

A Protein Chip Study on the Heart Failure With Recovered Ejection Fraction

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Research

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Abstract

Background: Characteristics of heart failure with recovered ejection fraction (HFrecEF) have not yet been fully understood. The objective of this study is to identify potential biomarkers for the left ventricular ejection fraction(LVEF) recovery.

Methods: Antibody microarrays were used to detect proteins in serum of healthy volunteers, patients with heart failure with reduced ejection fraction(HFrEF), and patients with HFrecEF, looking for specific proteins of HFrecEF patients.

Results:1000 proteins were detected in the sera of healthy volunteers, HFrEF patients and HFrecEF patients using antibody microarrays (three in each group). There were dozens of different proteins between each group. Based on the signal strength, fold changes, clinical significance and Venn diagram analysis, 11 proteins were selected to be detected in the sera of 10 healthy volunteers ,47 HFrEF patients and 22 HFrecEF patients using antibody microarrays. Serum concentrations of cysteine dioxygenase type 1 (CDO1) and growth/differentiation factor 8 (GDF-8) were significantly downregulated in HFrecEF patients compared with HFrEF patients. ROC curve analysis showed that the area under the CDO1 curve was 0.662(95%CI 0.517-0.808,P=0.031).The sensitivity of CDO1 was 77%, the specificity was 54%, and diagnostic cut-off points was 10198.5.The GDF-8 has no diagnostic value. Kaplan–Meier survival curves showed that the prognosis is better in HFrecEF patients than HFrEF patients about all cause death(P=0.011) and cardiovascular death(P=0.004).But we did not find that patients with low baseline CDO1 levels (<10198.5) had better outcomes than those with high CDO1 levels (\geq 10198.5).

Conclusions: This pilot study indicates that CDO1 is a potential biomarker of LVEF recovery, which needs to be confirmed by further studies.

Background

Nowadays measurement of left ventricular ejection fraction(LVEF) is an initial step in the management of heart failure (HF). We usually divide HF cases into two categories: HF with reduced EF (HFrEF) and HF with preserved EF (HFpEF). In recent years, evidence-based medicine has proved that in some patients with HFrEF, LVEF may recovered or even completely returned to normal after appropriate treatment,we call it heart failure with recoverd ejection fraction(HFrecEF). It is accompanied by improvement in quality of life and reduction in the rate of readmission and mortality, which were significantly different from those patients with heart failure whose ejection fraction was continuously reduced[1–3]. Notably, however, there exist relatively few strategies for early diagnosis of HFrecEF. Finding valuable biomarkers is helpful for early identification of HFrecEF. Cytokines, which can be produced by various types of cells, are thought to play important roles in the occurrence and development of HF[4]. These increased or decreased cytokines in systemic circulation may be potential candidates of biomarkers for HFrecEF. Compared to other detection techniques,antibody microarrays are a novel technology simultaneously detecting multiple proteins with the advantage of being high-throughput amenable[5] The purpose of this study is to find

biomarkers for recovery of LVEF through antibody microarrays so as to provide basis for early recognition of HFrecEF.

Materials And Methods

Patients, controls, echocardiography and follow-up

This is a retrospective cohort study, including patients who were hospitalized for heart failure in the department of cardiology of our hospital on January 1, 2012 and June 30, 2017, with retained blood samples after admission, LVEF \leq 40% at admission, and echocardiography reexamination after discharge. The definition of Heart failure is based on the 2013 ACCF/AHA guidelines for heart failure[6]. Except for patients with other serious systemic diseases, such as tumors, acquired immunodeficiency syndrome, etc.

All healthy controls were from people who had come to our hospital for a health checkup.

Echocardiographic images were obtained using Philips IE33 or GE Vivid 9 machines at Beijing Hospital. In healthy controls, echocardiographic examinations were to be performed on the day of the physical examination. Echocardiographic examinations were to be performed at least 2 time points in heart failure patients: on admission and after discharge. Subsequent LVEF measurements were made when the patient was in a stable condition. LVEF is assessed by quantitative 2D biplane volumetric Simpson method or M-mode from the parasternal views. According to the changes of LVEF, patients were divided into 2 groups: HFrecEF group (LVEF \leq 40% at admission, LVEF $>$ 40% and LVEF increased \geq 10% when the follow-up) and HFrefEF group (LVEF \leq 40% at admission, LVEF \leq 40% or LVEF $>$ 40% but LVEF increased $<$ 10% when the follow-up).

All patients were followed-up by outpatient clinic attendance, telephone contact, or review of the medical notes. Median follow-up time was 57(20,69) months, the time of death was taken as the last follow-up time for the patients who died, and the actual follow-up time was recorded for the other patients. The end-point events were all-cause death and cardiovascular death.

