



The association between transferrin saturation and all-cause mortality in chronic kidney disease: findings from Korean Cohort Study for Outcome in Patients with Chronic Kidney Disease

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Background: Transferrin saturation (TSAT) has been used as an indicator of iron deficiency. However, there is no consensus regarding its optimal range for patient with chronic kidney disease (CKD). We aimed to analyze the effect of TSAT on the prognosis of patients with non-dialysis CKD (NDCKD).

Methods: From 2011 to 2016, 2157 NDCKD patients with baseline TSAT measurements were followed for 10 years. Patients were divided into three groups based on baseline TSAT values: <25%, ≥25% and <45%, and ≥45%. All-cause mortality and 4-point major adverse cardiovascular events (MACE) were analyzed using multivariable Cox regression analysis. Other iron biomarkers and mortality were also analyzed.

Results: During a mean follow-up of 7.1 ± 2.9 years, 182 of a total of 2,157 patients (8.4%) died. Compared with the TSAT ≥25% and <45% group, the TSAT <25% group showed significantly increased all-cause mortality (hazard ratio [HR], 1.44; 95% confidence interval (CI), 1.02–2.03; $p = 0.04$). The occurrence of 4-point MACE was significantly increased in univariable analysis in the TSAT <25% group (HR, 1.48; 95% CI, 1.02–2.15; $p = 0.04$), but it was not significant in the multivariable analysis (HR, 1.38; 95% CI, 0.89–2.15; $p = 0.15$). Tertile comparisons of the iron-to-log-ferritin ratio showed increased mortality in the first tertile group.

Conclusion: TSAT <25% is an independent risk factor for all-cause mortality in patients with NDCKD and care should be taken to prevent TSAT values of <25%. Other indicators, such as serum iron and iron-to-log-ferritin ratio, may also be used to assess iron deficiency.

Keywords: Chronic kidney disease, Chronic renal insufficiency, Ferritins, Iron deficiencies, Mortality, Transferrin saturation

Received: November 1, 2023; **Revised:** December 18, 2023; **Accepted:** January 6, 2024

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Introduction

In patients with chronic kidney disease (CKD), anemia is a common complication that plays a major role in determining the prognosis. The lower the hemoglobin concentration, the higher the mortality, the faster the decline in renal function, the more severe the associated symptoms, and the lower the productivity of daily life [1-4].

Iron deficiency anemia is a leading cause of anemia, and the importance of iron deficiency (ID) itself is increasing regardless of the occurrence of anemia [5-7]. Serum ferritin and transferrin saturation (TSAT) are the most common tools used to evaluate ID. Ferritin is a marker of stored iron and TSAT is a marker of circulating iron, indicating iron availability to the tissue [8]. However, serum ferritin has limitations in diagnosing functional ID (FID) because it can be increased in the inflammatory state as an acute-phase reactant, independent of iron metabolism [9]. In CKD, where both absolute and FID are important, TSAT can be a useful marker for assessing low iron availability [5,7].

However, the exact ranges of serum biomarkers for iron supply are inconsistent. The 2012 Kidney Disease-Improving Global Outcomes (KDIGO) guidelines recommend iron supplementation when ferritin is less than 500 ng/mL and TSAT is less than 30% in both dialysis and non-dialysis CKD (NDCKD) patients [10]. However, subsequent studies have shown a better prognosis in a higher range than the current guidelines, with mortality rising again above a certain level of TSAT [11-14].

Serum iron is reduced in ID, even in the setting of inflammation, and its usefulness in evaluating ID is emerging [7,15]. However, in the progression of anemia serum iron begins to decrease later than stored iron and alone its concentrations do not indicate ID [7]. It may be helpful to evaluate the prognosis through the relationship between serum iron and ferritin as well as TSAT.

Therefore, we aimed to analyze the effect of TSAT on the prognosis of patients with NDCKD through all-cause mortality, cardiovascular outcomes, and its association with other iron biomarkers.

Methods

Study participants and design

The Korean Cohort Study for Outcome in Patients with Chronic Kidney Disease (KNOW-CKD) is a multicenter, prospective cohort study that enrolled pre-dialysis patients with CKD stages 1 to 5 in South Korea (NCT01630486 at <http://www.clinicaltrials.gov>). From 2011 to 2016, 2,238 CKD adult patients aged 20 to 75 years were recruited from nine clinical centers in university-affiliated hospitals. Details of the rationale and design of the KNOW-CKD study have been described previously [16]. A total of 2,157 patients were finally included in the analysis after excluding 69 patients without baseline TSAT values and 12 patients without data on follow-up duration (Supplementary Fig. 1, available online).

The study was conducted in accordance with the principles of the Declaration of Helsinki and informed consent was obtained from all participants. The study protocol was approved by the Institutional Review Board of each participating clinical center: Seoul National University Hospital (No. 1104-089-359), Seoul National University Bundang Hospital (No. B-1106/129-008), Severance Hospital (No. 4-2011-0163), Kangbuk Samsung Medical Center (No. 2011-01-076), The Catholic University of Korea, Seoul St. Mary's Hospital (No. KC11OIMI0441), Gachon University Gil Hospital (No. GIRBA2553), Nowon Eulji Medical Center (No. 201105-01), Chonnam National University Hospital (No. CNUH-2011-092), and Pusan Paik Hospital (No. 11-091).

