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Circulating Aminoterminal Propeptide of Type Ⅲ Procollagen as a Biomarker of Cardiovascular Events in Patients Undergoing Hemodialysis

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Aim: Type Ⅲ collagen abundantly exists in the cardiovascular system, including the aorta and heart. We prospectively investigated whether serum levels of aminoterminal propeptide of type Ⅲ procollagen (PⅢNP), a circulating biomarker of cardiovascular fibrosis, could predict cardiovascular events in patients undergoing hemodialysis.

Methods: Serum PⅢNP concentrations were measured in 244 patients undergoing maintenance hemodialysis (men, 126; women, 118; mean age, 64 ± 11 years; dialysis duration, 11.5 ± 7.8 years) by immunoradiometric assay in February 2005. The endpoint was cardiovascular events, and the patients were followed up until the endpoint was reached, or until January 31, 2011.

Results: During the follow-up for 4.7 ± 1.8 years, cardiovascular events occurred in 78 (30.3%) of 244 patients. Stepwise Cox hazard analysis revealed that cardiovascular events were associated with increased serum PⅢNP concentration (1 U/mL; hazard ratio, 1.616; $P=0.0001$). The median serum PⅢNP concentrations were higher in patients with cardiovascular events than in those without (2.30 ± 0.19 U/mL vs 1.30 ± 0.03 U/mL; $P<0.0001$). When the patients were assigned to subgroups based on serum PⅢNP cut-off value for cardiovascular events of 1.75 U/mL, defined by receiver operating characteristic analysis, cardiovascular event-free survival rates at 5 years were lower ($P=0.0001$) in the subgroup of serum PⅢNP ≥ 1.75 U/mL than in that of serum PⅢNP < 1.75 U/mL (31.9% vs 88.2%).

Conclusions: Serum PⅢNP could be a new biomarker for predicting the cardiovascular events in patients undergoing hemodialysis.

Key words: Aortic stiffness, Cardiovascular fibrosis, Hemodialysis, Left ventricular hypertrophy, Procollagen

Introduction

Fibrillar collagen is synthesized in cardiac fibroblasts as a procollagen[1]. The myocardium and coronary artery comprise type I and Ⅲ collagen as major fibrillar collagens. Increased collagen turnover plays an important role in determining the functional properties of the arterial vasculature and ventricular myocardium[2], and that in the coronary artery contributes to coronary intimal thickening and progression of coronary plaque. In human aorta, type I, Ⅲ, and V are the main collagens; particularly, more type Ⅲ than type I collagen is found in the aortic media[3]. The aminoterminal propeptide of type Ⅲ procollagen (PⅢNP) is an extension peptide of type Ⅲ procollagen, which is cleaved stoichiometrically during conversion from type Ⅲ procollagen to type Ⅲ collagen and liberated into the serum; serum PⅢNP is thought to be a biomarker of collagen type Ⅲ synthesis[4]. Among the many circulating molecules proposed as biomarkers of myocar-

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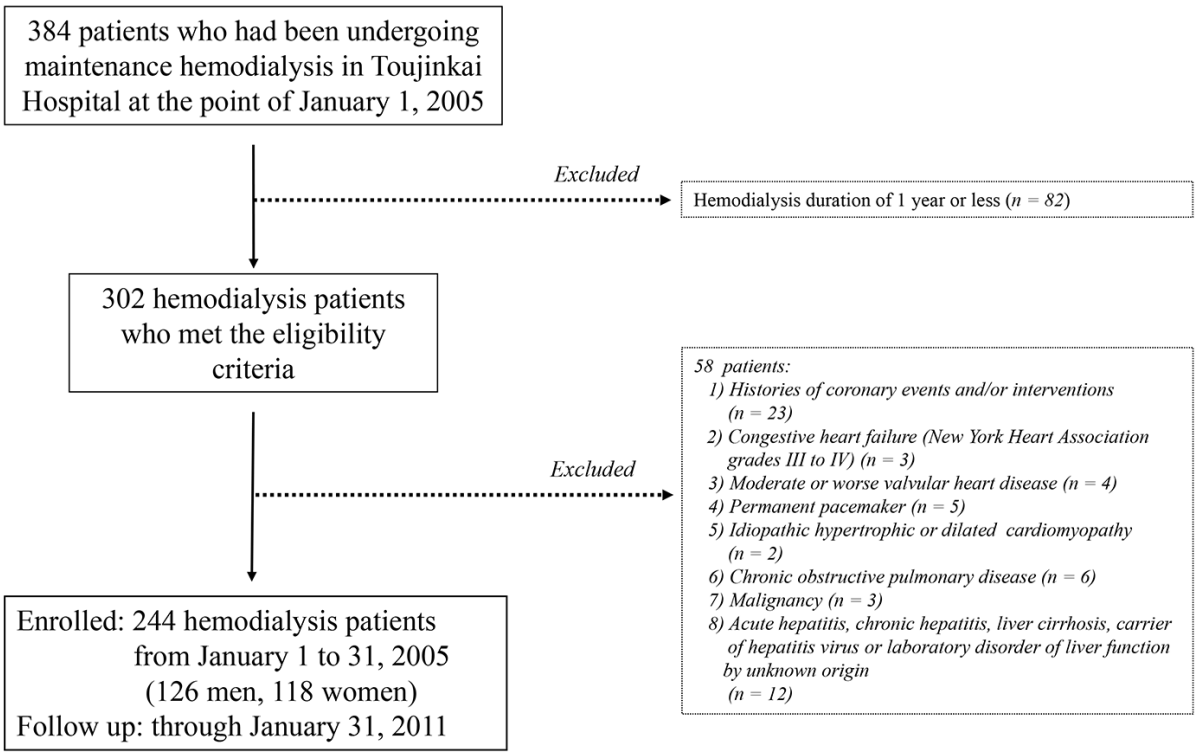