Antibody array assay

All patients and healthy controls were collected 5 ml of peripheral venous blood on an empty stomach in the morning. Blood samples were collected in a test tube containing serum separation glue. After being placed at room temperature for 60 minutes, centrifugation was conducted at a speed of 3000r/min for 15 minutes. The centrifuged serum was transferred into the 0.5 ml EP cryopreservation tube and stored at -80°C at ultra-low temperature.

First of all, 3 HFrecEF patients, 3 HFrefEF patients and 3 healthy controls were assayed for the relative expression of 1,000 human proteins. A RayBio G-Series Human Cytokine Antibody Array X00 kit was used

for protein detection in accordance with the manufacturer's instructions.

This antibody array simultaneously detects 1000 cytokines in a single experiment by utilizing a sandwich technique with 1000 antibody dots arranged in four duplicates printed onto the glass. Briefly, serum samples were diluted (1:2) and added into the array pools to incubate with capture antibodies overnight. After washing, the arrays were incubated with a biotin-conjugated anti-cytokine antibody mix for 2 h at room temperature. Cy3-conjugated streptavidin was added to bind with biotin from the detection antibodies and the fluorescent signal was detected using an InnoScan 300 Microarray Scanner (Innopsys, France). Signal values were captured with Mapix software. The data was normalized using positive control values from the array with the RayBiotech analysis tool, specifically designed to analyze the data of Human Cytokine Antibody Array X00 with Microsoft Excel technology.

Then, according to the signal strength, fold changes, clinical significance and Venn diagram analysis, we selected partial proteins from the differential proteins screened for the first time, and customized an antibody microarray including several selected proteins. More patients and healthy controls were assayed for the relative expression of the selected human proteins to find the biomarkers of patients with HFrecEF. The test method is the same as before.

Statistical Analysis

All array data analyses were performed using RayBio Analysis Tool software. Biostatistics and bioinformatics analysis included discriminatory protein analysis and data mining cluster analysis. Statistical differences between two groups were determined by Student's ttest. Fold change values of proteins were used as indicators of relative expression levels. Proteins defined as having significantly different expression levels between the groups had a P-value < 0.05 and a fold change ≥ 1.2 or ≤ 0.83 . Data mining cluster analysis was used to identify potential biomarkers by clustering all relevant proteins according to the similarity of their expression profiles using Cluster software version 3.0 (<http://cluster2.software.informer.com/3.0>).

Other data are described as mean \pm standard deviation for normally distributed data, median and interquartile range (IQR) for non-normal data and number(percentage) for categorical variables. Continuous variables were compared using one-way analysis of variance(LSD method was used for pairwise comparison) or Mann-Whitney *U* test, and categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. ROC(receiver operator characteristic) curve was used to determine whether the proteins had diagnostic value. ROC curve was depicted by area under curve (AUC) with 95% CI. To compare the survival rate between the groups, Kaplan–Meier survival curves were plotted with the parameters compared using the log-rank test. A P-value < 0.05 was considered to indicate statistical significance. All analyses were performed with SPSS version 23 (SPSS Inc., Chicago, IL, USA).

Results

Demographical parameters

First of all, a total of 9 participants were included for the detection of 1000 cytokines. There were 3 healthy controls, 3 HFrecEF patients and 3 HFrEF patients. Participants in these three groups were all females and they were matched in age. Dilated cardiomyopathy is the underlying disease in all patients with heart failure. There was no significant difference in clinical complications (Table 1).

Table 1
Clinical characteristics of 3 HFrecEF patients, 3 HFrEF patients and 3 healthy controls

Variables	HFrecEF (n = 3)	HFrEF (n = 3)	Control (n = 3)	P value
Age, mean ± SD, yr	68.0 ± 11.4	63.3 ± 5.8	65.7 ± 2.5	0.758
Female sex, No. (%)	3(100.0)	3(100.0)	3(100.0)	–
Body mass index, mean ± SD, kg/m ²	24.1 ± 2.4	24.2 ± 1.3	23.6 ± 3.1	0.953
NYHA class III or IV, No. (%)	3(100)	2(66.7)	–	1.000 ^a
Dilated cardiomyopathy, No. (%)	3(100)	3(100)	–	–
Hypertension, No. (%)	2(66.7)	2(66.7)	3(100.0)	1.000 ^a
Diabetes, No. (%)	1(33.3)	2(66.7)	1(33.3)	1.000 ^a
Atrial fibrillation, No. (%)	0(0.0)	1(33.3)	0(0.0)	1.000 ^a
Baseline LVEF, mean ± SD, %	29.7 ± 4.5	22.3 ± 8.7	63.3 ± 2.9 ^{*#}	< 0.001
Follow-up LVEF, mean ± SD, %	50.0 ± 5.0	31.0 ± 6.1	–	0.014
Hemoglobin, mean ± SD, g/L	132.3 ± 11.0	113.7 ± 8.3	141.0 ± 13.5 [#]	0.058
eGFR, mean ± SD, mL/min/1.73 m ²	87.3 ± 25.4	72.8 ± 6.2	96.0 ± 23.4	0.421
NT-proBNP, median (IQR), pg/mL	813(170,2330)	2077(312,2653)	–	0.7
*P < 0.05 vs HFrecEF				
#P < 0.05 vs HFrEF				
^a Fisher's Exact Test				
Abbreviations: HFrecEF, heart failure with recovered ejection fraction; HFrEF, heart failure with reduced ejection fraction; SD, standard deviation; No, number; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-B-type natriuretic peptide; IQR, interquartile range				