Data collection

Baseline demographic data including age, sex, comorbidities, anthropometric measurements, and medical history were recorded at enrollment. Blood samples were obtained after overnight fasting and sent to a central laboratory (Lab Genomic). The glomerular filtration rate was estimated by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine equation using serum creatinine levels in the central laboratory [17]. The spot urine albumin-to-creatinine ratio was used to measure urinary albumin excretion.

Study outcome

The primary outcome was all-cause mortality according to TSAT (%). Baseline TSAT was calculated as serum iron $\times$ 100/total iron-binding capacity (TIBC) values at enrollment. The secondary outcome was 4-point major adverse cardiovascular events (MACE). In this study, 4-point MACE was defined as cardiovascular death, nonfatal acute myocardial infarction, nonfatal stroke, and unstable angina requiring hospitalization. We also analyzed the differences in outcomes according to their relationship with serum ferritin, iron, and TSAT levels.

Statistical analysis

We categorized patients into three groups based on baseline TSAT level: <25%, $\geq$ 25% and <45% (reference group), and $\geq$ 45%. The three groups were classified by referring to the points showing a clear difference in mortality between adjacent sections. We performed a t-test for mortality between adjacent groups by dividing the TSAT level into 5% units, and there was a clear difference starting from 25% and 45%. In addition, analysis using the cubic spline curve according to TSAT showed that the adjusted hazard ratio (HR) began to rise from the section below 25% to 30% ([Supplementary Fig. 2](#), available online). Data are expressed as mean $\pm$ standard deviation or median (interquartile range, IQR) for continuous variables and as number (percentage) for categorical variables. Baseline characteristics and laboratory findings were compared using the analysis of variance or the Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables. Cumulative survival curves for all-cause mortality were generated using the Kaplan-Meier method, and between-group survival was compared using the log-rank test. A Cox proportional hazard regression model was used to assess the independent relationship between TSAT levels and all-cause mortality. The analyses were adjusted for baseline confounders, including age, sex, body mass index (BMI), mean blood pressure, underlying comorbidities, laboratory tests, erythropoietin-stimulating agent (ESA) use, and iron supplementation. Four models were designed and verified to satisfy the proportional hazards assumption of the Cox model using the Schoenfeld test. The results of the Cox analysis were presented as HRs and 95% confidence

intervals (CIs). We performed a subgroup analysis and subgroups were defined by age (<65 years vs. $\geq$ 65 years), sex (male vs. female), BMI (<25 kg/m² vs. $\geq$ 25 kg/m²), diabetes mellitus (DM) vs. non-DM, anemia vs. non-anemia, ferritin (<100 mg/dL vs. $\geq$ 100 mg/dL), high-sensitivity C-reactive protein (hsCRP; <0.5 mg/dL vs. $\geq$ 0.5 mg/dL), estimated glomerular filtration rate (eGFR; <30 mL/min/1.73 m² vs. $\geq$ 30 mL/min/1.73 m²), and serum hepcidin (<15 ng/mL vs. $\geq$ 15 ng/mL). Differences in mortality were analyzed for the combination of TSAT and serum ferritin and TSAT and serum iron. To examine the association between mortality and other serum iron profiles, such as serum iron and ferritin, we used the serum iron-to-log-ferritin ratio and classified the patients into three tertile groups (T1, T2, and T3). Ferritin values were normally distributed through the log and the ratio of serum iron-to-log-ferritin was obtained. and classified the patients into three tertile groups (T1, T2, and T3). Statistical analyses were performed using SPSS for Windows (version 27.0; IBM Corp.) and R version 4.3.1 (R Foundation for Statistical Computing).

Results

Baseline characteristics

Of the 2,157 patients, 644 patients were in the TSAT <25% group, 1,240 were in the TSAT $\geq$ 25% and <45% group (reference), and 273 were in the TSAT $\geq$ 45% group ([Supplementary Fig. 1](#), available online). The baseline demographic and clinical characteristics of the patients are shown in [Table 1](#). The mean age was 53.7 ± 12.2 years and the mean eGFR was 53.0 ± 30.7 mL/min per 1.73 m². The mean value of TSAT was $31.7\% \pm 12.1\%$ and the median value of ferritin was 99.9 ng/mL (IQR, 54.1–176.6 ng/mL). The incidence of DM ($p < 0.001$) and cardiovascular disease ($p = 0.03$) was higher in the lower TSAT group. There were no differences in the prescriptions of ESA and supplementary iron between the TSAT groups.

Primary outcome

During a mean follow-up duration of 7.1 ± 2.9 years, 182 patients (8.4%) died. The causes of death were evaluated in 120 patients, with 41 (deaths 34.2%) attributed to infection, 25 (20.8%) to malignancy, 23 (19.2%) to cardiovas-

