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Original article

Predictive value of serum myostatin for the severity and clinical outcome of heart failure

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ABSTRACT

Background: The exact relationship between serum myostatin and the severity and prognosis of chronic heart failure (CHF) is unclear. In this study, we investigated the association between serum myostatin and the severity and prognosis in patients with CHF.

Methods: Two hundred and eighty-eight CHF patients and 62 healthy controls were studied. Cardiac ultrasound and serum myostatin, N-terminal pro-B-type natriuretic peptide (NT-proBNP) and other parameters were detected. CHF patients were divided into 3 groups according to tertiles of NT-proBNP or myostatin levels respectively.

Results: Serum myostatin levels were higher in CHF patients than in controls. New York Heart Association (NYHA) class IV patients had the highest levels of serum myostatin among the four NYHA classes. Compared with the low tertile NT-proBNP group, serum myostatin levels were significantly higher in the moderate and high tertile groups (15.47 ± 4.25 vs. 14.18 ± 3.69 ng/mL, $p = .026$; 16.28 ± 5.34 vs. 14.18 ± 3.69 ng/mL, $p = .002$). During 51-months follow-up, of 173 patients there were 36 deaths. Compared to survivors, non-survivors had significantly higher serum myostatin (18.11 ± 4.52 vs. 14.85 ± 5.11 ng/mL, $p < .01$). Patients in the high tertile myostatin group had lower survival rate (73.95% vs. 93.75%; $p < .05$) and larger number of CHF rehospitalization than those in the low tertile group. Cox regression analysis showed that serum myostatin was an independent predictor of mortality.

Conclusions: Serum myostatin levels can reflect the severity of CHF and be a predictor of adverse prognosis in CHF patients.

1. Introduction

Patients with heart failure have high mortality and readmission rates. Chronic heart failure (CHF) is the leading cause of hospitalizations in the United States [1]. It is a clinical syndrome which may adversely influence skeletal muscle metabolism and provoke weight loss, and skeletal muscle atrophy and metabolic abnormalities occur even in patients with mild to moderate heart failure [2]. The onset of weight loss indicates that hormonal and immunological abnormalities have reached clinically relevant concentrations, and that the subsequent outlook of the patient is impaired [3]. So skeletal muscle mass is an independent prognostic indicator of survival in CHF patients [4].

Myostatin is a member of the transforming growth factor-β superfamily and serves as an important negative regulator of skeletal muscle growth [5], and inhibition of myostatin leads to increases in skeletal muscle mass [6]. Though the major site of myostatin expression is skeletal muscle, myostatin is also produced in heart [7]. By regulating skeletal muscle growth, myostatin is associated with heart failure. It is

Abbreviations: CHF, chronic heart failure; NYHA, New York Heart Association; NT-proBNP, N-terminal pro-B-type natriuretic peptide; LVEF, left ventricular ejection fraction; ROC, receiver operating characteristic; IHD, ischemic heart disease; AUC, area under the curve.

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Table 1
Baseline characteristics of controls and CHF patients with different NYHA classification.

Characteristics	Control	Group 1	Group 2	Group 3	p Value
	(n = 62)	(*n* = 102)	(*n* = 125)	(*n* = 61)	
Age, years	63.37 ± 10.16	66.08 ± 12.04	67.78 ± 14.00	63.15 ± 16.98	0.069
Female, *n* (%)	28 (45.16)	44 (43.14)	48 (38.40)	25 (40.98)	0.812
Concomitant disease					
Hypertension, *n* (%)	–	56 (54.90)	66 (52.80)	23 (37.70)	0.080
Ischemic heart disease, *n* (%)	–	54 (52.94)	49 (39.20)	23 (37.70)	0.065
Valvular heart diseases, *n* (%)	–	9 (8.82)	21 (16.80)	13 (21.31)	0.069
Medication use					
ACE inhibitors or ARBs, *n* (%)	–	56 (54.90)	72 (57.60)	40 (65.57)	0.399
Aldosterone antagonists, *n* (%)	–	28 (27.45)	71 (56.80)	45 (73.77)	< 0.001
Calcium channel blocker, *n* (%)	–	47 (46.08)	43 (34.40)	18 (29.51)	0.068
Beta-blockers, *n* (%)	–	57 (55.88)	58 (46.40)	26 (42.62)	0.196
Diuretics, *n* (%)	–	40 (39.22)	93 (74.40)	51 (83.61)	< 0.001
Digitalis, *n* (%)	–	9 (8.82)	41 (32.80)	23 (37.70)	< 0.001
Statin, *n* (%)	19 (30.65)	52 (50.98)	60 (48.00)	28 (45.90)	0.070
Clinical measurements					
Body mass index, kg/m^2	23.20 ± 1.25	23.24 ± 2.79	23.18 ± 2.81	22.83 ± 2.67	0.780
Fasting blood glucose, mmol/L	5.19 ± 0.66	5.79 ± 2.37	5.87 ± 2.19	5.99 ± 1.84	0.106
2 h PBG, mmol/L	6.85 ± 0.82	8.50 ± 3.46	9.81 ± 5.22	9.43 ± 3.25	< 0.001
Hemoglobin A1c,%	5.69 ± 0.52	6.21 ± 1.24	6.44 ± 1.71	6.27 ± 0.87	0.003
Total cholesterol, mmol/L	4.97 ± 0.40	4.37 ± 0.98	4.10 ± 1.11	4.29 ± 1.26	< 0.001
Low density lipoprotein, mmol/L	2.73 ± 0.54	2.52 ± 0.75	2.55 ± 0.83	2.70 ± 1.03	0.254
Triglyceride, mmol/L	1.31 ± 0.32	1.54 ± 1.07	1.30 ± 0.79	1.27 ± 0.75	0.079
High density lipoprotein, mmol/L	1.05 ± 0.17	1.04 ± 0.25	0.96 ± 0.29	1.04 ± 0.47	0.080
LVEF, %	58 (55–61)	57 (53–61)	45 (34–56)	46 (38–60)	< 0.001
Myostatin, ng/mL	12.18 ± 3.02	14.17 ± 4.01	15.27 ± 3.49	17.32 ± 6.36	< 0.001
NT-proBNP, pg/mL	79 (58–111)	494.25 (102.55–1764.2)	5482 (2175–12,316)	9824 (4054–20,500)	< 0.001

