

Inverse association of transferrin saturation with mortality risk in chronic kidney disease

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Abstract

Background: Transferrin saturation (TSAT) is an indicator of iron deficiency or overload, but its relationship with mortality in patients with different stages of chronic kidney disease (CKD) is unclear. We investigated the association of TSAT with mortality in CKD patients.

Methods: In 479 CKD patients (97 CKD3-4 patients, 298 CKD5 non-dialysis patients and 84 peritoneal dialysis patients; median age 58 years, 67% males, 33% cardiovascular disease, CVD, and 29% diabetes), biomarkers of iron status (plasma iron, TSAT, transferrin and ferritin), systemic inflammation (high sensitivity C-reactive protein, hsCRP, and interleukin-6, IL-6) and nutritional status were assessed. During median follow-up of 35.6 months, 139 (29%) patients died, and 176 (37%) patients underwent renal transplantation. Patients were stratified into Low (n=157) and Middle and high (n=322) TSAT tertile groups. All-cause and CVD mortality risk were analyzed by competing risk regression with renal transplantation as competing risk.

Results: TSAT (median 23%; interquartile range, 17-30%) was negatively associated with presence of diabetes and CVD, body mass index, hsCRP, IL-6, erythropoiesis stimulating agent (ESA) dose, erythropoietin resistance index (ERI) and iron supplementation, and positively associated with hemoglobin, ferritin and s-albumin. In competing risk analysis, low tertile of TSAT was independently associated with increased all-cause mortality risk (sHR=1.74, 95%CI 1.30-2.54) and CVD mortality risk (sHR=1.80, 95%CI 1.02-3.16) after fully adjusting for 1-standard deviation (SD) of age, sex, CKD stages, 1-SD of hemoglobin, 1-SD of ferritin, 1-SD of hsCRP, 1-SD of ESA dose and iron supplementation.

Conclusions: Lower TSAT indicating iron deficiency was independently associated with increased mortality risk in CKD patients, underlining that iron status should be considered when evaluating clinical outcomes of CKD patients.

Introduction

Anemia is common in patients with chronic kidney disease (CKD) and contributes to increased morbidity and mortality [1, 2]. Apart from inadequate erythropoietin secretion, a leading cause of anemia in CKD is impaired iron homeostasis [3], commonly presenting as either absolute or functional iron deficiency, affecting 24–85% of patients with CKD [4].

Iron deficiency as such has been shown to be associated with increased risk of mortality in some chronic conditions, including anemia, independently of potential confounders [5, 6]. But it is still unclear if iron dysmetabolism, especially iron deficiency alone, could directly impact the clinical outcome in patients with CKD.

Transferrin saturation (TSAT), an index that considers both plasma iron and its main transport protein transferrin, serves as a biochemical marker of iron availability in plasma. Together with plasma ferritin that reflects body iron stores, TSAT is routinely used to monitor iron therapy [7]. In patients with adequate

or excessive iron stores as reflected by elevated ferritin levels, a low TSAT is commonly associated with inflammation and hepcidin-induced functional iron deficiency.

Several studies have investigated the association between iron dysregulation and all-cause and cardiovascular mortality using TSAT values in CKD patients [8–10]. These studies indicated that low TSAT values are associated with higher mortality in CKD patients, independently of anemia. The level of TSAT that predicts an adverse outcome varied between studies, and therefore the optimal range remains to be defined. Consequently, the prognostic importance of TSAT for all-cause and cardiovascular mortality in patients with various stages of CKD needs to be evaluated further.

The purpose of our study was to evaluate clinical characteristics associated with TSAT levels, factors influencing TSAT in different CKD stages, and the independent potential of TSAT to predict all-cause and CVD mortality in patients with CKD.

Materials And Methods

Patients and study design

This study was conducted in 479 clinically stable CKD patients, including 97 CKD3-4 patients, 298 CKD5 non-dialysis (CKD5-ND) patients and 84 CKD5-peritoneal dialysis (CKD5-D) patients. The patients (age 19–87 years) were recruited from three cohorts briefly described below. All patients were followed until renal transplantation or death, or after finishing 60 months of follow-up. There were no patients lost for follow-up.

CKD3-4 patients (n = 97) were recruited from PRIMA cohort [11]. The median age was 59 years, 71% were males, 23% had cardiovascular disease (CVD), and 30% had diabetes mellitus (DM). The median estimated glomerular filtration rate [eGFR, according to the CKD-EPI (CKD Epidemiology Collaboration) equation [12]] was 25.2 (interquartile range, IQR, 15.4–33.5) ml/min/1.73². The etiologies of renal disease were diabetic nephropathy (10%), chronic glomerulonephritis (22%), hypertension and renovascular disease (2%), and others or unknown causes (66%). 14% patients used erythropoietin stimulating agent (ESA) and 15% of patients used iron supplementation.

CKD5-ND patients (n = 297) were recruited from the MIA (Malnutrition, Inflammation and Atherosclerosis) study [13]. The median age was 56 years, 65% were males, 38% had cardiovascular disease, and 31% had diabetes mellitus. Their median eGFR was 6.0 (IQR, 4.8–7.9) ml/min/1.73². Causes of CKD included diabetic nephropathy (27%), chronic glomerulonephritis (20%), hypertension and renovascular disease (23%), and others or unknown causes (30%). 86% of the patients used ESAs and median ESA dose was 6000 IU/week. 57% of patients used iron supplementation.