Fig. 1. Flow diagram of subject recruitment.

dial fibrosis in humans, only PⅢNP and the carboxy-terminal propeptide of type I procollagen (CIPC), formed during the extracellular conversion of type I procollagen into mature fibril-forming type I collagen, have been shown to be associated with myocardial fibrosis[5-8].

Aim

Left ventricular hypertrophy (LVH) and arterial stiffness, which significantly contribute to the occurrence of cardiovascular events, are the common findings in patients undergoing hemodialysis[9, 10]. Fibrosis in the cardiovascular system is involved in remodeling of the heart and vascular system, and it plays an important role in the genesis of LVH and arterial stiffness. Circulating PⅢNP, a biomarker of type Ⅲ collagen synthesis, has been shown to predict prognosis in patients with heart failure and ischemic heart disease[11, 12] or in the general population with idiopathic dilated cardiomyopathy[5, 6, 13]. In a previous study, the extent of myocardial fibrosis was associated with mortality in patients with dilated cardiomyopathy and heart failure undergoing hemodialysis[14]. Because patients undergoing hemodialysis have higher cardiovascular risks such as LVH or aortic stiffness than the general population, increased fibrosis in the cardiovascular system may cause long-term cardiovascular events, even in the absence of heart failure or dilated cardiomyopathy. In the present study, we aimed to prospectively investigate whether the serum levels of PⅢNP could predict the long-term cardiovascular events in patients without apparent heart failure or cardiomyopathy undergoing maintenance hemodialysis.

Methods

Patients

Fig. 1 is the flow diagram of subject recruitment. Patients with end-stage kidney disease undergoing maintenance hemodialysis for more than 1 year in Toujinkai Hospital, Japan, at the point of January 1, 2005, who met the inclusion criteria, were eligible for this study. Of the 384 patients undergoing hemodialysis, 302 met the eligibility criteria. Exclusion criteria were as follows: a history of coronary events and/or interventions; congestive heart failure of New York Heart Association grades Ⅲ to Ⅳ; moderate or worse valvular heart disease (aortic or mitral valvular areas ≤ 1.5 cm^2 for aortic or mitral stenosis, and Sellers grades Ⅲ or Ⅳ for aortic or mitral regurgitation); permanent pacemaker implantation; idiopathic hypertrophic or dilated cardiomyopathy; chronic obstructive pulmonary disease; malignancy; and acute hepatitis, chronic hepatitis, liver cirrhosis, or

carrier of hepatitis virus, or laboratory disorder of liver function by unknown origin. Of 302 patients who met the eligibility criteria, 58 were excluded based on the exclusion criteria. Consequently, 244 patients (men, 126; women, 118; mean age, 64 ± 11 years; dialysis duration, 11.5 ± 7.8 years) were enrolled in the study from January 1 to 31, 2005, and followed through January 31, 2011. Blood pressure was measured hourly during dialysis using a mercury sphygmomanometer; for study purposes, blood pressure was determined as the mean of the measurements obtained at the beginning of eight consecutive midweek hemodialysis sessions before enrollment. Histories of cigarette smoking and alcohol consumption were determined by a questionnaire. A smoking habit was defined as smoking 10 or more cigarettes per week. Alcohol consumption was defined as alcohol intake three times or more per week. The Ethics Committee for Human Research of Toujinkai Hospital approved this study. All patients provided written informed consent to all procedures associated with the study before participation. The study was performed in accordance with the principles of the Declaration of Helsinki and registered in the *ClinicalTrials.gov* (https://clinicaltrials.gov/): protocol identifier, NCT03391128.

Measurement of PⅢNP

Blood was collected for determination of serum PⅢNP concentration just before starting the first hemodialysis session of the week after the enrollment. Serum PⅢNP was determined by immunoradiometric assay (RIA-gnost PⅢP c.t., CIS Bio International, Saclay, France). The intra- and interassay coefficients of variation for this assay were 1.4%–2.9% and 2.5%–3.1%, respectively.