CHF: chronic heart failure; NYHA: New York Heart Association; ACE: angiotensin converting enzyme; ARB: angiotensin receptor blocker; PBG: postprandial blood glucose; LVEF: left ventricular ejection fraction; NT-proBNP: N-terminal pro-B-type natriuretic peptide. Values are mean ± SD, %, or median (interquartile range).

reported that myostatin is upregulated in the heart induced by pathological conditions such as after volume overload, which may then significantly contribute to serum levels [8–10]. However, few study investigated the relationship between serum myostatin levels and the severity of CHF. Furthermore, it is unknown that whether the levels of serum myostatin have a predictive role of outcome for CHF.

In this study, we investigated the differences in levels of serum myostatin in patients with different severity of heart failure and assessed the prognostic value of myostatin levels on death or recurrent hospital admission due to heart failure in CHF patients.

2. Materials and methods

2.1. Study population

Three hundred and forty-two CHF patients admitted to department of cardiology because of heart failure from January to August 2012 were consecutively enrolled after obtaining informed consent in 2 participating centers (Guangzhou First People's Hospital and Nanfang Hospital, China). Fifty-four patients were excluded according to exclusive criteria and finally 288 patients were analyzed. Patients with sepsis, malignancy, acute coronary syndrome, diabetes mellitus, muscle disease, thyroid and other metabolic diseases, estimated glomerular filtration rate < 30 mL/min/1.73 m^2, or severe hepatic disease were excluded. Consensus of two experienced clinical cardiologists was required for the classification of New York Heart Association (NYHA) functional classes. Follow-up events, including all-cause mortality and rehospitalization due to heart failure, were ascertained via hospital database, medical records and contact with patients or their family members. Sixty-two age-matched control subjects were recruited from the health management center and outpatient department in Guangzhou First People's Hospital. The study complied with the Declaration of Helsinki and was approved by the institutional ethics committee of Guangzhou First People's Hospital and Nanfang Hospital, China.

2.2. Biochemistry detection

Fasting venous blood was drawn into pryogen-free tubes without or with EDTA as anticoagulant respectively on the next day of admission. All subjects underwent oral glucose tolerance test with 75 g of oral anhydrous glucose as described previously [11]. After centrifuged at 3000*g* at 4 °C for 10 min, serum or plasma samples were obtained. All samples were stored at −80 °C until assayed. N-terminal pro-B-type natriuretic peptide (NT-proBNP) was analyzed with the Elecsys NT-proBNP immunoassay (Roche Diagnostics). Estimated glomerular filtration rate was calculated based on MDRD formula. Fasting blood glucose and postprandial blood glucose were measured using the direct chemiluminescence immunoassay (Siemens Healthcare Diagnostics Inc., USA). Myostatin was detected by enzyme linked immunosorbent assay (Wuhan USCN Business Co., Ltd., China) according to the manufacturer's protocol and its detection range was 0.78–50 ng/mL.

2.3. Echocardiographic measurement

Echocardiographic examinations were performed using a Siemens Sequoia 512 Encompass (Germany) device with a 2.5–3.5 MHz transducer. Images of the long-axis, short-axis and apical 4-chamber views were obtained. Left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) were measured according to the guideline [12]. Left ventricular ejection fraction (LVEF) was defined by the formula LVEF = (LVEDV − LVESV) / LVEDV. All of the echocardiograms were analyzed by the experienced sonographer.

2.4. Statistical analysis

Continuous normally distributed variables were expressed as mean ± SD, and medians were presented with the 25th to 75th percentiles for skewed continuous variables. Categorical variables were compared with Pearson's $\chi 2$ test. Differences between the mean or median values for continuous variables were evaluated with Kruskall-

