Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure.

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Short title: Plasma pentraxin 3 in heart failure

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ABSTRACT

Background: Pentraxin 3 (PTX3) is a novel inflammatory marker produced by endothelial cells, smooth muscle cells and macrophages. The purpose of the present study was to examine the clinical significance of plasma PTX3 levels in patients with heart failure.

Methods: We measured plasma PTX3 levels in 196 patients with heart failure and 60 control subjects without heart failure by sandwich ELISA. Patients were prospectively followed during a median follow-up period of 655 days with the end points of cardiac death or progressive heart failure requiring re-hospitalization. **Results:** Plasma PTX3 concentrations were higher in patients with heart failure than in control subjects ($P < 0.0001$) and increased as the severity of NYHA functional class advanced ($P < 0.0001$). A total of 63 cardiac events occurred during a follow-up period, and cardiac event free rate was markedly lower in patients with high PTX3 levels than in those with normal PTX3 levels (44.7% vs. 89.2%, $P < 0.0001$). The multivariate Cox proportional hazard analysis demonstrated that plasma PTX3 level, but not high sensitive C-reactive protein, was the independent predictor of cardiac events (hazard ratio 1.20, 95% confidence interval 1.03-1.40, $P = 0.0162$). Patients were divided into 4 groups based on plasma PTX3 values from 1st to 4th quartile. The highest 4th quartile of plasma PTX3 levels was associated with the highest risk of cardiac events

(9.23-fold compared to the 1st quartile).

Conclusions: Plasma PTX3 level provides important prognostic information for the risk stratification of patients with heart failure.

Key Words: Pentraxin 3, heart failure, inflammation, prognosis

INTRODUCTION

Activation of the inflammatory system plays a role in the pathogenesis of heart failure and causes increases in plasma levels of inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6.[1-3] Increased plasma levels of TNF-α and IL-6 in patients with heart failure are related to decreasing functional status and provide important prognostic information for morbidity and mortality.[4,5] One of the inflammatory markers, C-reactive protein (CRP), is produced in the liver in response to stimulation of various cytokines, mostly IL-6.[6,7] It has been reported that elevated high sensitive CRP (hs-CRP) has an independent prognostic value in heart failure patients.[8,9]

The classical short pentraxin, CRP and serum amyloid P, are the members of pentraxin superfamily. Pentraxin 3 (PTX3), which is one of the long pentraxin, conserves C-terminal pentraxin domain with the classical short pentraxins but differs for the presence of an unrelated long N-terminal domain. A variety of cell types can produce PTX3 upon exposure to primary inflammatory signals, such as IL-1, TNF-α, oxidized low density lipoprotein, and microbial moieties (e.g. lipopolysaccharide, lipoarabinomannans).[10,11] These cells include dendritic cells, mononuclear phagocytes, macrophages, smooth muscle cells, fibroblasts, and endothelial cells. Specifically, agonists for different members of the Toll-like receptor family can produce PTX3. Interestingly, IL-6 is a potent inducer of the

classic short pentraxin CRP in the liver,[12,13] but is generally a weak inducer of the long pentraxin PTX3.[14] Vascular endothelial cells produce PTX3 in human atherosclerotic lesions.[15] Plasma PTX3 levels are very low in normal conditions, but increase rapidly to 200-800 ng/ml 6-8 hrs after treatment with proinflammatory mediators and return to basal levels in 16-24 hrs.[10,16] Recently, it has been reported that PTX3 is an early indicator of acute myocardial infarction.[17,18] However, relationship between plasma PTX3 levels and heart failure has not been previously examined.

The purpose of the present study was to examine clinical significance of plasma PTX3 levels in patients with heart failure. We hypothesized that plasma PTX3 levels increase with advancing the severity of heart failure, and PTX3 provides important prognostic information. We also compared plasma PTX3 levels with hs-CRP levels and examined which inflammatory marker was a better predictor in patients with heart failure.

METHODS

Study subjects

We measured plasma PTX3 levels in 223 patients who admitted to the Yamagata University Hospital for the treatment of worsening heart failure or for diagnosis and pathophysiological investigations of heart failure or for therapeutic evaluation of heart failure from August 2002 to March 2005. The diagnosis of heart failure was based on the Framingham criteria for heart failure. We excluded 27 patients who had renal insufficiency characterized by a serum creatinine concentration > 2.0 mg/dl, or active systemic inflammatory diseases or collagen diseases, and who had experienced clinical or electrocardiographic evidence suggestive of acute coronary syndrome, or positive troponin T within 3 months preceding admission.[17-19] The remaining 196 patients (112 men and 84 women, mean age of 68.3 ± 13.3 years old) were characterized in the subsequent analysis. Sixty subjects matched for age without heart failure were served as control (34 men and 26 women, aged 66.1 ± 9.5 years old). Those subjects were diagnosed as normal by physical examinations, ECG, chest X-ray, and echocardiography. The study protocol was approved by the institutional review board of the Yamagata University, and the written informed consent was obtained from all participating patients.

The functional severity of heart failure was New York Heart Association (NYHA)

class I in 43, class II in 79, class III in 55, and class IV in 19 patients (Table 1). Hypertension, diabetes mellitus, and hyperlipidemia were identified in 104 (53%), 52 (27%), and 43 (22%) of patients, respectively. Hypertension was defined as elevated systolic pressure of ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or when patients had taken antihypertensive drugs. Diabetes mellitus was defined as an increased fasting plasma glucose concentration of ≥ 126 mg/dl, glycosylated hemoglobin of ≥ 6.4%, or when patients undergo treatment with insulin or hypoglycemic agents. Hyperlipidemia was defined as augmented total cholesterol level of ≥ 220 mg/dl, triglyceride ≥ 150 mg/dl, decreased high-density lipoprotein level of ≤ 40 mg/dl, or when patients had taken drugs of antihyperlipidemia. The etiologies of heart failure were identified as dilated cardiomyopathy in 55 (28%), ischemic heart disease in 50 (26%), valvular heart disease in 43 (22%), hypertensive heart disease in 26 (13%), and others in the remaining 22 (11%) patients.

Blood samples were obtained on admission to measure levels of serum hs-CRP, plasma PTX3, B-type natriuretic peptide (BNP), TNF-α, and other biochemical markers. Glomerular filtration rate (GFR) was estimated from the Modification of Diet in Renal Disease (MDRD) formula.[20] Two-dimensional echocardiography was performed by an experienced cardiologist within 1 week after admission. Physicians were kept blind to the results of the biochemical markers, and optimal medical therapy was performed

independently based on improvement in symptoms, physical examination findings, and pulmonary congestion on chest X-ray.

Endpoints and follow-up

All patients were followed up (mean 655 days, range 5-1600 days) after discharge. Events were centrally adjudicated using medical records, autopsy reports, death certificates, and witness statements. The endpoints were (1) cardiac death, defined as death from worsening heart failure or sudden cardiac death, and (2) re-hospitalization with worsening heart failure. Sudden cardiac death was defined as death without definite premonitory symptoms or signs and was established by the attending physician. Patients were contacted after the initial presentation by telephone interview performed by trained researchers.

