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Low Levels of Serum Ferritin Lead to Adequate Hemoglobin Levels and Good Survival in Hemodialysis Patients

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Key Words
Anemia · Hemodialysis · Serum ferritin · Mortality

Abstract
Background: The optimal level of serum ferritin (s-ft) for anemia control and good survival in hemodialysis (HD) patients remains unclear. A 10-year survey was performed to clarify the appropriate quantities of s-ft and investigate the relationships among s-ft, transferrin saturation (TSAT), and mortality in HD patients. ***Methods:*** HD outpatients (n = 125) treated with erythropoiesis-stimulating agents (ESA) were followed for 10 years. The ESA and low-dose iron supplement dosages were adjusted to maintain the hemoglobin (Hb) at 10–11 g/dl, according to Japanese guidelines. The Kaplan-Meier method, log-rank tests, and the Cox proportional hazards model were used for performing the statistical analyses. The interactions among the Hb, s-ft, and TSAT were analyzed using a multiple linear regression model. Patients with TSAT ≥20% were classified according to the s-ft cutoff values: group 1 (s-ft <30 ng/ml); group 2 (s-ft 30–80 ng/ml); group 3 (s-ft >80 ng/ml); TSAT <20% was a predictor of poor outcome. ***Results:*** The survival rate in group 2 was significantly higher than that in other groups (p = 0.013), and the Cox proportional hazards model analysis showed a good effect of low levels of s-ft on patients' survival. The multiple linear regression model showed a strong effect of s-ft on the Hb (log [s-ft], β-coefficient −0.45: 95% confidence interval −0.65 to −0.26, p < 0.001). ***Conclusion:*** This study revealed that low levels of s-ft have a beneficial effect on the outcome of HD patients receiving ESA. Thus, the optimal s-ft level might be lower than that established previously for these patients.

Introduction

Anemia is detected in more than 80% of patients under maintenance hemodialysis (HD) therapy (HD patients) [1] and has long influenced the quality of life of these patients. The annual loss of iron caused by dialysis therapy is said to be 1–2 g, and generally, HD patients tend to develop iron deficiency. Therefore, erythropoiesis-stimulating agents (ESA) and iron replacement therapy are used mainly to treat anemia. However, the optimal amount of iron in HD patients remains unknown and iron management standards using serum ferritin (s-ft), which is an index of stored iron, vary greatly among guidelines. In addition, ≤100 ng/ml of s-ft and ≤20% transferrin saturation (TSAT) are the criteria required for initiating iron replacement therapy [2]. According to the Japanese Society for Dialysis Therapy (JSDT) guidelines, a lower s-ft level is recommended as compared to most western guidelines, which recommend a considerably higher s-ft level than its cutoff value for the detection of

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iron deficiency (12–40 ng/ml) in healthy individuals [3–5]. Moreover, in HD patients, intravenous (IV) iron administration can reduce the required dose of ESA and is useful for maintaining the serum hemoglobin (Hb) [6]. Therefore, various guidelines recommend the non-physiological IV route for iron supplementation in HD patients.

However, excess iron produces strong oxidative stress through the Fenton reaction, which underlies the onset of various pathological processes, including arteriosclerosis [7–9], myocardial infarction [10], infection [11, 12], and malignant tumors [13, 14]. Moreover, such oxidative stress caused by IV iron administration has also been reported in HD patients [7, 15, 16], suggesting that excess iron in the body due to frequent iron administration may affect the prognosis of these patients. Moreover, a regulator of iron metabolism, hepcidin 25 (Hep25), was discovered recently [17]. Excess iron induces the production of Hep25, which inhibits iron absorption from the gastrointestinal tract and iron release from macrophages, thereby reducing the efficiency of iron use [18].

Therefore, we provided iron treatment to HD patients based on the hypothesis that it is more beneficial to maintain lower s-ft levels to avoid oxidative stress due to excess iron and to maintain the use of iron efficiently. This 10-year retrospective study was conducted to investigate the effect of body iron status on anemia treatment and patient survival rates, assess the validity of our iron management protocol, and reconsider the optimal body iron stores in HD patients.

Methods

Patients

Among the maintenance HD patients with identified outcomes who had attended the Maeda Institute of Renal Research (Kanagawa, Japan) between May 2002 and April 2013, 125 who had received ESA, during the 1-year period from May 2002 to April 2003, were enrolled in this study. Those who had acute heart disease, cerebrovascular disease, active infection, or malignant tumor at the initiation of the study were excluded. All patients were receiving 4- to 5-h HD sessions thrice a week.

All patients provided informed consent permitting data sampling and analysis at the time of initiation of the dialysis therapy. The protocol for the study was approved by the ethics committee of the Biomarker Society, INC, comprising 7 committees, including outside experts.

Methods

Blood was collected when HD was initiated at the beginning of the week. Hematologic indices [Hb, reticulocyte count (Ret)] were measured twice a month, and s-ft, serum iron (Fe) and total iron-binding capacity (TIBC) were measured once a month. TSAT was calculated from the values of Fe and TIBC (TSAT = Fe/TIBC/ 100). Furthermore, the Kt/V value was measured once a month using the single-pool method.

For the treatment of anemia, the dose of ESA was adjusted for obtaining an Hb goal of 10–11 g/dl, and s-ft <30 mg/dl was used as the criterion to initiate iron administration.

To investigate the relationship between the body iron status and the prognosis, the patients were grouped according to the mean s-ft and TSAT levels during the 1-year period from May 2002 to April 2003. Patients with TSAT values of <20% (n = 34) were defined as the TSAT <20% group, and those with TSAT ≥20% were classified into 3 groups according to the s-ft level: group 1 (s-ft <30 ng/ml; n = 15), group 2 (s-ft 30–80 ng/ml; n = 28), and group 3 (s-ft >80 ng/ml; n = 48). The cutoff points of the 20th percentile and 50th percentile were used for the s-ft level. The 10-year survival rates, HRs, and causes of death during the 10-year period from May 2003 to April 2013 were compared among the groups. Moreover, correlations among Hb, TSAT, and s-ft were investigated.

