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 procollagen³. 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 myocardium³, 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 media³. 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 synthesis⁴. Among the many circulating molecules proposed as biomarkers of myocardial fibrosis, type III collagen is a major component of the extracellular matrix in the myocardium and aorta. Type III collagen is a key component of the extracellular matrix in the myocardium and aorta.
dial fibrosis in humans, only PⅢNP and the carboxy-terminal propeptide of type I procollagen (C1PC), 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 (New York Heart Association grades III to IV)\(^1\); moderate or worse valvular heart disease\(^2\); permanent pacemaker\(^4\); idiopathic hypertrophic or dilated cardiomyopathy\(^5\); chronic obstructive pulmonary disease\(^6\); malignancy\(^7\); and acute hepatitis, chronic hepatitis, liver cirrhosis, or dial fibrosis in humans, only PⅢNP and the carboxy-terminal propeptide of type I procollagen (C1PC).
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 PIII NP
Blood was collected for determination of serum PIII NP concentration just before starting the first hemodialysis session of the week after the enrollment. Serum PIII NP was determined by immunoradiometric assay (RIA-gnost PIII P 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 PIII NP. We measured blood hemoglobin levels; serum concentrations of aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transference, total cholesterol, high-density lipoprotein cholesterol, triglyceride, albumin, calcium, inorganic phosphorus, intact parathyroid hormone, β2 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 PIII NP for cardiovascular events; thresholds were obtained from minimal false positive and false negative results, that is, by minimizing the expression (1 – specificity)² + (1 – 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-
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 \(PIIINP\) concentrations were distributed between 0.30 and 9.50 U/mL, with a median of 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.

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. 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/m² | 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  |
| Cardiacoracic 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/m² | 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 β2 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            |                    |                   |         |
| a1 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   |
| Nitrites, 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.

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 calcium 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).
In univariate Cox hazard analysis, cardiovascular events were positively associated with age, dialysis duration, cardiothoracic ratio, or serum P-IIINP 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 P-IIINP concentration, and an inverse association with diastolic blood pressure or LVEF (Table 3). Serum P-IIINP 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 P-IIINP concentration for cardiovascular events was de-
Myocardial fibrosis is of two types: focal and diffuse\(^2\). 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\(^2\)\(^-\)\(^3\)\(^-\)\(^0\). 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\(^3\)\(^\)\(^0\). 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-third as 1.75 U/mL (Fig. 3). When patients were assigned to subgroups based on this serum PI\(\text{IIINP}\) cut-off value, cardiovascular event-free survival rates at 5 years were lower in the subgroup of serum PI\(\text{IIINP}\) of 1.75 U/mL or more than in that of serum PI\(\text{IIINP}\) below 1.75 U/mL (31.9% vs 88.2%) (Fig. 4).

### Discussion

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

| Table 3. Multivariate Cox-hazard analysis for cardiovascular events. |
|----------------------------------|-----------------|----------------|
| Age (1 year)                     | 1.05            | 0.001          |
| Serum PI\(\text{IIINP}\) (1 U/mL)| 1.59            | 0.001          |
| Diastolic blood pressure before dialysis (1 mmHg) | 0.98 | 0.034          |
| Left ventricular ejection fraction (1%) | 0.98 | 0.024 |

\(\text{PI\(\text{IIINP}\), aminoterminal propeptide of type \(\text{III}\) procollagen.}\)

Myocardial fibrosis is of two types: focal and diffuse\(^3\)^\(^0\). 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\(^2\)\(^7\)\(^-\)\(^3\)\(^0\). 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\(^3\)\(^\)\(^1\). 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-
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![Graph showing Kaplan-Meier analysis of cardiovascular event-free survival rates differentiated by the threshold of serum PⅢNP concentration (1.75 U/ml).](image)

Log-Rank $P=0.0001$

**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$^{44}$. 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 arrhythmias 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$^{40}$. On the contrary, type III collagen is the most abundant form of collagen in the aortic wall$^{41}$; 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$^{29}$. 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 \( \text{PIIINP} \) 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 \( \text{PIIINP} \) 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 \( \text{PIIINP} \) 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 EPESUS study of patients with congestive heart failure after AMI, circulating \( \text{PIIINP} \) 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 \( \text{PIIINP} \) were correlated with cardiovascular mortality in patients with chronic heart failure in the RALES study.\(^{10}\) Serum \( \text{PIIINP} \) 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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