Human PTX3 ELISA

Human PTX3 ELISA system is obtained from Perseus Proteomics Inc. (Tokyo, Japan). This kit can be measured plasma PTX3 concentration linearly between 0.1 and 20 ng/ml. Plasma PTX3 levels were measured as previously reported.[19] The ELISA assay did not cross-react with the short pentraxins CRP and serum amyloid P.

Assay of BNP and TNF-α levels

Same plasma samples were used for measurements of plasma BNP concentrations (n = 196). BNP concentrations were measured using a commercially available specific radioimmunoassay for human BNP (Shiono RIA BNP assay kit, Shionogi Co. Ltd, Tokyo, Japan). TNF-α levels were also measured using commercially available high sensitive ELISA kit (Human TNF-α/TNFSF1A Immunoassay, R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Results are expressed as mean ± standard deviation (SD), and skewed variables are presented as median and inter-quartile range. A P value of 0.05 was considered as statistically significant. Significance between 2 groups was determined by unpaired Student's *t* test for continuous variables and by chi-square test for discrete variables. If data were not distributed normally, the Mann-Whitney's U test was used. Comparisons of the levels of PTX3 among NYHA functional classes were performed by the Kruskal-Wallis test. The Cox proportional hazard regression analysis and multiple logistic regression analysis were used to determine which variables were related significantly to cardiac events. Only

variables with *P* value less than 0.05 in the univariate Cox regression analysis were entered into the multivariate Cox regression analysis. Kaplan-Meier survival curves determined the time-dependent cumulative cardiac event free rates in patients stratified into 2 groups on the basis of the plasma PTX3 levels, and were analyzed by a log rank test. Statistical analysis was performed with a standard statistical program package (Stat View, version 5.0, SAS Institute Inc, Cary, NC, USA).

RESULTS

Clinical characteristics of study subjects and plasma PTX3 levels

The clinical characteristics, laboratory data, and medications of all study subjects are listed in Table 1. The mean left ventricular end diastolic diameter (LVEDD) and left ventricular ejection fraction (LVEF) were 56.6 mm and 45.2%, respectively. The median values of plasma BNP, serum hs-CRP, TNF-α and plasma PTX3 were 311.0 pg/ml, 0.253 mg/dl, 1.360 pg/ml and 3.7 ng/ml, respectively.

As shown in Figure 1, the concentration of plasma PTX3 levels was significantly higher in patients with heart failure than in control subjects, and increased with advancing NYHA functional class ($P < 0.0001$), especially in severe heart failure patients with NYHA class III and IV.

From the mean + 2SD value of 60 normal control subjects, the normal upper limit of plasma PTX3 level was determined as 4.0 ng/ml. Heart failure patients were divided into two groups: normal plasma PTX3 levels (< 4.0 ng/ml, n = 102) and high plasma PTX3 levels ($\geq$ 4.0 ng/ml, n = 94). High plasma PTX3 levels were present in 10 of 43 patients (23.3%) with NYHA class I, 28 of 79 patients (35.4%) with NYHA II, 39 of 55 patients (70.9%) with NYHA III, and 17 of 19 patients (89.5%) with NYHA IV ($P < 0.0001$).

Clinical characteristics were compared between patients with normal and high plasma

PTX3 groups (Table 2). NYHA functional class was more severe, and LVEF and estimated GFR were lower in high plasma PTX3 group than in normal PTX3 group. We found that plasma BNP ($P < 0.0001$) and serum hs-CRP ($P < 0.0001$) levels were higher in high plasma PTX3 group than in normal PTX3 group. Levels of plasma PTX3 and serum hs-CRP were weakly correlated by a simple linear regression analysis ($R = 0.300$, $P < 0.0001$).

Prognosis of subjects and plasma PTX3 levels

There were 63 cardiac events (24 cardiac deaths and 39 re-hospitalization) during a follow-up period in all heart failure patients. Clinical characteristics were compared between patients with and without cardiac events (Table 3). We found that plasma BNP ($P < 0.0001$), serum creatinine ($P = 0.0328$), uric acid ($P = 0.0039$), hs-CRP ($P = 0.0025$), TNF-α ($P = 0.0006$) and plasma PTX3 levels ($P < 0.0001$) were higher in the cardiac event group than in the event free group. Estimated GFR was lower in the cardiac event group than in the event free group ($P < 0.0001$). Echocardiography showed that LVEDD was larger and LVEF was lower in the cardiac event group than in the event free group ($P = 0.0010$ and $P = 0.0057$, respectively).

Cumulative event free survival curves were illustrated by the Kaplan-Meier method and compared by a log rank test (Figure 2). Cardiac event free rate was significantly lower

in the high PTX3 group than in the normal PTX3 group (44.7% vs. 89.2%, P<0.0001, Figure 2A). Rates of cardiac death (P < 0.0001) and re-hospitalization due to worsening heart failure (P < 0.0001) were significantly higher in patients with high PTX3 than in those with normal PTX3 levels (Figures 2B and 2C).

The univariate Cox proportional hazards analysis to predict cardiac events for PTX3 and other variables are shown in Table 4. An increase of one SD (6.22 ng/ml) in plasma PTX3 levels was a significant variable (hazard ratio 1.33, 95% confidence interval, 1.19-1.51, P < 0.0001). Furthermore, age, NYHA class, an increase of one SD in the LVEDD, LVEF, BNP, estimated GFR, uric acid, hs-CRP, and TNF-α were related significantly to cardiac events (Table 4).

Those variables with P values of less than 0.05 were entered into the multivariate Cox proportional hazard regression analysis (Table 5). With the multivariate Cox proportional hazard regression analysis, plasma PTX3 level, LVEDD, hs-CRP and BNP were the independent predictors of cardiac events among those variables as reported in Table 5.

We classified all heart failure patients into 4 groups according to plasma PTX3 levels: 1st quartile (< 2.2 ng/ml, n = 49), 2nd quartile (2.2-3.7 ng/ml, n = 49), 3rd quartile (3.7-6.5 ng/ml, n = 49) and 4th quartile (> 6.5 ng/ml, n = 49). As shown in Figure 3, the relative risk of all cardiac events was the highest in 4th quartile (9.23-fold compared to the 1st quartile).

Comparison of PTX3 with BNP

Plasma PTX3 levels were weakly correlated with BNP by a simple linear regression analysis (R = 0.241, P = 0.0007). Receiver operated characteristic (ROC) curves for PTX3 and BNP are shown in Figure 4. The area under the ROC of PTX3 was larger than that of BNP (0.8047 vs. 0.7107), suggesting that PTX3 was superior to BNP to predict adverse outcomes. The sensitivity and specificity to detect future cardiac events were 71.4% and 64.7% by BNP alone, 82.5% and 69.2% by PTX3 alone, and 93.7% and 52.6% by PTX3 + BNP.