Statistical Analysis

Analyses were performed with the SAS system software, version 9.3 (SAS Institute, Cary, N.C., USA). Data are presented as the mean ± standard deviation (SD) and as medians with interquartile ranges. One-way analysis of variance was used to compare the groups with respect to normally distributed continuous variables, and the Kruskal-Wallis H test was used for other skewed continuous variables. The Chi-square test was used to compare nominally scaled variables. The cumulative probabilities of time-to-event curves were estimated with the Kaplan-Meier product-limit function, and the difference among the curves was tested by the log-rank test. Furthermore, to evaluate the impact of the value of TSAT and s-ft on death from any cause, we applied the conventional Cox proportional hazards model with a fixed one-year group and an extended time-dependent Cox model using the value from the follow-up period.

The multivariable model included age and the following imbalanced covariates: systolic blood pressure, hemoglobin, Ret, total iron-binding capacity, and albumin. The proportional hazards assumption was confirmed by the log [–log (survival function)]. To evaluate the relationship of Hb with TSAT and s-ft, the Pearson product-moment correlation coefficient and multivariable linear regression model were used. The values of s-ft that were not normally distributed were log-transformed before performing the above parametric analysis. The influences of the profile, interaction, and multicollinearity in the multivariable model were examined using the regression diagnostic analysis.

Two-tailed p values of less than 0.05 were considered to indicate a statistically significant difference. All analyses were performed at an independent biostatistics and data center (STATZ Institute, Inc., Tokyo, Japan).

Limitations of the Study

This study had several limitations. Because this was a retrospective and observational study and the number of enrolled patients was small, there is the possibility that there may be other confounding factors that remain to be analyzed. Furthermore, because the patient's management was dependent on the attending physician, aside from using different targets for s-ft and Hb levels, there were variations in the management of the patients.

Table 1. Baseline characteristics

Variables	Total (n = 125)	TSAT <20% (n = 34)	TSAT >20%			p value
			group 1 (n = 15)	group 2 (n = 28)	group 3 (n = 48)	
TSAT, %	23.2±5.4	16.9±2.5	24.9±2.6	24.3±3.1	26.6±4.7	
s-ft, ng/ml*	86.0 [41.2–130.0]	87.1 [51.0–127.9]	22.0 [17.7–24.5]	45.3 [36.4–56.8]	129.2 [104.4–178.9]	
Age, years	56.2±11.4	56.9±9.7	56.8±9.5	51.6±11.1	58.2±12.7	0.099
Gender, n (%)						
Men	70 (56.0)	18 (52.9)	10 (66.7)	16 (57.1)	26 (54.2)	0.825
Women	55 (44.0)	16 (47.1)	5 (33.3)	12 (42.9)	22 (45.8)	
Primary diagnosis, n (%)						
Chronic glomerulonephritis	78 (62.4)	21 (61.8)	9 (60.0)	19 (67.9)	29 (60.4)	0.837
Diabetes nephropathy	26 (20.8)	7 (20.6)	4 (26.7)	6 (21.4)	9 (18.8)	
Renal sclerosis	9 (7.2)	2 (5.9)	–	2 (7.1)	5 (10.4)	
Polycystic Kidney	2 (1.6)	1 (2.9)	1 (6.7)	–	–	
RPGN	3 (2.4)	1 (2.9)	1 (6.7)	–	1 (2.1)	
SLE	4 (3.2)	1 (2.9)	–	–	3 (6.3)	
Other	2 (1.6)	1 (2.9)	–	1 (3.6)	–	
Unknown	1 (0.8)	–	–	–	1 (2.1)	
Duration of dialysis, years*	5.3 [2.5–11.1]	6.7 [3.1–14.1]	3.6 [1.6–12.0]	5.4 [2.1–9.8]	4.7 [2.3–10.2]	0.442
Systolic BP, mm Hg	156.4±20.9	160.8±24.3	161.8±22.8	147.4±15.0	156.8±19.4	0.049
Diastolic BP, mm Hg	85.8±14.0	89.9±14.1	83.5±14.1	80.7±14.7	86.6±12.9	0.062
Kt/V	1.32±0.22	1.31±0.21	1.33±0.25	1.33±0.25	1.31±0.19	0.969
Hemoglobin, g/dl	9.8±0.4	9.7±0.3	10.0±0.4	9.9±0.4	9.7±0.5	0.006
Reticlocyte, ‰	15.5±7.2	16.5±8.7	16.3±3.7	18.0±7.8	13.0±5.8	0.016
TIBC, μg/dl	243.9±37.3	257.3±40.2	259.5±40.2	246.3±29.9	228.1±32.8	<0.001
Urea nitrogen, mg/ml	71.7±14.9	68.4±12.9	69.6±17.3	73.3±13.5	73.9±16.1	0.359
Creatinine, mg/ml	11.5±2.3	11.3±2.3	11.5±1.8	12.2±2.8	11.3±2.1	0.352
Albumin, g/dl	3.8±0.3	3.9±0.2	3.7±0.3	3.9±0.3	3.8±0.3	0.049
Calcium, mg/dl	9.6±0.8	9.7±0.8	9.1±0.6	9.6±0.7	9.6±0.9	0.149
Phosphoric, mg/dl	5.9±1.3	5.9±1.2	6.0±1.4	6.0±1.5	5.8±1.2	0.894
C-reactive protein, mg/dl*	0.2 [0.1–0.3]	0.3 [0.1–0.5]	0.2 [0.1–0.6]	0.2 [0.1–0.3]	0.2 [0.1–0.2]	0.119

TSAT = Transferrin saturation; s-ft = serum ferritin; RPGN = rapid progressive glomerulonephritis; SLE = systemic lupus erythematosus; BP = blood pressure; TIBC = total iron-binding capacity. Group 1: s-ft <30 ng/ml; group 2: s-ft 30–80 ng/ml; group 3: s-ft >80 ng/ml. Plus minus value: mean ± SD. * median [interquartile range]. p value for the comparison among four groups.

