Table 3 Univariate and multivariate analyses of factors related to late onset of AKI

	Univariate analysis		Multivariate analysis	
	Odds ratio (95 % CI)	*p* value	Odds ratio (95 % CI)	*p* value
Age >75 years	1.64 (0.89–3.07)	0.111		
Male	1.47 (0.79–2.84)	0.229		
Systolic blood pressure <100 mmHg	2.45 (1.11–5.12)	0.028	1.33 (0.53–3.13)	0.529
Blood urea nitrogen >24 mg/dl	6.74 (3.30–15.2)	<0.001	4.69 (2.00–11.9)	<0.001
Creatinine >1.0 mg/dl	3.81 (1.97–7.87)	<0.001	1.54 (0.69–3.60)	0.299
B-type natriuretic peptide >500 pg/ml	1.71 (0.89–3.47)	0.107	0.93 (0.44–2.01)	0.840
Intravenous dobutamine	4.77 (2.50–9.07)	<0.001	3.34 (1.62–6.88)	0.002

AKI acute kidney injury, *CI* confidence intervals

and hyperactivity of the renin-angiotensin-aldosterone system and sympathetic nervous system [24, 25]. They increase the reabsorption of blood urea nitrogen in the renal tubules, resulting in an increase in blood urea nitrogen level. In addition, the necessity for intravenous administration of dobutamine may reflect severe hemodynamic abnormalities. Right heart failure following low cardiac output and high pulmonary arterial/venous pressure causes persistent congestion including increased renal venous pressure. Increasing renal venous pressure induces hyperactivity of not only the intrarenal but also the systemic renin-angiotensin-aldosterone system and sympathetic nervous system, leading to a further fall in glomerular filtration rate. Thus, our results suggest that patients with late onset of AKI in the pathophysiology and treatment of ADHF had the above systems activated due to severe hemodynamic abnormalities, leading to permanent renal dysfunction and adverse outcomes.

The present study has clinical implications. The changes in serum creatinine level in the pathophysiology and treatment of ADHF often fluctuate dynamically, and their causes may be different at onset time. However, assessment of changes in serum creatinine level at multiple time points during ADHF therapy, as was done in the present study, was not performed in previous studies. Therefore, we investigated the clinical importance of early or late onset of AKI defined by the AKI Network creatinine criteria [16], and we found that late onset of AKI, but not early onset of AKI, was linked to high mortality rate. Early onset of AKI might be caused by basal renal dysfunction and strict fluid control, and late onset of AKI might be caused by severe hemodynamic abnormalities. The relationship of AKI with mortality in patients with ADHF has remained unclear [10–15], and the present study, therefore, adds one piece of evidence that onset time of AKI may be useful for risk stratification of mortality in ADHF patients developing AKI. Knowledge of the clinical differences in AKI would be valuable for attending physicians to make clinical decisions.

There are also limitations. First of all, the present study was a retrospective observational study in a single center. Our findings need to be confirmed in large multicenter trials. Secondly, the present study had a survival bias, since we defined early or late onset of AKI according to the median onset time of 4.5 days. Thirdly, since the timing of laboratory measurements during hospitalization was left to the discretion of the treating physicians, the time interval of measurements of serum creatinine level was not just 48 h, resulting in potential underestimation of the incidence of AKI. Fourthly, AKI defined by the AKI Network criteria [16] includes changes in serum creatinine level and urine output criteria, but we used only the serum creatinine criterion. Fifthly, we could not obtain data for evaluating the influence of accurate volume depletion. Sixthly, several studies suggest that AKI on admission is associated with adverse outcomes in patients with ADHF [26, 27]. In the present study, CRRT was started on the 1st day of admission in all 4 patients. The patients required CRRT might have AKI at the time of admission and adverse outcomes. However, these patients were excluded, because we could not judge them as AKI suggested by the AKI Network creatinine criteria [16]. Thus, we did not assess the effect of AKI which occurred until the 1st day of admission. Finally, direct hemodynamic parameters during ADHF therapy were not obtained.

Conclusions

Late onset of AKI, but not early onset of AKI, was linked to adverse outcomes in patients with ADHF. Early onset of AKI might be caused by basal renal dysfunction and strict volume control, and late onset of AKI might be caused by severe hemodynamic abnormalities. Our findings suggest the different mechanisms of early and late onset of AKI and the usefulness of onset time for risk stratification of AKI in patients with ADHF. Although clinical differences in AKI were found, specific therapy has not been established. Further studies are needed to improve clinical outcomes in ADHF patients developing AKI.

Conflict of interest This work was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the Pharmaceuticals and Medical Devices Agency in Japan.

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Pathophysiological impact of serum fibroblast growth factor 23 in patients with nonischemic cardiac disease and early chronic kidney disease