Then, a total of 79 participants were included for further detection. There were 10 healthy controls, 22 HFrecEF patients and 47 HFrEF patients. The basic diseases of patients with heart failure included coronary heart disease, dilated cardiomyopathy, rheumatic heart disease, hypertension, etc. Most patients with heart failure were accompanied by hypertension, some patients with diabetes mellitus and atrial fibrillation. The healthy controls were the youngest among all participants. HFrEF patients had the highest proportion with coronary heart disease and old myocardial infarction, and the poorest renal function. Clinical characteristics of the 79 participants are shown in Table 2.

Table 2
Clinical characteristics of 47 HFrEF patients, 22 HFrecEF patients and 10 healthy controls

	HFrecEF	HFrEF	Control	
Variables	n = 22	n = 47	n = 10	P value
Age, mean ± SD, yr	64.6 ± 17.9	70.3 ± 11.4	58.3 ± 7.9 [#]	0.023
Female sex, No.(%)	13(59.1)	17(36.2)	6(60.0)	0.126
NYHA class III or IV, No.(%)	16(88.9)	34(77.3)	–	0.486
Body mass index, mean ± SD, kg/m ²	25.4 ± 5.2	25.3 ± 4.2	24.8 ± 2.9	0.928
Coronary heart disease, No.(%)	8(36.4)	32(68.1)	–	< 0.001
Acute myocardial infarction, No.(%)	5(22.7)	5(10.6)	–	0.162
Old myocardial infarction, No.(%)	2(9.1)	23(48.9)	–	< 0.001
Dilated cardiomyopathy, No.(%)	10(45.5)	12(25.5)	–	0.098
Rheumatic heart disease, No.(%)	1(4.5)	2(4.3)	–	1.000 ^a
Hypertension, No.(%)	16(72.7)	32(68.1)	6(60.0)	0.519
Diabetes, No.(%)	4(18.2)	15(31.9)	3(30.0)	0.488
Atrial fibrillation, No.(%)	5(22.7)	11(23.4)	0(0.0)	0.245 ^a
Baseline LVEF, mean ± SD, %	30.3 ± 6.3	29.0 ± 7.6	63.5 ± 2.4 ^{**}	< 0.001
Follow-up LVEF, mean ± SD, %	55.3 ± 5.0	29.5 ± 6.6	–	< 0.001
Hemoglobin, mean ± SD, g/dL	132.4 ± 34.0	129.2 ± 20.7	143.6 ± 17.3	0.258

*P < 0.05 vs HFrecEF

[#]P < 0.05 vs HFrEF

^aFisher's Exact Test

Abbreviations: HFrecEF, heart failure with recovered ejection fraction; HFrEF, heart failure with reduced ejection fraction; SD, standard deviation; No, number; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-B-type natriuretic peptide; IQR, interquartile range

	HFrEF	HFrEF	Control	
eGFR,mean ± SD, mL/min/1.73 m ²	82.5 ± 26.5	69.9 ± 23.3*	107.6 ± 24.5*#	< 0.001
NT-proBNP,median(IQR), pg/mL	1848 (711,3548)	1711(294,4878)	–	0.564
*P < 0.05 vs HFrEF				
#P < 0.05 vs HFrEF				
^a Fisher's Exact Test				
Abbreviations: HFrEF, heart failure with recovered ejection fraction; HFrEF, heart failure with reduced ejection fraction; SD,standard deviation;No,number;NYHA,New York Heart Association;LVEF,left ventricular ejection fraction;eGFR,estimated glomerular filtration rate;NT-proBNP,N-terminal pro-B-type natriuretic peptide;IQR, interquartile range				

Analysis of antibody microarrays

A total of 1000 known proteins (e.g. cytokines, chemokines, adipokine,growth factors, angiogenic factors, proteases, soluble receptors and soluble adhesion molecules) were measured in the sera of 3 HFrEF patients, 3 HFrEF patients and 3 healthy controls.

The results demonstrated that 52 proteins had significantly different expressions between HFrEF group and control group (Appendix Table 1). Serum mixture samples were arranged by similarities in the abundance of these 52 markers in the sera clustering algorithm, which produced two clusters that contained HFrEF and HFrEF individuals (Fig. 1A).