Results

Patients

This study included 125 patients. Their characteristics and clinical data at the initiation of the study are shown in table 1.

Of the 125 patients, 70 were male and 55 were female. The mean age was 56.2 ± 11.4 years, and the median duration of dialysis was 5.3 years. The underlying kidney diseases were chronic glomerulonephritis (78 patients, 62.4%), diabetic nephropathy (26 patients, 20.8%), nephrosclerosis (9 patients, 7.2%), and others (11 patients, 10.0%). There were no significant differences in the age, sex ratio, underlying kidney disease distribution, or dialysis duration among the 4 groups.

With regard to the clinical data, the Hb level was significantly higher in group 1 and significantly lower in group 3 and the TSAT <20% group (p = 0.006). The Ret was significantly higher in group 2 and significantly lower in group 3 as compared to the TSAT <20% group (p = 0.016). TIBC was significantly higher in group 1 and significantly lower in group 3 as compared to the TSAT <20% group (p < 0.001). Biochemical tests revealed only slightly significant differences (p = 0.049) in serum albumin (Alb), and no significant differences in dialysis efficiency, blood urea nitrogen, serum creatinine, serum calcium, serum inorganic phosphorus, and serum C-reactive protein (CRP) among the groups.

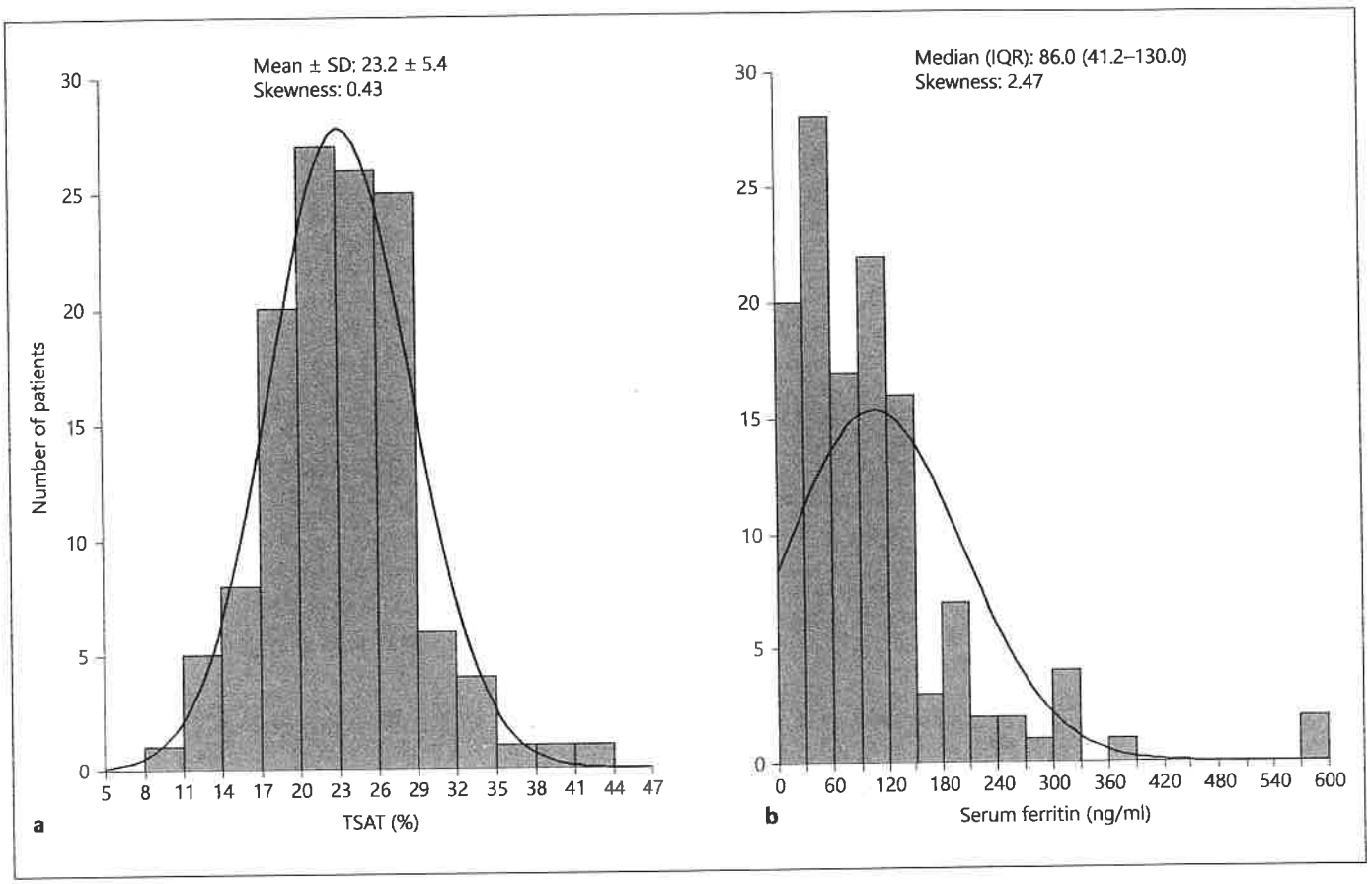

Fig. 1. Distribution of transferrin saturation (TSAT) levels (**a**) and serum ferritin (**b**).