CKD5-D patients (n = 84) were recruited from MIMICK2 (Mapping of Inflammation Markers in Chronic Kidney Disease 2) study [14]. Their median age was 64 years, 69% were males, 29% had cardiovascular disease, 24% had diabetes mellitus. The median residual renal function (RRF) was 2.5 (IQR, 1.2–3.8)

ml/min/1.73². The causes of CKD were diabetic nephropathy (12%), chronic glomerulonephritis (13%), hypertension and renovascular disease (11%), and others or unknown causes (64%). 94% patients used ESAs and median ESA dose was 5000 IU/week. 29% of patients used iron supplementation.

Patients' characteristics at baseline in these three cohorts are displayed in Table S1. The serum transferrin saturation (TSAT) percentage was calculated by dividing the serum iron level by total iron-binding capacity*100. Patients were divided into two groups based on the tertiles of TSAT: Low tertile group (n = 157), TSAT ranged from (min-max) 5.1 to 19.2%; and Middle and high tertile group (n = 322), TSAT ranged from (min-max) 19.2 to 83.8%.

Clinical, Anthropometric And Nutritional Evaluations

Cardiovascular disease was defined by medical history or clinical findings of ischemic cardiac disease, cerebrovascular or peripheral arterial disease. Smoking habits were recorded as current and non-smokers.

Anthropometric measurements were obtained at baseline. Body mass index (BMI) was calculated. Handgrip strength (HGS) was quantified in both hands using a Harpenden Handgrip Dynamometer (Yamar, Jackson, MI, USA). Values for HGS were expressed as percentage of healthy subjects, adjusted for sex. Lean body mass index (LBMI) and fat body mass index (FBMI) were calculated according to the method of Kyle et al. [15] from anthropometric data using four measurements of skinfold thickness [16], and were expressed as kg/m². Nutritional status was evaluated using the subjective global assessment (SGA) questionnaire [17]. Protein energy wasting (PEW) was defined as SGA score > 1 while a score of 0 indicated normal nutritional status. Blood pressure was reported as mean arterial blood pressure (MAP) defined as [diastolic pressure + (systolic pressure-diastolic pressure) / 3].

The erythropoietin resistance index (ERI) was calculated by dividing the weekly weight adjusted epoetin dose by the hemoglobin level at the time of investigation[18].

Biochemical Assessments

After overnight fasting, venous blood samples were collected at baseline. Concentrations of hemoglobin, high-sensitivity C-reactive protein (hsCRP), leucocyte count, serum creatinine, iron, ferritin, transferrin, serum albumin (bromocresol purple), cholesterol, triglycerides, intact parathyroid hormone (iPTH), calcium and phosphate were determined by routine methods at the Department of Laboratory Medicine, Karolinska University Hospital. Interleukin-6 (IL-6) and plasma tumor necrosis factor (TNF) were tested by enzyme-labeled chemiluminescent assay (Immulite, DPC, Los Angeles, CA) at our research laboratory.

Iron Deficiency

Functional iron deficiency (ID) was defined as serum ferritin ≥ 100 ng/mL and TSAT $< 20\%$; Absolute iron deficiency was defined as serum ferritin < 100 ng/mL and TSAT $< 20\%$. Patients with TSAT $\geq 20\%$ were considered not having iron deficiency [19].

Statistical Analyses

Data were expressed as median with interquartile range (IQR) or percentage or sub-distribution hazard ratio (sHR) or crude mortality rate, as appropriate. All tests were two-sided, and statistical significance was set at the level of $P < 0.05$. Comparisons between two groups were assessed with the non-parametric Wilcoxon test for continuous variables and Chi-square test for nominal variables. Comparisons between three or more groups were assessed with the non-parametric Kruskal-Wallis test for continuous variables and Chi-square test for nominal variables. Non-parametric Spearman rank test was performed to determine associations between variables. Multivariate logistic regression was used to analyze the factors associated with tertiles of TSAT. Survival during follow-up was analyzed by the competing risk regression model and the cumulative incidence curve [20]. Fine & Gray models were used [21] and adjusted for age, sex, CKD stages, hemoglobin, ferritin, hsCRP, ESA dose and iron supplementation. The sHR for TSAT were calculated with renal transplantation as a competing risk. We also performed restricted cubic spline in the flexible regression model to examine the non-linear association of TSAT level with all-cause mortality [22]. We used four knots to make a smooth curve. Spline curve was expressed as sub-hazard ratio for all-cause mortality, adjusting for age, sex, CKD stages, hemoglobin, ferritin, hsCRP, ESA dose and iron supplementation.

In sensitivity analyses, we used Cox proportional hazard model to further examine the association between lower TSAT and mortality across different subgroups of patients. All statistical analyses were performed using Stata 16.1 (Stata Corporation, College Station, TX, USA) and SAS version 9.4 (SAS Campus Drive, Cary, NC, USA).

Results

Baseline characteristics

The baseline demographics and clinical characteristics are shown in Table 1. Of 479 CKD patients (median age 58 years, males 67%), 158 patients (33%) had cardiovascular disease, and 140 patients (29%) had diabetes mellitus. The median values (IQR) were for TSAT 23 (17–30) %, iron 11 (8–15) $\mu\text{mol/L}$, ferritin 251 (121–408) $\mu\text{g/L}$, transferrin 2.0 (1.7–2.2) g/L and for hemoglobin 112 (101–123) g/L. 73% patients were on ESA treatment and 44% were on iron supplementation.

