Serum Cystatin C as a Biomarker of Cardiac Diastolic Dysfunction in Patients with Cardiac Disease and Preserved Ejection Fraction

Kazumasa Nosaka[1,2]*, Kazufumi Nakamura[1]**, Kengo Kusano[1], Norihisa Toh[1], Takeshi Tada[1], Toru Miyoshi[1,3], Masayuki Doi[2], Kunihisa Kohno[1], Hiroshi Morita[1,3], Hiroshi Ito[1]

[1]Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.
[2]Department of Cardiology, Kagawa Prefectural Central Hospital, Takamatsu, Japan.
[3]Department of Cardiovascular Therapeutics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.

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Email: *kn_orch@yahoo.co.jp, **ichibun@cc.okayama-u.ac.jp

ABSTRACT

Diastolic dysfunction of the heart is correlated with cardiac mortality. Serum cystatin C (CysC) is an endogenous marker of kidney function. It is not clear whether serum CysC is associated with diastolic dysfunction in patients with varying cardiac conditions with concomitant diastolic abnormalities and preserved ejection fraction (EF). We measured serum CysC levels in patients with those cardiac diseases and examined the relationships between serum CysC levels and diastolic function. We measured serum CysC and performed echocardiography in 124 consecutive patients with those cardiac diseases. Trans mitral flow (TMF) patterns surrogating diastolic function were categorized into 2 groups: a normal group and an abnormal group. Serum CysC and BNP showed a significant positive correlation. There were no significant differences in serum CysC among those cardiac diseases. Seventy-eight patients with cardiac disease and preserved EF (LVEF $\geq$ 50%) and without renal dysfunction (eGFR $\geq$ 60mL/min/1.73m^2) were examined. Multivariate linear regression analysis demonstrated that left atrium diameter and abnormal TMF patterns were independent determinants of serum CysC. Furthermore, patients with elevated serum CysC had poor prognosis. Serum CysC is associated with diastolic dysfunction in patients with various cardiac diseases and preserved EF. Serum CysC might be a biomarker of cardiac diastolic dysfunction in patients with preserved EF.

INTRODUCTION

Diastolic dysfunction, or heart failure preserved ejection fraction, is independently correlated with cardiac mortality [1-4]. However, a diagnostic method has not been established and its diagnosis is often difficult. Therefore, a reliable biomarker for its diagnosis is needed.

Worsening renal function also increases mortality and hospitalization, known as cardio-renal syndrome [5-7]. Cystatin C (CysC) is a novel endogenous marker of kidney function that obviates many of the limitations of serum creatinine as an endogenous marker of glomerular filtration rate (eGFR) [8-10]. Serum concentrations of CysC appear to be unaffected by age, sex, or muscle mass and are more sensitive to mild decrements in GFR than are serum concentrations of creatinine. Recently, high CysC concentrations were shown to be associated with increased incidence of systolic and diastolic heart failure in a community-based cohort [11] and to be associated with left ventricular diastolic dysfunction in patients with coronary artery disease and without heart failure [12] . Therefore, serum CysC may be a useful surrogate marker of diastolic dysfunction. However, it is not clear whether serum CysC is influenced by disease specificity and whether serum CysC is associated with diastolic dysfunction in patients with varying cardiac conditions with concomitant diastolic abnormalities and preserved ejection fraction (EF). We therefore measured serum CysC levels in patients with various cardiac diseases and examined the relationships between serum CysC levels and diastolic function.

MATERIALS AND METHODS

Subjects

We studied consecutive patients with 5 cardiac diseases including coronary artery disease, arrhythmias, cardiomyopathy, congenital heart disease, and valvular diseases who had been admitted to Okayama University Hospital (Okayama, Japan) during the period from January 2008 to May 2009. We excluded patients with acute coronary syndrome and acute heart failure. We measured serum levels of CysC, serum creatinine, thyroid-stimulating hormone (TSH), and brain natriuretic peptide (BNP). The patients underwent transthoracic echocardiography at rest on the same day. eGFR (mL/min/1.73m^2) was determined by the modified Modification of Diet and Renal Disease study formula (MDRD) for Japanese : eGFR = $194 \times (\text{age}^{-0.287}) \times (\text{serum creatinine}^{-1.094}) \times (0.739 \text{ if female})$ [13]. Apparent renal dysfunction was defined as eGFR < 60mL/min/1.73m^2. Informed consent was obtained from all

patients and this study was approved by our Institutional Review Board. The investigation also conforms to the principles outlined in the Declaration of Helsinki.