Biochemical and Hematological Determinations

Other blood samples were collected at the same hemodialysis sessions for the sampling of PⅢNP. We measured blood hemoglobin levels; serum concentrations of aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, total cholesterol, high-density lipoprotein cholesterol, triglyceride, albumin, calcium, inorganic phosphorus, intact parathyroid hormone, β_2 microglobulin, high-sensitivity C-reactive protein, or hemoglobin A1c; and plasma concentrations of B-type natriuretic peptide (BNP) or aldosterone. Plasma BNP and aldosterone concentrations were measured using commercially available immunoradiometric assay kits (Shionoria BNP kit, Shionogi, Osaka Japan; SPACS-S Aldosterone kit, Dai-ichi Radioisotope, Tokyo, Japan). The intra- and interassay coefficients of variation for determining BNP concentration were 5.3% and 5.9%, respectively.

Echocardiography

All patients underwent two-dimensionally guided echocardiography using a single ultrasonographic recorder (UF-8800, Fukuda Denshi, Tokyo, Japan) on a midweek non-dialysis day within 1 month after the enrollment. LV ejection fraction (LVEF) was quantified using the modified Simpson rule, and LV mass was normalized to body surface area, and is described herein as left ventricular mass index.

Endpoint

All 244 patients were followed up at Toujinkai Hospital. The endpoint was cardiovascular events: cardiovascular deaths, including death caused by acute myocardial infarction (AMI) or congestive heart failure, and sudden cardiac death (SCD); coronary interventions, including percutaneous coronary intervention or coronary artery bypass grafting; vasospastic angina identified by coronary angiography; malignant arrhythmias such as ventricular tachycardia or fibrillation; bradycardia needing permanent pacemaker implantation; congestive heart failure needing hospitalization; cardiac valvular disease needing operation; aortic aneurysm including rupture or dissection; or peripheral artery disease needing vascular bypass or leg amputation. SCD was defined as death within 24 h of the time that the victim was last seen alive in a normal state of health, and cardiac diseases such as malignant arrhythmias or acute coronary syndrome were considered the most frequent causes of death. Cerebrovascular accidents were ruled out by post-mortem examinations. Cardiologists in Toujinkai Hospital or Kyoto Second Red Cross Hospital diagnosed cardiac-derived death, and they did not know about the study protocol at the point of diagnosis.

Statistical Analysis

Continuous values were expressed as the median ± SE. Continuous variables were compared using Mann–Whitney *U* test. Categorical data were analyzed using the Chi-square test. Receiver operating characteristic analysis was performed to define the threshold of PⅢNP for cardiovascular events; thresholds were obtained from minimal false positive and false negative results, that is, by minimizing the expression $(1-\text{specificity})^2+(1-\text{sensitivity})^2$. The associations of clinical factors with cardiovascular events were analyzed using Cox hazard model. Stepwise Cox hazard analysis was performed among significant ($P<0.05$) factors in univariate analysis. Cardiovascular event-free survival rates were assessed using the Kaplan–Meier method and the log-rank test. A P value of <0.05 was considered statistically significant. Individuals who were blinded to all personal information about the patients statisti-