Table 1

Baseline demographic and biochemical characteristics of 479 CKD patients in relation to TSAT.

	Low tertile	Middle and high tertiles	P-value
	(n = 157)	(n = 322)	
Age (years)	58 (49–67)	58 (47–68)	0.72
Males, n (%)	104 (66.2)	217 (67.4)	0.84
CKD 3–4, n (%)	19 (12)	78 (24)	-
CKD5-ND ^a , n (%)	117 (75)	181 (56)	-
CKD5-D ^b , n (%)	21 (13)	63 (20)	-
Diabetes mellitus, n (%)	58 (36.9)	82 (25.5)	0.01
History of CVD, n (%)	68 (43.3)	90 (28)	< 0.001
Current smoker, n (%) (n = 414)	31 (23.5)	70(24.8)	0.81
MAP (mmHg) (n = 462)	107 (99–116)	106 (97–116)	0.47
Nutritional status			
PEW (SGA > 1), n (%) (n = 451)	48 (32.4)	80 (26.4)	0.18
Body mass index, (kg/m ²)	25.3 (22.4–28.5)	24.7 (22.3–27.9)	0.36
Lean body mass index, (kg/m ² , n = 440)	18.2 (16.3–19.5)	17.7 (16.0-19.3)	0.23
Fat body mass index, (kg/m ² , n = 440)	6.8(5.4–9.6)	7.0 (5.2–9.3)	0.79
%HGS of healthy subjects, (n = 443)	84 (63–97)	77 (67–101)	0.05
Laboratory variables			
S-Albumin (g/L)	33 (29–36)	35 (32–38)	< 0.001
Calcium (mmol/L)	2.3 (2.2–2.5)	2.4 (2.2–2.5)	0.24
Phosphate (mmol/L)	1.9 (1.5–2.2)	1.7 (1.3–2.1)	< 0.001
Ca × PO ₄ (mmol ² /L ²)	4.3 (3.5–5.3)	3.9 (3.1–4.9)	0.003
iPTH (ng/L), (n = 476)	230 (118–373)	189 (102–348)	0.15
Cholesterol (mmol/L, n = 478)	4.4 (3.6–5.3)	4.7 (4.0-5.7)	0.013
Triglyceride (mmol/L, n = 476)	1.7 (1.2–2.4)	1.7 (1.2–2.2)	0.80

	Low tertile	Middle and high tertiles	P-value
	(n = 157)	(n = 322)	
Biomarkers of inflammation			
Leucocyte count (10 ⁹ /L)	7.7 (6.4–9.3)	6.9 (5.6–8.5)	< 0.001
hsCRP (mg/L)	6.5 (2.4–13.0)	2.8 (1.0–8.0)	< 0.001
IL-6 (pg/ml, n = 402)	7.0 (3.8–13.2)	4.2 (2.5–7.2)	< 0.001
TNF (pg/ml, n = 238)	15.0 (12.1–18.1)	14 (11.2–16.8)	0.09
Medications			
β-blocker, n (%) (n = 472)	104 (67.5)	203 (63.8)	0.47
Ca-blocker, n (%) (n = 471)	80 (51.9)	137 (43.2)	0.08
ACEI/ARB, n (%)	109 (69.4)	219 (68.0)	0.83
Statins therapy, n (%)	71 (45.2)	99 (30.7)	0.002
Diuretics, n (%) (n = 464)	137 (89.0)	249 (80.3)	0.02
Phosphate binders, n (%) (n = 295)	97 (83.6)	150 (83.8)	0.97
Anemia related parameters			
Hemoglobin (g/L)	108 (98–116)	115 (104–126)	< 0.001
Iron (μmol/L)	7 (6–9)	13 (11–17)	< 0.001
Ferritin (μg/L)	175 (87–308)	288 (140–483)	< 0.001
Transferrin (g/L)	2.1 (1.8–2.3)	2.0 (1.7–2.2)	0.002
ESA use, n (%)	132 (84.1)	219 (68.0)	< 0.001
ESA dose, (IU/week) (n = 351)	6K (4-8K)	5K (4-8K)	0.012
ESA dose/BW, (IU/week/kg) (n = 351)	79.8(51.8-116.2)	70.1(46.0-103.4)	0.017
ERI, (IU/kg/week/g/dl) (n = 351)	7.8(4.9–11.4)	6.2(4.1–9.3)	0.002
Iron supplementation, n (%)	87 (55.4)	122 (37.9)	< 0.001
Intravenous iron therapy, n (%)	63 (40.1)	94 (29.2)	0.02
Oral iron therapy, n (%)	24 (15.3)	28 (8.7)	0.04
TSAT (%)	15.3 (12.3–17.2)	26.7(23.1–33.7)	< 0.001

	Low tertile	Middle and high tertiles	P-value
	(n = 157)	(n = 322)	
Values presented as median (interquartile range), number or percentage.			
ESA dose, ESA dose/BW and ERI were analyzed in patients used ESA therapy (n = 351).			
^a Defined as CKD 5 non dialysis patients. ^b Defined as CKD 5 peritoneal dialysis patients.			
Abbreviations: CVD, cardiovascular disease; MAP, mean arterial blood pressure; PEW, protein-energy wasting; SGA, subjective global assessment of nutritional status; % HGS, handgrip strength as percentage of the controls; iPTH, intact parathyroid hormone; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF, tumor necrosis factor; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ESA, erythropoiesis stimulating agents; BW, body weight; ERI, erythropoietin resistance index; TSAT, transferrin saturation.			

