Original Article

Differences in T cell-associated serum markers between ischemic cardiomyopathy and dilated cardiomyopathy

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Background: Ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM) have similar clinical manifestations but differ in pathogenesis. We aimed to identify T cell-associated serum markers that can be used to distinguish between ICM and DCM.

Methods: We identified differentially expressed genes (DEGs) with transcriptome sequencing data in GSE116250, and then conducted enrichment analysis of DEGs in the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. Protein-protein interaction (PPI) networks were used to analyze the relationship between T cells-related genes and identify hub genes. Enzyme-linked immunosorbent assay (ELISA) kits were used to detect T cell-associated proteins in serum, and receiver operating characteristic (ROC) curves were used to evaluate the diagnostic efficacy of these serum markers.

Results: Using the limma package and Venn plots, we found that the non-failing donors (NFD) and DCM groups shared many of the same DEGs and DEGs-enriched functions compared to the ICM group, which were involved in T cell activation and differentiation, among other functions. Subsequently, the immune cell score showed no difference between NFD and DCM, but they were significantly different from ICM patients in CD8 T cells CD4 T cells memory resting and activated, T cells follicular helper, and M1 macrophage. After analyzing T cell-associated DEGs, it was found that 4 DEGs encoding secreted proteins were highly expressed in the ICM group compared with the NFD and DCM groups, namely chemokine (C-C motif) ligand 21 (*CCL21*), interleukin (*IL*)-*1β*, lymphocyte-activation gene 3 (*LAG3*), and vascular cell adhesion molecule-1 (*VCAM-1*). Importantly, the serum levels of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in ICM patients were all significantly higher than those in DCM patients. The ROC curves showed that the area under the curve (AUC) values of serum *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* were 0.775, 0.868, 0.934, and 0.903, respectively.

Conclusions: We have identified four T cell-associated serum markers, *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*, as potential diagnostic serum markers that differentiate ICM from DCM.

Keywords: Ischemic cardiomyopathy (ICM); dilated cardiomyopathy (DCM); T cell; differentially expressed genes (DEGs); diagnosis

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Introduction

Heart failure (HF) is the terminal stage of the progression of various cardiovascular diseases, and is one of the main direct causes of death in patients with cardiovascular diseases, with reduced exercise tolerance, respiratory weakness and edema, and the mortality rate of patients within 5 years is as high as 60% (1,2). Ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM) are the two most important diseases that cause HF, and although the clinical manifestations of patients with these diseases are basically the same, the clinical treatment protocols are completely different due to their different pathogeneses (3,4). Therefore, differentiation of which cardiomyopathy is causing HF would be necessary but may not be possible in the emergency department.

Currently, coronary angiography (CAG) is the gold standard for differentiating between ICM and DCM (5,6). However, as an invasive test, CAG not only has certain risks, but is also very expensive, and many grassroots medical institutions are limited by equipment and personnel constraints to carry out CAG testing (7,8). Serological indicators are important auxiliary indicators for clinical differential diagnosis of various diseases, which have the advantage of rapidity and low equipment and personnel requirements. However, there are currently few serologic markers that can be used to distinguish between ICM and DCM, mainly due to the current lack of understanding of the pathogenesis of DCM. With the development of high-throughput sequencing, multiple pathological mechanisms related to the pathogenesis of DCM have been discovered, including infectious or non-infectious inflammation, autoimmune diseases, endocrine and metabolic disorders, poisoning, and genetic diseases (9,10). In addition, a number of new biomarkers for improving cardiac function, such as sodium/glucose cotransporter 2 (SGLT2), have also been screened (11,12). The pathogenesis of ICM is relatively clear, that is, myocardial injury caused by long-term ischemia and hypoxia of the myocardium caused by coronary arteriosclerosis (13). Therefore, pathogenesis-based serological indicators are the future of rapid differentiation between ICM and DCM.

In this study, we used transcriptome sequencing data from heart tissue in public databases (GSE116250, a set of RNA-sequencing data on cardiac tissue from patients with DCM and ICM) to analyze the differences in pathogenesis between ICM and DCM, and then looked for secreted proteins from the hub genes involved in the pathogenesis. Potential serological markers to distinguish between ICM and DCM were identified by detecting and comparing these differentially expressed genetically encoded secreted proteins in serum. We present this article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-901/rc).

Highlight box

Key findings

- Chemokine (C-C motif) ligand 21 (*CCL21*), interleukin *(IL)-1β*, lymphocyte-activation gene 3 (*LAG3*), and vascular cell adhesion molecule-1 (*VCAM-1*) were found to be able to distinguish between ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM).

What is known and what is new?

- Differentiating between ICM and DCM is critical for clinical management, and pathogenesis-based serological indicators are the future of rapid differentiation between ICM and DCM.
- Four serum markers were identified to distinguish between ICM and DCM.

What is the implication, and what should change now?

- We can quickly distinguish between ICM and DCM by measuring *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in serum without the need for cumbersome imaging tests. And this can help doctors quickly determine treatment protocols in an emergency clinic.

Methods

Patients and ethics statement

From January 2022 to December 2023 in Department of Cardiovascular Medicine, The First Affiliated Hospital of Bengbu Medical University, we enrolled 97 patients hospitalized for HF which was diagnosed according to 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic HF (14), including 48 patients with DCM and 49 patients with ICM. At the same time, we also enrolled 25 age- and sex-matched healthy volunteers. Demographic and clinical characteristics of all participants are shown in *Table 1*. The inclusion and exclusion criteria for patients with HF were as follows. Inclusion criteria: (I) patients with ICM are diagnosed with stenosis of at least 1 coronary artery by CAG; (II) patients with DCM are diagnosed by CAG without coronary artery stenosis; (III) complete clinical data. Exclusion criteria: (I) comorbid with other types of heart disease; (II) combined with severe liver and kidney insufficiency, hoof tissue disease, malignant tumors,

Table 1 Demographic and clinical characteristics of all participants

Variables	Healthy (N=25)	DCM (N=48)	ICM (N=49)	P value
Age (years)	55.32±6.71	56.24±6.35	56.27±7.03	0.42
Male	15 (60.00)	34 (70.83)	36 (73.46)	0.72
BMI (kg/m^2)	22.12±3.41	22.41±2.51	21.99±4.02	0.36
NYHA grade, n (%)				0.50
II	–	18 (37.50)	19 (38.78)	
III	–	24 (50.00)	20 (40.82)	
IV	–	6 (12.50)	10 (20.41)	

Data are presented as mean ± standard deviation or n (%). P value indicates difference between DCM group and ICM group. BMI, body mass index; NYHA, New York Heart Association; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy.

thyroid diseases, and severe infectious diseases; (III) record of invasive treatment within half a year; (IV) infectious diseases, drug addiction, or alcohol addiction; (V) pregnant or lactating females; (VI) diseases of the immune system or blood system. This study protocol was reviewed and annotated by the Ethics Committee of The First Affiliated Hospital of Bengbu Medical University (approval number [2023]ky039), and the study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all individual participants.

Cardiac transcriptome sequencing data analysis

There were 14 non-failing donors (NFD), 37 DCM patients, and 13 ICM patients who had contributed their left ventricle for transcriptome sequencing, and RNA-sequencing data had been uploaded to the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) by Sweet *et al.* and Yamaguchi *et al.* (15,16). We used the GEO2R tool on the National Center for Biotechnology Information (NCBI) platform to identify differentially expressed genes (DEGs) among ICM versus NFD, DCM versus NFD, and DCM versus ICM, and the Bioinformatics online tool (http://www.bioinformatics.com.cn) to visualize DEGs as volcano maps. At the same time, we functionally enriched DEGs in the Gene Ontology (GO) database (http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/) and visualized them using the Bioinformatics online tool. In addition, we extracted the data of gene expression, and visualization was performed using the heatmaps package in R software (https://bioconductor.org/packages/release/bioc/html/heatmaps.html). Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) algorithm was used to evaluate immune cells infiltration in the heart tissues (LM22 signature, 1,000 permutations) (17,18).