There are 40 proteins had significantly different expressions between HFrEF group and control group(Appendix Table 2). Serum mixture samples were arranged by similarities in the abundance of these 40 markers in the sera clustering algorithm, which produced two clusters that contained HFrEF and HFrEF individuals (Fig. 1B).

There are 26 proteins had significantly different expressions between HFrEF group and HFrEF group (Appendix Table 3). Serum mixture samples were arranged by similarities in the abundance of these 26 markers in the sera clustering algorithm, which produced two clusters that contained HFrEF and HFrEF individuals (Fig. 1C).

Table 3
Difference of 11 cytokines in the 3 groups

Cytokines	HFrecEF vs HFrEF		HFrecEF vs control		HFrEF vs control	
	P value	FC	P value	FC	P value	FC
CD01	0.01	0.79	0	0.62	0.05	0.78
GDF-8	0.03	0.52	0.04	0.4	0.5	0.76
Angiopoientin-4/ANG-4	0.61	1.28	0.36	0.51	0.17	0.4
B4GALT1	0.5	1.14	0.98	0.99	0.6	0.87
Flt-3L	0.27	1.22	0.98	0.99	0.39	0.82
GALECTIN-4	0.16	0.87	0.92	0.98	0.35	1.14
KELL	0.13	1.36	0.65	0.87	0.1	0.64
HO-1	0.09	0.76	0.53	0.86	0.56	1.13
TPP1	0.77	1.05	0.64	0.89	0.46	0.84
TSLP	0.48	0.68	0.41	0.51	0.71	0.76
BAI1	0.56	0.72	0.73	0.75	0.96	1.04

Abbreviations: HFrEF, heart failure with reduced ejection fraction; HFrecEF, heart failure with recovered ejection fraction; FC, foldchange; CD01, Cysteine dioxygenase type 1 ; GDF-8, Growth/differentiation factor 8; B4GALT1, Beta-1,4-galactosyltransferase 1; Flt-3L, Fms-related tyrosine kinase 3 ligand; KELL, Kell blood group glycoprotein ;HO-1, Heme oxygenase 1; TPP1, Tripeptidyl-peptidase 1; TSLP, Thymic stromal lymphopoietin; BAI1, Brain-specific angiogenesis inhibitor 1

According to the signal strength, fold changes, clinical significance and Venn diagram analysis, we hypothesized 11 specific HFrecEF biomarkers include cysteine dioxygenase type 1 (CD01), growth-differentiation factor 8 (GDF-8), Angiopoientin-4/ANG-4, beta-1,4-galactosyltransferase 1 (B4GALT1), fms related receptor tyrosine kinase 3 ligand (Flt-3L), GALECTIN-4, kell blood group glycoprotein (KELL), heme oxygenase 1 (HO-1), tripeptidyl peptidase 1 (TPP1), thymic stromal lymphopoietin (TSLP) and brain specific angiogenesis inhibitor 1 (BAI1) for further detection. A chip containing 11 proteins mentioned above was customized to test in 47 HFrEF patients, 22 HFrecEF patients and 10 healthy controls. Serum levels of were selected to be measured. Among the 11 proteins, CD01 and GDF-8 were found to be differentially expressed in patients with HFrecEF and HFrEF (Table 3 and Fig.2).

Analysis of sensitivity and specificity of serum biomarkers for HFrecEF

To validate whether CDO1 and GDF-8 may be used as biomarkers for predicting HFrecEF, ROC curves were used to analyze sensitivity and specificity. Area under ROC curve values for CDO1 was 0.663(95%CI:0.517–0.808)(Fig. 3A), which was statistically significant ($P = 0.031$). Area under ROC curve values for GDF-8 was 0.581 (95%CI: 0.414–0.747) (Fig. 3B), which was not statistically significant ($P = 0.282$). So CDO1 was deemed suitable biomarkers for the prediction of HFrecEF. CDO1 had a sensitivity of 77% and specificity of 54%. The correct diagnostic index corresponding to the cut-off point 10198.5 is the largest.

Clinical end-point and survival analyses

69 patients enrolled were followed-up by 57(20,69) months, all cause death was recorded in 17 patients (24.6%), cardiovascular death was recorded in 15 patients(21.7%). Kaplan-meier survival curves showed significantly lower risk of all-cause death ($P = 0.011$) and cardiovascular death ($P = 0.004$) in HFrecEF patients than in HFrEF patients (Fig. 4A, Fig. 4B).

Although the survival rate in patients with low baseline CDO1 levels (< 10198.5) seemed to be higher, we failed to find high baseline CDO1 levels (≥ 10198.5) as a significant predictor of all-cause death and cardiovascular death in the longer term follow-up duration by using the cut-off value based on ROC curve analysis(Fig. 4C, Fig. 4D).

