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NT-proBNP levels measured in health checkups during the preclinical stages of cardiac structural and functional abnormalities

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Abstract

Objectives: Stage B heart failure is defined as an asymptomatic abnormality of the heart structure or function. The circulating level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is elevated in symptomatic patients with left ventricular (LV) dysfunction caused by a structural or functional abnormality. This study investigated the association of the NT-proBNP level with echocardiography detected cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved LV systolic function (ejection fraction >50%).

Methods: We retrospectively studied 412 health examinees who underwent echocardiography and NT-proBNP test at a health-promotion center in Seoul, between January 2016 and December 2016. The left ventricular mass index, and left atrial dimension (LAD) were used as markers for structural abnormalities, and the mean e′ velocity and E/e′ ratio were used as markers for diastolic dysfunction. The plasma NT-proBNP level was measured using electrochemiluminescence immunoassay (DPC Immulite 2000 XPi, Siemens Healthcare Diagnostics, Tarrytown,

NY, USA).

Results: Subjects with preclinical structural abnormalities were older and had a higher body mass index (BMI), higher blood pressure, lower high-density lipoprotein cholesterol level, higher NT-proBNP level, and higher E/e' (P<0.05). Multivariate regression analysis indicated that the factors associated with a higher NT-proBNP level were being older, being female, and having a lower BMI, higher diastolic blood pressure, higher creatinine level, lower eGFR, and higher LAD (P<0.05).

Conclusion: The level of NT-proBNP was associated with preclinical cardiac structural abnormalities but not with diastolic dysfunction during asymptomatic health checkups.

Keywords: Heart failure, NT-proBNP, Echocardiography, Preclinical structural abnormalities, Diastolic dysfunction

Article Summary

The circulating level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is elevated in symptomatic patients with left ventricular (LV) dysfunction caused by a structural or functional abnormality. However, few studies have investigated the association between NT-proBNP levels and preclinical structural or functional heart abnormalities demonstrated by echocardiography performed as part of preventive screening programs in primary healthcare system. This study investigated the association of the NT-proBNP level with echocardiography-detected cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved left ventricular systolic function (LVEF >50%) during health checkups. In conclusion, the level of NT-proBNP was associated with preclinical cardiac structural abnormalities but not with diastolic dysfunction during asymptomatic health checkups.

Strengths and limitations of this study

- This is the first study to address the association of preclinical echocardiography-detected cardiac structural or diastolic abnormalities with

the NT-proBNP level in a primary healthcare setting.

- We suggest the optimal target subjects for screening stage B heart failure based on the NT-proBNP level in health checkups.
- The cross-sectional study design and the relatively small sample mean that further research is needed into the associated causal relationships.

INTRODUCTION

Heart failure (HF) is a clinical syndrome characterized by typical symptoms and signs caused by a structural and/or functional cardiac abnormalities that result in a reduced pumping capability of heart and its pre- and post-loading volumes, stasis of blood flow, and insufficiency of the blood and oxygen supplied to the main organs [1]. The incidence of cardiovascular diseases is increasing including in younger subjects due to changes in lifestyles and dietary patterns, and there have also been increases in the rates of progression to HF [2]. Systolic dysfunction is frequently present in community-dwelling individuals without recognized symptoms of HF [3, 4]. In addition, most subjects with diastolic dysfunction have a normal left ventricular ejection fraction (LVEF), with even moderate or severe isolated diastolic dysfunction being as common as systolic dysfunction [5–7]. Thus, the early recognition and treatment of preclinical structural or functional abnormalities represent a potentially powerful strategy for reducing the incidence of HF.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is produced within

myocytes and released into the circulation during increases in the ventricular and atrial pressures [8, 9]. The NT-proBNP level is therefore a useful diagnostic marker for cardiac insufficiency [10], ventricular dysfunction [11] and cardiomyopathy [12]. However, few studies have investigated the association between NT-proBNP levels and preclinical structural or functional heart abnormalities demonstrated by echocardiography performed as part of preventive screening programs in primary healthcare system.

This study investigated the association of the NT-proBNP level with echocardiography-detected cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved left ventricular systolic function (LVEF >50%) during health checkups.

METHODS

Subjects

We retrospectively studied 412 health examinees who underwent echocardiography and NT-proBNP test during health checkups at a health-promotion center in Seoul between January 2016 and December 2016. The self-reported personal medical history, subjective symptoms and signs, and life style information were obtained from all participants at time of health checkups. Their medical records were also reviewed. Participants who had valvular heart disease, acute myocardial infarction, stroke, renal dysfunction, LVEF < 50%, or clinical symptoms or signs of HF were excluded from this study.

This study was approved by the Institutional Review Board of Korea Association of Health Promotion (approval no. 130750-201807-HR-016).

Echocardiography

The echocardiographic investigations were carried out using a Philips/Hewlett-Packard Sono 5500 ultrasound device (Philips Ultrasound, Andover, MA, USA). M-

mode, two-dimensional, and hemodynamic Doppler images were acquired using a standardized protocol with a 3.5-MHZ transducer. LVEF was calculated using the modified Simpson method. An increased left ventricular mass index (LVMI; >115 g/m^2 in males and >95 g/m^2 in females), and an increased left atrial dimension (LAD; >41 mm in males and >39 mm in females) were used as markers for structural abnormalities [13]. The diastolic function was evaluated using the early diastolic mitral flow velocity (E), early relaxation velocity in tissue Doppler recordings (e') and the E/e' ratio. E/e' ≥13 and a mean e' septal and lateral wall velocity of <9 cm/s were used as markers for diastolic dysfunction [14].

NT-proBNP measurement

Venous blood was collected in an lithium-heparin tube. The plasma NT-proBNP level was measured using an electrochemiluminiscent immunoassay on a DPC Immulite 2000 XPi device (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The intra-assay and total variances were 3.4% and 4.7%, respectively, and the limit of detection was 10 pg/mL.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). Differences in the characteristics of the study subjects between with and without structural abnormalities and between with and without diastolic dysfunction were analyzed using Student's *t*-test or the chi-square test. Univariate and multivariate regression analyses were performed to determine the variables affecting an increased NT-proBNP level. Wilcoxon rank sum tests were performed to compare mean NT-proBNP levels between sexes, and between with and without structural abnormalities in each age groups, respectively. A *P* value of <0.05 was considered statistically significant.

Patient and public involvement

Patients were not involved in the recruitment to and conduct of the study. Results will be disseminated to study participants through annual information events.

RESULTS

This study enrolled 412 participants with a median age of 58 years (range 28–82 years). Echocardiography revealed structural abnormalities in 60 (14.6%) of the subjects, who were older (60.3±8.5 vs 57.2±9.3 years [mean±SD], *P*<0.017), and had a higher body mass index (BMI), higher blood pressure, lower high-density lipoprotein cholesterol (HDL-C) level. higher NT-proBNP level (65.6±109.7 vs 29.8±28.7 pg/mL, *P*=0.015), and higher E/e′ ratio (12.5±2.9 vs 11.1±3.3) in echocardiography (Table 1).

Diastolic dysfunction was detected using echocardiography in 283 (68.7%) of the subjects. These subjects were older and had a higher BMI, higher blood pressure, higher hemoglobin, higher triglycerides, and lower HDL-C levels. However, the NT-proBNP level did not differ significantly between subjects with and without diastolic dysfunction (Table 2).

In a multivariate model, being older, being female, and having a lower BMI, higher diastolic blood pressure, higher creatinine level, higher eGFR level and

higher LAD were associated with an increased NT-proBNP level (P<0.05). While sex was not associated with the NT-proBNP level in a univariate model, being female was associated with increased NT-proBNP level (P<0.01) in multivariate model (Table 3). Among subjects younger than 61 years (combining those aged ≤45 and 46–60 years), females showed higher NT-proBNP levels than males, while there was no significant difference between males and females among those aged ≥61 years (Figure 1). A structural abnormality defined by a higher LAD was associated with an increased NT-proBNP level (P<0.01) but the E/e' ratio and mean e' velosity were not associated with NT-proBNP. In subjects aged 46–60, and ≥61 years, those with structural abnormality showed a higher NT-proBNP level (P=0.014 and P=0.001, respectively) (Figure 2).

DISCUSSION

This study investigated the association between NT-proBNP levels with echocardiography-detected cardiac structural abnormalities and diastolic dysfunction in asymptomatic subjects with preserved left ventricular systolic function in a primary healthcare setting. Subjects with structural abnormalities that were not yet apparent, were older and had a higher BMI, higher blood pressure, lower HDL-C level, or greater impairment of diastolic function in echocardiography. Furthermore, we have demonstrated that NT-proBNP levels are associated with preclinical cardiac structural abnormalities but not with diastolic dysfunction.

We observed associations of diastolic dysfunction with cardiovascular risk factors such as higher BMI, blood pressure, blood glucose, and triglycerides, and lower HDL-C levels. These results are consistent with reports of the presence of diastolic dysfunction being closely associated with cardiovasular diseases [15–17]]. Furthermore, subjects with diastolic dysfunction exhibited elevated LVMI and LAD,

which indicate the presence of structural abnormalities. These findings support that a hypertrophied ventricle is more likely to exhibit diastolic dysfunction and a chronic increase in the left atrial pressure associated with diastolic dysfunction, and would be expected to lead to atrial enlargement [18].

NT-proBNP is released into the circulation in response to a stretched myocardium resulting from any cardiac structural abnormalities [19]. Hung et al. [20] demonstrated an association between any structural anomaly and NT-proBNP levels, which was consistent with our finding of the NT-proBNP level being associated with subclinical cardiac structural abnormalities. This means that an elevated circulating level of NT-proBNP is a predictor of systolic dysfunction. However, we found no association between preclinical diastolic dysfunction (PDD) and the circulating NT-proBNP level. These differences might be due to differences between the included subjects: our study subjects participated in routine health checkups and preexcluded any valvular heart disease or pulmonary hypertension, while the subjects included in the previous study had aortic root dilatation, ventricular hypertrophy, pulmonary hypertension or valvular heart

disease and presented at a tertiary referal center. Another previous study of PDD [21] found that while advanced diastolic dysfunction with a normal LVEF was independently associated with structural abnormalities (increased LVMI and left atrial volume index [LAVI]) and increased circulating NT-proBNP, the structural abnormalities and NT-proBNP levels did not differ between normal controls and patients with mild diastolic dysfunction.

Among subjects with a normal LVEF and no HF diagnosis, a higher severity of diastolic dysfunction was associated with a higher mean LVMI and LAVI [18]. Our subjects with diastolic dysfunction had relatively low mean LVMI or LAD values, and they are therefore likely to have had only mild diastolic dysfunction. Our observations suggest that the association between PDD and circulating NT-proBNP levels may vary according to the severity of diastolic dysfunction. Therefore, the screening of preclinicl structural or functional abnormalities based on NT-proBNP levels could be optimized by targeting subjects with potentially advanced diastolic dysfunction, which encompasses those who are older and have cardiovascular risk factors such as obesity, hypertension, higher blood glucose or

dyslipidemia in health checkups.

Our study have some limitations. Its cross-sectional design and relatively small sample mean that further research is needed into the associated causal relationships. Data on potential clinical correlates of diastolic dysfunction such as exercise tolerance were not included. Moreover, the echocardiopgraphic evaluation was performed using a minimal data set that did not include detailed echocardiographic data on diastolic function.

In conclusion, the findings of this study suggest that NT-proBNP levels are associated with preclinical cardiac structural abnormalities but not with diastolic dysfunction, which is applicable to stage-B HF from among the four categories defined by the American Ccollege of Cardiology and American Heart Association.

Figure 1. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 2. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

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Author Contributions All of the authors participated in designing this study. SC and SYK performed data collection. SK undertook the statistical analyses. EN, SYK, HC and SK analysed and interpreted the data. EN wrote the first draft of the manuscript, which was reviewed by all of the other authors, who also provided further contributions and suggestions.

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Competing interests None to declare.

Patient consent Obtained.

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Data sharing statement No additional data are available.

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Table 1 Characteristics of study subjects according to the presence of structural abnormalities

Variable	No structural abnormalities (N=352)	Structural abnormalities (N=60)	*P*
Age years	57.2±9.3	60.3±8.5	0.017
Sex, male, N (%)	192 (54.5)	36 (60.0)	0.432
BMI (kg/m^2)	24.2±3.2	26.5±2.7	0.000
SBP (mmHg)	118.9±13.6	125.9±11.1	0.000
DBP (mmHg)	72.3±8.8	76.4±8.7	0.003
Hemoglobin (g/L)	147±15	148±18	0.965
FBS (mmol/L)	5.96±1.43	6.17±1.60	0.289
TC (mmol/L)	5.45±1.09	5.21±0.97	0.110
TG (mmol/L)	1.33±0.93	1.59±1.19	0.125
HDL-C (mmol/L)	1.51±0.40	1.35±0.29	0.000
LDL-C (mmol/L)	3.33±1.03	3.16±1.00	0.236
Creatinine (μmol/L)	76.02±13.97	77.79±15.56	0.520
eGFR (mL/min/1.73m^2)	87.5±13.6	86.6±14.2	0.639
NT-proBNP (pg/mL)	29.8±28.7	65.6±109.7	0.015
LVEF (%)	66.9±6.4	67.1±6.0	0.807
LVMI (g/m^2)	72.2±13.9	89.5±15.4	0.000
LAD (mm)	33.8±3.8	40.9±6.0	0.000
E/e′	11.1±3.3	12.5±2.9	0.002
e′ (cm/s)	6.4±1.6	6.4±6.6	0.950

Continuous variables are expressed as mean±SD values and were compared using Student's *t*-test. Ccategorical variables are expressed as N (%) values and were analyzed using the chi-square test. NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 2. Characteristics of study subjects according to the presence of diastolic dysfunction

Variables	No diastolic dysfunction (N=129)	Diastolic dysfunction (N=283)	*P*
Age (year)	53.1±9.4	59.7±8.4	0.000
Sex, male, N (%)	68 (52.7)	160 (56.5)	0.469
BMI (kg/m^2)	23.3±3.1	25.1±3.1	0.000
SBP (mmHg)	115.6±12.2	121.9±13.6	0.000
DBP (mmHg)	70.4±8.1	74.6±8.9	0.000
Hemoglobin (g/L)	144±17	149±15	0.002
FBS (mmol/L)	5.68±1.12	6.13±1.57	0.001
TC (mmol/L)	5.35±1.06	5.44±1.08	0.452
TG (mmol/L)	1.18±0.83	1.46±1.03	0.004
HDL-C (mmol/L)	1.56±0.40	1.45±0.38	0.007
LDL-C (mmol/L)	3.25±0.98	3.33±1.05	0.499
Creatinine (μmol/L)	76.02±14.85	76.91±13.88	0.544
eGFR (mL/min/1.73m^2)	89.2±14.7	86.5±13.1	0.063
NT-proBNP (pg/mL)	38.8±73.6	33.3±36.1	0.306
LVEF (%)	66.1±6.6	67.3±6.2	0.081
LVMI (g/m^2)	70.2±14.7	76.8±15.3	0.000
LAD (mm)	34.1±5.3	35.2±4.7	0.040
E/e'	9.4±2.2	12.1±3.3	0.000
e' (cm/s)	8.5±4.3	5.4±1.0	0.000