Transthoracic echocardiography

Echocardiographic values were used to determine cardiac function. Echocardiologists were blinded to all of the patients' characteristics including results of blood tests. Echocardiographic studies were performed with Vivid 7 (GE, Milwaukee, Wisconsin) as previously described [14]. We obtained standard 2-dimensional parasternal long axis and apical 2- and 4-chamber views. Left ventricular diastolic dimension (LVDd), left ventricular systolic dimension (LVDs), and left atrium diameter (LAD) were measured by a parasternal long axis view. The ratio of mitral early inflow pattern (E) and atrial inflow pattern (A) and the value of early diastolic velocity derived from the septal mitral annulus by tissue doppler (E') were used as surrogates of diastolic function. Trans mitral flow (TMF) patterns were categorized into two groups by E/A ratio, mitral E velocity deceleration time (DT) and flow patterns of pulmonary veins (PV). A normal TMF pattern ($0.75 <$ E/A ratio < 1.5, DT > 140 msec and dominant systolic PV flow) was categorized into the normal group. An impaired relaxation pattern (E/A ratio < 0.75 and dominant systolic PV flow), pseudonormalization pattern ($0.75 <$ E/A ratio <1.5, DT > 140 msec and dominant diastolic pulmonary vein flow), restrictive pattern (E/A > 1.5, DT < 140 msec, and dominant diastolic pulmonary vein flow) were categorized into an abnormal group [2]. We defined LVEF of less than 50% as apparent cardiac systolic dysfunction.

Laboratory tests

Serum CysC was measured by an immunologic turbid metric assay (Nescoat GC Cystatin C, Alfresa Pharma, Japan) with reference ranges of 0.63 to 0.95 mg/L for men and 0.56 to 0.87 mg/L for women. BNP was measured by a standardized and widely validated immunonephelometric method (E-test TOSOH II, TOSOH, Japan) with a reference range from less than 18.4 pg/mL. Other laboratory parameters were measured using standard laboratory techniques with an automatic analyzer.

 Serum CysC is greatly influenced by thyroid function [15, 16]. We therefore measured TSH levels. If TSH level was out of the normal range (< 0.33 μg/mL or > 4.05 μg/mL), we excluded the patients even if the patients had no symptoms of thyroid dysfunction.

Statistical analysis

Data are expressed as means ± SD. Linear regression analysis was used to evaluate the associations between serum CysC, eGFR and echocardiographic values (LVEF, LVDd, LVDs, LAD, E/A ratio, E', and TMF patterns). The Mann-Whitney test and was used to evaluate differences among 2 TMF pattern groups. For comparison between 4 different TMF groups, statistical analysis was performed using one-way ANOVA with Bonferroni Dunn test. Statistical significance was defined as $P < 0.05$.

RESULTS

Patients' characteristics and levels of serum CysC

One hundred and fifty patients were admitted during the study period. Since serum CysC is influenced by thyroid function [15, 16], we excluded 26 patients with thyroid dysfunction; 5 patients had hyperthyroidism (TSH < 0.33 μg/mL) and 21 patients had hypothyroidism (TSH > 4.05 μg/mL). We evaluated 124 patients with cardiac disease and with normal thyroid function (Table I).