Discussion

Due to the high morbidity and mortality of HFrEF patients, it is necessary to adopt more effective strategies to optimize the clinical management of the disease, including diagnosis, definition of disease status, assessment of individual risk profiles, and development of individual treatment strategies. So the identification of reliable biomarkers for the prognosis of heart failure is necessary. Currently commonly used biomarkers for heart failure include B-type natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (NT-proBNP), ST2, Troponins, Matrix metalloproteinase, Galectin-3, C-reactive protein (CRP) et al[7]. They reflect, respectively, increased myocardial stress, damage to the myocardium, proliferation of the extracellular matrix, and inflammation. Although a number of biomarkers have been developed, there have been few reports of heart failure biomarkers that predict improvement in ejection fraction. Proteins are the main effectors of cellular function, and proteomics techniques are rapidly advancing to allow us to infer the overall state of biological systems by assessing changes in the expression of proteins in the system. In this study, antibody chips were used to detect protein expression profiles in serum of heart failure patients. Finally, it was found that there were two differential proteins in HFrEF patients and HFrecEF patients, respectively CDO1 and GDF-8.

GDF-8

Growth-differentiation factor 8(GDF-8), also known as myostatin (Mstn), was first isolated by McPherron et al in 1997[8]. GDF-8 is a protein belonging to the TGF- β superfamily[9]. It was first recognized as a

negative regulator of skeletal muscle mass. It is well known to be mainly expressed in skeletal muscles. [10].

GDF-8 was reported to be expressed in the myocardium for the first time in 1999, when Sharma et al found that GDF-8 was upregulated in cardiomyocytes after infarction in animal models[11]. Also, George et al found that plasma GDF-8 levels significantly increased in patients with heart failure[12]. The exact role GDF-8 plays in heart failure is not very clear until now. GDF-8 may play an active role in cardiac remodelling after injury. GDF-8 may act in an opposite fashion to limit unrestrained cellular growth, possibly to prevent the untoward effects of overcompensated myocardial growth as a homeostatic function. McKoy et al reported the effect of recombinant GDF-8 in cardiomyocytes isolated from rat myocardium at different developmental stages and showed that GDF-8 can act as an inhibitor of cardiomyocyte proliferation with the potential to limit cardiomyocyte hyperplastic growth by altering the cardiac cell cycle progression[13]

The results observed in this study are different from previous studies. GDF-8 levels decreased in heart failure patients compared to the normal control group, and further decreased in HFrecEF patients. This may be associated with patients with more underlying diseases, more influencing factors. In addition, GDF-8 in the HFrecEF group was down-regulated, possibly because the proliferation of cardiomyocytes in the HFrecEF group was less severe than that in the HFrEF group, resulting in less GDF-8 secretion than that in the HFrEF group. For now, that's just a theory, and larger studies are needed to confirm it. Although ROC curve analysis suggests that GDF-8 does not have the diagnostic value of HFrecEF, it is still worthy of our continued attention as many previous studies have confirmed the relationship between GDF-8 and heart failure.

CDO1

Cysteine dioxygenase 1 comes from the cysteine dioxygenase family. CDO1 is a metalloproteinase whose main function is to participate in cysteine regulation and taurine synthesis. It is a key enzyme in cysteine catabolism and mainly distributed in cytoplasm[14–16].

CDO1 catalyze cysteine metabolism by taking cysteine as substrate with high specificity. L-cysteine is converted into l-cysteine sulfonic acid in the presence of oxygen, and taurine is the final product of pathway. Taurine has a number of roles in the mammalian body, including maintaining heart function and protecting nerve cells from excitatory toxicity and ischemic injury. Myocardial levels of taurine fall in ischemia, hypoxia and cardiac failure, with the depletion correlated with the degree of mechanical dysfunction[17–19]. In addition, taurine has been demonstrated to abolish arrhythmias in guinea pig and rabbit hearts[20, 21], and to attenuate the development of hypertension in rat[22].

CDO1 has been linked to a wide variety of tumors. Methylation of cysteine dioxygenase type1 gene, a tumor suppressor gene, has been studied in various cancers. CDO1 promoter methylation may be a potentially valuable diagnostic biomarker for hepatocellular carcinoma[23], and it is also an independent risk factor for poor prognosis in patients with renal clear cell carcinoma[24]. CDO1 gene promoter

hypermethylation was more frequently observed in non-small cell lung cancer tissues compared with in normal lung tissues[25].

At present, although no studies have confirmed a direct relationship between CDO1 levels and heart failure, taurine, a metabolic end product of cysteine, is associated with heart function and has a positive effect on heart function. CDO1, on the other hand, have a highly specific cysteine substrate. Sensitivity and specificity analysis by ROC revealed that CDO1 may be used as biomarkers of HFrecEF. The exact reasons for this need to be confirmed by further research.

Conclusions

The present study used a microarray platform to detect 1,000 proteins to identify specific serum factors expressed in HFrecEF samples. This method was demonstrated to be effective in investigating dynamic alterations in protein profiles, and to select target proteins for further HFrecEF research. The results indicated that CDO1 expression were downregulated in HFrecEF patients, suggesting that CDO1 may be important in the pathological process of HFrecEF. CDO1 represented potential predictive and diagnostic markers for HFrecEF due to its high sensitivity and specificity. However, larger scale studies are required to confirm the diagnostic value of this marker.

