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ORIGINAL RESEARCH

Extracellular Superoxide Dismutase Is Associated With Left Ventricular Geometry and Heart Failure in Patients With Cardiovascular Disease

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BACKGROUND: Extracellular superoxide dismutase (Ec-SOD) is a major scavenger of reactive oxygen species. However, its relationships with abnormal left ventricular (LV) geometry patterns and heart failure (HF) are still unknown in patients with cardiovascular disease.

METHODS AND RESULTS: A cross-sectional study was carried out to evaluate the association of serum Ec-SOD activity with LV geometry, as well as HF in 1047 patients with cardiovascular disease. All participants underwent standard echocardiography examination and measurement of serum Ec-SOD activity. Overall, we found a significantly decreased trend of serum Ec-SOD activity from subjects with normal geometry (147.96±15.94 U/mL), subjects with abnormal LV geometry without HF (140.19±20.12 U/mL), and subjects with abnormal LV geometry and overt HF (129.32±17.92 U/mL) after adjustment for potential confounders (*P* for trend <0.001). The downward trends remained significant in the concentric hypertrophy and eccentric hypertrophy groups after stratification by different LV geometry patterns. Multinomial logistic regression analysis showed that each 10 U/mL increase in serum Ec-SOD activity was associated with a 16.5% decrease in the odds of concentric remodeling without HF (odds ratio [OR], 0.835; 95% CI, 0.736–0.948), a 40.4% decrease in the odds of concentric hypertrophy with HF (OR, 0.596; 95% CI, 0.486–0.730), a 16.1% decrease in the odds of eccentric hypertrophy without HF (OR, 0.839; 95% CI, 0.729–0.965) and a 34.0% decrease in the odds of eccentric hypertrophy with HF (OR, 0.660; 95% CI, 0.565–0.772).

CONCLUSIONS: Serum Ec-SOD activity was independently associated with abnormal LV geometry patterns with and without overt HF. Our results indicate that Ec-SOD might be a potential link between LV structure remodeling and the development of subsequent HF in patients with cardiovascular disease.

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Key Words: extracellular superoxide dismutase ■ heart failure ■ left ventricular geometry

Heart failure (HF) is a complicated clinical syndrome caused by structural and/or functional cardiac abnormalities[1] and has become a rapidly growing public health issue throughout the world. In 2016, there were an estimated 37.7 million people living with HF globally, and the rising prevalence with advancing age still exists.[2–4] Although the mortality of HF has been reported to have decreased in recent decades, the 1- and 5-year mortality rates remain high, at near 20% and 50%, respectively,[5] partly attributable to the lack of effective therapy. Thus, early diagnosis, preventing the onset, and delaying the progression of HF, is of great importance.

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CLINICAL PERSPECTIVE

What Is New?

- Serum extracellular superoxide dismutase activity was independently associated with abnormal left ventricular (LV) geometry patterns.
- Serum extracellular superoxide dismutase activity presented a significant gradual downward trend from normal LV geometry to abnormal LV geometry without heart failure, and finally, to abnormal LV geometry with overt heart failure in patients with cardiovascular disease.

What Are the Clinical Implications?

- Our findings indicate that extracellular superoxide dismutase might be a potential link in the progression of normal LV structure to LV structure remodeling, and further to heart failure in patients with cardiovascular disease, and serum extracellular superoxide dismutase activity could be added to current biomarkers for risk assessment, as well as clinical management of heart failure.

Nonstandard Abbreviations and Acronyms

ACCF/AHA	American College of Cardiology Foundation/American Heart Association
AUC	area under the curve
CH	concentric hypertrophy
CR	concentric remodeling
CVD	cardiovascular disease
Ec-SOD	extracellular superoxide dismutase
GLM	general linear model
HF	heart failure
hsTNT	high-sensitivity troponin T
LV	left ventricular
LVDd	left ventricular end-diastolic dimension
LVH	left ventricular hypertrophy
LVMI	left ventricular mass index
NG	normal geometry
NT-proBNP	N-terminal proB-type natriuretic peptide
NYHA	New York Heart Association
OR	odds ratio
SOD	superoxide dismutase

Although the pathogenic mechanism of HF is complex and has not been fully elucidated, accumulating evidence suggests that enhanced oxidative stress contributes to cardiac ventricular and vascular remodeling and promotes the progression of HF.[6–8] Oxidative stress is an imbalanced state of reactive oxygen species production and the antioxidant defense system.[9] The superoxide dismutase (SOD) family are the first-line antioxidant enzymes in oxidative stress modulation.[10] Extracellular superoxide dismutase (Ec-SOD) is the predominant isoform, accounting for >70% of the total SOD activity in the human cardiovascular system.[11,12] Previous studies have demonstrated that Ec-SOD activity is associated with endothelial function and long-term outcomes in patients with chronic HF with cardiomyopathy.[13,14]

Left ventricular (LV) remodeling is widely regarded as a crucial event in the progression of HF related to both cardiac geometry and function.[15,16] Compared with normal geometry (NG), concentric remodeling (CR), eccentric hypertrophy (EH), and concentric hypertrophy (CH) are LV structure remodeling phenotypes that have been well characterized via echocardiography examination.[17,18] Although epidemiologic studies have reported that abnormal LV geometry phenotypes were associated with an increased risk of HF incidence,[19] as well as worse outcomes in patients with HF independent of traditional measures of LV size and function,[20,21] the underlying mechanism from normal LV geometry to abnormal LV geometry and, finally, to HF remains unclear. In addition, substantial evidence indicates that oxidative stress contributes to cardiac remodeling via several mechanisms,[8,22–24] and Ec-SOD protects the heart against oxidative stress and ventricular remodeling in mice.[25,26] However, while Ec-SOD has been studied individually in cardiac remodeling animal models or patients with end-stage HF, few studies have directly explored the role of Ec-SOD in the progression from LV structure remodeling to HF status. Evidence from large-scale population studies of the association between Ec-SOD and the transition from LV structure remodeling to HF is scarce.

The present hospital-based observational study was conducted to evaluate the association between serum Ec-SOD activity and abnormal LV geometry patterns in patients with and without symptomatic HF and to determine whether Ec-SOD is a potential marker in the early stage of cardiac structure remodeling before symptomatic clinical HF is apparent.

METHODS

The data and study materials that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

The study participants were a subset from the Guangdong Cardiovascular Disease Cohort, a

hospital-based ongoing prospective cohort, which was designed to estimate the impact of genetic, social, and environmental factors on the development of cardiovascular disease (CVD). Participants were recruited in the Department of Cardiology of Sun Yat-sen Memorial Hospital from November 2017 to June 2019, during their visits for the diagnosis or treatment of CVD. All participants in this study were admitted to the hospital for at least 1 cardiovascular condition, such as coronary artery disease, hypertension, dilated cardiomyopathy, hypertrophic cardiomyopathy, cardiac arrhythmia, or cardiac valve disease. Patients were excluded from all analyses if they met any of the following criteria: (1) history of malignant tumors, thyroid dysfunction, infectious diseases, autoimmune diseases, severe hepatic disease, or end-stage renal disease; (2) current antioxidant therapy; or (3) missing or incomplete echocardiography parameters, laboratory measurements, clinical characteristics, or demographic characteristics. Informed consent was obtained from all participants, and the study protocol was approved by the institutional review board at Sun Yat-sen University.

Echocardiography and Definition of LV Geometry

Standard echocardiography examination was performed at the recruiting center by professionally trained ultrasound physicians according to current guidelines of the American Society of Echocardiography. LV ejection fraction was determined by Simpson's biplane method, and end-diastolic interventricular septum, LV end-diastolic posterior wall thickness, LV end-diastolic diameter (LVDd), and anteroposterior diameter of the left atrium were determined by M-mode. LV mass according to the American Society of Echocardiography was calculated as LV mass=0.8×1.04 [(end-diastolic interventricular septum +LVDd+LV end-diastolic posterior wall thickness)^3–(LVDd)^3]+0.6 g (equation according to Devereux). LV mass was divided by body surface area to obtain the left ventricular mass index (LVMI). Left ventricular hypertrophy (LVH) was defined as LVMI ≥115 g/m^2 for males and LVMI ≥95 g/m^2 for females. Relative wall thickness was calculated with the formula relative wall thickness=((2×LV end-diastolic posterior wall thickness)/LVDd). Normal geometry (NG) was defined as RWT ≤0.42 plus non-LVH, CR was defined as RWT >0.42 plus non-LVH, CH was defined as RWT >0.42 plus LVH, and EH was defined as RWT ≤0.42 plus LVH.[17]

Diagnostic Criteria of HF

HF was defined according to the 2016 European Society of Cardiology Guidelines for the diagnosis and treatment of acute and chronic heart failure.[1] Patients were diagnosed with HF if they met all of the following criteria: (1) typical symptoms or signs of heart failure; (2) elevated levels of NT-proBNP (N-terminal pro-B-type natriuretic peptide) >125 pg/mL; and (3) either relevant structural heart disease (LVH or left atrial enlargement [define as left atrial diameter >35 mm] according to echocardiography) or diastolic dysfunction.