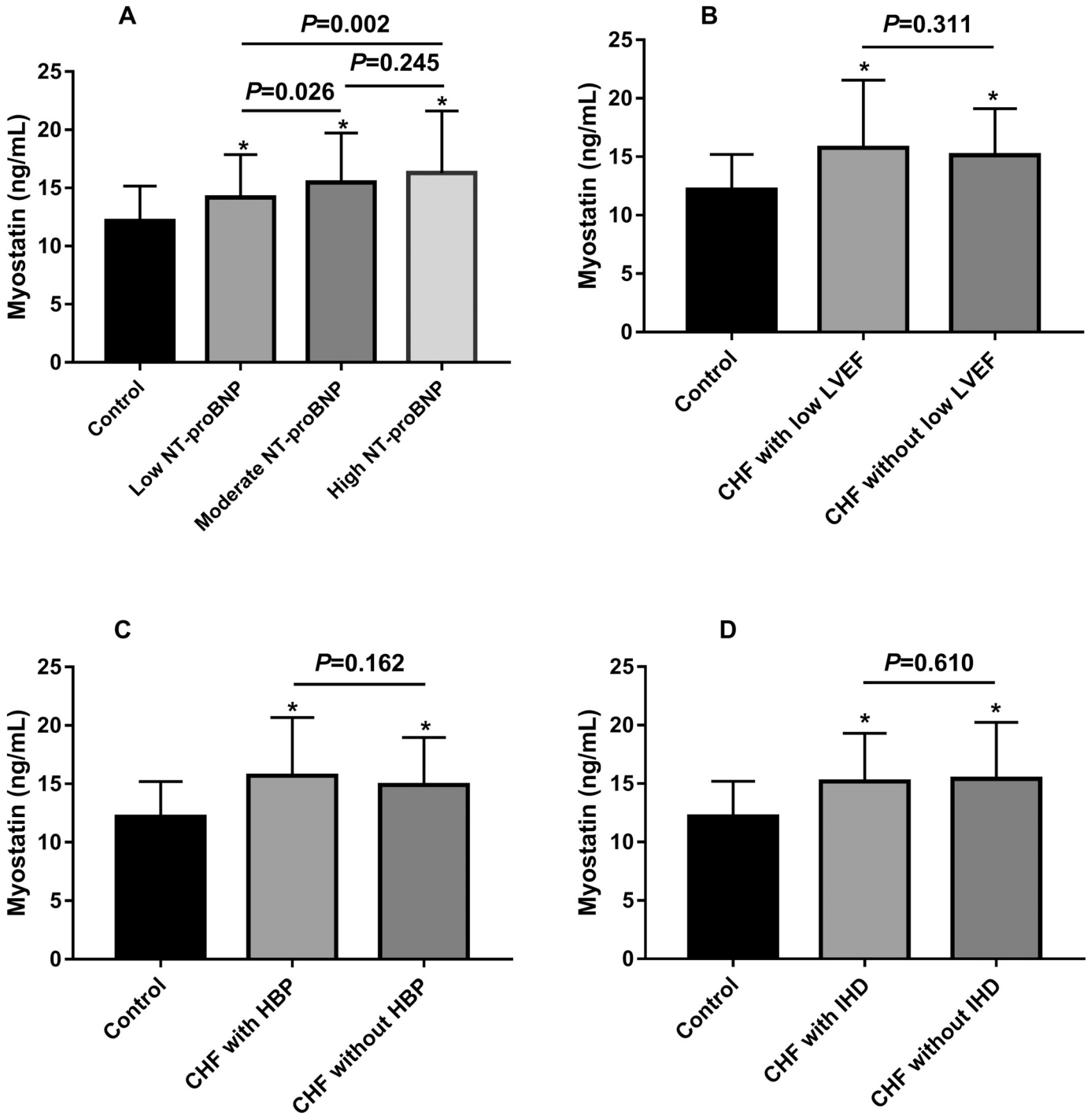

Fig. 1. Comparisons of serum myostatin levels between different types of CHF patients and controls. (A) Between patients with different NT-proBNP concentrations and controls. The groups were divided according to tertiles of NT-proBNP (NT-proBNP < 1501 pg/mL, 1501 pg/mL ≤ NT-proBNP < 6526 pg/mL, and ≥ 6526 pg/mL) in CHF patients. (B) Between patients with (15.76 ± 5.80 ng/mL, $n = 80$) or without low LVEF (≤ 40%) (15.14 ± 3.96 ng/mL, $n = 208$) and controls (12.18 ± 3.02 ng/mL, $n = 62$), (C) Between patients with (15.71 ± 4.97 ng/mL, $n = 145$) or without hypertension (14.96 ± 4.03 ng/mL, $n = 143$) and controls. (D) Between patients with (15.17 ± 4.12 ng/mL, $n = 117$) or without ischemic heart disease (15.45 ± 4.81 ng/mL, $n = 171$) and controls. *$p < .05$ vs. controls.

Wallis test or 1-way ANOVA with S-N-K analysis, as appropriate, and the between-group comparisons of normally distributed variables were evaluated with independent samples *t*-test. The relevance of parameters to predict mortality was investigated by Cox proportional hazards regression. Variables with a p value < .05 in univariable analysis were finally entered into the multivariable analysis. Events of death during follow-up were investigated with Kaplan–Meier analysis by Log rank test. Receiver operating characteristic (ROC) curve analyses were performed to evaluate the diagnostic efficiency of parameters to predict mortality. The best cut-off values were calculated from ROC curve analysis, where 'best' meant that the Youden index (sensitivity + specificity – 1) was maximal. p values were two-sided and considered significant when < 0.05. Statistical analyses were carried out using the software package SPSS version 17.0 (SPSS Inc., Chicago, IL).

