

# Role of anti-beta-1-adrenergic receptor antibodies in cardiac dysfunction in patients with cirrhotic cardiomyopathy

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## Research Article

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# Abstract

**Background and aim:** Cirrhotic cardiomyopathy (CCM) is a recognized complication of cirrhosis and is associated with poor outcomes, especially under challenges such as surgery/liver transplantation. However, the mechanism is not clear, and the treatment is not specific. The present study aimed to evaluate the role of anti- $\beta_1$ -adrenergic receptor antibodies (anti- $\beta_1$ -AR) in CCM.

**Methods:** We enrolled 3 groups: healthy controls, cirrhotic patients without CCM and patients with CCM. The serum anti- $\beta_1$ -AR and N-terminal (NT)-pro hormone brain natriuretic peptide (NT-proBNP) were detected by enzyme-linked immunosorbent assay (ELISA). Left ventricular ejection fraction (LVEF), fractional shortening (FS), the ratio of peak early (E wave) and atrial (A wave) flow velocities (E/A) and left ventricular posterior wall minor motion amplitude were measured by echocardiography.

**Results:** The anti- $\beta_1$ -AR levels in the CCM group were significantly higher than that in the non-CCM group ( $0.97 \pm 0.37$  vs  $0.74 \pm 0.37$  ng/mL,  $P < 0.05$ ); anti- $\beta_1$ -AR was positively correlated to NT-proBNP ( $r = 0.48$ ,  $P < 0.05$ ), negatively correlated to LVEF ( $r = -0.466$ ,  $P < 0.05$ ), FS ( $r = -0.488$ ,  $P < 0.05$ ) and E/A ( $r = -0.475$ ,  $P < 0.05$ ) in CCM patients. The area under the receiver-operating-characteristic (AUROC) of serum anti- $\beta_1$ -AR on CCM was 0.678 (95% CI 0.515-0.768). At a cutoff value of 0.669 ng/mL, the sensitivity of anti- $\beta_1$ -AR to diagnose CCM was 89.5%, and the specificity was 57%.

**Conclusion:** The anti- $\beta_1$ -AR level in the CCM group was significantly elevated compared with that in the non-CCM group, and this increase was correlated with cardiac function. Anti- $\beta_1$ -AR is a useful predictive biomarker for the presence of CCM and eventually may also have therapeutic implications.

## Introduction

Cirrhotic cardiomyopathy (CCM) is a syndrome of cardiac dysfunction in patients with cirrhosis, in the absence of prior heart disease [1-3]. Because of the reduced ventricular afterload in cirrhosis due to peripheral vasodilation, thus "auto-treating" the patient, the cardiac dysfunction in patients with CCM is latent (1), [4, 5]. However, when challenged, the cardiac dysfunction becomes overt. One of the main pathogenic mechanisms is abnormalities of the  $\beta$ -adrenergic receptor ( $\beta$ AR) pathway [6].

It is well known that  $\beta$ AR plays a major role in cardiac contraction [7]. In the heart,  $\beta$ AR are subdivided into either  $\beta_1$ - or  $\beta_2$ - subtypes. More than 60 years ago it was noticed that cardiac responsiveness of patients with cirrhosis to exogenous [8] infusions of catecholamines or endogenous production (i.e., caused by exercise) [9] is attenuated. However, the underlying mechanism of hyporesponsiveness was unclear then. Three decades ago, Lee and colleagues measured heart rate responsiveness to isoprenaline and myocardial  $\beta$ AR-binding characteristics in cirrhotic rats and found that compared with sham-operated controls, a significantly higher dose of isoprenaline was needed to raise basal heart rate by 50 beats/min ( $102 \pm 19$  vs.  $28 \pm 11$  ng/kg) in cirrhotic rats. They also found that myocardial  $\beta$ AR density was significantly lower in cirrhotic rats and the dissociation constant was higher. Subtype analysis demonstrated that the decreased total  $\beta$ AR density in cirrhotic heart was entirely due to selective

downregulation of the  $\beta$ 1-AR subtype. Moreover, the  $\beta$ AR affinity for agonist was not altered. They concluded that  $\beta$ AR downregulation is responsible for the myocardial hyporesponsiveness to catecholamines in the cirrhotic rat heart. However, the exact mechanism underlying the  $\beta$ AR downregulation in cirrhosis remains unclear.

Autoantibodies attack self cells/organs and cause diseases. Antibodies to glomerular basement membrane cause immune-complex crescentic nephritis [10]. anti- $\beta$ 1-AR binds to and constitutively stimulates the  $\beta$ 1-AR to cause  $\beta$ AR desensitization and downregulation [11]. Whether anti- $\beta$ 1-AR plays a role in the decreased density of  $\beta$ 1-AR and CCM is not clear. The present study therefore aimed to investigate the hypothesis that  $\beta$ 1-AR downregulation is due to the presence of increased activity of  $\beta$ 1-AR antibodies in patients with CCM.

## Methods

### Patients

The protocol was approved by the Beijing Youan Hospital ethics committee and conformed to the guidelines of the Helsinki Declaration. All patients provided written informed consent. The study was registered with the Chinese Clinical Trials No. ChiCTR 2000037730.