Definition of New York Heart Association Classes and American College of Cardiology Foundation/American Heart Association Stages of HF

The New York Heart Association (NYHA) Functional Classifications of HF and the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) stages of HF were defined by the 2013 ACCF/AHA Guideline for the Management of Heart Failure.[27] For ACCF/AHA stages of HF, patients at high risk for HF but with no structural heart disease or HF symptoms/signs were defined as stage A; patients who already had structural heart disease but without HF symptoms/signs were defined as stage B; patients who already had structural heart disease as well as prior or current symptoms/signs of HF were defined as stage C; patients with refractory HF requiring specialized interventions were defined as stage D. NYHA classes are based on the physical activity capacities of patients with HF. Patients were classified into different NYHA classes according to the following criteria: class I: ordinary physical activity does not cause symptoms of HF; class II: comfortable at rest, but ordinary physical activity causes symptoms of HF; class III: comfortable at rest, but less than ordinary physical activity causes symptoms of HF; class IV: unable to carry on any physical activity without symptoms of HF, or show symptoms of HF at rest. The NYHA classes and ACCF/AHA stages of HF were evaluated by ≥2 trained and experienced cardiologists after careful physical examinations at the same time of biomarker drawing in our study.

Data Collection

All participants were interviewed face to face to collect demographic information, medical history, medication use, behavioral habits, and risk factor prevalence. Smoking habits were classified into 2 groups: never or past smoking and current smoking. Current smoking was defined as at least 1 cigarette per day regularly for more than 6 months before recruitment. Current alcohol drinking was defined as drinking any type of alcoholic beverage at least once a week for more than half a year before recruitment.

Blood pressure, body weight, and height were measured by trained nurses on admission. Body mass index was defined as the weight in kilograms divided by the square of height in meters. Body surface area was calculated using the formula: body surface area=(body weight [kg]×height [cm]/3600)$^{1/2}$. The clinical diagnosis of all participants was marked by ≥2 professionally trained and experienced cardiologists after careful physical examinations.

Biomarker Measurements

Overnight fasting venous blood specimens were sampled the next morning after hospital admission. Blood specimens were sent to the central clinical laboratory of Sun Yat-sen Memorial Hospital within 2 hours and measured by trained technicians. Serum Ec-SOD activity was tested using the autoxidation of the pyrogallol method (Superoxide Dismutase Assay Kit, Fuyuan Biotechnology Co. Ltd., Fujian, China), following the manufacturer's instructions. Creatinine, hsCRP (high-sensitivity C-reactive protein), serum uric acid, and lactate dehydrogenase were determined using standard techniques by an automatic analyzer (Beckman Coulter chemistry analyzer AU5800, Beckman Coulter Co., Ltd, Tokyo, Japan). NT-proBNP and hsTNT (high-sensitivity troponin T) were measured by a fully automated electrochemiluminescence immunoassay system (Roche Cobas e601, Hoffmann-La Roche Ltd, Basel, Switzerland). Glycated hemoglobin was measured by high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA). The estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration Equation.

Statistical Analysis

Normally distributed data were expressed as the mean±SD, and variables with skewed distributions were reported as the median (interquartile range). One-way ANOVA or the Kruskal–Wallis H test was used for overall comparisons for continuous variables, and the least significant difference t test was used for pairwise comparisons. Categorical variables were expressed by frequency and percentages, and intergroup comparisons were analyzed by the chi-square test. The bivariate correlations between serum Ec-SOD activity and echocardiographic parameters and laboratory biomarkers were determined by Spearman correlation analysis, and a partial correlation analysis on ranks (Spearman correlation) was further conducted to calculate the correlation coefficients after controlling for potential covariates. Afterward, a multivariable-adjusted general linear model (GLM) was used to compare the differences in Ec-SOD between NG and the other 3 types of LV geometry and determine the association of NG, abnormal LV geometry without HF, and abnormal LV geometry with HF, as well as to compare the differences of Ec-SOD in patients with different NYHA classes or ACCF/AHA stages of HF, only variables identified significant in univariate analysis would be incorporated in further multivariable-adjusted GLM. Multinomial logistic regression analysis was performed to estimate the odds ratios (ORs) per 10 U/mL increase in serum Ec-SOD activity for CR no HF, CR+HF, CH no HF, CH+HF, EH no HF, and EH+HF, with NG patients as the reference, as well as for NYHA class and ACCF/AHA stages of HF, with the lowest class or stage as the references, using a forward stepwise procedure to select variables with the test level α=0.05. Binary logistic regression models were constructed to predict HF in patients with CR, CH, and EH, using a forward selection procedure to select variables with the test level α=0.05. After the patients were divided into the low and high Ec-SOD groups based on the median serum Ec-SOD activity in each type of LV geometry, 2 biomarkers reflecting myocardial stretch (NT-proBNP) and myocyte injury (hsTNT) were compared between the low and high Ec-SOD groups, and the Mann–Whitney *U* test and GLM were used to compare the differences of these 2 markers between the low and high Ec-SOD groups in different LV geometry patterns. Receiver operating characteristic (ROC) curve analysis was performed to test the potential ability of serum Ec-SOD activity to identify abnormal LV geometry with or without HF, using the bootstrap method to make comparisons of different areas under the curves (AUCs). All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL) and R (3.5.0). A 2-sided $P<0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics of the Study Patients

The baseline demographic and clinical characteristics of the 1047 recruited patients with CVD in this study are summarized in Table 1. The average age for all the participants was 59.8±13.1 years, and 531 (50.7%) of them were male. Among all of the participants, 269 (25.7%) patients had HF, 171 (16.7%) patients had nonobstructed coronary artery disease, 404 (38.6%) patients had obstructed coronary artery disease, and 159 (15.2%) patients had a percutaneous coronary intervention history. The rates of hypertension, diabetes mellitus, dilated cardiomyopathy/hypertrophic cardiomyopathy, atrial fibrillation, and valve disease in the total study population were 52.2%, 19.1%, 3.8%, 8.5%, and 6.4%, respectively. According to the LV geometry patterns, 409 patients (39.1%) had NG, 171