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Imazu M, Takahama H, Asanuma H, Funada A, Sugano Y, Ohara T, Hasegawa T, Asakura M, Kanzaki H, Anzai T, Kitakaze M. Pathophysiological impact of serum fibroblast growth factor 23 in patients with nonischemic cardiac disease and early chronic kidney disease. *Am J Physiol Heart Circ Physiol* 307: H1504–H1511, 2014. First published September 12, 2014; doi:10.1152/ajpheart.00331.2014.—Although the important role of fibroblast growth factor (FGF)23 on cardiac remodeling has been suggested in advanced chronic kidney disease (CKD), little is known about serum (s)FGF23 levels in patients with heart failure (HF) due to nonischemic cardiac disease (NICD) and early CKD. The present study aimed to investigate sFGF23 levels in NICD patients and identify the responsible factors for the elevation of sFGF23 levels. We prospectively measured sFGF23 levels in consecutive hospitalized NICD patients with early CKD (estimated glomerular filtration rate ≥ 40 ml·min^{-1}·1.73 m^{-2}) and analyzed the data of both echocardiography and right heart catheterization. Of the 156 NICD patients (estimated glomerular filtration rate range: 41–128 ml·min^{-1}·1.73 m^{-2}), the most severe HF symptom (New York Heart Association class III-IV, 53% vs. 33%, $P = 0.015$) was found in the above median sFGF23 (39.1 pg/ml) group compared with the below median sFGF23 group. sFGF23 levels were higher in patients with HF hospitalization history compared with those without HF [median: 46.8 (interquartile range: 38.8–62.7) vs. 34.7 (interquartile range: 29.6–42.4) pg/ml, $P < 0.0001$]. In the multivariate analysis, HF hospitalization was independently related to elevated sFGF23 levels ($P = 0.022$). Both systolic dysfunction and high plasma aldosterone concentration were identified as predictors of high sFGF23 levels ($P < 0.05$). Among the neurohormonal parameters, elevated sFGF23 levels were the only factor to predict a declining left ventricular ejection fraction ($P = 0.001$). These findings suggest that the progression of HF per se contributes to the elevation of sFGF23 levels even in the early stages of CKD, which leads to further myocardial dysfunction, potentially creating a vicious cycle.

fibroblast growth factor 23; heart failure; chronic kidney disease

FIBROBLAST GROWTH FACTOR (FGF)23, a phosphate-regulating hormone secreted from osteoblasts, promotes urinary phosphorus excretion and inhibits the activation of vitamin D in the presence of its cofactor, Klotho (1, 11, 21). Recent studies have suggested that an elevated circulating FGF23 level is an independent risk factor for mortality and morbidity in patients with chronic kidney disease (CKD) (8, 12) and a potential risk factor for cardiovascular events in a community-based population (10). Furthermore, a recent experimental study (4) has demonstrated that FGF23 directly promotes cardiomyocyte hypertrophy. Several clinical studies have also suggested the relationship of serum FGF23 levels with both cardiac dysfunction (25) and hypertrophy in CKD patients (7) and the association between serum FGF23 levels and clinical outcomes in outpatients with stable heart failure (HF) (20). Interestingly, the previous studies have suggested no relationship of FGF23 levels with the prevalence of coronary artery disease (CAD) (25) and coronary artery calcification (24). These clinical and experimental findings have the significant implication that FGF23 can directly influence cardiac function and structure other than the ischemic mechanisms. These findings facilitated us to hypothesize that FGF23 plays a role on the progression of cardiac dysfunction in patients with nonischemic cardiac disease (NICD). In addition, other studies (6, 28) have also reported that circulating FGF23 levels are elevated before the development of overt hyperphosphatemia in the early stages of CKD. These lines of evidence promoted us to test the idea that serum FGF23 levels are elevated even in early stages of CKD and affect the pathophysiology in HF patients. Furthermore, it is also crucial to identify the risk factor to elevated FGF23 levels in HF patients, although the determinants of serum FGF23 levels in NICD patients have not yet been completely identified in the clinical setting.

Accordingly, we measured serum FGF23 levels in NICD patients without advanced renal impairment, surveyed the relationship of serum FGF23 levels with cardiac structure, function, and the hemodynamic state, and sought to identify the determinants of serum FGF23 levels in NICD patients.

METHODS

Study design. This study was a prospective cross-sectional study of serum FGF23 levels in hospitalized patients with NICD at a single center.

NICD patients. Since previous studies have suggested that serum FGF23 levels increased marginally with declining renal function below 30–40 ml/min of glomerular filtration rate (GFR) (13, 28), we set the inclusion criteria for renal function as an estimated GFR (eGFR) of ≥40 ml·min^{-1}·1.73 m^{-2}. We could obtain written informed consents from a total of 181 consecutive patients admitted to our department between January and December 2012 (male patients: $n = 93$, 51.4%; female patients: $n = 88$, 48.6%). Patients with CAD were excluded from this study. All patients received coronary angiography or coronary computed tomography. CAD was defined by ≥75% narrowing in one or more coronary arteries or clinical history of myocardial infarction or coronary artery bypass surgery or percutaneous coronary intervention. Of the included patients, whether the HF episode met Framingham criteria was reviewed by two investigators

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(M. Imazu and H. Takahama) via medical records and confirmed that all patients met the criteria. Patients with cardiomyopathy underwent either myocardial biopsy or cardiac MRI for diagnosis. Diagnosis for cardiomyopathy was based on the definition of the World Health Organization/International Society and Federation Cardiology Task Force (23).