Screening of hub-genes analysis

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) online database (https://cn.string-db.org/) was used to analyze protein-molecule interactions, namely protein-protein interaction (PPI) networks. And then we exported score results of molecular interconnection to screen top 10 hub-genes.

Detection of serum CCL21, IL-1β, LAG3, and VCAM-1

In the early morning fasting condition, 5–10 mL of peripheral blood was collected from all participants and centrifuged (room temperature and 1,000 ×g) to obtain serum. The serum levels of chemokine (C-C motif) ligand 21 (*CCL21*), interleukin (*IL*)-*1β*, lymphocyte-activation gene 3 (*LAG3*), and vascular cell adhesion molecule-1 (*VCAM-1*) were determined using a human exodus 2 (*CCL21*) enzyme-linked immunosorbent assay (ELISA) kit (ab193759, Abcam, Cambridge, UK), human IL-1 beta ELISA kit (ab214025, Abcam), human LAG-3 ELISA kit (ab215061, Abcam), and human VCAM-1 ELISA kit (ab223591, Abcam), respectively.

Statistical analysis

Measurement data in this study were expressed as (mean ± standard deviation) and counting data were shown as n (%).

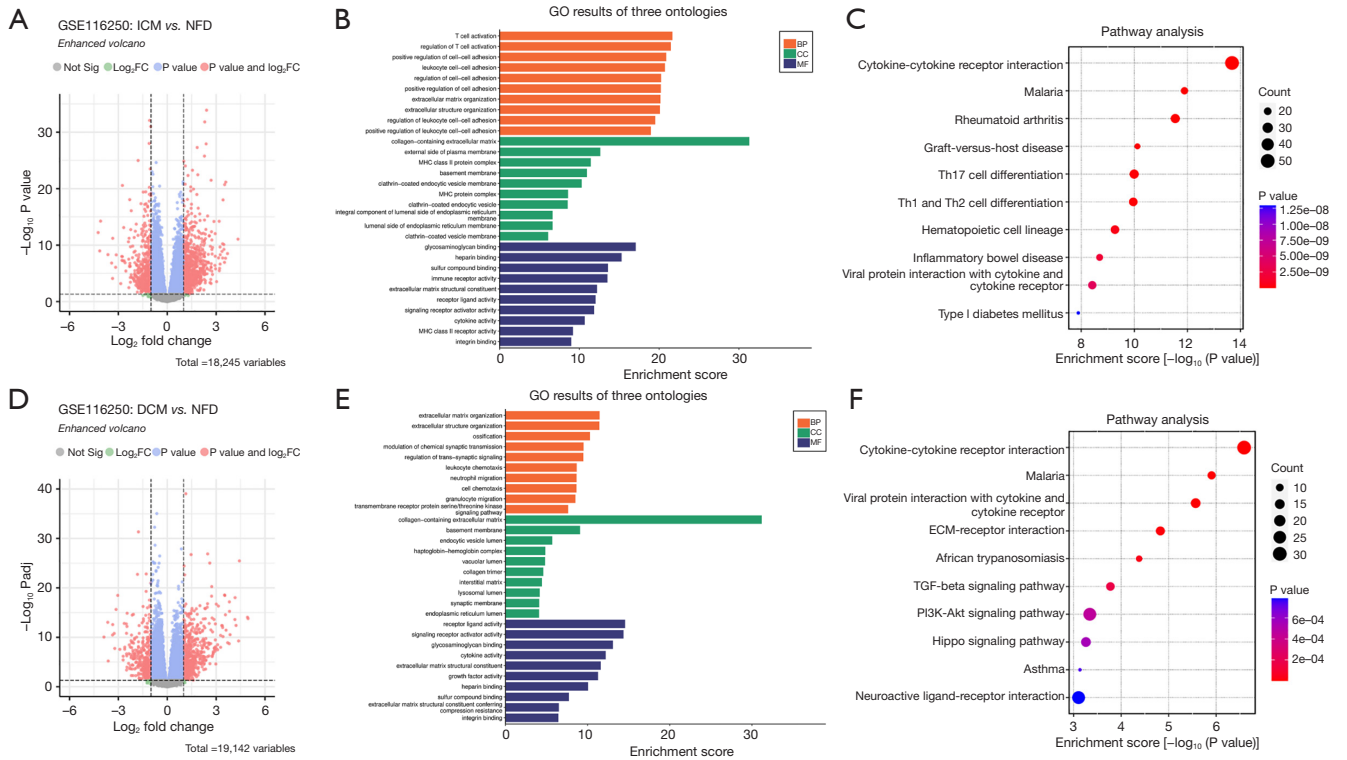


Figure 1 Identification and enrichment analysis of DEGs between ICM/DCM and NFD groups. (A) Volcano map of DEGs between ICM and NFD groups. (B,C) Enrichment analysis of DEGs between ICM and NFD groups in GO database (B) and KEGG database (C). (D) Volcano map of DEGs between DCM and NFD groups. (E,F) Enrichment analysis of DEGs between DCM and NFD groups in GO database (E) and KEGG database (F). ICM, ischemic cardiomyopathy; NFD, non-failing donors; FC, fold change; GO, Gene Ontology; BP, Biological Process; CC, Cell Component; MF, Molecular Function; MHC, major histocompatibility complex; DCM, dilated cardiomyopathy; ECM, extracellular matrix; TGF, transforming growth factor; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

The software SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The chi-square test was used to compare the differences in counting data between the two groups, and the Student's *t*-test was used to compare the differences in quantitative data between the two groups. One-way analysis of variance (ANOVA) was used for continuous data comparison among the three groups. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic efficacy of serum *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in patients with ICM. A P value <0.05 indicated a significant difference.

Results

Identification and enrichment analysis of DEGs versus NFD

Transcriptome sequencing was performed on the left ventricles of 14 NFD, 37 DCM, and 13 ICM. Firstly, we compared transcriptome data between ICM and NFD groups to identify DEGs in ICM patients, and there were 1,263 DEGs that met the screening requirements [$|\log_2$ fold change (FC)$|$ >1 and adjusted P<0.05], comprising 418 DEGs of down-regulation and 845 DEGs of up-regulation (*Figure 1A*). Functional enrichment analysis of DEGs in the GO database showed that there were altogether 1,986 features enriched, including T cell activation, leukocyte cell-cell adhesion, cytokine activity, cytokine receptor binding, immune receptor activity, receptor ligand activity, signaling receptor activator activity, and so on (*Figure 1B* and available online: https://cdn.amegroups.cn/static/public/jtd-24-901-1.xlsx). Besides, the results of enrichment analysis of DEGs in the KEGG database showed a total of 74 pathways, including cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, cell adhesion molecules, Th17 cell differentiation, and Th1 and Th2 cell differentiation,