Distribution of the Body Iron Status

In the histograms of TSAT and s-ft shown in figure 1, TSAT had an almost normal distribution with a mean value of 23.2% (skewness: 0.43), whereas the s-ft had a non-normal distribution with a median value of 86.0 ng/dl (skewness: 2.47).

Investigation of the 10-Year Survival Rate

Of the 125 patients, 44 (35.2%) died. The 10-year survival rate was highest in group 2 (90.3%), followed by that in group 1 (66.7%) and group 3 (60.4%), and lowest in the TSAT <20% group (50%) ($p = 0.013$; fig. 2). Moreover, in the Cox proportional hazards model analysis of the TSAT <20% group using death as the endpoint, univariate and multivariate analyses using the variables of age, sex, and Hb revealed significantly lower hazard ratios (HRs) in group 2: 0.16 (95% confidence interval (CI): 0.05–0.55, $p = 0.004$) and 0.18 (95% CI: 0.05–0.64, $p = 0.008$), respectively. In contrast, no significant differences were observed in HRs between groups 1 and 3 (fig. 3). In contrast, because the category of the iron status might have varied during the study period, we adopted the time-dependent Cox hazards models to analyze the survival rate. Again, the univariate and multivariate analyses showed significantly lower HRs in group 2: 0.21 (95% CI: 0.07–0.69, $p = 0.010$) and 0.25 (95% CI: 0.07–0.88, $p = 0.031$). This result also turned out to be positive; however, the CI was slightly wider for the survival rate ($p = 0.039$) in group 1, the group with s-ft <30 (fig. 3).

Investigation of the Causes of Death

The causes of death included heart failure in 7 patients (5.6%), myocardial infarction in 6 (4.8%), infection in 6 (4.8%), cerebrovascular disease in 5 (4.0%), and malignant tumor in 5 patients (4.0%). Of the 17 cases of death in the TSAT <20% group, in which the mortality rate was the highest, 2 patients (5.9%) died of myocardial infarction, 2 (5.9%) of cerebrovascular disease, 2 (5.9%) of malignant tumor, and 7 (20.6%) of other causes, resulting in a broad range of causes of death. Of the 19 deaths in

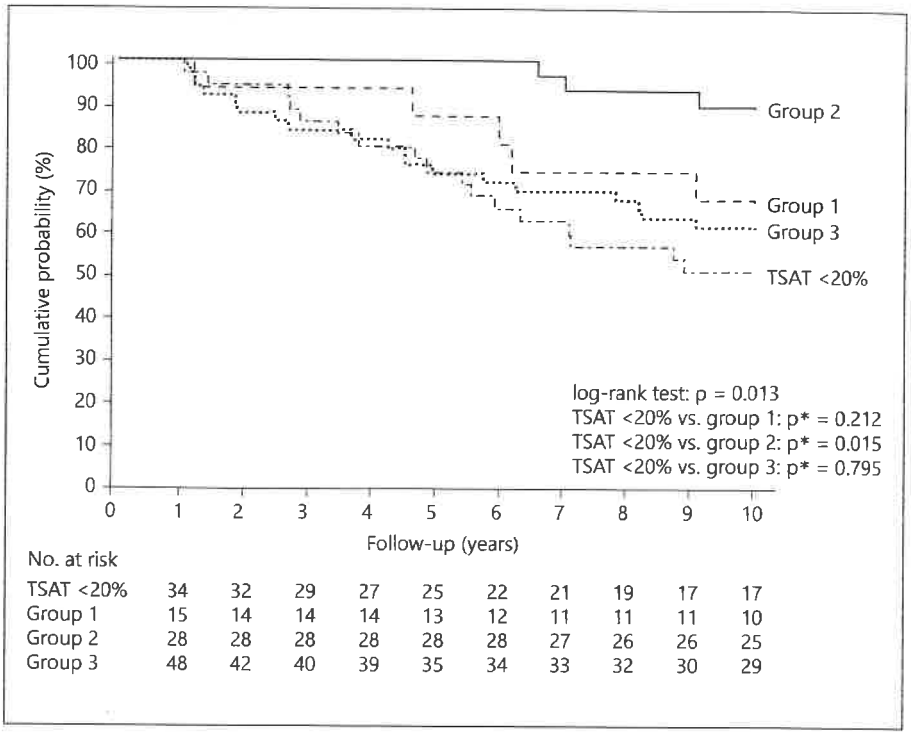

Fig. 2. Kaplan-Meier product-limit function estimates for time to all-cause death by transferrin saturation (TSAT) and serum ferritin (s-ft). The 10-year survival rate in group 2 was noted to be substantially higher ($p = 0.013$) than that in the other groups. * p value adjusted by the multivariable model including age and following imbalanced covariates: systolic blood pressure, hemoglobin, reticulocyte count, total iron-binding capacity, and albumin.

Group	No. of patients	No. of patients with death (%)	Univariable analysis: Unadjusted hazard ratio	(95% confidence interval)	p-value	Multivariable analysis: Adjusted hazard ratio	(95% confidence interval)	p-value
Conventional Cox proportional hazards model								
TSAT ≤20 [reference]	34	17 (50.0)	1.00			1.00		
s-ft <30 (TSAT >20)	15	5 (33.3)	0.58	(0.21–1.57)	0.281	0.50	(0.17–1.48)	0.212
s-ft 30–80 (TSAT >20)	28	3 (10.7)	0.16	(0.05–0.55)	0.004	0.20	(0.05–0.73)	0.015
s-ft >80 (TSAT >20)	48	19 (39.6)	0.76	(0.40–1.47)	0.419	1.11	(0.49–2.51)	0.795
Time-dependent Cox proportional hazards model								
TSAT ≤20 [reference]			1.00			1.00		
s-ft <30 (TSAT >20)			0.27	(0.06–1.12)	0.071	0.22	(0.05–0.93)	0.039
s-ft 30–80 (TSAT >20)			0.21	(0.07–0.69)	0.010	0.25	(0.07–0.88)	0.031
s-ft >80 (TSAT >20)			0.92	(0.45–1.89)	0.816	1.22	(0.57–2.58)	0.610