3. Results

The 288 CHF patients were classified into 3 groups as group 1

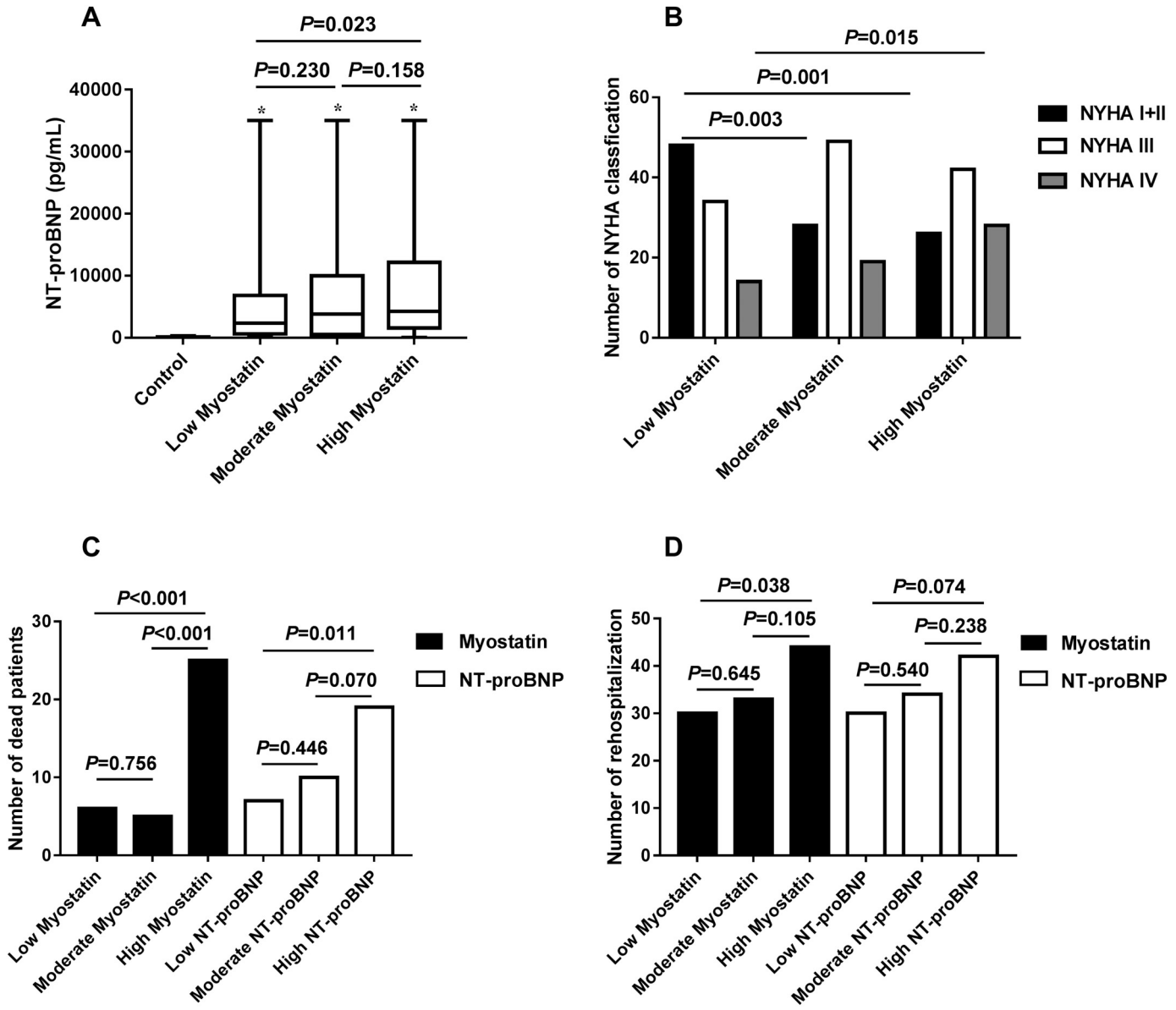

Fig. 2. Association of different serum myostatin levels with (A) NT-proBNP concentrations and (B) number of patients with different NYHA classification. Association of different serum myostatin or NT-proBNP levels with (C) number of dead patients and (D) heart failure-related hospitalizations. The groups were divided according to tertiles of myostatin (myostatin < 13.49 ng/mL, 13.49 ng/mL ≤ myostatin < 16.70 ng/mL, and ≥ 16.70 ng/mL) or NT-proBNP (NT-proBNP < 1501 pg/mL, 1501 pg/mL ≤ NT-proBNP < 6526 pg/mL, and ≥ 6526 pg/mL) in CHF patients. *p < .05 vs. controls.

(NYHA I and II classes), group 2 (NYHA III class) and group 3 (NYHA IV class) according to the NYHA classification. As Table 1 showed, compared with controls, levels of serum myostatin were significantly higher in three different CHF groups (all $p < .05$). Furthermore, serum myostatin levels were lower in group 1 than those in group 2 and group 3 (both p < .05). Among three CHF groups, group 3 possessed the highest levels of serum myostatin (17.32 ± 6.36 vs. 14.17 ± 4.01 ng/mL, $p < .001$; 17.32 ± 6.36 vs. 15.27 ± 3.49 ng/mL, $p = .005$).

CHF patients were divided into low, moderate and high groups according to tertiles of NT-proBNP (NT-proBNP < 1501 pg/mL, 1501 pg/mL ≤ NT-proBNP < 6526 pg/mL, and ≥ 6526 pg/mL). Compared with the low NT-proBNP group, levels of serum myostatin were significantly higher in the moderate and high groups (15.47 ± 4.25 vs. 14.18 ± 3.69 ng/mL, $p = .026$; 16.28 ± 5.34 vs. 14.18 ± 3.69 ng/mL, $p = .002$). Moreover, serum myostatin levels were significantly lower in controls than those in all three CHF groups (all $p < .05$, Fig. 1A).

Patients were subdivided into different groups according to the types of CHF: those with or without low LVEF (≤ 40%); with or without hypertension; with or without ischemic heart disease (IHD). Compared with controls, serum myostatin levels were significantly higher in patients with different types of CHF (all $p < .01$, Fig. 1B–D). However, there were no differences of serum myostatin levels between CHF patients with or without low LVEF, hypertension or IHD (Fig. 1B–D).

