Clinical Investigation

Association of Serum Zinc Level With Prognosis in Patients With Heart Failure

AKIOMI YOSHIHISA, MD, PhD, SATOSHI ABE, MD, TAKATOYO KIKO, MD, YUSUKE KIMISHIMA, MD, YU SATO, MD, SHUNSUKE WATANABE, MD, YUKI KANNO, MD, MAKIKO MIYATA-TATSUMI, MD, PhD, TOMOFUMI MISAKA, MD, PhD, TAKAMASA SATO, MD, PhD, SATOSHI SUZUKI, MD, PhD, MASAYOSHI OIKAWA, MD, PhD, ATSUSHI KOBAYASHI, MD, PhD, TAKAYOSHI YAMAKI, MD, PhD, HIROYUKI KUNII, MD, PhD, KAZUHIKO NAKAZATO, MD, PhD, TAKAFUMI ISHIDA, MD, PhD, AND YASUCHIKA TAKEISHI, MD, PhD

Fukushima, Japan

ABSTRACT

Background: Zinc is an essential cofactor for energy transfer and physiological heart function, has antioxidant properties, and is involved in multiple signaling pathways. We aimed to investigate the associations between serum zinc levels with prognosis, as well as underlying cardiac function and exercise capacity, in patients with heart failure (HF).

Methods and Results: We measured serum zinc levels in 968 consecutive hospitalized patients with decompensated HF, who were divided into 3 groups based on serum zinc levels (μg/dL): first (zinc ≥75, n = 323), second (62≤ zinc <75, n = 322), and third (zinc <62, n = 323) tertiles. We examined cardiac function and exercise capacity and followed up on all patients. Although cardiac function did not differ among the 3 groups, peak oxygen consumption was significantly lower in the third tertile than in the first and second tertiles (peak oxygen consumption, 14.2 vs 15.9 and 15.2 mL/kg/min, $P = .010$). In the Kaplan-Meier analysis (mean duration of follow-up 1103 days), cardiac and all-cause mortality was highest in the third tertile compared with the first and second tertiles. In the Cox proportional hazard analysis, serum zinc level was a predictor of cardiac and all-cause mortality. In the subgroup analysis, there were no interactions concerning associations between serum zinc levels with prognosis and other important variables, including age, gender, comorbidities, medications, other micronutrient levels, B-type natriuretic peptide, and left ventricular ejection fraction. The associations between zinc levels with mortality were consistent in all subgroups.

Conclusion: Decreased serum zinc levels are associated with high mortality, accompanied by impaired exercise capacity. (*J Cardiac Fail 2018;24:375–383*)

Key Words: Serum zinc levels, cardiac function, exercise capacity, prognosis.

Heart failure (HF) is a major cause of death among the elderly in many countries, and has become a significant public health issue.[1,2] Micronutrients such as zinc (Zn) are essential cofactors for energy transfer and physiological heart function, have antioxidant properties, and are involved in multiple signaling pathways.[3–7] Zinc plays a role in antioxidant defense and the regulation of various metalloproteases (eg, angiotensin-converting enzyme, matrix metalloproteinases, copper [Cu]/Zn superoxide dismutase).[7,8] Zinc deficiency may occur in patients with HF because of reduced dietary intake, decreased Zn absorption from gastrointestinal edema, impaired motility, or intestinal Zn losses or side effects of several HF medications.[9] Several studies have reported that blood Zn concentrations are lower in patients with HF than in control

From the Department of Cardiovascular Medicine, Fukushima Medical University, Fukushima, Japan.

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Reprint requests: Akiomi Yoshihisa, MD, PhD, Department of Cardiovascular Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima, 960-1295, Japan. Tel: +81 24 547 1190; Fax: +81 24 548 1821. E-mail: yoshihis@fmu.ac.jp.

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subjects.[5] Little is known, however, about the clinical associations between serum Zn levels with HF prognosis.[3–6]

The aim of the current study was to investigate the associations between Zn levels with HF prognosis and the underlying clinical and pathophysiological parameters (eg, cardiac function, exercise capacity).