We divided the patients based on TSAT into two groups, Low tertile group (n = 157) and Middle and high tertile group (n = 322). While nutritional status was similar among the two groups, patients with lower TSAT levels had more comorbidities (CVD and DM). TSAT level was significantly associated positively with hemoglobin, ferritin, serum albumin, cholesterol and negatively with phosphate, leucocyte count, hsCRP and IL-6. Users of statins, diuretics, ESAs and iron supplementation had lower TSAT levels. In patients treated with ESAs, those with lower TSAT levels had significantly higher ESA dosages, ESA dose/body weight and ERI. There were no significant differences in TSAT levels that were related to usage of Ca-blockers, β -blockers, renin-angiotensin system (RAS) blockers and phosphate binders.

Figure 1 shows that among patients with lower TSAT levels, 27.7% had absolute iron deficiency and 72.3% functional iron deficiency, while in patients with higher TSAT levels 1.2% had absolute iron deficiency and 5.9% functional iron deficiency.

The baseline demographics and clinical characteristics in the three cohorts, CKD3-4, CKD5-ND and CKD5-D are shown in Table S1. The prevalence of PEW, inflammatory status and use of ESAs increased with decline of renal residual function, and other anemia-related parameters also differed significantly between the three CKD stage groups. Thus, compared with other patients, CKD5-ND patients had the lowest levels of TSAT and hemoglobin, and the highest levels of ERI, in addition to having the highest proportion of iron supplementation whereas several anemia-related parameters showed more normal values in CKD5-D patients, such as hemoglobin, ERI, ferritin and TSAT.

There were no significant differences in iron status and prevalence of ESA use and iron supplementation between males and females except that males had more comorbidities (CVD and DM). Compared with non-diabetic CKD patients, diabetic CKD patients had significantly higher prevalence of cardiovascular diseases and iron supplementation, but lower serum iron, ferritin and TSAT values. There was no difference in prevalence of ESA use between diabetic and non-diabetic CKD patients.

Univariate Correlations Of Factors Associated With TSAT

Univariate associations between TSAT and other parameters are shown in Table S2. TSAT was negatively associated with DM, CVD, BMI, mean arterial blood pressure, phosphate, leucocyte count, hsCRP, IL-6, use of ESA, ESA dose, iron supplementation and intravenous iron therapy, and positively associated with hemoglobin, ferritin, s-albumin and cholesterol. A negative relationship was found between ERI and TSAT levels ($\rho = -0.16$, $P = 0.004$, shown in Figure S1).

Multivariate Analysis Of Factors Associated With TSAT

Table 2 shows the results of multivariate logistic regression analysis to identify predictors of TSAT levels. 1-standard deviation (SD) higher hemoglobin (OR, 0.71; 95% CI, 0.56–0.91), ferritin (OR, 0.41; 95% CI, 0.30–0.56), hsCRP (OR, 1.53; 95% CI, 1.19–1.97) and ESA dose (OR, 1.41; 95% CI, 1.13–1.77) were found to be independently associated with TSAT after adjustments for diabetes, history of CVD and iron supplementation.

Table 2

Multivariate logistic regression analysis of factors associated with low versus middle and high tertiles of TSAT in CKD patients (n = 479, Pseudo r = 0.15).

	OR (95%CI)	P-value
1-SD increase of hemoglobin, g/L	0.71 (0.56–0.91)	0.006
1-SD increase of ferritin, $\mu\text{g/L}$	0.41 (0.30–0.56)	< 0.001
Diabetes mellitus	1.38 (0.87–2.18)	0.18
History of CVD	1.56 (0.99–2.44)	0.053
1-SD increase of hsCRP, mg/L	1.53 (1.19–1.97)	< 0.001
1-SD increase of ESA dose, IU/week	1.41 (1.13–1.77)	0.003
Iron supplementation, yes or no	1.28 (0.81–2.04)	0.29
Abbreviations: CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; ESA, erythropoiesis stimulating agents.		

Crude Mortality Rate/1000 Person-years For All-cause And CVD Mortality

Crude all-cause mortality rate/1000 person-years was for the low tertile of TSAT 12.4 (95% CI, 9.7–16.0), the middle tertile 7.0 (95% CI, 5.1–9.4), and high tertile 6.0 (95% CI, 4.3–8.3) deaths/1000 person-years (shown in Figure S2A). Crude CVD mortality rate/1000 person-years was for the low tertile of TSAT 5.6

(95% CI, 3.9–8.1), middle tertile 2.8 (95% CI, 1.8–4.5), and high tertile 2.4 (95% CI, 1.4–4.0) deaths/1000 person-years (shown in Figure S2B).

Associations Between TSAT Levels And All-cause Mortality

During follow-up for a median of 35.6 months, 139 (29%) of the patients died and 176 (37%) patients underwent renal transplantation. Multivariate competing risk analysis for all-cause mortality and CVD mortality took renal transplantation into account.

