ORIGINAL ARTICLE

Serum autotaxin as a novel prognostic marker in patients with non-ischaemic dilated cardiomyopathy

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Abstract

Aims   Autotaxin (ATX) promotes myocardial inflammation, fibrosis, and the subsequent cardiac remodelling through lysophosphatidic acid production. However, the prognostic impact of serum ATX in non-ischaemic dilated cardiomyopathy (NIDCM) has not been clarified. We investigated the prognostic impact of serum ATX in patients with NIDCM.

Methods and results   We enrolled 104 patients with NIDCM (49.8 ± 13.4 years, 76 men). We divided the patients into two groups using different cutoffs of median serum ATX levels for men and women: high-ATX group and low-ATX group. Cardiac events were defined as a composite of cardiac death and heart failure resulting in hospitalization. Median ATX level was 203.5 ng/mL for men and 257.0 ng/mL for women. Brain natriuretic peptide levels [224.0 (59.6–689.5) pg/mL vs. 96.5 (40.8–191.5) pg/mL, *P* = 0.010] were higher in the high-ATX group than low-ATX group, whereas high-sensitivity C-reactive protein and collagen volume fraction levels in endomyocardial biopsy samples were not significantly different between the two groups. Kaplan–Meier survival analysis revealed that the event-free survival rate was significantly lower in the high-ATX group than low-ATX group (log-rank; *P* = 0.007). Cox proportional hazard analysis revealed that high-ATX was an independent determinant of composite cardiac events. In both sexes, serum ATX levels did not correlate with high-sensitivity C-reactive protein levels and collagen volume fraction but had a weak correlation with brain natriuretic peptide levels (men; spearman's rank: 0.274, *P* = 0.017, women; spearman's rank: 0.378, *P* = 0.048).

Conclusion   High serum ATX levels can be associated with increasing adverse clinical outcomes in patients with NIDCM. These results indicate serum ATX may be a novel biomarker or therapeutic target in NIDCM.

Introduction

A variety of biomarkers have been proposed as non-invasive and useful tools for prognostication and risk stratification in heart failure (HF).[1] The causes of myocardial remodelling of non-ischaemic dilated cardiomyopathy (NIDCM) are diverse, and inflammation and subsequent fibrosis are considered as one of the causes.[2] Actually, inflammatory markers such as interleukin-6 (IL-6), tumour necrosis factor-α (TNFα), high-sensitivity C-reactive protein (hsCRP), and soluble suppression of tumorigenesis-2 (sST2) have been reported to be determinants of poor prognosis in patients with NIDCM.[3,4] In addition, myocardial fibrosis is associated with myocardial remodelling and sudden cardiac death,[5,6] and the fibrosis marker galectin-3 has been shown to be a useful biomarker in risk stratification.[7] However, the only biomarker that are routinely used in clinical practice and recommended in Class I in the HF guideline is brain natriuretic peptide (BNP), which correlates with pulmonary capillary wedge pressure (PCWP) and sensitively reflects the presence and severity of the ventricular wall stress.[1] Therefore, exploratory researches are needed to identify new potential biomarkers that may serve as therapeutic targets for HF and cardiomyopathy.

Autotaxin (ATX) has a lysophospholipase D activity that is strongly involved in the extracellular synthesis of lysophosphatidic acid (LPA). Most ATXs are considered to be derived from adipose tissue and are expressed in the blood, cerebrospinal fluid, lungs, and urine. LPA causes various physiological and pathophysiological effects through G protein-coupled cell surface receptors, namely, LPA1–6,[8] which have been reported to correlate well with ATX levels; however, LPA levels easily change depending on time even after serum is separated.[9] LPA receptors are also expressed in the myocardium and vascular endothelium,[10] and ATX promotes the release of cytokines and other substances through LPA, which causes inflammation and fibrosis. The ATX/LPA axis has attracted attention in non-cardiac diseases, such as breast cancer, rheumatoid arthritis, idiopathic pulmonary fibrosis (IPF), and liver fibrosis,[11,12] although studies on the ATX/LPA axis in the heart are limited.

The relationship between the ATX/LPA axis and cardiovascular diseases, such as arteriosclerosis, aortic valve sclerosis, and cardiac dysfunction, was studied in mice.[8] In humans, a correlation between serum ATX levels and plasma BNP levels[13] and an increase in ATX and LPA levels was observed in patients with acute myocardial infarction (AMI).[14] Furthermore, high-ATX levels were reported to be a poor prognostic factor in patients with sepsis, a systemic inflammation.[15] However, little is known about the impact of ATX levels on future cardiac events in patients with NIDCM. We hypothesize that the ATX/LPA axis may be involved in the prognosis of NIDCM. Therefore, we investigated the prognostic impact of serum ATX levels in patients with NIDCM.