Table 1. Baseline Characteristics of Study Participants

Variables	Normal Geometry (n=409)	Concentric Remodeling (n=171)	Concentric Hypertrophy (n=175)	Eccentric Hypertrophy (n=292)	Total (n=1047)	*P* Value
Demographic characteristics						
Age, y	53.1±13.1	61.8±12.2	64.9±10.7	64.4±11.3	59.8±13.1	<0.001
Male	209 (51.1)	100 (58.5)	82 (46.9)	140 (47.9)	531 (50.7)	0.107
BMI, kg/m^2	23.29±3.07	24.76±3.25	25.57±3.82	24.39±3.42	24.22±3.43	<0.001
BSA, m^2	1.65±0.16	1.71±0.18	1.69±0.18	1.65±0.18	1.67±0.17	0.001
SBP, mm Hg	126±18	133±20	139±23	133±22	131±21	<0.001
DBP, mm Hg	78±11	81±12	80±12	79±12	79±12	0.121
Pause, bpm	75±13	81±15	76±14	80±16	76±14	<0.001
Smoking	88 (21.5)	38 (22.2)	43 (24.6)	59 (20.0)	228 (21.8)	0.654
Drinking	17 (4.2)	20 (11.7)	16 (9.1)	22 (7.5)	75 (7.2)	0.008
Echocardiographic parameters						
LA, mm	31 (29–33)	34 (30–37)	37 (34–39)	39 (35–49)	34 (31–38)	<0.001
IVSd, mm	9 (8–9)	10 (10–11)	12 (11–13)	10 (9–11)	10 (9–11)	<0.001
LVDd, mm	47 (44–49)	44 (42–46)	48 (46–50)	54 (55–60)	48 (45–51)	<0.001
LVPW, mm	8 (8–9)	10 (10–11)	11 (11–12)	10 (10–11)	9 (8–10)	<0.001
LVM, g	128 (114–148)	153 (132–204)	212 (175–241)	200 (176–243)	163 (131–203)	<0.001
LVMI, g/m^2	79 (70–88)	91 (81–98)	122 (107–135)	121 (107–141)	97 (80–119)	<0.001
RTW	0.36 (0.34–0.39)	0.45 (0.43–0.48)	0.46 (0.44–0.49)	0.36 (0.31–0.39)	0.39 (0.35–0.43)	<0.001
LVEF, %	69 (65–72)	68 (65–71)	66 (63–70)	61 (43–67)	67 (62–70)	<0.001
Comorbidities						
HF	0 (0.0)	17 (9.9)	74 (42.3)	178 (61.0)	269 (25.7)	<0.001
CAD						<0.001
NC	246 (60.1)	73 (42.7)	55 (31.4)	98 (33.6)	472 (45.1)	
NOCAD	71 (17.4)	22 (12.9)	50 (17.1)	128 (17.4)	171 (16.3)	
OCAD	92 (22.5)	76 (44.4)	92 (52.6)	144 (49.3)	404 (38.6)	
Hypertension	133 (32.5)	103 (60.2)	147 (84.0)	164 (56.2)	547 (52.2)	<0.001
Diabetes mellitus	40 (9.8)	41 (24.0)	53 (30.3)	66 (22.6)	200 (19.1)	<0.001
DCM/HCM	0 (0.0)	2 (1.2)	5 (2.9)	33 (11.3)	40 (3.8)	<0.001
PCI history	27 (6.6)	27 (15.8)	46 (26.3)	59 (20.2)	159 (15.2)	<0.001
AF	5 (1.5)	17 (9.9)	20 (11.4)	47 (16.1)	89 (8.5)	<0.001
Valve disease	8 (2.0)	5 (2.9)	11 (6.3)	43 (14.7)	67 (6.4)	<0.001
Medication usage						
ACEI/ARB	36 (8.8)	40 (23.4)	54 (30.9)	61 (20.9)	191 (18.2)	<0.001
Beta-blocker	34 (8.3)	31 (18.1)	44 (25.1)	65 (22.3)	174 (16.6)	<0.001
Antidiabetic	26 (6.4)	33 (19.3)	44 (25.1)	41 (27.0)	144 (13.8)	<0.001
Diuretic agents	8 (2.0)	7 (4.1)	11 (6.3)	31 (10.6)	57 (5.4)	<0.001
Statin	41 (10.0)	34 (19.9)	37 (21.1)	63 (21.6)	175 (16.7)	<0.001
Clinical biomarkers						
hsCRP, mg/L	0.92 (0.41–1.90)	1.17 (0.53–1.33)	1.50 (0.69–4.01)	1.50 (0.63–3.65)	1.11 (0.50–2.91)	<0.001
HbA1c, %	5.6 (5.3–5.9)	5.9 (5.5–6.4)	6.0 (5.7–6.2)	5.9 (5.6–6.3)	5.8 (5.4–6.2)	<0.001
Uric acid, μmol/L	347 (294–461)	378 (308–476)	381 (316–457)	412 (322–509)	372 (304–450)	<0.001
NT-proBNP, pg/mL	40.5 (21.7–64.9)	69.6 (30.3–176.0)	101.8 (54.4–373.6)	316.8 (78.6–1406.2)	65.4 (32.2–202.7)	<0.001
hsTNT, pg/mL	4.9 (3.9–6.5)	7.8 (5.5–11.6)	9.7 (6.4–17.8)	11.8 (7.3–23.9)	7.0 (4.7–12.5)	<0.001
LDH, U/L	169 (152–187)	173 (153–199)	187 (164–216)	185 (166–222)	177 (157–202)	<0.001

(*Continued*)

Table 1. Continued

Variables	Normal Geometry (n=409)	Concentric Remodeling (n=171)	Concentric Hypertrophy (n=175)	Eccentric Hypertrophy (n=292)	Total (n=1047)	*P* Value
eGFR, mL/min	86.92±16.91	77.66±18.53	75.09±21.74	71.22±20.14	79.41±20.21	<0.001
Ec-SOD, U/mL	147.96±15.74	139.46±17.98	135.12±24.08	133.63±18.00	140.48±19.62	<0.001

Values are mean±SD, n (%), or median (interquartile range). Significance tests for comparisons by group based on one-way analysis of variance for normal distribution continuous variables, and Kruskal–Wallis H test for skewed distribution continuous variables; χ^2 test for categorical variables. ACEI indicates angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin-receptor blocker; BMI, body mass index; BSA, body surface area; CAD, coronary artery disease; DBP, diastolic blood pressure; DCM, dilated cardiomyopathy; Ec-SOD, extracellular superoxide dismutase; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycosylated hemoglobin A_1; HCM, hypertrophic cardiomyopathy; HF, heart failure; hsCRP, high-sensitivity C-reactive protein; hsTNT, high sensitivity troponin T; IVSd, interventricular septum; LA, left atrial diameter; LDH, lactate dehydrogenase; LVDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LVMI, left ventricular mass index; LVPW, left ventricular posterior wall thickness; NC, normal coronary artery; NOCAD, nonobstructive coronary artery disease; NT-proBNP, N-terminal pro-B type natriuretic peptide; OCAD, obstructive coronary artery disease; PCI, percutaneous coronary stent implantation; RTW, relative wall thickness; and SBP, systolic blood pressure.

patients (16.3%) had CR, 292 patients (27.9%) had EH, and 175 patients (16.7%) had CH (Figure S1). Compared with patients with abnormal LV geometry, patients in the NG group were younger, had lower systolic blood pressure and lower rates of obstructed coronary artery disease, hypertension, diabetes mellitus, dilated cardiomyopathy/hypertrophic cardiomyopathy, percutaneous coronary intervention history, atrial fibrillation, and valve disease. The serum Ec-SOD activities were normally distributed in the study patients with a mean level of 140.48±19.62 U/mL, which suggests that there were no carriers of *R213G* in our study population, since *R213G* carriers would have very high serum Ec-SOD activity and would appear as outliers (Figure S2). The serum Ec-SOD activities decreased in a stepwise manner from NG patients (147.96±15.74 U/mL) to CR patients (139.46±17.98 U/mL), to EH patients (135.12±24.08 U/mL) and to CH patients (133.63±18.00 U/mL). Serum Ec-SOD activity was inversely associated with LVMI, left atrial diameter, end-diastolic interventricular septum, LVDd, LV mass, LV ejection fraction, NT-proBNP, hsTNT and hsCRP (Table S1). NYHA class did not affect serum Ec-SOD activity after adjusting for covariates (Figure S3); serum Ec-SOD activity was not an independent influencing factor for the ACCF/AHA stages of HF (Figure S4).

Serum Ec-SOD Activity in Different LV Geometry Patterns

Compared with the NG group, serum Ec-SOD activity in the CR, CH, and EH groups were markedly declined (*P* for trend <0.001) after adjusting for covariates. (Figure 1). The estimated average levels of Ec-SOD by multivariable-adjusted GLM were 144.23±3.89 U/mL for the NG group, 140.21±5.29 U/mL for the CR group, 137.73±5.53 U/mL for the CH group, and 137.79±4.45 U/mL for the EH group after controlling for potential confounders.