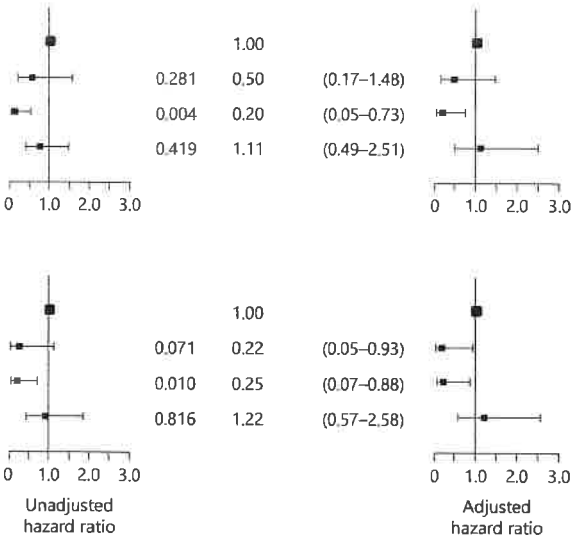

Fig. 3. The conventional Cox proportional hazards model and time-dependent Cox proportional hazards model. Adjusted hazard ratios were obtained by the multivariable model including age and following imbalanced covariates: systolic blood pressure, hemoglobin, reticulocyte count, total iron-binding capacity, and albumin.

Table 2. Cause of death according to the value of transferrin saturation and serum ferritin

Variables, n (%)	Total (n = 125)	TSAT <20% (n = 34)	TSAT >20%			p value
			group 1 (n = 15)	group 2 (n = 28)	group 3 (n = 48)	
All deceased patients	44 (35.2)	17 (50.0)	5 (33.3)	3 (10.7)	19 (39.6)	
Cause of death						
Heart failure	7 (5.6)	1 (2.9)	1 (6.7)	–	5 (10.4)	0.350
Myocardial infarction	6 (4.8)	2 (5.9)	2 (13.3)	–	2 (4.2)	
Infectious disease	6 (4.8)	1 (2.9)	1 (6.7)	2 (7.1)	2 (4.2)	
Cerebrovascular disease	5 (4.0)	2 (5.9)	–	–	3 (6.3)	
Malignancy	5 (4.0)	2 (5.9)	1 (6.7)	1 (3.6)	1 (2.1)	
Sudden death	3 (2.4)	1 (2.9)	–	–	2 (4.2)	
Other	10 (8.0)	7 (20.6)	–	–	3 (6.3)	
Unknown	2 (1.6)	1 (2.9)	–	–	1 (2.1)	

TSAT = Transferrin saturation. p value for the comparison among four groups.

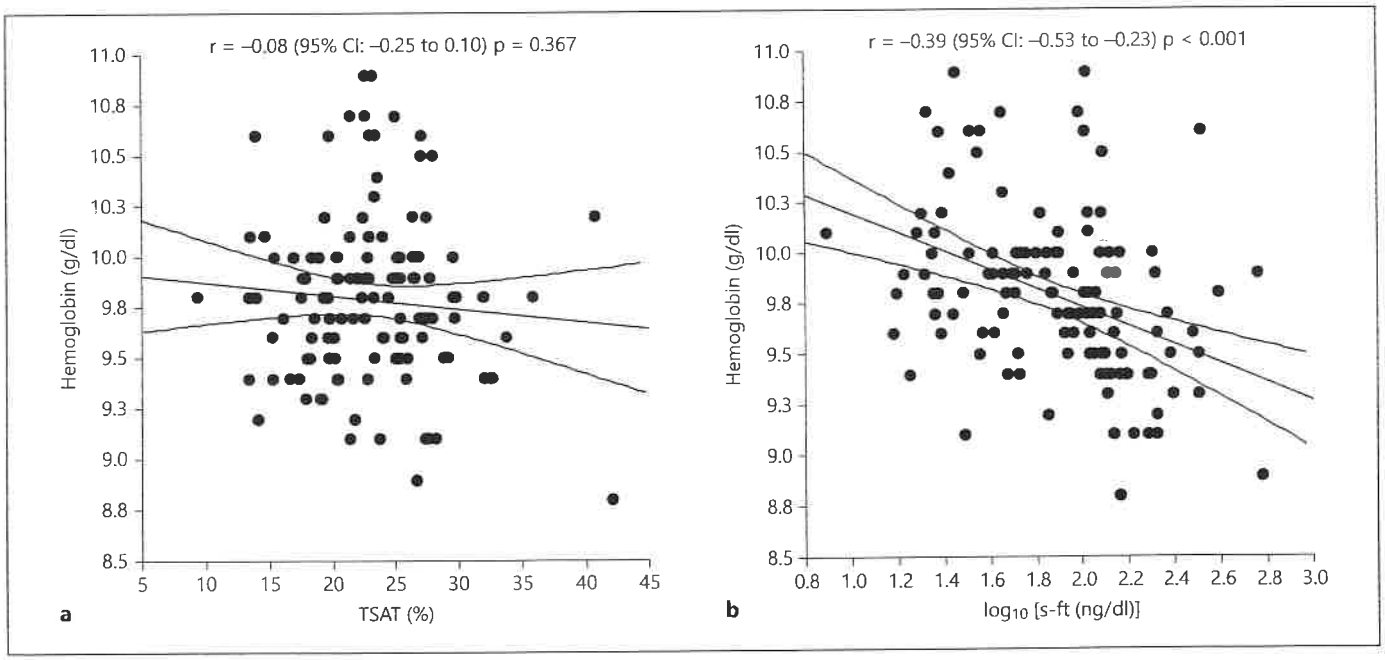

Fig. 4. Correlation between hemoglobin and transferrin saturation (TSAT; **a**), serum ferritin (s-ft; **b**).