Subset analysis in patients with NYHA class III/IV

There were 74 patients with NYHA class III and IV in the present study. Those patients were divided into high PTX3 group (n = 55) and normal PTX3 group (n = 19). Event free rates were compared between high and normal PTX3 groups (Figure 5). Kaplan-Meier curves demonstrated that high PTX3 group had significantly lower cardiac event free rate than normal PTX3 group (P = 0.0193) in patients with NYHA class III and IV as shown in Figure 5.

Discussion

We reported the new important findings in this study; (1) PTX3, which is one of the pentraxin superfamily and a new vascular inflammatory marker, was increased in patients with heart failure, and plasma PTX3 levels increased with advancing NYHA functional class (Figures 1), (2) patients with abnormally high plasma PTX3 levels more frequently had cardiac events than patients with normal PTX3 levels (Figure 2), and (3) PTX3 remained an independent predictor of cardiac events by the multivariate Cox proportional hazard analysis (Table 5) ~~and the multiple logistic regression analysis (Table 6)~~ when compared with other well-known predictors of clinical outcomes in heart failure.

Heart failure is systemic disorder which affects not only cardiovascular and musculoskeletal systems but also inflammation and immune systems.[4,5,21,22] There is now substantial evidence to suggest that activation of the inflammatory and immune systems may play an important role in the heart failure process.[21-24] Recently, several numbers of biochemical novel factors, inflammatory markers and cytokines (e.g. hs-CRP, TNF-α, IL-6, tissue factor) have been identified that are related to prognosis and risk in patients with heart failure.[8,9,25] CRP, which is one of the short pentraxin, is synthesized in the liver in response to various inflammatory signals and cytokines, mostly IL-6. In contrast to short pentraxin CRP, the long pentraxin including PTX3 is produced only in small amounts in the liver, but

much more in dendritic cells, endothelial cells, smooth muscle cells, macrophages, and fibroblasts in response to primary inflammatory signals. PTX3 binds the complement component C1q and activates the classical pathway of complement activation.[26] In addition, PTX3 binds fibroblast growth factor 2 and inhibits activation of smooth muscle cells, neointima formation, and angiogenesis after arterial injury.[27] PTX3 is also expressed in human advanced atherosclerotic plaques.[15] There was not a close correlation between PTX3 and CRP in the present study as reported in other studies.[17] Therefore, PTX3 provides important diagnostic and prognostic information independently from CRP, and is more sensitive and immediate inflammatory marker than CRP in heart failure patients.

There were some limitations in the present study. The use of angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers and β-blockers was relatively low in this study (Table 1). There are some reasons for these low rates; (1) many of study subjects had preserved left ventricular systolic function (LVEF > 40%) in the present study, and (2) numbers of patients with ischemic heart failure were relatively low (26%) in this study population compared to Western countries. The prevalence of ischemic heart disease was lower than in comparable European or North American cohorts. To generalize our findings with PTX3 in heart failure, assessment of a new biomarker should be performed in a larger population. Ideally, findings in this study would be independently and

prospectively confirmed in another cohort. Multi-center trial with a large scale study population is necessary to validate the clinical importance of PTX3 in the future.

Conclusion

Plasma PTX3 level was increased in patients with heart failure and independently associated with an increased risk for cardiac events. These data indicate that plasma PTX3 level is a novel promising marker to provide useful prognostic information for clinical outcomes in patients with heart failure.

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Figure legends

Figure 1. Plasma levels of PTX3 in study population. We determined the normal upper limit of plasma PTX3 levels from mean + 2SD value of 60 control subjects (4.0 ng/ml, dash line). *P < 0.01 vs. control, #P < 0.05 vs. NYHA I, ##P < 0.01 vs. NYHA I, $P < 0.01 vs. NYHA II

Figure 2. Kaplan-Meier analysis for all cardiac events (A), cardiac deaths (B), and re-hospitalization (C) between patients with high and normal plasma PTX3 levels.

Figure 3. Quartile analysis of plasma PTX3 levels and relative risk for all cardiac events in heart failure patients. *P < 0.05, **P < 0.01 vs. 1st Quartile, #P < 0.05 vs. 2nd Quartile.

Figure 4. Receiver operated characteristic (ROC) curves for PTX3 and BNP. The area under the ROC (AUC) of PTX3 was larger than that of BNP (0.8047 vs. 0.7107).

Figure 5. Kaplan-Meier analysis for all cardiac events between patients with high and normal plasma PTX3 levels in NYHA class III/IV.

Table 1. Clinical Characteristics of 196 Patients With Chronic Heart Failure

	All patients (n=196)
Age (years)	68.3 ± 13.3
Gender (Male / Female)	112 / 84
NYHA functional class (I / II / III / IV)	43 / 79 / 55 / 19
Hypertension	104 (53%)
Diabetes mellitus	52 (27%)
Hyperlipidemia	43 (22%)
Etiology of chronic heart failure	
Dilated cardiomyopathy	55 (28%)
Ischemic heart disease	50 (26%)
Valvular heart disease	43 (22%)
Hypertensive heart disease	26 (13%)
Others	22 (11%)
Echocardiography	
LVEDD (mm)	56.6 ± 9.8
LVEF (%)	45.2 ± 18.1
BNP* (pg/ml)	311.0 (764.0)
Serum creatinine (mg/dl)	0.97 ± 0.49
Estimated GFR (ml/min/1.73m^2)	64.0±32.2
Uric acid (mg/dl)	6.3 ± 2.0
Na (mEq/l)	141.1 ± 3.0
hs-CRP* (mg/dl)	0.253 (0.688)
TNF-α* (pg/ml)	1.360 (1.446)
Pentraxin 3* (ng/ml)	3.7 (4.3)
Pharmacotherapy (at discharge)	
ACE inhibitors or ARBs	138 (73%)
β-blockers	95 (50%)
Ca channel blockers	32 (17%)
Diuretics	124 (65%)
Digitalis	55 (29%)
Statins	31 (16%)

NYHA, New York Heart Association; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; BNP, B-type natriuretic peptide; GFR, glomerular filtration

rate; hs-CRP, high sensitive C-reactive protein; TNF, tumor necrosis factor; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker. *Skewed data are reported as median (inter-quartile range).