Methods

Study population

We enrolled 104 consecutive patients with NIDCM between September 2006 and December 2019 for this single-centre prospective observational study. All patients underwent laboratory measurements, 12-lead electrocardiography, echocardiography, coronary angiography, right heart catheterization, and endomyocardial biopsy for a definitive diagnosis of dilated cardiomyopathy under the individual stable condition of HF. NIDCM was defined as left ventricular ejection fraction (LVEF) < 50% on echocardiography or left ventriculography in the absence of coronary artery diseases, primary valvular heart disease, active myocarditis, significant hypertension, excessive alcohol intake, congenital heart diseases, pericardial diseases, or secondary cardiac muscle disease caused by any known systemic condition.[16,17] We excluded patients with cancer, inflammatory disease, or chronic liver disease. In addition, patients regularly taking steroids and anti-inflammatory agents were excluded to avoid a bias in drug-induced changes in serum ATX levels.[18] The study protocol was approved by the Ethics Review Board of our institute (approval No. 2017-0031), and written informed consent was obtained from all participants.

Evaluation of serum autotaxin levels

Blood samples were collected from the peripheral veins of the participants during cardiac catheterization at baseline. The samples were immediately centrifuged at 3000 rpm for 10 min at 4°C. Supernatants were stored at −80°C until ATX measurement. Serum ATX levels were assessed using a sandwich ELISA kit (Quantikine ELISA kit, R&D Systems, Minneapolis, MN, USA). The ATX levels were measured twice, and the average level was used for the analysis.

Echocardiography

Standard M-mode, two-dimensional and Doppler echocardiography, and tissue Doppler imaging were performed (Vivid 7 system, GE Healthcare, WI, USA) by trained physicians or professional echocardiographers, according to the guidelines of the American Society of Echocardiography.[19] LVEF was assessed using modified Simpson's method. Early diastolic filling velocity (E) was measured using pulsed-wave Doppler. Early diastolic mitral annular tissue velocity (e′) was assessed at the septal side of the mitral annulus in the apical four-chamber view.

Cardiac catheterization and endomyocardial biopsy

To assess baseline haemodynamic status, all patients underwent biventricular cardiac catheterization analyses under each stable condition. Right heart catheterization was performed using a 7-Fr triple-lumen Swan–Ganz thermodilution pulmonary artery catheter (Edwards Life Science Co., Irvine, CA, USA) through the internal jugular vein. Cardiac index (CI) was determined using the Fick method.

An endomyocardial biopsy was performed to exclude secondary cardiomyopathies.[20] Several biopsy specimens were obtained from the right side of the interventricular septum using a 6-Fr cardiac bioptome catheter (Myocardial Biopsy Forceps, Technowood®, Tokyo, Japan). All specimens were immediately fixed in 10% buffered formalin.

Calculation of collagen volume fraction

Collagen volume fraction (CVF) was calculated using picrosirius red staining to evaluate myocardial fibrosis.[21,22] The stained sections were photographed at a high magnifi-

cation (×200). Nine fields from each stained section were combined and analysed using BZ-X800 (Keyence, Osaka, Japan). CVF was calculated as the percentage of collagen area divided by total myocardial area excluding subendocardial or perivascular areas. All samples were analysed by another investigator who was blinded to the clinical information, including the results of serum ATX concentration.

Patient classification and clinical follow-up

All patients were divided into two groups: high-ATX and low-ATX groups. Considering the sex-dependent difference in serum ATX levels, patients of each sex with serum ATX above the median value were assigned to the high-ATX group and those below the median value were assigned to the low-ATX group. Skilled cardiologists followed up all patients under the global guidelines.[1] A cardiac event was defined as a composite of cardiac deaths and unscheduled hospitalizations for worsening HF requiring diuretics or inotropic therapy. Data on clinical events were collected from the medical records or via telephone interview.

Statistical analysis

All statistical analyses were performed using the JMP Pro version 15.0 software (SAS Institute, Cary, NC, USA). Continuous variables analysed using the Shapiro–Wilk normality test were presented as mean ± standard deviation or median with interquartile range. Categorical variables were expressed as numbers with percentages. Data were compared between the two groups using Student's *t* test for parametric variables or the Mann–Whitney *U* test for nonparametric variables. Categorical variables were compared using the nonparametric Fisher's exact test. Kaplan–Meier survival analysis with log-rank testing was performed to assess event-free survival. The Cox proportional hazards model was used to assess univariate and multivariable covariates for cardiac events. We limited the number of variables to avoid model overfitting. The associations between ATX levels and other variables were analysed using Spearman's rank correlation coefficients. Statistical significance was set at $P < 0.05$.

