute coronary syndromes [53].

Nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) may function as an inducer of apoptosis. The NLRP3 inflammatory body is a multimeric protein complex composed of NLRP3, apoptosis-related speck-like protein, and pro-caspase-1 [54]. It has been reported that in the case of AMI and heart ischemia, the formation of NLRP3 inflammatory bodies is increasingly triggered, which in turn damages the function and viability of myocardial cells [55,56]. Moreover, NLRP3 inflammasome inhibitors can effectively reduce the amount of myocardial necrosis during AMI [57–59]. These results indicate that the NLRP3 inflammasome has potential as a biomarker of AMI [60] and as a promising therapeutic target in the treatment of AMI [61].

Chemokine (C-X-C motif) ligand 2 (CXCL2), a member of the CXC chemokine family, is produced by activated

monocytes and neutrophils and expressed at the site of inflammation. In the inflammatory response, CXCL2 acts as a powerful neutrophil chemoattractant to recruit and adhere to neutrophils. Recent studies have shown that inflammation is an essential pathological process in the development of AMI, and CXCL2 plays a vital role in the development of AMI [62]. CXCL2 is highly expressed in the infarcted and marginal regions of myocardial infarction [63]. CXCL2 overexpression induces the rapid accumulation of neutrophils to the injury site. It releases proteolytic enzymes to directly damage the surrounding cells, causing ROS damage and leading to the formation of myocardial infarction [64]. Increased expression of CXCL2 in myocardial infarction will aggravate the acute inflammatory response after myocardial injury and promote heart rupture. In contrast, suppression of CXCL2 chemoattractant expression can regulate neutrophil recruitment to the injured myocardium and cardiac fibroblasts and relieve myocardial injury [65]. In the KEGG pathway analysis, we found that CXCL2 was enriched in cytokine-cytokine receptor

Table 5: GO and KEGG pathway enrichment analyses of DEGs in the most significant module

Pathway ID	Pathway description	Count	p-value
GO:0006954	Inflammatory response	7	1.01×10^{-8}
GO:0030593	Neutrophil chemotaxis	5	2.70×10^{-8}
GO:0045087	Innate immune response	6	1.25×10^{-6}
GO:0050786	RAGE receptor binding	3	1.39×10^{-5}
GO:0005576	Extracellular region	7	3.13×10^{-5}
GO:0051092	Positive regulation of NF- κ B transcription factor activity	4	3.94×10^{-5}
hsa04621	NOD-like receptor signaling pathway	2	3.21×10^{-2}

interaction, the NOD-like receptor signaling pathway, and the TNF signaling pathway. Thus, CXCL2 plays a significant role in myocardial infarction through the above pathways and can be a potential therapeutic target.

Dendritic cell-associated c-type lectin 1 (Dectin-1, also named CLEC7A) is an innate immune pattern recognition receptor that recognizes β -glucan on the cell wall. In a recent study, researchers demonstrated that upregulation of Dectin-1 could promote myocardial injury, inflammation, and fibrosis and then damage myocardial function. Dectin-1 can also induce activation of the NF- κ B/NLRP3 axis in infarcted myocardium [66]. Dectin-1 promotes myocardial ischemia-reperfusion injury by regulating macrophage polarization and neutrophil infiltration [67]. These studies revealed a new link between Dectin-1 in cardiac muscle cells and cardiac remodeling, suggesting that targeting the Dectin-1/NF- κ B/NLRP3 signaling pathway may be a promising treatment for AMI.