Methods

Subjects and Study Protocol

This was a prospective observational study of 1079 consecutive decompensated patients with HF who were discharged from Fukushima Medical University Hospital between 2010 and 2015. The diagnosis of decompensated HF was made by several cardiologists based on the definition in the HF guidelines.[1,2] Blood samples were obtained at hospital discharge. Patients without any data on their Zn levels (n = 36), those who had acute coronary syndrome (n = 31), and those who received dialysis (n = 44) were excluded. The included patients (n = 968) were divided into 3 groups based on Zn level (μg/dL): first (Zn ≥75, n = 323), second (62≤ Zn < 5, n = 322), and third (Zn <62, n = 323) tertiles. We compared clinical features, laboratory data, and the parameters of echocardiography and cardiopulmonary exercise testing among the 3 groups. We evaluated several comorbidities that often coexist and/or are associated with adverse prognosis in patients with HF. Hypertension was defined as the recent use of antihypertensive drugs, or a systolic blood pressure of >140 mmHg, and/or a diastolic pressure of >90 mmHg. Diabetes was defined as the recent use of insulin or antidiabetic drugs, a fasting blood glucose value >126 mg/dL, and/or a hemoglobinA1c value >6.5%. Dyslipidemia was defined as the recent use of cholesterol-lowering drugs, a triglyceride value >150 mg/dL, a low-density lipoprotein cholesterol value >140 mg/dL, and/or a high-density lipoprotein cholesterol value <40 mg/dL. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate of <60 mL•min•1.73 cm^2 according to the Modification of Diet in Renal Disease formula.[10] Anemia was defined as a hemoglobin level <12.0 g/dL in females and <13.0 g/dL in males.[1] Atrial fibrillation was identified by electrocardiogram performed during hospitalization and/or from medical records.

The patients were followed until 2017 for cardiac and all-cause mortality. Cardiac death was confirmed by independent experienced cardiologists as death either from worsened HF, ventricular fibrillation documented by electrocardiograph or implantable devices, sudden cardiac death, or acute coronary syndrome. All-cause death included death from respiratory failure, infection, sepsis, cancer, renal failure, stroke, digestive hemorrhage, and others. Status and dates of death were obtained from the patients' medical records. If these data were unavailable, status was ascertained by a telephone call to a patient's referring hospital physician. Different physicians, who were blinded to the analyses of this study, conducted an investigation. We were able to follow-up all patients, and written informed consent was obtained from all study subjects. The study protocol was approved by the ethical committee of Fukushima Medical University, and the investigation conforms to the principles outlined in the Declaration of Helsinki. Reporting of the study conforms to Strengthening the Reporting of Observational Studies in Epidemiology along with references to Strengthening the Reporting of Observational Studies in Epidemiology and the broader Enhancing the Quality and Transparency of Health Research guidelines.[11]

Measurement of Serum Zn Levels

Serum Zn was measured by using 5-Br-PAPS absorptiometry (ACCURAS AUTO Zn, Shino-test Corporation, Kanagawa, Japan). Absorptiometry was performed by clinical laboratory technologists at Fukushima Medical University Hospital.

Echocardiography

Echocardiography was performed using standard techniques[12,13] by experienced echocardiographers who were blinded to Zn levels. The echocardiographic parameters investigated included left ventricular ejection fraction (LVEF), left atrial volume, the ratio of early transmitral flow velocity to mitral annular velocity (mitral valve E/e′), inferior vena cava diameter, right ventricular fractional area change, and tricuspid regurgitation pressure gradient. The LVEF was calculated using a modification of the Simpson method. Mitral valve E/e′ was calculated by transmitral Doppler flow and tissue Doppler imaging. The right ventricular fractional area change was defined as follows: (end-diastolic area — end systolic area)/end-diastolic area × 100.[12,13] All recordings were performed on ultrasound systems (ACUSON Sequoia, Siemens Medical Solutions USA, Inc., Mountain View, CA).

Cardiopulmonary Exercise Testing

To estimate the severity of heart failure, exercise capacity and its use in the cardiac rehabilitation and education of patients with HF, cardiopulmonary exercise testing was encouraged by hospital physicians as much as possible during hospitalization. Of the 968 patients, 322 (33.3%) underwent cardiopulmonary exercise testing. The patients underwent incremental symptom-limited exercise testing before discharge using an upright cycle ergometer with a ramp protocol (Strength Ergo 8, Fukuda Denshi Co. Ltd., Tokyo, Japan). Breath-by-breath oxygen consumption (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE) were measured during exercise using an AE-300S respiratory monitor (Minato Medical Science, Osaka, Japan).[14,15] Peak VO_2 was measured as an average of the last 30 s of exercise. Ventilatory response to exercise (the slope of the relationship between ventilation and carbon dioxide production, VE/VCO_2 slope) was calculated as the regression slope relating VE to CO_2 from the start of exercise until the respiratory compensation point (the time at which ventilation is stimulated by CO_2 output and end-tidal CO_2 tension begins to decrease).[14,15] The ventilatory anaerobic threshold was calculated using the V-slope method.