In multivariate competing risk analysis for all-cause mortality, after adjusting for 1-SD of age, sex, CKD stages, 1-SD of hemoglobin, 1-SD of ferritin, 1-SD of hsCRP, 1-SD of ESA dose and iron supplementation, lower TSAT (low tertile of TSAT) was significantly associated with higher all-cause mortality risk when compared to higher TSAT (middle and high tertile of TSAT): sHR, 1.74; 95% CI, 1.20–2.51; $P = 0.003$ (Fig. 2A). We performed competing risk analysis for patients with ESA therapy. After adjustments for the same confounders, the results showed that patients with lower TSAT levels had significantly higher risk of all-cause mortality (sHR, 1.74; 95% CI, 1.16–2.60; $P = 0.007$; $n = 351$).

In spline curve analysis using a flexible parametric model of TSAT as a continuous variable in 479 patients, TSAT showed an inverse association with all-cause mortality after adjusting for age, sex, CKD stages, hemoglobin, ferritin, hsCRP, ESA dose and iron supplementation (Figure S3).

Sensitivity analyses showed that lower TSAT was associated with higher all-cause mortality across different subgroups of age, sex, anemia, inflammation, and dialysis therapy but not in those with ferritin $> 500 \mu\text{g/L}$, SGA = 0 (i.e., well-nourished) and without ESA therapy (Fig. 3).

Associations Between TSAT Levels And CVD Mortality

In multivariate competing risk analysis for CVD mortality, after fully adjusted, lower TSAT was associated with significantly higher CVD mortality risk: sHR, 1.80; 95% CI, 1.02–3.16; $P = 0.042$ (Fig. 2B).

Discussion

This study shows that TSAT is inversely associated with mortality risk in CKD patients indicating that iron deficiency contributes to poor clinical outcomes. Iron deficiency exerts adverse effects on the cardiovascular system and has been demonstrated to negatively affect cardiac performance [23–26]. Previous studies have shown that iron deficiency increases mortality risk, independently of anemia in both heart failure [24, 25] and in CKD populations [9]. Our observation that low TSAT associated with increased mortality risk irrespective of hemoglobin status corroborates these findings. The importance of iron status is supported by a randomized clinical trial among 2141 hemodialysis patients showing that high-dose iron therapy administered proactively was superior to a low-dose iron regimen and associated with significantly lower risk of death or nonfatal adverse cardiovascular events [27]. While details for iron

therapy were not available, our study suggests that iron status as reflected by TSAT should be considered when evaluating risk factors in various CKD stages, with or without anemia. Whether iron deficiency with or without anemia in CKD should be treated as a distinct entity to improve survival and reduce cardiovascular morbidity deserves further studies.

Since treatment of iron deficiency is commonly guided by both TSAT and serum ferritin levels, we performed a sensitivity analysis with cutoffs for serum ferritin of < 100 µg/L, < 300 µg/L, < 500 µg/L and ≥ 500 µg/L which demonstrated that a low level of TSAT remained significantly associated with all-cause mortality when applying most of the applied ferritin cutoffs besides serum ferritin ≥ 500 µg/L. These results are in line with results from a study by Eisenga et al. [8] which demonstrated that a low TSAT (< 10%) remained a predictor of mortality when serum ferritin was below 500 µg/L. When serum ferritin ≥ 500 µg/L, it may reflect iron overload.

In our study, 73% patients were on ESA treatment, and TSAT was negatively correlated with ESA dose and ERI. The inverse relation between TSAT and ERI was also presented in a prospective and observational study including 1710 hemodialysis patients [28]. CKD is largely considered as an inflammatory state and inflammation is one of the major causes of ESA resistance, as indicated by independent association of ERI with hsCRP [29]. Systemic inflammation - and CKD - associate with increased concentrations of hepcidin [30]. Raised hepcidin levels further impair the ferroportin-mediated release of iron from enterocytes and the reticuloendothelial system [31], leading to high tissue ferritin but inadequate circulating iron available for erythropoiesis. In CKD patients, hypo-responsiveness to ESA can to some extent be explained by functional iron deficiency. A low TSAT level, which reflects either insufficient iron mobilization or depleted iron stores, was observed to be related to higher hsCRP level and ESA dose in our study, indicating interactions between inflammation, iron dysregulation and hypo-responsiveness to ESA.

The interpretation of iron biomarkers is affected by inflammation, a hallmark feature and a well-established mortality risk factor in CKD, as inflammation directly affects the levels of most iron biomarkers [32], including TSAT. Inflammation also accelerates the process of atherosclerosis, vascular calcification, and other causes of CVD [33, 34]. Thus, when assessing iron biomarkers as risk factors, inflammation is a considerable confounder. In our study, even though hsCRP was strongly associated with TSAT, lower TSAT was still independently associated with both all-cause and CVD mortality risk after adjusting for inflammation. Previous findings suggest as well that mortality risk associated with iron deficiency is independent from inflammation [8, 9].

In the present study, 29% patients were diabetic and had significantly lower serum TSAT levels. As presence of diabetes and its related metabolic syndrome are pro-inflammatory conditions, TSAT level may decrease due to a persistent microinflammatory state [35], and this may have contributed to the negative association between TSAT and diabetes

Several previous studies have focused on the relationship between iron indicators, especially TSAT, and mortality in CKD and dialysis patients [8–10, 36–38]. In one study including 975 CKD patients, TSAT < 10% was strongly associated with the risk of adverse outcomes [8]. In a pre-dialysis cohort (n = 32,489)

the highest risk of mortality was seen among those with the lowest quantile of TSAT (range = 0.36–11%) [9]. In incident dialysis patients with anemia, TSAT \leq 20% was a significant independent risk factor for adverse clinical outcome [37]. In the present study, low tertile (range = 5.1%-19.2%) was associated with higher risk of both all-cause as well as CVD mortality. So far, the definition of a specific accurate and reliable lower threshold of TSAT that would be useful for predicting adverse clinical outcomes has not been agreed upon; however, as concluded present study and several previous studies there is a robust inverse relationship between TSAT levels and mortality.