group 3 showing high s-ft levels, 5 patients (10.4%) died of heart failure, 3 (6.3%) of cerebrovascular disease, and 2 (4.2%) of myocardial infarction with heart failure being the common cause of death. In contrast, in group 2, which had the lowest mortality, 2 patients died of infection, 1 of a malignant tumor, and none died of cardiovascular disease. However, there were no significant differences in the frequency of each cause of death among the groups (table 2).

Correlations among the Hb, TSAT, and s-ft

Correlations among the Hb, TSAT, and s-ft were investigated using linear regression models. The results revealed the absence of any correlation between Hb and TSAT. However, univariate and multivariate analyses, adjusted for age and sex, showed a significantly negative correlation between Hb and log (s-ft [ng/ml]), with β-coefficients of –0.46 (95% CI: 0.65–0.26, $p < 0.001$) and –0.45 (95% CI: 0.65–0.26, $p < 0.001$), respectively (fig. 4).

Table 3. Relationship between hemoglobin and transferrin saturation, and serum ferritin in the linear regression model

Dependent variable	Independent variables	Univariable analysis			Multivariable analysis		
		unadjusted regression β-coefficient	(95% CI)	p value	adjusted regression β-coefficient	(95% CI)	p value
Hemoglobin, g/dl	TSAT (per 1% increase)	−0.01	(−0.02 to 0.01)	0.367	−0.00	(−0.02 to 0.01)	0.836
	$\log_{10}$ (s-ft) (per 1% increase)	−0.46	(−0.66 to −0.27)	<0.001	−0.37	(−0.65 to −0.26)	0.004
						Corrected model R^2	0.146

TSAT = Transferrin saturation; s-ft = serum ferritin.

CI denotes confidence interval. Adjusted β-coefficient were obtained by the multivariable model including age, systolic blood pressure, reticulocyte, total iron-binding capacity and albumin.

Discussion

The prevailing iron management standards for dialysis patients in western countries are as follows: the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines state that 'there is insufficient evidence to recommend routine administration of IV iron if the s-ft level is greater than 500 ng/ml' [19] and the European Renal Best Practice guidelines state that 'if the s-ft level is greater than 800 ng/ml, IV iron administration should be discontinued' [20]. In contrast, JSDT states that '≤100 ng/ml of s-ft and ≤20% of TSAT' are the criteria for initiating iron replacement therapy. Thus, a considerably lower level of s-ft is recommended for HD patients in Japan as compared to that in western countries. However, even 100 ng/ml of s-ft, which is the s-ft cutoff point for the initiation of iron administration according to JSDT guidelines, represents an increased iron content [21], and is associated with the impaired functioning [22] of polymorphonuclear leukocytes in maintenance HD patients. Therefore, according to the findings of our study even 100 ng/ml of s-ft may be an excessive cutoff value for the initiation of iron administration in HD patients. Thus, we set the s-ft goal for the initiation of iron therapy at <60 ng/ml, based on the report stating that the polymorphonuclear leukocytes function was only mildly impaired in patients with s-ft levels <60 ng/ml [22]. We measured the s-ft and TSAT once a month and carefully managed the iron administration. Furthermore, the cutoff value of s-ft for the diagnosis of iron deficiency is 12 ng/ml in the absence of infection or inflammatory disease [3–5] and the s-ft level decreases by 50–75% from the baseline when recombinant human erythropoietin is used [23, 24]. Therefore, an s-ft value of <30 ng/ml was used as the criterion for initiating iron administration in patients receiving ESA. Because the iron requirements differ between patients receiving and not receiving ESA, in this study, we only investigated the requirements of patients receiving ESA.

TSAT <20% is representative of insufficient and inefficient iron use [25–27]. TSAT ≤20% is one of the criteria for initiating iron administration according to JSDT guidelines and is associated with poor prognosis [28], suggesting that disease conditions that reduce the efficiency of iron use may affect patient prognosis. Therefore, patients with TSAT <20% were grouped as the TSAT <20% group, and the optimal s-ft level was investigated in patients with TSAT ≥20%. In this study, patients with acute infection and malignant tumors, conditions generally associated with reduced iron use efficiency [29], were excluded from this study, and there were no significant differences in the serum CRP, serum Alb, or dialysis efficiency between the TSAT <20% group and the other groups. Thus, the TSAT <20% group had a median s-ft level of 87.1 (51.0–127.9) ng/ml, but a mean TSAT of only 16.9 ± 2.5%, suggesting that this group had a lower iron use efficiency than the other groups. Therefore, it is considered necessary to prioritize the improvement of the iron use efficiency over iron administration in the treatment of anemia in HD patients.

To investigate the optimal s-ft level in the TSAT ≥20% group, in which the iron use efficiency was considered to be maintained, the prognosis was compared among three groups classified according to the s-ft levels, using the Kaplan-Meier method and Cox proportional hazards models. Both analyses showed a significantly superior prognosis in group 2. Analysis using the conventional Cox proportional hazards models revealed that in the TSAT ≥20% group, both excessively low and high s-ft levels were associated with a poor prognosis, as compared to

s-ft levels in the intermediate range. Moreover, TSAT and s-ft may be associated with variations of the doses of ESA and iron; thus, these may have been important confounders during the period of this study. To analyze these time-dependent confounders, we adopted the time-dependent Cox hazards model. The adjusted hazard ratio in the time-dependent model was positive for s-ft <30 ($p = 0.039$). Although a comparison of the results between the conventional and time-dependent models showed slight differences, both showed a good survival prognosis for group 2.