Table 1. Baseline characteristics

Characteristic	Total	TSAT group			p-value
		<25%	≥25% and <45%	≥45%	
No. of patients	2,157	644	1,240	273	
Age (yr)	53.7 ± 12.2	53.8 ± 11.8	54.1 ± 12.3	51.2 ± 12.7	0.002
Male sex	1,324 (61.4)	334 (51.9)	785 (63.3)	205 (75.1)	<0.001
Primary cause of CKD					<0.001
Diabetes mellitus	499 (23.1)	171 (26.6)	297 (24.0)	31 (11.4)	
Glomerulonephritis	782 (36.3)	222 (34.5)	431 (34.8)	129 (47.3)	
Polycystic kidney disease	348 (16.1)	106 (16.5)	198 (16.0)	44 (16.1)	
Hypertension	395 (18.3)	107 (16.6)	234 (18.9)	54 (19.8)	
Others	133 (6.2)	38 (5.9)	80 (6.5)	15 (5.5)	
Body mass index (kg/m ²)	24.6 ± 3.4	24.7 ± 3.5	24.6 ± 3.4	24.4 ± 3.1	0.34
SBP (mmHg)	127.8 ± 16.2	128.5 ± 16.7	127.7 ± 16.1	127.0 ± 15.8	0.38
DBP (mmHg)	77.0 ± 11.1	77.1 ± 11.7	76.8 ± 10.8	77.1 ± 10.9	0.84
MBP (mmHg)	93.9 ± 11.7	94.2 ± 12.2	93.8 ± 11.4	93.8 ± 11.6	0.72
Comorbidities					
Diabetes mellitus	728 (33.8)	259 (40.3)	421 (34.0)	48 (17.6)	<0.001
Hypertension	2068 (95.9)	610 (94.7)	1,197 (96.5)	261 (95.6)	0.17
Cardiovascular disease	283 (13.1)	98 (15.2)	161 (13.0)	24 (8.8)	0.03
Prescription					
Iron, IV or oral	316 (14.7)	96 (14.9)	191 (15.5)	29 (10.7)	0.13
ESA	163 (7.6)	56 (8.7)	93 (7.5)	14 (5.1)	0.17
ACEi/ARBs	1,845 (85.5)	548 (85.1)	1,063 (85.7)	234 (85.7)	0.93
Diuretics	685 (31.8)	228 (35.4)	394 (31.8)	63 (23.1)	0.001
Statin	1,112 (51.6)	321 (49.8)	654 (52.7)	137 (50.2)	0.44
Antiplatelet drug	644 (29.9)	200 (31.1)	381 (30.7)	63 (23.1)	0.03
Anticoagulant	29 (1.3)	11 (1.7)	13 (1.0)	5 (1.8)	0.38
Laboratory test					
White blood cell (×10 ³ /mm ³)	6.6 ± 1.9	6.9 ± 2.0	6.5 ± 1.9	6.4 ± 1.7	<0.001
Hemoglobin (g/dL)	12.8 ± 2.0	12.4 ± 1.9	12.9 ± 2.0	13.5 ± 2.0	<0.001
Iron (μg/dL)	92.6 ± 35.1	59.6 ± 17.2	97.4 ± 21.5	148.3 ± 34.6	<0.001
TIBC (μg/dL)	297.5 ± 52.3	315.2 ± 57.0	293.0 ± 47.8	276.1 ± 48.1	<0.001
TSAT (%)	31.7 ± 12.1	19.1 ± 4.7	33.3 ± 5.3	53.9 ± 9.7	<0.001
Ferritin (ng/mL)	99.9 (54.1–176.6)	69.4 (36.6–126.1)	109.8 (62.5–186.1)	127.0 (74.8–221.3)	<0.001
Creatinine (mg/dL)	1.6 ± 1.0	1.6 ± 1.1	1.6 ± 0.9	1.6 ± 1.1	0.84
eGFR (mL/min per 1.73 m ²)	53.0 ± 30.7	51.2 ± 30.7	52.1 ± 30.2	61.3 ± 31.9	<0.001
Albumin (g/dL)	4.2 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.1 ± 0.5	0.52
hsCRP (mg/dL)	0.6 (0.2–1.7)	1.1 (0.3–2.7)	0.5 (0.2–1.3)	0.6 (0.3–1.3)	<0.001
Uric acid (mg/dL)	7.0 ± 1.9	7.0 ± 2.0	7.1 ± 1.9	6.9 ± 1.9	0.26
Total cholesterol (mg/dL)	174.2 ± 39.3	172.4 ± 37.3	174.2 ± 39.0	178.3 ± 44.3	0.12
LDL-C (mg/dL)	97.0 ± 31.9	94.6 ± 30.0	97.2 ± 32.0	101.4 ± 35.0	0.01
HDL-C (mg/dL)	49.2 ± 15.5	47.7 ± 14.8	49.6 ± 15.4	51.2 ± 17.0	0.003
Triglyceride (mg/dL)	157.7 ± 98.8	168.9 ± 108.7	153.1 ± 94.6	152.7 ± 91.0	0.003
Fasting glucose (mg/dL)	100 (92–114)	100 (91–118)	100 (91–114)	98 (93–106)	0.09
Phosphorus (mg/dL)	3.7 ± 0.7	3.7 ± 0.7	3.7 ± 0.7	3.5 ± 0.6	<0.001
Corrected calcium (mg/dL)	9.0 ± 0.4	9.0 ± 0.4	9.0 ± 0.5	9.0 ± 0.4	0.99
UACR (g/g)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.5–0.8)	0.62

Data are expressed as number only, mean ± standard deviation, number (%), or median (interquartile range).

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ESA, erythropoietin-stimulating agent; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IV, intravenous; LDL-C, low-density lipoprotein cholesterol; MBP, mean blood pressure; SBP, systolic blood pressure; TIBC, total iron-binding capacity; TSAT, transferrin saturation; UACR, urine albumin-to-creatinine ratio.