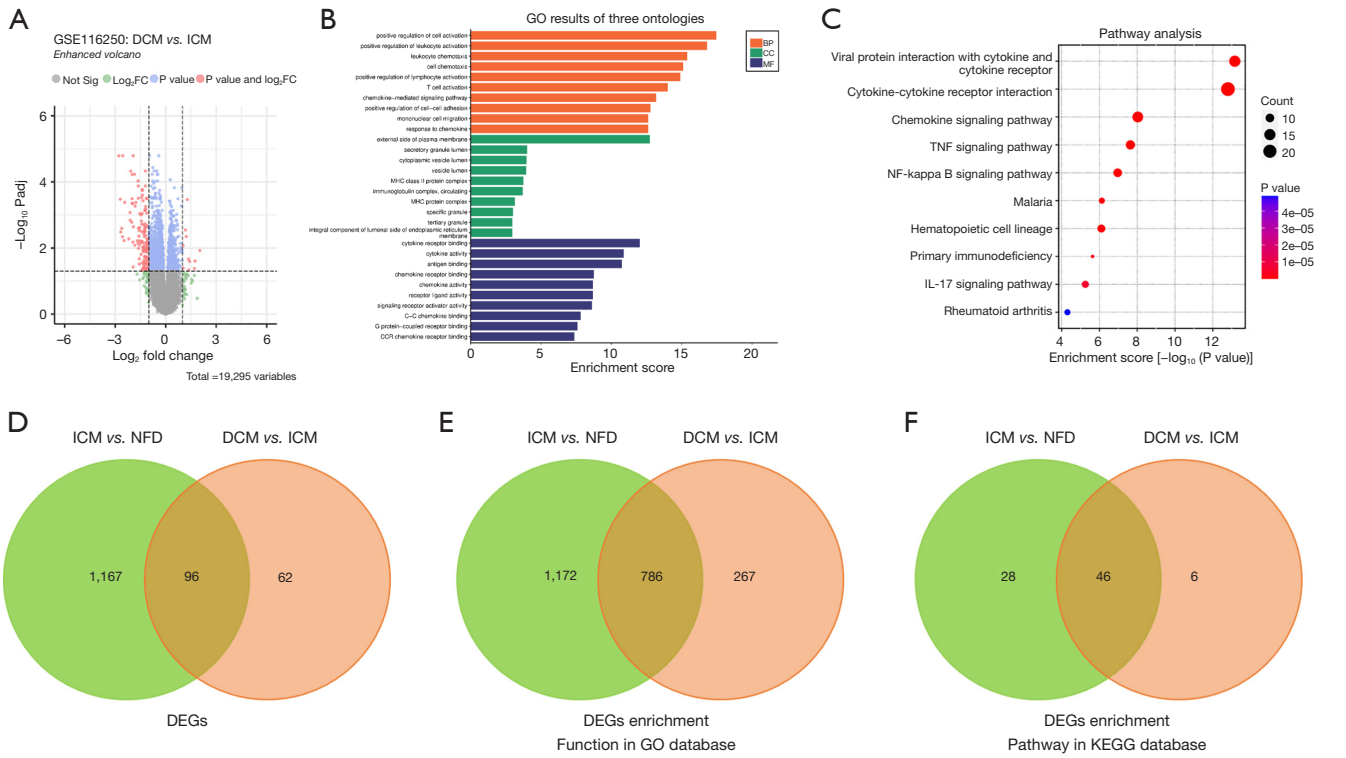


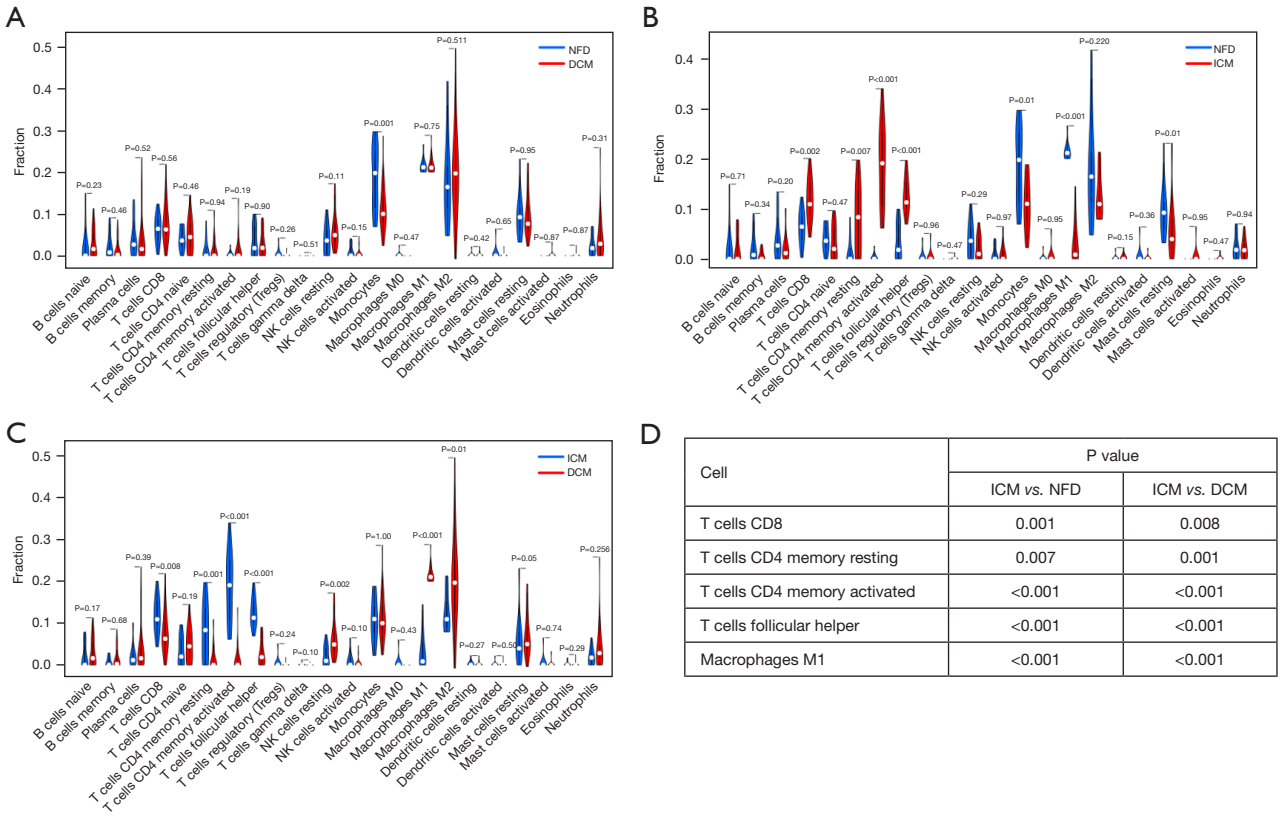
Figure 2 Identification and enrichment analysis of DEGs between ICM and DCM groups. (A) Volcano map of DEGs between ICM and DCM groups; (B,C) enrichment analysis of DEGs between ICM and DCM groups in GO database (B) and KEGG database (C). (D-F) Intersection of DEGs (D) and enrichment analysis results in GO database (E) and KEGG database (F) between different comparisons in GSE116250. DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; GO, Gene Ontology; BP, Biological Process; CC, Cell Component; MF, Molecular Function; MHC, major histocompatibility complex; CCR, C-C chemical affinity receptor; TNF, tumor necrosis factor; NFD, non-failing donors; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

among others (*Figure 1C* and available online: https://cdn.amegroups.cn/static/public/jtd-24-901-1.xlsx).

In addition, there were 956 DEGs between DCM versus NFD that met the screening requirements (|$\log_2$ FC| >1 and adjusted P<0.05), comprising 341 DEGs of down-regulation and 615 DEGs of up-regulation (*Figure 1D*). Functional enrichment analysis of DEGs in the GO database showed that altogether 1,328 features were enriched, including extracellular matrix organization, extracellular structure organization, modulation of chemical synaptic transmission, regulation of trans-synaptic signaling and cellular calcium ion homeostasis, and so on (*Figure 1E* and available online: https://cdn.amegroups.cn/static/public/jtd-24-901-2.xlsx). Besides, the results of enrichment analysis of DEGs in the KEGG database showed a total of 36 pathways, including cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, ECM-receptor interaction, African trypanosomiasis, and transforming growth factor (TGF)-beta signaling pathway (*Figure 1F* and available online: https://cdn.amegroups.cn/static/public/jtd-24-901-2.xlsx).