Statistical Analysis

Normal distribution was assessed through the Kolmogorov-Smirnov test. Normally distributed data are presented as mean ± standard deviation, non-normally distributed data are presented as median (interquartile range), and categorical variables are expressed as numbers and percentages. The chi-square test was used for comparisons of categorical variables. We used the analysis of variance for continuous variables, followed by the Tukey post hoc test. The Kaplan-Meier method was used for presenting the event rate and the log-rank test was used for initial comparisons. Univariable and multivariable Cox proportional hazard analyses were used to evaluate serum Zn levels (1 μg/dL increase) as a predictor of cardiac and all-cause mortality. In addition, to assess the potential heterogeneity of associations between Zn levels and both cardiac and all-cause mortality, we conducted subgroup analyses. A value of $P < .05$ was considered significant for all comparisons. These analyses were performed using a statistical software package (SPSS, version 22.0, IBM, Armonk, NY).

Results

The average Zn level of the present study's population was 68.2 ± 15.9 (range 19.0–145.0 μg/dL). Comparisons of the clinical characteristics are shown in **Table 1**. Age, systolic blood pressure, prevalence of hypertension, CKD, anemia, and usage of diuretics were significantly highest in the third tertile. In contrast, we found no significant difference in gender, diastolic blood pressure, prevalence of dyslipidemia, atrial fibrillation, or usage of other medications among the groups.

The comparisons of parameters of other laboratory data and echocardiography and cardiopulmonary exercise tests are shown in Table 2. Sodium, calcium, iron, low-density lipoprotein, and high-density lipoprotein were significantly lower, whereas C-reactive protein and troponin I were significantly higher in the third tertile than in the first and second tertiles. In contrast, potassium, chloride, magnesium, and B-type natriuretic peptide (BNP) did not differ among the groups. Although no echocardiographic parameters differed between the groups, peak VO_2 was significantly lower and VE/VCO_2 was higher in the third than in the first and second tertiles (Fig. 1).

In the follow-up period (mean 1103 ± 628 days), 184 deaths (90 cardiac deaths and 94 noncardiac deaths) occurred. In the Kaplan-Meier analysis (Fig. 2), cardiac and all-cause mortality was highest in the third compared with the first and second tertiles. Cox proportional hazard analyses of association of serum Zn level, which are presented as categorical variable (tertiles) and continuous variable (per 10 μg/dL decrease), with cardiac and all-cause mortality are presented in Table 3. In the univariable Cox proportional hazard analysis, serum Zn was predictor of cardiac and all-cause mortality. To prepare for potential confounding and avoid overfitting considering the number of the events, 2 bivariate models were analyzed: model 1 for cardiac mortality (demographic parameters that are generally known to affect the risk of mortality in patients with HF and/or were different factors among the groups presented in Table 1; age, gender, body mass index, New York Heart Association functional class, presence of hypertension, diabetes, CKD, anemia, usages of diuretics and inotropic agents) and model 2 for cardiac mortality and all-cause mortality (model 1 plus other laboratory data generally

Table 1. Comparisons of Clinical Characteristics of Patients Among Zinc Tertiles (N = 968)

	First Tertile Zinc ≥75 (n = 323)	Second Tertile 62≤ Zinc <75 (n = 322)	Third Tertile Zinc <62 (n = 323)	*P* Value
Zinc (μg/dL)	84.9 ± 9.7 (range 75–145)	68.4 ± 3.8 (range 62–74)[A]	51.2 ± 9.2 (range 19–61)[A,B]	<.001
Demographics				
Age (y)	62.4 ± 14.9	65.9 ± 13.2[A]	71.4 ± 13.4[A,B]	<.001
Male gender (n, %)	204 (63.2)	198 (61.5)	206 (63.8)	.824
Body mass index (kg/m^2)	23.8 ± 3.9	23.2 ± 4.1	23.0 ± 4.4	.026
Systolic blood pressure (mmHg)	124.1 ± 25.9	128.8 ± 33.5	132.2 ± 33.0[A]	.005
Diastolic blood pressure (mmHg)	72.0 ± 18.3	73.6 ± 22.8	73.9 ± 22.1	.446
NYHA functional class I/ II/III/ IV	87 (26.9)/231 (71.5)/5 (1.5)/0	73 (22.7)/240 (74.5)/9 (2.8)/0	65 (20.1)/244 (75.5)/14 (4.3)/ 0	.091
Comorbidity				
Hypertension (n, %)	208 (64.4)	233 (72.4)	242 (74.9)	.009
Diabetes (n, %)	111 (34.4)	129 (40.1)	140 (43.3)	.061
Dyslipidemia (n, %)	262 (81.1)	250 (77.6)	251 (77.7)	.466
Chronic kidney disease (n, %)	155 (48.0)	170 (52.8)	216 (66.9)	<.001
Anemia (n, %)	124 (38.4)	146 (45.3)	227 (70.3)	<.001
Atrial fibrillation (n, %)	114 (35.3)	131 (40.7)	121 (37.5)	.365
Medication				
RAS inhibitors (n, %)	242 (74.9)	236 (73.3)	239 (74.0)	.894
β-blockers (n, %)	250 (77.4)	257 (79.8)	258 (79.9)	.678
Diuretics (n, %)	175 (54.2)	204 (63.4)	240 (74.3)	<.001
Inotropic agents (n, %)	31 (9.6)	35 (10.9)	49 (15.2)	.072
Mineral corticoid receptor antagonists (n, %)	130 (40.2)	129 (40.1)	150 (46.4)	.175
Calcium channel blockers (n, %)	111 (34.4)	113 (35.1)	107 (33.1)	.868