From January 2016 to December 2018, 352 patients with cirrhosis were consecutively screened in Youan Hospital. The diagnosis of cirrhosis was based on clinical features, and laboratory, ultrasonography, and histology examinations. The inclusion criteria were age 18-65 and presence of cirrhosis. Patients with the following conditions that may impact cardiac function were excluded: fever, systemic inflammatory response syndrome, severe anemia (hemoglobin < 60g/L), alcoholic liver disease, cardiovascular diseases (hypertension, coronary artery disease, rheumatic heart disease, pericarditis, myocarditis and valvular heart disease), thyroid diseases, renal dysfunction, acute or chronic respiratory diseases, diabetes mellitus, and malignance. Patients who had taken drugs such as  $\beta$ -blockers, calcium channel blockers, and angiotensin-converting enzyme inhibitors within the past week were also excluded (Fig. 1). The diagnosis of CCM was based on the Montreal 2005 World Congress of Gastroenterology criteria [12]. The patients were divided into 3 groups: CCM group, cirrhotic non-CCM group, and age and sex-matched healthy volunteer group (n=20).

Amongst the 352 screened cases, 125 patients were considered for enrolment, whereas 227 patients were excluded due to the presence of alcoholic hepatitis, coronary artery disease, hypertension, and hepatocellular carcinoma (Fig. 1). Of the 125 patients, 19 had CCM (CCM group), and 106 had non-CCM. After propensity matching for sex, age, and Child-Pugh score, 76 of the 106 cases were selected for the non-CCM group. The average age in the CCM group was  $58.4 \pm 9.1$  years; 12 were males and 7 females. The etiologies were HCV-cirrhosis (n=9), HBV-cirrhosis (n=7), primary biliary cholangitis (PBC, n=3). We also enrolled 20 sex and age matched healthy volunteers.

The demographics, physical exams, electrocardiography (ECG), echocardiography, routine blood tests were recorded. Echocardiography was performed by an experienced cardiologist (ID) on a iE33 ultrasonography (Philips Medical Systems, Best, the Netherlands) with structural and functional parameters measured according to current guidelines. Child-Pugh score, Model for End-Stage Liver Disease (MELD) score and MELD-Na score were used to evaluate the severity of the cirrhosis. Compensated cirrhosis was defined as liver is scarred but still able to perform most its basic functions, whereas decompensated was characterized by the presence of ascites, jaundice, variceal hemorrhage, or hepatic encephalopathy.

Natriuretic drug history was carefully evaluated to avoid the effect of these agents on clinical variables and ultrasonography. Patients were examined while on a low sodium diet and, where applicable, at least 48 hours after paracentesis. The serum was separated for anti- $\beta$ 1-AR, NT-proBNP and troponin I detection. The blood samples were also sent to relevant laboratories to test routine biochemistry, liver and kidney function, electrolytes, and coagulation function.

### **Diagnosis of cirrhotic cardiomyopathy**

According to the Montreal 2005 diagnostic criteria [12], systolic dysfunction was defined as ejection fraction  $<55\%$ , and diastolic dysfunction was defined as two of the three indices: the ratio of peak early (E wave) and atrial (A wave) flow velocities (E/A; early/late diastolic filling velocities) ratio (age corrected)  $< 1.0$ ; prolonged mitral deceleration time (DT;  $>200$  ms); and prolonged isovolumic relaxation time ( $>80$  ms). The supportive criteria were: [corrected QT interval](#) (QTc)  $> 0.440$ s; increased B-type natriuretic peptide (BNP) and pro-B-type natriuretic peptide (pro BNP); and increased troponin I.

### **Serum anti- $\beta$ 1-AR assay**

The serum from all the participants were saved at  $-80^{\circ}\text{C}$ . The serum level of anti- $\beta$ 1-AR was measured by enzyme-linked immunosorbent assay (ELISA). The ELISA kit was purchased from Blue Gene for Life Science (Shanghai, China). The samples and reagents in the ELISA kit were recovered to room temperature, each sample was tested in duplicate. Standards or samples (100  $\mu\text{l}$ ) were incubated with 50  $\mu\text{l}$  horseradish peroxidase at  $37^{\circ}\text{C}$  for 1 hour. After washing for 5 times, the substrate A and B (50  $\mu\text{l}$ ) were added, and the plate was incubated in dark at  $37^{\circ}\text{C}$  for 15 min. The stop solution was added, and the plate was read at 450nm wavelength.

### **Serum NT-proBNP, troponin I assay**

The serum NT-proBNP and troponin I were measured by enzyme-linked immunosorbent assay (ELISA). The ELISA kits were purchased from Abcam, (ab263877, ab200016, Cambridge, UK.). The samples and reagents in the ELISA kits were recovered to room temperature, each sample was tested in duplicate. All the reagents, samples, and standards were prepared as instructed. First, 50  $\mu\text{L}$  standards or samples were added to appropriate wells, then, 50  $\mu\text{L}$  antibody cocktail were added to all wells and incubated at room temperature for 1 hour. After washing for three times with 350  $\mu\text{L}$  1X wash buffer. 3,3',5,5'-

Tetramethylbenzidine (TMB, 100  $\mu$ l) was added and incubated for 10 min, the stop solution (100  $\mu$ l) was added, and the plate was read at 450nm wavelength.

## Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc, Chicago, IL, USA). Normal distribution data are expressed as mean  $\pm$  standard deviation, and skewed data are expressed as median (inter quartile range, IQR). Comparisons between different groups were analyzed by a Kruskal-Wallis or Mann-Whitney test. Spearman correlations were applied. Logistic regression was performed with the backward method for multivariable analysis (the entry probability for stepwise:  $P < 0.05$ ). The diagnostic performance of an assay was assessed by the analysis of receiver-operating characteristic (ROC) curve. The assessment of sensitivity and specificity was determined using selected cutoff value.  $P < 0.05$  was considered statistically significant.

## Results

### Cardiac function parameters

NT-proBNP in the CCM group was significantly higher than that in the non-CCM group ( $213 \pm 56$  vs  $159 \pm 112$  pg/mL,  $P < 0.05$ ). There were no statistically significant differences in other laboratory tests or cirrhosis complications between the two groups (Table 1). In the CCM group, the left ventricular ejection fraction (LVEF), Fractional shortening (FS), and E/A ratio were significantly lower and left ventricular posterior wall minor motion amplitude (LVPWA) was significantly higher than those in the non-CCM group, ( $P < 0.05$ ) (Table 2).

### Serum anti- $\beta$ AR

The anti- $\beta$ 1-AR levels in the CCM group were significantly higher than that in non-CCM group ( $0.97 \pm 0.37$  vs  $0.74 \pm 0.37$  ng/mL,  $P < 0.05$ ); this value was  $0.12 \pm 0.06$  ng/mL in healthy controls. We also divided all patients according to the severity of cirrhosis and found that anti- $\beta$ 1-AR levels were not significantly different between compensated and decompensated groups ( $P > 0.05$ ). (Fig. 2).

### Relationship between serum anti- $\beta$ 1-AR and heart function

In the CCM group, serum anti- $\beta$ 1-AR level was negatively correlated with LVEF ( $r = -0.466$ ,  $P < 0.05$ ) and FS ( $r = -0.488$ ,  $P < 0.05$ ) which represents cardiac systolic function (Fig 3), and negatively correlated with the diastolic function index E/A ( $r = -0.475$ ,  $P < 0.05$ ) . which represents cardiac diastolic function. However, there was no significant correlation with the electrocardiographic QTc interval ( $r = 0.02$ ,  $P = 0.938$ ). In addition, Serum anti- $\beta$ 1-AR in the CCM group showed a significant correlation with NT-proBNP ( $r = 0.48$ ,  $P < 0.05$ ), but had no significant correlation with troponin I ( $r = 0.053$ ,  $P = 0.833$ ) (Table 3).

### Relationship between serum anti- $\beta$ 1-AR and liver function or portal hypertension

In the CCM group, serum anti- $\beta$ 1-AR had no significant correlation with ALT ( $r = 0.267$ ,  $P = 0.269$ ), AST ( $r = 0.369$ ,  $P = 0.120$ ), bilirubin ( $r = 0.275$ ,  $P = 0.255$ ), albumin, ( $r = -0.137$ ,  $P = 0.576$ ), creatinine ( $r = -0.219$ ,  $P = 0.367$ ), Child-Pugh score ( $r = 0.021$ ,  $P = 0.933$ ), MELD score ( $r = 0.089$ ,  $P = 0.718$ ) or MELD-Na score ( $r = 0.015$ ,  $P = 0.885$ ), but did negatively correlate with portal vein diameter ( $r = -0.609$ ,  $P < 0.05$ ) and splenic vein diameter ( $r = -0.527$ ,  $P < 0.05$ , Table 4).

### Diagnostic value of serum anti- $\beta$ 1-AR CCM

The AUROC of serum anti- $\beta$ 1-AR on CCM was 0.678 (95% CI 0.515-0.768). If the cutoff value was set at 0.669 ng/mL, the sensitivity was 89.5%, and the specificity was 57% for diagnosis of CCM.

## Discussion

To our knowledge, this is the first study to demonstrate that the serum anti- $\beta$ 1-AR was increased in patients with cirrhotic cardiomyopathy. We also found that anti- $\beta$ 1-AR levels correlated with cardiac systolic function (LVEF, fractional shortening) and diastolic function (E/A ratio). It is thought that NT-proBNP correlates with left ventricular systolic and diastolic dysfunction in several noncirrhotic cardiac conditions [13]. NT-proBNP also correlates with cardiac dysfunction in cirrhotic patients [14]. Our data revealed that anti- $\beta$ 1-AR was correlated with the serum level of NT-proBNP in CCM patients. All these data suggested that anti- $\beta$ 1-AR is a useful biomarker of CCM.

Patients with CCM show overt cardiac dysfunction when challenged [12]. Lee and coworkers demonstrated that the desensitization of the cirrhotic rat heart to  $\beta$ AR stimulation is caused, at least in part, by downregulation of cardiac  $\beta$ AR density. It thus seems reasonable to hypothesize that: 1) the long-term activation of the sympathetic nervous system induces downregulation of  $\beta$ AR and 2) alteration in  $\beta$ AR metabolism in the cirrhotic environment contributes to the defect in  $\beta$ -adrenergic system function [15].