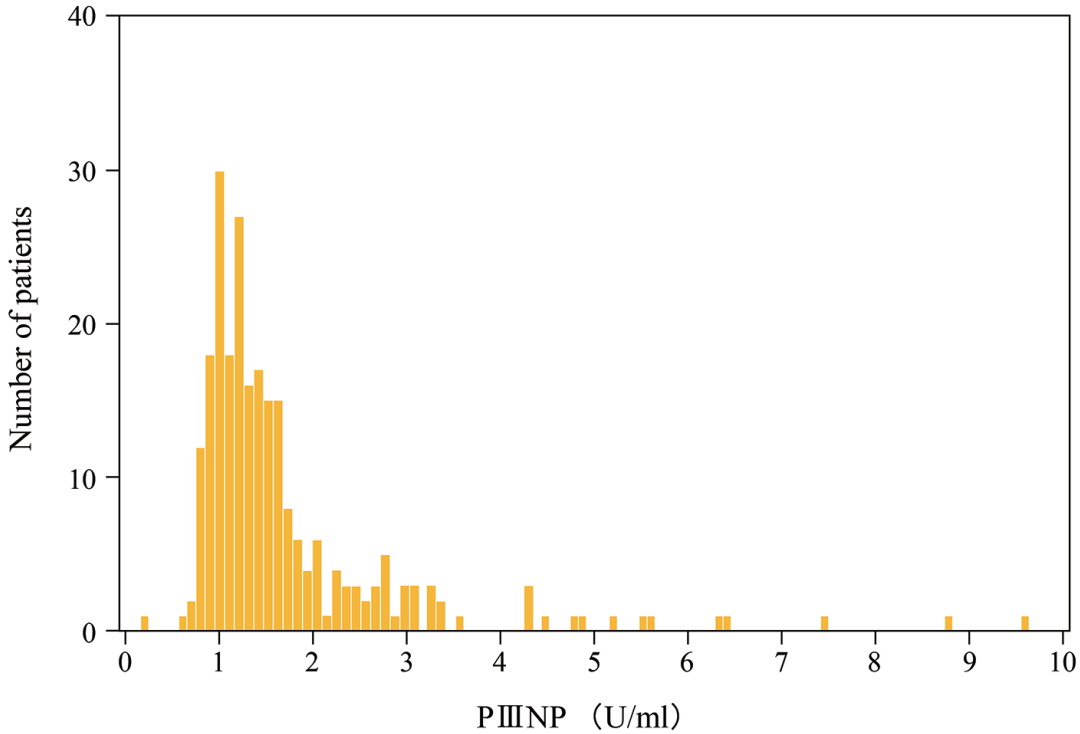

Fig. 2. Histogram of serum PⅢNP concentrations. Serum PⅢNP concentrations distributed between 0.30 and 9.50 U/mL, and the median value was 1.40 ± 0.08 U/mL for all subjects. In the Japanese population, the reference interval for PⅢNP in this assay kit is 0.30 to 0.80 U/mL; the serum PⅢNP concentrations of patients undergoing hemodialysis would be higher than those of normal population.

cally analyzed all data. All statistical analyses were performed using IBM SPSS Statistics software, version 23.

Results

All 244 patients had been followed up in Toujinkai Hospital until the endpoint was reached, or until January 31, 2011 (end of the study). During follow-up for 4.7 ± 1.8 years, all-cause death occurred in 62 patients (25.4%), as follows: cardiovascular deaths (n = 20: 14 SCD, 3 AMI deaths, 2 heart failure deaths, and 1 rupture of dissecting aortic aneurysm), malignancy (n = 11), infection (n = 10), cerebrovascular accidents (n = 7:4 cerebral bleeding and 3 cerebral infarction), digestive system diseases (n = 6), respiratory failure (n = 4), liver failure (n = 1), leukemia (n = 1), multiple organ failure (n = 1), and senility (n = 1). Cardiovascular death was 31.7% of all-cause death. Cardiovascular events occurred in 78 (32%) of 244 patients, as follows: cardiac deaths (n = 19), non-fatal AMI (n = 1), obstructive coronary artery disease needing percutaneous coronary intervention (n = 22), vasospastic angina identified by angiography (n = 3), heart failure needing hospitalization (n = 21), bradycardia needing pacemaker implantation (n = 7:5 sick sinus syndrome and 2 complete atrioventricular block), dissecting aortic aneurysm (n = 2), aortic valvular stenosis needing valve replacement (n = 1), peripheral artery disease needing bypass surgery (n = 1), and leg amputation (n = 1). One of the two patients with dissecting aneurysm died immediately after the diagnosis.

Serum PⅢNP

Serum PⅢNP concentrations were distributed between 0.30 and 9.50 U/mL, with a median of 1.40 ± 0.08 U/mL for all subjects (**Fig. 2**). To evaluate reproducibility of serum PⅢNP levels in this population, we remeasured the serum PⅢNP concentrations after 1 month of the first measurement in 30 of the participants; serum PⅢNP concentrations (mean ± SD) did not differ during 1 month: 1.83 ± 1.02 U/mL versus 1.85 ± 1.03 U/mL. In the Japanese population, the reference interval for PⅢNP in this assay kit is 0.30−0.80 U/mL; the serum PⅢNP concentrations of patients undergoing hemodialysis would be higher than those of normal population, as previously reported[15]. Serum PⅢNP concentration was positively correlated with dialysis periods (r = 0.46, P < 0.001), serum cal-

Table 1. Clinical characteristics in the groups with or without cardiovascular events.