NYHA, New York Heart Association; RAS, renin-angiotensin-aldosterone system.
[A]$P < 0.01$ vs first tertile and [B]$P < 0.01$ vs second tertile.

Table 2. Comparisons of Parameters of Laboratory Data, Echocardiography and Cardiopulmonary Exercise Tests Among Zinc Tertiles (N = 968)

	First Tertile Zinc ≥75 (n = 323)	Second Tertile 62≤ Zinc <75 (n = 322)	Third Tertile Zinc <62 (n = 323)	*P* Value
Laboratory data				
Sodium (mmol/L)	139.6 ± 3.3	139.0 ± 3.5	138.1 ± 4.2[B,C]	<.001
Potassium (mmol/L)	4.2 ± 0.5	4.2 ± 0.5	4.2 ± 0.7	.513
Chloride (mmol/L)	103.5 ± 3.7	103.0 ± 4.1	103.4 ± 4.5	.358
Magnesium (mg/dL)	1.78 ± 0.22	1.77 ± 0.24	1.76 ± 0.29	.363
Calcium (mg/dL)	9.28 ± 0.51	9.08 ± 0.57[B]	8.61 ± 0.63[B,D]	<.001
Iron (μg/dL)	92.8 ± 43.5	80.3 ± 41.0[B]	62.0 ± 36.7[B,D]	<.001
LDL (mg/dL)	112.5 ± 33.7	100.3 ± 34.3[B]	89.6 ± 30.6[B,D]	<.001
HDL (mg/dL)	48.9 ± 14.8	53.0 ± 23.7	44.2 ± 18.1[D]	<.001
C-reactive protein (mg/dL)[‖]	0.06 (0.03–0.13)	0.07 (0.03–0.16)	0.13 (0.04–0.43)[B,D]	<.001
BNP (pg/mL)[‖]	79.0 (27.4–161.7)	88.0 (41.1–193.9)	106.5 (56.1–263.2)	.147
Troponin I (ng/mL)[‖]	0.018 (0.017–0.040)	0.026 (0.017–0.053)	0.040 (0.019–0.112)[B,D]	<.001
Echocardiography				
LVEF (%)	50.9 ± 16.9	50.4 ± 15.9	50.4 ± 15.1	.942
RV-FAC (%)	39.4 ± 14.2	42.7 ± 15.1	41.5 ± 13.3	.100
Inferior vena cava diameter (mm)	14.3 ± 4.4	15.1 ± 4.9	15.0 ± 5.1	.133
TRPG (mmHg)	20.1 ± 11.3	20.2 ± 12.1	19.9 ± 10.0	.970
Cardiopulmonary exercise test (n = 322)	n = 126	n = 111	n = 85	
Peak VO_2 (mL•kg•min)	15.9 ± 4.1	15.2 ± 4.1 [A]	14.2 ± 3.9 [B,C]	.010
VE/VCO_2 slope	33.5 ± 8.2	36.1 ± 8.5 [A]	36.7 ± 8.8 [A,C]	.013

BNP, B-type natriuretic peptide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; peak VO_2, breath-by-breath oxygen consumption; RV-FAC, right ventricular fractional area change; TRPG, tricuspid regurgitation pressure gradient; VE/VCO_2 slope, slope of the relationship between ventilation and carbon dioxide production.

[A]$P < .05$ and [B]$P < .01$ vs first tertile, [C]$P < .05$ and [D]$P < .01$ vs second tertile.