Biomarker measurements. Patients underwent a blood test for measurements of serum levels of FGF23, creatinine, calcium, phosphate, intact parathoroid hormone, troponin T, IL-6, TNF-α, plasma renin activity, and plasma levels of aldosterone in the present study before being discharged from the hospital. Blood sampled from the patients was placed in tubes with EDTA, and the serum was separated and frozen in plastic tubes at −80°C until analysis. The serum FGF23 level was measured with a chemiluminescence enzyme immunoassay (Kyowa Medex, Tokyo, Japan) as previously described (26). Both serum concentrations of IL-6 and TNF-α were measured with immu-

Table 1. *Baseline characteristics in NICD patients*

Characteristics	Below Median Group	Above Median Group	*P* Value
n	78	78	
Demographic data			
Age, yr	66 (IQR: 55–74)	57 (IQR: 43–69)	0.011
Women/men, %	60/40	40/60	0.010
New York Heart Association class III-IV, %	33	53	0.015
History			
HF hospitalization, %	23	60	<0.0001
Hypertension, %	50	37	0.106
Diabetes mellitus, %	21	27	0.324
Stroke, %	12	10	0.797
Atrial fibrillation, %	22	40	0.015
Etiology of NICD			<0.0001
Primary cardiomyopathy, *n*	19	38	0.235
Idiopathic dilated cardiomyopathy, *n*	9	24	
Hypertrophic cardiomyopathy, *n*	9	14	
Arrhythmogenic right ventricular cardiomyopathy, *n*	1	0	
Secondary cardiomyopathy, *n*	5	11	0.036
Amyloidosis, *n*	0	4	
Myocarditis, *n*	3	1	
Cardiac sarcoidosis, *n*	1	0	
Other, *n*	1	6	
Valvular disease, *n*	51	21	0.070
Aortic stenosis, *n*	21	4	
Aortic regurgitation, *n*	7	1	
Mitral stenosis, *n*	6	1	
Mitral regurgitation, *n*	10	8	
Tricuspid regurgitation, *n*	0	1	
Postvalve replacement, *n*	7	6	
Hypertensive heart disease with HF, *n*	3	8	
Physical findings			
Systolic blood pressure, mmHg	116 (IQR: 107–126)	104 (IQR: 93–118)	<0.0001
Heart rate, beats/min	65 (IQR: 58–76)	70 (IQR: 62–77)	0.081
Body mass index, kg/m^2	22.5 (IQR: 20.0–25.7)	22.1 (IQR: 19.9–25.3)	0.970
Medications			
β-Blockers, %	41	69	0.0004
Angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, %	46	64	0.024
Loop diuretics, %	22	62	<0.0001
Aldosterone antagonists, %	9	58	<0.0001
Statins, %	32	23	0.210
Laboratory data			
Phosphate, mg/dl	3.6 (IQR: 3.2–3.9)	3.7 (IQR: 3.3–4.1)	0.258
Intact parathyroid hormone, pg/dl	52 (IQR: 40–68)	56 (IQR: 41–80)	0.137
eGFR, ml·min^{-1}·1.73 m^{-2}	73 (IQR: 63–83)	69 (IQR: 55–80)	0.060
Troponin T, ng/ml	0.010 (IQR: 0.008–0.014)	0.015 (IQR: 0.009–0.023)	0.027
TNF-α, pg/ml	0.81 (IQR: 0.40–1.61)	1.36 (IQR: 0.85–2.05)	0.008
IL-6, pg/ml	1.51 (IQR: 0.92–2.66)	2.16 (IQR:1.29–3.19)	0.024
Plasma renin activity, ng·ml^{-1}·h^{-1}	1.4 (IQR: 0.4–3.8)	6.5 (IQR: 1.2–13.6)	0.0003
Plasma aldosterone concentration, ng/dl	12.6 (IQR: 8.5–16.3)	14.4 (IQR: 8.7–29.9)	0.032
Brain natriuretic peptide, pg/ml	81 (IQR: 41–162)	154 (IQR: 55–289)	0.007
TmP/GFR, mg/dl	3.29 (IQR: 2.90–3.55)	3.19 (IQR: 2.66–3.81)	0.797
FGF23, pg/ml	31.1 (IQR: 27.6–35.3)	51.3 (IQR: 43.2–61.5)	
Echocardiography data			
LVEDV index, ml/m^2	103 (IQR: 84–139)	128 (IQR: 90–165)	0.098
LVEF, %	63 (IQR: 45–68)	38 (IQR: 23–60)	<0.0001
Relative wall thickness	0.37 (IQR: 0.29–0.48)	0.33 (IQR: 0.25–0.41)	0.101
Left atrial volume index, ml/m^2	51 (IQR: 38–69)	57 (IQR: 49–71)	0.109

Values are numbers of patients (*n*), medians with interquartile ranges (IQRs), or percentages. The below median group comprised patients with a less than median value of serum fibroblast growth factor (FGF)23 level (39.1 pg/ml); the above median group comprised patients with a greater than median value of serum FGF23 level. NICD, nonischemic cardiac disease; HF, heart failure; eGFR, estimated glomerular filtration rate (eGFR); TmP/GFR, tubular maximal reabsorption rate of phosphate to GFR; LVEDV, left ventricular (LV) end-diastolic volume; LVEF, LV ejection fraction.