When divided into 3 groups according to tertiles of serum myostatin. Those with myostatin < 13.49 ng/mL were included in the low myostatin group; 13.49 ng/mL ≤ myostatin < 16.70 ng/mL in the moderate group; and ≥ 16.70 ng/mL in the high group. High myostatin group possessed higher NT-proBNP (median, 4266 pg/mL [interquartile range (IQR) 1569 to 12,146] vs. 2366 pg/mL [IQR 620 to 6803], $p = .023$), larger number of NYHA IV (28 vs. 14, $p = .015$) and smaller number of NYHA I-II class (26 vs. 48, $p = .001$) patients than low myostatin group (Fig. 2A and B).

Of the 288 CHF patients, 173 patients had clinical follow-up data. The mean follow-up duration was 51.40 ± 15.01 months. There was no significant difference of serum myostatin levels between patients with and without follow-up data (15.53 ± 5.12 vs. 15.11 ± 3.37 ng/

Table 2
Baseline characteristics of CHF patients according to survival.

Characteristics	Survivors	Nonsurvivors	*p* Value
	(*n* = 137)	(*n* = 36)	
Age, years	68.87 ± 13.62	67.53 ± 12.95	0.596
Female, *n* (%)	57 (41.60)	14 (38.8)	0.768
NYHA classification			
NYHA I, *n* (%)	28 (20.44)	4 (11.11)	0.200
NYHA II, *n* (%)	24 (17.52)	7 (19.44)	0.789
NYHA III, *n* (%)	60 (43.80)	16 (44.44)	0.944
NYHA IV, *n* (%)	25 (18.25)	9 (25.00)	0.364
Clinical measurements			
Body mass index, kg/m^2	23.29 ± 2.70	22.53 ± 3.14	0.146
Fasting blood glucose, mmol/L	5.72 ± 2.04	5.70 ± 1.89	0.969
2 h PBG, mmol/L	8.77 ± 3.33	8.76 ± 3.23	0.996
Hemoglobin A1c,%	6.28 ± 1.12	6.14 ± 1.04	0.506
Total cholesterol, mmol/L	4.21 ± 1.16	4.40 ± 1.01	0.370
Low density lipoprotein, mmol/L	2.52 ± 0.87	2.71 ± 0.81	0.249
Triglyceride, mmol/L	1.30 ± 0.71	1.36 ± 0.97	0.710
High density lipoprotein, mmol/L	1.01 ± 0.28	0.99 ± 0.25	0.605
LVEF, %	53 (40–59)	54 (34–62)	0.894
Myostatin, ng/mL	14.85 ± 5.11	18.11 ± 4.52	0.001
NT-proBNP, pg/mL	2551 (689.25–7218)	6826 (1753–14,832)	0.008

CHF: chronic heart failure; NYHA: New York Heart Association; PBG: postprandial blood glucose; LVEF: left ventricular ejection fraction; NT-proBNP: N-terminal pro-B-type natriuretic peptide. Values are mean ± SD, %, or median (interquartile range).

mL, $p = .443$). During follow-up there were 36 deaths, 71 recurrent hospital admissions due to heart failure. Six CHF patients died (6.25%) and 30 were rehospitalized (31.25%) in the low myostatin group, 5 died (5.21%) and 33 rehospitalized (34.38%) in the moderate group, and 25 died (26.05%) and 43 rehospitalized (44.79%) in the high group. Among three groups, the number of patients who died or were rehospitalized for heart failure in the high myostatin group was the largest (both $p < .05$, Fig. 2C and D). Similar to the results obtained in three myostatin levels groups, the number of dead patients in the high NT-proBNP group ($n = 19$) was the largest in three different NT-proBNP groups (19 vs. 10 vs. 7, Fig. 2C and D).

As Table 2 showed that age and NYHA classification had no significant difference between survivor and nonsurvivor groups, but nonsurvivors had significantly elevated levels of NT-proBNP and serum myostatin (both $p < .01$, Fig. 3A and B). When compared to those without rehospitalization, CHF patients with rehospitalization due to heart failure during follow-up period had significantly higher serum myostatin (16.31 ± 5.69 vs. 14.26 ± 3.87 ng/mL, $p = .011$) and NT-proBNP levels (median, 4328 pg/mL [IQR 1279 to 11,846] vs. 2094 pg/mL [IQR 266 to 4974], p = .001, Fig. 3C and D).

Kaplan–Meier event curves were used to assess the differences of three myostatin groups in clinical outcomes. The log rank test indicated that the Kaplan–Meier event curves were significantly different among 3 groups (Log Rank, Chi^2 = 14.942, $p < .001$). Patients in the high tertile myostatin group had lower survival than those in the low and moderate tertile groups (Fig. 4A).

Cox regression analysis was performed to determine which biomarkers can predict mortality in patients with CHF. As demonstrated in Table 3, in univariable analysis myostatin, NT-proBNP, blood glucose and systolic blood pressure all predicted mortality. However, the predictive role of serum myostatin (HR =1.049, $p = .007$) and systolic blood pressure (HR =0.981, $p = .020$) on mortality still persisted in a multivariable analysis adjusting for other potential confounders.

ROC analysis was used to evaluate the diagnostic value of serum myostatin to predict mortality in patients with CHF. As shown in Table 4, area under the curve (AUC) was higher for myostatin (0.716) than for NT-proBNP (0.643) and for B-type natriuretic peptide (0.625). The AUC was 0.716 for the combination of NT-proBNP and myostatin, and it < 0.5 indicated that lower systolic blood pressure was related with worse prognosis. The best cut-off value to predict the mortality of patients with CHF was 6413 pg/mL for NT-proBNP and 16.80 ng/mL for myostatin (Fig. 4B, Table 4).