[‖]Data are presented as median (interquartile range).

known to affect the risk of mortality in patients with HF and/or were different factors among the groups presented in Table 2; sodium, calcium, iron, C-reactive protein, BNP, and troponin I). Serum Zn level was an independent predictor of cardiac and all-cause mortality in model 1; however, serum Zn level was not an independent predictor of all-cause mortality in model 2.

Furthermore, to assess the potential heterogeneity of associations between Zn levels with both cardiac and all-cause mortality, we conducted subgroup analyses (Tables 4 and 5). There were no interactions among associations between serum Zn levels with prognosis and other important variables, including age, gender, comorbidities, medications, other micronutrients levels, BNP, and LVEF. Thus, the association between Zn levels with mortality was consistent in all subgroups.

Discussion

To the best of our knowledge, the present study is the first to report that HF patients with lower serum Zn levels have higher mortality rates, lower levels of other micronutrients (sodium, calcium and iron), increased inflammation and myocardial damage (C-reactive protein and troponin I), and impaired exercise capacity. Reduction in serum Zn, as well as sodium, calcium, and iron, may occur in patients with HF because of reduced dietary intake, decreased absorption from gastrointestinal edema, impaired motility, or intestinal Zn losses.[9,16] Additionally, merged diabetes and CKD, as well as the side effects of diuretics, leads to Zn excretion of excessive amounts in the urine.[9,16] Concordant with these data, the present study reports that the prevalence of diabetes and CKD, as well as the use of diuretics,

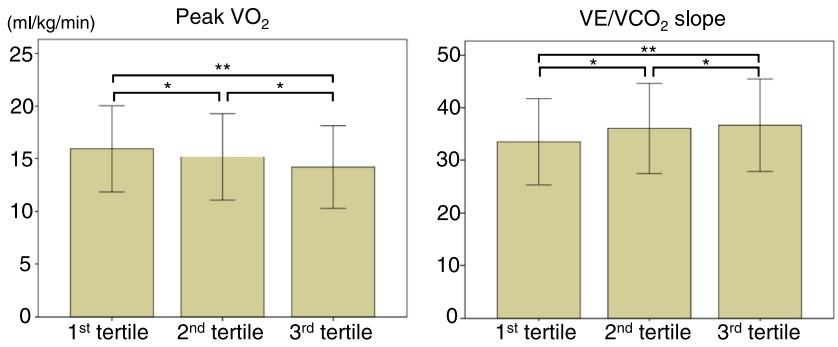

Fig. 1. Comparisons of peak VO_2 and VE/VCO_2 slope among tertiles. VE/VCO_2, relationship between ventilation and carbon dioxide production; VO_2, peak oxygen consumption.

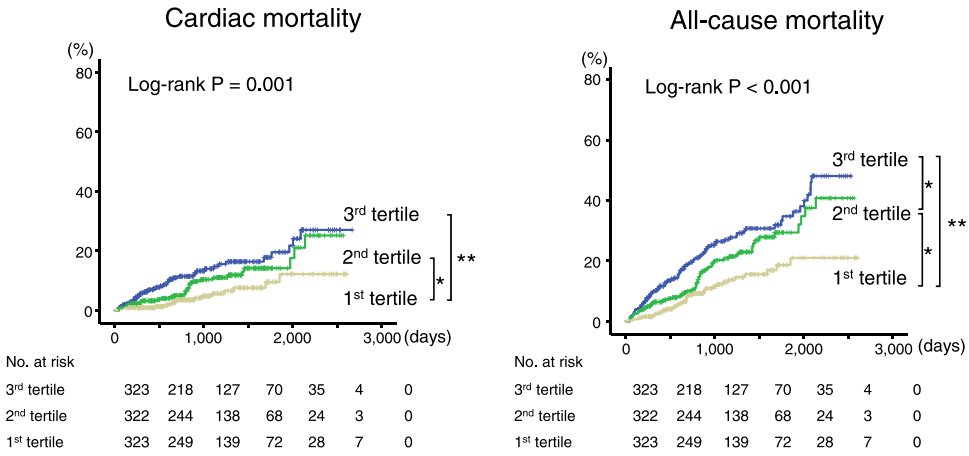

Fig. 2. Kaplan-Meier analysis for cardiac and all-cause mortality for both normal zinc and zinc-deficient groups. *$P < .05$; **$P < .01$.

were significantly higher in the reduced Zn group. Another possible underlying pathophysiologic mechanism of Zn deficiency is renin angiotensin aldosterone system activation with a marked increase in fecal and urinary Zn excretion,[16] which can be blocked by an aldosterone receptor antagonist. Use of renin angiotensin aldosterone system inhibitors and mineral corticoid receptor antagonists did not differ among the 3 groups in the present study, however.