Serum Ec-SOD Activity in Patients With NG and Patients With Abnormal LV Geometry With and Without Overt HF

In GLM analysis, overall, the serum Ec-SOD activity levels were significantly lower in the patients with any types of abnormal LV geometry but no HF (140.19±20.12 U/mL, *P*<0.01) and in the patients with abnormal LV geometry plus HF (129.32±17.92 U/mL, *P*<0.001) compared with the patients in the NG group (147.96±15.94 U/mL). After stratification by the different patterns of abnormal LV geometry, patients with CR+HF showed no difference in serum Ec-SOD activity compared with the NG group. As shown in Figure 2, there were substantial downward trends of serum Ec-SOD activity from NG (147.96±15.94 U/mL) to CH with no HF (140.34±17.81 U/mL) and to CH+HF (128.00±15.12 U/mL) (*P* for trend <0.01), and from NG to EH with no HF (140.22±15.03 U/mL) to EH+HF (129.40±18.51 U/mL) (*P* for trend <0.01).

Association Between Serum Ec-SOD Activity and the Presence of Abnormal LV Geometry With and Without Overt HF

Multinomial logistic regression analysis showed that each 10 U/mL increase in serum Ec-SOD activity was associated with a 16.5% decrease in the odds of CR without HF (OR, 0.835; 95% CI, 0.736–0.948), a 40.4% decrease in the odds of CH with HF (OR, 0.596; 95% CI, 0.486–0.730), a 16.1% decrease in the odds of EH without HF (OR, 0.839; 95% CI, 0.729–0.965) and a 34.0% decrease in the odds of EH with HF (OR, 0.660; 95% CI, 0.565–0.772), with the NG group as the reference (Figure 3).

Association Between Serum Ec-SOD Activity and the Risk of HF in Patients With CR, CH, and EH

On simple logistic regression analysis, higher serum Ec-SOD activity was associated with lower rates of

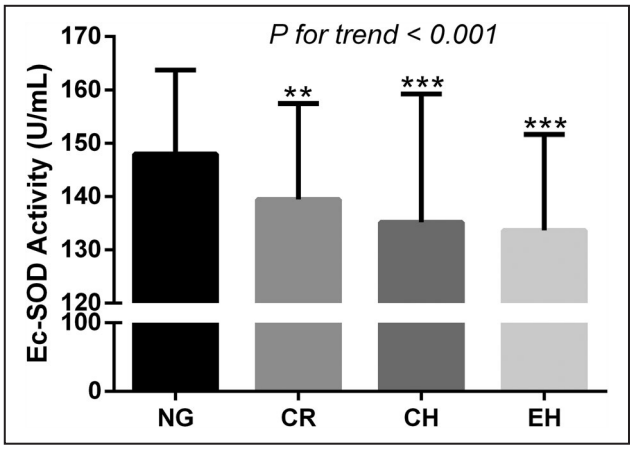

Figure 1. Serum extracellular superoxide dismutase (Ec-SOD) activity in subjects with different left ventricular geometry patterns.
The mean serum Ec-SOD activity in subject with normal geometry (NG, n=409), concentric remodeling (CR, n=171), concentric hypertrophy (CH, n=292) and eccentric hypertrophy (EH, n=175). ***P*<0.01 vs NG; ****P*<0.001 vs NG. *P* values were calculated using the multivariable-adjusted general linear model, adjusted by age, sex, smoking, hypertension, diabetes mellitus, coronary artery disease, dilated cardiomyopathy/hypertrophic cardiomyopathy, PCI history, atrial fibrillation, valve disease, drug use (including angiotensin-converting enzyme inhibitor/qngiotensin receptor blocker, beta-blocker, and diuretic agents), glycated hemoglobin, high-sensitivity C-reactive protein, estimated glomerular filtration rate, and uric acid, all covariates included in the multivariable-adjusted general linear model were significant in univariate analysis.

HF in the CH group (OR, 0.684; 95% CI, 0.555–0.844; *P*<0.001) and EH group (OR, 0.691; 95% CI, 0.594–0.803; *P*<0.001). After adjustment for age, sex, and body mass index, the ORs for HF remained significant for each 10 U/mL serum Ec-SOD activity increase in the CH group (OR, 0.710; 95% CI, 0.574–0.878; *P*=0.002) and the EH group (OR, 0.714; 95% CI, 0.611–0.834; *P*<0.001). However, when the covariates were selected by the forward stepwise procedure, serum Ec-SOD activity was eliminated in the final logistic models in the CR and CH groups but remained independently significant in the EH group (OR, 0.823; 95% CI, 0.682–0.993; *P*=0.042) (Table 2).

Comparison of HF Markers in Patients With Different LV Geometry With Low and High Ec-SOD Activity

In patients with NG, no difference was found in NT-proBNP or hsTNT between these 2 groups. In patients with CR without HF, NT-proBNP was elevated in the low Ec-SOD group (OR, 74.8; 95% CI, 27.8–166.0; *P*=0.030) compared with the high Ec-SOD group (OR, 46.6; 95% CI, 19.7–96.2) even after controlling for confounders by GLM. In patients with CH plus HF, NT-proBNP and hsTNT were significantly higher in the low Ec-SOD group (OR, 161.4; 95% CI, 62.7–951.4) before adjustment for covariates, but they were not significant after adjustment. In the EH group, we found that NT-proBNP was markedly elevated in the low Ec-SOD group, in subjects both with and without overt HF, before and after adjusting for covariates (Table 3).

Diagnostic Ability of Serum Ec-SOD Activity for Patients With Abnormal LV Geometry With and Without Overt HF

Serum Ec-SOD activity showed mild but significant diagnostic ability to distinguish patients in the CR no HF group (AUC, 0.626; 95% CI, 0.573–0.678; sensitivity, 49.4%; specificity, 71.6%), CH no HF group (AUC, 0.652; 95% CI, 0.595–0.712; sensitivity, 53.6%; specificity, 71.6%), and EH no HF group (AUC, 0.655; 95% CI, 0.600–0.711; sensitivity, 52.6%; specificity, 69.4%) from the patients in the NG group. When tested together with NT-proBNP and hsTNT, 2 conventional risk markers of cardiac function and damage, serum Ec-SOD activity added significant improvement in diagnosis performance beyond these 2 markers in distinguishing normal geometry from concentric remodeling without HF (AUC, 0.703; 95% CI, 0.566–0.879; *P* for bootstrap method=0.015) (Table 4).In addition, compared with NG, patients with CR plus HF could be identified by Ec-SOD with a sensitivity of 64.7% and a specificity of 83.1% (AUC, 0.723; 95% CI, 0.566–0.879); patients with CH plus HF could be identified by Ec-SOD with a sensitivity of 68.9% and a specificity of 80.0% (AUC, 0.819; 95% CI, 0.769–0.868); and patients with EH plus HF could be identified by Ec-SOD with a sensitivity of 74.7% and a specificity of 71.6% (AUC, 0.789; 95% CI, 0.747–0.832). Furthermore, compared with CH no HF, patients with CH plus HF could be identified by Ec-SOD with a sensitivity of 58.1% and a specificity of 73.3% (AUC, 0.696; 95% CI, 0.618–0.775); compared with EH no HF, patients with EH plus HF could be identified by Ec-SOD with a sensitivity of 55.1% and a specificity of 78.1% (AUC, 0.692; 95% CI, 0.631–0.753) (Figure 4).