	Events (−) (n=166)	Events (+) (n=78)	P value
Male gender, n (%)	82 (49.4)	44 (56.4)	0.367
Age, years	63.0 ± 0.8	66.0 ± 1.2	0.005
Dialysis duration, months	108.0 ± 6.4	147.0 ± 12.6	0.032
Diabetes, n (%)	51 (30.7)	27 (34.6)	0.543
Body mass index, kg/m^2	18.3 ± 0.3	18.9 ± 0.5	0.882
Smoking, n (%)	54 (32.5)	25 (32.1)	0.941
Alcohol, n (%)	80 (48.2)	32 (41.0%)	0.295
Systolic blood pressure before dialysis, mmHg	145.0 ± 1.3	142.0 ± 1.8	0.102
Diastolic blood pressure before dialysis, mmHg	79.0 ± 0.8	73.0 ± 1.4	<0.001
Cardiothoracic ratio, %	51.0 ± 0.3	52.0 ± 0.6	0.077
Left ventricular ejection fraction, %	69.6 ± 0.9	68.4 ± 1.6	0.109
Left ventricular mass index, g/m^2	116.3 ± 4.4	118.1 ± 4.5	0.670
Serum aspartate aminotransferase, IU/L	17.0 ± 0.2	17.0 ± 0.5	0.926
Serum alanine aminotransferase, IU/L	19.0 ± 0.2	18.0 ± 0.6	0.529
Serum γ-glutamyl transferase, IU/L	22.0 ± 0.2	22.0 ± 0.5	0.796
Blood hemoglobin, g/dL	10.0 ± 0.1	10.3 ± 0.1	0.219
Serum albumin, g/dL	4.0 ± 0.03	3.8 ± 0.05	0.003
Serum calcium, mg/dL	8.9 ± 0.1	9.0 ± 0.1	0.075
Serum inorganic phosphorus, mg/dL	5.3 ± 0.1	5.6 ± 0.1	0.497
Serum intact parathyroid hormone, pg/mL	210.0 ± 13.3	140.0 ± 28.0	0.046
Serum C-reactive protein, mg/L	2.9 ± 0.2	3.9 ± 0.4	0.098
Serum hemoglobin A1c, %	6.1 ± 0.1 (n=51)	5.6 ± 0.2 (n=27)	0.440
Serum total cholesterol, mg/dL	170.0 ± 3.1	163.0 ± 4.2	0.299
Serum high-density lipoprotein cholesterol, mg/dL	35.0 ± 0.6	36.0 ± 0.7	0.911
Serum triglyceride, mg/dL	126.0 ± 5.2	132.0 ± 6.7	0.942
Serum β_2 microglobulin, ng/mL	41.8 ± 0.4	40.7 ± 0.4	0.580
Serum PⅢNP, U/mL	1.30 ± 0.03	2.30 ± 0.19	<0.001
Plasma B-type natriuretic peptide, pg/mL	283.0 ± 29.5	276.0 ± 34.5	0.655
Plasma aldosterone, pg/mL	121.0 ± 28.8	116.0 ± 15.7	0.272
Medications			
α_1 blockers, n (%)	25 (15.1)	6 (7.7)	0.107
β blockers, n (%)	35 (21.1)	16 (20.5)	0.918
Calcium channel blockers, n (%)	42 (25.3)	17 (21.8)	0.551
ACE inhibitors, n (%)	21 (12.7)	7 (9.0)	0.401
ARB, n (%)	34 (20.5)	16 (20.5)	0.996
Nitrates, n (%)	7 (4.2)	4 (5.1)	0.748
Antiplatelet drugs, n (%)	54 (32.5)	15 (19.2)	0.032
Anticoagulants (%)	4 (2.4)	1 (1.3)	0.562
Statins, n (%)	10 (6.0)	4 (5.1)	0.779
Vitamin D, (%)	90 (54.2)	44 (56.4)	0.509

PⅢNP, aminoterminal propeptide of type Ⅲ procollagen; ACE, angiotensin Ⅰ converting enzyme; ARB, angiotensin Ⅱ type-1 receptor blocker.

cium concentration (r=0.24, P<0.001), calcium-inorganic phosphate product (r=0.15, P=0.017) or cardiovascular events (r=0.54, P<0.001), and inversely correlated with diabetes mellitus (r=−0.17, P=0.009) or diastolic blood pressure (r=−0.16, P=0.014).

Cardiovascular Events and PⅢNP

Patients with cardiovascular events had longer dialysis duration; higher age or serum PⅢNP concentration; and lower diastolic blood pressure, serum albumin, or intact parathyroid concentration, or administration ratio of antiplatelet drugs than those without

Table 2. Univariate Cox-hazard analysis for cardiovascular events.