Chronic inflammation plays an essential role in the onset of atherosclerosis and its cardiovascular-related diseases. The immune response is a vital initiating factor of the inflammatory response, and the complement system is an important component of natural immunity and participates in the development of atherosclerosis [68]. The complement system is composed of more than 30 plasma proteins. After complement activation, active fragments with inflammatory mediators are generated, such as the production of various proinflammatory factors (including C3a, C4a, and C5a). These cytokines can stimulate cell degranulation and release histamine, as well as vasoactive substances that enhance vascular permeability and stimulate visceral smooth muscle contraction. C5a is the most active of these substances. C5a first binds to its receptor (C5a receptor; C5aR) so that it can be activated and can activate C5aR to exert its functions. C5a can also regulate the expression of inflammatory mediators, promote the accumulation of chemotaxis neutrophils, induce neutrophil degranulation, and cause inflammatory damage to vascular endothelial cells [69]. The C5a-C5aR1 axis seems to be involved in the occurrence of atherosclerosis and CHD [70]. Experimental studies have shown that when the C5a/C5aR1 axis is attenuated, the infarct size and inflammation will decrease [71]. In the *in vivo* AMI mouse model, the lack of circulating leukocyte C5aR1 led to a reduction in infarct size and improved clinical outcomes [72].

Triggering receptor expressed on myeloid cells 1 (TREM-1) is a vital signal receptor expressed on neutrophils and monocytes, and plays an important role in systemic infection. Current evidence suggests that TREM-1 may be a useful biomarker for predicting neonatal sepsis

and a biomarker of clinical diagnosis and prognosis for sepsis [73] and other types of acute and chronic inflammation-related diseases [74]. Inflammation is one of the most important complication factors of CHDs. The study found that TREM-1 is involved in the pathogenesis of acute and chronic cardiovascular diseases, such as AMI and atherosclerosis, and shows the prospect of using the TREM-1 pathway as a potential target for understanding and managing cardiovascular disease prospects [75].

Additionally, we conducted hierarchical clustering analysis of hub genes. The results show that these hub genes can distinguish myocardial infarction samples from non-myocardial infarction samples and may become diagnostic biomarkers. Studies have shown that neopterin is an independent predictor of all-cause and cardiovascular mortality. Serum neopterin concentration is associated with adverse cardiovascular events in patients with chronic stable angina pectoris, and this correlation is closely related to the severity of CHD [76]. Neopterin may therefore be a useful marker of patient's risk of future coronary events [77]. The main mechanism of AMI in patients with CHD is the rupture of unstable atherosclerotic plaque mediated by inflammatory cells, followed by intra-plaque or subplaque bleeding leading to thrombosis and lumen occlusion. As an inflammatory marker of macrophage immune activation, neopterin level can reflect the change in plaque stability, so it has a good prediction effect on the occurrence and prognosis of myocardial infarction in patients with CHD. Alteration of S100A8/S100A9, IL1RN, NLRP3, CXCL2, and C5AR1 is involved in inflammation and atherosclerosis, which constitute the crucial mechanisms of AMI formation, indicating that these genes may play essential roles in the progression and prognosis of AMI by regulating the concentration of neopterin.

It is worth noting that there have been articles that study DEGs in AMI. However, the results of those articles are somewhat different from ours. Compared with other AMI studies, this study performed a complete bioinformatics analysis of DEGs of AMI through statistical methods. We were able to provide more reliable results for the following reasons: (1) The selected four datasets are all based on the same platform. They can be combined into one dataset for normalized analysis, which increased the credibility of the results. (2) During the investigation, the original data were processed with background correction and missing value supplementation to ensure that the information was valid and reliable. (3) The sample size of previous articles is relatively small. We selected 4 datasets with a sample size of 209, and the DEGs selected were more accurate. Of course, it is also important to verify these results in the subsequent experiments.

In summary, our study used bioinformatics methods to analyze the gene chips of a large-sample myocardial infarction population and control population and identified DEGs that may be involved in the occurrence and development of AMI. The study provides reliable molecular biomarkers for AMI screening, diagnosis, and prognosis; it also provides a basis for exploring new therapeutic targets for AMI. Compared with other studies on AMI, the innovation and advantage of this study lie in the use of multichip joint analysis and the use of the same platform, with a larger sample size. However, no experimental verification is the limitation of this study. Thus, to verify the current results, further studies are needed to elucidate the biological function of these genes in AMI.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