Screening DEGs to identify ICM

First of all, there were 158 DEGs between DCM and ICM that met the screening requirements (|$\log_2$ FC| >1 and adjusted P<0.05), comprising 147 DEGs of down-regulation and 11 DEGs of up-regulation (*Figure 2A*). Functional enrichment analysis of DEGs in the GO database showed that altogether 1,053 features were enriched, including positive regulation of cell activation, positive regulation of leukocyte activation, leukocyte chemotaxis, cell chemotaxis, positive regulation of lymphocyte activation, T cell activation, and so on (*Figure 2B* and Table S1). Moreover, the results of enrichment analysis of DEGs in the KEGG database showed a total of 52 pathways, including cytokine-



Cell	P value	
	ICM *vs.* NFD	ICM *vs.* DCM
T cells CD8	0.001	0.008
T cells CD4 memory resting	0.007	0.001
T cells CD4 memory activated	<0.001	<0.001
T cells follicular helper	<0.001	<0.001
Macrophages M1	<0.001	<0.001

Figure 3 The score of immune cells in GSE116250. (A-C) Comparison of immune cell scores of NFD and DCM (A), NFD and ICM (B), ICM and DCM (C). (D) Summary of immune cells with significant differences. NFD, non-failing donors; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; NK, natural killer.

cytokine receptor interaction, chemokine signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway, IL-17 signaling pathway, Th17 cell differentiation, and Th1 and Th2 cell differentiation (*Figure 2C* and Table S1). Next, we found that 96 of the 158 DEGs between DCM and ICM were also differentially expressed between ICM and NFD (*Figure 2D*). Importantly, most of the features (786 of 1,053 features) that were enriched with DEGs between DCM and ICM in the GO database were also present between ICM and NFD (*Figure 2E* and Figure S1), and the same was revealed by the enrichment analysis results of DEGs in the KEGG database (46 of 52 pathways) (*Figure 2F*).

Immune infiltration analysis in GSE116250

We analyzed the score of immune cells in GSE116250, and found that there was no significant difference between the NFD group and DCM group (*Figure 3A*), yet a significant difference in T cells CD8, T cells CD4 memory resting and activated, T cells follicular helper, monocytes, macrophage M1, and mast cells resting between the NFD group and ICM group (*Figure 3B*), and a significant difference in T cells CD8, T cells CD4 memory resting and activated, T cells follicular helper, NK cells resting, macrophage M1, and macrophage M2 (*Figure 3C*). Importantly, there was a significant difference in T cells CD8, T cells CD4 memory resting and activated, T cells follicular helper, and macrophage M1 between ICM and NFD or DCM (*Figure 3D*).

Hub genes associated with T cells

Compared with the ICM group, there were 75 DEGs related to T cells in the NFD group, 25 DEGs related to T cells in the DCM group, and 17 DEGs related to T cells in the NFD and DCM groups (*Figure 4A*). Analysis of the expression of these 17 DEG-related T cells showed that the expression of all of them in the ICM group were

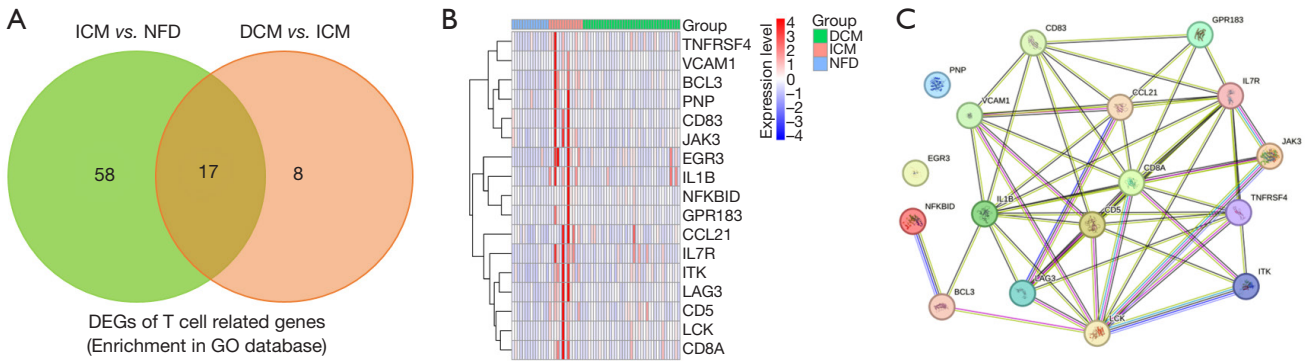


Figure 4 The enrichment pathway analysis of T cells-related hub genes. (A) Intersection of DEGs related to T cells between different comparisons in GSE116250; (B) heatmap of hub genes related to T cells in GSE116250; (C) protein-protein interaction networks of hub-genes related to T cells in GSE116250. ICM, ischemic cardiomyopathy; NFD, non-failing donors; DCM, dilated cardiomyopathy; DEGs, differentially expressed genes; GO, Gene Ontology.

higher than they were in the NFD group and DCM group (*Figure 4B*). At last, to screen hub genes, we created a PPI network with these 17 DEGs related T cells using the STRING database (*Figure 4C* and Table S2), and found that two genes were free outside the PPI network (*PNP* and *EGR3*), and the top ten genes with the highest link scores were *CCL21*, *IL-1B*, *LAG3*, *VCAM-1*, *CD8A*, *CD5*, *IL7R*, *LCK*, *TNFRSF4*, and *ITK*.

Serum levels of protein related to T cells

Among the top ten hub genes related to T cells, there were four genes encoding proteins that could be secreted into serum, namely *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*. Therefore, we collected 25 healthy controls, 48 patients with DCM, and 49 patients with ICM to determine the serum levels of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*. The results showed that there was no significant difference in the serum levels of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* between healthy people and DCM patients, whereas the serum levels of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in ICM patients were all significantly higher than these in DCM patients (*Figure 5*).

Diagnostic efficacy of T cell-associated serum markers for ICM

The ROC curve was used to distinguish between ICM and DCM patients, and we found that the area under the curve of the ROC curve (AUC) for serum *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in 48 patients with DCM and 49 patients with ICM was 0.775, 0.868, 0.934, and 0.903, respectively. At the optimal cut-off value of the ROC curve, the sensitivity of serum *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in the diagnosis of ICM patients were 73.47%, 71.43%, 79.17%, and 81.63%, and the specificity were 77.08%, 85.42%, 91.67%, and 93.75%, respectively (*Figure 6* and *Table 2*).