Continuous variables are expressed as mean±SD values and were compared using Student's *t*-test. Ccategorical variables are expressed as N (%) values and were analyzed using the chi-square test. NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 3. Regression analysis of the variables affecting an increased NT-proBNP level

Variables	Univariate model	Multivariate model
Age (year)	0.202**	0.303**
Sex, male	-0.043	0.980**
BMI (kg/m^2)	-0.009	-0.154**
SBP (mmHg)	0.026	-0.144*
DBP (mmHg)	0.066	0.167*
Hb (g/L)	0.001	-0.043
FBS (mmol/L)	-0.080	-0.048
TC (mmol/L)	-0.111*	0.789
TG (mmol/L)	-0.056	-0.369
HDL-C (mmol/L)	-0.046	-0.310
LDL-C (mmol/L)	-0.075	-0.825
Creatinine (μmol/L)	0.219**	1.411**
eGFR (mL/min/1.73m^2)	-0.240**	0.814**
LVEF (%)	-0.002	0.012
LVMI (g/m^2)	0.167**	0.086
LAD (mm)	0.298**	0.341**
E/e'	0.017	-0.039
e' (cm/s)	-0.021	0.015

Model significance; * P<0.05, ** P<0.01

NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

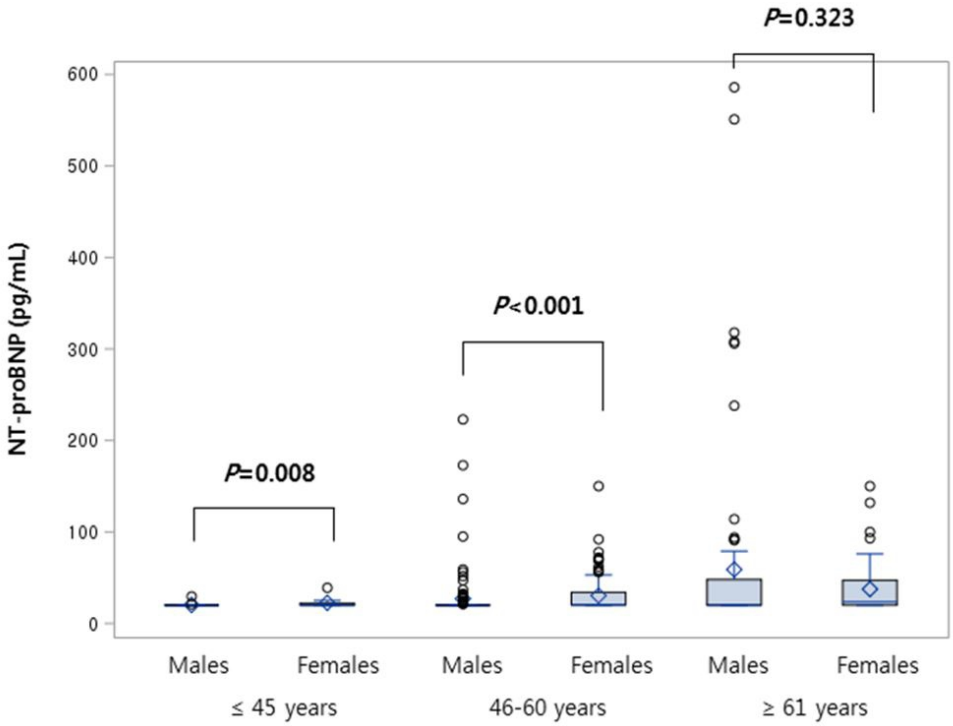

Figure 1 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

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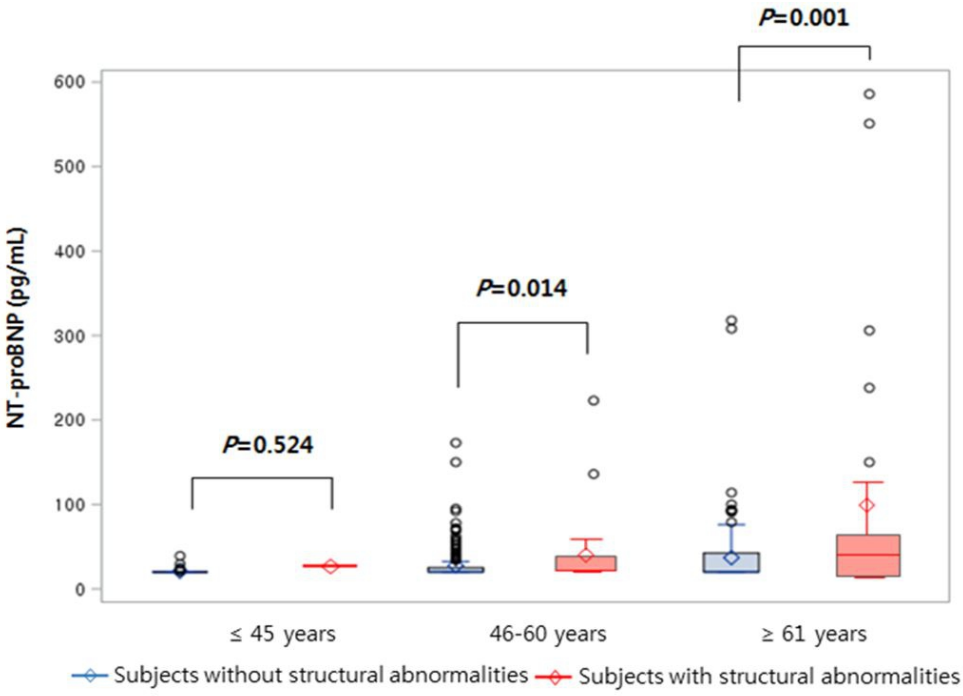

Figure 2 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 2 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

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NT-proBNP levels measured in health checkups with preserved ejection fraction during the preclinical stages of cardiac structural and functional abnormalities: a retrospective and cross-sectional study

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NT-proBNP levels measured in health checkups with preserved ejection fraction during the preclinical stages of cardiac structural and functional abnormalities: a retrospective and cross-sectional study

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ABSTRACT

Objectives: Stage B heart failure is defined as an asymptomatic abnormality of the heart structure or function. The circulating level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is elevated in symptomatic patients with left ventricular (LV) dysfunction caused by a structural or functional abnormality. This study investigated the association of the NT-proBNP level with echocardiography detected cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved LV systolic function (ejection fraction >50%).

Methods: We retrospectively studied 652 health examinees who underwent echocardiography and NT-proBNP test at a health-promotion center in Seoul, between January 2016 and September 2018. The left ventricular mass index (LVMI), and left atrial dimension (LAD) were used as markers for structural abnormalities, and the mean e' velocity and E/e' ratio were used as markers for diastolic dysfunction. The plasma NT-proBNP level was measured using electrochemiluminescence immunoassay (DPC Immulite 2000 XPi, Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Results: Subjects with preclinical structural abnormalities were older and had a higher body mass index (BMI), higher blood pressure, lower high-density lipoprotein cholesterol level, higher NT-proBNP level, and higher E/e' (P<0.05). Multivariate regression analysis indicated that the factors associated with a higher NT-proBNP level were being older, being female, and having a lower BMI, higher creatinine level, higher LVMI and higher LAD (P<0.01).

Conclusion: The level of NT-proBNP was associated with preclinical cardiac structural abnormalities but not with diastolic dysfunction during asymptomatic health checkups.

Keywords: Heart failure, NT-proBNP, Echocardiography, Preclinical structural abnormalities, Diastolic dysfunction

Strengths and limitations of this study

- This is the first study to address the association of early stage of preclinical echocardiography-detected cardiac structural or diastolic abnormalities with the NT-proBNP level in a primary healthcare setting.
- We suggest the optimal target subjects with potentially cardiac structural abnormality, which encompasses those who are older and have cardiovascular risk factors such as obesity, hypertension, or dyslipidemia for screening early stage B heart failure based on the NT-proBNP level in health checkups.
- The cross-sectional study design and the relatively small sample mean that further research is needed into the associated causal relationships.

INTRODUCTION

Heart failure (HF) is a clinical syndrome characterized by typical symptoms and signs caused by a structural and/or functional cardiac abnormalities that result in a reduced pumping capability of heart and its pre- and post-loading volumes, stasis of blood flow, and insufficiency of the blood and oxygen supplied to the main organs [1]. The incidence of cardiovascular diseases is increasing including in younger subjects due to changes in lifestyles and dietary patterns, and there have also been increases in the rates of progression to HF [2]. Systolic dysfunction is frequently present in community-dwelling individuals without recognized symptoms of HF [3, 4]. In addition, most subjects with diastolic dysfunction have a normal left ventricular ejection fraction (LVEF), with even moderate or severe isolated diastolic dysfunction being as common as systolic dysfunction [5–7]. Thus, the early recognition and treatment of preclinical structural or functional abnormalities represent a potentially powerful strategy for reducing the incidence of HF.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is produced within myocytes and released into the circulation during increases in the ventricular and atrial pressures [8, 9]. The NT-proBNP level is therefore a useful diagnostic marker for cardiac insufficiency [10], ventricular dysfunction [11] and cardiomyopathy [12]. However, few studies have investigated the association between NT-proBNP levels and early stage of preclinical structural or functional heart abnormalities demonstrated by echocardiography performed as part of preventive screening programs in primary healthcare system.

This study investigated the association of the NT-proBNP level with echocardiography-detected early stage of cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved left ventricular systolic function (LVEF >50%) during health checkups.

METHODS

Subjects

We retrospectively studied consecutive health examinees aged over 18-year old who underwent echocardiography and NT-proBNP test during health checkups at a health-promotion center in Seoul between January 2016 and September 2018. The study design was retrospective and cross-sectional. The self-reported personal medical history, subjective symptoms and signs, and life style information were obtained from all participants at time of health checkups. Their medical records were also reviewed. Our study subjects had no previous cardiac surgery or diagnosed heart disease and they presented with preserved left ventricular systolic function (LVEF>50%) determined by echocardiography. Subjects who had echocardiography-detected valvular heart disease, atrial fibrillation, acute myocardial infarction, stroke, renal dysfunction, pregnancy, echocardiography-detected LVEF < 50%, or clinical symptoms or signs of HF were excluded from this study. After exclusion, the final sample size was 652 (361 males and 291 females: aged 28–82)

This study was approved by the Institutional Review Board of Korea Association of Health Promotion (approval no. 130750-201807-HR-016).

Echocardiography

The echocardiographic investigations were carried out using a Philips/Hewlett-Packard Sono 5500 ultrasound device (Philips Ultrasound, Andover, MA, USA). M-mode, two-dimensional, and hemodynamic Doppler images were acquired using a standardized protocol with a 3.5-MHZ transducer. LVEF was calculated using the modified Simpson method [13]. An increased left ventricular mass index (LVMI; >115 g/m^2 in males and >95 g/m^2 in females), and an

increased left atrial dimension (LAD; >41 mm in males and >39 mm in females) were used as markers for structural abnormalities [14]. The diastolic function was evaluated using the early diastolic mitral flow velocity (E), early relaxation velocity in tissue Doppler recordings (e') and the E/e' ratio. E/e' ≥13, and a mean e' septal and lateral wall velocity of <9 cm/s were used as markers for diastolic dysfunction [15]. Structural cardiac abnormality was defined as the increased LVMI (LVMI; >115 g/m^2 in males and >95 g/m^2 in females) and/or increased LAD (LAD; >41 mm in males and >39 mm in females). Cardiac diastolic dysfunction was defined as an E/e' ≥13 and/or a mean e' septal and lateral wall <9 cm/s.

NT-proBNP measurement

Venous blood was collected in an lithium-heparin tube. The plasma NT-proBNP level was measured using an electrochemiluminescent immunoassay on a DPC Immulite 2000 XPi device (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The intra-assay and total variances were 3.4% and 4.7%, respectively, and the limit of detection was 10 pg/mL.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). Differences in the characteristics of the study subjects were analyzed according to the presence of structural abnormalities using Student's *t*-test or the chi-square test. In addition, differences in the characteristics of the study subjects were analyzed according to the presence of diastolic dysfunction using Student's *t*-test or the chi-square test. Among those with one of structural abnormality or diastolic dysfunction or both abnormality, differences in the characteristics of the study subjects were analyzed using ANOVA. Univariate (crude) and multivariate (adjusted) regression analyses were performed to determine the variables affecting an increased NT-proBNP level. Variables considered in the analysis included age, sex, body mass index (BMI),

systolic blood pressure, diastolic blood pressure, hemoglobin, fasting blood glucose, triglyceride, HDL-cholesterol, LDL-cholesterol, cretinine, LVEF, LVMI, LAD, E/e' and e'. We used multiple linear regression model to control for effects of included age, sex, BMI, blood pressure, Hb, FBS, blood lipid, creatinine, LVEF, LVMI, LAD, E/e' and e' (Model 1). Additional regression model adjusted for age, sex, BMI, creatinine, LVMI, and LAD was used to identify increased NT-proBNP level in multivariate model (Model 2). Wilcoxon rank sum tests were performed to compare mean NT-proBNP levels between sexes, and between with and without structural abnormalities in each age groups, respectively. In addition, Wilcoxon rank sum test was used to compare those with diastolic dysfunction to those without diastolic dysfunction. Logarithmic transformations were applied to NT-proBNP level. Area under the receiver operating curve (AUROC) was calculated to measure the performance of NT-proBNP in predicting the cardiac structural abnormalities. A *P* value of <0.05 was considered statistically significant.

Patient and public involvement

Patients were not involved in the recruitment to and conduct of the study. Results will be disseminated to study participants through annual information events.

RESULTS

There were a total of 652 study subjects with a median age of 58 years (range 28–82 years). Echocardiography revealed structural abnormalities in 92 (14.1%) of the subjects, who were older (60.8±8.8 vs 57.7±8.9 years [mean±SD], $P<0.017$), and had a higher body mass index (BMI), higher blood pressure, lower high-density lipoprotein cholesterol (HDL-C) level. higher NT-proBNP level (55.8±91.5 vs 29.6±27.5 pg/mL, $P=0.015$), and higher E/e' ratio (13.0±3.5 vs 11.0±3.4) in echocardiography (Table 1).

Diastolic dysfunction was detected using echocardiography in 470 (72.0%) of the subjects. These subjects were older and had a higher BMI, higher blood pressure, higher blood glucose, higher triglycerides, and lower HDL-C levels. However, the NT-proBNP level did not differ significantly between subjects with and without diastolic dysfunction (Table 2). Although subjects with both structural abnormality and diastolic dysfunction were older, higher LVMI, higher E/e' compared to one of structural abnormality or diastolic dysfunction, NT-proBNP was higher in those with structural abnormality only (data not shown).