	Hazard ratio	95% CI	*P* value
Male gender (0=female; 1=male)	1.42	0.91−2.23	0.122
Age (1 year)	1.04	1.02−1.06	<0.001
Dialysis duration (1 month)	1.00	1.00−1.01	0.002
Diabetes mellitus (0=no; 1=yes)	1.21	0.76−1.93	0.424
Body mass index (1 kg/m^2)	0.98	0.93−1.04	0.554
Smoking habit (0=no; 1=yes)	1.02	0.63−1.64	0.941
Alcohol consumption (0=no; 1=yes)	0.78	0.50−1.23	0.288
Systolic blood pressure before dialysis (1 mmHg)	0.99	0.97−1.00	0.055
Diastolic blood pressure before dialysis (1 mmHg)	0.96	0.95−0.98	<0.001
Cardiothoracic ratio (1%)	1.05	1.00−1.11	0.040
Left ventricular ejection fraction (1%)	0.98	0.96−1.00	0.010
Left ventricular mass index (1 g/m^2)	1.00	0.99−1.00	0.342
Serum aspartate aminotransferase (1 IU/L)	0.94	0.86−1.03	0.172
Serum alanine aminotransferase (1 IU/L)	0.97	0.90−1.05	0.433
Serum γ-glutamyl transferase (1 IU/L)	1.01	0.93−1.10	0.859
Blood hemoglobin (1 g/dL)	1.13	0.84−1.36	0.191
Serum albumin (1 g/dL)	0.37	0.21−0.64	<0.001
Serum calcium (1 mg/dL)	1.18	0.90−1.54	0.233
Serum inorganic phosphorus (1 mg/dL)	1.04	0.85−1.26	0.714
Serum intact parathyroid hormone (1 pg/mL)	1.00	1.00−1.00	0.849
Serum C-reactive protein (1 mg/L)	1.08	0.99−1.18	0.097
Serum hemoglobin A1c (1%)	1.15	0.83−1.64	0.425
Serum total cholesterol (1 mg/dL)	1.00	0.99−1.00	0.099
Serum high-density lipoprotein cholesterol (1 mg/dL)	0.99	0.96−1.03	0.700
Serum triglyceride (1 mg/dL)	1.00	1.00−1.00	0.783
Serum β_2 microglobulin (1 ng/mL)	0.99	0.94−1.03	0.560
Plasma B-type natriuretic peptide (1 pg/mL)	1.00	1.00−1.00	0.667
Plasma aldosterone (1 pg/mL)	1.00	1.00−1.00	0.138
Serum PIIINP (1 U/mL)	1.53	1.40−1.68	<0.001
Medications			
α_1 blockers (0=no; 1=yes)	0.52	0.23−1.19	0.123
β blockers (0=no; 1=yes)	0.99	0.57−1.72	0.984
Calcium channel blockers (0=no; 1=yes)	0.81	0.47−1.38	0.437
ACE inhibitors (0=no; 1=yes)	0.74	0.34−1.61	0.446
ARB (0=no; 1=yes)	0.99	0.57−1.71	0.965
Nitrates (0=no; 1=yes)	1.22	0.45−3.33	0.701
Antiplatelet drugs (0=no; 1=yes)	0.60	0.34−1.06	0.079
Anticoagulation drugs (0=no; 1=yes)	0.70	0.10−5.02	0.721
Statins (0=no; 1=yes)	0.82	0.30−2.25	0.704
Vitamin D (0=no; 1=yes)	1.17	0.75−1.84	0.485

PⅢNP, aminoterminal propeptide of type Ⅲ procollagen; ACE, angiotensin Ⅰ converting enzyme; ARB, angiotensin Ⅱ type-1 receptor blocker.

(Table 1). In univariate Cox hazard analysis, cardiovascular events were positively associated with age, dialysis duration, cardiothoracic ratio, or serum PⅢNP concentration, and inversely associated with diastolic blood pressure, LVEF, or serum albumin concentration (Table 2). Stepwise Cox hazard analysis among these factors revealed a positive association of cardiovascular events with age or serum PⅢNP concentration, and an inverse association with diastolic blood pressure or LVEF (Table 3). Serum PⅢNP concentration was also positively associated with cardiac death (hazard ratio, 1.32; 95% CI, 1.02−1.70; *P*=0.032). In receiver operating characteristic analysis, the threshold of serum PⅢNP concentration for cardiovascular events was de-

Table 3. Multivariate Cox-hazard analysis for cardiovascular events.

	Hazard ratio	95% CI	*P* value
Age (1 year)	1.05	1.02−1.07	<0.001
Serum PⅢNP (1 U/mL)	1.59	1.43−1.77	<0.001
Diastolic blood pressure before dialysis (1 mmHg)	0.98	0.96−1.00	0.034
Left ventricular ejection fraction (1%)	0.98	0.96−1.00	0.024

PⅢNP, aminoterminal propeptide of type Ⅲ procollagen.

termined as 1.75 U/mL (**Fig. 3**). When patients were assigned to subgroups based on this serum PⅢNP cut-off value, cardiovascular event-free survival rates at 5 years were lower in the subgroup of serum PⅢNP of 1.75 U/mL or more than in that of serum PⅢNP below 1.75 U/mL (31.9% vs 88.2%) (**Fig. 4**).

Discussion

Various studies have indicated that circulating PⅢNP levels are associated with LV remodeling induced by hypertension, myocardial infarction, heart failure[4, 5, 7, 13, 16-18], or aortic stiffness[18, 19] and prognosis including mortality[6, 11, 12, 20, 21]. However, there are few studies showing the relationship between circulating PⅢNP and cardiovascular events in patients undergoing hemodialysis. To the best of our knowledge, this study is the first to show the independent association of serum PⅢNP concentration with cardiovascular event occurrence in patients undergoing hemodialysis. Because serum PⅢNP levels are reportedly elevated in acute or chronic liver diseases and correlate positively with serum aminotransferase or bilirubin levels[22], patients with liver disease were excluded from this study. In addition, laboratory data of serum aminotransferases were not related with serum PⅢNP levels in the participants of our study. Because serum PⅢNP concentrations did not change during 1 month in our study and did not vary throughout the day in the study by Saggese *et al.*[23], it may be concluded that serum PⅢNP concentrations remain stable in patients undergoing hemodialysis. The molecular weight of PⅢNP is 42000 Da; PⅢNP would not be removed by hemodialysis, but partly removed by hemodialysis filtration, although we had not evaluated the changes of serum PⅢNP before and after dialysis. Because serum PⅢNP is reportedly cleared from the blood via hepatobiliary elimination and does not depend on renal function[24, 25], predialysis serum PⅢNP concentrations are thought to directly indicate PⅢNP turnover. In addition, because collagen type Ⅲ abundantly exists in the cardiovascular system[2, 3], increased serum PⅢNP levels indicating advanced turnover of collagen type Ⅲ may be a risk factor for cardiovascular events in this population.