DISCUSSION

In this study, which included 1047 patients with CVD, we demonstrated that serum Ec-SOD activity presented a significant gradual downward trend from normal LV geometry to abnormal LV geometry without HF, and finally, to abnormal LV geometry with overt HF. After adjusting for demographic and clinical covariates, this association remained significant in patients with concentric hypertrophy and eccentric hypertrophy. To our knowledge, this is the first study to investigate the association of serum Ec-SOD

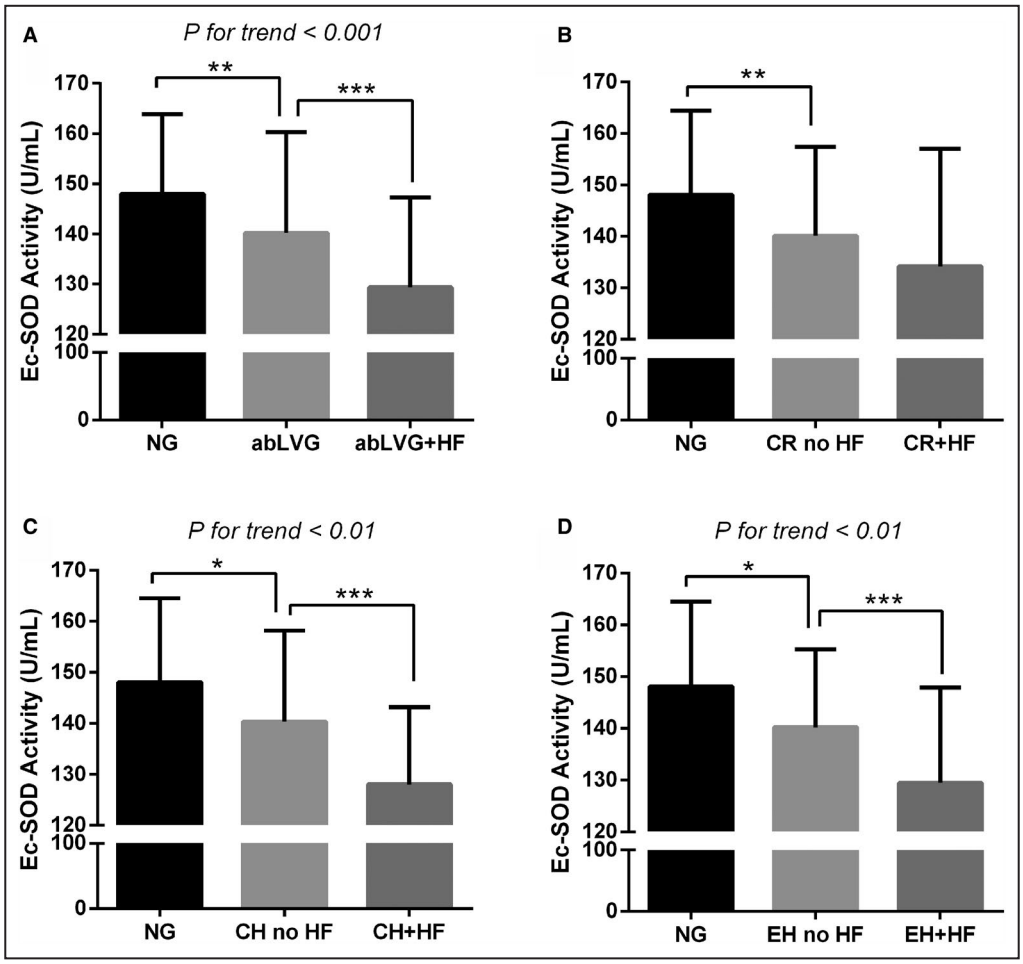

Figure 2. Serum Ec-SOD activity in patients with normal left ventricular (LV) geometry and abnormal LV geometry with and without HF.

A, Differences of serum Ec-SOD activities among subjects with NG, subjects with all types of abnormal LV geometry without HF (abLVG, including CR, CH, and EH subject without HF), and subjects with abnormal LV geometry plus heart failure (abLVG+HF); **B**, differences of serum Ec-SOD activities among subjects with NG, subjects with CR but without HF (CR no HF), and subjects with CR and overt HF (CR+HF); **C**, differences of serum Ec-SOD activities among subjects with NG, subjects with CH but without HF (CH no HF), and subjects with CH and overt HF (CH+HF); **D**, differences of serum Ec-SOD activities among subjects with NG, subjects with EH but without HF (EH no HF) and subjects with EH and overt HF (EH+HF). abLVG indicates abnormal left ventricular geometry; CH, concentric hypertrophy; CR, concentric remodeling; Ec-SOD, extracellular superoxide dismutase; EH, eccentric hypertrophy; HF, heart failure; and NG, normal geometry. *P<0.05; **P<0.01; ***P<0.001; P values were calculated using the same multivariable-adjusted general linear model as Figure 1. Least significant difference t test was used for pairwise comparison.

activity with LV geometry and heart failure in a large population. Our study indicates that declining serum Ec-SOD activity might be an independent risk factor for the presence of abnormal LV geometry patterns and subsequent heart failure, especially in patients with LV hypertrophy.

Adverse LV structure remodeling is considered an intermediate phenotype of HF, given the high incidence of HF events observed among individuals with abnormal LV structure.[19,20,28] de Simone et al[29] provided strong evidence that concentric LV hypertrophy is a risk factor for the development of HF independent of myocardial infarction and overload pressure, suggesting that mechanisms other than myocardial ischemia and hemodynamic load may play key roles in the development of HF in individuals with abnormal LV structure. However, the underlying pathophysiological mechanism in the progression of normal LV geometry to LV structure remodeling and eventually to HF remains to be elucidated.

Accumulating evidence derived from animal studies has demonstrated that Ec-SOD plays an important role in the development of HF. Ec-SOD gene-deficient mice developed more LV hypertrophy in response to

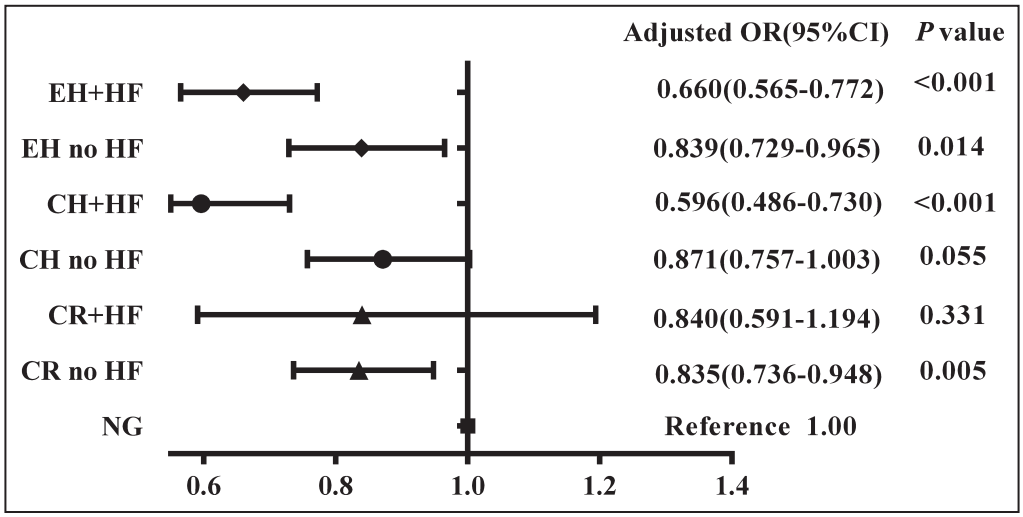

Figure 3. ORs of different LV geometry patterns with and without HF by 10 U/mL serum Ec-SOD activity increase.
Multivariable-adjusted odds ratios (95% CI) for the presence of different LV geometry patterns with and without HF per 10 U/mL serum Ec-SOD activity increase were calculated through multinomial logistic regression model, using forward stepwise procedure to select variables. CH indicates concentric hypertrophy; CR, concentric remodeling; Ec-SOD, extracellular superoxide dismutase; EH, eccentric hypertrophy; HF, heart failure; NG, normal geometry; and ORs, odds ratios.

overload pressure and showed greater oxidative stress and myocardial fibrosis associated with activation of the mitogen-activated protein kinase signaling cascades.[25,26] Overexpression of Ec-SOD in the hearts of transgenic mice helps to protect cardiac function from ischemia-reperfusion injury.[30] A genetic variant with a substitution in the heparin-binding domain of Ec-SOD (Ec-SOD-*R213G*) was associated with excessive oxidative stress, endothelial dysfunction,[31] increased risk of ischemic heart disease, and more severe HF.[32] The Ec-SOD *R213G* mutation is present in 4% to 6% of the Asian population, and the plasma EC-SOD levels are 10-fold or higher in mutation carriers than in noncarriers.[33,34] It has been speculated that such an increase results from the accelerated release of EC-SOD from the interstitial matrix.[33,35] In noncarriers, higher serum EC-SOD comes from higher tissue EC-SOD, which is the effective part to protect against oxidative stress in tissue.[36,37] In the present study, the serum EC-SOD activities were normally distributed, and there were no patients with very high serum EC-SOD activity levels, suggesting that there was no carriers of the Ec-SOD *R213G* mutation in our study; thus, the negative correlation of serum EC-SOD activity and the severity of the heart phenotype may not be influenced by the Ec-SOD *R213G* mutation.