Discussion

ICM is a heart disease mainly caused by narrowing or blockage of the coronary arteries, whereas DCM is caused by an enlarged and deformed heart. Although patients with ICM and DCM have similar clinical presentations, their pathogenesis and pathology are completely different. In terms of pathogenesis, ICM is caused by the lack of oxygen to the heart muscle cells due to insufficient blood supply to the coronary arteries, which causes a series of pathological changes (19,20), whereas DCM is caused by the breakdown of elastic fibers in the heart wall, causing the heart to gradually continuously expand (9,21). In addition, myocardial ischemia is a direct cause of ICM, and there are many causes of DCM (13), which is the main reason why ICM and DCM are difficult to distinguish (7,8). Herein, we found that compared with NFD heart tissue, the DEGs function of ICM patients was mainly enriched in T cell activation and differentiation, cytokines, and cell-cell adhesion, whereas the DEGs function of DCM patients was mainly enriched in extracellular matrix organization, extracellular structure organization, modulation of chemical synaptic transmission, and so on. Therefore, these results indicated that there is a difference between

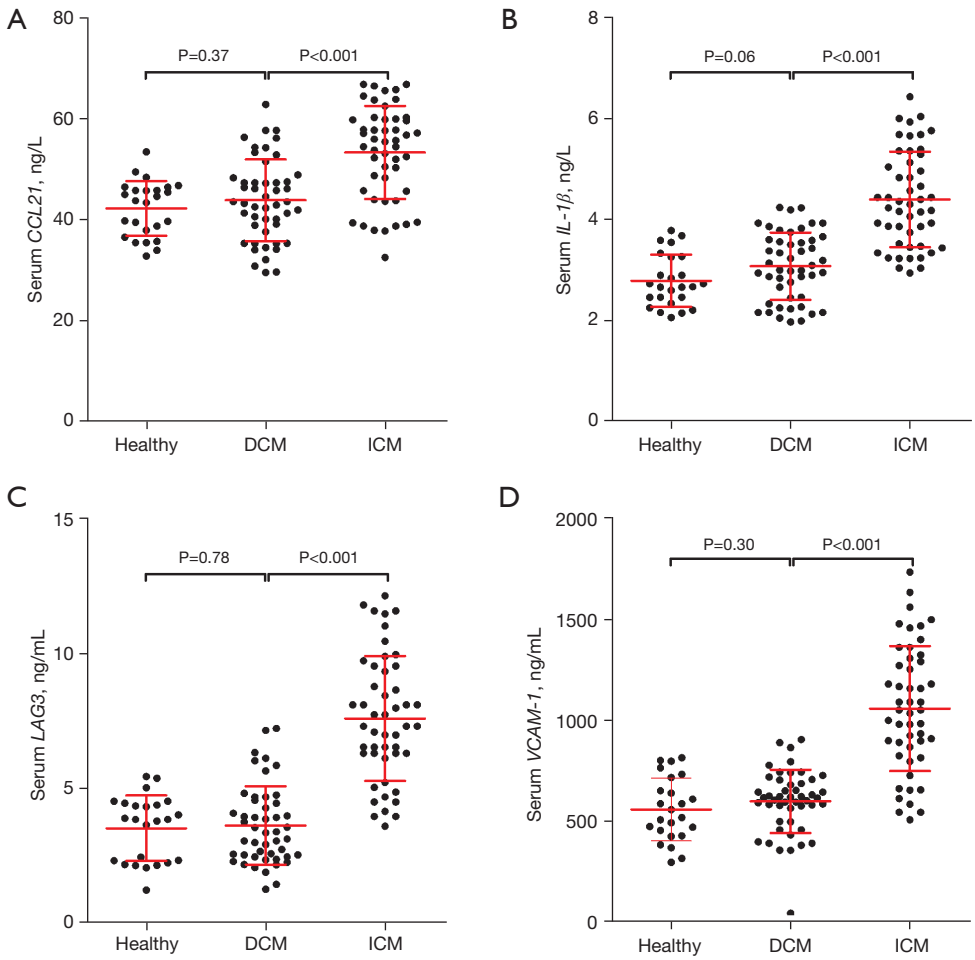


Figure 5 Serum levels of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*. (A-D) ELISA kit was used to determine the serum level of *CCL21* (A), *IL-1β* (B), *LAG3* (C), and *VCAM-1* (D) in participants in the NFD, DCM, and ICM groups. DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; *CCL21*, chemokine (C-C motif) ligand 21; *IL-1β*, interleukin-1β; *LAG3*, lymphocyte-activation gene 3; *VCAM-1*, vascular cell adhesion molecule-1; ELISA, enzyme-linked immunosorbent assay; NFD, non-failing donors.

cardiac histopathological changes in patients with ICM and DCM, and that it is feasible to quickly distinguish between ICM and DCM using serological indicators based on pathogenesis.

In order to explore the differences in the pathogenesis and pathological changes of ICM and DCM, we further compared the transcriptome sequencing data of cardiac tissues of ICM and DCM patients, and found that many DEGs in the heart tissues of DCM patients were also differentially expressed in NFD compared with ICM. Analysis of these intersecting DEGs and the functions enriched by these DEGs revealed that immune cells (especially T cells and leukocytes), chemokines, cytokines, and inflammation are the most common characteristics of these DEGs. In recent years, mounting studies have confirmed that the immune microenvironment is essential for the occurrence and progression of ICM (22-24); Bansal *et al.* found that pro-inflammatory T lymphocytes were crucial for cardiac remodeling in patients with ICM (23), and study by Xia *et al.* suggested that the unique regulatory T lymphocyte population of cardiac tissue infiltration had a protective effect on the heart of patients with myocardial infarction (24). As previously reported, inflammation is a risk factor for the development of cardiovascular disease (25), and some biomarkers associated with inflammation can be used to assess the clinical prognosis of patients with HF (26). At the same time, based on the results of the above transcriptome sequencing data, we shifted our focus to the immune

microenvironment of cardiac tissue in patients with ICM and DCM. The results of immune cell scoring showed that although there was no difference between NFD and DCM, they were significantly different from ICM patients in CD8 T cells CD4 T cells memory resting and activated, T cells follicular helper, and M1 macrophage, suggesting that the activation/differentiation of T cells and macrophage polarization in the immune microenvironment of cardiac tissue may be key to differentiating ICM from DCM. Since macrophage polarization is non-specific, and that acute or chronic inflammatory responses are considered a common factor in the pathogenesis of ICM and DCM, we will focus on studying T cell-related genes in future work. Returning to the functional enrichment results of differential DEGs, we selected the gene set of DEGs associated with T cells for further analysis. Finally, four T cell-related genes encoding secreted proteins were identified, namely *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*.

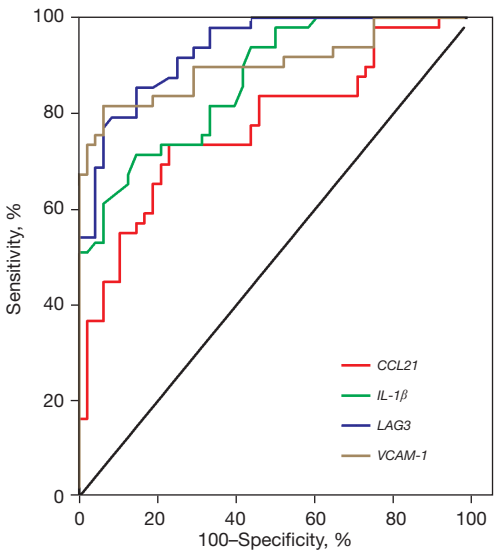


Figure 6 ROC curve of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in the serum for distinguishing between ICM and DCM. *CCL21*, chemokine (C-C motif) ligand 21; *IL-1β*, interleukin-1β; *LAG3*, lymphocyte-activation gene 3; *VCAM-1*, vascular cell adhesion molecule-1; ROC, receiver operating characteristic; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy.

CCL21 is a chemokine that acts on T cells but has no chemotaxis activity against B cells and macrophages (27,28). At the same time, *CCL21* promotes T cell migration by binding to the PSGL-1 glycoprotein on T cells. *IL-1*, also known as lymphocyte-stimulating factor, mainly exists in two forms: *IL-1α* and *IL-1β*, and their main function is to promote the activation, proliferation and differentiation of thymic T cells (29). *LAG-3* protein is an inhibitory receptor on antigen-activated T cells, which, when bound to *FGL1*, transmits inhibitory signals that negatively regulate the proliferation, activation, effector function, and homeostasis of $CD8^+$ and $CD4^+$ T cells (30). *VCAM-1* is a ligand for very late antigen 4 (*VLA-4*) present on leukocytes, and its mediated cell-to-cell adhesion is essential for T cell activation and leukocyte recruitment to the site of inflammation (31).