As expected, most anemia-related parameters differed between the different stages of CKD. As compared to patients with CKD stage 3–4, the CKD5-ND group showed marked impairment of most parameters, reflecting most likely the decline of renal function and complications linked to this such as inflammation, poor nutritional status and an increased burden of comorbidities. However, most anemia-related parameters improved in CKD5-D patients, such as hemoglobin, iron, ferritin, TSAT and ERI, suggesting that anemia treatment, dialysis therapy per se or factors related to dialysis initiation had a positive effect.

The present study has some limitations that should be considered when interpreting the results. First, this study was observational in nature and cannot prove causality. Second, only baseline demographic and clinical characteristics were used for the analyses; we did not analyze the impact of changes over time of TSAT and other iron parameters on clinical outcomes. Hepcidin levels were not measured in the present cohort. Third, covariates including peptic ulcer disease, gastrointestinal bleed or blood transfusions, which may influence anemia and iron markers were not considered in present study. Fourth, we included different groups of CKD patients. However, the impact of the heterogeneity between the groups was reduced by adjusting for CKD stage in the statistical analyses, and despite inclusion of patients with different stages of CKD, we could demonstrate an independent effect of TSAT on mortality, which may reinforce the conclusion of the study. Strengths of the study include detailed comprehensive phenotyping and that all patients were followed up in a uniform way, with no patients lost to follow up.

In conclusion, low TSAT was inversely and independently associated with increased mortality in CKD patients emphasizing the importance of TSAT when assessing iron status in CKD. Further studies are warranted to elucidate whether serial monitoring, rather than a single measurement of TSAT, can add value to TSAT as a prognosticator of clinical outcomes in CKD.

List Of Abbreviations

ACEI	Angiotensin-converting enzyme inhibitor
ARB	Angiotensin II receptor blocker
CKD	Chronic kidney disease
CVD	Cardiovascular disease
BMI	Body mass index
BW	Body weight
DM	Diabetes mellitus
ESA	Erythropoiesis stimulating agent
ERI	Erythropoietin resistance index
HGS	Handgrip strength
hsCRP	High sensitivity C-reactive protein
ID	Iron deficiency
iPTH	Intact parathyroid hormone
IQR	Interquartile range
IL-6	Interleukin-6
MAP	Mean arterial blood pressure
ND	Non-dialysis
PD	Peritoneal dialysis
PEW	Protein energy wasting
SD	Standard deviation
sHR	Sub-distribution hazard ratio
SGA	Subjective global assessment
TSAT	Transferrin saturation
TNF	Tumor necrosis factor

Declarations

Ethics approval and consent to participate

The Ethics Committee of the Karolinska Institutet, Stockholm, Sweden, approved study protocols. The studies were conducted in adherence to the declaration of Helsinki. Informed written consent was

obtained from each participant.

Consent for publication

Not applicable

Availability of data and materials

The data used and analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

Baxter Novum is the result of a grant from Baxter Healthcare to Karolinska Institutet. BL and IF are employed by Baxter Healthcare Corporation. KD is employed by Renapharma AB. None of the other authors declare any conflicts of interest.

Funding

Not applicable

Authors' contributions

XL and ARQ designed the present study, performed statistical analyses, interpreted the results, prepared figures and tables, and drafted the manuscript. The PRIMA, MIA and MIMICK cohort studies were designed by PS, OH and PB. All authors contributed to data collection, revised the manuscript, and approved the final version of the manuscript.