Results

Baseline clinical characteristics of the patients

Baseline clinical characteristics of the patients are summarized in *Table 1*. Of 104 patients, 76 (73.1%) were men with a mean age of 49.8 ± 13.4 years. Most patients were clinically

stable; 89.4% were classified as New York Heart Association (NYHA) functional Class I or II. Serum ATX levels were 203.5 (169.7–267.0) ng/mL for men and 257.0 (210.6–341.4) ng/mL for women, showing a significant difference ($P = 0.003$) (*Figure 1*). We found significant differences in heart rate (79.5 ± 12.8 bpm vs. 71.2 ± 11.7 bpm, $P < 0.001$), BNP concentration [224.0 (59.6–689.5) pg/mL vs. 96.5 (40.8–191.5) pg/mL, $P = 0.010$], and PCWP [14.5 (10.0–21.5) mmHg vs. 11.5 (7.3–16.8) mmHg, $P = 0.016$] between the high-ATX and low-ATX groups, respectively. By contrast, no significant differences were determined between the groups in age, medications used, comorbidities, haemoglobin levels, renal function, liver enzyme levels, hsCRP, CVF, and electrocardiogram findings. Furthermore, there was no significant difference in haemodynamic parameters except for PCWP.

Cumulative event-free survival

The patients were followed up for 4.6 ± 3.0 years. During follow-up, cardiac events occurred in 23 patients: 17 of 52 (32.7%) in the high-ATX group and 6 of 52 (11.5%) in the low-ATX group (*Table 2*). Cardiac death occurred only in the high-ATX group [in 7 (13.5%) patients]. Twenty-three HF hospitalizations occurred in 17 of 52 (32.7%) patients in the high-ATX group and 6 of 52 (11.5%) in the low-ATX group. Kaplan–Meier survival analysis revealed that the composite event-free survival rate was significantly lower in the high-ATX group (log-rank: $P = 0.007$) (*Figure 2A*). Specifically, both the rate of cardiac death and the rate of worsening HF were higher in the high-ATX group (log-rank: $P = 0.006$ and $P = 0.007$, respectively) (*Figure 2B and 2C*).

Cox proportional hazards model for cardiac events

Table 3 shows the results from Cox proportional hazards regression analyses for composite cardiac events. In univariate Cox proportional hazards analyses, high-ATX [hazard ratio (HR): 3.376, $P = 0.006$], Hb (HR: 0.758, $P = 0.020$), BNP (per 10 pg/mL increment, HR: 1.013, $P < 0.001$), LVEF (per 5% increment, HR: 0.789, $P = 0.014$), and PCWP (HR: 1.113, $P < 0.001$) were identified as determinants of composite cardiac events. In multivariate analyses, high-ATX was an independent determinant of composite cardiac events in all models. In our results, PCWP was correlated with BNP levels ($r = 0.602$, $P < 0.001$), and therefore, we could not examine adjusted models using both factors simultaneously.

Table 1 Baseline characteristics

	Total (n = 104)	High-ATX (n = 52)	Low-ATX (n = 52)	P
Autotaxin, ng/mL	220.7 (176.1–275.4)	274.8 (242.9–333.6)	177.1 (150.7–200.4)	<0.001
Age, years	49.8 ± 13.4	49.3 ± 13.8	50.4 ± 13.1	0.680
Male, n (%)	76 (73.1)	38 (73.1)	38 (73.1)	1.000
BMI, kg/m^2	22.5 (20.6–27.0)	22.2 (20.2–26.6)	23.0 (21.0–29.3)	0.146
SBP, mmHg	116 (100–131)	113 (98–129)	118 (100–140)	0.555
DBP, mmHg	73.3 ± 13.2	75.0 ± 13.1	71.7 ± 13.2	0.217
Heart rate, bpm	75.3 ± 12.9	79.5 ± 12.8	71.2 ± 11.7	<0.001
NYHA functional Class I/II/III/IV, n	60/33/10/1	28/15/8/1	32/18/2/0	0.144
Family history of NIDCM, n (%)	14 (13.5)	6 (11.5)	8 (15.4)	0.775
Hypertension, n (%)	24 (23.1)	13 (25.0)	11 (21.2)	0.816
Diabetes mellitus, n (%)	25 (24.0)	15 (28.9)	10 (19.2)	0.359
Hyperlipidaemia, n (%)	38 (36.5)	15 (28.9)	23 (44.2)	0.154
ACE-I/ARB, n (%)	92 (88.5)	44 (84.6)	48 (92.3)	0.358
Beta-blockers, n (%)	97 (93.3)	46 (88.5)	51 (98.1)	0.112
Aldosterone antagonists, n (%)	68 (65.4)	34 (65.4)	34 (65.4)	1.000
Diuretics, n (%)	68 (65.4)	36 (69.2)	32 (61.5)	0.537
Haemoglobin, g/dL	14.3 ± 1.9	14.0 ± 2.0	14.6 ± 1.6	0.089
Creatinine, mg/dL	0.89 (0.70–1.10)	0.88 (0.70–1.10)	0.89 (0.71–1.10)	0.920
eGFR, mL/min/1.73 m^2	68 (57–85)	66 (57–86)	70 (56–82)	0.923
AST, IU/L	24 (18–31)	25 (17–35)	22 (18–28)	0.355
ALT, IU/L	20 (15–31)	20 (14–34)	20 (16–26)	0.969
BNP, pg/dL	122.5 (56.3–382.8)	224.0 (59.6–689.5)	96.5 (40.8–191.5)	0.010
hsCRP, mg/dL	0.10 (0.04–0.24)	0.11 (0.04–0.29)	0.08 (0.03–0.18)	0.244
Atrial fibrillation, n (%)	12 (11.5)	7 (13.5)	5 (9.6)	0.760
LBBB, n (%)	18 (17.3)	11 (21.2)	7 (13.5)	0.438
LVDd, mm	64.3 ± 9.1	63.6 ± 10.0	65.1 ± 8.1	0.383
LVDs, mm	54.6 ± 11.1	53.9 ± 12.2	55.4 ± 10.0	0.514
E/eʹ ratio	13.9 (10.3–19.8)	14.9 (10.5–20.9)	13.8 (10.1–19.7)	0.511
LVEF, %	30.6 (23.8–40.6)	30.6 (23.0–41.9)	30.8 (24.5–40.2)	0.902
CI, L/min/m^2	2.34 (2.01–3.03)	2.36 (1.93–3.22)	2.34 (2.03–2.69)	0.760
PCWP, mmHg	13.0 (8.3–19.0)	14.5 (10.0–21.5)	11.5 (7.3–16.8)	0.016
RAP, mmHg	6.0 (3.3–8.0)	6.5 (4.0–8.0)	5.5 (3.0–7.0)	0.138
CVF, %	12.2 (8.9–15.5)	12.0 (9.6–15.3)	12.4 (8.8–15.6)	0.862