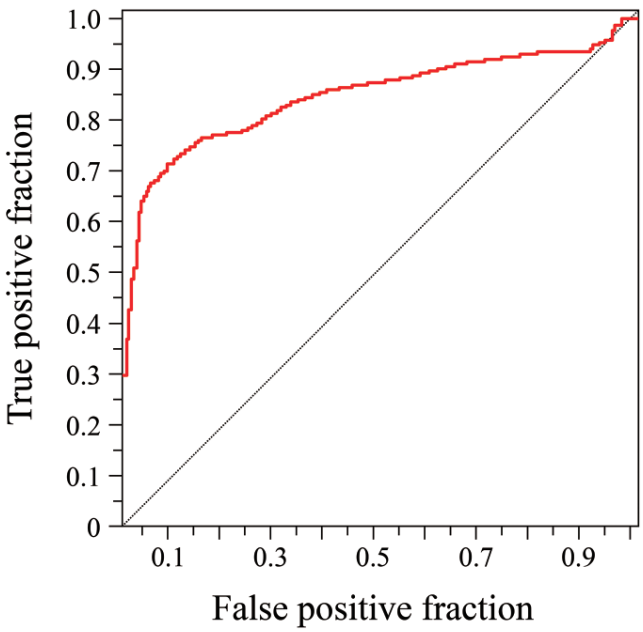

Fig. 3. Receiver operating characteristic curves to determine the threshold of serum PⅢNP concentration for cardiovascular events. The area under the curve was 0.842.

Myocardial fibrosis is of two types: focal and diffuse[26]. Focal fibrosis replaces dead cardiomyocytes and forms scars. Diffuse fibrosis occurs in the interstitial and perivascular space, and it is involved in the various pathophysiological and clinical features of chronic cardiac diseases: increased LV stiffness and diastolic dysfunction, impaired LV systolic dysfunction, arrhythmias, and impaired coronary flow reserve[27-30]. In addition, increased collagen turnover is associated with the functional properties of the arterial vasculature. Increased arterial stiffness due to medial fibrosis contributes to LVH, myocardial ischemia, and microcirculatory disturbance in the heart[31]. Diffuse fibrosis in the heart and vasculature affect each other to promote the occurrence of cardiovascular events.

In the present study, SCD was the main cause of cardiac death. Sudden death occurs more frequently in patients undergoing hemodialysis compared with general population: SCD accounts for approximately one-

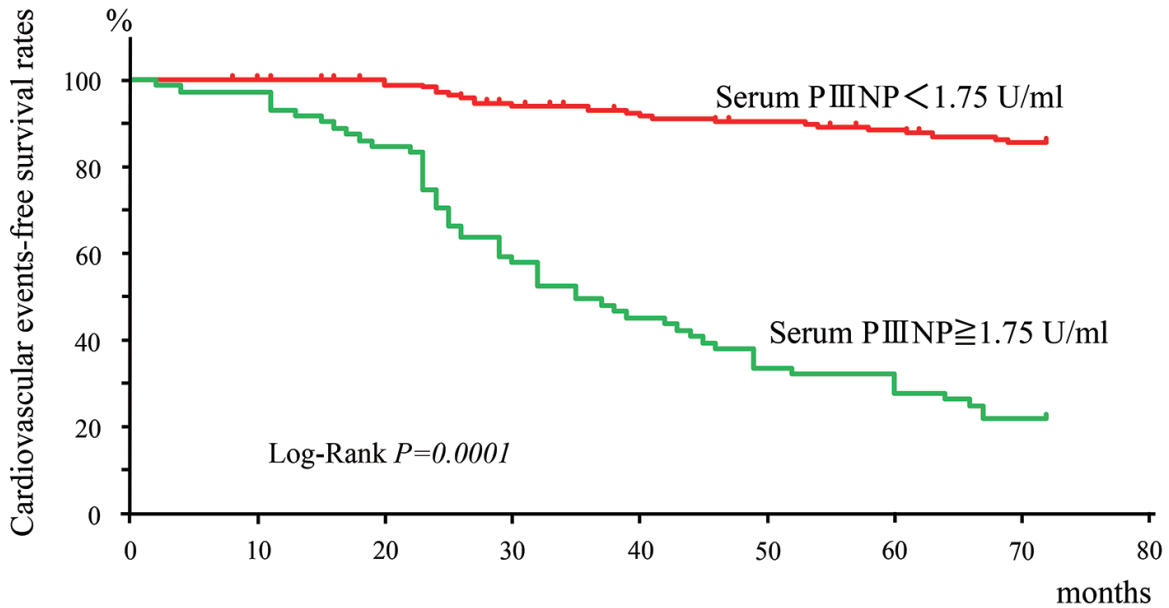

Fig. 4. Kaplan−Meier analysis of cardiovascular event-free survival rates differentiated by the threshold of serum PⅢNP concentration (1.75 U/mL).