To verify the value of the 4 T cell-associated genes identified above in differentiating between ICM and DCM, we recruited NFDs, DCM patients, and ICM patients to measure their serum levels. We found that the serum levels of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* in ICM patients were significantly higher than those in DCM patients, and the ROC curves showed that serum levels of *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1* had good diagnostic efficacy in the diagnosis of ICM. All in all, we identified four T cell-associated serum markers that could be used to distinguish between ICM and DCM.

Based on cardiac transcriptome data, we identified four differentially expressed genes in ICM patients and DCM patients, which encode secreted proteins. We further

Table 2 Diagnostic efficacy of T cell-associated serum markers to distinguish between ICM and DCM

Index	AUC (95% CI)	Cutoff value	Specificity (%)	Sensitivity (%)
CCL2	0.775 (0.681–0.868)	48.20 ng/L	77.08	73.47
IL-1β	0.868 (0.800–0.936)	3.835 ng/L	85.42	71.43
LAG3	0.934 (0.890–0.979)	6.075 ng/mL	91.67	79.17
VCAM-1	0.903 (0.839–0.967)	794.9 ng/mL	93.75	81.63

ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; CCL21, chemokine (C-C motif) ligand 21; IL-1β, interleukin-1β; LAG3, lymphocyte-activation gene 3; VCAM-1, vascular cell adhesion molecule-1; AUC, area under the curve; CI, confidence interval.

identified these four protein levels secreted into the serum, i.e., serum *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*, that can be used to distinguish between ICM and DCM. However, it should be noted that the smaller sample size is a limitation of this study, and the inclusion of more patients will be more meaningful for the validation of the results of this study. All in all, we have identified 4 T cell-associated serum markers, *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*, as potential diagnostic serum markers that differentiate ICM from DCM.

Conclusions

All in all, results in the present study indicated that four T cell-associated serum markers had been identified as potential diagnostic serum markers that differentiate ICM from DCM, that is, *CCL21*, *IL-1β*, *LAG3*, and *VCAM-1*.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-901/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-901/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-901/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study protocol was reviewed and annotated by the Ethics Committee of The First Affiliated Hospital of Bengbu Medical University (approval number [2023]ky039). Informed consent was taken from all individual participants.