Data are shown as mean ± SD or median (interquartile range).
ACE-I, angiotensin converting enzyme inhibitor; ATX, autotaxin; ALT, alanine aminotransferase; ARB, angiotensin II receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; BNP, brain natriuretic peptide; CI, cardiac index; CVF, collagen volume fraction; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; LBBB, left bundle branch block; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; NIDCM, non-ischaemic dilated cardiomyopathy; NYHA, New York Heart Association; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; SBP, systolic blood pressure.

Figure 1 Serum autotaxin levels in men and women. Serum autotaxin levels in women were significantly higher than those in men (P = 0.003). ATX, autotaxin.
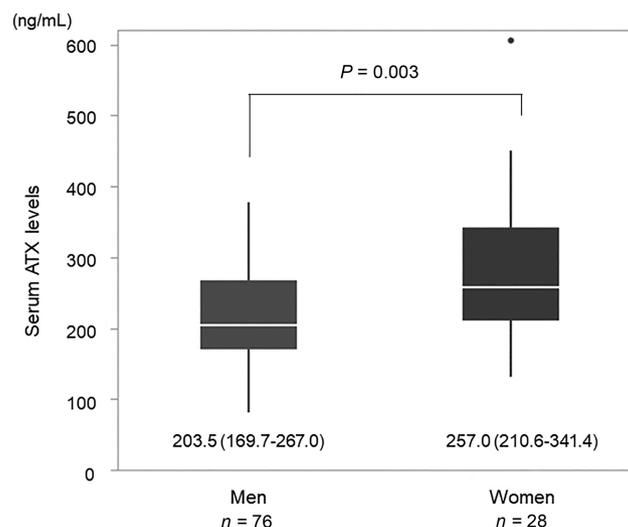
Table 2 Numbers of cardiac events during follow-up

	Total (n = 104)	High-ATX (n = 52)	Low-ATX (n = 52)
Composite cardiac events, n (%)	23 (22.1)	17 (32.7)	6 (11.5)
Cardiac death, n (%)	7 (6.7)	7 (13.5)	0
HF hospitalization, n, (%)	23 (22.1)	17 (32.7)	6 (11.5)

ATX, autotaxin; HF, heart failure.
Composite cardiac events were defined as cardiac deaths and HF hospitalizations.