In a multivariate model, being older, being female, and having a lower BMI, higher creatinine level, higher LVMI, and higher LAD were associated with an increased NT-proBNP level ($P<0.01$). While sex was not associated with the NT-proBNP level in a univariate model, being female was associated with increased NT-proBNP level ($P=0.002$) in multivariate model (Table 3). Among subjects in all age ranges (aged ≤45, 46–60 years, and aged ≥61 years), females showed higher NT-proBNP levels than males (Figure 1). A structural abnormality defined by higher LVMI and/or higher LAD was associated with an increased NT-proBNP level ($P<0.01$) but the E/e' ratio and mean e' velosity were not associated with NT-proBNP. In subjects aged 46–60, and ≥61 years, those with structural abnormality showed a higher NT-

proBNP level (P=0.002 and P=0.004, respectively) (Figure 2).

Figure 3 shows the receiver operating curve for assessing the performance of NT-proBNP in predicting cardiac structural abnormalities. AUROC was 0.625 (95% CI: 0.566–0.684). The cutoff for NT-proBNP at 21.0 pg/mL had sensitivity of 51.1% (95% CI: 40.9%–61.3%), specificity of 69.3% (95% cI: 65.5%–73.1%), positive predictive value of 21.5% (95% CI: 16.0–26.9%) and negative predictive value (NPV) of 89.6% (95% CI: 86.7%–92.5%) in predicting cardiac structural abnormalities.

DISCUSSION

This study investigated the association between NT-proBNP levels with echocardiography-detected early stage of cardiac structural abnormalities and diastolic dysfunction in asymptomatic subjects with preserved left ventricular systolic function in a primary healthcare setting. Subjects with structural abnormalities that were not yet apparent, were older and had a higher BMI, higher blood pressure, lower HDL-C level, or greater impairment of diastolic function in echocardiography. Furthermore, we have demonstrated that NT-proBNP levels are associated with preclinical cardiac structural abnormalities but not with diastolic dysfunction.

We observed associations of diastolic dysfunction with cardiovascular risk factors such as higher BMI, blood pressure, blood glucose, and triglycerides, and lower HDL-C levels. These results are consistent with reports of the presence of diastolic dysfunction being closely associated with cardiovascular diseases [16–18]. Furthermore, subjects with diastolic dysfunction exhibited elevated LVMI and LAD, which indicate the presence of structural abnormalities. These findings support that a hypertrophied ventricle is more likely to exhibit diastolic dysfunction and a chronic increase in the left atrial pressure associated with diastolic dysfunction, and would be expected to lead to atrial enlargement [19].

NT-proBNP is released into the circulation in response to a stretched myocardium resulting from any cardiac structural abnormalities [20]. Hung et al. [21] demonstrated an association between any structural anomaly and NT-proBNP levels, which was consistent with our finding of the NT-proBNP level being associated with subclinical cardiac structural abnormalities. Our AUROC suggest a threshold level of 21.0 pg/mL to exclude early stage of subclinical structural abnormalities in health checkups. Compared to previously recommended cutoff value of 32.8 pg/mL [21], our newly proposed cutoff value is much lower with fairly acceptable NPV.

However, we found no association between preclinical diastolic dysfunction (PDD) and the circulating NT-proBNP level. These differences might be due to differences between the included subjects: our study subjects participated in routine health checkups and pre-excluded any echocardiography-detected valvular heart disease or pulmonary hypertension, while the subjects included in the previous study had aortic root dilatation, ventricular hypertrophy, pulmonary hypertension or valvular heart disease and presented at a tertiary referal center. Another previous study of PDD [22] found that while advanced diastolic dysfunction with a normal LVEF was independently associated with structural abnormalities (increased LVMI and left atrial volume index [LAVI]) and increased circulating NT-proBNP, the structural abnormalities and NT-proBNP levels did not differ between normal controls and patients with mild diastolic dysfunction.

Among subjects with a normal LVEF and no HF diagnosis, a higher severity of diastolic dysfunction was associated with a higher mean LVMI and LAVI [19]. Our subjects with diastolic dysfunction had relatively low mean LVMI or LAD values, and they are therefore likely to have had only mild diastolic dysfunction. Our observations suggest that the association between PDD and circulating NT-proBNP levels may vary according to the severity of diastolic dysfunction. Therefore, the screening of preclinical structural or functional abnormalities based on NT-proBNP levels could be optimized by targeting subjects with potentially advanced diastolic dysfunction, which encompasses those who are older and have cardiovascular risk factors such as obesity, hypertension, higher blood glucose or dyslipidemia in health checkups.

Our study has some limitations. Its cross-sectional design and relatively small sample mean that further research is needed into the associated causal relationships. Nevertheless, power calculation using G* power 3.1 with effect size f^2 of 0.2 (recommended by Cohen) showed the

efficiency of sample size as the power of 0.85, which seems to be available in drawing conclusions of this study. Data on potential clinical correlates of diastolic dysfunction such as exercise tolerance were not included. Moreover, the echocardiographic evaluation was performed using a minimal data set that did not include detailed echocardiographic data on diastolic function.

In conclusion, the findings of this study suggest that NT-proBNP levels are associated with preclinical cardiac structural abnormalities but not with diastolic dysfunction, which is applicable to very early stage-B HF from among the four categories defined by the American College of Cardiology/American Heart Association [23].

Figure 1. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 2. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 3. Receiver operating characteristic curve and cutoff value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting cardiac structural abnormalities.

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performed data collection. SK undertook the statistical analyses. EN, SYK, HC and SK analysed and interpreted the data. EN wrote the first draft of the manuscript, which was reviewed by all of the other authors, who also provided further contributions and suggestions.

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Patient consent Obtained.

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Open access

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Table 1 Characteristics of study subjects according to the presence of structural abnormalities

Variables	No Structural abnormality (N=560)	Structural abnormality (N=92)	P Value
Age (year)	57.7 ± 8.9	60.8 ± 8.8	0.017
Gender Male, N (%)	309 (55.2)	52 (56.5)	0.432
BMI (kg/m2)	24.2 ± 3.0	26.9 ± 3.5	<0.001
SBP (mmHg)	119.8 ± 13.7	126.6 ± 12.1	<0.001
DBP (mmHg)	73.7 ± 8.9	76.9 ± 8.6	0.003
Hb (g/L)	147.7 ± 14.6	146.3 ± 16.5	0.965
FBS (mmol/L)	5.9 ± 1.5	6.2 ± 1.5	0.289
TC (mmol/L)	5.4 ± 1.1	5.2 ± 1.0	0.110
TG (mmol/L)	1.4 ± 0.9	1.6 ± 1.1	0.125
HDL-C (mmol/L)	1.5 ± 0.4	1.4 ± 0.4	<0.001
LDL-C (mmol/L)	3.2 ± 1	3.1 ± 0.9	0.236
Creatinine (μmol/L)	78.5 ± 15.2	76.9 ± 15.7	0.520
eGFR (mL/min/1.73m2)	85.3 ± 14.7	87.3 ± 18.4	0.639
NT-proBNP (pg/mL)	29.6 ± 27.5	55.8 ± 91.5	0.015
LVEF (%)	66 ± 6.5	64.9 ± 7.3	0.807
LVMI (g/m2)	71.7 ± 13.3	88.9 ± 18.1	<0.001
LAD (mL/m2)	33.7 ± 3.9	40.8 ± 5.5	<0.001
E/e’	11 ± 3.4	13 ± 3.5	0.002
e’ (cm/s)	6.2 ± 1.6	5.9 ± 5.4	0.950

Continuous variables are expressed as mean±SD values and were compared using Student’s *t*-test.
Ccategorical variables are expressed as N (%) values and were analyzed using the chi-square test.
NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-

cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 2. Characteristics of study subjects according to the presence of diastolic dysfunction

Variables	No diastolic dysfunction (N=182)	Diastolic dysfunction (N=470)	*P* Value
Age (year)	52.6 ± 8.8	60.3 ± 8.1	<0.001
Gender Male, N (%)	93 (51.1)	268 (57.0)	0.172
BMI (kg/m2)	23.4 ± 3	25 ± 3.2	<0.001
SBP (mmHg)	116.2 ± 12.6	122.6 ± 13.7	<0.001
DBP (mmHg)	71.4 ± 8.6	75.3 ± 8.8	<0.001
Hb (g/L)	144 ± 15.2	148.9 ± 14.5	<0.001
FBS (mmol/L)	5.6 ± 1	6.1 ± 1.6	<0.001
TC (mmol/L)	5.4 ± 1	5.4 ± 1.1	0.958
TG (mmol/L)	1.2 ± 0.8	1.5 ± 1	<0.001
HDL-C (mmol/L)	1.6 ± 0.4	1.5 ± 0.4	<0.001
LDL-C (mmol/L)	3.2 ± 1	3.2 ± 1	0.996
Creatinine (μmol/L)	76.3 ± 15.5	79 ± 15.2	0.040
eGFR (mL/min/1.73m2)	88.9 ± 15.7	84.3 ± 15	0.001
NT-proBNP (pg/mL)	35.9 ± 63.5	32.3 ± 32.9	0.476
LVEF (%)	65.2 ± 6.9	66.1 ± 6.5	0.131
LVMI (g/m2)	69.5 ± 14.2	75.9 ± 15.3	<0.001
LAD (mL/m2)	33.9 ± 5.1	35 ± 4.7	0.007
E/e'	9.4 ± 2.1	12 ± 3.6	<0.001
e' (cm/s)	8.4 ± 3.6	5.3 ± 1	<0.001

Continuous variables are expressed as mean±SD values and were compared using Student's *t*-test. Ccategorical variables are expressed as N (%) values and were analyzed using the chi-square test. NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-

cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 3. Regression analysis of the variables affecting an increased NT-proBNP level

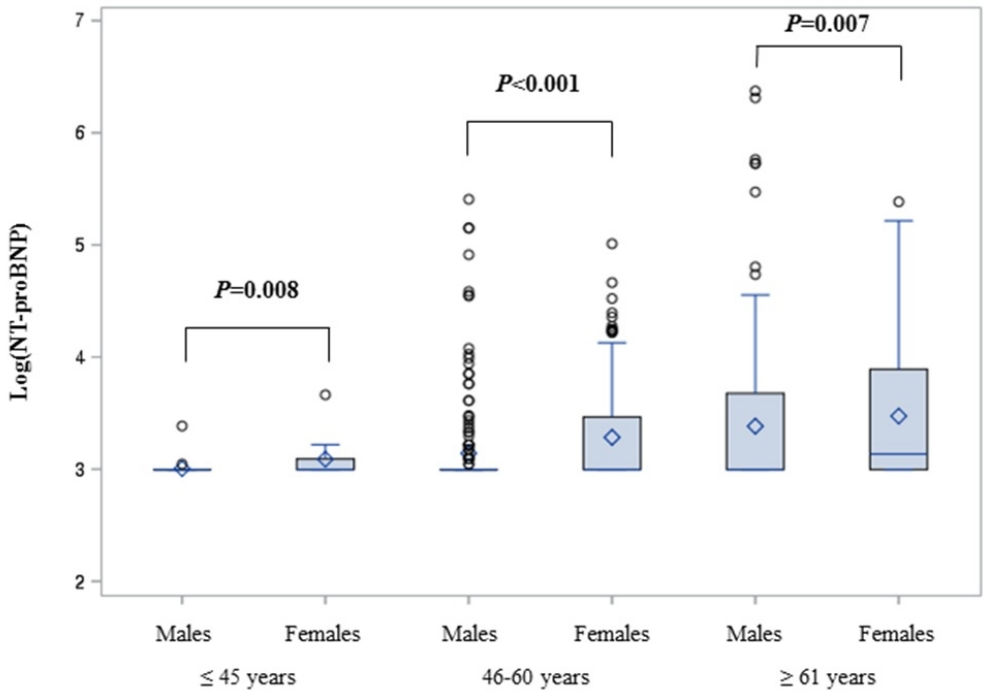
Variables	Univariate model				Multivariate model							
					Model 1				Model 2			
	Coeff.	95% CI	*P*-value	R^2	Coeff.*	95% CI	*P*-value	R^2	Coeff.	95% CI	*P*-value	R^2
Age (year)	0.97	(0.6–1.33)	<0.001	0.040	0.58	(0.15– 1.01)	0.008	0.153	0.51	(0.13– 0.89)	0.009	0.140
Sex (ref: female)	-0.52	(-7.27– 6.23)	0.879	<0.001	-17.31	(-28.12– -6.51)	0.002		-19.47	(-28.5– -10.45)	<0.001	
BMI (kg/m2)	-0.26	(-1.3– 0.78)	0.626	<0.001	-1.97	(-3.2 –-0.74)	0.002		-2.34	(-3.47– -1.21)	<0.001	
SBP (mmHg)	0.14	(-0.1–0.39)	0.258	0.002	-0.25	(-0.61– 0.1)	0.165					
DBP (mmHg)	0.27	(-0.11– 0.64)	0.164	0.003	0.48	(-0.06– 1.01)	0.081					
Hb (g/L)	-0.14	(-0.37– 0.08)	0.216	0.002	-0.15	(-0.45– 0.15)	0.338					
FBS (mmol/L)	-0.97	(-3.22– 1.28)	0.396	0.001	-0.44	(-2.71– 1.84)	0.707					
TG (mmol/L)	-2.34	(-5.81– 1.13)	0.186	0.003	-2.01	(-5.8– 1.78)	0.298					
HDL-C (mmol/L)	-1.72	(-10.2– 6.77)	0.691	<0.001	-0.76	(-9.82– 8.31)	0.870					
LDL-C (mmol/L)	-3.62	(-6.96– -0.27)	0.034	0.007	-2.95	(-6.2– 0.3)	0.075					
Creatinine (μmol/L)	0.36	(0.14– 0.58)	0.001	0.016	0.70	(0.4– 0.99)	<0.001		0.68	(0.4 –0.97)	<0.001	
LVEF (%)	-0.01	(-0.51– 0.49)	0.968	<0.001	0.12	(-0.37– 0.6)	0.637					
LVMI (g/m2)	0.47	(0.26– 0.69)	<0.001	0.028	0.28	(0.04– 0.52)	0.022		0.30	(0.07– 0.53)	0.012	
LAD (mL/m2)	2.11	(1.43– 2.79)	<0.001	0.054	2.58	(1.79– 3.37)	<0.001		2.63	(1.85– 3.41)	<0.001	
E/e’	0.65	(-0.33– 1.63)	0.191	0.003	-0.14	(-1.24– 0.97)	0.806					
e’ (cm/s)	-0.54	(-1.87– 0.8)	0.430	0.001	0.38	(-1.04– 1.81)	0.597					

*Regression coefficient: the usual interpretation of a regression coefficient is the average change in the outcome variable when the corresponding predictor variable is changed by one unit.

