1 **SUPPLEMENTAL MATERIAL**

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3 **The utility of collagen-derived peptides as markers of organ injury in patients with acute**

4 **heart failure: A prospective cohort study**

5 Nagao et al. Collagen markers in acute heart failure

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1   **Supplemental Methods**

2   **Participating centers**

3   Osaka Red Cross Hospital, Osaka, Japan,

4   Hyogo Prefectural Amagasaki General Medical Center, Amagasaki, Japan,

5   Kurashiki Central Hospital, Kurashiki, Japan

6   **Measurements of markers**

7   We used commercially available radioimmunoassay kits for PIIINP (CIS Bio International,

8   Codolet, France) and P4NP 7S (Sceti Medical Labo, Tokyo, Japan) assays; the lower limits of

9   detection for PIIINP and P4NP 7S were 0.2 U/mL and 1.25 ng/mL, respectively, and the

10 coefficient of variation was <5%. When necessary, the serum was stored at $\leq-30°C$ until

11 assayed following the established protocol used in the clinical setting. Based on the nationwide

12 standardization of laboratory measurements for medical care in Japan (Multianalyte

13 Conventional Reference Material [MacRM]),[17] the following were used as cutoff values of

14 normal LFTs: total bilirubin (T-Bil), 1.5 mg/dL; aspartate aminotransferase (AST), 30 IU/L;

15 alkaline phosphatase (ALP), 322 IU/L; γ-glutamyltransferase (γ-GTP), 64 IU/L in males and

16 32 IU/L in females. We calculated %changes of (%Δ) markers during hospitalization as

17 (discharge value – admission value)/admission value×100.

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1   **Supplemental Table 1. Correlation of PIIINP and P4NP 7S with clinical parameters**

Variables	PIIINP		P4NP 7S	
	Coefficient	*P*	Coefficient	*P*
T-Bil, mg/dl	−0.2	0.0001	0.4	<0.0001
AST, U/L	−0.12	0.016	0.28	<0.0001
ALP, U/L	0.073	0.16	0.16	0.002
γ-GTP, U/L	−0.14	0.011	0.41	<0.0001
Albumin, g/dl	−0.1	0.059	0.045	0.38
eGFR, mL/min/1.73 m^2	−0.44	<0.0001	−0.07	0.0063
BNP, pg/ml	0.095	0.071	0.25	<0.0001
PIIINP, U/mL	N/A		0.24	<0.0001
P4NP 7S, ng/mL	0.24	<0.0001	N/A	

2   ALP, Alkaline phosphatase; AST, aspartate aminotransferase; BNP, brain natriuretic peptide;

3   eGFR, estimated glomerular filtration rate; γ-GTP, γ-glutamyltransferase; PIIINP, N-terminal

4   propeptide of procollagen type III; P4NP 7S, 7S domain of the collagen type IV N-terminal

5   propeptide; T-Bil, total bilirubin.

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1 **Supplemental Table 2. Univariate and multivariate COX analyses of the 365-day**

2 **incidence of cardiac death and admission for HF**

Variable	Unadjusted HR (95%CI)	P	Adjusted HR (95%CI)	P	Adjusted HR (95%CI)	P
			Model 1		Model 2	
Age (per 1 year)	1.02 (1.00-1.04)	0.04	1.01 (0.99-1.03)	0.44	1.01 (0.99-1.03)	0.2
Male	1.14 (0.79-1.66)	0.48	1.39 (0.94-2.05)	0.1	1.37 (0.93-2.02)	0.11
EF <40%	0.84 (-.57-1.22)	0.36	1.02 (0.68-1.52)	0.9	1.04 (0.69-1.55)	0.86
eGFR (per 1 mL/min/1.73 m^2)	0.97 (0.96-0.98)	<0.001	0.97 (0.96-0.98)	<0.001	0.97 (0.96-0.98)	<0.001
PIIINP (high versus low)	1.19 (0.83-1.73)	0.34	**0.78 (0.52-1.17)**	**0.23**	-	
P4NP 7S (high versus low)	1.20 (0.83-1.73)	0.34	-		**1.50 (1.02-2.21)**	**0.04**

3 EF, ejection fraction; HF, heart failure; HR, hazard ratio

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Supplemental Table 3. Crude and adjusted HR associated with abnormal

LFTs for cardiac death and admission for HF

	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
T-Bil	1.26 (0.44–2.78)	0.63	1.58 (0.55–3.57)	0.36
AST	0.72 (0.42–1.15)	0.17	0.86 (0.50–1.40)	0.56
ALP	1.17 (0.73–1.79)	0.51	1.37 (0.86–2.12)	0.18
γ-GTP	0.70 (0.45–1.05)	0.08	0.89 (0.56–1.39)	0.62

1   In the multivariable Cox proportional hazards models, age, male, ejection fraction (EF) <40%,

2   and estimated glomerular filtration rate (eGFR), were incorporated as risk-adjusting variables.