Figure 2 Survival analysis for cardiac events. Kaplan–Meier curves of composite cardiac events (A), cardiac deaths (B), and heart failure hospitalizations (C). The event-free probability for composite cardiac events was significantly lower in high-ATX group (log-rank; *P* = 0.007). The composite cardiac event was defined as cardiac deaths and hospitalizations for worsening heart failure. HF, heart failure; ATX, autotaxin.
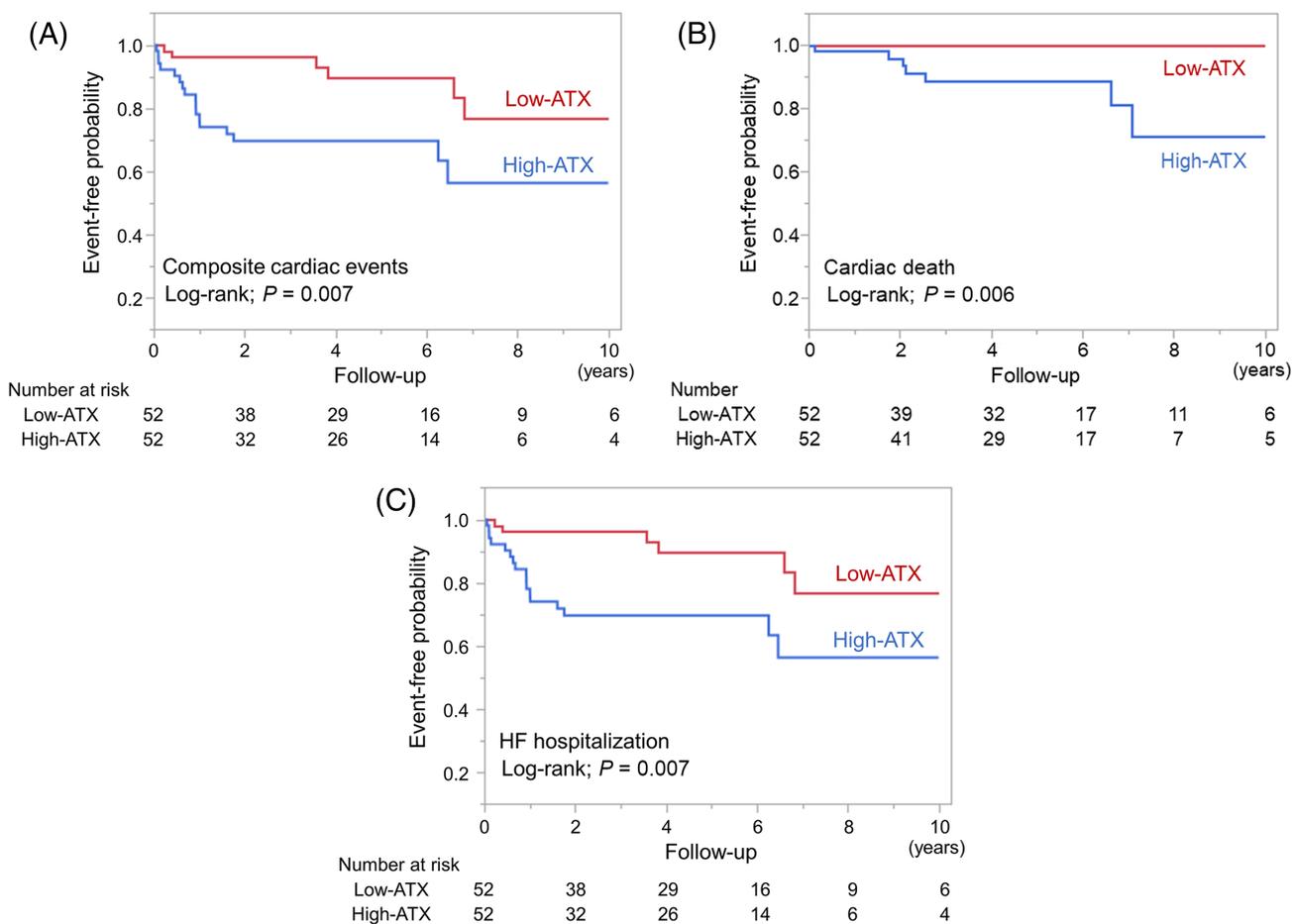
Best cutoff value of serum autotaxin levels for cardiac events

The best cutoff value of serum ATX levels for cardiac events was 240.4 ng/mL for men (sensitivity: 68.4%, specificity: 75.4%, area under the curve (AUC): 0.686) and 295.2 ng/mL for women (sensitivity: 75.0%, specificity: 75.0%, AUC: 0.740), respectively. Kaplan–Meier survival analysis revealed that composite cardiac event-free survival rates were significantly lower in the high-ATX subgroups for both sexes (men: log-rank: $P < 0.001$, HR: 5.119, $P < 0.001$, women: Log-rank: $P = 0.024$, HR: 9.037, $P = 0.038$) (*Figure 3A and 3B*).

Table 3 Univariate and multivariate Cox proportional hazards analysis for composite cardiac events

Variable	Univariate hazard ratio (95% confidence interval)	P	Variable	Multivariate hazard ratio (95% confidence interval)	P
Age (per 5 year increment)	0.942 (0.809–1.096)	0.437	Model 1		
High-ATX	3.376 (1.399–9.376)	0.006	High-ATX	2.578 (1.028–7.312)	0.042
Hb (g/dL)	0.758 (0.598–0.957)	0.020	BNP (per 10 pg/mL increment)	1.012 (1.005–1.018)	<0.001
eGFR (per 10 mL/min/1.73m^2)	0.924 (0.784–1.086)	0.341	Model 2		
hsCRP (mg/dL)	1.619 (0.857–2.423)	0.114	High-ATX	3.083 (1.270–8.361)	0.012
BNP (per 10 pg/mL increment)	1.013 (1.007–1.019)	<0.001	PCWP (mmHg)	1.114 (1.053–1.181)	<0.001
LVEF (per 5% increment)	0.789 (0.646–0.954)	0.014	Model 3		
LVDd (mm)	1.033 (0.991–1.076)	0.126	High-ATX	3.287 (1.355–9.163)	0.008
PCWP (mmHg)	1.113 (1.055–1.175)	<0.001	LVEF (per 5% increment)	0.806 (0.666–0.964)	0.018
CI (per 0.1 L/min/m^2)	0.956 (0.897–1.015)	0.147	Model 4		
HR (bpm)	1.003 (0.971–1.036)	0.862	High-ATX	2.951 (1.206–8.277)	0.017
CVF (%)	1.061 (0.977–1.138)	0.146	Hb (g/dL)	0.799 (0.632–1.007)	0.057
			Model 5		
			High-ATX	2.598 (1.038–7.396)	0.041
			BNP (per 10 pg/mL increment)	1.010 (1.003–1.017)	0.009
			LVEF (per 5% increment)	0.901 (0.727–1.118)	0.340
			Model 6		
			High-ATX	3.081 (1.266–8.444)	0.012
			PCWP (mmHg)	1.101 (1.033–1.176)	0.003
			LVEF (per 5% increment)	0.923 (0.749–1.133)	0.442