4. Discussion

In the present study, patients with CHF (including different types of CHF) possessed higher levels of serum myostatin compared with controls. CHF patients with higher NT-proBNP and NYHA classification had higher levels of serum myostatin. During follow-up, patients with high serum myostatin levels had a markedly elevated died or rehospitalization rate. In addition, serum myostatin was the independent predictive factor of survival in CHF patients. The findings showed that serum myostatin was closely associated with the CHF severity and the prognosis of CHF patients.

Loss in body weight was associated with the CHF severity, and independently related to poor clinical outcomes [13]. Because muscle wasting in patients with CHF yielded increased sympathetic activation, vasoconstriction, endothelial dysfunction, and finally worsening of left ventricular function [14]. Furthermore, despite a decrease in body mass index, waist circumference, which is related with myostatin levels [15], can continue to increase in old adults [16]. Testa G et al. found that waist circumference was associated with a 5% increased risk of long-term mortality for each 1-cm increase in waist circumference in elderly individuals with CHF [17], because fluid retention due to heart failure may less impact it [18]. As an important negative regulator of skeletal muscle growth, myostatin was associated with muscle wasting, heart failure and may causally contribute to pathogenesis of CHF [19].

Consistent with previous studies [7–9], our results showed that CHF patients (including different types of CHF) had higher levels of serum myostatin than controls, meaning that serum myostatin elevated when heart failure occurred. The increase in myostatin activation in CHF is most likely stimulated by myocardiocyte stretch and increased myocardial stress [10]. Elevated cardiac stress in CHF likely induces physiologically meaningful myocardial myostatin expression and release [20], which contributes to the elevation of serum levels. Moreover, basic studies also indicated that myostatin levels in the plasma were significantly increased in mice with overload-induced heart failure [21]. All these indicated that elevated myostatin may suggest the existence of heart failure.

However, recently Furihata T et al. reported that serum myostatin levels were decreased in CHF patients [22], which was different from most previous studies. As the authors speculated that the levels of serum myostatin might depend on the various conditions including the severity of CHF and treatment including exercise therapy. In our study, most CHF patients admitted to hospital were in decompensated state. But in their study, all CHF patients were already compensated and about 70% of them performed exercise training when they tested [22]. It was known that exercise training can lead to a significant reduction in myostatin protein and mRNA expression in both basic and clinical research of CHF [23,24]. Maybe exercise training and compensated state of heart failure influenced the elevation of serum myostatin levels, because the regulation of the myostatin levels was disease-dependent. In some neuromuscular diseases the myostatin pathway is shut down at mRNA level, leading to low levels of circulating myostatin [25].

In our study CHF patients with elevated NT-proBNP or NYHA classification possessed higher levels of serum myostatin, and Gruson D et al. also showed that myostatin correlated with biomarkers related to heart failure severity [9], suggesting that myostatin was associated with the severity of heart failure. Serious heart failure patients are characterized by significant decrease of cardiac function and serious fatigue. Myostatin may involve in the process of heart failure. It inhibits protein