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Figures

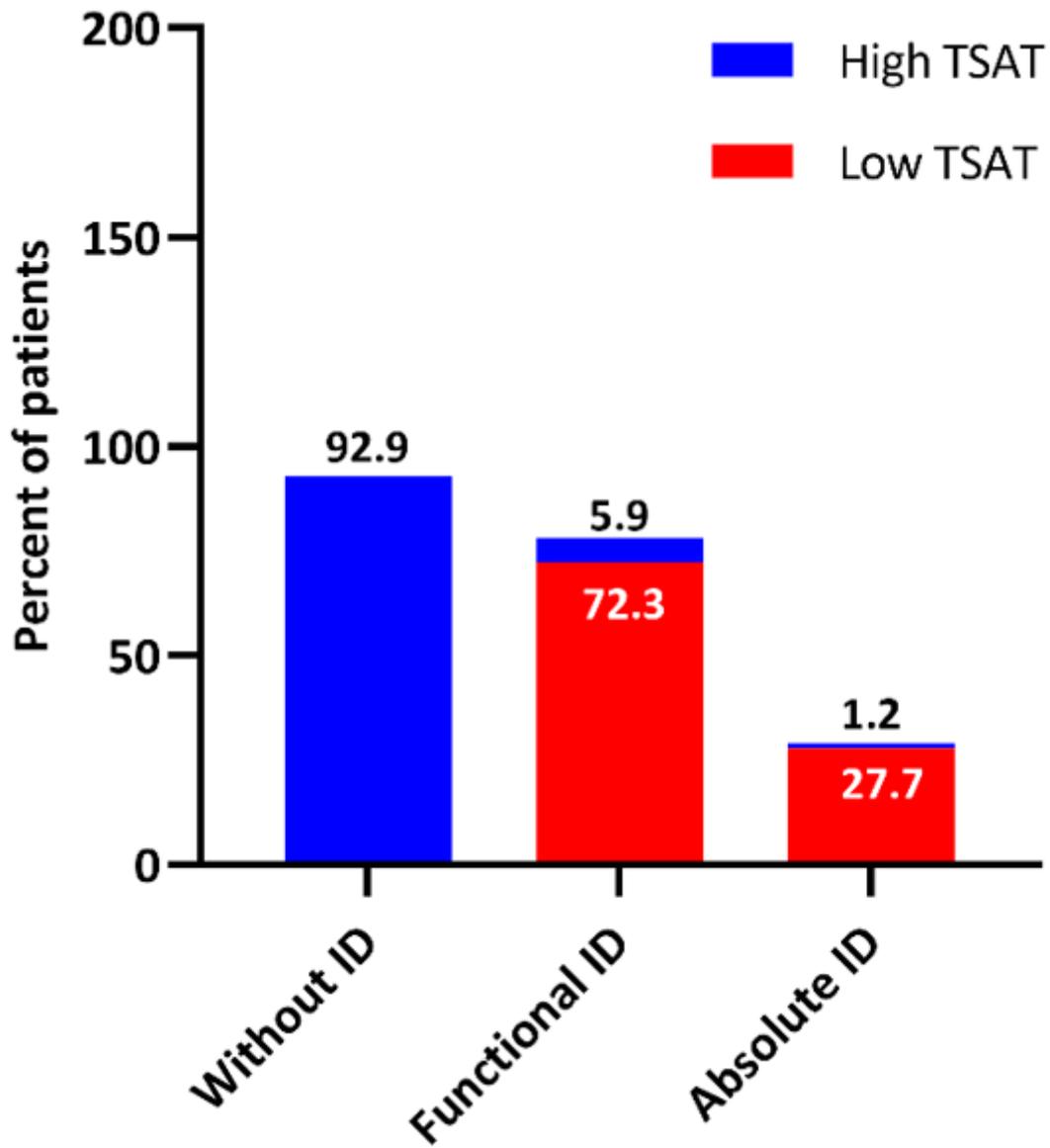
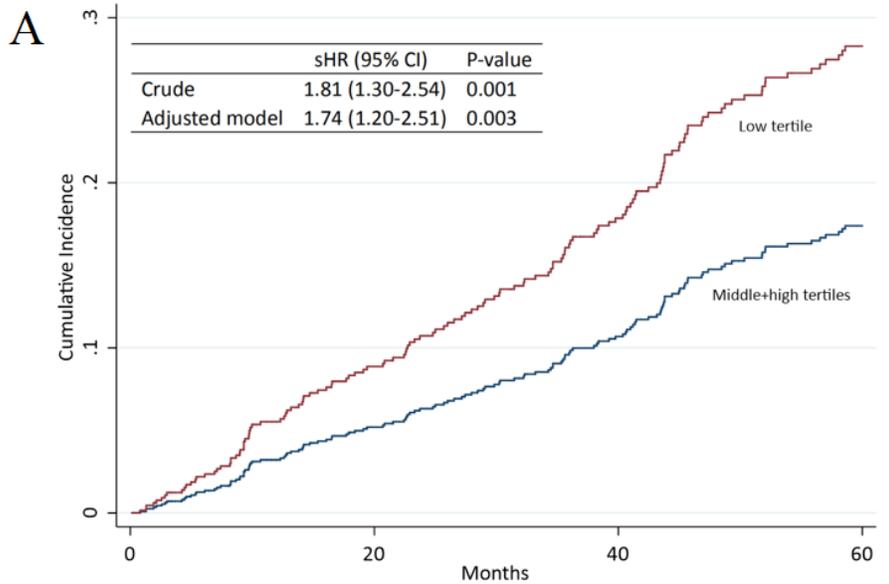
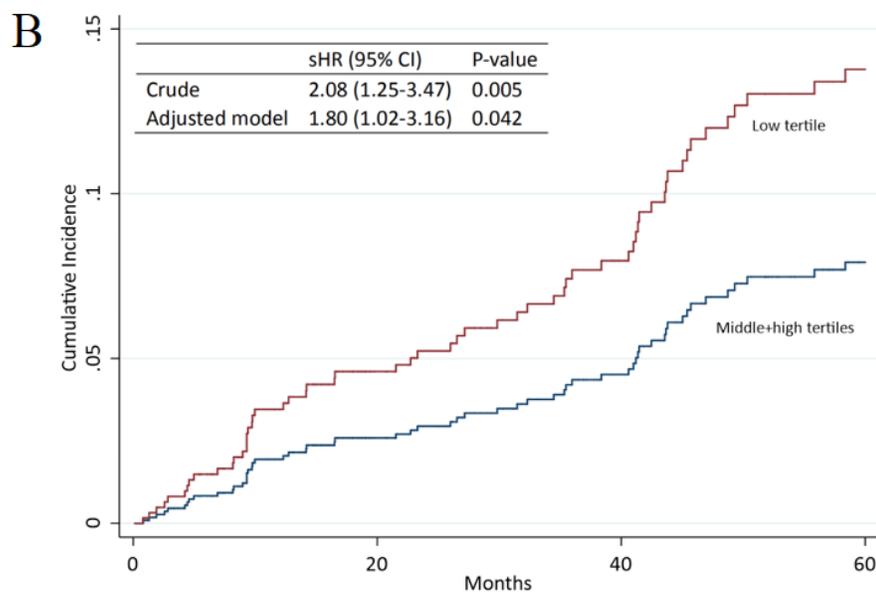


Figure 1

Distribution of iron deficiency (ID) in Low vs. Middle and high TSAT groups.