Declarations

Authors' contributions

Hua Wang and Yao Luo conceived the study, designed the trial, and obtained research funding. Hua Wang, Yao Luo, Jianping Cai, and Jiefu Yang supervised the conduct of the trial and data collection. Ke Chai and Minghui Du undertook recruitment of participating centers and patients and managed the data, including quality control. Tong Liu and Yao Luo provided statistical advice on study design and analyzed the data; Jiefu Yang chaired the data oversight committee. Yao Luo drafted the manuscript, and all authors contributed substantially to its revision. Hua Wang takes responsibility for the paper as a whole. All authors have read and approved the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Beijing Hospital. The participants or their family members provided verbal informed consent for participation.

Consent for publication

Not applicable.

Conflict of interests

The authors declare that there is no conflict of interest.

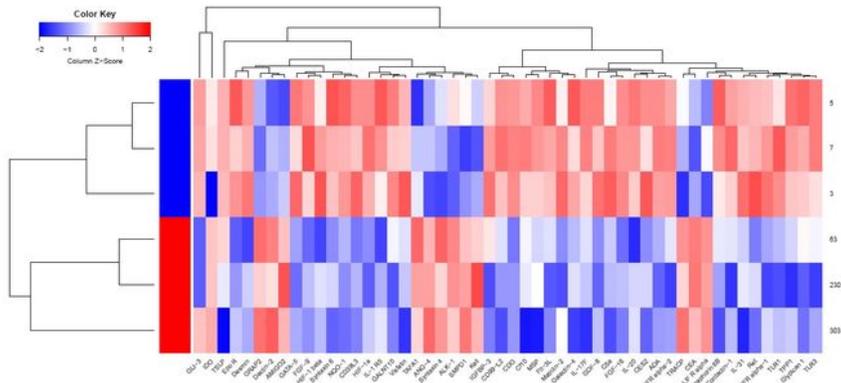
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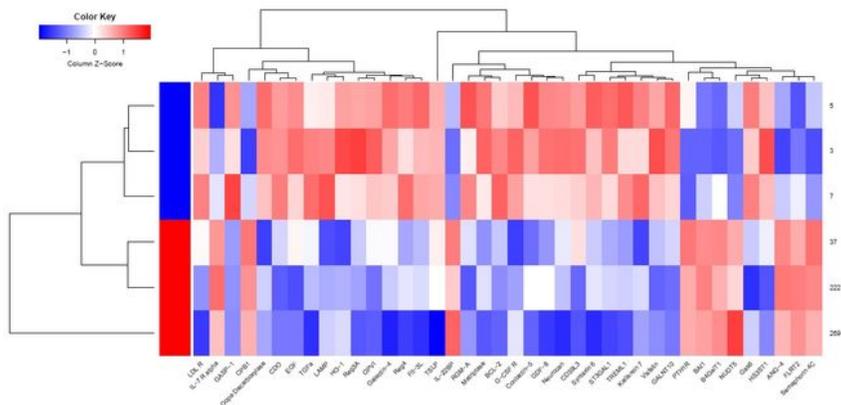
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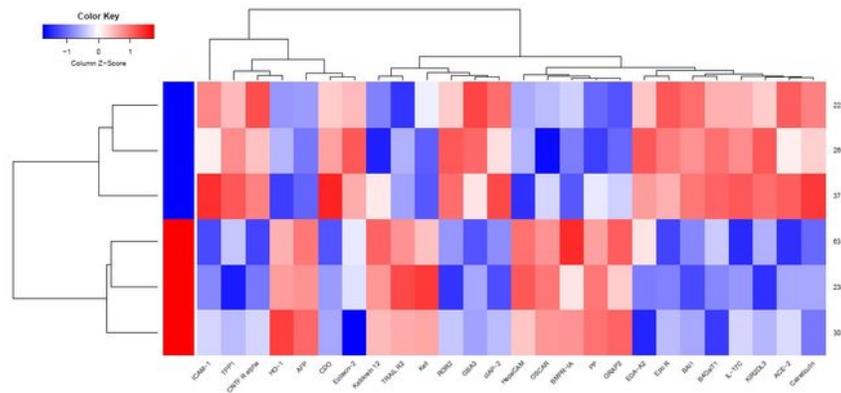
Figures



1a



1b



269 37, HFrEF patients).(C) Samples are clustered into HFrecEF and HFrecEF categories (Clinical diagnosis of serum sample donor: 222 269 37, HFrEF patients; 63 230 303, HFrecEF patients). Abbreviations: HFrecEF, heart failure with recovered ejection fraction;HFrEF,heart failure with reduced ejection fraction.