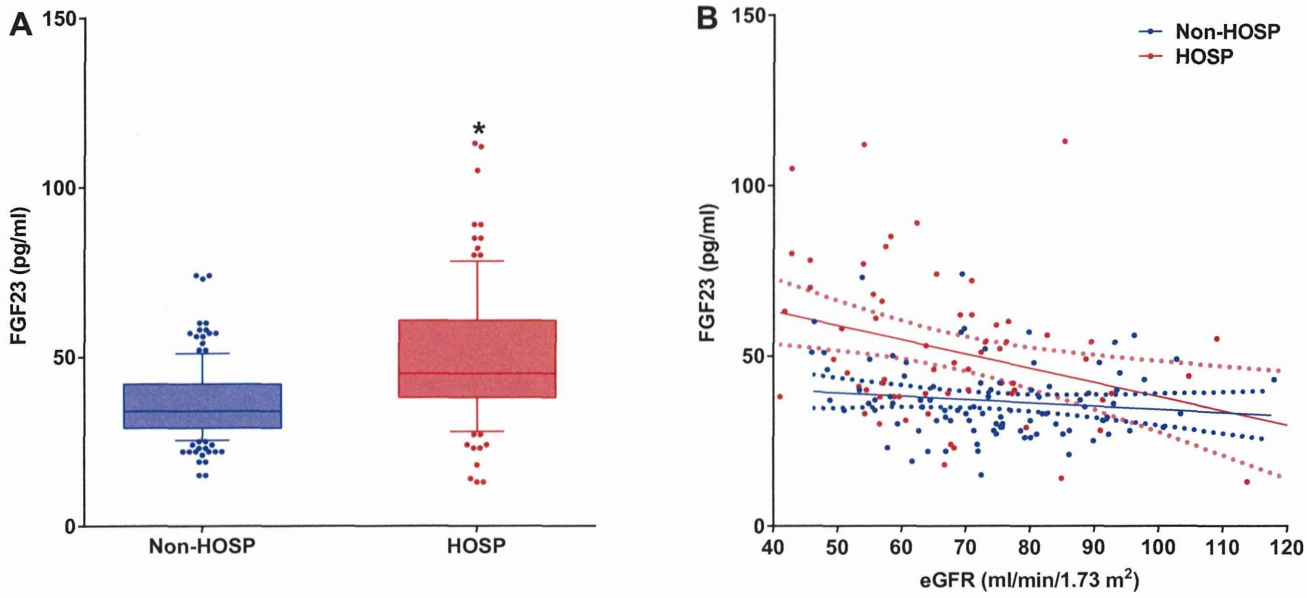

Fig. 1. Serum fibroblast growth factor (FGF)23 levels in patients with nonischemic cardiac disease (NICD). *A*: serum FGF23 levels in NICD patients. Red and blue squares show patients with a history of heart failure hospitalization (HOSP group) and without any history of HF hospitalization (non-HOSP group), respectively. *$P < 0.0001$, non-HOSP group vs. HOSP group. Values are shown as medians and 5–95% distribution. *B*: relationships between estimated glomerular filtration rate (eGFR) and serum FGF23 levels in NICD patients with and without a history of heart failure hospitalization. Graphs indicate the correlations between serum FGF23 levels and eGFR. Red and blue plots and lines show patients in the HOSP and non-HOSP groups, respectively. There was no difference in the relationship between eGFR and serum FGF23 levels in the non-HOSP group ($r = 0.129$, $P = 0.224$). In contrast, a steeper relationship between eGFR and serum FGF23 levels was found in patients in the HOSP group ($r = 0.342$, $P = 0.005$ in the HOSP group, group difference, $P = 0.042$).

noassays (R&D Systems). The plasma intact parathoroid hormone level was measured by an electrochemiluminescence immunoassay (Roche Diagnostics, Tokyo, Japan). eGFR (in ml·min^{-1}·1.73 m^{-2}) was calculated according to the following published equation for Japanese individuals: 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ (× 0.739 for women) (18). Both plasma renin activity and aldosterone concentration were measured by radioimmunoassays (Fujirebio, Tokyo, Japan). Urinary creatinine and phosphate concentrations were obtained via medical records. As previously described (2), the tubular reabsorption of phosphate (TRP) was calculated using the following equation: $1 - (U_P/P_P) \times (P_{Cr}/U_{Cr})$, where U_P and P_P are urine and plasma phosphate concentrations, respectively, and P_{Cr} and U_{Cr} are urine and plasma creatinine concentrations, respectively. The renal tubular maximum reabsorption rate of phosphate to GFR (TmP/GFR) was calculated according to the following equation: TRP × P_P (if TRP ≤ 0.86) or 0.3 × TRP/[1 − (0.8 × TRP)] × P_P (if TRP > 0.86).

Echocardiography. We retrospectively reviewed the data of echocardiography of the enrolled patients via their medical records. Left ventricular (LV) dimensions, left atrium volume, and wall thickness were measured according to American Society of Echocardiography guidelines (16). LV ejection fraction (LVEF) was measured using the Simpson biplane method or the semiquantitative two-dimensional visual estimate method as previously described (22). LV end-diastolic volume and mass were calculated using the Teichholz and Devereux formula (3, 27), respectively.

Right heart catheterization. The indication of right heart catheterization (RHC) was determined by the need of disease managements for an assessment for the HF severity for hospitalized patients. We collected the data from all enrolled patients who underwent RHC. Standard RHC was performed using a Swan-Ganz catheter (Goodman, Tokyo, Japan). Cardiac output was calculated with the direct Fick method as O_2 consumption divided by the arteriovenous O_2 difference, as previously described (15). Briefly, O_2 consumption was obtained by a respiratory gas analyzer (Aeromonitor AE-300S, Minato Medical Science, Osaka, Japan). Levels of hemoglobin, O_2 saturation, and Po_2 (arterial and venous Po_2) were measured by a blood gas analyzer (OSM3, Radiometer, Copenhagen, Denmark). Blood from a vein was sampled from the pulmonary artery.