We classified all patients into 5 disease groups according to their cardiac disease: coronary artery disease, arrhythmias, cardiomyopathy, congenital heart disease, and valvular disease. There were no significant differences in serum CysC among the 5 disease groups (P = NS) (Figure 1). Serum CysC and eGFR showed a significant negative correlation (r = -0.71, P < 0.001) (Figure 2A), and serum CysC and BNP showed a significant positive correlation (r = 0.43, P < 0.001) (Figure 2B). Serum CysC levels were not influenced by disease specificity in this study group.

Relationships between CysC and echocardiac parameters in patients with cardiac disease

Results of univariate and multivariate linear regression analyses of relationships between CysC and echocardiac parameters are shown in Table II. In univariate analysis, LVDd, LVDs, LVEF, LAD, E/A, E' and abnormal TMF patterns were significantly associated with serum CysC. Moreover, multivariate regression analysis demonstrated that abnormal TMF patterns were independently correlative to elevated levels of serum CysC (β = 0.286, P < 0.01). There was a significant difference in serum CysC between normal and abnormal groups of TMF patterns (P < 0.001) and there were significant differences in serum CysC between patients with normal TMF pattern and patients with impaired relaxation pattern, pseudonormalization pattern or restrictive pattern (P < 0.01) (Figure 3). There was no significant difference among abnormal TMF groups.

Relationships between serum CysC and echocardiac parameters in patients with cardiac disease

and preserved ejection fraction and without renal dysfunction

Seventy-eight patients with cardiac disease and preserved ejection fraction (LVEF ≥ 50%) and without renal dysfunction (eGFR ≥ 60mL/min/1.73m^2) were examined (Table III). Table IV shows relationships between serum CysC and echocardiac parameters in the 78 patients. In univariate linear regression analysis, LAD, E/A, E' and TMF patterns, surrogates of cardiac diastolic function, were significantly associated with serum CysC. However, LVDd, LVDs and LVEF, surrogates of cardiac systolic function, were not associated with serum CysC. Multivariate linear regression analysis demonstrated that LAD and abnormal TMF patterns were independent correlates of serum CysC (LAD: β = 0.362, P < 0.01; abnormal TMF patterns: β = 0.328, P < 0.05).

Prognostic Significance of Serum Cystatin C

Serum CysC is also correlated with prognosis in the patients with cardiac diseases. We defined the combined end point as all-cause mortality and admission for heart failure. The median follow up period was 24 months (range: 3-36 months). In the Kaplan-Meier survival curves (Figure 4), there were significant differences between normal CysC patients and higher CysC patients (Men; > 0.95mg/dL, Women; > 0.87mg/dL). Patients with elevated serum CysC had poor prognosis.

DISCUSSION

The major new findings of this work are that serum CysC was independently correlative to diastolic parameters even in the setting of normal kidney function, that serum CysC was not influenced by disease specificity, and that serum CysC was associated with diastolic dysfunction in patients with varying cardiac conditions with concomitant diastolic abnormalities and preserved ejection fraction and without renal dysfunction.

 CysC is regulated in cardiac remodeling. Cheng et al. reported that CysC mRNA and protein levels are increased in hearts of rats and humans with hypertension-induced left ventricular hypertrophy (LVH) [18]. The precise mechanisms are not known, but these regulations may be associated with elevation of CysC in patients with diastolic dysfunction.

 Furthermore, patients with elevated serum CysC have poor prognosis. Previous studies showed that CysC was associated with the incidence of systolic and diastolic heart failure in a community-based cohort [11] and that CysC was associated with more advanced left ventricular diastolic dysfunction in patients with coronary artery disease and without

heart failure [12] and in patients with chronic systolic heart failure [17]. Our study also suggested that CysC will become a surrogate biomarker of cardiac diastolic dysfunction in patients with various cardiac diseases and preserved ejection fraction.

There were no significant differences in serum CysC among various cardiac diseases including coronary artery disease, arrhythmias, cardiomyopathy, congenital heart disease, and valvular disease. However, CysC was significantly correlated with eGFR and BNP. Therefore, serum CysC may be a common biomarker of cardio-renal dysfunction in patients with various cardiac diseases.