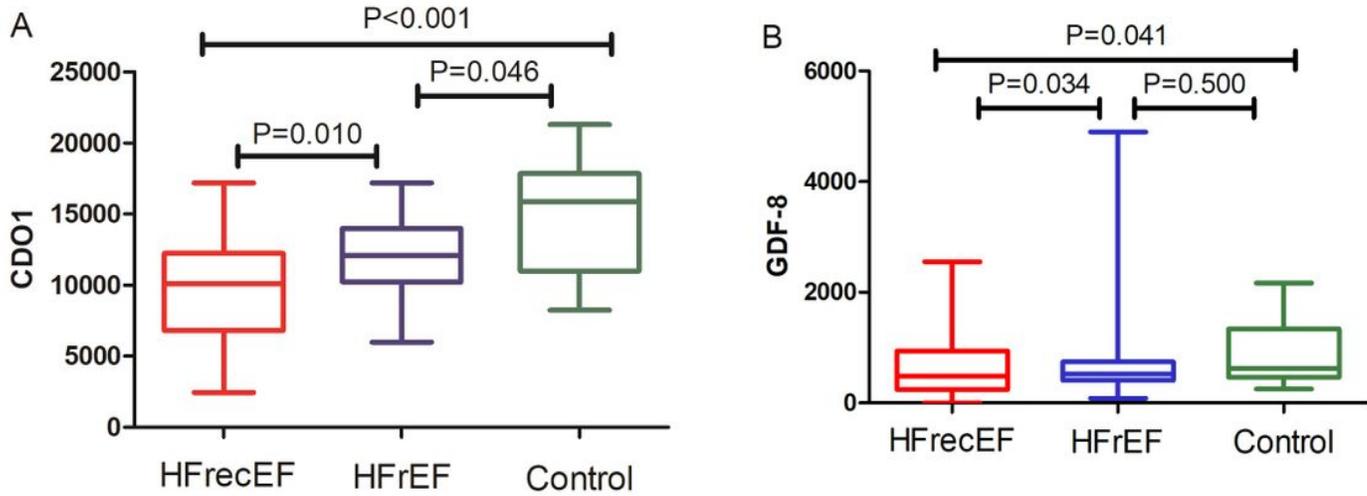


Figure 2

Boxplot of antibody array data .(A)Boxplot of CDO1.(B) Boxplot of GDF-8. Abbreviations:CDO1,Cysteine dioxygenase type 1;GDF-8, Growth/differentiation factor 8;HFrecEF, heart failure with recovered ejection fraction; HFrEF, heart failure with reduced ejection fraction .

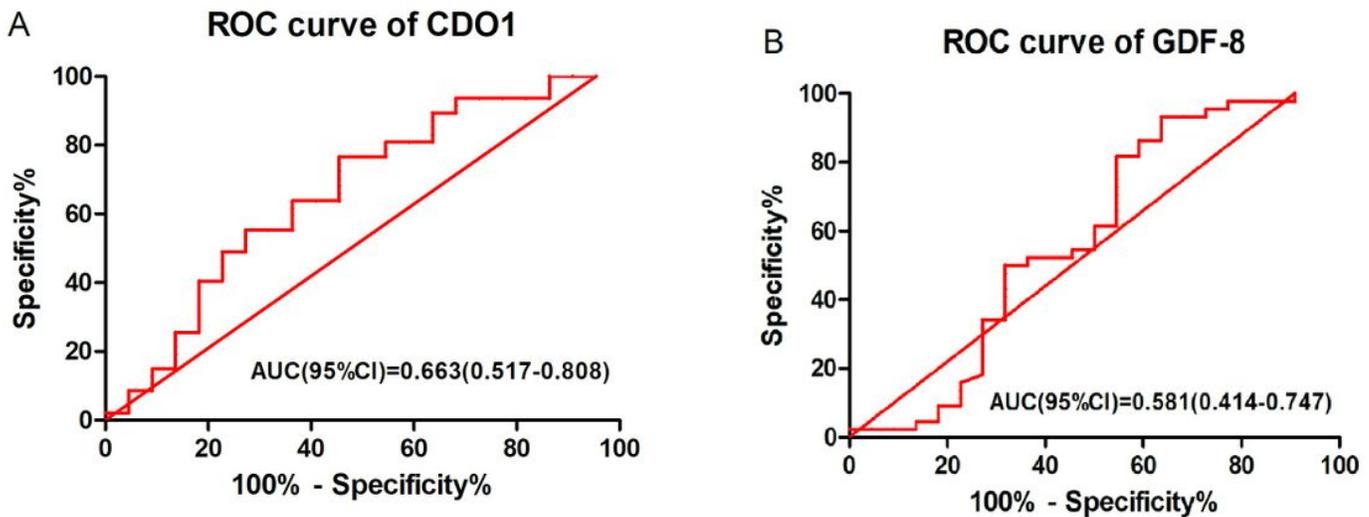


Figure 3

ROC curve.(A) ROC curve for CDO1.(B)ROC curve for GDF-8. Abbreviations:CDO1, Cysteine dioxygenase type 1 ; GDF-8, Growth/differentiation factor 8; ROC, receiver operating characteristics; AUC, area under curve; CI: confidence interval.