In that respect, there is no previous study on the relationship between anti- $\beta$ AR antibody and  $\beta$ AR status in patients with CCM. However, in patients with dilated cardiomyopathy, Patel et al. demonstrated that the anti- $\beta$ 1-AR binds to and constitutively stimulates the  $\beta$ 1-AR to cause  $\beta$ AR desensitization and downregulation [11]. Limas et al. found that the serum from patients with idiopathic dilated cardiomyopathy inhibits the binding of [ $^3$ H]dihydroalprenolol to rat cardiac membranes [16], and the inhibition could be prevented by preincubating the sera with anti-human IgG.

Using peptide corresponding to the second extracellular loop of the  $\beta$ 1-AR to immunize rats, the animals developed anti- $\beta$ 1-AR. These rats developed dilated cardiomyopathy. Furthermore, after infusing the serum from antibody-positive rats to healthy, antibody-negative rats, the latter also developed dilated cardiomyopathy [17]. These studies strongly suggest a causal role for anti- $\beta$ 1-AR in the development of dilated cardiomyopathy.

Autoantibodies attack self cells/organs and play a pathogenic role in several conditions. Myasthenia gravis [18], Graves' disease [19] and insulin-resistant diabetes [20] are well-documented examples. Besides the desensitization of the  $\beta_1$ -AR, anti- $\beta_1$ -AR also affect cardiac systolic function which indicates that anti- $\beta_1$ -AR is a cardiodepressant in antibody-positive patients. Stork et al found that the anti- $\beta_1$ -AR increases all-cause and cardiovascular mortality risk in patients with dilated cardiomyopathy [21].

Autoantibodies are common in patients with cirrhosis. Immuno-regulatory dysfunction contributes to liver diseases such as autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis. Furthermore, patients with cirrhosis of other etiologies also have been documented with autoantibody-related conditions. Gilman et al. found that autoantibodies, such as antinuclear antibody, antimitochondrial antibody, antismooth muscle antibody and/or antiliver kidney microsomal antibody, are positive in some patients with hepatitis C infection [22] Karpova et al. demonstrated that increases of autoantibodies (anti-intestinal antigens) are associated with degenerative changes in the intestinal mucosa in patients with cirrhosis [23, 24].

The mechanism of the anti- $\beta_1$ AR production in our study is not clear. One possibility is that cardiomyocyte injury releases the  $\beta_1$ -AR from the cell membrane to the circulation, which acts as an autoantigen stimulating the immune system to form anti- $\beta_1$ -AR. Our previous study demonstrated that myocardial apoptosis is increased in a rat model of CCM [25]. We believe that anti- $\beta_1$ -AR and cardiac injury thus comprise a 'vicious circle'. Blocking or weakening this circle may be therapeutically beneficial in CCM. Muller and colleagues [24, 26] used immunoglobulin adsorption to decrease the amount of anti- $\beta_1$ -AR in dilated cardiomyopathy. They achieved a therapeutic effect with an improvement of *New York Heart Association* (NYHA) class, decreased markers of oxidative stress, and reduction in hospital stay. Those results raise the intriguing possibility that immunoglobulin adsorption may be a new strategy for the treatment of CCM patients with high levels of anti- $\beta_1$ -AR antibodies.

Besides the correlation between anti- $\beta_1$ -AR and cardiac function, we also found that anti- $\beta_1$ -AR was correlated with portal hypertension, but not with the severity of liver dysfunction. This is not surprising given that portal hypertension is a hemodynamic/cardiovascular perturbation while liver dysfunction depends on many factors including metabolism, fibrosis, cell energetics and redox status.

The exact prevalence of CCM in cirrhotic patients remains unclear but recent estimates place it around 60% [27, 28]. In our study it was only 15%. The explanation for the lower prevalence in our study is likely patient selection. Most of our patients were Child-Pugh class A, as decompensated class B or C patients generally were excluded due to infection, bleeding, or other complications of advanced liver disease. Most evidence suggests that the prevalence of CCM correlates with the extent of liver failure in cirrhosis [29].

Since there is no specific biomarker for the diagnosis of CCM, we evaluated the predictive value of anti- $\beta_1$ -AR for CCM using ROC curves and found that the level of anti- $\beta_1$ AR showed a significant predictive value for CCM. However, because of our small sample size, these results should be confirmed by larger studies.

A limitation of this study is that we used the old 2005 Montreal diagnostic criteria[12]; the new proposed criteria were published after we finished this study [3]. However, whether the new criteria are superior to the old ones remains unknown. Singh and colleagues in a preliminary study compared these two criteria in cirrhotic patients waiting for liver transplantation [30]. They found that 8% of their cohorts had CCM according to the new criteria, while the percentage was 74% according to the old criteria [30]. Since approximately 7%-21% of deaths after liver transplantation result from cardiovascular complications [12], it is possible that the new CCC criteria may be too restrictive to apply to advanced-cirrhotic patients. Another recent publication compared the two diagnostic criteria in 122 patients with cirrhosis[28]. They found that the overall prevalence of cirrhotic cardiomyopathy was similar for the old criteria (67.2%), compared to the new (55.7%, P=0 .9). The diagnostic criteria thus need to be standardized and validated in further studies to determine which better correlate with relevant outcomes such as mortality and cardiovascular morbidity. Until such time as the issue of which criteria are best is resolved, which will take several years, we believe that the present study based on the Montreal criteria remains valuable in the cirrhotic cardiomyopathy literature.