Zinc is essential for the immune system,[17] and its reduction leads to impairment in the activity of the thymus and thymic hormones.[18] In addition, Zn is associated with the healing process and is implicated in the antioxidant defense

Table 3. Cox Proportional Hazard Model of Cardiac Event and All-Cause Mortality in Heart Failure

	HR	95% CI	*P* Value
Cardiac mortality (90 event/n = 968)			
Zinc (categorical variable) unadjusted			
First tertile	Ref		
Second tertile (vs first tertile)	2.013	1.093–3.707	.025
Third tertile (vs first tertile)	2.913	1.615–5.256	<.001
Zinc (categorical variable) adjusted model 1*			
First tertile	Ref		
Second tertile (vs first tertile)	1.907	1.034–3.515	.039
Third tertile (vs first tertile)	2.162	1.189–3.933	.021
Zinc (continuous variable) unadjusted			
Per zinc 10 μg/dL decrease	1.259	1.105–1.438	.001
Zinc (continuous variable) adjusted model 1*			
Per zinc 10 μg/dL decrease	1.233	1.020–1.331	.046
All-cause mortality (184 event/n = 968)			
Zinc (categorical variable) unadjusted			
First tertile	Ref		
Second tertile (vs first tertile)	1.922	1.269–2.911	.002
Third tertile (vs first tertile)	2.615	1.745–3.919	<.001
Zinc (categorical variable) adjusted model 1*			
First tertile	Ref		
Second tertile (vs first tertile)	1.517	0.993–2.317	.054
Third tertile (vs first tertile)	1.540	1.009–2.349	.045
Zinc (categorical variable) adjusted model 2†			
First tertile	Ref		
Second tertile (vs first tertile)	1.145	0.710–1.845	.179
Third tertile (vs first tertile)	1.344	0.855–2.111	.120
Zinc (continuous variable) unadjusted			
Per zinc 10 μg/dL decrease	1.268	1.138–1.370	<.001
Zinc (continuous variable) adjusted model 1*			
Per zinc 10 μg/dL decrease	1.221	1.010–1.231	.030
Zinc (continuous variable) adjusted model 2†			
Per zinc 10 μg/dL decrease	1.030	0.904–1.149	.182

CI, confidence interval; HR, hazard ratio; Ref, reference; other abbreviations as in **Table 1**.

*Adjusted model 1: adjusted for age, gender, body mass index, systolic blood pressure, NYHA functional class, presence of hypertension, diabetes, chronic kidney disease, anemia, usage of diuretics, and inotropic agents.

†Adjusted model 2: model 1 plus adjusted for sodium, calcium, iron, C-reactive protein, BNP, and troponin I.

Table 4. Subgroup Analysis for Associations Between Zinc Levels and Cardiac Mortality

Factor	Subgroup	n	HR	95% CI	*P* Value	Interaction *P* Value
Zinc level (10 μg/dL decrease)	**Total**	**968**	**1.259**	**1.105–1.438**	**.001**	
Age	≥75	325	1.268	1.040–1.553	.020	.610
	<75	643	1.172	0.980–1.411	.083	
Gender	Male	608	1.243	1.072–1.452	.005	.836
	Female	360	1.293	1.010–1.676	.045	
Body mass index	≥22.8	482	1.138	0.904–1.424	.256	.194
	<22.8	486	1.318	1.116–1.553	<.001	
NYHA class	I/II	940	1.293	1.105–1.480	<.001	.091
	III/IV	28	0.809	0.544–1.219	.322	
Hypertension	Present	683	1.318	1.116–1.553	.001	.442
	Absent	285	1.184	0.951–1.495	.138	
Diabetes	Present	380	1.161	0.970–1.411	.107	.306
	Absent	588	1.344	1.116–1.613	.002	
Dyslipidemia	Present	763	1.243	1.083–1.438	.002	.800
	Absent	205	1.318	0.932–1.842	.125	
CKD	Present	541	1.138	0.980–1.331	.100	.077
	Absent	427	1.495	1.138–1.931	.003	
Anemia	Present	497	1.116	0.951–1.318	.163	.114
	Absent	471	1.424	1.105–1.860	.007	
Atrial fibrillation	Present	366	1.318	1.072–1.598	.007	.556
	Absent	602	1.219	1.020–1.452	.029	
RAS inhibitors	Present	717	1.195	1.030–1.397	.020	.216
	Absent	251	1.424	1.127–1.842	.004	
β-blockers	Present	765	1.231	1.072–1.424	.004	.541
	Absent	203	1.344	0.990–1.860	.059	
Diuretics	Present	619	1.231	1.062–1.424	.008	.703
	Absent	349	1.318	1.001–1.741	.050	
Inotropic agents	Present	115	1.161	0.942–1.452	.162	.503
	Absent	853	1.268	1.072–1.509	.006	
Mineral corticoid receptor antagonists	Present	409	1.195	1.020–1.397	.029	.362
	Absent	559	1.357	1.083–1.708	.007	
Calcium channel blockers	Present	331	1.370	1.072–1.741	.010	.370
	Absent	637	1.207	1.041–1.411	.015	
Sodium	≥139	500	1.411	1.161–1.724	.001	.093
	<139	468	1.127	0.951–1.357	.173	
Potassium	≥4.2	475	1.438	1.172–1.774	.001	.106
	<4.2	493	1.161	0.980–1.384	.082	
Chloride	≥104	460	1.553	1.219–1.967	<.001	.119
	<104	508	1.116	0.951–1.318	.171	
Magnesium	≥1.80	499	1.268	1.030–1.524	.022	.952
	<1.80	469	1.268	1.051–1.509	.012	
Calcium	≥8.8	481	1.127	0.860–1.495	.365	.586
	<8.8	487	1.231	1.062–1.452	.008	
Iron	≥48	485	1.161	0.904–1.495	.243	.536
	<48	483	1.280	1.083–1.509	.003	
BNP	≥90.8	484	1.219	1.041–1.424	.012	.510
	<90.8	484	1.344	1.020–1.774	.035	
Troponin I	≥0.029	482	1.243	1.072–1.452	.004	.318
	<0.029	486	1.072	0.825–1.384	.589	
LVEF	≥50	500	1.344	1.051–1.708	.020	.473
	<50	468	1.207	1.030–1.424	.017	