Previous population studies have reported that the serum activities of the SOD family, including manganese SOD, copper/zinc-containing SOD, and total SOD, were predictors of worse long-term clinical outcome in nonischemic dilated cardiomyopathy patients, which is a frequent cause of HF.[14] Reduced Ec-SOD activity was reported to be closely associated with increased vascular oxidative stress and endothelial dysfunction in patients with chronic HF.[38] A later small sample case-control study including 38 patients with chronic HF and 12 controls validated this association and found that it might be related to serum uric acid.[13] However, only a few small-sample studies based on populations have paid attention to the association between

Table 2. Association Between Ec-SOD Activity and the Risk of HF Presence in CR, CH, and EH Patients

Group	Model 1		Model 2		Model 3	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
CR	0.830 (0.623–1.105)	0.202	0.997 (0.719–1.382)	0.985	…	…
CH	0.684 (0.555–0.844)	<0.001	0.710 (0.574–0.878)	0.002	…	…
EH	0.691 (0.594–0.803)	<0.001	0.714 (0.611–0.834)	<0.001	0.823 (0.682–0.993)	0.042

Model 1, simple logistic regression. Model 2, multiple logistic regression adjusted for age, sex, body mass index. Model 3, multiple logistic regression using a forward stepwise procedure to select variables. CH indicates concentric hypertrophy; CR, concentric remodeling; Ec-SOD, extracellular superoxide dismutase; EH, eccentric hypertrophy; HF, heart failure; and OR, odds ratio.

Table 3. Comparisons of HF Markers in Subjects According to the Serum Ec-SOD Activity

Group	Variables	Low Ec-SOD	High Ec-SOD	Unadjusted *P* Value	Adjusted *P* Value
NG	NT-proBNP	41.8 (21.2–69.7)	38.1 (19.1–61.3)	0.128	0.888
	hs-TNT	5.2 (4.0–7.0)	4.9 (3.9–7.0)	0.607	0.397
CR no HF	NT-proBNP	74.8 (27.8–166.0)	46.6 (19.7–96.2)	0.043*	0.030*
	hs-TNT	8.4 (5.5–13.8)	6.9 (5.2–10.1)	0.064	0.035
CR+HF	NT-proBNP	781.5 (354.9–2546.0)	466.3 (165.9–722.6)	0.098	0.966
	hs-TNT	17.04 (11.7–30.5)	15.6 (10.3–76.7)	0.884	0.231
Total CR	NT-proBNP	88.7 (34.6–290.8)	50.7 (24.0–129.0)	0.010*	0.051
	hs-TNT	9.2 (5.7–15.9)	0.71 (0.54–10.77)	0.027*	0.980
CH no HF	NT-proBNP	61.3 (40.2–83.5)	56.8 (31.1–87.4)	0.634	0.966
	hs-TNT	7.8 (5.4–10.9)	7.3 (5.4–11.1)	0.942	0.777
CH+HF	NT-proBNP	578.8 (265.9–1513.0)	321.7 (201.2–711.1)	0.036*	0.089
	hs-TNT	17.6 (11.9–32.1)	17.7 (8.0–43.2)	0.879	0.531
Total CH	NT-proBNP	161.4 (62.7–954.1)	75.6 (42.9–175.7)	<0.001*	0.025*
	hs-TNT	12.0 (7.5–18.6)	8.4 (5.9–17.0)	0.019*	0.224
EH no HF	NT-proBNP	67.7 (36.3–101.2)	52.3 (30.0–80.9)	0.046*	0.020*
	hs-TNT	9.8 (5.5–16.7)	4.8 (6.9–11.2)	0.082	0.336
EH+HF	NT-proBNP	1275.5 (471.5–4773.2)	617.5 (222.9–1305.0)	0.001*	0.011*
	hs-TNT	22.5 (13.9–37.2)	14.0 (8.1–21.5)	<0.001*	0.102
Total EH	NT-proBNP	731.4 (156.6–3198.0)	116.9 (49.3–591.5)	<0.001*	<0.001*
	hs-TNT	17.9 (10.52–33.02)	8.9 (5.9–17.0)	<0.001*	0.016*

Unadjusted *P* value was calculated by the Mann–Whitney *U* test. Adjusted *P* value was calculated by multivariable-adjusted general linear model adjusted by potential confounders (covariates were same as Figure 1). CH indicates concentric hypertrophy; CR, concentric remodeling; Ec-SOD, extracellular superoxide dismutase; EH, eccentric hypertrophy; HF, heart failure; hsTNT, high-sensitivity troponin T; NG, normal geometry; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

*$P<0.05$.

circulating Ec-SOD activity and nonischemic dilated cardiomyopathy in patients with end-stage HF. There is still limited information about circulating Ec-SOD activity in patients with HF and its precursor condition, LV geometry remodeling. The present study extends this information in 4 aspects in a large Chinese population for the first time. First, serum Ec-SOD activity decreased in patients with CVD with abnormal LV geometry patterns without HF, including patients with concentric remodeling, concentric hypertrophy, and eccentric hypertrophy, and the declining levels presented more obviously in patients with LV hypertrophy than in patients with simple concentric remodeling. Second, serum Ec-SOD activity decreased more notably in patients with abnormal LV geometry plus overt HF than in those without HF. Third, we demonstrated that even after adjustment for demographic and clinical covariates, serum Ec-SOD activity gradually declined from normal LV geometry to abnormal LV geometry and finally to HF in patients with concentric hypertrophy and eccentric hypertrophy but not in patients with concentric remodeling. Fourth, serum Ec-SOD activity showed significant improvement

Table 4. AUC of ROC Analysis in Identifying Abnormal LV Geometry Without HF

	NG vs CR No HF AUC (95% CI)	NG vs CH No HF AUC (95% CI)	NG vs EH No HF AUC (95% CI)
Ec-SOD	0.626 (0.573–0.678)	0.652 (0.592–0.712)	0.635 (0.579–0.692)
NT-proBNP	0.621 (0.562–0.680)	0.649 (0.589–0.708)	0.655 (0.600–0.711)
hsTNT	0.688 (0.638–0.738)	0.691 (0.635–0.747)	0.669 (0.610–0.728)
NT-proBNP+hsTNT	0.664 (0.608–0.719)	0.694 (0.636–0.751)	0.686 (0.631–0.742)
Ec-SOD+NT-proBNP+hsTNT	0.703 (0.652–0.754)	0.718 (0.660–0.776)	0.711 (0.656–0.765)
P value	0.015	0.182	0.137

P value was calculated using bootstrap method to make comparisons of the AUC between NT-proBNP+hsTNT and Ec-SOD+NT-proBNP+hsTNT. AUC indicates area under the curve; CH, concentric hypertrophy; CR, concentric remodeling; Ec-SOD, extracellular superoxide dismutase; EH, eccentric hypertrophy; HF, heart failure; hsTNT, high-sensitivity troponin T; NG, normal geometry; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and ROC, receiver operating characteristic curve.