- [1] Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. *Lancet*. 2017 Jan 14;389(10065):197–210. doi: 10.1016/S0140-6736(16)30677-8.
- [2] Shapiro MD, Fazio S. From lipids to inflammation: new approaches to reducing atherosclerotic risk. *Circ Res*. 2016;118(4):732–49. doi: 10.1161/CIRCRESAHA.115.306471.
- [3] Nadir MA, Struthers AD. Family history of premature coronary heart disease and risk prediction. *Heart*. 2011;97(8):684. doi: 10.1136/hrt.2011.222265.
- [4] Wang Y, Li J, Zheng X, Jiang Z, Hu S, Wadhera RK, et al. Risk factors associated with major cardiovascular events 1 year after acute myocardial infarction. *JAMA Netw Open*. 2018;1(4):e181079. doi: 10.1001/jamanetworkopen.2018.1079.
- [5] Aydin S, Ugur K, Aydin S, Sahin İ, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. *Vasc Health Risk Manag*. 2019;15:1–10. doi: 10.2147/VHRM.S166157.
- [6] Lin F, Gong X, Yu P, Yue A, Meng Q, Zheng L, et al. Distinct circulating expression profiles of long noncoding RNAs in heart failure patients with ischemic and nonischemic dilated cardiomyopathy. *Front Genet*. 2019;10:1116. doi: 10.3389/fgene.2019.01116.
- [7] Edgar R, Domrachev M, Lash AE. Gene expression omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res*. 2002;30(1):207–10. doi: 10.1093/nar/30.1.207.
- [8] Rothman AMK, Morton AC, Chico TJ, Crossman DC, Milo M. Peripheral whole blood microRNA signature differentiates between myocardial infarction and unstable angina. *Heart*. 2011;97(20):e7. doi: 10.1136/heartjnl-2011-300920b.16.
- [9] Suresh R, Li X, Chiriac A, Goel K, Terzic A, Perez-Terzic C, et al. Transcriptome from circulating cells suggests dysregulated pathways associated with long-term recurrent events following first-time myocardial infarction. *J Mol Cell Cardiol*. 2014;74:13–21. doi: 10.1016/j.jmcc.2014.04.017.
- [10] Muse ED, Kramer ER, Wang H, Barrett P, Parviz F, Novotny MA, et al. A whole blood molecular signature for acute myocardial infarction. *Sci Rep*. 2017;7(1):12268. doi: 10.1038/s41598-017-12166-0.
- [11] Fan L, Meng H, Guo X, Li X, Meng F. Differential gene expression profiles in peripheral blood in Northeast Chinese Han people with acute myocardial infarction. *Genet Mol Biol*. 2018;41(1):59–66. doi: 10.1590/1678-4685-GMB-2017-0075.
- [12] Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics*. 2012;28(6):882–3. doi: 10.1093/bioinformatics/bts034.
- [13] Gautier L, Cope L, Bolstad BM, Irizarry RA. Affy-analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics*. 2004;20(3):307–15. doi: 10.1093/bioinformatics/btg405.
- [14] Ritchie ME, Phipson B, Wu DI, Hu Y, Law CW, Shi W, et al. LIMMA powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. doi: 10.1093/nar/gkv007.
- [15] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. *Nat Genet*. 2000;25(1):25–9. doi: 10.1038/75556.
- [16] Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28(1):27–30. doi: 10.1093/nar/28.1.27.
- [17] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44–57. doi: 10.1038/nprot.2008.211.
- [18] Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607–13. doi: 10.1093/nar/gky1131.
- [19] Smoot ME, Ono K, Ruscheinski J, Wang P-L, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics*. 2011;27(3):431–2. doi: 10.1093/bioinformatics/btq675.
- [20] Chin C-H, Chen S-H, Wu H-H, Ho C-W, Ko M-T, Lin C-Y. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol*. 2014;8(Suppl 4):S11. doi: 10.1186/1752-0509-8-S4-S11.
- [21] Oliveira JB, Soares AASM, Sposito AC. Inflammatory response during myocardial infarction. *Adv Clin Chem*. 2018;84:39–79. doi: 10.1016/bs.acc.2017.12.002.
- [22] Burke GM, Genuardi M, Shappell H, D'Agostino RB, Magnani JW. Temporal associations between smoking and cardiovascular disease, 1971 to 2006 (from the framingham heart study). *Am J Cardiol*. 2017 Nov 15;120(10):1787–91. doi: 10.1016/j.amjcard.2017.07.087.
- [23] Huang S, Frangogiannis NG. Anti-inflammatory therapies in myocardial infarction: failures, hopes and challenges. *Br J Pharmacol*. 2018;175(9):1377–400. doi: 10.1111/bph.14155.
- [24] Fang L, Moore X-L, Dart AM, Wang L-M. Systemic inflammatory response following acute myocardial infarction. *J Geriatr*