Table 2. Comparisons of Clinical Characteristics between Patients with Normal and High Plasma Pentraxin 3 Levels

	Normal PTX3 levels (<4.0 ng/ml) (n=102)	High PTX3 levels (≥4.0 ng/ml) (n=94)	P value
Age (years)	67.7 ± 11.6	69.1 ± 15.0	0.4489
Gender (Male / Female)	59 / 43	53 / 41	0.8365
NYHA functional class (I / II / III / IV)	33 / 51 / 16 / 2	10 / 28 / 39 / 17	<0.0001
Hypertension	61 (61%)	43 (46%)	0.0488
Diabetes mellitus	32 (32%)	20 (21%)	0.1097
Hyperlipidemia	25 (25%)	18 (19%)	0.3649
Etiology oh chronic heart failure			0.7575
Dilated cardiomyopathy	25 (25%)	30 (32%)	
Ischemic heart disease	25 (25%)	25 (26%)	
Valvular heart disease	25 (25%)	18 (19%)	
Hypertensive heart disease	15 (15%)	11 (12%)	
Others	12 (11%)	10 (11%)	
Echocardiography			
LVEDD (mm)	54.8 ± 9.1	57.7 ± 10.3	0.0817
LVEF (%)	50.9 ± 17.9	42.3 ± 16.9	0.0002
BNP* (pg/ml)	147.0 (356.2)	722.0 (1087.0)	<0.0001
Serum creatinine (mg/dl)	0.96 ± 0.56	0.97 ± 0.40	0.8634
Estimated GFR (ml/min/1.73m^2)	68.7 ± 31.5	54.2 ± 31.6	0.0034
Uric acid (mg/dl)	6.0 ± 1.9	6.5 ± 2.1	0.1010
Na (mEq/l)	141.3 ± 2.5	140.0 ± 3.4	0.5491
hs-CRP* (mg/dl)	0.063 (0.241)	0.516 (2.011)	<0.0001
TNF-α* (pg/ml)	1.143 (1.064)	1.296 (1.005)	0.0527

Abbreviations as in Table 1

Table 3. Comparisons of Clinical Characteristics between Patients With and Without Cardiac Events

	Event-free (n=133)	Cardiac event (n=63)	P value
Age (years)	66.6 ± 13.7	71.9 ± 11.6	0.0082
Gender (Male / Female)	77 / 56	35 / 28	0.7573
NYHA functional class (I / II / III / IV)	40 / 59 / 26 / 8	3 / 20 / 29 / 11	<0.0001
Hypertension	76 (57%)	28 (44%)	0.0962
Diabetes mellitus	36 (27%)	16 (25%)	0.8046
Hyperlipidemia	28 (21%)	15 (24%)	0.6631
Etiology oh chronic heart failure			0.3497
Dilated cardiomyopathy	34 (26%)	21 (33%)	
Ischemic heart disease	32 (24%)	18 (29%)	
Valvular heart disease	32 (24%)	11 (17%)	
Hypertensive heart disease	18 (13%)	8 (13%)	
Others	17 (13%)	5 (8%)	
Echocardiography			
LVEDD (mm)	54.4 ± 8.5	59.5 ± 11.5	0.0010
LVEF (%)	49.8 ± 17.5	42.0 ± 18.2	0.0057
BNP* (pg/ml)	198.3 (449.2)	809.0 (981.3)	<0.0001
Serum creatinine (mg/dl)	0.92 ± 0.48	1.08 ± 0.50	0.0328
Estimated GFR (ml/min/1.73m^2)	72.2 ± 32.7	50.3 ± 26.2	<0.0001
Uric acid (mg/dl)	6.0 ± 2.0	6.9 ± 1.9	0.0039
Na (mEq/l)	141.3 ± 2.5	140.8 ± 3.8	0.2561
hs-CRP* (mg/dl)	0.078 (0.483)	0.411 (0.970)	0.0025
TNF-α* (pg/ml)	1.150 (1.064)	1.562 (1.828)	0.0006
Pentraxin 3* (ng/ml)	2.99 (2.95)	6.22 (5.59)	<0.0001

Abbreviations as in Table 1

Table 4. Results of the Univariate Cox Proportional Hazard Analysis

Variables	Hazard ratio	95% confidence interval	P value
Age, per 5-years increase	1.19	1.06-1.33	0.0033
Male vs. Female	1.10	0.67-1.80	0.7206
NYHA I/II vs. III/IV	3.50	2.09-5.85	<0.0001
Presence of			
Hypertension	0.73	0.45-1.21	0.2220
Diabetes mellitus	0.92	0.52-1.62	0.7730
Hyperlipidemia	1.40	0.78-2.50	0.2576
Echocardiography			
LVEDD, per 9.8 mm increase	1.57	1.21-2.02	0.0006
LVEF, per 18.1 % increase	0.68	0.52-0.90	0.0055
BNP, per 762 pg/ml increase	1.39	1.00-2.14	<0.0001
Estimated GFR, per 32.2 ml increase	0.58	0.43-0.80	0.0005
Uric acid, per 2.0 mg/dl increase	1.42	1.13-1.79	0.0028
Na, per 3.0 mEq/l increase	0.89	0.69-1.15	0.3718
hs-CRP, per 1.35 mg/dl increase	1.25	1.04-1.49	0.0164
TNF-α, per 1.02 pg/ml increase	1.50	1.20-1.87	0.0003
Pentraxin 3, per 6.22 ng/ml increase	1.33	1.19-1.51	<0.0001

Abbreviations as in Table 1

Table 5. Results of the Multivariate Cox Proportional Hazard Analysis

Variables	Hazard ratio	95% confidence interval	P value
Age, per 5-years increase	1.16	0.98-1.38	0.0904
LVEDD, per 9.8mm increase	1.47	1.06-2.05	0.0207
LVEF, per 18.1% increase	0.99	0.69-1.40	0.9467
BNP, per 762 pg/ml increase	1.25	1.00-2.14	0.0493
Estimated GFR, per 32.2 ml increase	1.12	0.70-1.83	0.6424
Uric acid, per 2.0 mg/dl increase	1.20	0.90-1.59	0.1989
hs-CRP, per 1.35 mg/dl increase	1.24	1.01-1.53	0.0418
TNF-α, per 1.02 pg/ml increase	1.16	0.88-1.53	0.2971
Pentraxin 3, per 6.22 ng/ml increase	1.20	1.03-1.40	0.0162