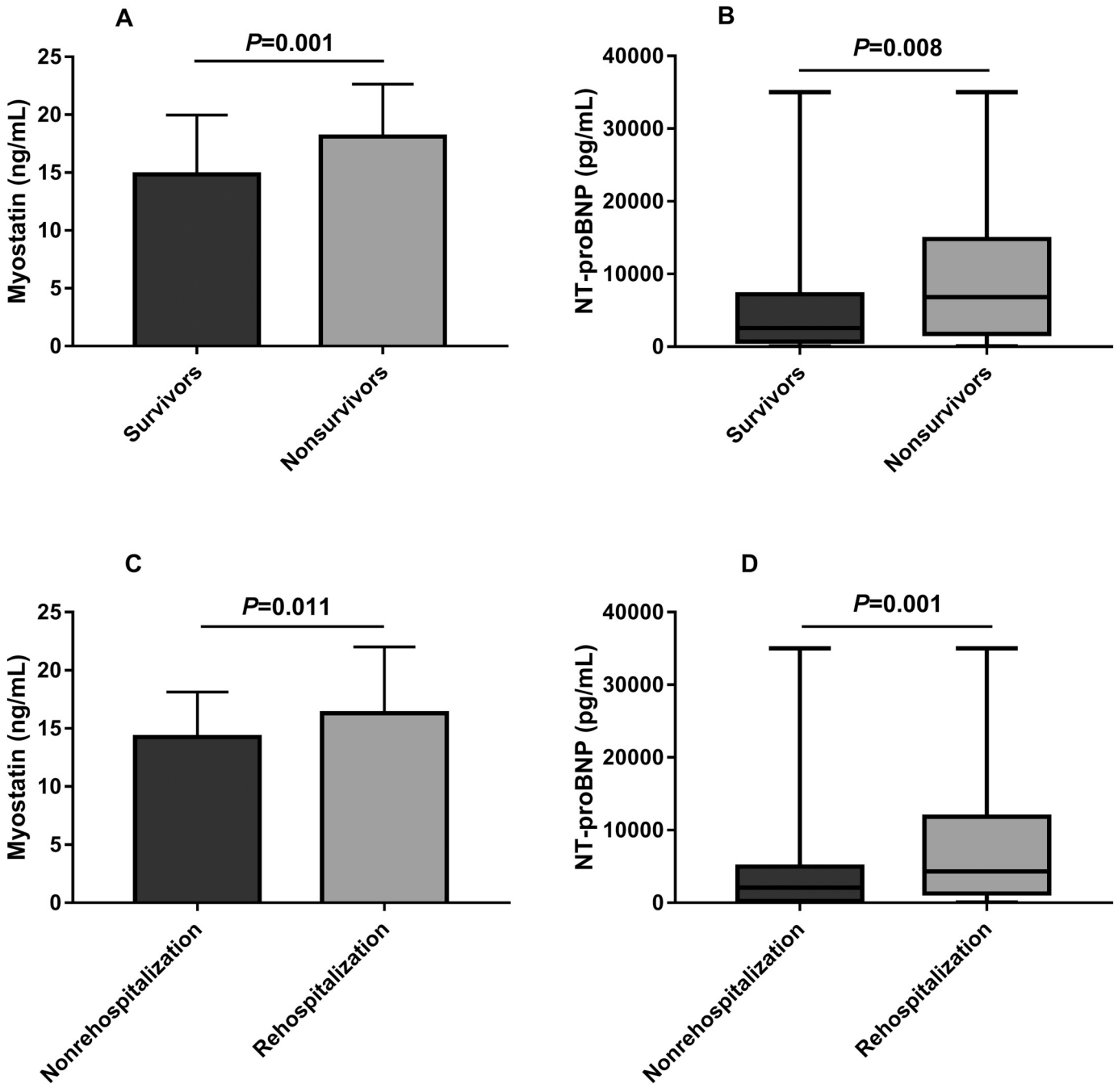

Fig. 3. Serum myostatin and NT-proBNP levels in survivor and nonsurvivor groups (A, B), nonrehospitalization and rehospitalization groups (C, D).

synthesis via inhibiting p70S6k and rpS6 [26], and genetic deletion of myostatin from the heart protects from skeletal muscle atrophy [21]. Furthermore, contractile force generation of myofibers can be mediated by myostatin, because myostatin knockout mice show an increase in the number of fast glycolytic myofibers which are subjective to typically fatigue quickly and a corresponding decrease in slow fibers which are resistant to fatigue [27,28]. Absence of myostatin potentially improves the cardiac function after myocardial infarction by limiting extent of fibrosis [29]. In this view, myostatin seems to be a potential indicator of the severity of heart failure. However, Zamora E et al. thought that there was no relationship between the myostatin or myostatin propeptide levels and any parameters of disease severity (eg NYHA functional class, LVEF and NT-proBNP) or prognosis in patients with CHF [30]. Maybe, in their study the very low number of CHF patients enrolled and low events rate affected this relationship.

During follow-up, nonsurvivors and patients with rehospitalization due to heart failure had significantly elevated levels of serum myostatin in our study. Furthermore, the dead rate was nearly four times higher in the high myostatin group (26.05%) than in the low (6.25%) and moderate group (5.21%). It showed significant association between high myostatin levels and increased adverse outcomes in CHF patients. But in Olson KA et al. study, higher growth differentiation factor (GDF) 11/myostatin levels were associated with lower risk of cardiovascular events and death, and GDF11/ myostatin had cardioprotective properties. Maybe in their study patients were stable ischaemic heart disease and less likely to have impaired left ventricular relaxation or NYHA class III or IV CHF symptoms [31]. Moreover, they did not discriminate GDF11 from myostatin. Though GDF11 shares 90% sequence identity with myostatin [32], the variation trend of their levels are not the same in different physiological and pathological conditions when accurately

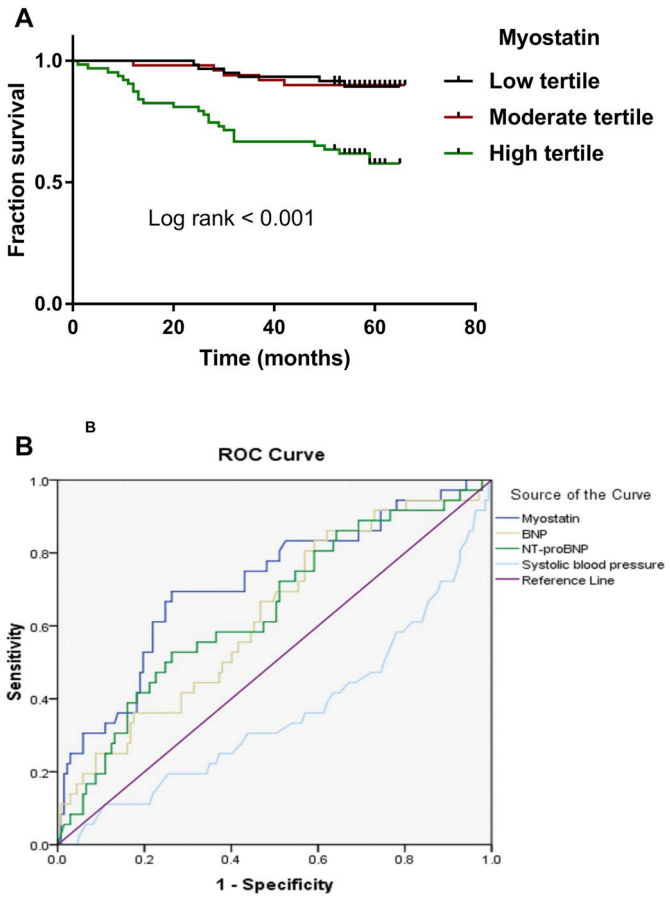

Fig. 4. (A) Kaplan-Meier estimates of survival in patients with heart failure according to tertiles of serum myostatin. (B) Receiver operating characteristic curve analysis of serum myostatin, B-type natriuretic peptide, N-terminal pro-B-type natriuretic peptide and systolic blood pressure to predict 5-years mortality in patients with heart failure.

resolving GDF11 from myostatin [33], suggesting that the roles and functions between GDF11 and myostatin may not be congruent [34].