Clinical outcomes. After the enrollment in this study, we investigated cardiovascular death, heart transplantation, implantation of a LV assist device, and rehospitalization for HF over 1 yr through medical chart review or a letter. Cardiovascular events were defined as a composite of cardiovascular death, implantation of a LV assist device, or rehospitalization for HF.

Ethics. Written informed consent was obtained from all subjects. This study was approved by our institutional ethics committee and was conducted in accordance with the Declaration of Helsinki.

Statistical analysis. Data are expressed as medians and interquartile ranges (IQRs). Between-group differences were compared with a χ^2-test for categorical variables. Student's *t*-test (normalized distributed data) or Wilcoxon's rank sum test (non-normalized distributed data) was used for the comparison of continuous variables between two groups. Pearson's correlation coefficient analysis or Spearman's rank correlation coefficient analysis and linear regression were used to assess the relationships between FGF23 levels and other variables. The multiple linear regression model was used to test multiple covariates. All variables with $P < 0.10$ in univariate analysis were selected and performed into the multivariable models. All tests were

Table 2. *Comparison of serum FGF23 levels in non-HF and HF patients*

Nonhospitalized group versus hospitalized group	Adjusted Mean Difference	95% Confidence Interval	*P* Value
Model 1	−7.260	−9.877, −4.642	<0.0001
Model 2	−6.190	−8.802, −3.578	<0.0001
Model 3	−3.989	−6.771, −1.206	0.005

Model 1 was adjusted for age and sex, *model 2* was adjusted for age, sex, and eGFR, and *model 3* was adjusted for age, sex, eGFR, and use of loop diuretics (yes = 1).

Table 3. *Univariate and multivariate analyses of serum FGF23 levels in laboratory data*

	Univariate Analysis			Multivariate Analysis		
Variables	Regression coefficient, pg/ml	95% confidence interval	*P* value	Regression coefficient, pg/ml	95% confidence interval	*P* value
eGFR	−0.329	−0.495, −0.163	0.0001	0.237	−0.510, 0.037	0.089
Troponin T	235.8	2.741, 468.9	0.047	70.40	−185.9, 326.7	0.587
TNF-α	2.227	0.260, 4.194	0.027	2.715	−0.538, 5.968	0.101
IL-6	0.628	−0.580, 1.837	0.306			
White blood cell count	0.0003	−0.0013, 0.0019	0.747			
C-reactive protein	2.579	−5.967, 11.13	0.552			
Plasma aldosterone concentration	0.384	0.194, 0.573	0.0001	0.345	0.122, 0.567	0.003

n = 156 patients total. In the multivariate model, age and sex were adjusted.

two-tailed, and P values of <0.05 were considered significant. These analyses were performed with JMP 10 (SAS Institute, Cary, NC).

RESULTS

Relationship between clinical features of NICD-induced HF patients and serum FGF23 levels. We excluded 25 CAD patients from this study according to the aim of this study and the criteria. Of the remaining 156 patients, we divided the patients into above and below median FGF23 levels (39.1 pg/ml), as shown in Table 1 (above or below median groups). Between these two groups, higher prevalence of New York Heart Association (NYHA) class III-IV at admission (53% vs. 33%, P = 0.015) and HF hospitalization (60% vs. 23%, P < 0.0001) were frequently observed in the above median group. We also observed a higher proportion of patients with cardiomyopathy (62% vs. 28%, P < 0.0001) and atrial fibrillation (40% vs. 22%, P = 0.015) and a lower systolic blood pressure [104 (IQR: 93–118) vs. 116 (IQR: 107–126) mmHg, P < 0.0001] in the above median group. Loop diuretics were frequently used in the above median group compared with the below median group (62% vs. 22%, P < 0.0001). Aldosterone antagonists were frequently used in the above median group compared with the below median group (58% vs. 9%, P < 0.0001). eGFR was on the statistical border between the two groups (P = 0.060). Blood levels of troponin T, TNF-α, IL-6, and aldosterone were higher in the above median group than in the below median group (Table 1). Echocardiography revealed a lower degree of LVEF in the above median group [38 (IQR: 23–60)% vs. 63 (45–68)%, P < 0.0001]. There were no significant differences regarding the LV end-diastolic volume index, relative wall thickness, and left atrial volume index between the groups. The subtype of cardiac disease is shown in Table. 1. There was no patient with a history of kidney transplant or kidney disease. TmP/GFR was measured in 104 patients, and we found no differences between the above and below median FGF23 groups (Table 1). In the analysis between serum FGF23 levels and physiological variables, serum FGF23 levels were related to mean blood pressure (r = −0.281, P = 0.003) but were not related to heart rate (r = 0.090, P = 0.267) and body mass index (r = −0.017, P = 0.830).

Serum FGF23 levels in NICD-induced HF patients. We investigated serum FGF23 levels in patients with or without a history of HF hospitalization (hospitalized or nonhospitalized groups, respectively). Serum FGF23 levels were higher in the hospitalized group compared with the nonhospitalized group [34.7 (IQR: 29.6–42.4) vs. 46.8 (IQR: 38.8–62.7), P < 0.0001; Fig. 1*A*]. Figure 1*B* shows the relationship between eGFR and FGF23 levels in the hospitalized and nonhospitalized groups. There were no differences in the relationship between eGFR and serum FGF23 levels in the nonhospitalized group (r = 0.129, P = 0.224). In contrast, as eGFR decreased, serum FGF23 levels were elevated at a higher rate for the hospitalized group than for the nonhospitalized group (r = 0.342, P = 0.005 in the hospitalized group, group difference, P = 0.042). Table 2 shows the multivariate linear model of serum FGF23 levels in these groups. Serum FGF23 levels were higher in the hospitalized group even after adjustments of age, sex, eGFR, and the use of loop diuretics. TmP/GFR did not differ between the hospitalized and nonhospitalized groups.