Model 1: adjust for age, sex, BMI, blood pressure, Hb, FBS, blood lipid creatinine

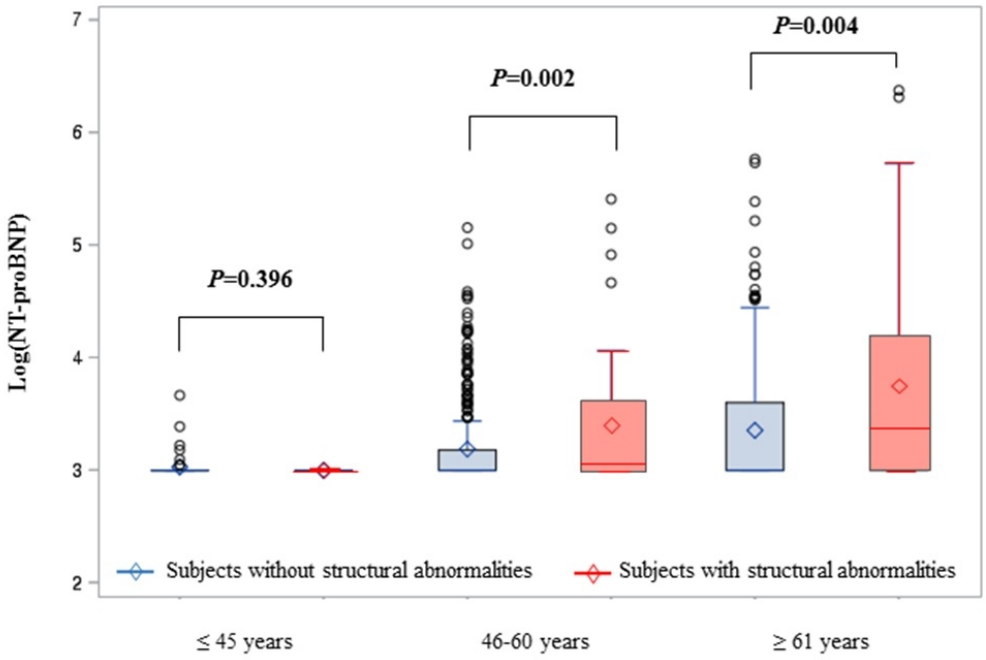
Model 2: adjusted for age, sex, BMI, creatinine, LVMI, and LAD

NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e’, early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e’, mean e’ septal and lateral wall velocit.



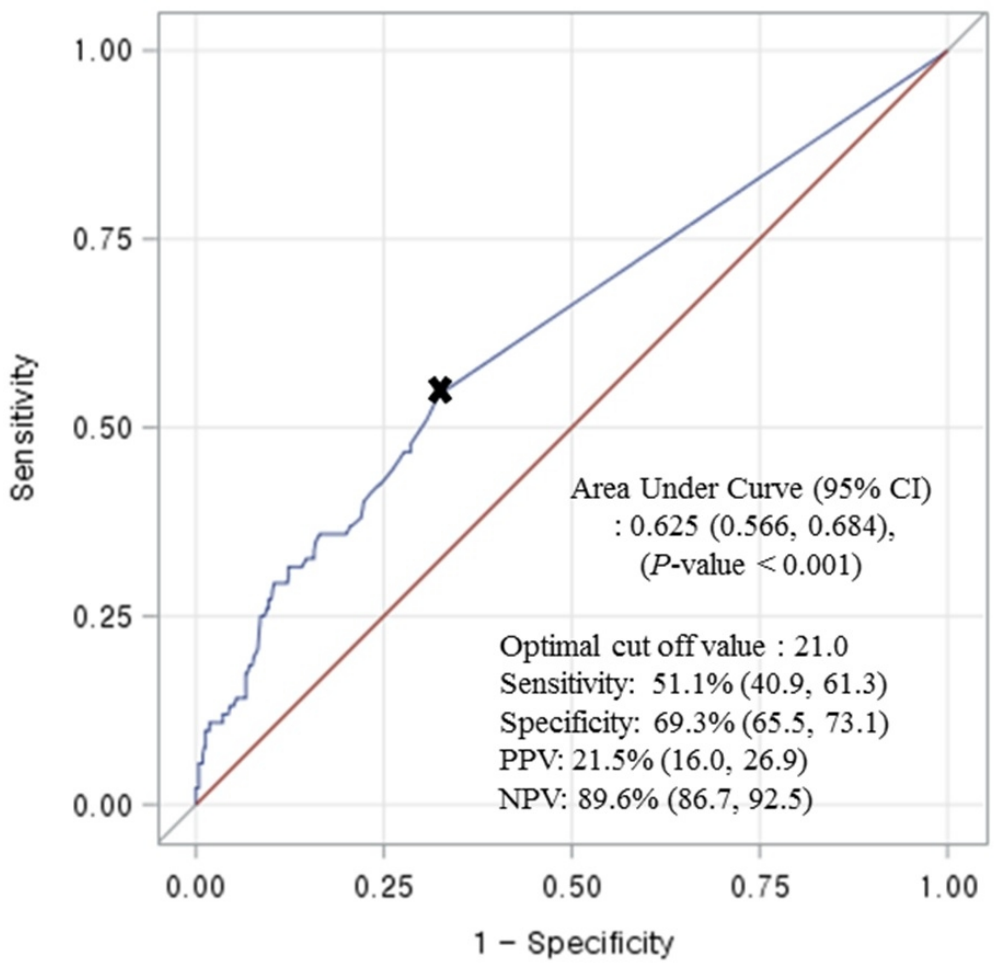
N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

256x180mm (96 x 96 DPI)



N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

256x171mm (96 x 96 DPI)



Receiver operating characteristic curve and cutoff value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting cardiac structural abnormalities.

241x233mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5,6*
Bias	9	Describe any efforts to address potential sources of bias	Not relevant
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	5,6
		(*c*) Explain how missing data were addressed	Not relevant
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Not relevant
		(*e*) Describe any sensitivity analyses	No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Not relevant
		(b) Give reasons for non-participation at each stage	Not relevant
		(c) Consider use of a flow diagram	Not relevant
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	Not relevant
Outcome data	15*	Report numbers of outcome events or summary measures	7,8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make	Not relevant

		clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	Not relevant
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not relevant
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not relevant
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Plasma NT-proBNP levels are associated with cardiac structural abnormalities, but not with diastolic dysfunction, in asymptomatic health promotion centre attendees with preserved ejection fraction: a retrospective cross-sectional study

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Title page

Plasma NT-proBNP levels are associated with cardiac structural abnormalities, but not with diastolic dysfunction, in asymptomatic health promotion centre attendees with preserved ejection fraction: a retrospective cross-sectional study

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Keywords: Heart failure, NT-proBNP, Echocardiography, Preclinical structural abnormalities, Diastolic dysfunction

Word counts: 2,061

ABSTRACT

Objectives: Stage B heart failure is defined as an asymptomatic abnormality of the heart structure or function. The circulating level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is elevated in symptomatic patients with left ventricular (LV) dysfunction caused by a structural or functional abnormality. This study investigated the association of the NT-proBNP level with echocardiography detected cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved LV systolic function (ejection fraction >50%).

Methods: We retrospectively studied 652 health examinees who underwent echocardiography and NT-proBNP test at a health-promotion center in Seoul, between January 2016 and September 2018. The left ventricular mass index (LVMI), and left atrial dimension (LAD) were used as markers for structural abnormalities, and the mean e' velocity and E/e' ratio were used as markers for diastolic dysfunction. The plasma NT-proBNP level was measured using electrochemiluminescence immunoassay (DPC Immulite 2000 XPi, Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Results: Subjects with preclinical structural abnormalities were older and had a higher body mass index (BMI), higher blood pressure, lower high-density lipoprotein cholesterol level, higher NT-proBNP level, and higher E/e' (P<0.05). Multivariate regression analysis indicated that the factors associated with a higher NT-proBNP level were older age, female sex, and lower BMI, higher creatinine level, higher LVMI and higher LAD (P<0.01).

Conclusion: Diastolic dysfunction is not associated with higher NT-proBNP levels, whereas, preclinical cardiac structural abnormalities as well as older age, female sex, lower BMI, and higher creatinine level are associated with higher NT-proBNP levels.

Strengths and limitations of this study

- This is the first study to address the association of early stage of preclinical echocardiography-detected cardiac structural or diastolic abnormalities with the NT-proBNP level in a primary healthcare setting.
- The target subjects with potential cardiac structural abnormalities for screening early stage B heart failure would be selected based on the NT-proBNP levels in health checkups.
- The cross-sectional study design and the relatively small sample mean that further research is needed into the associated causal relationships.

INTRODUCTION

Heart failure (HF) is a clinical syndrome characterized by typical symptoms and signs caused by a structural and/or functional cardiac abnormalities that result in a reduced pumping capability of heart and its pre- and post-loading volumes, stasis of blood flow, and insufficiency of the blood and oxygen supplied to the main organs [1]. The incidence of cardiovascular diseases is increasing including in younger subjects due to changes in lifestyles and dietary patterns, and there have also been increases in the rates of progression to HF [2]. Systolic dysfunction is frequently present in community-dwelling individuals without recognized symptoms of HF [3, 4]. In addition, most subjects with diastolic dysfunction have a normal left ventricular ejection fraction (LVEF), with even moderate or severe isolated diastolic dysfunction being as common as systolic dysfunction [5–7]. Thus, the early recognition and treatment of preclinical structural or functional abnormalities represent a potentially powerful strategy for reducing the incidence of HF.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is produced within myocytes and released into the circulation during increases in the ventricular and atrial pressures [8, 9]. The NT-proBNP level is therefore a useful diagnostic marker for cardiac insufficiency [10], ventricular dysfunction [11] and cardiomyopathy [12]. An assay for measuring the NT-proBNP level may also be applied for detecting asymptomatic subclinical cardiac structural or functional impairment. However, few studies have investigated the association between NT-proBNP levels and the early stage of preclinical structural or functional heart abnormalities, which are checked by echocardiography performed as a part of preventive screening programs in the primary healthcare system.

This study investigated the association of the NT-proBNP level with echocardiography-

detected early stage of cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved left ventricular systolic function (LVEF >50%) during health checkups.

METHODS

Subjects

We retrospectively studied consecutive health examinees aged over 18-year old who underwent echocardiography and NT-proBNP test during health checkups at a health-promotion center in Seoul between January 2016 and September 2018. The study design was retrospective and cross-sectional. The self-reported personal medical history, subjective symptoms and signs, and life style information were obtained from all participants at time of health checkups. Their medical records were also reviewed. Inclusion criteria were preserved left ventricular systolic function (LVEF >50%) determined by echocardiography, no previous cardiac surgery or diagnosed heart disease. Subjects who had echocardiography-detected valvular heart disease, atrial fibrillation, acute myocardial infarction, stroke, renal dysfunction, pregnancy, echocardiography-detected LVEF < 50%, or clinical symptoms or signs of HF were excluded from this study. After exclusion, the final sample size was 652 (361 males and 291 females: aged 28–82)

This study was approved by the Institutional Review Board of Korea Association of Health Promotion (approval no. 130750-201807-HR-016).

Echocardiography

The echocardiographic investigations were carried out using a Philips/Hewlett-Packard Sono 5500 ultrasound device (Philips Ultrasound, Andover, MA, USA). M-mode, two-dimensional, and hemodynamic Doppler images were acquired using a standardized protocol with a 3.5-

MHZ transducer. LVEF was calculated using the modified Simpson method [13]. An increased left ventricular mass index (LVMI; >115 g/m^2 in males and >95 g/m^2 in females), and an increased left atrial dimension (LAD; >41 mm in males and >39 mm in females) were used as markers for structural abnormalities [14]. Cardiac diastolic dysfunction was defined as an early diastolic mitral flow velocity (E)/early relaxation velocity in tissue Doppler recordings (e') ≥13 and a mean e' septal and lateral wall <9 cm/s. [15].

NT-proBNP measurement

Venous blood was collected in an lithium-heparin tube. The plasma NT-proBNP level was measured using an electrochemiluminescent immunoassay on a DPC Immulite 2000 XPi device (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The intra-assay and total variances were 3.4% and 4.7%, respectively, and the limit of detection was 10 pg/mL.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). Differences in the characteristics of the study subjects were analyzed according to the presence of structural abnormalities using Student's *t*-test or the chi-square test. In addition, differences in the characteristics of the study subjects were analyzed according to the presence of diastolic dysfunction using Student's *t*-test or the chi-square test. Differences in the characteristics of those with structural abnormality, diastolic dysfunction, or both abnormalities were analyzed using ANOVA. Univariate (crude) and multivariate (adjusted) regression analyses were performed to determine the variables affecting an increased NT-proBNP level. Variables considered in the analysis included age, sex, body mass index (BMI), systolic blood pressure, diastolic blood pressure, hemoglobin (Hb), fasting blood glucose (FBS), triglyceride, HDL-cholesterol, LDL-cholesterol, creatinine, LVEF, LVMI, LAD, E/e' and e'. We used multiple

linear regression model to control for effects of included age, sex, BMI, blood pressure, Hb, FBS, blood lipid, creatinine, LVEF, LVMI, LAD, E/e' and e' (Model 1). Additional regression model adjusted for age, sex, BMI, creatinine, LVMI, and LAD was used to identify increased NT-proBNP level in multivariate model (Model 2). Wilcoxon rank sum tests were performed to compare mean NT-proBNP levels between sexes, and between with and without structural abnormalities in each age groups, respectively. In addition, Wilcoxon rank sum test was used to compare those with diastolic dysfunction to those without diastolic dysfunction. Logarithmic transformations were applied to NT-proBNP level. Area under the receiver operating curve (AUROC) was calculated to measure the performance of NT-proBNP in predicting the cardiac structural abnormalities. A *P* value of <0.05 was considered statistically significant.

Patient and public involvement

Patients were not involved in the recruitment to and conduct of the study. Results will be disseminated to study participants through annual information events.

RESULTS

Six hundred and fifty-two of the 876 eligible subjects were analyzed (Figure 1). The median age of the study subjects was 58 years (range 28–82 years). Echocardiography revealed structural abnormalities in 92 (14.1%) of the subjects, who were older (60.8±8.8 vs 57.7±8.9 years [mean±SD], $P<0.017$), and had a higher BMI, higher blood pressure, lower high-density lipoprotein cholesterol (HDL-C) level, higher NT-proBNP level (55.8±91.5 vs 29.6±27.5 pg/mL, $P=0.015$), and higher E/e' ratio (13.0±3.5 vs 11.0±3.4) in echocardiography (Table 1).

Diastolic dysfunction was detected using echocardiography in 470 (72.0%) of the subjects. These subjects were older and had a higher BMI, higher blood pressure, higher FBS, higher triglycerides, and lower HDL-C levels. However, the NT-proBNP level did not differ significantly between subjects with and without diastolic dysfunction (Table 2). Although subjects with both structural abnormality and diastolic dysfunction were older, higher blood pressure, higher FBS, higher triglyceride, higher LVMI, higher E/e', and lower e' compared to one of structural abnormality or diastolic dysfunction, NT-proBNP was higher in subjects with structural abnormality only (Table 3).