In conclusion, anti- $\beta_1$ -AR was increased in CCM patients. Anti- $\beta_1$ -AR may play an important role in the desensitization of  $\beta_1$ -AR in patients with CCM. Furthermore, the presence of Anti- $\beta_1$ AR may increase cardiovascular complications in cirrhotic patients. Anti- $\beta_1$ -AR are also valuable as a potential biomarker to diagnose CCM. Using immunological methods to reduce/diminish Anti- $\beta_1$ -AR levels may represent a promising new therapeutic strategy for patients with cirrhotic cardiomyopathy.

## Declarations

**Data Availability:** Upon request

**Animal Research (Ethics):** N/A

**Consent to Participate (Ethics):** All patients provided written informed consent.

**Consent to Publish (Ethics):**N/A

**Plant Reproducibility:** N/A

**Clinical Trials Registration:** Chinese Clinical Trials No. ChiCTR 2000037730

**Author Contribution:** Lixia Ma, Xiaohui Liu, Qingshan Wu: conducted the study, and collected and/or interpreted data; Jing Zhang, designed the study; Jing Zhang, Hongqun Liu, Samuel S Lee: drafted and revised the manuscript. All authors have approved the final draft.

**Conflict of Interest** The authors have no conflicts to declare.

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## Tables

**Table 1. Characteristics of the patients**

Parameters	Total(n=95)	no CCM (n=76)	CCM(n=19)	<i>P</i> value
Male,n(%)	59(62.1)	47(61.8)	12(63.2)	0.831
Age (years)	56.6±8.3	56.3±8.3	57.7±8.2	0.335
Cirrhosis Etiologies				0.461
Hepatitis B, n(%)	38(40.0)	31(40.8)	7(36.8)	
Hepatitis C, n(%)	45(47.4)	36(47.4)	9(47.4)	
primary biliary cholangitis, n(%)	9(9.5)	6 (7.9)	3(15.8)	
Others, n(%)	3 (3.1)	3 (3.9)	-	
MAP (mmHg)	85.3(81.3-94.6)	84.8(80.0-94.9)	86.7(83.3-94.3)	0.381
HR (bpm)	76.3(66.0-84.0)	75.0(66.0-85.0)	75.0(65.0-83.2)	0.934
White blood cell (×10 <sup>9</sup> /L)	3.53(2.09-5.06)	3.52(1.94-4.97)	3.99(2.24-5.23)	0.632
Hemoglobin [g/dL]	104.1±32	106.2±32.2	96.0±33.4	0.621
Platelet (×10 <sup>9</sup> /L)	65.0(41.0-102.0)	62.0(39.0-97.2)	74.0(48.0-129.0)	0.185
ALT (U/L)	32.8 (19.4-48.5)	25.8 (15.4-38.5)	39.1 (29.5-57.1)	0.552
AST (U/L)	32.1 (27.6-78.1)	39.1 (29.2-57.1)	29.0 (25.8-84.0)	0.379
Bilirubin (mmol/L)	28.6 (17.6-48.1)	29.3 (17.6-49.4)	25.9 (18.4-33.7)	0.500
Albumin (g/dL)	32.4±5.8	32.6±5.6	31.4±6.7	0.401
γ-glutamyl transpeptidase (U/L)	32.7 [18.1-62.4]	30.7 (17.5-62.0)	36.9 (20.1-101.1)	0.308
Alkaline phosphatase (U/L)	86.7 (64.0-116.1)	87.5 (66.3-119.5)	74.0 (57.0-106.1)	0.329
Na (mmol/L)	139.3(136.5-141.0)	139.4(136.9-140.8)	137.5(135.0-141.3)	0.308
K (mmol/L)	3.8 [3.5-4.0]	3.8(3.5-4.0)	3.9(3.3-4.1)	0.892
Creatinine (μmol/L)	59(49.5-69.3)	60.5(49.6-70.5)	54(47.5-64.9)	0.437
Urea nitrogen (mmol/L)	4.8(4.4-6.3)	5.2(4.1-6.3)	4.4(3.8-5.7)	0.211

NT-proBNP (pg/mL)	140(108-198)	119(108-160)	220(160-250)	0.001
cTNI (pg/mL)	0.273(0.271-0.275)	0.273 (0.271-0.275)	0.273(0.271-0.277)	0.930
INR	1.3(1.2-1.5)	1.3(1.2-1.5)	1.2(1.1-1.5)	0.725
Ascites, n (%)	72(75.7)	64(59.8)	8(42.1)	0.218
Spontaneous peritonitis, n (%)	16(16.8)	13(17.1)	3(15.7)	0.961
HE, n (%)	15(15.7)	14(12.9)	1(5.3)	0.550
Portal vein diameter (mm)	12.0(11.0-14.0)	12.0(11.2-14.0)	13.0 (11.0-15.0)	0.709
Spleen vein diameter (mm)	10(8.0-12.0)	10.0(8.0-12.0)	8(7.0-12.0)	0.120
Spleen thickness (mm)	51.7±12.5	53.1±12.7	46.2±10.3	0.320
Child-Pugh grade				0.166
A, n (%)	63(66.3)	50(65.8)	13(68.4)	
B, n (%)	17(17.9)	14(18.4)	3(15.8)	
C, n (%)	15(15.8)	12(15.8)	3(15.8)	
MELD score (range)	7.3(4.5-11.1)	7.3(4.5-11.1)	7.7(3.5-9.7)	0.717
MELD-Na score (range)	7.8(4.7-11.1)	7.5(4.7-11.2)	8.1(5.1-10.2)	0.932
Anti β1-AR (ng/mL)	0.79±0.38	0.74±0.37	0.97±0.37	0.026