CKD, chronic kidney disease; other abbreviations as in Tables 1, 2 and 3.

and regulation of various metalloproteinases, which are a family of functionally related Zn-containing enzymes that denature and degrade myofibrillar collagen and other components of the extracellular matrix.[7,9] Resultant Zn deficiency causes stress-induced damage to cardiomyocytes and an immunostimulatory state with activated immune cells elaborating inflammatory cytokines, thus contributing to the systemic illness of HF.[16] Furthermore, inflammation and oxidative stress promotes cardiomyocyte damage.[16,19] Consistent with our data, it has been reported that serum Zn levels do not have a correlation with LVEF, but with renal function, C-reactive protein, and cardiac troponins.[7] Additionally, reactive oxygen species (ROS) and oxidative stress have been reported to be implicated in the widely recognized wasting and weakness of skeletal muscles that develops with aging, commonly referred to as sarcopenia.[20] Copper and Zn play major roles in the oxidant/antioxidant mechanism. Cu/Zn-superoxide dismutase (Cu/Zn-SOD) is an antioxidant mechanism.[721] Zinc deficiency is associated with a decline in Cu/Zn-SOD activity,[22] and prolonged exposure to ROS from a lack of Cu/Zn-SOD leads to neuromuscular dysfunction and ultimately results in muscle weakness and atrophy.[23] Metallothioneins are proteins that are involved in intracellular Zn storage and transport and act as an antioxidant that is extremely efficient in

Table 5. Subgroup Analysis for Associations Between Zinc Deficiency and All-Cause Mortality