fourth of all-cause mortality in patients undergoing dialysis[32, 33]. The main cause of SCD is believed to be hemodynamic collapse due to malignant arrhythmia such as ventricular fibrillation in the setting of structural heart disease[34]. Arrhythmias were reportedly responsible for 78% of all cardiac deaths or 29% of all-cause mortality in patients undergoing hemodialysis[35]. A triggering event or condition interacts with the underlying structural heart disease to produce fatal arrhythmia. LVH, the most frequent cardiac abnormality in patients undergoing dialysis[36], causes electrical remodeling of the heart[37], and ventricular arrhythmia is increased in patients with echocardiographically identified LVH compared with those without[38]. Advanced myocardial fibrosis, an important component of LVH, might be associated with fatal arrhythmias such as ventricular tachycardia/fibrillation via the mechanism of localized conduction disturbance and spiral reentry[39].

In addition, obstructive coronary artery disease accounted for a large percentage of cardiovascular events. Because the main fibrillar collagen in coronary plaque is type I collagen, type Ⅲ collagen is thought not to be involved in the progression of coronary plaque[3]. On the contrary, type Ⅲ collagen is the most abundant form of collagen in the aortic wall[3]; increased collagen turnover of medial layers of the aorta decreases vascular distensibility, leading to increased arterial stiffness, as described earlier. Increased arterial stiffness contributes to causing LVH, including myocardial fibrosis[27, 28]; it easily leads to impaired coronary blood flow[30]. In pathological studies, intramyocardial arteriolar thickening, reduced capillary density, and myocardial fibrosis are unique findings in the heart of patients undergoing hemodialysis[40, 41]; these myocardial abnormalities can potentially cause myocardial microcirculatory disturbance. These characteristics in patients undergoing hemodialysis result in increased susceptibility of myocardial cells to reduced myocardial blood supply in patients undergoing hemodialysis than in non-dialysis patients. Advanced collagen turnover in the aorta may be involved in the early detection of myocardial ischemia based on obstructive coronary artery disease.

Not only LVH but also LV systolic function is deeply involved in the prognosis of patients undergoing hemodialysis. Previous studies have indicated that LVEF examined by echocardiography could detect the high-risk group of hemodialysis population[42, 43]. Myocardial fibrosis and myocardial ischemia are likely involved in reduced LV systolic function, as described earlier. In the present study, LVEF and serum PⅢNP were independently associated with the occurrence of cardiovascular events. We may have to pay attention to protecting patients undergoing hemodialysis with low LVEF and high serum PⅢNP concentration of 1.75 U/mL or more from future cardiovascular events.

This study has several limitations. The long observation duration (mean period: 4.7 years) could have introduced various biases on the results. We considered 19 sudden deaths as cardiac death, although a coronary origin was not clearly determined. We could not completely eliminate the possibility of hyperkalemia or some other cause for these deaths. The tissue and

organ origins of circulating PⅢNP have not been identified yet. It is well known that circulating BNP is a useful biomarker for predicting cardiovascular events; however, plasma BNP was not associated with cardiovascular events in this study. Plasma BNP concentrations ranged from 23 to 3930 pg/mL, and the coefficient of variation of plasma BNP was 102%; the extraordinarily wide distribution of plasma BNP concentrations might be involved with no relationship between plasma BNP and cardiovascular events. Because BNP samples were obtained just before starting the first hemodialysis session of the week after the enrollment, and not on the non-dialysis day like echocardiography, the temporary increase in volume load in some patients might have contributed to the wide distribution of plasma BNP concentrations and lack of association between plasma BNP and cardiovascular events. Finally, the predictive value of serum PⅢNP concentration could not be defined because of the relatively small sample size. A larger patient population is needed to establish the clinical implications and prognostic value of this method.

Conclusion

Higher levels of circulating PⅢNP may be associated with increased collagen turnover or fibrosis of the cardiovascular tissues, leading to sclerosis of the vascular systems and myocardial remodeling or overload. In the EPHESUS study of patients with congestive heart failure after AMI, circulating PⅢNP levels did not correlate with cardiovascular events or mortality for a mean follow-up of 16 months[12]. On the contrary, high basal values of PⅢNP were correlated with cardiovascular mortality in patients with chronic heart failure in the RALES study[10]. Serum PⅢNP concentrations may be able to predict more accurately long-term cardiovascular events in chronic conditions such as heart failure or end-stage kidney disease than early-phase events such as AMI.

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COI

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