Although there were no significant differences, presumably because of the small number of patients, investigation of the causes of death revealed that heart failure tended to be more common in group 3 (10.4%) than that in group 2 (0%) as a cause of death, suggesting that death from heart failure could be reduced by decreasing the s-ft level. Ischemic changes due to arteriosclerosis seemed to account for the great majority of causes of heart failure. Drűeke et al. reported that oxidative stress is positively correlated with the s-ft levels and promotes arteriosclerosis in HD patients [9]. Moreover, Kiechi et al. also showed that the risk of arteriosclerosis increases with increasing s-ft levels [8]. Therefore, the avoidance of high s-ft levels could lead to reduced oxidative stress and inhibit the progression of arteriosclerosis. In contrast, the results of this study showed that the mortality was also high in group 1, even with low s-ft levels. Moreover, there were a broad range of causes of death in group 1, including heart failure, infection, malignant tumor, and myocardial infarction. Iron deficiency exacerbates heart failure [30], reduces immune response [12, 31], and induces carcinogenesis [32, 33]. Therefore, it would seem better to avoid excessively low s-ft levels.

The trend of increasing s-ft levels has been observed worldwide [34], but the amount of iron in the liver, which is a good index of excess iron, becomes excessive if the s-ft level is higher than 100–200 ng/ml in HD patients [35, 36]. Excess iron is said to promote endothelial dysfunction, cardiovascular lesions, immunological abnormalities, among pathological states [37], suggesting that the avoidance of excess iron and maintenance of iron at adequate levels to avoid iron deficiency-associated dysfunction led to a good prognosis of the patients in this study.

The presence of inflammation makes it difficult to achieve TSAT ≥20% and increases s-ft levels [25–27], suggesting that it is indispensable to lower inflammatory responses. Our data showed a median CRP level in our patient series of 0.2 mg/dl, indicating that the incidence of inflammatory events in our study was low.

Moreover, low serum CRP levels were also reported by Hasuike and Kuragano et al. [38, 39] who described that 'patients with s-ft levels of <100 ng/ml showed a good prognosis'.

Furthermore, in this study, we investigated the influence of TSAT and s-ft on the Hb level and showed that the Hb level was unaffected by TSAT, but decreased significantly with increasing s-ft levels. Usually, a decrease of s-ft and TSAT induces an Hb level in the iron deficiency range, but an increase of both parameters does not necessarily result in the elevation of Hb levels. Therapeutic intervention by ESA and iron modify the anemic and iron storage parameters in a complex manner, particularly in renal anemia. According to one previous report, there was no relationship between the Hb level and s-ft in the high serum s-ft level range that was induced by iron therapy [28]. However, at a lower range of s-ft levels, there appears to be a tendency for Hb to be higher in the group with lower s-ft levels (0–49 ng/ml) as compared with that in the group with higher s-ft levels [40]. It is hypothesized that there may be a relationship between reduced hematopoiesis and increased iron storage, suggesting invalid or inefficient iron usage in this group. In contrast, TSAT reflects the available amount of iron and has been defined as a marker of iron supplementation when its values are below 20%, which may have affected the survival ratio in this study. However, we showed that no significant correlation exists between Hb and TSAT levels. This finding was consistent with a previous report that showed the absence of any significant correlation between the Hb and TSAT in three groups, which was based on the TSAT value (<20%, 20–40%, and >40% [28]. Hb levels of patients in the TSAT <20% group were at a lower level because of the limited iron availability. However, we did not find that patients in the TSAT >20% group had a necessarily positive relationship with TSAT levels probably because the hematopoietic efficacy was not sufficient to utilize adequate amounts of iron. That is, Hb levels in HD patients may not be dependent only on the available iron storage but may be affected by the hematopoietic status under external erythropoietin treatment.

Data from this study are not sufficient to clarify the relationship between the Hb level and the conventional iron markers, s-ft and TSAT. Although we could not yet measure the data at the initiation of this study, measurement of hepcidin, one of the modulators of iron homeostasis, may have provided useful information to disclose the iron and Hb relationship in this study. In HD patients, the serum levels of Hep25 are positively correlated with the s-ft levels and tend to increase at lower s-ft levels than

that in healthy individuals [41]. Therefore, excessive increase of the s-ft levels may induce Hep25 and inhibit efficient use of iron, consequently inhibiting hematopoiesis. Data at the initiation of the study showed that both Hb and Ret were significantly lower in group 3, suggesting the reduced hematopoiesis despite the higher body iron levels in this group, as compared to the other groups. Moreover, analyses in the Japanese Dialysis Outcomes and Practice Patterns Study (DOPPS) phase II and III studies showed that Hb was the highest at s-ft levels of 0–49 ng/ml, decreased with an increase in the s-ft levels, and showing further significant decrease at s-ft levels of ≥499 ng/ml [40], consistent with our results. Taken together, excessive iron suppresses hematopoiesis and conversely, reduced erythropoiesis induces the increment of iron storage, mediated in part by hepcidin.