Predictor of serum FGF23 levels in NICD-induced HF patients. Table 3 shows the relationships between serum FGF23 levels and other laboratory markers. eGFR, troponin T, TNF-α, and plasma aldosterone concentration were related to FGF23 levels in the univariate analysis. In the multivariate analysis, plasma aldosterone concentration was the only factor related to serum FGF23 levels (P = 0.003). Table 4 shows the relationship of serum FGF23 levels with ventricular structural and functional values as assessed by echocardiography. In the multivariate analysis, LVEF was the only factor related to serum FGF23 levels (P = 0.020). Table 5 shows the multivariate analysis for the relationship of serum FGF23 levels with history of HF hospitalization, plasma aldosterone concentration, LVEF, and use of diuretics after adjusting for age, sex,

Table 4. *Univariate and multivariate analyses of FGF23 in echocardiography data*

	Univariate Analysis			Multivariate Analysis		
Variables	Regression coefficient, pg/ml	95% confidence interval	*P* value	Regression coefficient, pg/ml	95% confidence interval	*P* value
LVEDV index	0.083	0.030, 0.136	0.003	0.032	−0.054, 0.118	0.462
LVEF	−0.348	−0.500, −0.197	<0.0001	−0.251	−0.461, −0.040	0.020
Relative wall thickness	−15.66	−33.82, 2.496	0.090	11.35	−11.84, 34.54	0.334

In the multivariate model, age, sex, and eGFR were adjusted.

Table 5. *Multivariate analysis of FGF23*

Variables	Multivariate Analysis		
	Regression coefficient, pg/ml	95% confidence interval	*P* value
History of HF hospitalization	−4.330	−8.250, −0.411	0.031
Plasma aldosterone concentration	0.236	0.041, 0.431	0.018
LVEF	−0.141	−0.344, 0.063	0.174
Use of loop diuretics	−3.042	−0.629, 0.201	0.066

n = 156 patients total. In the multivariate model, age, sex, and eGFR were adjusted.

and eGFR. The history of HF hospitalization (P = 0.031) and plasma aldosterone concentration (P = 0.018) were independently related to serum FGF23 levels in this model.

Relationships of systolic function with serum FGF23 levels and other laboratory markers. To test the correlation of LVEF with the laboratory data, including serum FGF23 levels, we performed multivariate regression analyses. The results are shown in Table 6. In the multivariate model, after adjusting for age and sex, serum FGF23 levels were correlated with LVEF (P = 0.001), whereas no relationships with LVEF were found in eGFR, TNF-α, and plasma aldosterone concentration.

Relationship between serum FGF23 levels and the hemodynamic state. Table 7 shows the central hemodynamic data obtained through RHC in 127 patients. In the above median FGF23 group, the cardiac index was significantly lower compared with the below median group [2.4 (IQR: 1.9–2.7) vs. 2.7 (IQR: 2.4–3.1) $l \cdot min^{-1} \cdot m^{-2}$, P = 0.001]. Table 8 shows the relationship between serum FGF23 levels and hemodynamic values as assessed by RHC. In the multivariate analysis, the cardiac index was the only factor related to serum FGF23 levels (P = 0.018).

Predictive value of serum FGF23 levels for clinical outcomes. During the 1-yr followup term, cardiovascular events occurred in 15 patients (cardiac death: 1 patient, implantation of a LV assist device: 2 patients, and rehospitalization for HF: 12 patients). Kaplan-Meier analysis showed that the frequency of cardiovascular events were higher in the above median FGF23 group than in the below median FGF23 group (P = 0.005; Fig. 2). Even after adjustments for age, sex, and eGFR, the predictability of serum FGF23 levels for cardiovascular events persisted (Table 9).

DISCUSSION

The present study showed that serum FGF23 levels were elevated in NICD patients who had a HF hospitalization history compared with patients without any HF hospitalization history. Although it is known that the circulating FGF23 level is influenced by other variables, such as renal function (28), we prospectively measured serum FGF23 levels in patients without advanced renal impairment and confirmed the pathophysiological importance of serum FGF23 levels in patients with HF history even in the early stages of CKD. The multivariate analysis in the present study revealed that HF hospitalization history was the strongest predictor for the elevation of serum FGF23 levels in NICD. The present study also revealed that serum FGF23 levels are tightly related to plasma aldosterone levels and that systolic dysfunction and low cardiac output are tightly related to serum FGF23 levels. Taken together, we raise the possibility that such pathogenesis and risk factors for the development of HF are the determinants of serum FGF23 levels in NICD patients. In addition, among the neurohormonal parameters measured in the present study, an elevated serum FGF23 level was the only factor to predict a declining LVEF level. Furthermore, even after adjustments for age, sex, and eGFR, the serum FGF23 level was a strong predictor for future cardiovascular events in the present study. Consistent with previous studies reporting that circulating FGF23 levels can affect cardiac structure and function, our findings suggest that the progression of HF contributes to the elevation of circulating FGF23 levels, which leads to further myocardial dysfunction, potentially creating a vicious cycle.