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Supplementary

Table S1 Enrichment analysis of DEGs between DCM and ICM groups

ID	Description	GeneRatio	BgRatio	P value	P.adjust	Q value	Gene ID	Count
hsa04060	Cytokine-cytokine receptor interaction	22/86	295/8223	1.58E-13	1.38E-11	1.02E-11	CXCL10/CCL3/CCL8/TNFSF14/IL1RN/IL1B/CSF3/IL10/CCR5/CCR1/CCL13/OSM/CCL4/CCL2/CXCL2/IL7R/IL2RG/TNFRSF4/LIF/CCR7/CCR2/CCL21	22
hsa04061	Viral protein interaction with cytokine and cytokine receptor	15/86	100/8223	6.87E-14	1.2E-11	8.89E-12	CXCL10/CCL3/CCL8/TNFSF14/IL10/CCR5/CCR1/CCL13/CCL4/CCL2/CXCL2/IL2RG/CCR7/CCR2/CCL21	15
hsa04062	Chemokine signaling pathway	14/86	192/8223	9.27E-09	5.41E-07	4E-07	CXCL10/CCL3/CCL8/CCR5/CCR1/CCL13/JAK3/CCL4/CCL2/CXCL2/CCR7/CCR2/ITK/CCL21	14
hsa04668	TNF signaling pathway	11/86	114/8223	2.28E-08	9.99E-07	7.39E-07	SOCS3/CXCL10/IL1B/VCAM1/MMP9/JUNB/CCL2/CXCL2/PTGS2/BCL3/LIF	11
hsa04064	NF-kappa B signaling pathway	10/86	104/8223	1.08E-07	3.77E-06	2.79E-06	TNFSF14/IL1B/VCAM1/GADD45B/CCL13/CCL4/CXCL2/PTGS2/LCK/CCL21	10
hsa04640	Hematopoietic cell lineage	9/86	99/8223	7.87E-07	1.97E-05	1.46E-05	IL1B/CSF3/HLA-DQA1/IL7R/HLA-DQB1/CD8A/CD5/CD8B/HLA-DRB5	9
hsa04630	JAK-STAT signaling pathway	9/86	166/8223	5.44E-05	0.000807	0.000597	SOCS3/CSF3/IL10/JAK3/OSM/IL7R/IL2RG/MYC/LIF	9
hsa05166	Human T-cell leukemia virus 1 infection	9/86	222/8223	0.00049	0.004287	0.003172	FOSL1/HLA-DQA1/JAK3/ZFP36/HLA-DQB1/IL2RG/MYC/LCK/HLA-DRB5	9
hsa05163	Human cytomegalovirus infection	9/86	225/8223	0.00054	0.004502	0.003331	CCL3/IL1B/CCR5/CCR1/CCL4/CCL2/PTGER2/PTGS2/MYC	9
hsa04151	PI3K-Akt signaling pathway	9/86	354/8223	0.011351	0.049663	0.036743	EREG/CSF3/JAK3/OSM/IL7R/NR4A1/THBS1/IL2RG/MYC	9
hsa04657	IL-17 signaling pathway	8/86	94/8223	5.52E-06	0.000107	7.93E-05	CXCL10/FOSL1/IL1B/CSF3/MMP9/CCL2/CXCL2/PTGS2	8
hsa05144	Malaria	7/86	50/8223	7.42E-07	1.97E-05	1.46E-05	IL1B/CSF3/VCAM1/IL10/ACKR1/CCL2/THBS1	7
hsa05323	Rheumatoid arthritis	7/86	93/8223	4.9E-05	0.000807	0.000597	CCL3/IL1B/HLA-DQA1/CCL2/CXCL2/HLA-DQB1/HLA-DRB5	7
hsa05142	Chagas disease	7/86	102/8223	8.85E-05	0.001191	0.000881	CCL3/IL1B/SERPINE1/IL10/BDKRB2/CCL2/GNA15	7
hsa04659	Th17 cell differentiation	7/86	108/8223	0.000127	0.001588	0.001175	IL1B/HLA-DQA1/JAK3/HLA-DQB1/IL2RG/LCK/HLA-DRB5	7
hsa04145	Phagosome	7/86	152/8223	0.001018	0.007124	0.005271	HLA-DQA1/FCAR/HLA-DQB1/MSR1/THBS1/MPO/HLA-DRB5	7
hsa04514	Cell adhesion molecules	7/86	157/8223	0.00123	0.008276	0.006123	VCAM1/HLA-DQA1/HLA-DQB1/CD8A/CD8B/HLA-DRB5/SLITRK4	7
hsa05164	Influenza A	7/86	171/8223	0.002011	0.012133	0.008977	SOCS3/CXCL10/IL1B/HLA-DQA1/CCL2/HLA-DQB1/HLA-DRB5	7
hsa05169	Epstein-Barr virus infection	7/86	202/8223	0.005076	0.026127	0.01933	CXCL10/HLA-DQA1/GADD45B/JAK3/HLA-DQB1/MYC/HLA-DRB5	7
hsa05340	Primary immunodeficiency	6/86	38/8223	2.32E-06	5.07E-05	3.75E-05	JAK3/IL7R/CD8A/IL2RG/CD8B/LCK	6
hsa05321	Inflammatory bowel disease	6/86	65/8223	5.53E-05	0.000807	0.000597	IL1B/HLA-DQA1/IL10/HLA-DQB1/IL2RG/HLA-DRB5	6
hsa05140	Leishmaniasis	6/86	77/8223	0.000144	0.001676	0.00124	IL1B/HLA-DQA1/IL10/PTGS2/HLA-DQB1/HLA-DRB5	6
hsa04612	Antigen processing and presentation	6/86	78/8223	0.000154	0.001688	0.001249	HLA-DQA1/HLA-DQB1/IFI30/CD8A/CD8B/HLA-DRB5	6
hsa04658	Th1 and Th2 cell differentiation	6/86	92/8223	0.000381	0.003708	0.002743	HLA-DQA1/JAK3/HLA-DQB1/IL2RG/LCK/HLA-DRB5	6
hsa05150	Staphylococcus aureus infection	6/86	96/8223	0.00048	0.004287	0.003172	HLA-DQA1/IL10/FCAR/C5AR1/HLA-DQB1/HLA-DRB5	6
hsa05135	Yersinia infection	6/86	137/8223	0.003026	0.017082	0.012638	IL1B/IL10/CCL2/CD8A/CD8B/LCK	6
hsa05152	Tuberculosis	6/86	180/8223	0.011211	0.049663	0.036743	IL1B/HLA-DQA1/IL10/HLA-DQB1/ITGAX/HLA-DRB5	6
hsa05167	Kaposi sarcoma-associated herpesvirus infection	6/86	194/8223	0.015759	0.06566	0.048579	CCR5/CCR1/ZFP36/CXCL2/PTGS2/MYC	6
hsa05417	Lipid and atherosclerosis	6/86	215/8223	0.024768	0.090301	0.066809	CCL3/IL1B/VCAM1/MMP9/CCL2/CXCL2	6
hsa04610	Complement and coagulation cascades	5/86	86/8223	0.001995	0.012133	0.008977	SERPINE1/BDKRB2/C5AR1/PLAUR/ITGAX	5
hsa04625	C-type lectin receptor signaling pathway	5/86	104/8223	0.004551	0.024132	0.017854	IL1B/EGR3/IL10/PTGS2/BCL3	5
hsa04660	T cell receptor signaling pathway	5/86	104/8223	0.004551	0.024132	0.017854	IL10/CD8A/CD8B/LCK/ITK	5
hsa05145	Toxoplasmosis	5/86	112/8223	0.006221	0.031107	0.023014	HLA-DQA1/IL10/CCR5/HLA-DQB1/HLA-DRB5	5
hsa04380	Osteoclast differentiation	5/86	128/8223	0.010779	0.04964	0.036726	SOCS3/FOSL1/IL1B/JUNB/LCK	5
hsa05322	Systemic lupus erythematosus	5/86	136/8223	0.013747	0.058677	0.043412	HLA-DQA1/IL10/GRIN2A/HLA-DQB1/HLA-DRB5	5
hsa05310	Asthma	4/86	31/8223	0.000283	0.002912	0.002155	HLA-DQA1/IL10/HLA-DQB1/HLA-DRB5	4
hsa05330	Allograft rejection	4/86	38/8223	0.000628	0.004993	0.003694	HLA-DQA1/IL10/HLA-DQB1/HLA-DRB5	4
hsa05332	Graft-versus-host disease	4/86	42/8223	0.000922	0.007016	0.005191	IL1B/HLA-DQA1/HLA-DQB1/HLA-DRB5	4
hsa04940	Type I diabetes mellitus	4/86	43/8223	0.001009	0.007124	0.005271	IL1B/HLA-DQA1/HLA-DQB1/HLA-DRB5	4
hsa04672	Intestinal immune network for IgA production	4/86	49/8223	0.001651	0.010702	0.007918	HLA-DQA1/IL10/HLA-DQB1/HLA-DRB5	4
hsa05320	Autoimmune thyroid disease	4/86	53/8223	0.002211	0.012897	0.009542	HLA-DQA1/IL10/HLA-DQB1/HLA-DRB5	4
hsa04933	AGE-RAGE signaling pathway in diabetic complications	4/86	100/8223	0.02046	0.083266	0.061604	IL1B/SERPINE1/VCAM1/CCL2	4
hsa05146	Amoebiasis	4/86	102/8223	0.021829	0.086822	0.064235	IL1B/IL10/CXCL2/GNA15	4
hsa04620	Toll-like receptor signaling pathway	4/86	104/8223	0.023254	0.090301	0.066809	CXCL10/CCL3/IL1B/CCL4	4
hsa04670	Leukocyte transendothelial migration	4/86	114/8223	0.03122	0.1115	0.082494	VCAM1/MMP9/RHOH/ITK	4
hsa04650	Natural killer cell mediated cytotoxicity	4/86	131/8223	0.048053	0.161716	0.119646	SH2D1A/CD48/HCST/LCK	4
hsa05143	African trypanosomiasis	3/86	37/8223	0.006641	0.032282	0.023884	IL1B/VCAM1/IL10	3
hsa05219	Bladder cancer	3/86	41/8223	0.008842	0.041821	0.030941	MMP9/THBS1/MYC	3
hsa04978	Mineral absorption	3/86	60/8223	0.02464	0.090301	0.066809	MT1A/MT1M/MT2A	3
hsa05416	Viral myocarditis	3/86	60/8223	0.02464	0.090301	0.066809	HLA-DQA1/HLA-DQB1/HLA-DRB5	3
hsa04115	p53 signaling pathway	3/86	73/8223	0.040684	0.142393	0.105349	SERPINE1/GADD45B/THBS1	3
hsa04623	Cytosolic DNA-sensing pathway	3/86	75/8223	0.043524	0.149348	0.110495	CXCL10/IL1B/CCL4	3

ICM, ischemic cardiomyopathy; NFD, non-failing donors; DEGs, differentially expressed genes.

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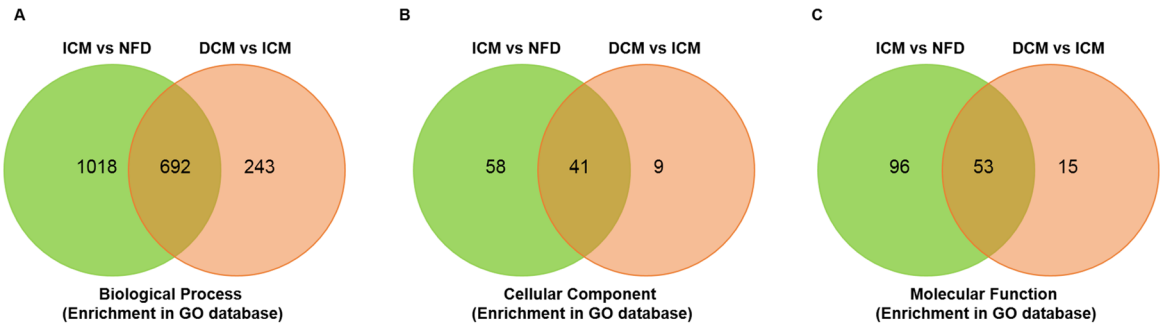

Figure S1 Intersection of enrichment analysis results in biological process (A), cellular component (B) and molecular function (C) of GO database between different comparisons in GSE116250. ICM, ischemic cardiomyopathy; NFD, non-failing donors; DCM, dilated cardiomyopathy; GO, Gene Ontology.