^aCorrected calcium = 0.8 × (normal albumin – patient's albumin) + serum calcium.

cular disease, and 31 (25.8%) to other causes. For patients with TSAT categories of <25%, ≥25% and <45%, and ≥45%, the mortality rate was 11.0%, 7.7%, and 5.5%, respectively. Cardiovascular death was more common in the TSAT <25% group than in other groups (Supplementary Fig. 3, available online). Kaplan-Meier analysis showed higher all-cause mortality in the TSAT <25% group than in the other groups and a decrease with higher TSAT (log-rank $p = 0.005$) (Fig. 1). Four models of multivariable Cox analysis revealed that the TSAT <25% was associated with higher mortality risk, even after adjustment for hemoglobin, ferritin, ESA use, and iron supplementation (HR, 1.44; 95% CI, 1.00–2.03; $p = 0.04$) (Table 2). The all-cause mortality

showed a trend toward decrease with higher TSAT, but no statistically significant difference at TSAT ≥45% (HR, 0.90; 95% CI, 0.50–1.64; $p = 0.73$). Supplementary Fig. 3 (available online) shows the restricted cubic spline curve for the multivariable Cox regression analysis of all-cause mortality according to TSAT. The results of the subgroup analyses are shown in Supplementary Table 1 (available online). In the subgroup analysis of patients with BMI <25 kg/m², serum ferritin <100 ng/mL, eGFR ≥30 mL/min per 1.73 m², and hepcidin <15 ng/mL, the HRs of the TSAT <25% group showed significantly increased mortality compared with the TSAT ≥25% and <45% group.

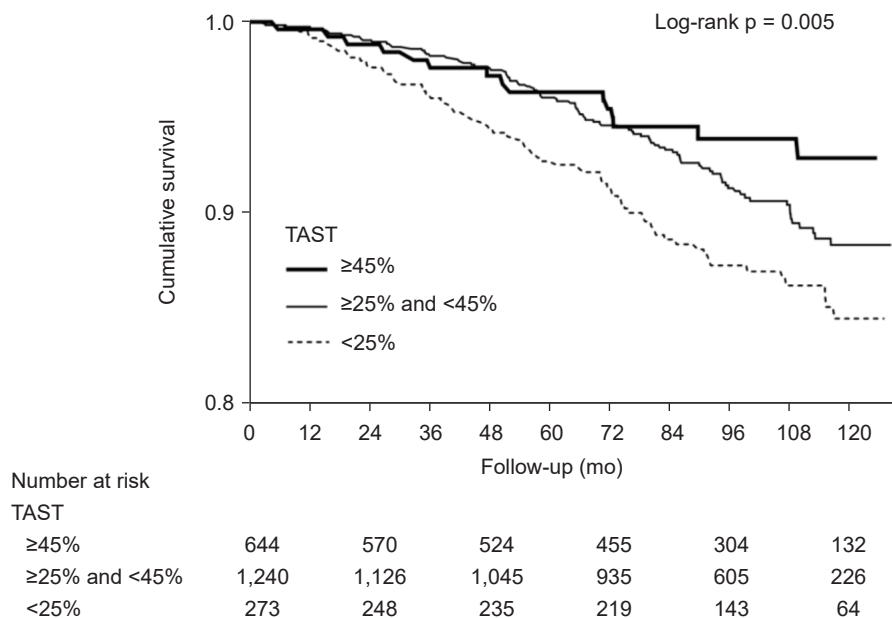


Figure 1. Kaplan-Meier survival curve for all-cause mortality according to baseline TSAT value. TSAT, transferrin saturation.

Table 2. HRs for all-cause mortality by TSAT levels

TSAT	Events, n (%)	Model 1 ^a		Model 2 ^b		Model 3 ^c		Model 4 ^d	
		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
<25%	71 (11.0)	1.49 (1.09–2.02)	0.01	1.67 (1.23–2.27)	0.001	1.45 (1.04–2.03)	0.03	1.44 (1.02–2.03)	0.04
≥25% and <45%	96 (7.7)	Reference		Reference		Reference		Reference	
≥45%	15 (5.5)	0.69 (0.40–1.18)	0.17	0.78 (0.45–1.35)	0.38	0.93 (0.04–2.03)	0.80	0.90 (0.50–1.64)	0.73

CI, confidence interval; HR, hazard ratio; TSAT, transferrin saturation.

^aUnadjusted. ^bAdjusted for age, sex, body mass index, mean blood pressure. ^cModel 2 + adjusted for underlying comorbidities (diabetes mellitus, hypertension, and cardiovascular disease), estimated glomerular filtration rate, corrected calcium, phosphate, uric acid, albumin, low-density lipoprotein cholesterol, log-high-sensitivity C-reactive protein, and log-urine albumin-to-creatinine ratio. ^dModel 3 + adjusted for hemoglobin, log-ferritin, erythropoietin-stimulating agent use, and iron supplementation.

Secondary outcome

For patients with TSAT categories of <25%, ≥25% and <45%, and ≥45%, the incidence of 4-point MACE was 12.9%, 9.7%, and 5.9%, respectively. Similar to the all-cause mortality outcomes, 4-point MACE decreased in the higher TSAT group (log-rank $p = 0.03$) (Fig. 2). In the Cox regression analysis, the TSAT <25% group had increased MACE compared with the TSAT ≥25% and <45% group in univariable analysis (HR, 1.48; 95% CI, 1.02–2.15; $p = 0.04$). However, this was not significant in the multivariable analysis (HR,

1.38; 95% CI, 0.89–2.15; $p = 0.15$). The TSAT ≥45% group also showed no significant difference in MACE compared with the TSAT ≥25% and <45% group (HR, 0.72; 95% CI, 0.32–1.60; $p = 0.42$) (Table 3).