Abbreviations as in Table 1

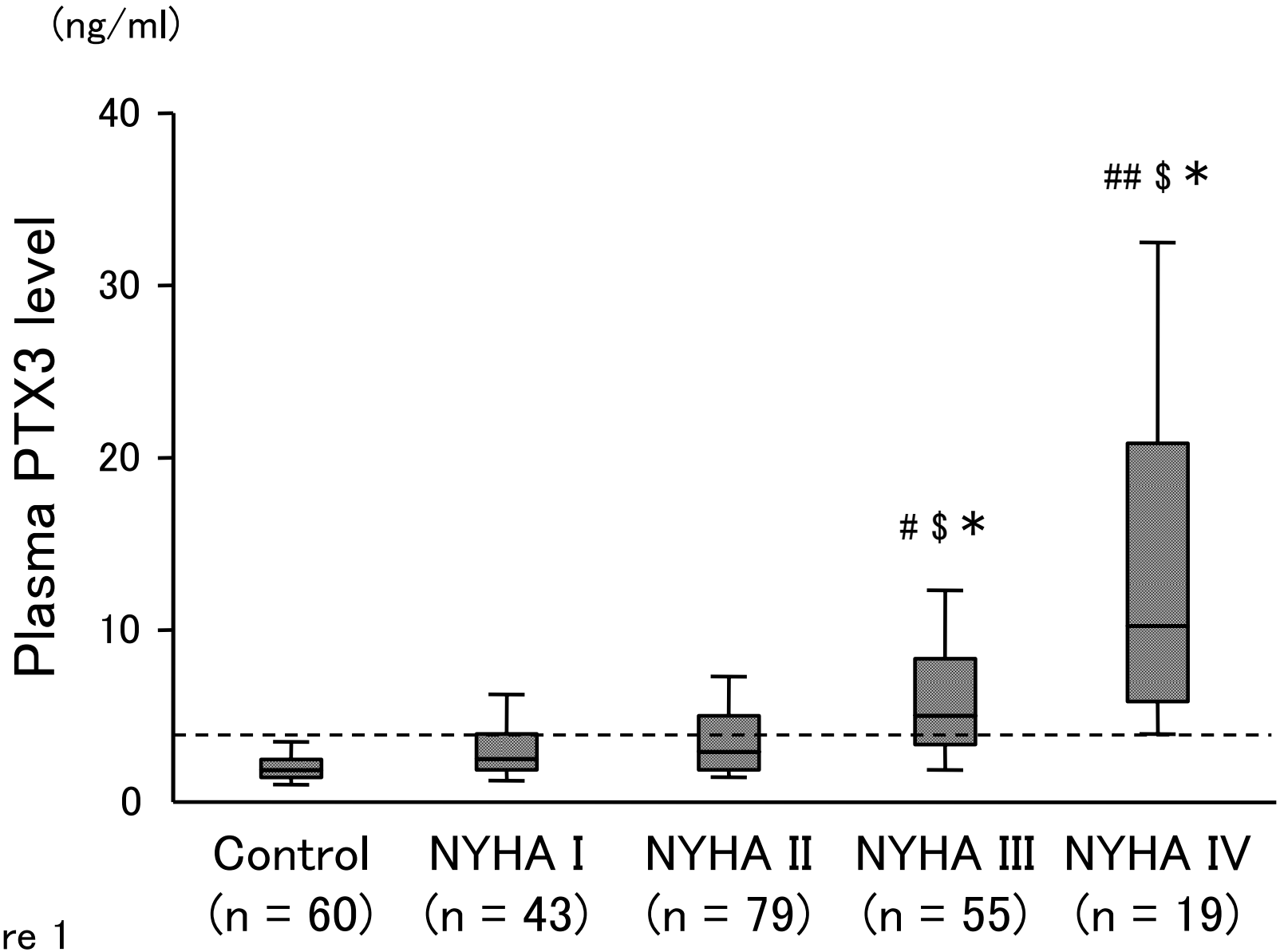

(ng/ml)
Plasma PTX3 level
40
30
20
10
0
$ *
$ *
Control
(n = 60)
NYHA I
(n = 43)
NYHA II
(n = 79)
NYHA III
(n = 55)
NYHA IV
(n = 19)

Figure 1

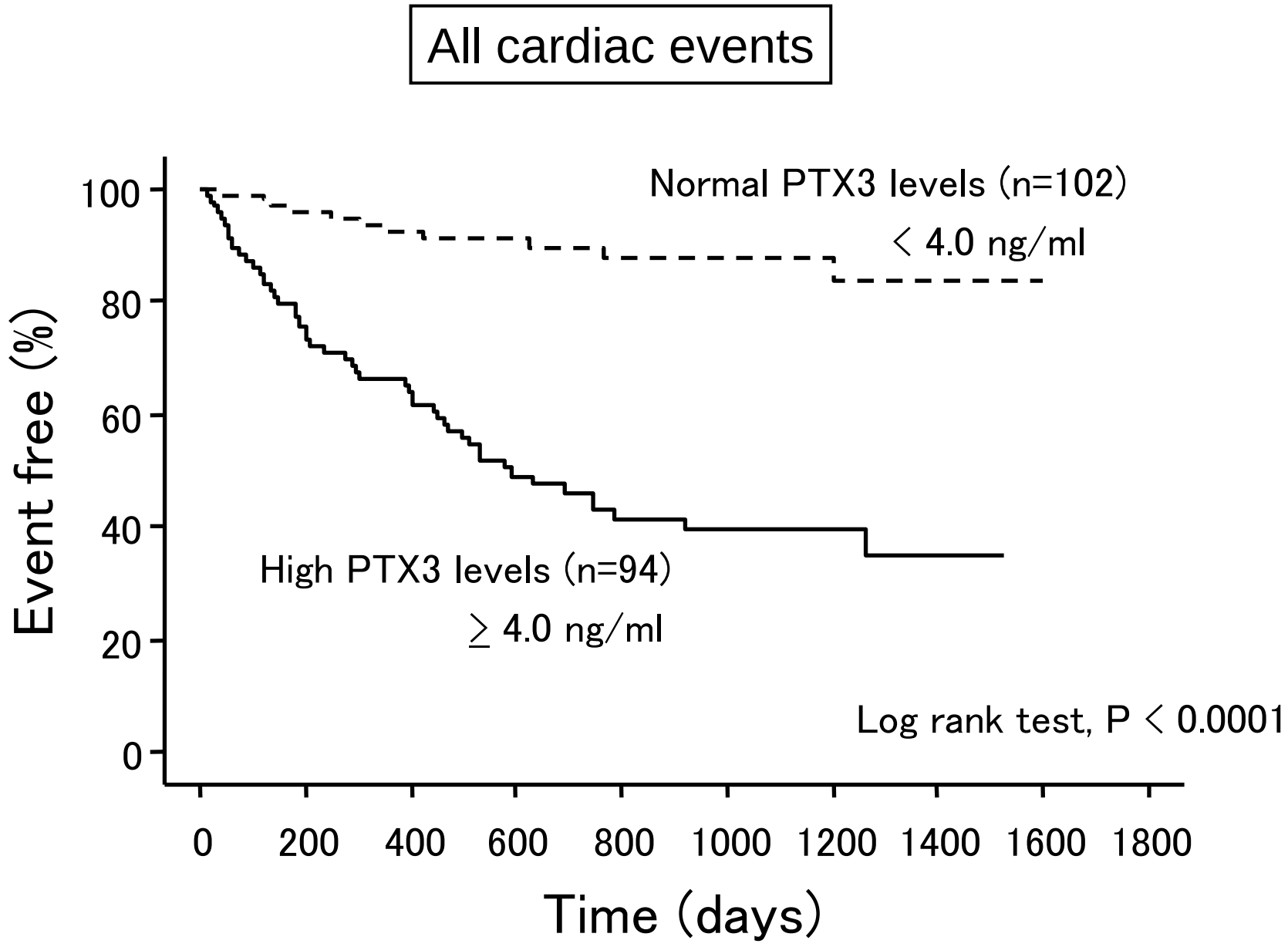

All cardiac events
Normal PTX3 levels (n=102)
< 4.0 ng/ml
High PTX3 levels (n=94)
≥ 4.0 ng/ml
Log rank test, P < 0.0001
Event free (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (days)