CysC is correlated with abnormal TMF patterns showing cardiac diastolic dysfunction in patients with various cardiac diseases and preserved ejection fraction and without renal dysfunction. The mechanism of elevation of CysC in those patients remains unclear. Worsening of heart function leads to kidney injury and/or dysfunction [7]. Elevation of CysC in those patients may reflect a small change in kidney function due to cardiac dysfunction. Nejat et al. showed that some biomarkers of acute kidney injury (AKI) including serum CysC were increased in pre-renal acute injury [19]. Further studies are needed to clarify this point.

In conclusion, serum CysC was shown to be associated with diastolic dysfunction in patients with varying cardiac conditions with concomitant diastolic abnormalities and preserved ejection fraction and without renal dysfunction. Patients with elevated serum CysC had poor prognosis. These results suggest that serum CysC is a biomarker of cardiac diastolic dysfunction in patients with preserved ejection fraction.

STUDY LIMITATIONS

This study was started in January 2008, and we therefore evaluated diastolic function by using the algorithm in 2003 [2]. Further studies are needed to evaluate diastolic function by new algorithms.

The subjects of this study included subjects with a current smoking habit and subjects using glucocorticoids, which have been reported to cause false elevation of serum CysC [20, 21]. Careful assessment is needed to use serum CysC as a biomarker of cardiac diastolic function.

DISCLOSURERS

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TABLE I. Patients' Characteristics

Number of Patients	124
Demographics	
Age, years	62 ± 16
Male, n (%)	78 (62)
Hypertension, n (%)	71 (57)
Diabetes Mellitus, n (%)	26 (21)
Dyslipidemia, n (%)	69 (56)
Current smoking, n (%)	20 (16)
Heart failure, n (%)	9 (7)
NYHA I/II/III/IV	1/5/3/0
Cardiac diseases	
Coronary artery disease, n (%)	58 (47)
CSS I/II/III/IV	38/18/2/0
OMI, n (%)	11 (19)
Arrhythmias, n (%)	39 (31)
Cardiomyopathy, n (%)	10 (8)
Congenital heart disease, n (%)	10 (8)
Valvular disease, n (%)	7 (6)
Medications	
Aspirin, n (%)	43 (35)
ACEI/ARBs, n (%)	50 (40)
β-blockers, n (%)	40 (32)
CCBs, n (%)	36 (29)
Diuretics, n (%)	33 (27)
Statins, n (%)	37 (30)
Glucocorticoids, n (%)	5 (4)
Measurements	
eGFR (mL/min/1.73m^2)	68 ± 21
Serum creatinine (mg/dL)	0.84 ± 0.26
Serum cystatin C (mg/L)	1.06 ± 0.36
BNP (pg/mL)	106 ± 192
Echocardiographic measurements	
LVEF (%)	64 ± 12
LVDd (mm)	48 ± 8
LVDs (mm)	31 ± 10
LAD (mm)	39 ± 9

E/A	1.0 ± 0.5
E' (cm/sec)	6.8 ± 2.8

Data are shown as the mean ± SD or as number (percentage). eGFR, estimated glomerular filtration rate; BNP, plasma concentration of brain natriuretic peptide; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic diameter; LVDs, left ventricular systolic diameter; LAD, left atrium diameter; E/A, ratio of early to late mitral valve flow velocity; E', early diastolic velocity of the mitral annulus.

TABLE II. Univariate and multivariate regression analyses for serum CysC in patients with cardiac disease

Variable	Univariate Analysis		Multivariate Analysis	
	r	P	β	P
LVDd (mm)	0.232	< 0.05	0.013	0.70
LVDs (mm)	0.269	< 0.01	-0.025	0.63
EF (%)	-0.266	< 0.01	-0.017	0.39
LAD (mm)	0.274	< 0.01	0.002	0.71
E/A	-0.260	< 0.01	-0.018	0.83
E' (cm/sec)	-0.286	< 0.01	-0.003	0.87
TMF patterns	0.410	< 0.001	0.286	< 0.01

Multivariate analysis included significant factors (P < 0.05) of univariate analysis. LVDd, left ventricular diastolic diameter; LVDs, left ventricular systolic diameter; LVEF, left ventricular ejection fraction; LAD, left atrium dimension; E/A, ratio of early to late mitral valve flow velocity; E', early diastolic velocity of the mitral annulus; TMF patterns, trans mitral flow patterns.