Serum FGF23 levels in NICD-induced HF patients. There has been less evidence of circulating FGF23 levels in HF patients with preserved renal function. This study confirmed that in NICD patients, even in the early stages of CKD, serum FGF23 levels are elevated in those with a history of HF hospitalization compared with those without HF hospitalization history (Fig. 1). We also confirmed higher serum FGF23 levels in HF patients even accounting for age, sex, eGFR, and the use of diuretics (Table 2). The determinants of serum FGF23 levels have not been completely clarified in previous studies of HF patients. Our findings suggest that NICD-induced HF hospitalization itself is one of the strong determinants of serum FGF23 levels even in a population with relatively preserved renal function. Figure 1*B* shows that there was a steeper negative slope between serum FGF23 levels and eGFR in the hospitalized group compared with the nonhospitalized group, suggesting that serum FGF23 levels are elevated in HF patients even in the early stages of CKD. Although the responsible factors for the elevation of serum FGF23 levels in HF patients have been remained uncertain in previous studies, we identified high plasma aldosterone concentration (Table 3), low LVEF (Table 4), and low cardiac index (Table 8) as predictors for the elevation of serum FGF23 levels even after accounting for eGFR. Whether low cardiac output itself independently contributes to the elevation of serum FGF23 levels from renal function still remains controversial. Interestingly, we also found that in the above median FGF23 group, the percentage of patients who took a loop diuretics was higher than in the below median group (P < 0.0001; Table 1), and we can raise the possibility that decreased renal perfusion by diuretics affects serum FGF23 levels. Consisted with these findings, a previous study (9) has suggested the relationship between the use of diuretics and circulating FGF23 levels. As a speculation, we also suggest that decreasing renal perfusion

Table 6. *Multivariate linear model of LVEF in laboratory data*

Variables	Multivariate Analysis		
	Regression coefficient, pg/ml	95% confidence interval	*P* value
eGFR	0.116	−0.109, 0.341	0.308
TNF-α	0.664	−1.308, 2.635	0.506
Plasma aldosterone concentration	−0.145	−0.359, 0.070	0.184
FGF23	−0.340	−0.531, −0.150	0.001

In the multivariate model, age and sex were adjusted.

Table 7. *Right heart catheterization data*

	Below Median Group	Above Median Group	*P* Value
Right atrial pressure, mmHg	4 (IQR: 2–5)	4 (IQR: 2–5)	0.553
Mean pulmonary artery pressure, mmHg	17 (IQR: 14–20)	18 (IQR: 13–25)	0.207
Pulmonary capillary wedge pressure, mmHg	10 (IQR: 7–12)	11 (IQR: 6–17)	0.345
Cardiac index, $l \cdot min^{-1} \cdot m^{-2}$	2.7 (IQR: 2.4–3.1)	2.4 (IQR: 1.9–2.7)	0.001

Values are medians with IQRs; n = 67 patients in the below median group and 60 patients in the above median group.

induced by a low cardiac output state is one of the key factors affecting serum FGF23 levels. Although eGFR in the present study was relatively preserved (median eGFR: 73 and 69 $ml \cdot min^{-1} \cdot m^{-2}$ in below and above median FGF23 groups, respectively), the findings of steeper correlation between serum FGF23 levels and eGFR in the hospitalized group also support the hypothesis. Our findings also suggest a positive relationship between serum FGF23 levels and neurohormone activation or inflammatory markers, such as TNF-α. These findings are consistent with those of previous study (1) with the respect to the relationship between inflammatory markers and FGF23 levels in CKD patients, suggesting that inflammation is associated with an elevation of circulating FGF23 levels in HF patients. Our findings also demonstrate that there was no longer a relationship between TNF-α and serum FGF23 levels in multivariate analysis (Table 3), with a possible explanation that there might be an interplay between several factors, such as neurohormonal activation and inflammation, for elevating serum FGF23 levels. Interestingly, a more recent study (17) has raised the possibility of endogenous Klotho expression in arteries. Inflammatory cytokines, such as TNF-α, downregulate Klotho expression in the kidney through NF-κB (19) or suppress the expression of both Klotho and FGF receptor in an in vitro study (17). It is worth considering the interplay among neurohormonal activation, inflammatory cytokines, vascular Klotho, and circulating FGF23 levels in patients with HF, and further investigation will be necessary to solve it. Taken together, our results could not explain the mechanism of elevating serum FGF23 levels by a single factor. Indeed, in multivariate analysis (Table 5), LVEF was not correlated with serum FGF23 levels. In addition, we found that aldosterone antagonists were frequently used in the above median group compared with the below median group (58% vs. 9%, $P < 0.001$). This might also influence the higher plasma aldosterone concentration in the above median group. We suggest that such complex interaction or underlying conditions predisposing to the development of HF contribute to the elevation of serum FGF23 levels in NICD patients.

Between the hospitalized and nonhospitalized groups, the differences of serum FGF23 levels were statistically significant, but small. Because of such low circulating levels of FGF23, it is worth to consider whether FGF23 is a "pure" endocrine factor to play a role on cardiac remodeling. Interestingly, some studies (5, 14) have suggested the presence of circulating progenitor cells, which are capable of differentiating into osteoblasts. These findings facilitated us to the further investigation to elucidate the relationship between circulating progenitor cells and serum FGF23 levels. The relationships between such a low level of circulating FGF23 and FGF23 signaling in cardiomyocytes or the endothelium and the interaction with vascular Klotho have also remained uncertain; further investigation will be needed to determine their relationships.