3   ALP, alkaline phosphatase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase; T-

4   Bil, total bilirubin.

1 **Supplemental Table 4. Difference in markers at discharge and %Δmarkers during hospitalization between the patients**

2 **with and without 90-day events**

	Markers at discharge			%Δmarkers		
	90-day cardiac death and HF hospitalization		*P*	90-day cardiac death and HF hospitalization		*P*
	No	Yes		No	Yes	
T-Bil	0.6 (0.5–0.9)	0.7 (0.5–0.9)	0.43	-20.0% (-37.5–0)	-22.2% (-38.7–1.8)	0.84
AST	22 (17–29)	23 (17–28)	0.68	-29.0% (-88.6–0)	-12.0% (-42.2–13.2)	0.0037
ALP	237 (195–303)	225 (171–309)	0.31	-7.1% (-18.9–5.3)	-1.8% (-17.1–11.5)	0.31
γ-GTP	33 (19–67)	26 (17–87)	0.50	-10.4% (-29.0–9.1)	-3.8% (-18.9–11.6)	0.09
PIIINP	0.71 (0.57–0.93)	0.72 (0.61–0.90)	0.52	-0.5% (-12.9–16.4)	-5.1% (-17.9–10.0)	0.13
P4NP 7S	5.0 (4–6.4)	5.6 (4.4–7.1)	0.042	-16.1% (-27.1–-5.4)	-11.5% (-26.1–3.5)	0.053

1   ALP, alkaline phosphatase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase; HF, heart failure; PIIINP, N-terminal propeptide of

2   procollagen type III; P4NP 7S, 7S domain of the collagen type IV N-terminal propeptide; T-Bil, total bilirubin.
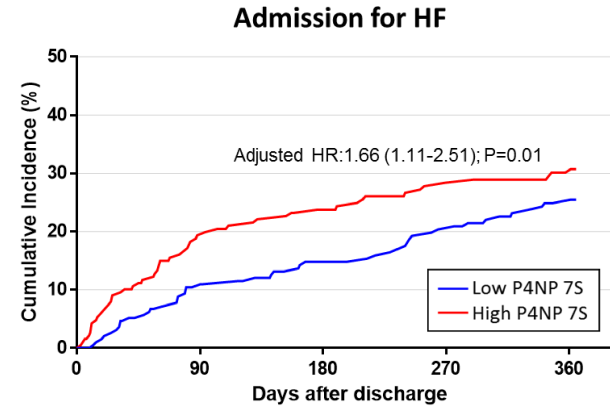
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1   **Supplemental Figure 1**

2   **A**   **B**
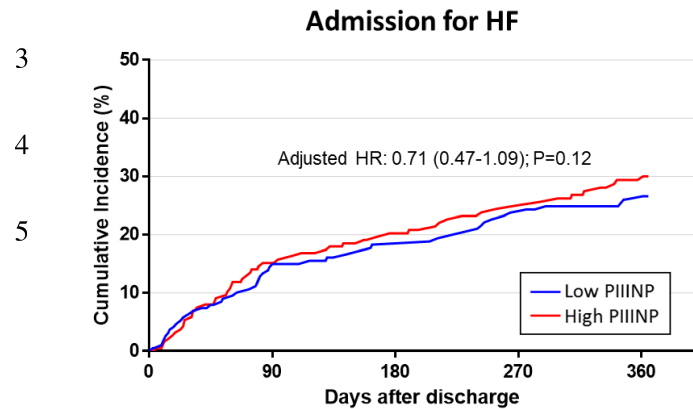
1   **C**   **D**

Cardiac death

Interval	0 day	90 days	180 days	365 days
Low PIIINP				
N of patients at risk	193	165	158	143
N of patients with event		6	8	13
Cumulative incidence		3.3%	4.5%	7.6%
High PIIINP				
N of patients at risk	191	152	137	109
N of patients with event		4	7	11
Cumulative incidence		2.8%	4.2%	7.2%