In a univariate model, older age, lower LDL-C level, higher creatinine level, higher LVMI, and higher LAD were associated with an increased NT-proBNP level. In a multivariate model, older age, female sex, lower BMI, higher creatinine level, higher LVMI, and higher LAD were associated with an increased NT-proBNP level ($P<0.01$). While sex was not associated with the NT-proBNP level in a univariate model, female sex was associated with increased NT-proBNP level ($P=0.002$) in multivariate model (Table 4). Among subjects in all age ranges (aged ≤45, 46–60 years, and aged ≥61 years), females showed higher NT-proBNP levels than males (Figure 2). A structural abnormality defined by higher LVMI and/or higher LAD was

associated with an increased NT-proBNP level (P<0.01) but the E/e' ratio and mean e' velocity were not associated with NT-proBNP level. In subjects aged 46–60, and ≥61 years, those with structural abnormality showed a higher NT-proBNP level (P=0.002 and P=0.004, respectively) (Figure 3).

Figure 4 shows the receiver operating curve for assessing the performance of NT-proBNP in predicting cardiac structural abnormalities. AUROC was 0.625 (95% CI: 0.566–0.684). The cutoff for NT-proBNP at 21.0 pg/mL had sensitivity of 51.1% (95% CI: 40.9%–61.3%), specificity of 69.3% (95% cI: 65.5%–73.1%), positive predictive value of 21.5% (95% CI: 16.0–26.9%) and negative predictive value (NPV) of 89.6% (95% CI: 86.7%–92.5%) in predicting cardiac structural abnormalities.

DISCUSSION

This study investigated the association between NT-proBNP levels with echocardiography-detected early stage of cardiac structural abnormalities and diastolic dysfunction in asymptomatic subjects with preserved left ventricular systolic function in a primary healthcare setting. Subjects with structural abnormalities that were not yet apparent, were older and had a higher BMI, higher blood pressure, lower HDL-C level, or greater impairment of diastolic function in echocardiography. Furthermore, we have demonstrated that diastolic dysfunction is not associated with higher NT-proBNP levels, whereas, preclinical cardiac structural abnormalities as well as older age, female sex, lower BMI, and higher creatinine level are associated with higher NT-proBNP levels.

We observed associations of diastolic dysfunction with cardiovascular risk factors such as higher BMI, blood pressure, FBS, and triglycerides, and lower HDL-C levels. These results are consistent with reports of the presence of diastolic dysfunction being closely associated with cardiovascular diseases [16–18]. Furthermore, subjects with diastolic dysfunction exhibited elevated LVMI and LAD, which indicate the presence of structural abnormalities. These findings support that a hypertrophied ventricle is more likely to exhibit diastolic dysfunction and a chronic increase in the left atrial pressure associated with diastolic dysfunction, and would be expected to lead to atrial enlargement [19].

NT-proBNP is released into the circulation in response to a stretched myocardium resulting from any cardiac structural abnormalities [20]. Hung et al. [21] demonstrated an association between any structural anomaly and NT-proBNP levels, which was consistent with our finding of the NT-proBNP level being associated with subclinical cardiac structural abnormalities. Our AUROC suggest a threshold level of 21.0 pg/mL to exclude early stage of subclinical structural

abnormalities in health checkups. Compared to previously recommended cutoff value of 32.8 pg/mL [21], our newly proposed cutoff value is much lower with fairly acceptable NPV. However, we found no association between preclinical diastolic dysfunction (PDD) and the circulating NT-proBNP level. These differences might be due to differences between the included subjects: our study subjects participated in routine health checkups and pre-excluded any echocardiography-detected valvular heart disease or pulmonary hypertension, while the subjects included in the previous study had aortic root dilatation, ventricular hypertrophy, pulmonary hypertension or valvular heart disease and presented at a tertiary referral center. Another previous study of PDD [22] found that while advanced diastolic dysfunction with a normal LVEF was independently associated with structural abnormalities (increased LVMI and left atrial volume index [LAVI]) and increased circulating NT-proBNP, the structural abnormalities and NT-proBNP levels did not differ between normal controls and patients with mild diastolic dysfunction.

Among subjects with a normal LVEF and no HF diagnosis, a higher severity of diastolic dysfunction was associated with a higher mean LVMI and LAVI [19]. Our subjects with diastolic dysfunction had relatively low mean LVMI or LAD values, and they are therefore likely to have had only mild diastolic dysfunction. Our observations suggest that the association between PDD and circulating NT-proBNP levels may vary according to the severity of diastolic dysfunction. Therefore, the screening of preclinical structural or functional abnormalities based on NT-proBNP levels could be optimized by targeting subjects with potentially advanced diastolic dysfunction, which encompasses those who are older and have cardiovascular risk factors such as obesity, hypertension, higher blood glucose or dyslipidemia in health checkups.

Our study has some limitations. Its cross-sectional design and relatively small sample mean

that further research is needed into the associated causal relationships. Nevertheless, power calculation using G* power 3.1 with effect size f^2 of 0.2 (recommended by Cohen) showed the efficiency of sample size as the power of 0.85, which seems to be available in drawing conclusions of this study. Data on potential clinical correlates of diastolic dysfunction such as exercise tolerance were not included. Moreover, the echocardiographic evaluation was performed using a minimal data set that did not include detailed echocardiographic data on diastolic function. American Society of Echocardiography 2016 guidelines recommended the four variables for identifying diastolic dysfunction: annular e' velocity, average E/e' ratio, LA maximum volume index, and peak TR velocity. And the average E/e' ratio, which was used in the present study, was recommended for simplification on the basis of the writing group's collective expert opinion [15]. Inclusion of other variables will be considered to differentiate between normal and abnormal diastolic function in the future study.

In conclusion, the findings of this study suggest that NT-proBNP levels are associated with preclinical cardiac structural but not with diastolic dysfunction, which is applicable to early stage-B HF among the four categories defined by the American College of Cardiology/American Heart Association [23].

Figure 1. Study flow diagram.

Figure 2. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 3. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines

within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 4. Receiver operating characteristic curve and cutoff value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting cardiac structural abnormalities.

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Author Contributions All of the authors participated in designing this study. SC and SYK performed data collection. SK undertook the statistical analyses. EN, SYK, HC and SK analysed and interpreted the data. EN wrote the first draft of the manuscript, which was reviewed by all of the other authors, who also provided further contributions and suggestions.

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Competing interests None to declare.

Patient consent Obtained.

Ethics approval This study was approved by the Institutional Review Board of the Korea Association of Health Promotion (approval no. 130750-201807-HR-016).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open access

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Table 1 Characteristics of study subjects according to the presence of structural abnormalities

Variables	No Structural abnormality (N=560)	Structural abnormality (N=92)	*P* Value
Age (year)	57.7 ± 8.9	60.8 ± 8.8	0.017
Gender Male, N (%)	309 (55.2)	52 (56.5)	0.432
BMI (kg/m^2)	24.2 ± 3.0	26.9 ± 3.5	<0.001
SBP (mmHg)	119.8 ± 13.7	126.6 ± 12.1	<0.001
DBP (mmHg)	73.7 ± 8.9	76.9 ± 8.6	0.003
Hb (g/L)	147.7 ± 14.6	146.3 ± 16.5	0.965
FBS (mmol/L)	5.9 ± 1.5	6.2 ± 1.5	0.289
TC (mmol/L)	5.4 ± 1.1	5.2 ± 1.0	0.110
TG (mmol/L)	1.4 ± 0.9	1.6 ± 1.1	0.125
HDL-C (mmol/L)	1.5 ± 0.4	1.4 ± 0.4	<0.001
LDL-C (mmol/L)	3.2 ± 1	3.1 ± 0.9	0.236
Creatinine (μmol/L)	78.5 ± 15.2	76.9 ± 15.7	0.520
eGFR (mL/min/1.73m^2)	85.3 ± 14.7	87.3 ± 18.4	0.639
NT-proBNP (pg/mL)	29.6 ± 27.5	55.8 ± 91.5	0.015
LVEF (%)	66 ± 6.5	64.9 ± 7.3	0.807
LVMI (g/m^2)	71.7 ± 13.3	88.9 ± 18.1	<0.001
LAD (mL/m^2)	33.7 ± 3.9	40.8 ± 5.5	<0.001
E/e’	11 ± 3.4	13 ± 3.5	0.002
e’ (cm/s)	6.2 ± 1.6	5.9 ± 5.4	0.950

Continuous variables are expressed as mean±SD values and were compared using Student’s *t*-test. Categorical variables are expressed as N (%) values and were analyzed using the chi-square test. NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-

cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 2 Characteristics of study subjects according to the presence of diastolic dysfunction

Variables	No diastolic dysfunction (N=182)	Diastolic dysfunction (N=470)	*P* Value
Age (year)	52.6 ± 8.8	60.3 ± 8.1	<0.001
Gender Male, N (%)	93 (51.1)	268 (57.0)	0.172
BMI (kg/m^2)	23.4 ± 3	25 ± 3.2	<0.001
SBP (mmHg)	116.2 ± 12.6	122.6 ± 13.7	<0.001
DBP (mmHg)	71.4 ± 8.6	75.3 ± 8.8	<0.001
Hb (g/L)	144 ± 15.2	148.9 ± 14.5	<0.001
FBS (mmol/L)	5.6 ± 1	6.1 ± 1.6	<0.001
TC (mmol/L)	5.4 ± 1	5.4 ± 1.1	0.958
TG (mmol/L)	1.2 ± 0.8	1.5 ± 1	<0.001
HDL-C (mmol/L)	1.6 ± 0.4	1.5 ± 0.4	<0.001
LDL-C (mmol/L)	3.2 ± 1	3.2 ± 1	0.996
Creatinine (μmol/L)	76.3 ± 15.5	79 ± 15.2	0.040
eGFR (mL/min/1.73m^2)	88.9 ± 15.7	84.3 ± 15	0.001
NT-proBNP (pg/mL)	35.9 ± 63.5	32.3 ± 32.9	0.476
LVEF (%)	65.2 ± 6.9	66.1 ± 6.5	0.131
LVMI (g/m^2)	69.5 ± 14.2	75.9 ± 15.3	<0.001
LAD (mL/m^2)	33.9 ± 5.1	35 ± 4.7	0.007
E/e'	9.4 ± 2.1	12 ± 3.6	<0.001
e' (cm/s)	8.4 ± 3.6	5.3 ± 1	<0.001

Continuous variables are expressed as mean±SD values and were compared using Student's *t*-test. Categorical variables are expressed as N (%) values and were analyzed using the chi-square test. NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-

cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 3 Characteristics of study subjects according to the presence of structural abnormalities and/or diastolic dysfunction

Variables	Normal [a] (N=167)		Structural abnormality [b] (N=15)		Diastolic dysfunction [c] (N=393)		Structural abnormality & Diastolic dysfunction [d] (N=77)		*P*-Value	Multiple comparison
Age (year)	52.5	± 8.8	54.8	± 8.9	59.9	± 8	62	± 8.3	<0.001	a,b < c,d
Gender Male, N (%)	82	(49.1)	11	(73.3)	227	(57.8)	41	(53.3)	0.129	-
BMI (kg/m^2)	23.2	± 3	25.9	± 2.1	24.6	± 2.9	27.1	± 3.7	<0.001	a < d
SBP (mmHg)	115.7	± 12.6	121.3	± 11.5	121.6	± 13.8	127.6	± 12	<0.001	a < d
DBP (mmHg)	71.2	± 8.7	73	± 8	74.8	± 8.8	77.6	± 8.5	<0.001	a,b < d
Hb (g/L)	143.7	± 15.1	147.3	± 16.6	149.4	± 14.1	146.1	± 16.6	<0.001	-
FBS (mmol/L)	5.6	± 1	5.5	± 1	6.1	± 1.6	6.4	± 1.5	<0.001	b < d
TC (mmol/L)	5.4	± 1	5.1	± 1.3	5.4	± 1.1	5.3	± 0.9	0.631	-
TG (mmol/L)	1.2	± 0.8	0.9	± 0.7	1.4	± 1	1.7	± 1.1	<0.001	b < d
HDL-C (mmol/L)	1.6	± 0.4	1.5	± 0.5	1.5	± 0.4	1.4	± 0.4	0.001	a > d
LDL-C (mmol/L)	3.2	± 1	3.2	± 1	3.3	± 1	3.1	± 0.9	0.618	-
Creatinine (μmol/L)	75.4	± 15.1	85.5	± 18.2	79.7	± 15.2	75.2	± 14.7	0.001	a,d < b
eGFR (mL/min/1.73m^2)†	89.5	± 15.8	82.3	± 13.1	83.5	± 13.9	88.3	± 19.2	<0.001	b < a
NT-proBNP (pg/mL)†	27.1	± 16	132.9	± 195.2	30.7	± 31.1	40.8	± 40	0.005	a,c,d < b
LVEF (%)†	65.3	± 6.3	63.8	± 12	66.3	± 6.6	65.2	± 6.2	0.272	-
LVMI (g/m^2)†	67.8	± 12.8	88	± 16.2	73.3	± 13.1	89.1	± 18.5	<0.001	a,c < b,d
LAD (mL/m^2)†	33	± 3.9	43.5	± 6.2	34	± 3.8	40.2	± 5.2	<0.001	a,c < d < b
E/e'†	9.3	± 2.1	10.7	± 2.4	11.8	± 3.5	13.4	± 3.5	<0.001	a < b,c <d
e' (cm/s)†	8.2	± 1.1	11	± 12.3	5.4	± 0.9	4.9	± 1.2	<0.001	d,c < a < b

†Welch's ANOVA (not equal variance) was used.

NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 4 Regression analysis of the variables affecting an increased NT-proBNP level

Variables	Univariate model				Multivariate model							
					Model 1				Model 2			
	Coeff.*	95% CI	*P*-value	R^2	Coeff. *	95% CI	*P*-value	R^2	Coeff. *	95% CI	*P*-value	R^2
Age (year)	0.97	(0.6–1.33)	<0.001	0.040	0.58	(0.15– 1.01)	0.008	0.153	0.51	(0.13– 0.89)	0.009	0.140
Sex (ref: female)	-0.52	(-7.27– 6.23)	0.879	<0.001	-17.31	(-28.12– -6.51)	0.002		-19.47	(-28.5– -10.45)	<0.001	
BMI (kg/m^2)	-0.26	(-1.3– 0.78)	0.626	<0.001	-1.97	(-3.2 –-0.74)	0.002		-2.34	(-3.47– -1.21)	<0.001	
SBP (mmHg)	0.14	(-0.1–0.39)	0.258	0.002	-0.25	(-0.61– 0.1)	0.165					
DBP (mmHg)	0.27	(-0.11– 0.64)	0.164	0.003	0.48	(-0.06– 1.01)	0.081					
Hb (g/L)	-0.14	(-0.37– 0.08)	0.216	0.002	-0.15	(-0.45– 0.15)	0.338					
FBS (mmol/L)	-0.97	(-3.22– 1.28)	0.396	0.001	-0.44	(-2.71– 1.84)	0.707					
TG (mmol/L)	-2.34	(-5.81– 1.13)	0.186	0.003	-2.01	(-5.8– 1.78)	0.298					
HDL-C (mmol/L)	-1.72	(-10.2– 6.77)	0.691	<0.001	-0.76	(-9.82– 8.31)	0.870					
LDL-C (mmol/L)	-3.62	(-6.96– -0.27)	0.034	0.007	-2.95	(-6.2– 0.3)	0.075					
Creatinine (μmol/L)	0.36	(0.14– 0.58)	0.001	0.016	0.70	(0.4– 0.99)	<0.001		0.68	(0.4 –0.97)	<0.001	
LVEF (%)	-0.01	(-0.51– 0.49)	0.968	<0.001	0.12	(-0.37– 0.6)	0.637					
LVMI (g/m^2)	0.47	(0.26– 0.69)	<0.001	0.028	0.28	(0.04– 0.52)	0.022		0.30	(0.07– 0.53)	0.012	
LAD (mL/m^2)	2.11	(1.43– 2.79)	<0.001	0.054	2.58	(1.79– 3.37)	<0.001		2.63	(1.85– 3.41)	<0.001	
E/e’	0.65	(-0.33– 1.63)	0.191	0.003	-0.14	(-1.24– 0.97)	0.806					
e’ (cm/s)	-0.54	(-1.87– 0.8)	0.430	0.001	0.38	(-1.04– 1.81)	0.597					

*Regression coefficient: the usual interpretation of a regression coefficient is the average change in the outcome variable when the corresponding predictor variable is changed by one unit.

Model 1: adjust for age, sex, BMI, blood pressure, Hb, FBS, blood lipid creatinine

Model 2: adjusted for age, sex, BMI, creatinine, LVMI, and LAD

NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e’, early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e’, mean e’ septal and lateral wall velocity.

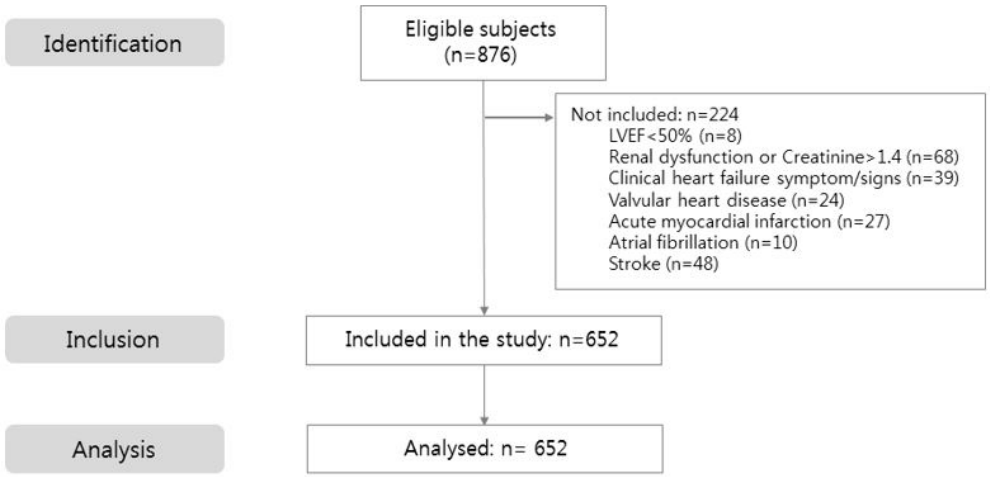

Figure 1 Study flow diagram.

Figure 1 Study flow diagram.

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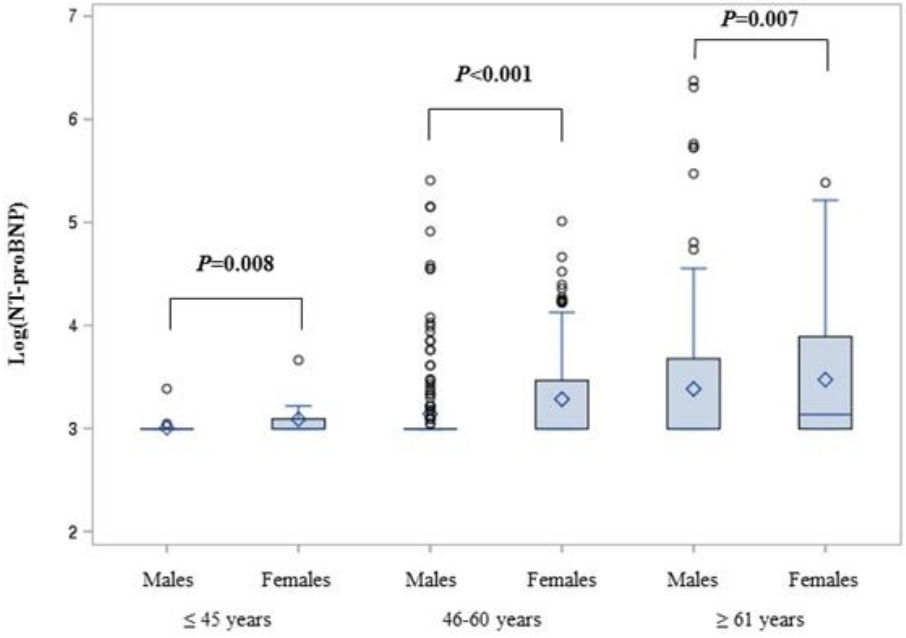

Figure 2 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 2 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

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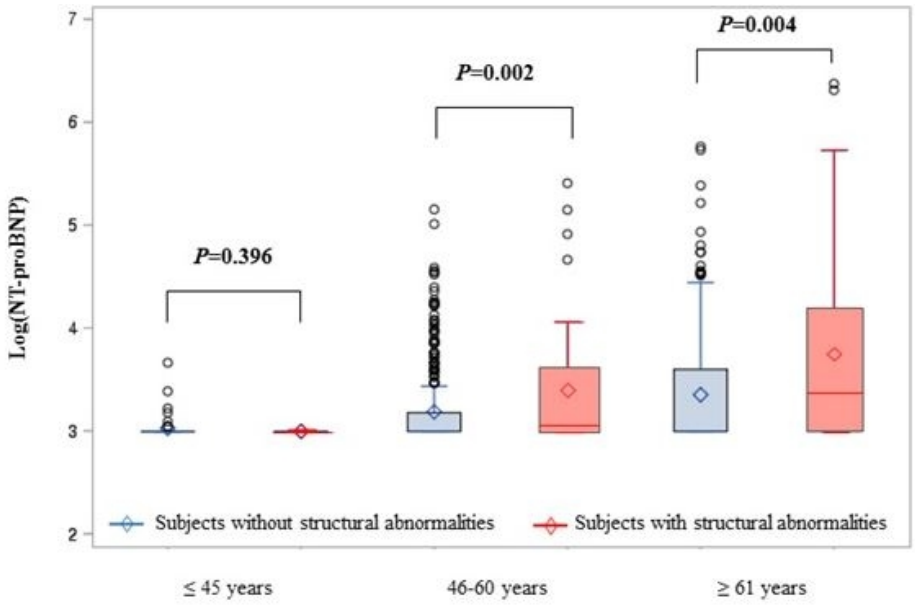

Figure 3 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 3 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

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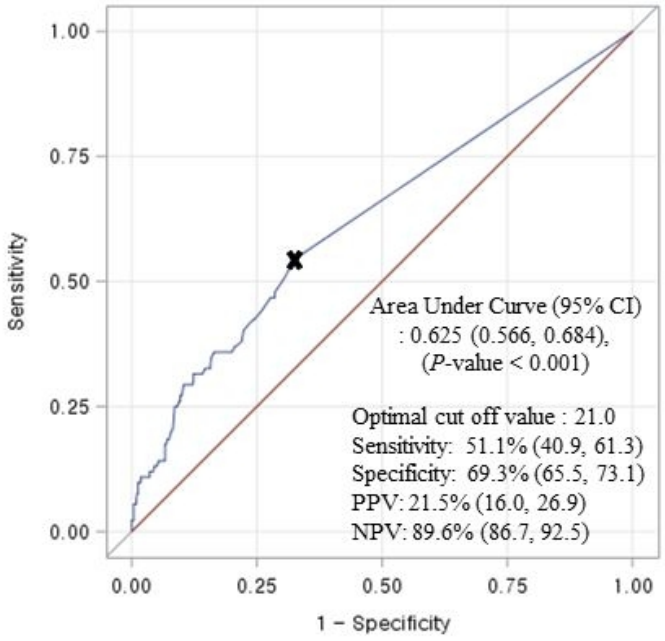

Figure 4 Receiver operating characteristic curve and cutoff value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting cardiac structural abnormalities.

Figure 4 Receiver operating characteristic curve and cutoff value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting cardiac structural abnormalities.

162x115mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5,6*
Bias	9	Describe any efforts to address potential sources of bias	Not relevant
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	5,6
		(*c*) Explain how missing data were addressed	Not relevant
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Not relevant
		(*e*) Describe any sensitivity analyses	No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Not relevant
		(b) Give reasons for non-participation at each stage	Not relevant
		(c) Consider use of a flow diagram	Not relevant
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	Not relevant
Outcome data	15*	Report numbers of outcome events or summary measures	7,8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make	Not relevant

		clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	Not relevant
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not relevant
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not relevant
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Plasma NT-proBNP levels associated with cardiac structural abnormalities in asymptomatic health examinees with preserved ejection fraction: a retrospective cross-sectional study

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Title page

Plasma NT-proBNP levels associated with cardiac structural abnormalities in asymptomatic health examinees with preserved ejection fraction: a retrospective cross-sectional study

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Word counts: 2,061

ABSTRACT

Objectives: Stage B heart failure is defined as an asymptomatic abnormality of the heart structure or function. The circulating level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) is elevated in symptomatic patients with left ventricular (LV) dysfunction caused by a structural or functional abnormality. This study investigated the association of the NT-proBNP level with echocardiography detected cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved LV systolic function (ejection fraction >50%).

Methods: We retrospectively studied 652 health examinees who underwent echocardiography and NT-proBNP test at a health-promotion center in Seoul, between January 2016 and September 2018. The left ventricular mass index (LVMI), and left atrial dimension (LAD) were used as markers for structural abnormalities, and the mean e' velocity and E/e' ratio were used as markers for diastolic dysfunction. The plasma NT-proBNP level was measured using electrochemiluminescence immunoassay (DPC Immulite 2000 XPi, Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Results: Subjects with preclinical structural abnormalities were older and had a higher body mass index (BMI), higher blood pressure, lower high-density lipoprotein cholesterol level, higher NT-proBNP level, and higher E/e' (P<0.05). Multivariate regression analysis indicated that the factors associated with a higher NT-proBNP level were older age, female sex, and lower BMI, higher creatinine level, higher LVMI and higher LAD (P<0.01).

Conclusion: Diastolic dysfunction is not associated with higher NT-proBNP levels, whereas, preclinical cardiac structural abnormalities as well as older age, female sex, lower BMI, and higher creatinine level are associated with higher NT-proBNP levels.

Strengths and limitations of this study

- This is the first study to address the association of early stage of preclinical echocardiography-detected cardiac structural or diastolic abnormalities with the NT-proBNP level in a primary healthcare setting.
- The target subjects with potential cardiac structural abnormalities for screening early stage B heart failure would be selected based on the NT-proBNP levels in health checkups.
- The cross-sectional study design and the relatively small sample mean that further research is needed into the associated causal relationships.

INTRODUCTION

Heart failure (HF) is a clinical syndrome characterized by typical symptoms and signs caused by a structural and/or functional cardiac abnormalities that result in a reduced pumping capability of heart and its pre- and post-loading volumes, stasis of blood flow, and insufficiency of the blood and oxygen supplied to the main organs [1]. The incidence of cardiovascular diseases is increasing including in younger subjects due to changes in lifestyles and dietary patterns, and there have also been increases in the rates of progression to HF [2]. Systolic dysfunction is frequently present in community-dwelling individuals without recognized symptoms of HF [3, 4]. In addition, most subjects with diastolic dysfunction have a normal left ventricular ejection fraction (LVEF), with even moderate or severe isolated diastolic dysfunction being as common as systolic dysfunction [5–7]. Thus, the early recognition and treatment of preclinical structural or functional abnormalities represent a potentially powerful strategy for reducing the incidence of HF.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is produced within myocytes and released into the circulation during increases in the ventricular and atrial pressures [8, 9]. The NT-proBNP level is therefore a useful diagnostic marker for cardiac insufficiency [10], ventricular dysfunction [11] and cardiomyopathy [12]. An assay for measuring the NT-proBNP level may also be applied for detecting asymptomatic subclinical cardiac structural or functional impairment. However, few studies have investigated the association between NT-proBNP levels and the early stage of preclinical structural or functional heart abnormalities, which are checked by echocardiography performed as a part of preventive screening programs in the primary healthcare system.

This study investigated the association of the NT-proBNP level with echocardiography-

detected early stage of cardiac structural or diastolic abnormalities in asymptomatic subjects with preserved left ventricular systolic function (LVEF >50%) during health checkups.

METHODS

Subjects

We retrospectively studied consecutive health examinees aged over 18-year old who underwent echocardiography and NT-proBNP test during health checkups at a health-promotion center in Seoul between January 2016 and September 2018. The study design was retrospective and cross-sectional. The self-reported personal medical history, subjective symptoms and signs, and life style information were obtained from all participants at time of health checkups. Their medical records were also reviewed. Inclusion criteria were preserved left ventricular systolic function (LVEF >50%) determined by echocardiography, no previous cardiac surgery or diagnosed heart disease. Subjects who had echocardiography-detected valvular heart disease, atrial fibrillation, acute myocardial infarction, stroke, renal dysfunction, pregnancy, echocardiography-detected LVEF < 50%, or clinical symptoms or signs of HF were excluded from this study. After exclusion, the final sample size was 652 (361 males and 291 females: aged 28–82)

This study was approved by the Institutional Review Board of Korea Association of Health Promotion (approval no. 130750-201807-HR-016).

Echocardiography

The echocardiographic investigations were carried out using a Philips/Hewlett-Packard Sono 5500 ultrasound device (Philips Ultrasound, Andover, MA, USA). M-mode, two-dimensional, and hemodynamic Doppler images were acquired using a standardized protocol with a 3.5-

MHZ transducer. LVEF was calculated using the modified Simpson method [13]. An increased left ventricular mass index (LVMI; >115 g/m^2 in males and >95 g/m^2 in females), and an increased left atrial dimension (LAD; >41 mm in males and >39 mm in females) were used as markers for structural abnormalities [14]. Cardiac diastolic dysfunction was defined as an early diastolic mitral flow velocity (E)/early relaxation velocity in tissue Doppler recordings (e') ≥13 and a mean e' septal and lateral wall <9 cm/s. [15].