Factor	Subgroup	n	HR	95% CI	*P* Value	Interaction *P* Value
Zinc level (10 μg/dL decrease)	**Total**	**968**	**1.268**	**1.138–1.370**	**<.001**	
Age	≥75	325	1.207	1.051–1.384	.006	.917
	<75	643	1.184	1.041–1.357	.012	
Gender	Male	608	1.280	1.149–1.424	<.001	.459
	Female	360	1.184	1.010–1.411	.045	
Body mass index	≥22.8	482	1.219	1.051–1.438	.012	.810
	<22.8	486	1.268	1.116–1.411	<.001	
NYHA class	I/II	940	1.280	1.161–1.397	<.001	.152
	III/IV	28	0.801	0.562–1.149	.230	
Hypertension	Present	683	1.268	1.127–1.397	<.001	.877
	Absent	285	1.231	1.041–1.495	.019	
Diabetes	Present	380	1.195	1.051–1.370	.009	.444
	Absent	588	1.305	1.138–1.480	<.001	
Dyslipidemia	Present	763	1.293	1.161–1.424	<.001	.336
	Absent	205	1.138	0.923–1.411	.224	
CKD	Present	541	1.195	1.072–1.344	.002	.450
	Absent	427	1.293	1.083–1.524	.004	
Anemia	Present	497	1.161	1.041–1.293	.009	.464
	Absent	471	1.268	1.030–1.524	.022	
Atrial fibrillation	Present	366	1.318	1.127–1.524	<.001	.468
	Absent	602	1.207	1.083–1.370	.001	
RAS inhibitors	Present	717	1.219	1.094–1.370	<.001	.351
	Absent	251	1.344	1.138–1.583	.001	
β-blockers	Present	765	1.231	1.105–1.370	<.001	.404
	Absent	203	1.357	1.116–1.660	.002	
Diuretics	Present	619	1.219	1.105–1.370	<.001	.716
	Absent	349	1.280	1.041–1.553	.017	
Inotropic agents	Present	115	1.105	0.923–1.344	.275	.151
	Absent	853	1.293	1.161–1.424	<.001	
Mineral receptor blockers	Present	409	1.184	1.051–1.344	.004	.224
	Absent	559	1.331	1.149–1.538	<.001	
Calcium channel blockers	Present	331	1.331	1.138–1.553	<.001	.316
	Absent	637	1.207	1.083–1.357	.001	
Sodium	≥139	500	1.357	1.172–1.568	<.001	.104
	<139	468	1.161	1.030–1.318	.017	
Potassium	≥4.2	475	1.411	1.219–1.660	<.001	.091
	<4.2	493	1.172	1.041–1.318	.009	
Chloride	≥104	460	1.370	1.172–1.613	<.001	.107
	<104	508	1.172	1.051–1.318	.006	
Magnesium	≥1.80	499	1.280	1.116–1.452	<.001	.790
	<1.80	469	1.231	1.083–1.397	.001	
Calcium	≥8.8	481	1.127	0.942–1.370	.181	.316
	<8.8	487	1.243	1.116–1.397	<.001	
Iron	≥48	485	1.231	1.030–1.480	.024	.866
	<48	483	1.207	1.082–1.357	.001	
BNP	≥90.8	484	1.219	1.094–1.370	<.001	.712
	<90.8	484	1.293	1.072–1.538	.006	
Troponin I	≥0.029	482	1.231	1.105–1.370	<.001	.306
	<0.029	486	1.105	0.923–1.331	.272	
LVEF	≥50	500	1.370	1.184–1.568	<.001	.199
	<50	468	1.172	1.041–1.331	.009	

Abbreviations as in Tables 1, 2 and 4.

scavenging or quenching various free radicals or ROS.[24] Modifications of metallothioneins are associated with skeletal muscle atrophy and sarcopenia.[25] These mechanisms may be associated with lower serum Zn levels as well as higher C-reactive protein and troponin I, and impaired exercise capacity in the present study.

With regard to managing Zn deficiency, Zn supplementation could be a reasonable therapeutic target in patients with HF. It has been reported that $ZnSO_4$ 50 to 100 mg/day was recommended as a supplement for Zn^{2+} deficiency.[16] It also has been reported that comprehensive micronutrient supplementation including not only Zn, but also Cu, selenium, vitamin A, vitamin B6, vitamin D, carnitine, and coenzyme Q10, causes LV reverse remodeling and improves LVEF.[26] The potential effects of Zn supplementation on the prognosis of HF remain controversial; thus, further investigation of supplementation is required to prove causation.

Study Strengths and Limitations

To the best of our knowledge, this is the first study to show the association of Zn deficiency with adverse prognosis in patients with HF, taking into consideration a multifaceted background, including comorbidities, medications, other minerals,

echocardiographic data, and exercise capacity. We were able to follow-up all patients. The causes of death were accurately confirmed by our experienced cardiologists.

The present study also has several limitations. First, as a prospective cohort study of a single center with a relatively small number of patients, the present results may not be a representative of the general population. Although we performed our analysis using both multivariable Cox proportional hazard analyses and subgroup analyses under the consideration of multiple confounding factors, we cannot rule out residual confounding variables, and the effects of differences in patient background among the 3 groups might not be completely adjusted. Second, because the present study included only variables at hospital discharge, we did not consider changes in medical parameters or postdischarge treatment. Third, because this was a cross-sectional and prospective observational study without intervention for Zn deficiency (Zn supplementation), the causal relationships and mechanisms of Zn deficiency on inflammation, myocardial damage, exercise capacity, and worse prognosis could not be fully explained. Therefore, the present results should be viewed as preliminary, and further studies with a larger population are needed.

Conclusion

Decreased serum Zn levels are associated with higher mortality, lower levels of other micronutrients (sodium, calcium, and iron), inflammation (C-reactive protein), ongoing myocardial damage (troponin I), and impaired exercise capacity.

Disclosures

None declared.

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