Association of transferrin saturation with other iron biomarkers: ferritin and iron levels

Differences in all-cause mortality were analyzed for the combination of TSAT and serum ferritin and TSAT and serum iron. Supplementary Fig. 4 (available online) shows

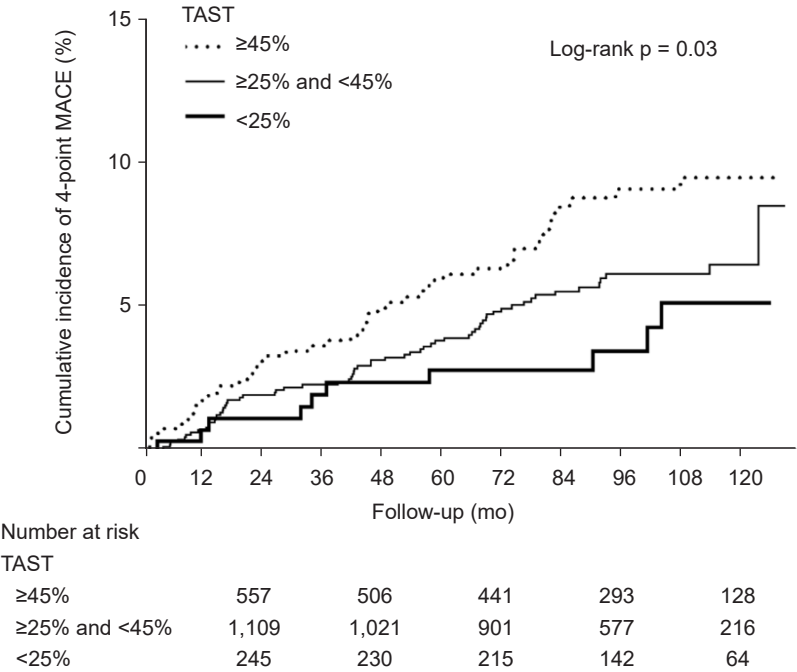


Figure 2. Cumulative incidence of 4-point MACE by baseline TSAT value. MACE, major adverse cardiovascular event; TSAT, transferrin saturation.

Table 3. HRs for 4-point MACE by TSAT levels

TSAT	Events, n (%)	Model 1 ^a		Model 2 ^b		Model 3 ^c		Model 4 ^d	
		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
<25%	48 (7.5)	1.48 (1.02–2.15)	0.04	1.67 (1.14–2.43)	0.008	1.44 (0.95–2.18)	0.08	1.38 (0.89–2.15)	0.15
≥25% and <45%	65 (5.2)	Reference		Reference		Reference		Reference	
≥45%	10 (3.7)	0.68 (0.35–1.32)	0.25	0.77 (0.40–1.50)	0.45	0.72 (0.32–1.58)	0.41	0.72 (0.32–1.60)	0.42

CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiovascular event; TSAT, transferrin saturation.
^aUnadjusted. ^bAdjusted for age, sex, body mass index, mean blood pressure. ^cModel 2 + adjusted for underlying comorbidities (diabetes mellitus, hypertension, and cardiovascular disease), estimated glomerular filtration rate, corrected calcium, phosphate, uric acid, albumin, low-density lipoprotein cholesterol, log-high-sensitivity C-reactive protein, and log-urine albumin-to-creatinine ratio. ^dModel 3 + adjusted for hemoglobin, log-ferritin, erythropoietin-stimulating agent use, and iron supplementation.

the HR of all-cause mortality according to serum ferritin and iron levels using a restricted cubic spline plot. The serum ferritin level was divided by 100 ng/mL, which is the current general reference value for ID in NDCKD, and the iron level was divided by 80 $\mu\text{g/dL}$ based on the restricted cubic spline of the HR. Kaplan-Meier analysis showed that mortality was highest in the group with TSAT <25% and ferritin <100 ng/mL (Fig. 3A). In a multivariable Cox analysis with the TSAT $\geq$ 25% and ferritin $\geq$ 100 ng/mL group as the reference, the adjusted HR was highest when TSAT <25% and ferritin <100 ng/mL (HR, 1.69; 95% CI, 1.12–2.56; $p = 0.01$) (Fig. 3B). The TSAT <25% and ferritin $\geq$ 100 ng/mL group was the next highest, but it was not statistically

significant. For TSAT and iron, the Kaplan-Meier analysis showed higher mortality in the two iron <80 $\mu\text{g/dL}$ groups (Fig. 3C). In Cox analysis with the TSAT $\geq$ 25% and iron $\geq$ 80 $\mu\text{g/dL}$ group as the reference, the adjusted HR was highest in the TSAT <25% and iron <80 $\mu\text{g/dL}$ group (HR, 1.67; 95% CI, 1.13–2.46; $p = 0.009$) (Fig. 3D). The TSAT $\geq$ 25% and iron <80 $\mu\text{g/dL}$ group had the next highest, but this was not statistically significant.