- Cardiol. 2015;12(3):305–12. doi: 10.11909/j.issn.1671-5411.2015.03.020.
- [25] Nicholson C, Hrabětová S. Brain extracellular space: the final frontier of neuroscience. *Biophys J*. 2017;113(10):2133–42. doi: 10.1016/j.bpj.2017.06.052.
- [26] Boulanger CM, Loyer X, Rautou P-E, Amabile N. Extracellular vesicles in coronary artery disease. *Nat Rev Cardiol*. 2017 May;14(5):259–72. doi: 10.1038/NRCARDIO.2017.7.
- [27] Jansen F, Nickenig G, Werner N. Extracellular vesicles in cardiovascular disease: potential applications in diagnosis, prognosis, and epidemiology. *Circ Res*. 2017;120(10):1649–57. doi: 10.1161/CIRCRESAHA.117.310752.
- [28] Zarkasi KA, Jen-Kit T, Jubri Z. Molecular understanding of the cardiomodulation in myocardial infarction and the mechanism of vitamin E protections. *Mini Rev Med Chem*. 2019;19(17):1407–26. doi: 10.2174/1389557519666190130164334.
- [29] Wang S, Song R, Wang Z, Jing Z, Wang S, Ma J. S100A8/A9 in inflammation. *Front Immunol*. 2018;9:1298. doi: 10.3389/fimmu.2018.01298.
- [30] Schiopu A, Cotoi OS. S100A8 and S100A9: DAMPs at the crossroads between innate immunity, traditional risk factors, and cardiovascular disease. *Mediators Inflamm*. 2013;2013:828354. doi: 10.1155/2013/828354.
- [31] Sreejit G, Nooti SK, Athmanathan B, Nagareddy PR. S100A8/A9 in myocardial infarction. *Methods Mol Biol*. 2019;1929:739–54. doi: 10.1007/978-1-4939-9030-6_46.
- [32] Katashima T, Naruko T, Terasaki F, Fujita M, Otsuka K, Murakami S, et al. Enhanced expression of the S100A8/A9 complex in acute myocardial infarction patients. *Circ J*. 2010;74(4):741–8. doi: 10.1253/circj.cj-09-0564.
- [33] Li Y, Chen B, Yang X, Zhang C, Jiao Y, Li P, et al. S100a8/a9 signaling causes mitochondrial dysfunction and cardiomyocyte death in response to ischemic/reperfusion injury. *Circulation*. 2019;140(9):751–64. doi: 10.1161/CIRCULATIONAHA.118.039262.
- [34] Du CQ, Yang L, Han J, Yang J, Yao XY, Hu XS, et al. The elevated serum S100A8/A9 during acute myocardial infarction is not of cardiac myocyte origin. *Inflammation*. 2012;35(3):787–96. doi: 10.1007/s10753-011-9375-8.
- [35] Frangogiannis NG. S100A8/A9 as a therapeutic target in myocardial infarction: cellular mechanisms, molecular interactions, and translational challenges. *Eur Heart J*. 2019;40(32):2724–6. doi: 10.1093/eurheartj/ehz524.
- [36] Saito T, Hojo Y, Ogoyama Y, Hirose M, Ikemoto T, Katsuki T, et al. S100A12 as a marker to predict cardiovascular events in patients with chronic coronary artery disease. *Circ J*. 2012;76(11):2647–52. doi: 10.1253/circj.cj-12-0093.
- [37] Garlanda C, Riva F, Bonavita E, Mantovani A. Negative regulatory receptors of the IL-1 family. *SemImmunology*. 2013;25(6):408–15. doi: 10.1016/j.smim.2013.10.019.
- [38] Mantovani A, Barajon I, Garlanda C. IL-1 and IL-1 regulatory pathways in cancer progression and therapy. *Immunol Rev*. 2018;281(1):57–61. doi: 10.1111/imr.12614.
- [39] Boraschi D, Italiani P, Weil S, Martin MU. The family of the interleukin-1 receptors. *Immunol Rev*. 2018;281(1):197–232. doi: 10.1111/imr.12606.
- [40] Peters VA, Joesting JJ, Freund GG. IL-1 receptor 2 (IL-1R2) and its role in immune regulation. *Brain Behav Immun*. 2013;32:1–8. doi: 10.1016/j.bbi.2012.11.006.