Figure 2A

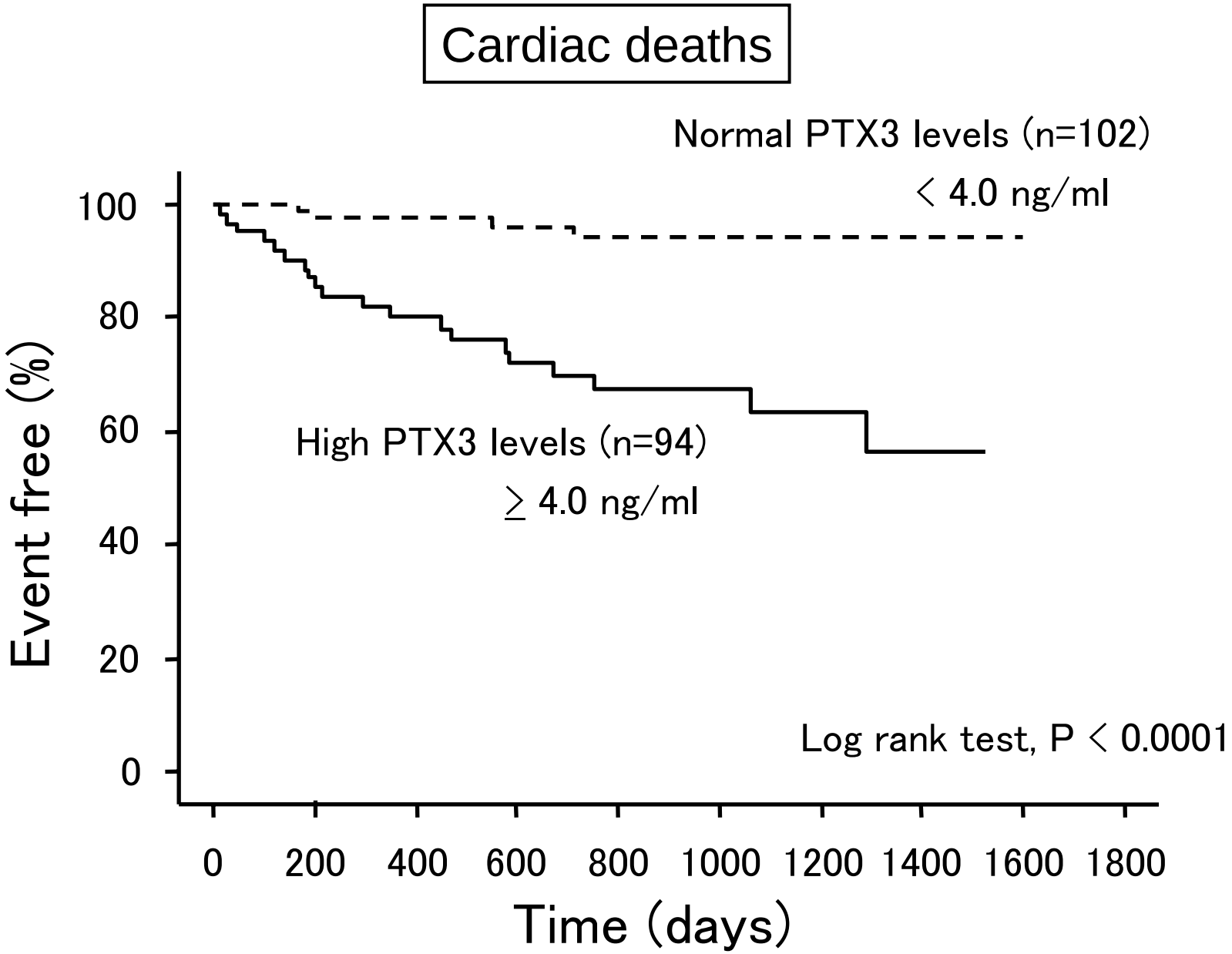

Cardiac deaths
Normal PTX3 levels (n=102)
< 4.0 ng/ml
High PTX3 levels (n=94)
≥ 4.0 ng/ml
Log rank test, P < 0.0001
Event free (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (days)