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Table S2 PPI network link scores of 17 T cell-related genes

#Node1	Node2	Node1_string_id	Node2_string_id	Neighborhood_on_chromosome	Gene_fusion	Phylogenetic_cooccurrence	Homology	Coexpression	Experimentally_determined_interaction	Database_annotated	Automated_textmining	Combined_score
CD8A	LCK	9606.ENSP00000386559	9606.ENSP00000477713	0	0	0	0	0.471	0.903	0.9	0.998	0.999
IL7R	JAK3	9606.ENSP00000306157	9606.ENSP00000432511	0	0	0	0	0.128	0.295	0.8	0.919	0.988
CD5	CD8A	9606.ENSP00000342681	9606.ENSP00000386559	0	0	0	0	0.342	0	0	0.946	0.963
CD5	LCK	9606.ENSP00000342681	9606.ENSP00000477713	0	0	0	0	0.455	0.51	0	0.854	0.957
CD8A	LAG3	9606.ENSP00000386559	9606.ENSP00000203629	0	0	0	0	0.441	0.064	0	0.907	0.947
CD8A	IL7R	9606.ENSP00000386559	9606.ENSP00000306157	0	0	0	0	0.214	0	0	0.935	0.946
CD8A	IL1B	9606.ENSP00000386559	9606.ENSP00000263341	0	0	0	0	0.06	0	0	0.92	0.922
ITK	LCK	9606.ENSP00000398655	9606.ENSP00000477713	0	0	0.101	0.851	0.559	0	0.5	0.631	0.917
CCL21	CD8A	9606.ENSP00000259607	9606.ENSP00000386559	0	0	0	0	0.108	0	0	0.852	0.862
JAK3	LCK	9606.ENSP00000432511	9606.ENSP00000477713	0	0	0	0.673	0.159	0.045	0.4	0.73	0.852
IL1B	VCAM1	9606.ENSP00000263341	9606.ENSP00000294728	0	0	0	0	0.089	0	0	0.821	0.83
IL7R	LCK	9606.ENSP00000306157	9606.ENSP00000477713	0	0	0	0	0.177	0	0	0.778	0.809
IL7R	ITK	9606.ENSP00000306157	9606.ENSP00000398655	0	0	0	0	0.514	0	0	0.601	0.798
CD8A	TNFRSF4	9606.ENSP00000386559	9606.ENSP00000368538	0	0	0	0	0.08	0	0	0.789	0.797
LAG3	TNFRSF4	9606.ENSP00000203629	9606.ENSP00000368538	0	0	0	0	0.124	0	0	0.778	0.797
CD8A	VCAM1	9606.ENSP00000386559	9606.ENSP00000294728	0	0	0	0	0.101	0	0	0.776	0.79
CD8A	ITK	9606.ENSP00000386559	9606.ENSP00000398655	0	0	0	0	0.487	0.045	0	0.581	0.776
IL1B	IL7R	9606.ENSP00000263341	9606.ENSP00000306157	0	0	0	0	0.195	0	0	0.712	0.758
CD83	IL1B	9606.ENSP00000368450	9606.ENSP00000263341	0	0	0	0	0.223	0	0	0.663	0.727
CD83	CD8A	9606.ENSP00000368450	9606.ENSP00000386559	0	0	0	0	0.06	0	0	0.72	0.726
CD5	IL7R	9606.ENSP00000342681	9606.ENSP00000306157	0	0	0	0	0.208	0	0	0.667	0.725
BCL3	IL1B	9606.ENSP00000164227	9606.ENSP00000263341	0	0	0	0	0.213	0	0	0.657	0.719
IL1B	TNFRSF4	9606.ENSP00000263341	9606.ENSP00000368538	0	0	0	0	0.128	0	0	0.657	0.688
CD5	ITK	9606.ENSP00000342681	9606.ENSP00000398655	0	0	0	0	0.498	0	0	0.4	0.686
IL7R	LAG3	9606.ENSP00000306157	9606.ENSP00000203629	0	0	0	0	0.064	0	0	0.667	0.675
CCL21	VCAM1	9606.ENSP00000259607	9606.ENSP00000294728	0	0	0	0	0.159	0.096	0	0.58	0.653
CD8A	JAK3	9606.ENSP00000386559	9606.ENSP00000432511	0	0	0	0	0.133	0.045	0	0.581	0.623
IL7R	VCAM1	9606.ENSP00000306157	9606.ENSP00000294728	0	0	0	0	0.105	0	0	0.585	0.612
CCL21	LAG3	9606.ENSP00000259607	9606.ENSP00000203629	0	0	0.234	0	0.069	0.099	0	0.459	0.606
CD5	LAG3	9606.ENSP00000342681	9606.ENSP00000203629	0	0	0	0	0.177	0.263	0	0.396	0.602
IL7R	TNFRSF4	9606.ENSP00000306157	9606.ENSP00000368538	0	0	0	0	0.078	0	0	0.583	0.599
CCL21	IL1B	9606.ENSP00000259607	9606.ENSP00000263341	0	0	0	0	0.086	0	0	0.573	0.593
CCL21	GPR183	9606.ENSP00000259607	9606.ENSP00000365596	0	0	0	0	0.135	0	0	0.543	0.588
BCL3	NFKBID	9606.ENSP00000164227	9606.ENSP00000380109	0	0	0.12	0.69	0.085	0	0	0.512	0.572
LAG3	LCK	9606.ENSP00000203629	9606.ENSP00000477713	0	0	0	0	0.188	0.081	0	0.449	0.553
CCL21	CD83	9606.ENSP00000259607	9606.ENSP00000368450	0	0	0	0	0.106	0	0	0.508	0.541
IL1B	JAK3	9606.ENSP00000263341	9606.ENSP00000432511	0	0	0	0	0.111	0	0	0.488	0.526
GPR183	IL7R	9606.ENSP00000365596	9606.ENSP00000306157	0	0	0	0	0.276	0	0	0.359	0.516
CD5	VCAM1	9606.ENSP00000342681	9606.ENSP00000294728	0	0	0	0	0.092	0.095	0	0.431	0.491
IL1B	LAG3	9606.ENSP00000263341	9606.ENSP00000203629	0	0	0	0	0.067	0	0	0.474	0.488
IL1B	LCK	9606.ENSP00000263341	9606.ENSP00000477713	0	0	0	0	0.109	0	0	0.435	0.475
CD83	GPR183	9606.ENSP00000368450	9606.ENSP00000365596	0	0	0	0	0.329	0	0	0.247	0.474
CCL21	IL7R	9606.ENSP00000259607	9606.ENSP00000306157	0	0	0	0	0.115	0	0	0.419	0.464
CD8A	GPR183	9606.ENSP00000386559	9606.ENSP00000365596	0	0	0	0	0.198	0	0	0.356	0.461
BCL3	LCK	9606.ENSP00000164227	9606.ENSP00000477713	0	0	0	0	0	0.331	0	0.2	0.442
CD83	IL7R	9606.ENSP00000368450	9606.ENSP00000306157	0	0	0	0	0.097	0	0	0.399	0.434
CD5	IL1B	9606.ENSP00000342681	9606.ENSP00000263341	0	0	0	0	0.062	0	0	0.418	0.43
CD5	CD83	9606.ENSP00000342681	9606.ENSP00000368450	0	0	0	0	0.11	0	0	0.38	0.425
LCK	TNFRSF4	9606.ENSP00000477713	9606.ENSP00000368538	0	0	0	0	0.062	0.052	0	0.396	0.416
CD83	VCAM1	9606.ENSP00000368450	9606.ENSP00000294728	0	0	0	0	0.123	0	0	0.362	0.416
LCK	VCAM1	9606.ENSP00000477713	9606.ENSP00000294728	0	0	0	0	0.107	0.102	0	0.329	0.416
CD5	TNFRSF4	9606.ENSP00000342681	9606.ENSP00000368538	0	0	0	0	0.151	0	0	0.323	0.401

PPI, protein-protein interaction.

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