- [41] Autenshlyus A, Arkhipov S, Mikhailova E, Marinkin I, Arkhipova V, Varaksin N. The relationship between cytokine production, CSF2RA, and IL1R2 expression in mammary adenocarcinoma, tumor histopathological parameters, and lymph node metastasis. *Technol Cancer Res Treat*. 2019;18:1533033819883626. doi: 10.1177/1533033819883626.
- [42] Liu J, Yang Y, Li H, Liu Y, Sun Y, Wu J, et al. IL1R2 polymorphisms are associated with increased risk of esophageal cancer. *Curr Mol Med*. 2020;20(5):379–87. doi: 10.2174/1566524019666191025091204.
- [43] Niu F, Wang T, Li J, Yan M, Li D, Li B, et al. The impact of genetic variants in IL1R2 on cervical cancer risk among Uyghur females from China: a case-control study. *Mol Genet Genomic Med*. 2019;7(1):e00516. doi: 10.1002/mggg.3516.
- [44] Xiong Z, Sun Y, Wu J, Niu F, Jin T, Li B. Genetic polymorphisms in IL1R1 and IL1R2 are associated with susceptibility to thyroid cancer in the Chinese Han population. *J Gene Med*. 2019;21(6):e3093. doi: 10.1002/jgm.3093.
- [45] Lian Z, Lv F-F, Yu J, Wang J-W. The anti-inflammatory effect of microRNA-383-3p interacting with IL1R2 against homocysteine-induced endothelial injury in rat coronary arteries. *J Cell Biochem*. 2018;119(8):6684–94. doi: 10.1002/jcb.26854.
- [46] Pou J, Martínez-González J, Rebollo A, Rodríguez C, Rodríguez-Calvo R, Martín-Fuentes P, et al. Type II interleukin-1 receptor expression is reduced in monocytes/macrophages and atherosclerotic lesions. *Biochim Biophys Acta*. 2011;1811(9):556–63. doi: 10.1016/j.bbalip.2011.05.014.
- [47] Chen DQ, Kong XS, Shen XB, Huang MZ, Zheng JP, Sun J, et al. Identification of differentially expressed genes and signaling pathways in acute myocardial infarction based on integrated bioinformatics analysis. *Cardiovasc Ther*. 2019;2019:8490707. doi: 10.1155/2019/8490707.
- [48] Zhang R, Ji Z, Qu Y, Yang M, Su Y, Zuo W, et al. Clinical value of ARG1 in acute myocardial infarction patients: Bioinformatics-based approach. *Biomed Pharmacother*. 2020;121:109590. doi: 10.1016/j.biopha.2019.109590.
- [49] Spinello A, Vecile E, Abbate A, Dobrina A, Magistrato A. How can Interleukin-1 receptor antagonist modulate distinct cell death pathways? *J Chem Inf Model*. 2019;59(1):351–9. doi: 10.1021/acs.jcim.8b00565.
- [50] Abbate A, Van Tassell BW, Biondi-Zoccai G, Kontos MC, Grizzard JD, Spillman DW, et al. Effects of interleukin-1 blockade with anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction from the virginia commonwealth university-Anakinra remodeling trial (2) (VCU-ART2) pilot study. *Am J Cardiol*. 2013;111(10):1394–400. doi: 10.1016/j.amjcard.2013.01.287.
- [51] Toldo S, Schatz AM, Mezzaroma E, Chawla R, Stallard TW, Stallard WC, et al. Recombinant human interleukin-1 receptor antagonist provides cardioprotection during myocardial ischemia reperfusion in the mouse. *Cardiovasc Drugs Ther*. 2012;26(3):273–6. doi: 10.1007/s10557-012-6389-x.
- [52] van Minkelen R, Wettinger SB, de Visser MC, Vos HL, Reitsma PH, Rosendaal FR, et al. Haplotypes of the interleukin-1 receptor antagonist gene, interleukin-1 receptor antagonist mRNA levels and the risk of myocardial infarction. *Atherosclerosis*. 2009;203(1):201–5. doi: 10.1016/j.atherosclerosis.2008.06.029.
- [53] Morton AC, Rothman AM, Greenwood JP, Gunn J, Chase A, Clarke B, et al. The effect of interleukin-1 receptor antagonist