CCM, cirrhotic cardiomyopathy; MAP, mean arterial pressure; HR heart rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; NT-proBNP, N-Terminal pro-brain natriuretic peptide; cTNI, cardiac troponin 1; HE, hepatic encephalopathy; MELD, model of end-stage liver disease; anti- β1-AR, anti-beta-1-adrenergic receptor antibodies; *P* values are the comparison between no CCM and CCM.

**Table 2. Comparison of cardiac function in patients with and without CCM**

Parameters	Total	no CCM (n=76)	CCM (n=19)	<i>P</i> value
QTc (ms)	397.9±27.5	398.1±26.8	397.2±31.1	0.906
E/A ratio	0.88(0.76-1.26)	1.02 (0.78-1.30)	0.76 (0.63-0.88)	<b>0.001</b>
LVEF (%)	64.3±6.9	68.3±6.5	54.2±7.7	<b>0.016</b>
LA (mm)	34.1±4.2	34.0±4.1	34.6±4.5	0.594
LVIDd (mm)	48.1±6.4	47.9±5.4	48.3±6.7	0.736
LVIDs (mm)	30.1±5.7	29.1±4.5	32.2±7.5	0.089
FS (%)	36.7±4.5	38.8±4.5	35.3±5.7	<b>0.004</b>
LVPWA (mm)	9.7±1.5	9.9±1.5	8.9±1.5	<b>0.007</b>
IVS (mm)	10.4(9.0-11.0)	9.9(9.0-11.0)	11.0(9.0-11.0)	0.784

CCM, cirrhotic cardiomyopathy; QTc, rate-corrected QT interval; E/A ratio, early/late diastolic filling velocities ratio; LVEF, left ventricular ejection fraction; LA, left atrial diameter; LVIDd, end-diastole left ventricular diameter; LVIDs, end-systolic left ventricular diameter; FS, left ventricular fractional shortening; LVPWA, left ventricular posterior wall minor motion amplitude; IVS, interventricular septum; *P* values are the comparison between no CCM and CCM groups.

**Table 3. Correlation of anti-β1-AR with cardiac function**

Parameters	anti $\beta$ 1-AR	
	R	Pvalue
MAP	-0.094	0.701
HR	-0.071	0.778
QTc	0.033	0.896
E/A ratio	-0.475	<b>0.047</b>
LVEF	-0.466	<b>0.044</b>
LA	0.173	0.523
LVIDd	-0.245	0.312
LVIDs	-0.393	0.096
FS	-0.488	<b>0.034</b>
LVPWA	0.338	0.157
IVS	0.143	0.559
NT-proBNP	0.480	<b>0.037</b>
cTNI	0.090	0.724