In addition, a clinical study (7) has suggested that circulating FGF23 levels are associated with the degree of LV hypertrophy in patients with CKD. In this study, we determined that serum FGF23 levels were significantly correlated with LV systolic function and cardiac output but were not related to the degree of cardiac hypertrophy. Because the stages of CKD in our patients were relatively early, these differences from previous studies also raise the possibilities that CKD stages and serum FGF23 levels are correlated with the cardiac phenotype. In accordance with this finding, the phosphate reabsorption, as indicated by TmP/GFR, did not differ between the above and below median groups in the present study. This finding suggests that serum FGF23 levels were relatively lower than advanced CKD patients because of our focus on NICD patients with early stages of CKD.

Limitations. We acknowledge that our study has some limitations that merit noting. First, this study was a single-center study, which poses a possible bias risk regarding HF severity and etiologies. Second, although differences in the etiologies of NICD were found between the below and above median FGF23 groups, the present study included valvular disease patients with milder HF symptoms, who hospitalized for an elective catheter test. These variances influence the differences of HF etiologies between the below and above median FGF23 groups. Age differences were observed between the above and below median FGF23 groups. Even after accounting for the age differences in the multivariate models, we confirmed that

Table 8. *Univariate and multivariate analyses of FGF23 in right heart catheterization data*

	Univariate Analysis			Multivariate Analysis		
Variables	Regression coefficient, pg/ml	95% confidence interval	*P* value	Regression coefficient, pg/ml	95% confidence interval	*P* value
Right atrial pressure	1.547	0.494, 2.601	0.004	0.612	−0.594, 1.820	0.317
Mean pulmonary artery pressure	0.503	0.120, 0.885	0.010	0.501	−0.095, 1.097	0.098
Pulmonary capillary wedge pressure	0.612	0.083, 1.142	0.024	−0.551	−1.450, 0.347	0.227
Cardiac index	−6.697	−10.73, −2.669	0.001	−4.532	−0.894, −0.126	0.044

n = 127 patients total. In the multivariate model, age, sex, and eGFR were adjusted.

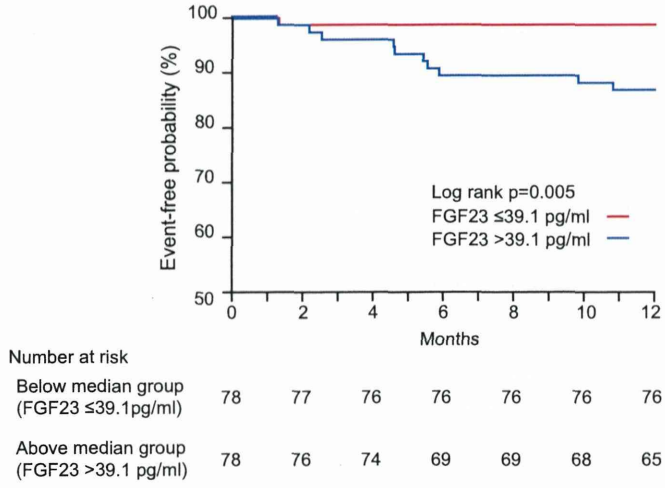

Fig. 2. Kaplan-Meier analysis for cardiovascular events in the below and above median FGF23 groups. Red and blue lines show the below and above median FGF23 groups, respectively. Kaplan-Meier analysis showed that the frequency of cardiovascular events was higher in the above median FGF23 group than in the below median FGF23 group ($P = 0.005$).

serum FGF23 levels were higher in patients with HF hospitalization history. We also demonstrated the predictive value of serum FGF23 levels for clinical outcome even after accounting for age differences in the multivariate models. There were a few patients with extreme outlier values, as shown in Fig. 1*B*. No confounding factor for the elevation of serum FGF23 levels, such as a past history of bone metabolic disease, was found in patients. However, after the exclusion of patients with outlier values, a similar tendency was found ($r = 0.411$, $P = 0.0008$). Urine tests were not performed in all enrolled patients, because the results of urine tests were taken from a part of clinical routine tests. Nevertheless, the subanalysis for TmP/GFR might provide us some information regarding relative levels of serum FGF23 levels.

Conclusions. Serum FGF23 levels were elevated in NICD patients with a history of HF hospitalization even though their renal function was relatively preserved. We demonstrated that the underlying substrates of HF, such as cardiac dysfunction and neurohormonal activation, are associated with an elevation of serum FGF23 levels in our patients and suggest that the progression of HF itself contributes to the elevation of circulating FGF23 levels even in early CKD, which leads to further myocardial dysfunction, potentially creating a vicious cycle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.I., H.T., A.F., Y.S., T.O., T.H., and M.A. analyzed data; M.I., H.T., A.F., Y.S., T.O., T.H., and M.A. interpreted results of experiments; M.I. and H.T. prepared figures; M.I., H.T., and M.K. drafted manuscript; H.T. and M.K. conception and design of research; H.A., T.H., H.K., T.A., and M.K. edited and revised manuscript; M.K. approved final version of manuscript.

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Table 9. *Predictive value of serum FGF23 levels for cardiovascular events*

Above Median Value Versus Below Median Value	Hazard Ratios	95% Confidence Interval	*P* Value
Unadjusted	1.05	1.03, 1.07	<0.0001
Model 1	1.05	1.03, 1.08	<0.0001
Model 2	1.06	1.03, 1.09	<0.0001

Model 1 was adjusted for age and sex, and *model 2* was adjusted for age, sex, and eGFR.

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