- therapy on markers of inflammation in non-ST elevation acute coronary syndromes: the MRC-ILA heart study. *Eur Heart J.* 2015;36(6):377–84. doi: 10.1093/eurheartj/ehu272.
- [54] Man SM, Kanneganti T-D. Gasdermin D: the long-awaited executioner of pyroptosis. *Cell Res.* 2015;25(11):1183–4. doi: 10.1038/cr.2015.124.
- [55] Toldo S, Abbate A. The NLRP3 inflammasome in acute myocardial infarction. *Nat Rev Cardiol.* 2018;15(4):203–14. doi: 10.1038/nrcardio.2017.161.
- [56] Gao R, Shi H, Chang S, Gao Y, Li X, Lv C, et al. The selective NLRP3-inflammasome inhibitor MCC950 reduces myocardial fibrosis and improves cardiac remodeling in a mouse model of myocardial infarction. *Int Immunopharmacol.* 2019;74:105575. doi: 10.1016/j.intimp.2019.04.022.
- [57] Van Hout GP, Bosch L, Ellenbroek GH, De Haan JJ, Van Solinge WW, Cooper MA, et al. The selective NLRP3-inflammasome inhibitor MCC950 reduces infarct size and preserves cardiac function in a pig model of myocardial infarction. *Eur Heart J.* 2017;38(11):828–36. doi: 10.1093/eurheartj/ehw247.
- [58] Buckley LF, Libby P. Inhibiting NLRP3 Inflammasome activity in acute myocardial infarction: a review of pharmacologic agents and clinical outcomes. *J Cardiovasc Pharmacol.* 2019;74(4):297–305. doi: 10.1097/FJC.0000000000000701.
- [59] Chen G, Song X, Lin D, Xu P. Isofraxidin alleviates myocardial infarction through NLRP3 inflammasome inhibition. *Inflammation.* 2020;43:712–21. doi: 10.1007/s10753-019-01158-z.
- [60] Bullón P, Cano-García FJ, Alcocer-Gómez E, Varela-López A, Roman-Malo L, Ruiz-Salmerón RJ, et al. Could NLRP3-inflammasome be a cardiovascular risk biomarker in acute myocardial infarction patients? *Antioxid Redox Signal.* 2017;27(5):269–75. doi: 10.1089/ars.2016.6970.
- [61] Mauro AG, Bonaventura A, Mezzaroma E, Quader M, Toldo S. NLRP3 Inflammasome in acute myocardial infarction. *J Cardiovasc Pharmacol.* 2019;74(3):175–87. doi: 10.1097/FJC.0000000000000717.
- [62] Guo L-Y, Yang F, Peng L-J, Li Y-B, Wang A-P. CXCL2, a new critical factor and therapeutic target for cardiovascular diseases. *Clin Exp Hypertens.* 2020;42(5):428–37. doi: 10.1080/10641963.2019.1693585.
- [63] Taleb S, Tedgui A, Mallat Z. IL-17 and Th17 cells in atherosclerosis: subtle and contextual roles. *Arterioscler Thromb Vasc Biol.* 2015;35(2):258–64. doi: 10.1161/ATVBAHA.114.303567.
- [64] Al-Amran FF, Shahkolahi M. Oxytocin ameliorates the immediate myocardial injury in heart transplant through down regulation of the neutrophil dependent myocardial apoptosis. *Heart Views.* 2014;15(2):37–45. doi: 10.4103/1995-705X.137493.
- [65] Mylonas KJ, Turner NA, Bageghni SA, Kenyon CJ, White CI, McGregor K, et al. 11 β -HSD1 suppresses cardiac fibroblast CXCL2, CXCL5 and neutrophil recruitment to the heart post MI. *J Endocrinol.* 2017;233(3):315–27. doi: 10.1530/JOE-16-0501.