NT-proBNP measurement

Venous blood was collected in an lithium-heparin tube. The plasma NT-proBNP level was measured using an electrochemiluminescent immunoassay on a DPC Immulite 2000 XPi device (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The intra-assay and total variances were 3.4% and 4.7%, respectively, and the limit of detection was 10 pg/mL.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). Differences in the characteristics of the study subjects were analyzed according to the presence of structural abnormalities using Student's *t*-test or the chi-square test. In addition, differences in the characteristics of the study subjects were analyzed according to the presence of diastolic dysfunction using Student's *t*-test or the chi-square test. Differences in the characteristics of those with structural abnormality, diastolic dysfunction, or both abnormalities were analyzed using ANOVA. Univariate (crude) and multivariate (adjusted) regression analyses were performed to determine the variables affecting an increased NT-proBNP level. Variables considered in the analysis included age, sex, body mass index (BMI), systolic blood pressure, diastolic blood pressure, hemoglobin (Hb), fasting blood glucose (FBS), triglyceride, HDL-cholesterol, LDL-cholesterol, creatinine, LVEF, LVMI, LAD, E/e' and e'. We used multiple

linear regression model to control for effects of included age, sex, BMI, blood pressure, Hb, FBS, blood lipid, creatinine, LVEF, LVMI, LAD, E/e' and e' (Model 1). Additional regression model adjusted for age, sex, BMI, creatinine, LVMI, and LAD was used to identify increased NT-proBNP level in multivariate model (Model 2). Wilcoxon rank sum tests were performed to compare mean NT-proBNP levels between sexes, and between with and without structural abnormalities in each age groups, respectively. In addition, Wilcoxon rank sum test was used to compare those with diastolic dysfunction to those without diastolic dysfunction. Logarithmic transformations were applied to NT-proBNP level. Area under the receiver operating curve (AUROC) was calculated to measure the performance of NT-proBNP in predicting the cardiac structural abnormalities. A *P* value of <0.05 was considered statistically significant.

Patient and public involvement

Patients were not involved in the recruitment to and conduct of the study. Results will be disseminated to study participants through annual information events.

RESULTS

Six hundred and fifty-two of the 876 eligible subjects were analyzed (Figure 1). The median age of the study subjects was 58 years (range 28–82 years). Echocardiography revealed structural abnormalities in 92 (14.1%) of the subjects, who were older (60.8±8.8 vs 57.7±8.9 years [mean±SD], $P<0.017$), and had a higher BMI, higher blood pressure, lower high-density lipoprotein cholesterol (HDL-C) level, higher NT-proBNP level (55.8±91.5 vs 29.6±27.5 pg/mL, $P=0.015$), and higher E/e' ratio (13.0±3.5 vs 11.0±3.4) in echocardiography (Table 1).

Diastolic dysfunction was detected using echocardiography in 470 (72.0%) of the subjects. These subjects were older and had a higher BMI, higher blood pressure, higher FBS, higher triglycerides, and lower HDL-C levels. However, the NT-proBNP level did not differ significantly between subjects with and without diastolic dysfunction (Table 2). Although subjects with both structural abnormality and diastolic dysfunction were older, higher blood pressure, higher FBS, higher triglyceride, higher LVMI, higher E/e', and lower e' compared to one of structural abnormality or diastolic dysfunction, NT-proBNP was higher in subjects with structural abnormality only (Table 3).

In a univariate model, older age, lower LDL-C level, higher creatinine level, higher LVMI, and higher LAD were associated with an increased NT-proBNP level. In a multivariate model, older age, female sex, lower BMI, higher creatinine level, higher LVMI, and higher LAD were associated with an increased NT-proBNP level ($P<0.01$). While sex was not associated with the NT-proBNP level in a univariate model, female sex was associated with increased NT-proBNP level ($P=0.002$) in multivariate model (Table 4). Among subjects in all age ranges (aged ≤45, 46–60 years, and aged ≥61 years), females showed higher NT-proBNP levels than males (Figure 2). A structural abnormality defined by higher LVMI and/or higher LAD was

associated with an increased NT-proBNP level (P<0.01) but the E/e' ratio and mean e' velocity were not associated with NT-proBNP level. In subjects aged 46–60, and ≥61 years, those with structural abnormality showed a higher NT-proBNP level (P=0.002 and P=0.004, respectively) (Figure 3).

Figure 4 shows the receiver operating curve for assessing the performance of NT-proBNP in predicting cardiac structural abnormalities. AUROC was 0.625 (95% CI: 0.566–0.684). The cutoff for NT-proBNP at 21.0 pg/mL had sensitivity of 51.1% (95% CI: 40.9%–61.3%), specificity of 69.3% (95% cI: 65.5%–73.1%), positive predictive value of 21.5% (95% CI: 16.0–26.9%) and negative predictive value (NPV) of 89.6% (95% CI: 86.7%–92.5%) in predicting cardiac structural abnormalities.

DISCUSSION

This study investigated the association between NT-proBNP levels with echocardiography-detected early stage of cardiac structural abnormalities and diastolic dysfunction in asymptomatic subjects with preserved left ventricular systolic function in a primary healthcare setting. Subjects with structural abnormalities that were not yet apparent, were older and had a higher BMI, higher blood pressure, lower HDL-C level, or greater impairment of diastolic function in echocardiography. Furthermore, we have demonstrated that diastolic dysfunction is not associated with higher NT-proBNP levels, whereas, preclinical cardiac structural abnormalities as well as older age, female sex, lower BMI, and higher creatinine level are associated with higher NT-proBNP levels.

We observed associations of diastolic dysfunction with cardiovascular risk factors such as higher BMI, blood pressure, FBS, and triglycerides, and lower HDL-C levels. These results are consistent with reports of the presence of diastolic dysfunction being closely associated with cardiovascular diseases [16–18]. Furthermore, subjects with diastolic dysfunction exhibited elevated LVMI and LAD, which indicate the presence of structural abnormalities. These findings support that a hypertrophied ventricle is more likely to exhibit diastolic dysfunction and a chronic increase in the left atrial pressure associated with diastolic dysfunction, and would be expected to lead to atrial enlargement [19].

NT-proBNP is released into the circulation in response to a stretched myocardium resulting from any cardiac structural abnormalities [20]. Hung et al. [21] demonstrated an association between any structural anomaly and NT-proBNP levels, which was consistent with our finding of the NT-proBNP level being associated with subclinical cardiac structural abnormalities. Our AUROC suggest a threshold level of 21.0 pg/mL to exclude early stage of subclinical structural

abnormalities in health checkups. Compared to previously recommended cutoff value of 32.8 pg/mL [21], our newly proposed cutoff value is much lower with fairly acceptable NPV. However, we found no association between preclinical diastolic dysfunction (PDD) and the circulating NT-proBNP level. These differences might be due to differences between the included subjects: our study subjects participated in routine health checkups and pre-excluded any echocardiography-detected valvular heart disease or pulmonary hypertension, while the subjects included in the previous study had aortic root dilatation, ventricular hypertrophy, pulmonary hypertension or valvular heart disease and presented at a tertiary referral center. Another previous study of PDD [22] found that while advanced diastolic dysfunction with a normal LVEF was independently associated with structural abnormalities (increased LVMI and left atrial volume index [LAVI]) and increased circulating NT-proBNP, the structural abnormalities and NT-proBNP levels did not differ between normal controls and patients with mild diastolic dysfunction.

Among subjects with a normal LVEF and no HF diagnosis, a higher severity of diastolic dysfunction was associated with a higher mean LVMI and LAVI [19]. Our subjects with diastolic dysfunction had relatively low mean LVMI or LAD values, and they are therefore likely to have had only mild diastolic dysfunction. Our observations suggest that the association between PDD and circulating NT-proBNP levels may vary according to the severity of diastolic dysfunction. Therefore, the screening of preclinical structural or functional abnormalities based on NT-proBNP levels could be optimized by targeting subjects with potentially advanced diastolic dysfunction, which encompasses those who are older and have cardiovascular risk factors such as obesity, hypertension, higher blood glucose or dyslipidemia in health checkups.

Our study has some limitations. Its cross-sectional design and relatively small sample mean

that further research is needed into the associated causal relationships. Nevertheless, power calculation using G* power 3.1 with effect size f^2 of 0.2 (recommended by Cohen) showed the efficiency of sample size as the power of 0.85, which seems to be available in drawing conclusions of this study. Data on potential clinical correlates of diastolic dysfunction such as exercise tolerance were not included. Moreover, the echocardiographic evaluation was performed using a minimal data set that did not include detailed echocardiographic data on diastolic function. American Society of Echocardiography 2016 guidelines recommended the four variables for identifying diastolic dysfunction: annular e' velocity, average E/e' ratio, LA maximum volume index, and peak TR velocity. And the average E/e' ratio, which was used in the present study, was recommended for simplification on the basis of the writing group's collective expert opinion [15]. Inclusion of other variables will be considered to differentiate between normal and abnormal diastolic function in the future study.

In conclusion, the findings of this study suggest that NT-proBNP levels are associated with preclinical cardiac structural but not with diastolic dysfunction, which is applicable to early stage-B HF among the four categories defined by the American College of Cardiology/American Heart Association [23].

Figure 1. Study flow diagram.

Figure 2. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 3. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines

within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

Figure 4. Receiver operating characteristic curve and cutoff value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting cardiac structural abnormalities.

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Author Contributions All of the authors participated in designing this study. SC and SYK performed data collection. SK undertook the statistical analyses. EN, SYK, HC and SK analysed and interpreted the data. EN wrote the first draft of the manuscript, which was reviewed by all of the other authors, who also provided further contributions and suggestions.

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Competing interests None to declare.

Patient consent Obtained.

Ethics approval This study was approved by the Institutional Review Board of the Korea Association of Health Promotion (approval no. 130750-201807-HR-016).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open access

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Table 1 Characteristics of study subjects according to the presence of structural abnormalities

Variables	No Structural abnormality (N=560)	Structural abnormality (N=92)	*P* Value
Age (year)	57.7 ± 8.9	60.8 ± 8.8	0.017
Gender Male, N (%)	309 (55.2)	52 (56.5)	0.432
BMI (kg/m^2)	24.2 ± 3.0	26.9 ± 3.5	<0.001
SBP (mmHg)	119.8 ± 13.7	126.6 ± 12.1	<0.001
DBP (mmHg)	73.7 ± 8.9	76.9 ± 8.6	0.003
Hb (g/L)	147.7 ± 14.6	146.3 ± 16.5	0.965
FBS (mmol/L)	5.9 ± 1.5	6.2 ± 1.5	0.289
TC (mmol/L)	5.4 ± 1.1	5.2 ± 1.0	0.110
TG (mmol/L)	1.4 ± 0.9	1.6 ± 1.1	0.125
HDL-C (mmol/L)	1.5 ± 0.4	1.4 ± 0.4	<0.001
LDL-C (mmol/L)	3.2 ± 1	3.1 ± 0.9	0.236
Creatinine (μmol/L)	78.5 ± 15.2	76.9 ± 15.7	0.520
eGFR (mL/min/1.73m^2)	85.3 ± 14.7	87.3 ± 18.4	0.639
NT-proBNP (pg/mL)	29.6 ± 27.5	55.8 ± 91.5	0.015
LVEF (%)	66 ± 6.5	64.9 ± 7.3	0.807
LVMI (g/m^2)	71.7 ± 13.3	88.9 ± 18.1	<0.001
LAD (mL/m^2)	33.7 ± 3.9	40.8 ± 5.5	<0.001
E/e’	11 ± 3.4	13 ± 3.5	0.002
e’ (cm/s)	6.2 ± 1.6	5.9 ± 5.4	0.950

Continuous variables are expressed as mean±SD values and were compared using Student’s *t*-test. Categorical variables are expressed as N (%) values and were analyzed using the chi-square test. NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-

cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 2 Characteristics of study subjects according to the presence of diastolic dysfunction

Variables	No diastolic dysfunction (N=182)	Diastolic dysfunction (N=470)	*P* Value
Age (year)	52.6 ± 8.8	60.3 ± 8.1	<0.001
Gender Male, N (%)	93 (51.1)	268 (57.0)	0.172
BMI (kg/m^2)	23.4 ± 3	25 ± 3.2	<0.001
SBP (mmHg)	116.2 ± 12.6	122.6 ± 13.7	<0.001
DBP (mmHg)	71.4 ± 8.6	75.3 ± 8.8	<0.001
Hb (g/L)	144 ± 15.2	148.9 ± 14.5	<0.001
FBS (mmol/L)	5.6 ± 1	6.1 ± 1.6	<0.001
TC (mmol/L)	5.4 ± 1	5.4 ± 1.1	0.958
TG (mmol/L)	1.2 ± 0.8	1.5 ± 1	<0.001
HDL-C (mmol/L)	1.6 ± 0.4	1.5 ± 0.4	<0.001
LDL-C (mmol/L)	3.2 ± 1	3.2 ± 1	0.996
Creatinine (μmol/L)	76.3 ± 15.5	79 ± 15.2	0.040
eGFR (mL/min/1.73m^2)	88.9 ± 15.7	84.3 ± 15	0.001
NT-proBNP (pg/mL)	35.9 ± 63.5	32.3 ± 32.9	0.476
LVEF (%)	65.2 ± 6.9	66.1 ± 6.5	0.131
LVMI (g/m^2)	69.5 ± 14.2	75.9 ± 15.3	<0.001
LAD (mL/m^2)	33.9 ± 5.1	35 ± 4.7	0.007
E/e'	9.4 ± 2.1	12 ± 3.6	<0.001
e' (cm/s)	8.4 ± 3.6	5.3 ± 1	<0.001

Continuous variables are expressed as mean±SD values and were compared using Student's *t*-test. Categorical variables are expressed as N (%) values and were analyzed using the chi-square test. NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-

cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 3 Characteristics of study subjects according to the presence of structural abnormalities and/or diastolic dysfunction