TABLE III. Patients with cardiac disease and preserved ejection fraction and without renal dysfunction

Number of Patients	78	P values (compare with all patients)
Age, years	58 ± 17	NS
Male, n (%)	52 (67)	NS
Hypertension, n (%)	36 (46)	NS
Diabetes mellitus, n (%)	13 (17)	NS
eGFR (mL/min/1.73m^2)	84 ± 18	< 0.001
Serum creatinine (mg/dL)	0.71 ± 0.13	< 0.001
Serum cystatin C (mg/L)	0.88 ± 0.17	< 0.001
BNP (pg/mL)	53 ± 74	< 0.01
LVEF (%)	69 ± 9	< 0.01
LVDd (mm)	46 ± 6	< 0.05
LVDs (mm)	28 ± 6	< 0.01
LAD (mm)	37 ± 8	NS
E/A	1.1 ± 0.6	NS
E' (cm/sec)	7.2 ± 3.0	NS

Data are shown as the mean $\pm$ SD or as number (percentage). eGFR, estimated glomerular filtration rate; BNP, plasma concentration of brain natriuretic peptide; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic diameter; LVDs, left ventricular systolic diameter; LAD, left atrium diameter; E/A, ratio of early to late mitral valve flow velocity; E', early diastolic velocity of the mitral annulus; NS, not significant.

TABLE IV. Univariate and multivariate regression analyses for serum cystatin C in patients without apparent renal and systolic cardiac dysfunction

Variable	Univariate Analysis		Multivariate Analysis	
	r	P	β	P
LVDd (mm)	0.029	0.80		
LVDs (mm)	-0.038	0.74		
EF (%)	0.082	0.48		
LAD (mm)	0.460	< 0.001	0.362	< 0.01
E/A	-0.360	< 0.01	-0.101	0.49
E' (cm/sec)	-0.441	< 0.001	0.023	0.89
Abnormal TMF patterns	0.491	< 0.001	0.328	< 0.05