ATX, autotaxin; BNP, brain natriuretic peptide; CI, cardiac index; CVF, collagen volume fraction; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HR, heart rate; hsCRP, high-sensitivity C-reactive protein; LVDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; PCWP, pulmonary capillary wedge pressure.

Figure 3 Survival analysis for cardiac events using best cutoff value of serum autotaxin levels. The best cutoff value of serum ATX levels for cardiac events was 240.4 ng/mL for men and 295.2 ng/mL for women. Kaplan–Meier curves of composite cardiac events for men (A) and for women (B). The event-free probability for composite cardiac events was significantly lower in the high-ATX subgroups for both sexes (men: log-rank; $P < 0.001$, women: log-rank; $P = 0.024$). ATX, autotaxin.
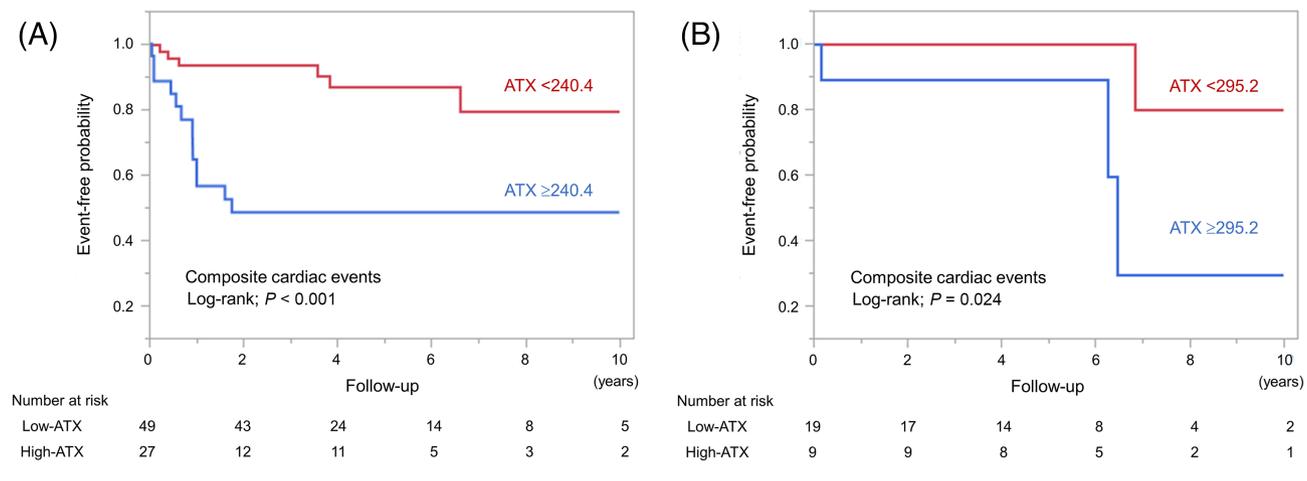
## Correlations between serum autotaxin levels and other variables

The correlations between serum ATX levels and other variables are shown in *Figure 4*. Serum ATX levels were significantly correlated with BNP levels (men; Spearman's rank correlation coefficient (rs) = 0.274, $P = 0.017$; women; rs = 0.378, $P = 0.048$). However, we could not find significant correlations between serum ATX levels and hsCRP or CVF in either sex,

although ATX has been reported to be associated with inflammation and fibrosis.[12–14]

Discussion

To our knowledge, this is the first study to investigate the impact of serum ATX levels on the prognosis of patients with