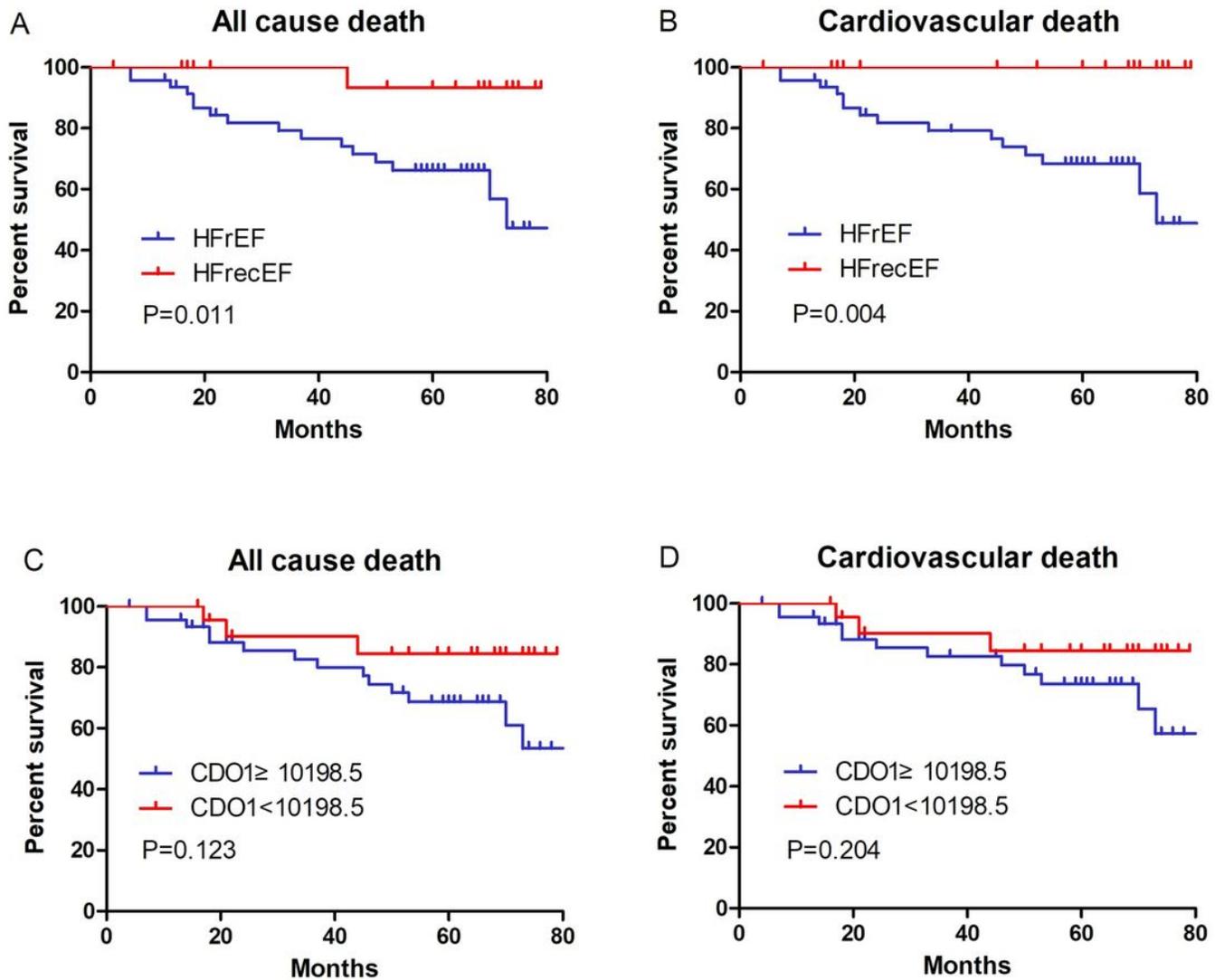


Figure 4

Kaplan–Meier curves in heart failure patients.(A) Kaplan–Meier curves for all-cause death in heart failure patients according to changes in ejection fraction.(B) Kaplan–Meier curves for cardiovascular death in heart failure patients according to changes in ejection fraction.(C) Kaplan–Meier curves for all-cause death in heart failure patients according to CDO1 level.(D)Kaplan–Meier curves for cardiovascular death in heart failure patients according to CDO1 level. Abbreviations:CDO1,Cysteine dioxygenase type 1;HFrecEF, heart failure with recovered ejection fraction; HFrEF, heart failure with reduced ejection fraction

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