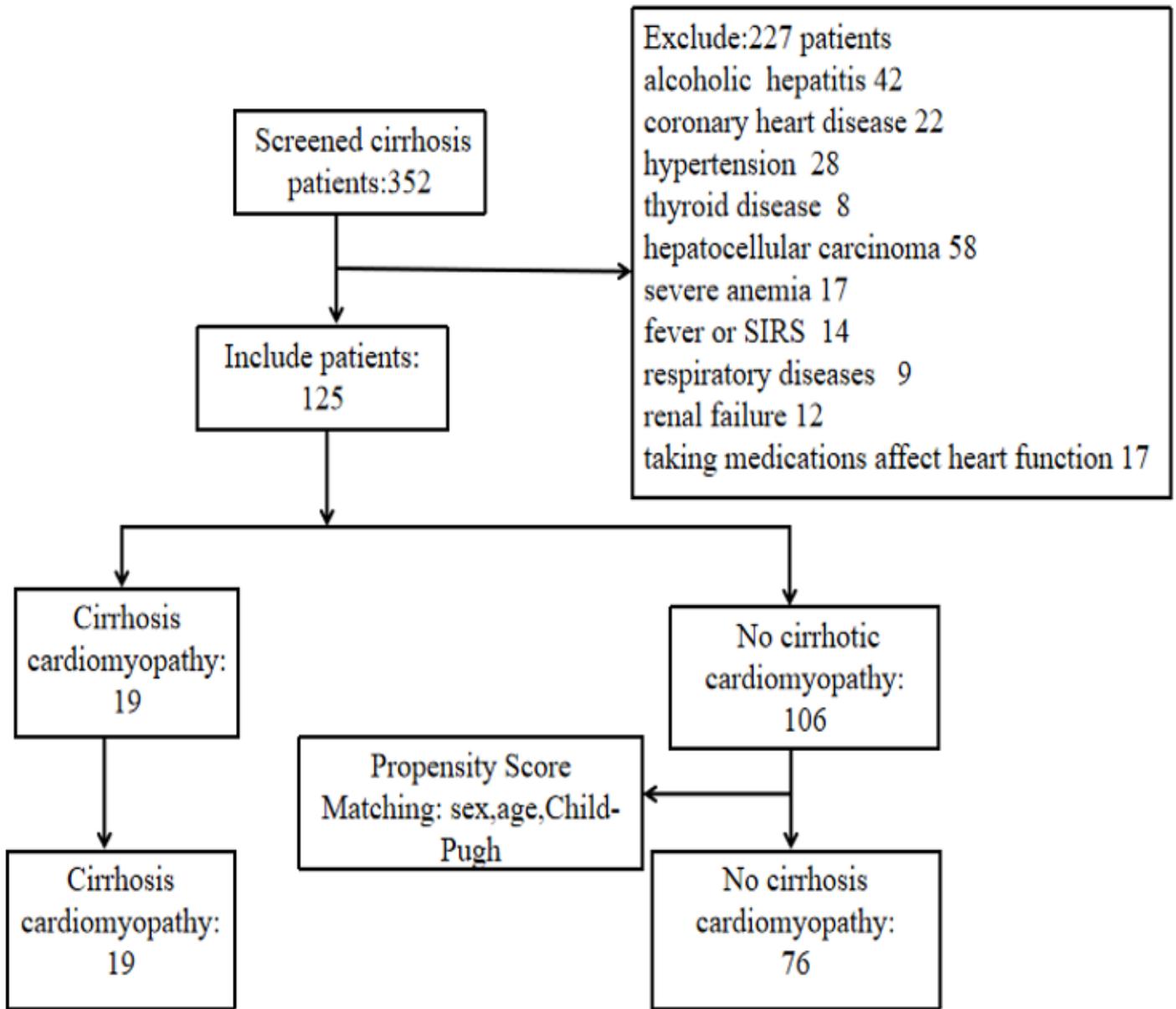
anti-  $\beta$ 1-AR, anti-beta-1-adrenergic receptor antibodies; MAP, mean arterial pressure; HR, heart rate; QTc, corrected QT interval; E/A ratio, early/late diastolic filling velocities ratio; LVEF, left ventricular rejection fraction; LA, left atrial diameter; LVIDd, end-diastole left ventricular diameter; LVIDs, end-systolic left ventricular diameter; FS, left ventricular fractional shortening; LVPWA, left ventricular posterior wall minor motion amplitude; IVS, interventricular septum; NT-proBNP, N-Terminal pro-brain natriuretic peptide; cTNI, cardiac troponin 1.

**Table 4. Correlation of anti - $\beta$ 1-AR with liver variables**

Parameters	R	Pvalue
ALT	0.267	0.269
AST	0.369	0.120
Bilirubin	0.275	0.255
Albumin	-0.137	0.576
Creatinine	-0.219	0.367
Child-Pugh score	0.021	0.933
MELD score	0.089	0.718
MELD-Na score	0.015	0.885
Portal vein diameter	-0.609	<b>0.006</b>
Spleen vein diameter	-0.527	<b>0.020</b>
Spleen thickness	-0.448	0.054

anti-  $\beta$ 1-AR, anti-beta-1-adrenergic receptor antibodies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MELD, model of end-stage liver disease.