Figure 4 Correlations between serum autotaxin levels and other variables in men and women. ATX, autotaxin; BNP, brain natriuretic peptide; CI, cardiac index; CVF, collagen volume fraction; hsCRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure.
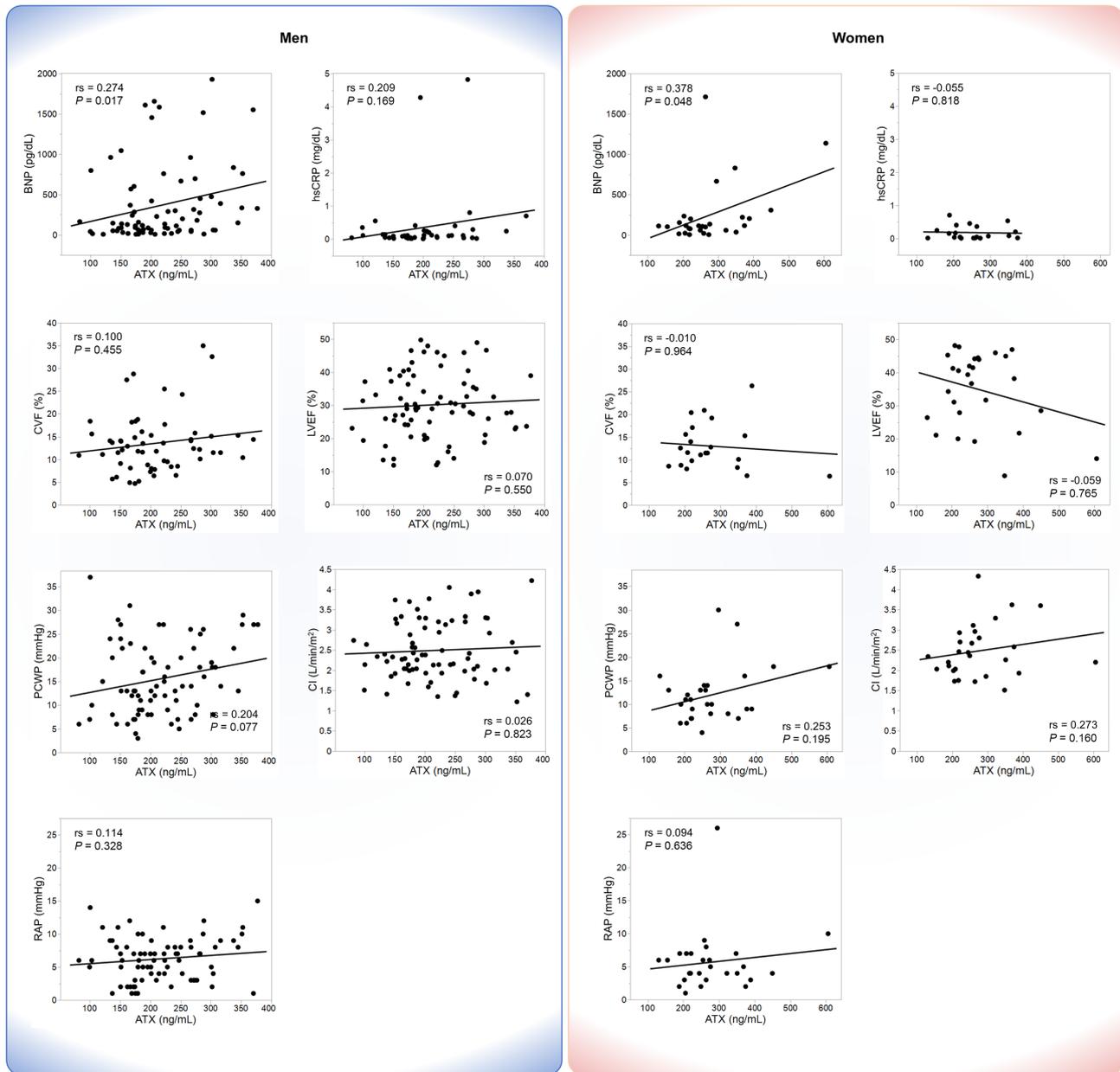
NIDCM. The main findings of this study were as follows: (i) The composite cardiac event-free rate was significantly lower in the high-ATX group than in the low-ATX group. In addition, both the rate of cardiac death and the rate of hospitalization for HF were significantly higher in the high-ATX group. (ii) High-ATX levels are an independent determinant of future cardiac events. (3) In our study, serum ATX levels correlated with BNP levels, but not with hsCRP and CVF, in both sexes.

Our results indicate that serum ATX level may be a useful prognostic determinant in patients with NIDCM. We found

no significant differences in age, medications used, comorbidities, haemoglobin level, liver enzyme levels, renal function, and haemodynamic parameters between the two groups (excluding PCWP). Nevertheless, the cardiac event rate was higher in the high-ATX group. hsCRP levels have been reported as independent prognostic determinants in NIDCM and generally increase as HF progresses.[3,23–25] Myocardial fibrosis is also a prognostic determinant.[5,6] However, hsCRP and CVF were not significant determinants in our results. This might be because most patients had mild HF symptoms with

NYHA functional Class I or II in our settings. We believe that serum ATX levels may be more useful for risk stratification than hsCRP levels or myocardial fibrosis in patients with NIDCM who had symptoms of mild HF.