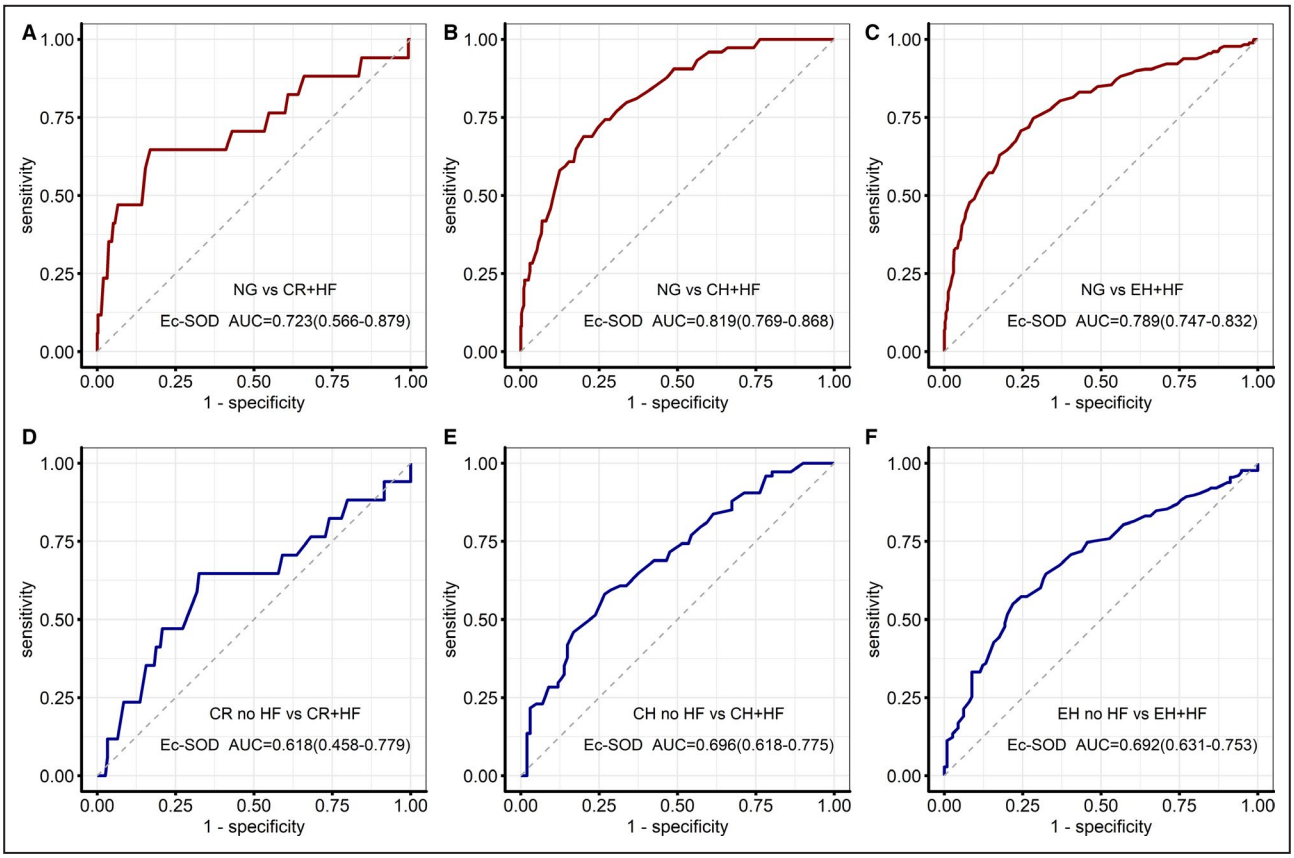

Figure 4. Receiver operating characteristic curves of serum Ec-SOD activity in the identification of abnormal LV geometry patients with HF.

A, Rreceiver operating characteristic curve analysis of serum Ec-SOD activity for distinguishing subjects in CR+HF group from subjects in NG group; **B**, for distinguishing subjects in CH+HF group from subjects in NG group; **C**, for distinguishing subjects in EH+HF group from subjects in NG group; **D**, for distinguishing subjects in CR+HF group from subjects in CR no HF group; **E**, for distinguishing subjects in CH+HF group from subjects in CH no HF group; **F**, for distinguishing subjects in EH+HF group from subjects in EH no HF group. AUC indicates area under the curve; CH, concentric hypertrophy; CR, concentric remodeling; Ec-SOD, extracellular superoxide dismutase; EH, eccentric hypertrophy; HF, heart failure; and NG, normal geometry.

in the ability to distinguish patients with concentric remodeling without HF from those with normal LV geometry beyond NT-proBNP, which has been widely used for HF management, outcome prediction, and risk assessment.[39–41] Additionally, in patients with eccentric hypertrophy but without overt HF, subjects with lower serum Ec-SOD activity were in higher HF risk since they also had higher NT-proBNP. Taken together with previous studies, we speculate that serum Ec-SOD might be a link in the progression of normal LV geometry to LV structure remodeling and further HF, and decreased serum Ec-SOD activity might contribute to alterations in LV structure and the onset of HF in patients with CVD. Further studies are required to elucidate the mechanism behind these associations.

There were some limitations in this study. First, it is important to stress that the cross-sectional design could not determine causal relationships between declining serum Ec-SOD activity and LV structure alteration or HF. However, we analyzed the associations in a large population and the evidence was strengthened by considering a variety of established confounders. Additionally, since we analyzed Ec-SOD activity in venous blood instead of in heart tissue, the activity level might not be as specific it would have been in myocardial biopsies. It is impossible to obtain heart tissue in routine clinical practice; thus, testing Ec-SOD activity in circulation might be more receptive and helpful in evaluating these complex phenotypes.

In summary, we observed that serum Ec-SOD activity declined gradually and significantly from normal LV geometry to abnormal LV geometry without HF, and finally, to abnormal LV geometry with overt HF. Our results suggest that Ec-SOD may be an independent link between LV structure remodeling and the development of subsequent HF.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Materials

Table S1
Figures S1–S4

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Supplemental Material

Table S1. Correlation coefficients between Ec-SOD and Echocardiographic parameters or laboratory biomarkers.

	Bivariate correlation		Partial correlation	
Variables	***r***	***P***	***r***	***P***
LVMI	-0.352	<0.001	-0.163	<0.001
RTW	-0.077	0.012	-0.007	0.821
LA	-0.330	<0.001	-0.139	<0.001
IVSd	-0.243	<0.001	-0.095	0.002
LVDd	-0.169	<0.001	-0.089	0.006
LVPWd	-0.214	<0.001	-0.063	0.050
LVM	-0.308	<0.001	-0.147	<0.001
LVEF	0.189	<0.001	0.149	<0.001
NT-proBNP	-0.393	<0.001	-0.216	<0.001
hsTNT	-0.335	<0.001	-0.118	<0.001
LDH	-0.184	<0.001	-0.087	0.007
Uric acid	-0.170	<0.001	-0.092	0.004
hsCRP	-0.271	<0.001	-0.195	<0.001
eGFR	0.389	<0.001	0.150	<0.001
HbA1c	-0.257	<0.001	-0.122	<0.001

Bivariate correlation coefficients were calculated using Spearman correlation analysis.

Partial correlation coefficients were calculated using Partial Spearman correlation analysis controlling for age, sex, BMI, smoking, drinking, hypertension, diabetes, coronary artery disease, Dilated cardiomyopathy/Hypertrophic cardiomyopathy, PCI history, Atrial fibrillation, Valve disease, drug use (including ACEI/ARB, Beta-blocker, Anti-diabetes, Diuretic agents and statin). Abbreviations are consistent with Table 1.

Figure S1. Flow chart of the study population in this study.

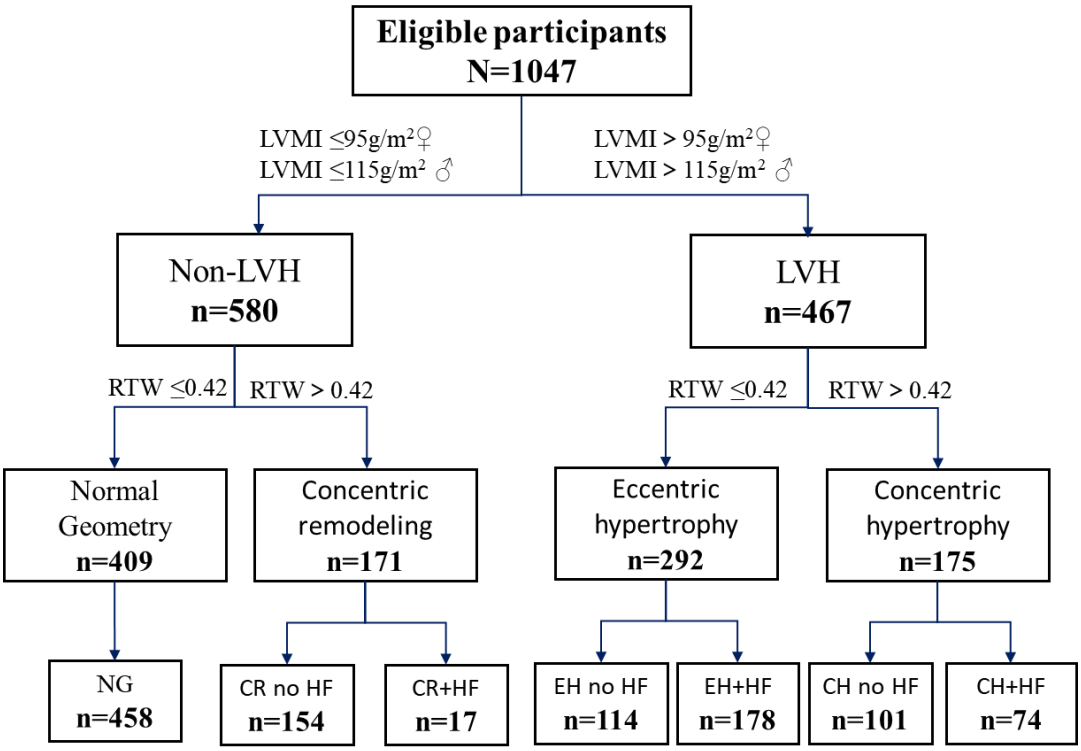


Figure S2. Distribution of serum Ec-SOD activity in total study population.