Variables	Normal [a] (N=167)		Structural abnormality [b] (N=15)		Diastolic dysfunction [c] (N=393)		Structural abnormality & Diastolic dysfunction [d] (N=77)		*P*-Value	Multiple comparison
Age (year)	52.5	± 8.8	54.8	± 8.9	59.9	± 8	62	± 8.3	<0.001	a,b < c,d
Gender Male, N (%)	82	(49.1)	11	(73.3)	227	(57.8)	41	(53.3)	0.129	-
BMI (kg/m^2)	23.2	± 3	25.9	± 2.1	24.6	± 2.9	27.1	± 3.7	<0.001	a < d
SBP (mmHg)	115.7	± 12.6	121.3	± 11.5	121.6	± 13.8	127.6	± 12	<0.001	a < d
DBP (mmHg)	71.2	± 8.7	73	± 8	74.8	± 8.8	77.6	± 8.5	<0.001	a,b < d
Hb (g/L)	143.7	± 15.1	147.3	± 16.6	149.4	± 14.1	146.1	± 16.6	<0.001	-
FBS (mmol/L)	5.6	± 1	5.5	± 1	6.1	± 1.6	6.4	± 1.5	<0.001	b < d
TC (mmol/L)	5.4	± 1	5.1	± 1.3	5.4	± 1.1	5.3	± 0.9	0.631	-
TG (mmol/L)	1.2	± 0.8	0.9	± 0.7	1.4	± 1	1.7	± 1.1	<0.001	b < d
HDL-C (mmol/L)	1.6	± 0.4	1.5	± 0.5	1.5	± 0.4	1.4	± 0.4	0.001	a > d
LDL-C (mmol/L)	3.2	± 1	3.2	± 1	3.3	± 1	3.1	± 0.9	0.618	-
Creatinine (μmol/L)	75.4	± 15.1	85.5	± 18.2	79.7	± 15.2	75.2	± 14.7	0.001	a,d < b
eGFR (mL/min/1.73m^2)†	89.5	± 15.8	82.3	± 13.1	83.5	± 13.9	88.3	± 19.2	<0.001	b < a
NT-proBNP (pg/mL)†	27.1	± 16	132.9	± 195.2	30.7	± 31.1	40.8	± 40	0.005	a,c,d < b
LVEF (%)†	65.3	± 6.3	63.8	± 12	66.3	± 6.6	65.2	± 6.2	0.272	-
LVMI (g/m^2)†	67.8	± 12.8	88	± 16.2	73.3	± 13.1	89.1	± 18.5	<0.001	a,c < b,d
LAD (mL/m^2)†	33	± 3.9	43.5	± 6.2	34	± 3.8	40.2	± 5.2	<0.001	a,c < d < b
E/e'†	9.3	± 2.1	10.7	± 2.4	11.8	± 3.5	13.4	± 3.5	<0.001	a < b,c <d
e' (cm/s)†	8.2	± 1.1	11	± 12.3	5.4	± 0.9	4.9	± 1.2	<0.001	d,c < a < b

†Welch's ANOVA (not equal variance) was used.

NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e', early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e', mean e' septal and lateral wall velocity.

Table 4 Regression analysis of the variables affecting an increased NT-proBNP level

Variables	Univariate model				Multivariate model							
					Model 1				Model 2			
	Coeff.*	95% CI	*P*-value	R^2	Coeff. *	95% CI	*P*-value	R^2	Coeff. *	95% CI	*P*-value	R^2
Age (year)	0.97	(0.6–1.33)	<0.001	0.040	0.58	(0.15– 1.01)	0.008	0.153	0.51	(0.13– 0.89)	0.009	0.140
Sex (ref: female)	-0.52	(-7.27– 6.23)	0.879	<0.001	-17.31	(-28.12– -6.51)	0.002		-19.47	(-28.5– -10.45)	<0.001	
BMI (kg/m^2)	-0.26	(-1.3– 0.78)	0.626	<0.001	-1.97	(-3.2 –-0.74)	0.002		-2.34	(-3.47– -1.21)	<0.001	
SBP (mmHg)	0.14	(-0.1–0.39)	0.258	0.002	-0.25	(-0.61– 0.1)	0.165					
DBP (mmHg)	0.27	(-0.11– 0.64)	0.164	0.003	0.48	(-0.06– 1.01)	0.081					
Hb (g/L)	-0.14	(-0.37– 0.08)	0.216	0.002	-0.15	(-0.45– 0.15)	0.338					
FBS (mmol/L)	-0.97	(-3.22– 1.28)	0.396	0.001	-0.44	(-2.71– 1.84)	0.707					
TG (mmol/L)	-2.34	(-5.81– 1.13)	0.186	0.003	-2.01	(-5.8– 1.78)	0.298					
HDL-C (mmol/L)	-1.72	(-10.2– 6.77)	0.691	<0.001	-0.76	(-9.82– 8.31)	0.870					
LDL-C (mmol/L)	-3.62	(-6.96– -0.27)	0.034	0.007	-2.95	(-6.2– 0.3)	0.075					
Creatinine (μmol/L)	0.36	(0.14– 0.58)	0.001	0.016	0.70	(0.4– 0.99)	<0.001		0.68	(0.4 –0.97)	<0.001	
LVEF (%)	-0.01	(-0.51– 0.49)	0.968	<0.001	0.12	(-0.37– 0.6)	0.637					
LVMI (g/m^2)	0.47	(0.26– 0.69)	<0.001	0.028	0.28	(0.04– 0.52)	0.022		0.30	(0.07– 0.53)	0.012	
LAD (mL/m^2)	2.11	(1.43– 2.79)	<0.001	0.054	2.58	(1.79– 3.37)	<0.001		2.63	(1.85– 3.41)	<0.001	
E/e’	0.65	(-0.33– 1.63)	0.191	0.003	-0.14	(-1.24– 0.97)	0.806					
e’ (cm/s)	-0.54	(-1.87– 0.8)	0.430	0.001	0.38	(-1.04– 1.81)	0.597					

*Regression coefficient: the usual interpretation of a regression coefficient is the average change in the outcome variable when the corresponding predictor variable is changed by one unit.

Model 1: adjust for age, sex, BMI, blood pressure, Hb, FBS, blood lipid creatinine

Model 2: adjusted for age, sex, BMI, creatinine, LVMI, and LAD

NT-proBNP, N-terminal pro-B-type natriuretic peptide; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; FBS, fasting blood sugar; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LAD, left atrial dimension; E/e’, early diastolic mitral inflow velocity/early diastolic mitral annular velocity; e’, mean e’ septal and lateral wall velocity.

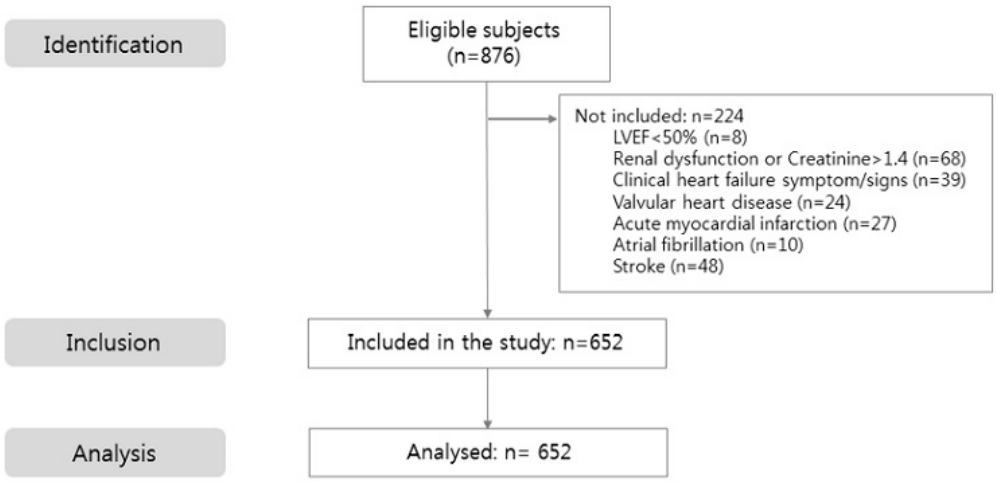

Figure 1 Study flow diagram.

196x95mm (96 x 96 DPI)

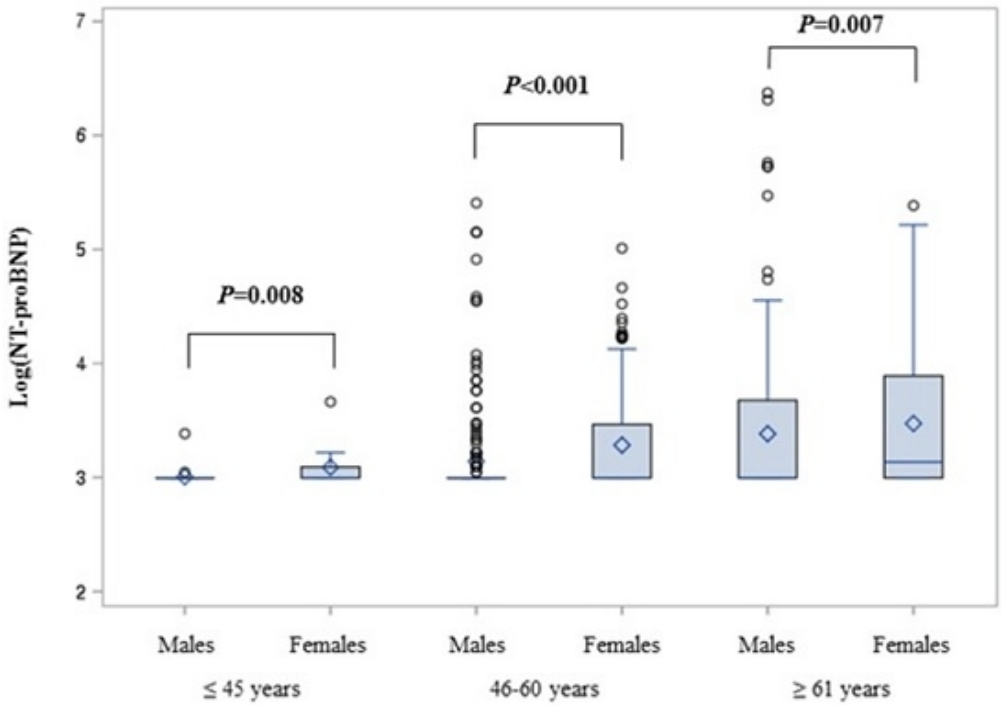

Figure 2 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels according to sex and age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

144x102mm (96 x 96 DPI)

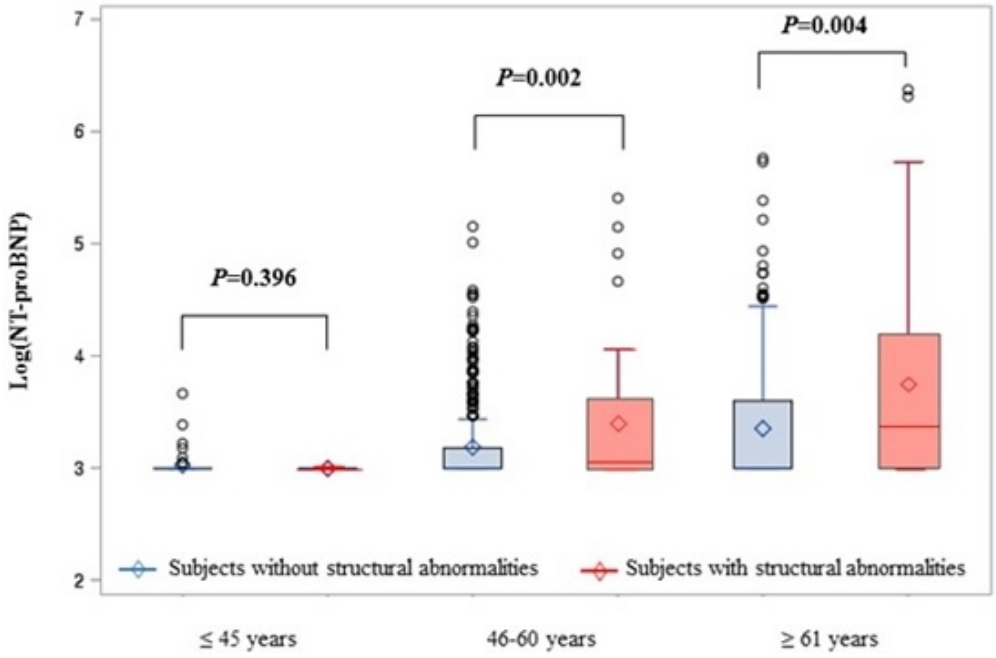

Figure 3 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels of subjects with and without structural abnormalities according to age categories. Box limits and horizontal lines within boxes represent interquartile ranges and the median, respectively. The upper and lower whiskers indicate the 97.5th and 2.5th percentiles, respectively.

145x95mm (96 x 96 DPI)

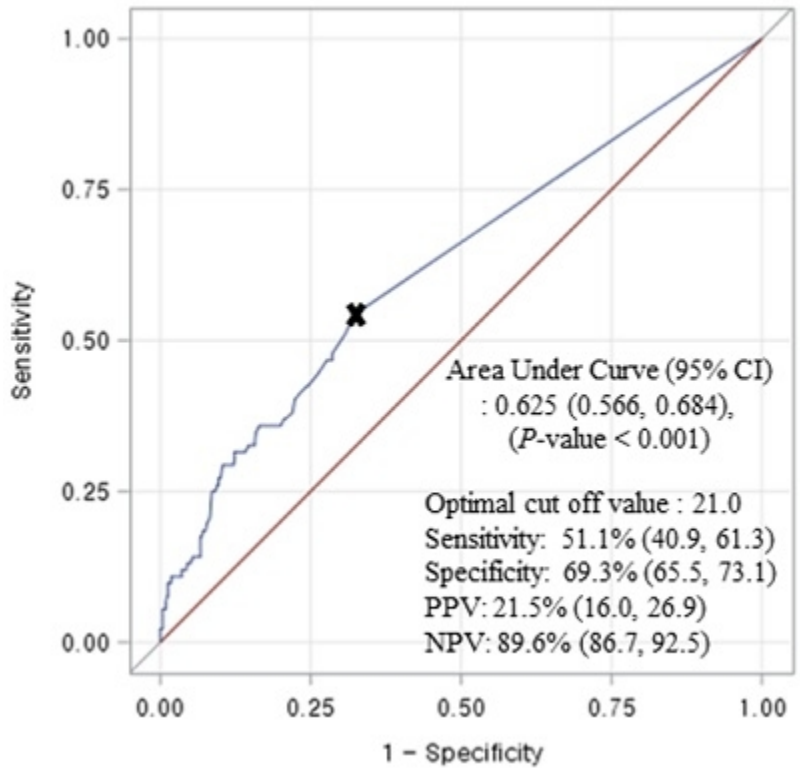

Figure 4 Receiver operating characteristic curve and cutoff value of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in predicting cardiac structural abnormalities.

106x101mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5,6*
Bias	9	Describe any efforts to address potential sources of bias	Not relevant
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5,6
		(*b*) Describe any methods used to examine subgroups and interactions	5,6
		(*c*) Explain how missing data were addressed	Not relevant
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Not relevant
		(*e*) Describe any sensitivity analyses	No
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Not relevant
		(b) Give reasons for non-participation at each stage	Not relevant
		(c) Consider use of a flow diagram	Not relevant
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	Not relevant
Outcome data	15*	Report numbers of outcome events or summary measures	7,8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make	Not relevant

		clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	Not relevant
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not relevant
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not relevant
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.