Although gender differences in NIDCM and its mechanisms have not been fully investigated and remain largely unestablished, Cannatà *et al*. reported that women with NIDCM have a better prognosis, despite receiving the same treatment as men and having more poor prognostic factors such as age, left ventricular dilation, and LBBB.[26] Our study showed that women had higher ATX levels despite lower LVEF and higher PCWP (*Supporting information Table S1*). In addition, there was no difference in composite events between men and women. Differences in ATX levels between men and women have been reported. We believe that it necessary to use different cutoffs when considering ATX levels as a prognostic predictor for NIDCM.

In this study, we showed a significant correlation between serum ATX levels and plasma BNP levels. Our results were consistent with those of another study.[13] We also found a significant correlation in both sexes. However, serum ATX levels did not correlate with haemodynamic parameters, such as PCWP, CI, and right atrial pressure. Nevertheless, care should be taken when interpreting these results. Our setting had a large number of patients with NIDCM with mild HF symptoms; therefore, the results may be different in patients with more severe symptoms.

The ATX induces inflammation through LPA, which is involved in maintaining vascular endothelial function, increasing vascular permeability, and vascular tone.[8,14,27] In addition, ATX promotes inflammation and fibrosis, resulting in organ remodelling. Furthermore, elevated serum ATX levels were observed in patients with sepsis, and a good correlation was observed between serum ATX and angiopoetin-2 levels ($P < 0.001$, Spearman's rank: 0.518). Angiopoietin-2 has been found to be elevated by inflammation and in patients with HF. In addition, it has been reported to be associated with vascular endothelial dysfunction and is a prognostic factor in patients with cardiogenic shock.[15,28,29] The relationship between vascular endothelial function and HF has also been reported.[23,30] Elevated ATX might also increase the levels of other inflammatory substances, which might have affected the prognosis.

The ATX is reported to be mainly produced in the adipose tissue; however, studies have not established the direct or indirect effects of adipose tissue-derived ATX on cardiac function. We showed that circulating ATX may be associated with poor prognosis in NIDCM, although the contribution of each organ to ATX synthesis in patients with advanced HF is unknown. However, in mice with cardiac dysfunction due to a high-fat diet, cardiac ATX mRNA levels reportedly did not increase; however, visceral fat ATX mRNA levels and circulating ATX levels increased.[31] Furthermore, HF induces the production of cytokines, such as IL-6 or TNFα, which cause chronic systemic organ inflammation, including that of the adipose tissue.[2] Therefore, as HF progresses, inflammation might be induced in the adipose tissue and ATX synthesis might be promoted, resulting in an increase in circulating ATX, which can further adversely affect the heart, thereby affecting cardiac remodelling and resulting in poor prognosis. We believe that this may be a new target for improving cardiac outcomes in patients with NIDCM.

In mice, an ATX inhibitor (PF8380) was reported to markedly decrease ATX activity and serum LPA, IL6, and TNFα levels. Furthermore, the inhibitors suppressed increase in scar size, structural remodelling, and cardiac dysfunction.[14] LPA3 might be involved in myocardial hypertrophy and injury in rats,[32] and a deficiency of lipid phosphate phosphatase 3 (LPP3), which decomposes LPA3, might cause myocardial remodelling in mice.[33] In addition, suppression of LPA3 reportedly attenuated myocardial fibrosis,[34] and LPP3 knockout mice showed higher systemic and cardiac inflammation after AMI, decreased recovery of cardiac function, and increased remodelling and scar size.[14]

In humans, an increase in plasma ATX and plasma LPA levels was observed after AMI, although the effect of ATX inhibitors on heart disease remains unclear. By contrast, ATX inhibitors have been investigated as new targets of treatment for IPF. In humans, ATX inhibitors have shown good results in Phase II trials for IPF, and phase III trials are ongoing.[35,36] Furthermore, the ATX/LPA axis is attracting attention as a new therapeutic target for muscular dystrophy, which causes cardiac dysfunction with inflammation and fibrosis.[37] Thus, future studies may prove that the ATX/LPA axis is closely associated with human cardiac function and circulatory disorders, leading to a novel therapeutic target for NIDCM.

This study had a few limitations. First, this study was a single-centre study with a small sample size. Second, almost all patients were classified as NYHA functional Class I or II. It is unclear whether these results can be generalized to more advanced patients. However, we believe that it is significant to clarify that prognostic stratification by serum ATX levels is available even in asymptomatic or mildly symptomatic patients. Third, we did not evaluate the time-course of serum ATX levels, which may change over time. Further investigations with a larger cohort of patients and observing changes in serum ATX levels over time are needed to establish the importance of the ATX/LPA axis in patients with NIDCM. Fourth, we performed some invasive investigations to diagnose NIDCM. Not all NIDCM patients who visited our hospital participated in this study. Thus, it is difficult to eliminate selection bias completely.

In conclusion, high serum ATX levels are associated with increasing adverse clinical outcomes in patients with NIDCM. Serum ATX may become a novel biomarker for risk stratification or a target for improving patient outcome in NIDCM.

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Conflict of interest

Funding

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Baseline characteristics and cardiac events by gender.

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