## Figures



**Figure 1**

Flowchart of patient selection

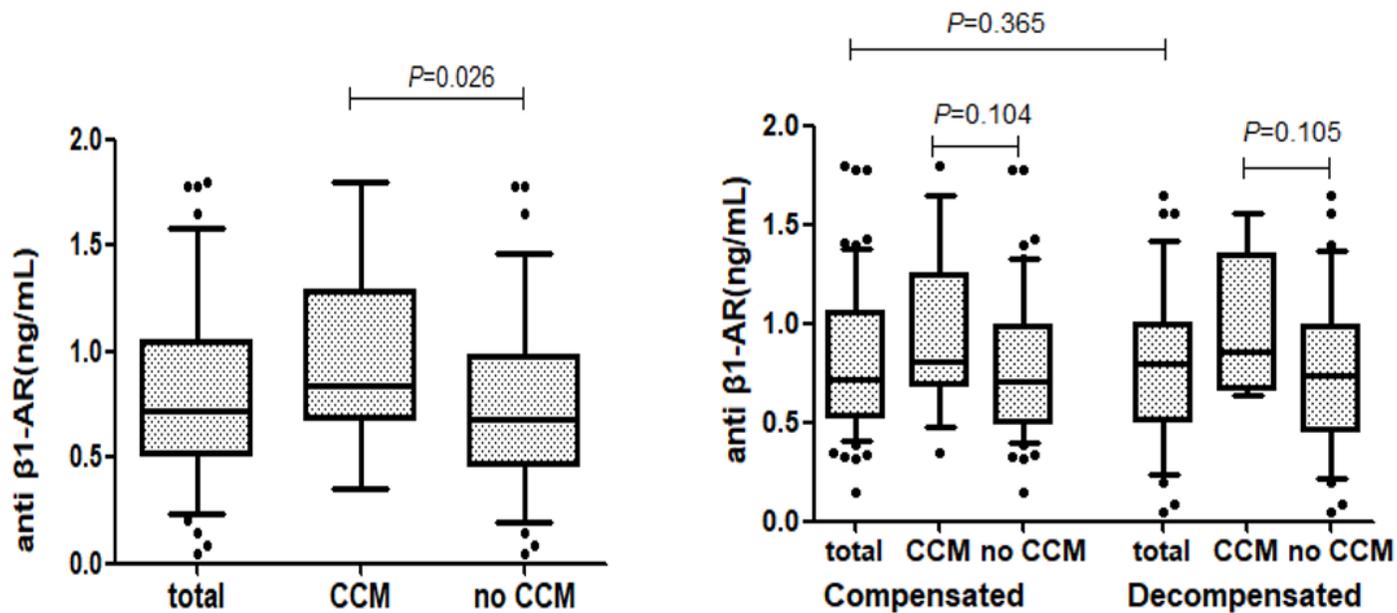
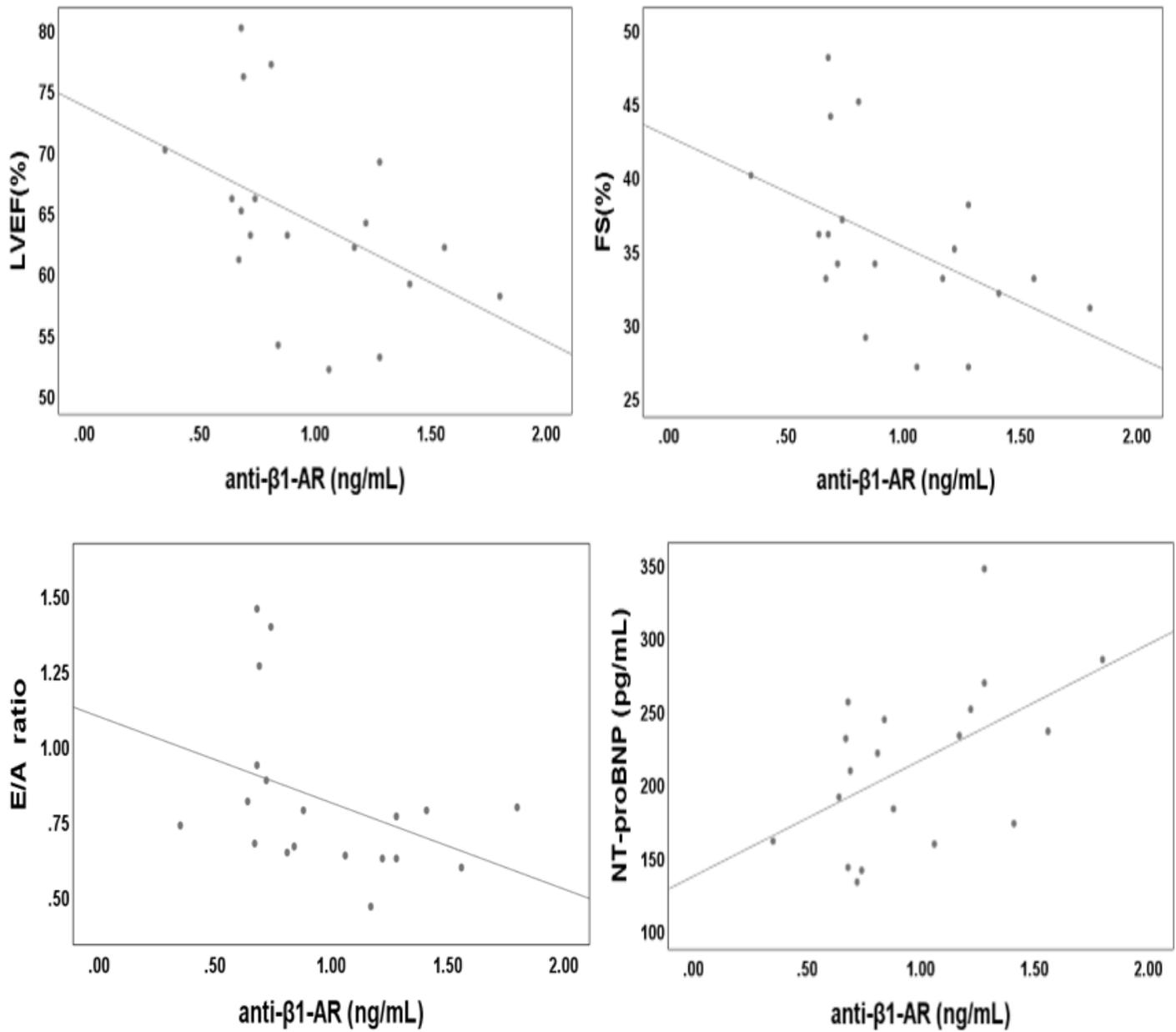


Figure 2

Serum anti-β1-AR levels in patients with and without cirrhotic cardiomyopathy. Serum anti-β1-AR levels in patients with and without CCM and in compensated or decompensated patients. (A) Serum anti-β1-AR levels were higher in CCM than the nonCCM group. (B) There was no difference of serum anti-β1-AR levels in decompensated as compared to compensated patients.



**Figure 3**

Relationship between serum anti-β1-AR and LVEF, FS, and NT-proBNP

## Supplementary Files

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