Adjusted model: Adjustment for 1-SD of age, sex, CKD stages, 1-SD of hemoglobin, 1-SD of ferritin, 1-SD of hsCRP, 1-SD of ESA dose and iron supplementation.



Adjusted model: Adjustment for 1-SD of age, sex, CKD stages, 1-SD of hemoglobin, 1-SD of ferritin, 1-SD of hsCRP, 1-SD of ESA dose and iron supplementation.

Figure 2

A. Cumulative incidence curves of 5-years all-cause mortality in relation to tertiles of TSAT levels (n=479). Figure inset show sHR for association of Low vs. Middle and high tertiles of TSAT with mortality in crude and adjusted model. B. Cumulative incidence curves of 5-years CVD mortality in relation to tertiles of TSAT levels (n=479). Figure inset show sHR for association of Low vs. Middle and high tertiles of TSAT with mortality in crude and adjusted model.

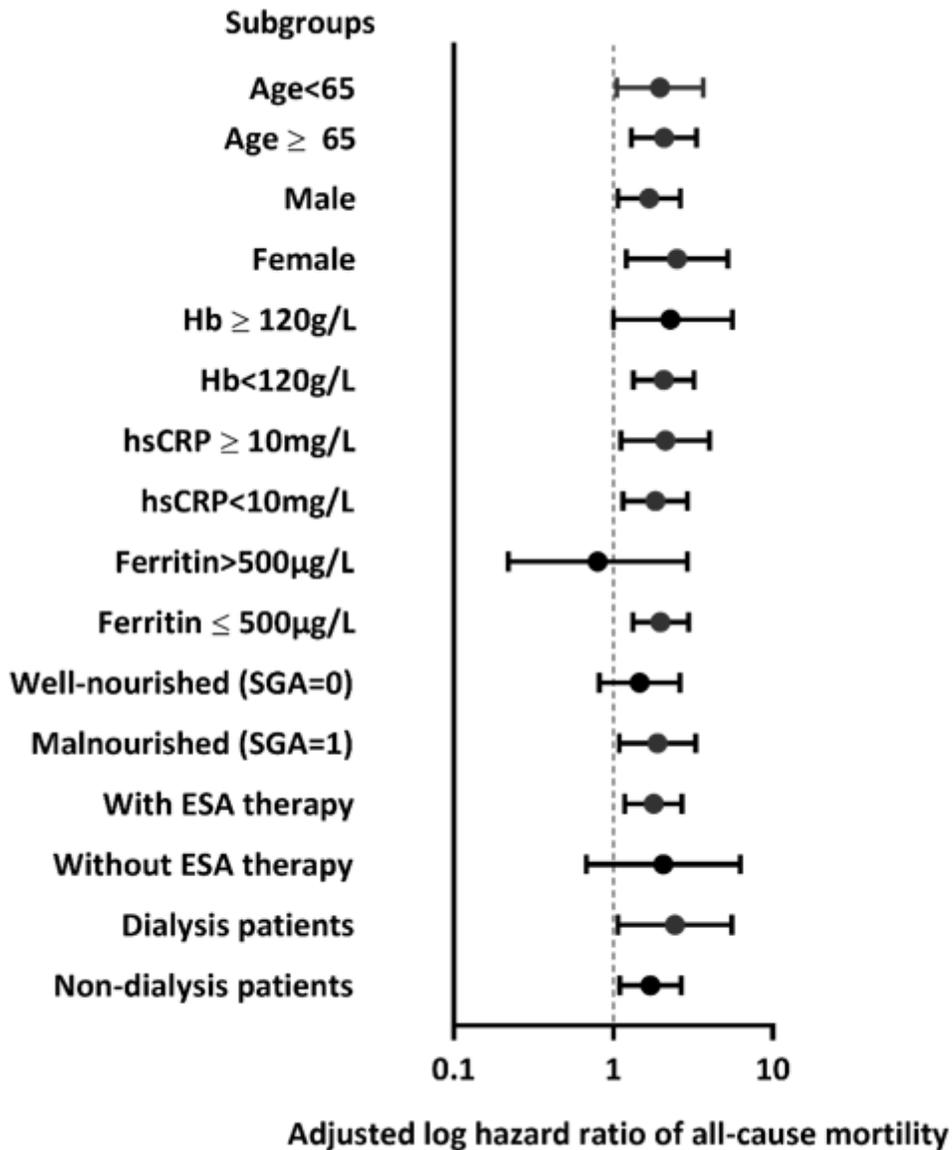


Figure 3

Adjusted log-HRs of all-cause mortality associated with lower TSAT across subgroups of age, sex, anemia, inflammation, ferritin, nutritional status, ESA therapy and dialysis therapy. Models were adjusted for 1-SD of age, sex, CKD stages, 1-SD of hemoglobin, 1-SD of hsCRP, 1-SD of ferritin, ESA therapy and iron supplementation.

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