- [66] Li X, Bian Y, Pang P, Yu S, Wang X, Gao Y, et al. Inhibition of Dectin-1 in mice ameliorates cardiac remodeling by suppressing NF- κ B/NLRP3 signaling after myocardial infarction. *Int Immunopharmacol.* 2020;80:106116. doi: 10.1016/j.intimp.2019.106116.
- [67] Fan Q, Tao R, Zhang H, Xie H, Lu L, Wang T, et al. Dectin-1 contributes to myocardial ischemia/reperfusion injury by regulating macrophage polarization and neutrophil infiltration. *Circulation.* 2019;139(5):663–78. doi: 10.1161/CIRCULATIONAHA.118.036044.
- [68] Speidl WS, Kastl SP, Huber K, Wojta J. Complement in atherosclerosis: friend or foe? *J Thrombosis Haemost.* 2011 Mar;9(3):428–40. doi: 10.1111/j.1538-7836.2010.04172.x.
- [69] Yan C, Gao H. New insights for C5a and C5a receptors in sepsis. *Front Immunol.* 2012;3:368. doi: 10.3389/fimmu.2012.00368.
- [70] An G, Ren G, An F, Zhang C. Role of C5a-C5aR axis in the development of atherosclerosis. *Sci China Life Sci.* 2014;57(8):790–4. doi: 10.1007/s11427-014-4711-5.
- [71] Ajona D, Zandueta C, Corrales L, Moreno H, Pajares MJ, Ortiz-Espinosa S, et al. Blockade of the complement C5a/C5aR1 axis impairs lung cancer bone metastasis by CXCL16-mediated effects. *Am J Respir Crit Care Med.* 2018;197(9):1164–76. doi: 10.1164/rccm.201703-0660OC.
- [72] De Hoog VC, Timmers L, Van Duijvenvoorde A, De Jager SC, Van Middelaar BJ, Smeets MB, et al. Leucocyte expression of complement C5a receptors exacerbates infarct size after myocardial reperfusion injury. *Cardiovasc Res.* 2014;103(4):521–9. doi: 10.1093/cvr/cvu153.
- [73] Bellos I, Fitrou G, Daskalakis G, Thomakos N, Papantoniou N, Pergialiotis V. Soluble TREM-1 as a predictive factor of neonatal sepsis: a meta-analysis. *Inflamm Res.* 2018;67(7):571–8. doi: 10.1007/s00011-018-1149-4.
- [74] Bosco MC, Raggi F, Varesio L. Therapeutic potential of targeting TREM-1 in inflammatory diseases and cancer. *Curr Pharm Des.* 2016;22(41):6209–33. doi: 10.2174/1381612822666160826110539.
- [75] Kouassi KT, Gunasekar P, Agrawal DK, Jadhav GP. TREM-1; Is it a pivotal target for cardiovascular diseases? *J Cardiovasc Dev Dis.* 2018;5(3):45. doi: 10.3390/jcdd5030045.
- [76] Grammer TB, Fuchs D, Boehm BO, Winkelmann BR, Maerz W. Neopterin as a predictor of total and cardiovascular mortality in individuals undergoing angiography in the Ludwigshafen risk and cardiovascular health study. *Clin Chem.* 2009;55(6):1135–46. doi: 10.1373/clinchem.2008.118844.
- [77] Avanzas P, Arroyo-Espliguero R, Quiles J, Roy D, Kaski JC. Elevated serum neopterin predicts future adverse cardiac events in patients with chronic stable angina pectoris. *Eur Heart J.* 2005;26(5):457–63. doi: 10.1093/eurheartj/ehi111.