Multivariate analysis included significant factors (P < 0.05) of univariate analysis. LVDd, left ventricular diastolic diameter; LVDs, left ventricular systolic diameter; LVEF, left ventricular ejection fraction; LAD, left atrium dimension; E/A, ratio of early to late mitral valve flow velocity; E', early diastolic velocity of the mitral annulus; TMF patterns, trans mitral flow patterns.
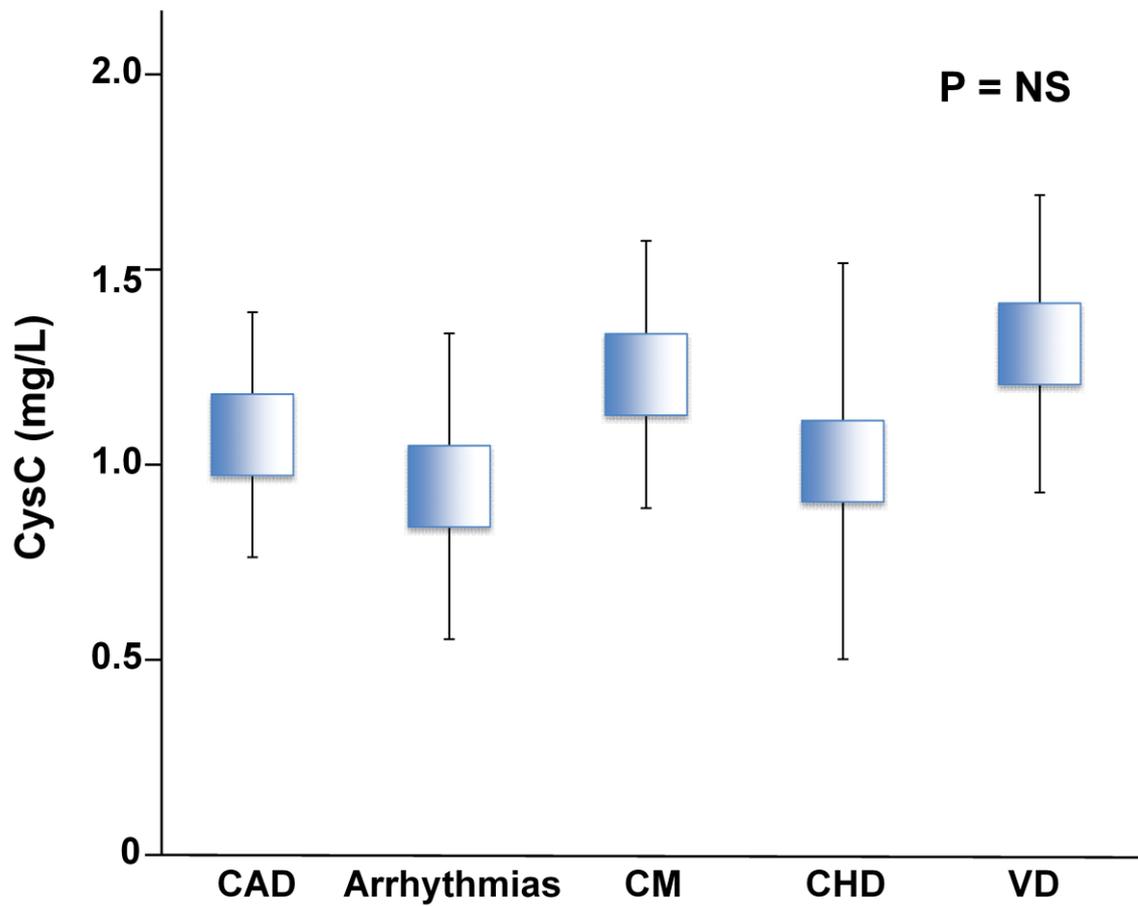
Figure 1. Distribution of serum CysC levels among diseases. Patients were classified into 5 groups by their cardiac disease: coronary artery disease (CAD), arrhythmias, cardiomyopathy (CM), congenital heart disease (CHD), and valvular disease (VD). Analysis of variance showed no significant differences in serum CysC levels (P=NS). Data are means ± SD.