Iron-to-log-ferritin ratio

Cox regression analysis of the iron-to-log-ferritin ratio showed a U-shaped distribution, with the lowest mortality

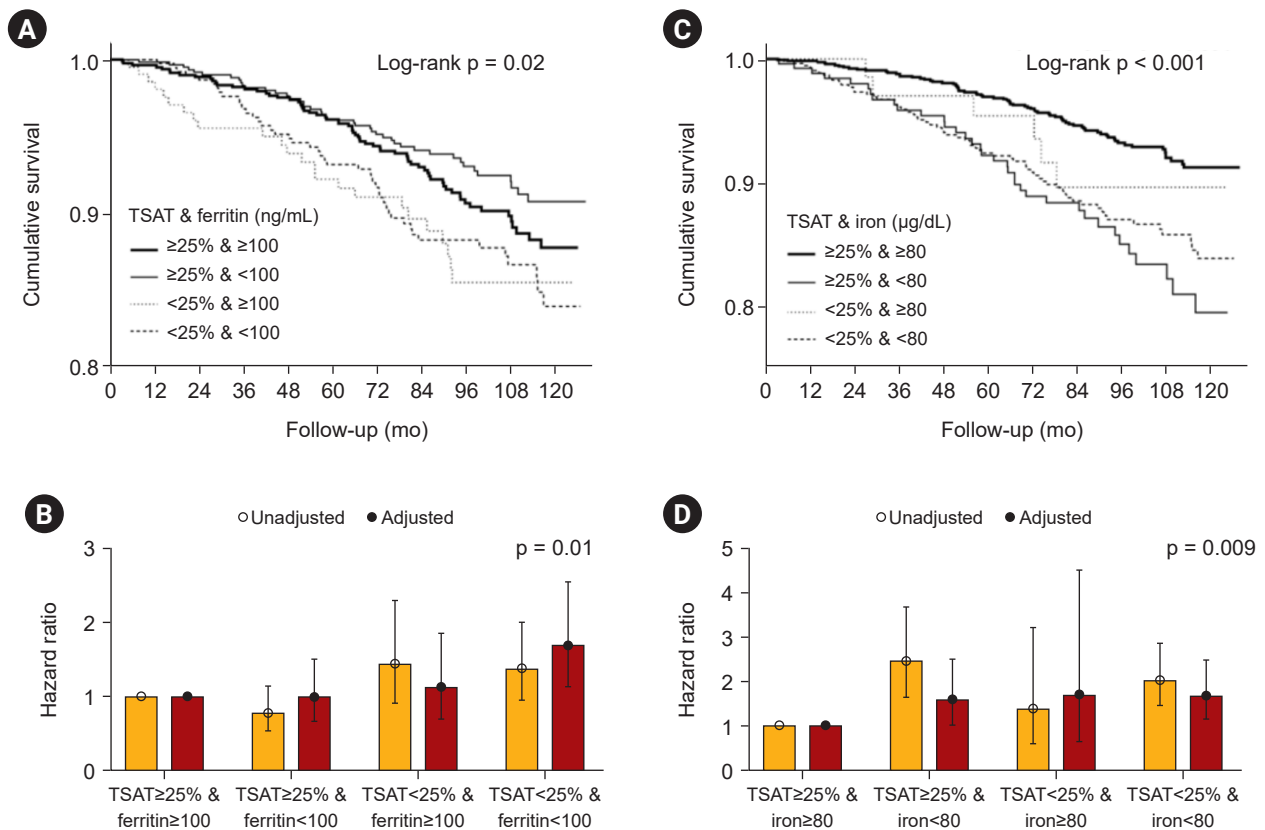


Figure 3. Kaplan-Meier survival curve and Cox analysis for all-cause mortality according to iron biomarkers: serum ferritin and iron. Kaplan-Meier survival curve according to TSAT and ferritin (A) and TSAT and iron (C). Unadjusted and adjusted hazard ratio for TSAT and ferritin (B) and TSAT and iron (D). In the Cox analysis, adjustment for TSAT and ferritin groups included age, sex, BMI, MBP, underlying comorbidities, estimated glomerular filtration rate (eGFR), albumin, uric acid, phosphate, corrected calcium, low-density lipoprotein cholesterol (LDL-C), log high-sensitivity C-reactive protein (log-hsCRP), log urine albumin-to-creatinine ratio (log-UACR), hemoglobin, total iron-binding capacity (TIBC), erythropoietin-stimulating agent (ESA) use, and iron supplementation. Adjustments for the TSAT and iron groups included age, sex, body mass index, mean blood pressure, underlying comorbidities, eGFR, albumin, uric acid, phosphate, corrected calcium, LDL-C, log-hsCRP, log-UACR, hemoglobin, TIBC, log-ferritin, ESA use, and iron supplementation. TSAT, transferrin saturation.

in the second tertile group (T2) (Fig. 4A). The HR of the first tertile group (T1) was significantly increased compared with T2 as the reference group (HR, 1.71; 95% CI, 1.16–2.51; $p = 0.007$) (Fig. 4B). There was also an increase in mortality in the third tertile (T3), but this was not statistically significant. The median values of the iron biomarkers in the T2 range were as follows: TSAT, 30% (IQR, 25%–35%); iron, 90 $\mu\text{g/dL}$ (IQR, 79–101 $\mu\text{g/dL}$); and ferritin, 108 ng/mL (IQR, 60–176 ng/mL).

Discussion

In our study, the TSAT <25% group had a significantly higher mortality rate than the $\geq 25\%$ and <45% group. Overall, all-cause mortality decreased with increasing TSAT, but there was no significance at TSAT 45% or higher. The incidence of 4-point MACE was significantly increased in the univariable analysis in the TSAT <25% group, but the significance was lost after multivariable adjustment. The 4-point MACE incidence tended to decrease as TSAT increased, but the difference was not statistically significant.