Abnormal energy metabolism and dysfunction of mitochondria have an adverse effect on the outcome of heart failure. As an important regulator of myocardial metabolism and growth, myostatin suppresses AMP-activated kinase (AMPK) activity in cardiomyocytes via transforming growth factor-β-activated kinase 1 (TAK1) to influence the cardiac metabolic pattern [5]. It also acts on glucose metabolism inducing the expression of glucose transporters and on lipid metabolism inducing the activity of lipolytic enzymes that affect fatty acid oxidation [35]. So increased myostatin may deteriorate cardiac energy metabolism and mitochondria function, which contributes to adverse prognosis in patients with heart failure.

However, some studies indicated that myostatin may have active roles in modulating mitochondrial function. Deficiency of myostatin can cause many other metabolic changes such as a decrease in mitochondrial content, disturbance in mitochondrial respiratory function with a decline in the respiratory control ratio in intermyofibrillar mitochondria and involve in the process of muscle contraction [36,37]. Myostatin absence also significantly alters the mitochondrial lipidome, which may be related to the impairment in mitochondrial function and the significant susceptibility to fatigue [37]. Furthermore, myostatin can inhibit AKT to block insulin-like growth factors induced hypertrophy of myocytes [38,39]. Considering these findings, it is hypothesized that increasing myostatin may be a potential means of feedback regulation to ameliorate the abnormal energy metabolism, dysfunction of mitochondria and cardiac hypertrophy which occurred during heart failure. So we thought that the more severe the heart failure the more elevated the levels of myostatin. By regulating myocardial energy metabolism, myostatin suppresses AMPK activity in cardiomyocytes via TAK1 to prevent appearance of a fetal metabolic pattern, thereby stabilizing the metabolic status of cardiomyocytes and restricting cardiac hypertrophy [5]. In this view, overexpression of myostatin tended to improve cardiac contractility and inhibit cardiac hypertrophy when heart failure occurred.

Several limitations should be discussed. First, the study enrolled a relatively low number of patients in only two centers and results need to be confirmed in larger population and multicenter. Second, we did not get the data of waist circumference at baseline when the CHF patients were recruited, and we did not evaluate the possible correlations between waist circumference, myostatin and heart failure. Third, in our study the missing rate was relatively high and the mortality was low. Parts of missing patients were due to the change of contact information. It was possible that some missing patients may have adverse outcomes. Finally, it was supposed that elevated serum myostatin may come out of the failing myocardium, but cardiac myostatin levels were not performed in our study because of no myocardial biopsy. Maybe skeletal muscle was also the important source of serum myostatin. A further investigation of the myocardial biopsy in these populations would be helpful.

In conclusions, serum myostatin levels were significantly elevated in

Table 3
Univariable and multivariable Cox regression analysis to predict mortality in patients with CHF.

	Clinical: univariable			Clinical: multivariable		
	HR	(95% CI)	*P* value	HR	(95% CI)	p value
Male	1.125	0.576–2.199	0.730			
Age	0.994	0.971–1.017	0.585			
Rehospitalization	1.085	0.922–1.278	0.327			
NYHA class	1.253	0.889–1.766	0.198			
Myostatin	1.060	1.025–1.096	0.001	1.049	1.013–1.085	0.007
NT-proBNP	1.000	1.000–1.000	0.016			
LVEF	0.993	0.968–1.019	0.599			
Hypertension	0.606	0.312–1.177	0.139			
Coronary heart disease	1.210	0.630–2.326	0.567			
Blood glucose	0.294	0.090–0.959	0.042			
Systolic blood pressure	0.978	0.962–0.994	0.008	0.981	0.966–0.997	0.020
Diastolic blood pressure	0.974	0.947–1.002	0.072			
Heart rate	0.977	0.954–1.001	0.065			
eGFR	1.002	0.987–1.018	0.773			

CHF: chronic heart failure; HR: hazard ratio; CI: confidence interval; NYHA: New York Heart Association; NT-proBNP: N-terminal pro-B-type natriuretic peptide; LVEF: left ventricular ejection fraction; CAD: coronary heart disease; eGFR: estimated glomerular filtration rate.

Table 4
Receiver operating characteristic curve analysis for the prediction of survival.

Variable	AUC (95% CI)	Cut-off	Specificity (%)	Sensitivity (%)	p value
Myostatin, ng/mL	0.716 (0.618–0.815)	16.80	73.7	69.4	< 0.001
BNP, ng/L	0.625 (0.525–0.727)	101.4	40.9	83.3	0.020
NT-proBNP, pg/mL	0.643 (0.541–0.745)	6413	73.7	52.8	0.008
Systolic blood pressure, mm Hg	0.359 (0.251–0.467)	121	47.2	25.2	0.009

AUC: area under the curve; BNP: B-type natriuretic peptide; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

CHF patients. Moreover, the more severe the heart failure, the higher the serum myostatin concentrations. During follow-up, CHF patients with high serum myostatin had a markedly elevated dead or re-hospitalization rate. In addition, serum myostatin was the independent predictive factor of survival in CHF patients. These findings suggest that the serum myostatin levels can reflect the severity of CHF and be a predictor of adverse prognosis in CHF patients.

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Declarations of interest

None.

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