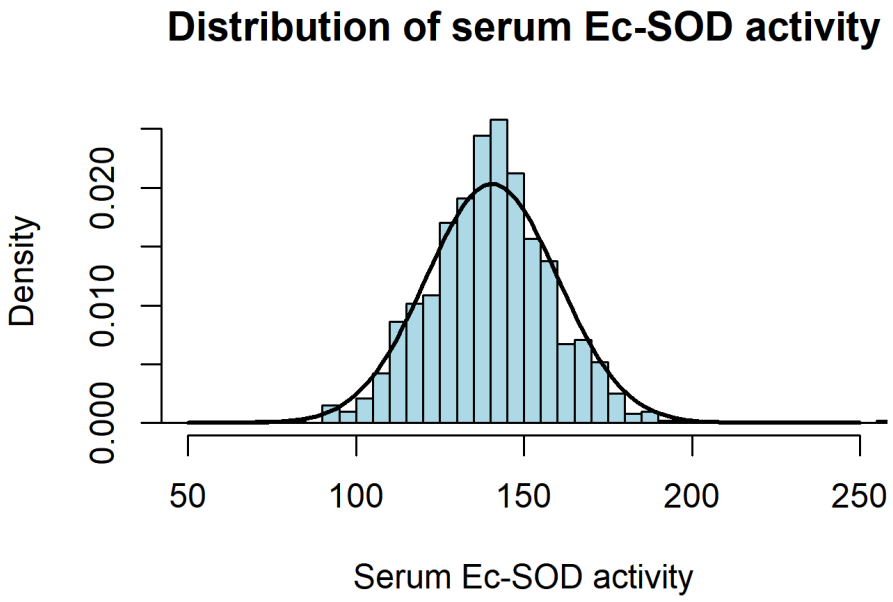
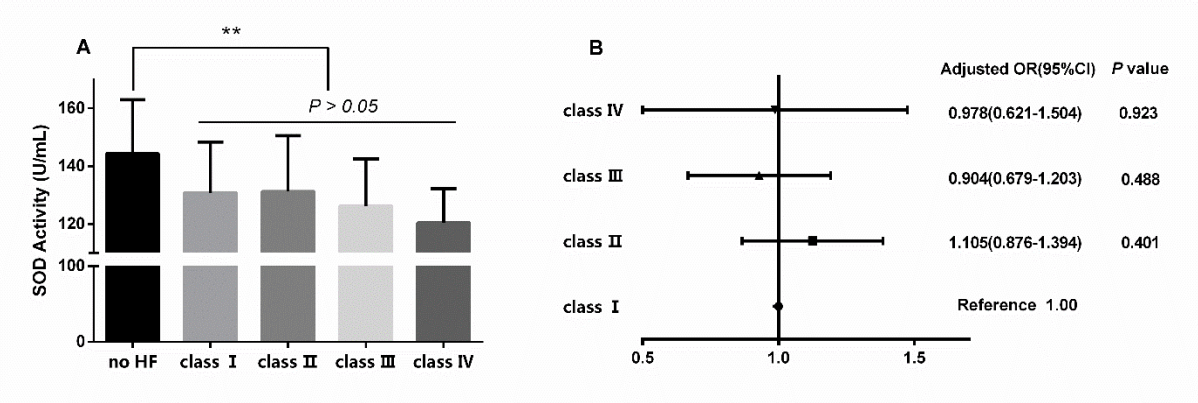
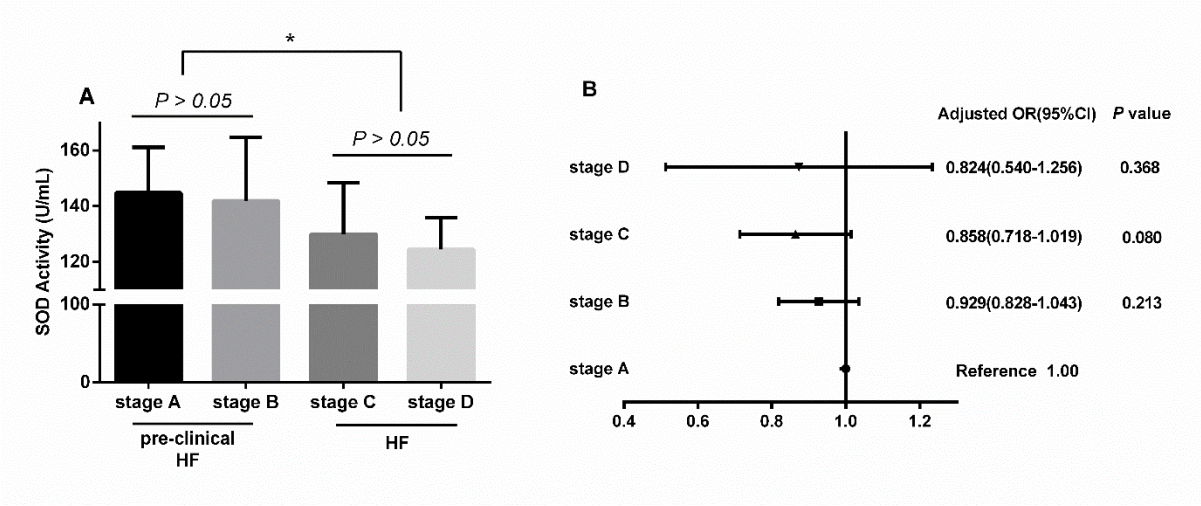


Figure S3. **Association between serum Ec-SOD activity and NYHA class.**



Panel A, Difference of serum Ec-SOD activity in patients with different NYHA class. **Panel B**, Odds ratio of subjects in different NYHA class by 10 U/mL serum Ec-SOD activity increase. ***P*<0.01. *P* values for Panel A were calculated using the multivariable-adjusted general linear model, adjusted by age, sex, smoking, hypertension, diabetes, coronary artery disease, Dilated cardiomyopathy/Hypertrophic cardiomyopathy, PCI history, Atrial fibrillation, Valve disease, drug use (including ACEI/ARB, Beta-blocker, and Diuretic agents), HbA1c, hsCRP, eGFR, and uric acid. Multivariable-adjusted Odds ratios (95% confidence interval) in Panel B were calculated using multinomial logistic regression model, adjusted by the same covariates as Panel A. When using forward stepwise procedure to select variables, serum Ec-SOD activity was removed from the ultimate multinomial logistic regression model.

Figure S4. **Association between serum Ec-SOD activity and ACCF/AHA Stages of HF.**



Panel A, Difference of serum Ec-SOD activity in patients with different Stages. **Panel B**, Odds ratio of subjects in different stages by 10 U/mL serum Ec-SOD activity increase. *P<0.05. P values for Panel A were calculated using the multivariable-adjusted general linear model, adjusted by age, sex, smoking, hypertension, diabetes, coronary artery disease, Dilated cardiomyopathy/Hypertrophic cardiomyopathy, PCI history, Atrial fibrillation, Valve disease, drug use (including ACEI/ARB, Beta-blocker, and Diuretic agents), HbA1c, hsCRP, eGFR, and uric acid. Multivariable-adjusted Odds ratios (95% confidence interval) in Panel B were calculated using multinomial logistic regression model, adjusted by the same covariates as Panel A. When using forward stepwise procedure to select variables, serum Ec-SOD activity was removed from the ultimate multinomial logistic regression model.