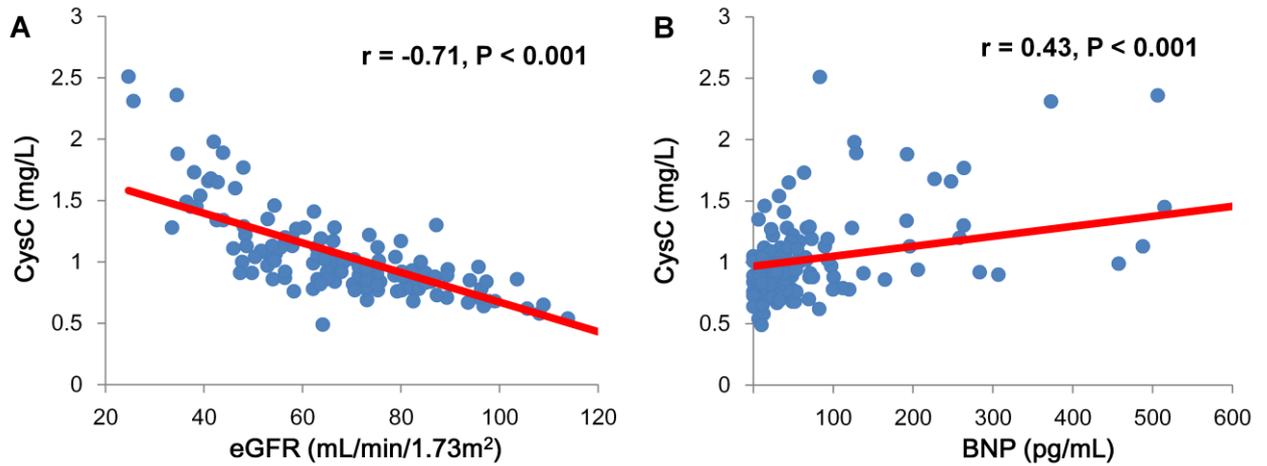
Figure 2. Relationships of serum CysC with eGFR and BNP. A and B show significant correlations between serum CysC and serological markers by regression analysis. A shows a negative significant correlation between serum CysC and eGFR (r = -0.71, P < 0.001). B shows a positive significant correlation between serum CysC and BNP (r = 0.43, P < 0.001).
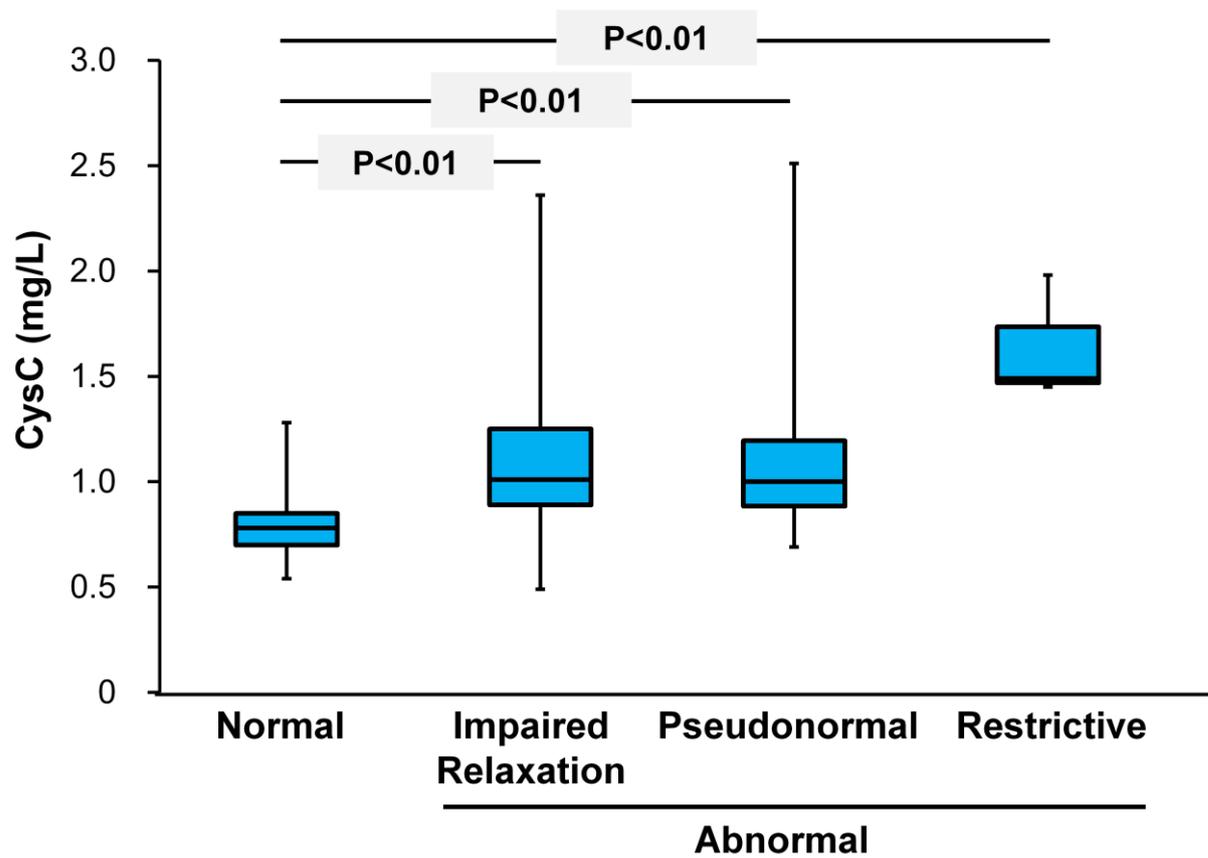
Figure 3. Serum levels of CysC in groups of TMF patterns. Trans mitral flow (TMF) patterns surrogating diastolic function were categorized into two groups by E/A ratio, mitral E velocity