Various studies on TSAT have been published since the 2012 KDIGO guidelines. The PIVOTAL trial, a randomized controlled trial (RCT) in patients with CKD on dialysis (HDCKD), showed that high-dose intravenous (IV) iron administered proactively (with a ferritin cutoff of 700 ng/mL or TSAT 40%) was superior to low-dose IV iron administered reactively (administered to maintain ferritin 200 ng/mL and TSAT 20%) [14]. Observational studies have suggested relatively clear optimal ranges. In patients with NDCKD, mortality has been shown to be higher in TSAT <10%–17% [12,18–20], with increased mortality above 45%–55% [12,21]. In the 2021 CKDopps study, all-cause mortality and MACE were the lowest in the TSAT 35%–45% range and increased again above 46% [12]. In patients with HDCKD, TSAT <20% was associated with higher mortality and was a predictor of coronary artery calcium scoring >400 [22–24], with the best survival at 30%–50% [2].

Our results are consistent with those of the CKDopps study, which showed an increase in all-cause mortality below 25% when the TSAT 25%–35% group was used as a reference [12]. However, there was no upper cutoff for

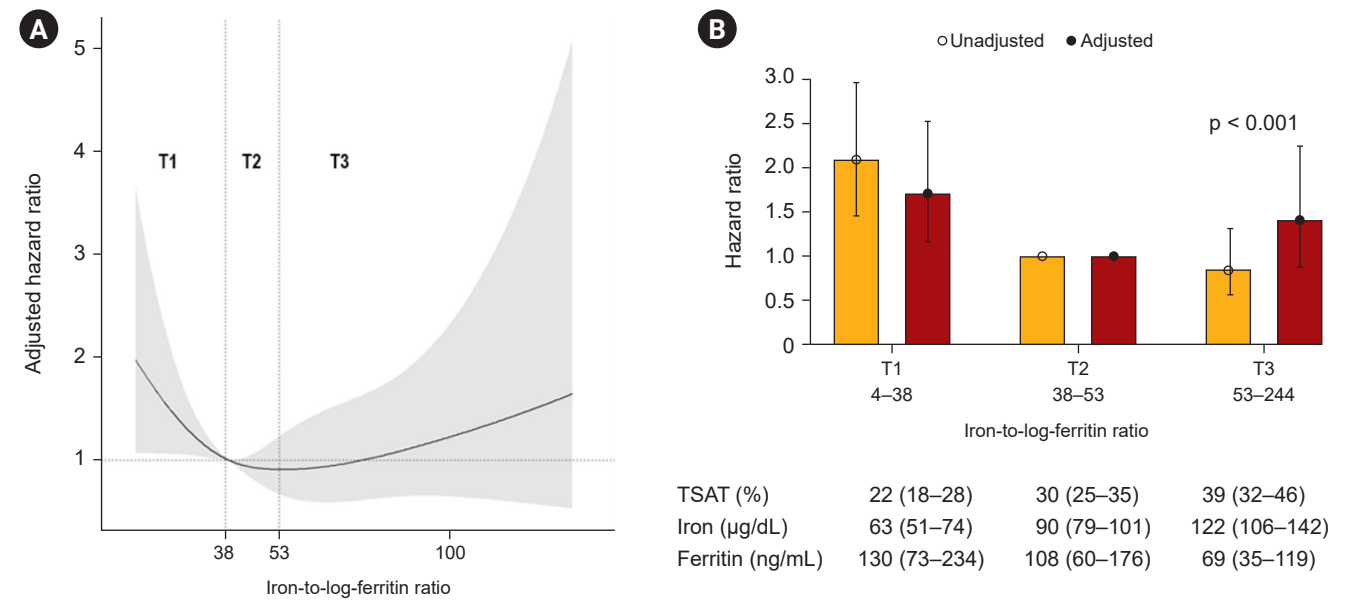


Figure 4. Adjusted hazard ratio of all-cause mortality by iron-to-log-ferritin ratio. (A) Restricted cubic spline curve for the hazard ratio for iron-to-log-ferritin ratio using multivariable Cox regression analysis. (B) Hazard ratio and values of iron biomarkers by iron-to-log-ferritin ratio tertiles. Iron-to-log-ferritin ratio was divided into tertiles (T1, T2, and T3, respectively) and compared with T2 as the reference group. The median value (interquartile range) of TSAT, serum iron, and ferritin according to each tertile section are indicated under the column. TSAT, transferrin saturation.

TSAT, and the HR continued to decrease. Nevertheless, caution is required in concluding that the higher the TSAT, the lower the mortality rate. Malignancy accounted for half of the deaths in the TSAT $\geq 45\%$ group, and high TSAT and iron supply may contribute to increase oxidative stress and the risk of malignancy [25,26]. The average age of our participants was 53 years old, and in particular, the TSAT $\geq 45\%$ group averaged 51 years old, which is younger than the average ages of 69 years [12] and 61 years [2] in previous studies. Therefore, if the incidence of malignancy increases with age due to longer follow-up periods, all-cause mortality in the high TSAT range may increase. In the subgroup analysis, the TSAT $< 25\%$ group had a higher HR in the non-obese subgroup, relatively preserved renal function, and lower serum ferritin and hepcidin levels. This suggests that the impact of a low TSAT is more pronounced in patients with better overall health. Under inflammatory conditions, such as CKD or obesity, cytokines increase ferritin and hepcidin expression [27]. In a controlled environment of chronic inflammation, which may contribute to mortality, the impact of low TSAT levels may be more apparent.

Of the 182 deaths, only 35 patients (1.6%) were confirmed as cardiovascular deaths, making comparisons between groups difficult. [Supplementary Table 2](#) (available online) shows the echocardiographic parameters. Lower TSAT has been shown to be associated with increased left ventricular geometric abnormalities, and increased E/E' although in the normal range. This is consistent with previous findings that ID and anemia are associated with diastolic dysfunction and increased left ventricular mass [28,29]. Although there was no significant difference in 4-MACE incidence, avoiding TSAT $< 25\%$ may be beneficial in preventing cardiac dysfunction.