Figure 2B

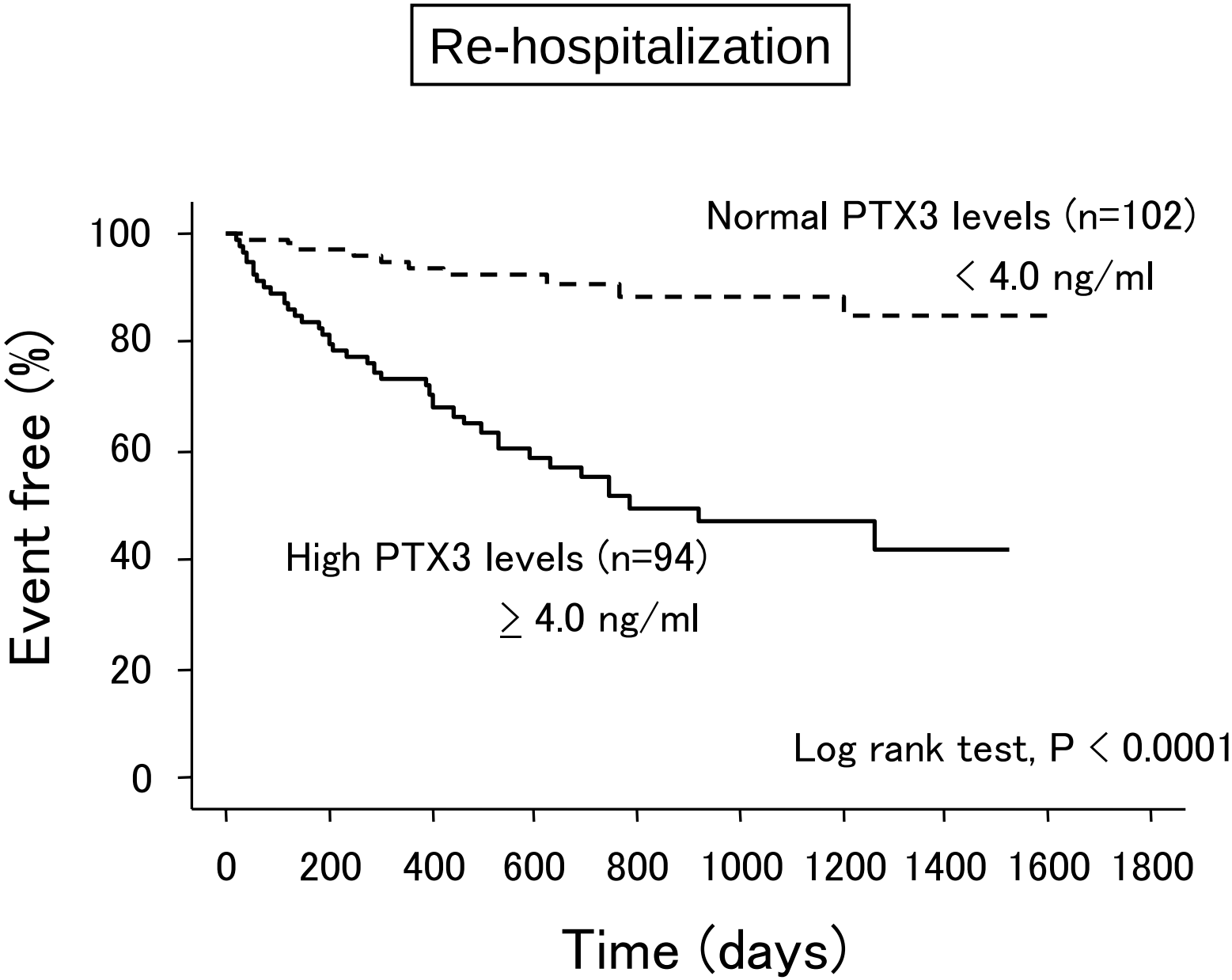
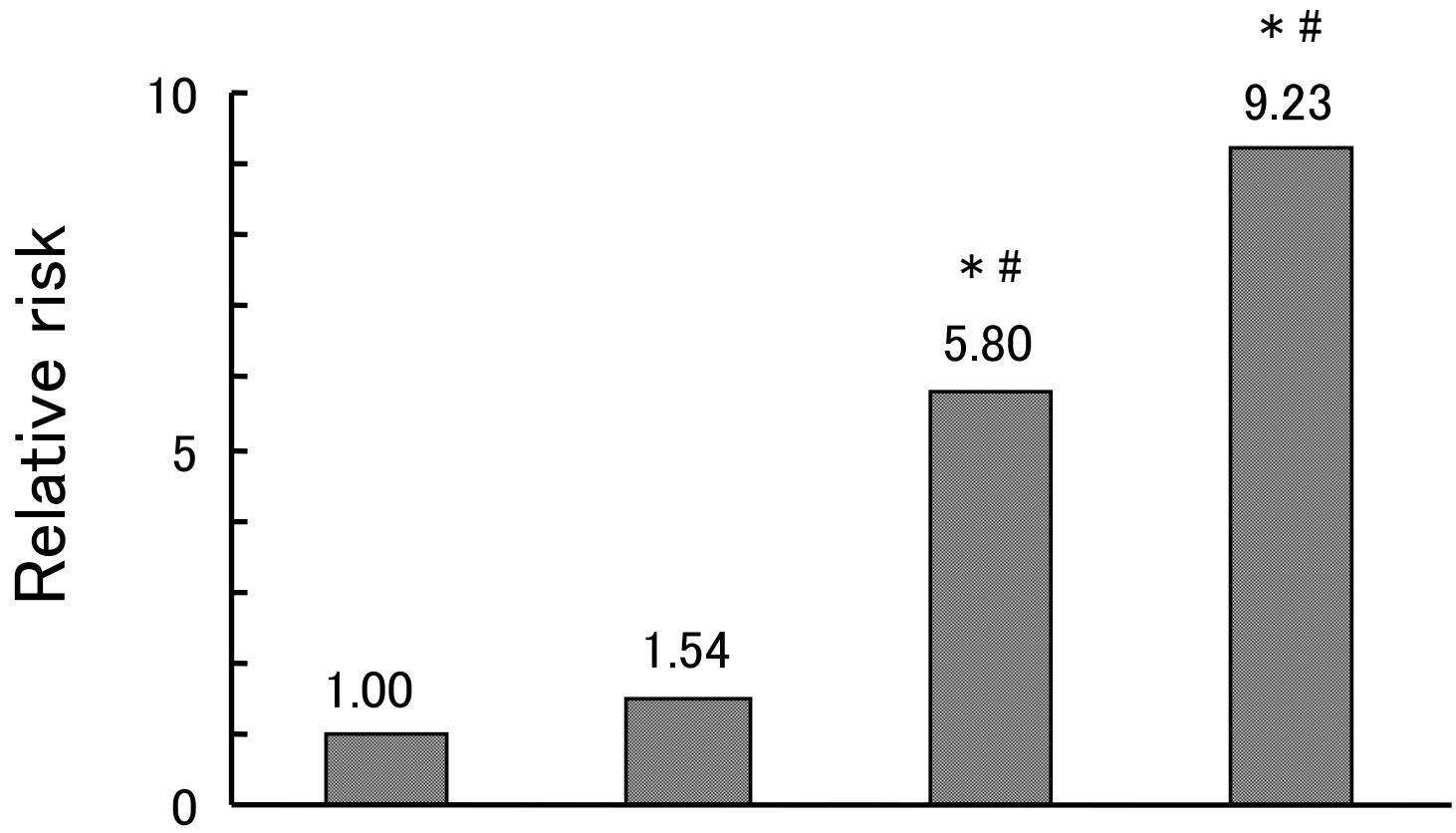

Re-hospitalization
Normal PTX3 levels (n=102)
< 4.0 ng/ml
High PTX3 levels (n=94)
≥ 4.0 ng/ml
Log rank test, P < 0.0001
Event free (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (days)

Figure 2C



	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile
Range of PTX3 (ng/ml)	(< 2.2)	(2.2–3.7)	(3.7–6.5)	(> 6.5)
All cardiac events	4/49	7/49	21/49	31/49