deceleration time and flow patterns of pulmonary veins: a normal group and an abnormal group including impaired relaxation, pseudonormalization and restrictive pattern. Serum CysC level was significantly higher in the impaired relaxation, pseudonormalization and restrictive pattern groups than in the normal pattern group ($P < 0.01$).
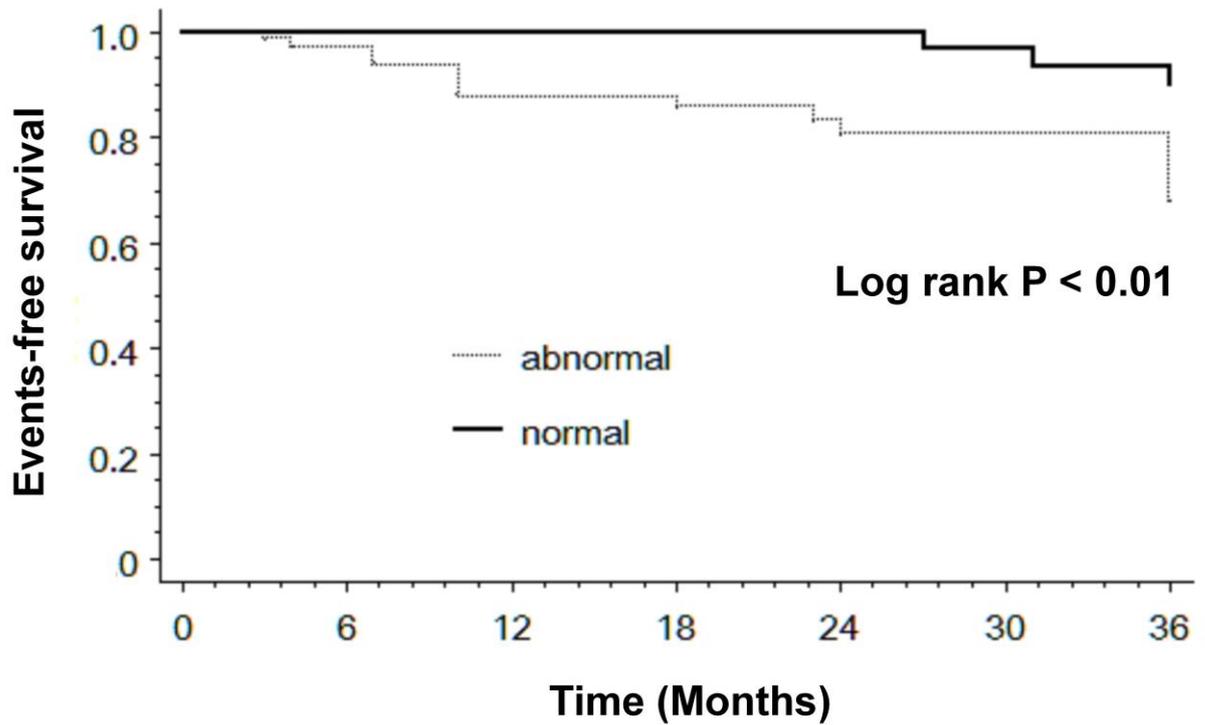
Figure 4. All-cause mortality and admission for heart failure between normal CysC patients and higher CysC patients. Kaplan-Meier curves for higher CysC patients (Men; > 0.95mg/dL, Women; > 0.87mg/dL) and normal CysC patients. Mortality and/or admission for heart failure at 3 years 94.5% versus 77.9%. Log rank P < 0.01.