Cardiac death
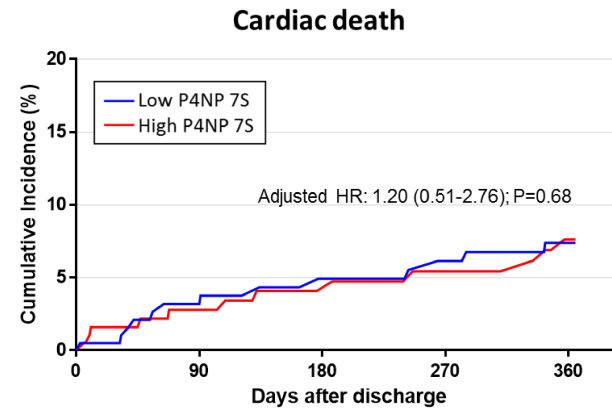
Interval	0 day	90 days	180 days	365 days
Low P4NP 7S				
N of patients at risk	200	167	154	126
N of patients with event		6	9	13
Cumulative incidence		3.2%	4.9%	7.4%
High P4NP 7S				
N of patients at risk	190	147	136	115
N of patients with event		5	7	12
Cumulative incidence		2.8%	4.1%	7.6%

1   **E**
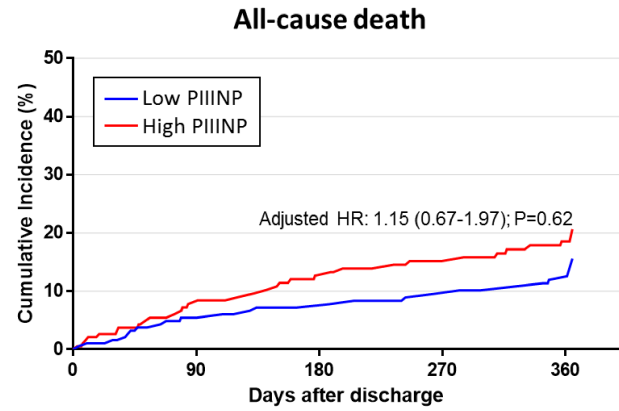
All-cause death

Adjusted HR: 1.15 (0.67-1.97); P=0.62

Interval	0 day	90 days	180 days	365 days
Low PIIINP				
N of patients at risk	193	165	158	143
N of patients with event		11	14	28
Cumulative incidence		6.0%	7.7%	16.1%
High PIIINP				
N of patients at risk	191	155	142	115
N of patients with event		15	23	35
Cumulative incidence		8.3%	13.1%	21.1%

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All-cause death

Adjusted HR: 1.07 (0.63-1.79); P=0.8
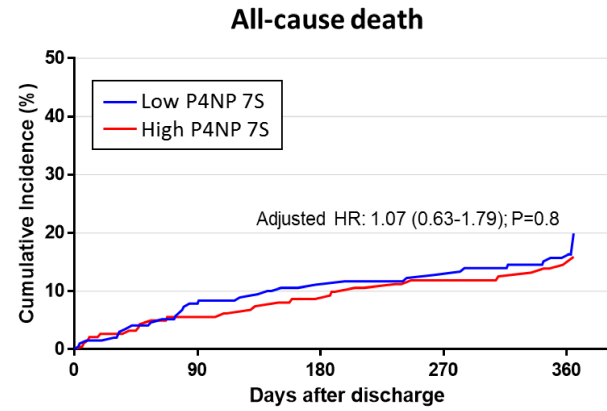
Interval	0 day	90 days	180 days	365 days
Low P4NP 7S				
N of patients at risk	200	170	160	138
N of patients with event		16	22	37
Cumulative incidence		8.3%	11.6%	20.4%
High P4NP 7S				
N of patients at risk	190	155	145	125
N of patients with event		11	16	27
Cumulative incidence		6.1%	9.1%	16.4%

1   **Supplemental Figure Legends**

2   **Supplemental Figure 1. The Kaplan–Meier curves for secondary outcome measures**

3   **across PIIINP (A, C and E) and P4NP 7S (B, D and F).**

4   The seconday outcome measures were defined as admission for HF (A and B), cardiac death

5   (C and D) and all-cause death (E and F). HF, heart failure; HR, hazard ratio; PIIINP, N-terminal

6   propeptide of procollagen III; P4NP 7S, 7S domain of the collagen type IV N-terminal

7   propeptide.

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