Figure 3

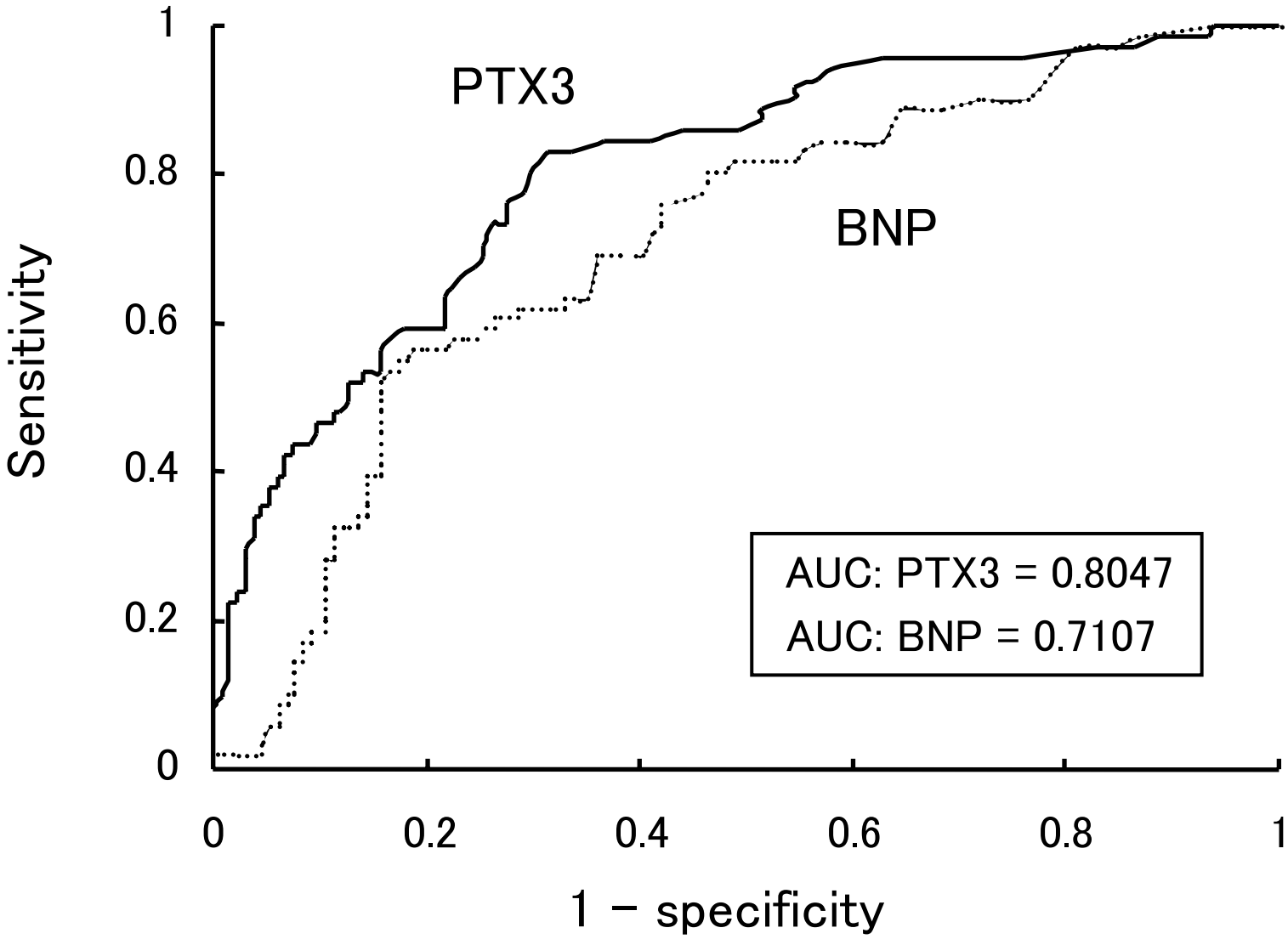

PTX3
BNP
AUC: PTX3 = 0.8047
AUC: BNP = 0.7107
Sensitivity
1 − specificity
0
0.2
0.4
0.6
0.8
1

Figure 4

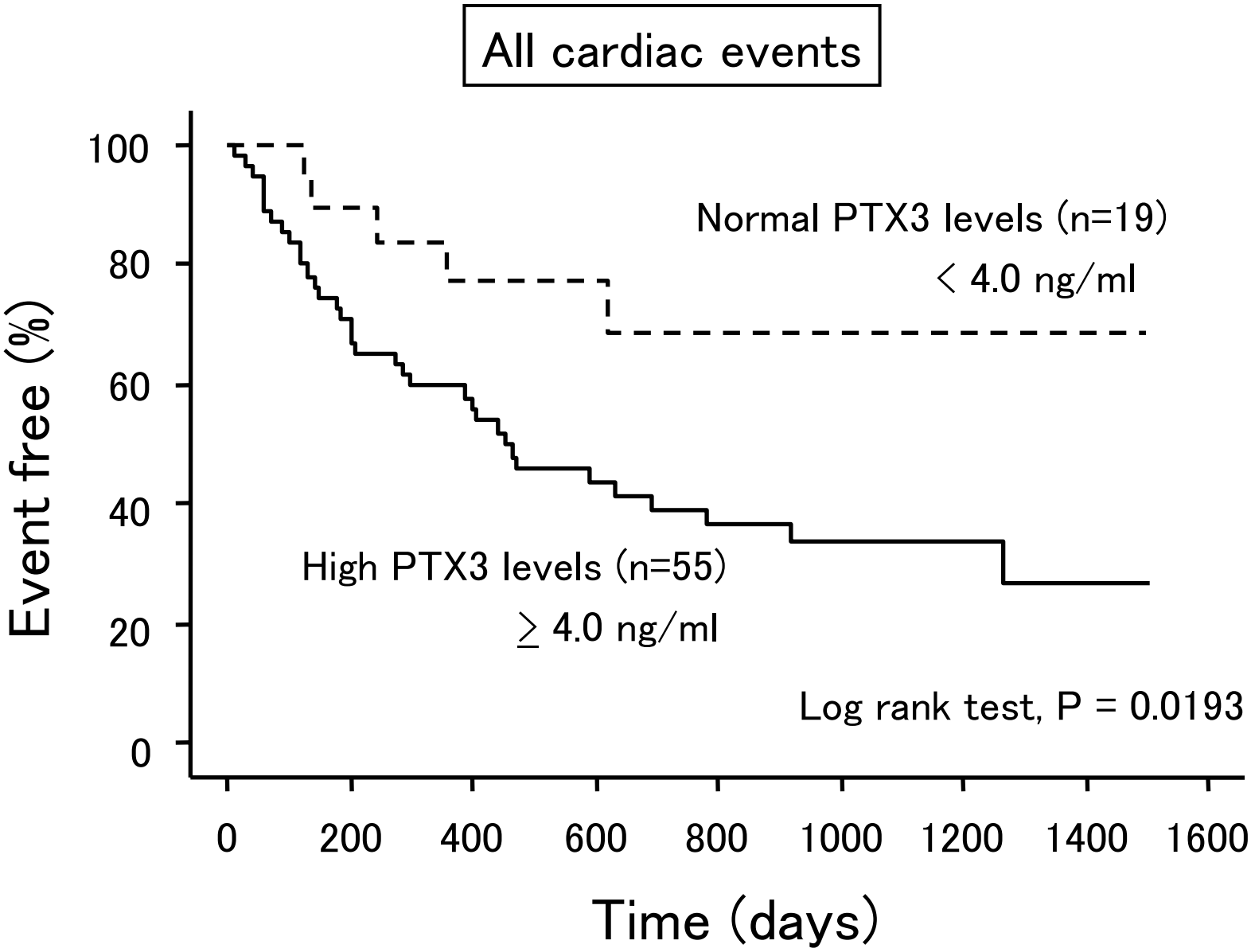

All cardiac events
Event free (%)
100
80
60
40
20
0
Normal PTX3 levels (n=19)
< 4.0 ng/ml
High PTX3 levels (n=55)
≥ 4.0 ng/ml
Log rank test, P = 0.0193
0
200
400
600
800
1000
1200
1400
1600
Time (days)

Figure 5