ID can be classified as absolute ID (AID) or FID. AID is a condition in which iron stores in the whole body are insufficient and iron is reduced. In contrast, FID refers to a condition in which the body is not deficient in iron but cannot use iron for hematopoiesis and is mainly related to chronic inflammation [7]. In CKD, the use of ESAs increases iron demand by stimulating hematopoiesis; however, insufficient iron mobilization worsens FID [30–33]. At the KDIGO conference in 2019, AID was defined as TSAT $< 20\%$ and ferritin < 100 ng/mL, and FID was defined as TSAT $< 20\%$ and ferritin > 100 ng/mL in NDCKD. However, the definition of ID in CKD patients is still not consistently used [8]. Pre-

vious studies have also been conducted on serum ferritin levels. The FIND-CKD RCT indicated that IV iron targeting higher ferritin (400–600 ng/mL) delayed the need for other anemia management than low ferritin (100–200 ng/mL). Other observational studies have shown no directional association, but mortality increased at levels above 250 [19], 300 [12], or 500 [34].

In our study, regardless of the ferritin level, the group with low TSAT had a poor prognosis, and the AID group had a higher mortality rate than the FID group. For TSAT $\geq 25\%$, the prognosis was better with ferritin < 100 than ≥ 100 ng/mL, and a similar result was found in a heart failure study [35]. This may be related to the implications of high ferritin because ferritin is always low in AID; however, when minimal iron is available, ferritin is regulated by non-iron-dependent factors, such as inflammation [36]. Therefore, when TSAT $\geq 25\%$, that is, when there is no shortage of iron supply, the prognosis may be better if the ferritin is low.

Under inflammatory conditions, cytokines upregulate ferritin and hepcidin and downregulate ferroportin, a transmembrane exporter of iron. Iron absorption in the gastrointestinal tract and its dissociation from macrophages and hepatocytes is impaired, limiting its supply to plasma and causing FID [7,27,32,37]. Because of this pathophysiology, even if ferritin levels are high in CKD, the possibility of FID cannot be ruled out [32,38]. In contrast, TSAT is more useful for identifying low plasma iron availability in both AID and FID [5,7]. In a study evaluating bone marrow aspiration in patients with heart failure, a low TSAT alone showed the best performance in diagnosing ID [39]. However, because TSAT can also be affected by inflammatory conditions, its limitations and the usefulness of serum iron have been noted [15,32,40]. In our study, the two groups with low iron levels, regardless of TSAT, had the worst prognoses. In patients with CKD, the value of TSAT required to achieve a serum iron level increases as a result of decreased TIBC [15]. Therefore, high TSAT levels have limitations as a stand-alone marker to exclude ID, and should also be considered a marker of inflammation in CKD [41]. In contrast, a low TSAT can be associated with low iron or high TIBC, both of which reflect the pathophysiology of ID.

As serum iron begins to deplete later than stored iron during the progression of anemia [7], the prognosis of

ID was also analyzed using the iron-to-log-ferritin ratio, including ferritin, in our study. It showed a U-shape with the T2 range as a reference and a statistically significant increase in mortality in the T1 range. This can be interpreted as a poor prognosis in inflammatory conditions with decreased iron and high ferritin levels. In the T2 segment, each biomarker ranged from TSAT 25% to 35%, ferritin 60 to 176 ng/mL, and iron 79 to 101 µg/dL. These ranges were within the low mortality interval in our study. Based on the above results, serum iron and iron-to-log-ferritin ratios would be helpful in addition to TSAT when evaluating ID in patients with CKD.

Our study has several strengths. A long-term analysis was conducted on a large cohort of patients with CKD, and the relationship between TSAT and other iron biomarkers provided an overall insight into ID and its effects. However, this study also has its limitations. The relatively small number of patients with TSAT $\geq 45\%$ may have contributed to the low incidence of the outcomes. In addition, iron status may vary according to ethnicity [42], but this study may have limitations in that it was conducted in a single ethnic group. Another limitation is that although we adjusted for multiple factors, this was an observational study, and confounding factors may remain.

In conclusion, TSAT $< 25\%$ was an independent risk factor for all-cause mortality in patients with NDCKD compared to the TSAT $\geq 25\%$ and $< 45\%$ group. Care should be taken to prevent TSAT values of $< 25\%$. In the treatment of ID in patients with CKD, other indicators, such as serum iron and iron-to-log-ferritin ratio, may also be used to assess ID.

Conflicts of interest

Tae-Hyun Yoo is the Editor-in-Chief of *Kidney Research and Clinical Practice* and was not involved in the review process of this article. All authors have no other conflicts of interest to declare.

Funding

This work was supported by the Research Program funded by the Korea Disease Control and Prevention Agency (2011E3300300, 2012E3301100, 2013E3301600, 2013E3301601, 2013E3301602, 2016E3300200, 2016E3300201, 2016E3300202, 2019E320100, 2019E320101, 2019E320102,

2022-11-007), a New Faculty Research Grant of Pusan National University, 2023 (No. 202310510001), and the Bio&Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. RS-2023-00223764).

Data sharing statement

The data presented in this study are available from the corresponding author upon reasonable request.

Authors' contributions

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Funding acquisition: HJK, KHO, SHS

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