The patients in this study were cared for according to the guidelines of JSDT, except for the target s-ft levels; therefore, it is not easy to refer to or compare with the data from western countries. Longitudinal data collection from representative dialysis units of countries participating in DOPPS were used as reference and basal data for analyzing the study data. In particular, the data in the Japanese population reported by J-DOPPS are reliable and served as a basic reference for the analysis in this study. Hb levels at the initiation of this study were lower than those reported for western countries. However, DOPPS phase I study (1996–2001) showed that the mean Hb was 9.7 g/dl in Japan [40] and the values of Hb in this study were higher than the average levels in Japan despite the low s-ft levels. The values of Hb were low, probably because no guideline for the management of anemia had been published in Japan when this study was initiated in May 2002. Subsequently, guidelines for the treatment of anemia were published by JSDT in 2004, setting a goal for Hb of 10–11 g/dl [42], which is lower than that recommended in western countries. This may be because, in Japan, blood samples are collected with the patient in the recumbent position at the beginning of the week when dialysis is initiated, while in western countries, blood samples are collected with the patient in the sitting position in the middle of the dialysis week. Although numerical values were not shown, Hb ≥10 g/dl was achieved in approximately 80–90% of the patients despite low s-ft levels in the second half of this study, suggesting that Hb can be well maintained even with low s-ft levels.

In the present study, we found that the 10-year survival rate was extremely high in the TSAT ≥20% group and the group with s-ft levels in the range of 30–80 ng/ml, and that the s-ft levels affected the prognosis in HD patients receiving ESA. Although considerably lower than that in western countries, the upper-limit s-ft level of 80 ng/ml seems reasonable, considering that abnormalities associated with excess iron appear in polymorphonuclear leukocytes and in the liver at s-ft levels of >100 ng/ml in HD patients. It seems necessary to maintain s-ft levels at a much lower threshold of ≥30 ng/ml to avoid iron deficiency during treatment with ESA, because a higher amount of iron is used. Furthermore, the results also showed that it is better to maintain lower s-ft levels during anemia treatment.

Our findings suggest that the optimal s-ft level in the HD patients receiving ESA may be considerably lower than that recommended currently. There is a possibility that high s-ft levels induced by excessive iron storage may impede efficient hematopoiesis and also affect patient prognosis. Because the s-ft level that was associated with the best prognosis was near the levels observed during iron deficiency, detailed control of the s-ft level would seem necessary.

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Disclosure Statement

All the authors declared no conflict of interests.

References

1 Pisoni RL, Bragg-Gresham JL, Young EW, et al: Anemia management and outcomes from 12 countries in the Dialysis Outcomes and Practice Patterns Study (DOPPS). Am J Kidney Dis 2004;44:94–111.

2 Tsubakihara Y, Nishi S, Akiba T, et al: 2008 Japanese Society for Dialysis Therapy: guidelines for renal anemia in chronic kidney disease. Ther Apher Dial 2010;14:240–275.

3 Ali MA, Luxton AW, Walker WH: Serum ferritin concentration and bone marrow iron stores: a prospective study. Can Med Assoc J 1978;118:945–946.

4 Punnonen K, Irjala K, Rajamaki A: Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 1997;89:1052–1057.

5 Mast AE, Blinder MA, Gronowski AM, et al: Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. Clin Chem 1998;44:45–51.

6 Eschbach JW, Egrie JC, Downing MR, et al: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. N Engl J Med 1987;316:73–78.

7 Reis KA, Guz G, Ozdemir H, et al: Intravenous iron therapy as a possible risk factor for atherosclerosis in end-stage renal disease. Int Heart J 2005;46:255–264.
8 Kiechl S, Willeit J, Egger G, et al: Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. Circulation 1997;96:3300–3307.
9 Drüeke T, Witko-Sarsat V, Massy Z, et al: Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. Circulation 2002;106:2212–2217.
10 Klipstein-Grobusch K, Koster JF, Grobbee DE, et al: Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam Study. Am J Clin Nutr 1999;69:1231–1236.
11 Brookhart MA, Freburger JK, Ellis AR, et al: Infection risk with bolus versus maintenance iron supplementation in hemodialysis patients. J Am Soc Nephrol 2013;24:1151–1158.
12 Sengoelge G, Sunder-Plassmann G, Hörl WH: Potential risk for infection and atherosclerosis due to iron therapy. J Ren Nutr 2005; 15:105–110.
13 Kato J, Miyanishi K, Kobune M, Nakamura T, et al: Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol 2007;42:830–836.
14 Kobayashi H, Yamada Y, Kanayama S, et al: The role of iron in the pathogenesis of endometriosis. Gynecol Endcrinol 2009;25:39–52.
15 Roob JM, Khoschsorur G, Tiran A, et al: Vitamin E attenuates oxidative stress induced by intravenous iron in patients on hemodialysis. J Am Soc Nephrol 2000;11:539–549.
16 Anraku M, Kitamura K, Shintomo R, et al: Effect of intravenous iron administration frequency on AOPP and inflammatory biomarkers in chronic hemodialysis patients: a pilot study. Clin Biochem 2008;41:1168–1174
17 Park CH, Valore EV, Waring AJ, et al: Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001;276:7806–7810.
18 Ganz T: Hepcidin – a regulator of intestinal iron absorption and iron recycling by macrophages. Best Pract Res Clin Haematol 2006; 108:1381–1387.
19 KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease. Am J Kidney Dis 2006;47(suppl 3):S58–S70.
20 Lacatelli F, Covic A, Eckardt KU, et al: Anaemia management in patients with chronic kidney disease: a position statement by the Anaemia Working Group of European Renal Best Practice. Nephrol Dial Transplant 2009; 24:348–354.
21 Otaki Y, Nakanishi T, Hasuike Y, et al: Defective regulation of iron transporters leading to iron excess in the polymorphonuclear leukocytes of patients on maintenance hemodialysis. Am J Kidney Dis 2004;43:1030–1039.
22 Patruta SI, Edlinger R, Sunder-Plassmann G, et al: Neutrophil impairment associated with iron therapy in hemodialysis patients with functional iron deficiency. J Am Soc Nephrol 1998;9:655–663.
23 Skikne BS, Cook JD: Effect of enhanced erythropoiesis on iron absorption. J Lab Clin Med 1992;120:746–751.
24 Major A, Mathez-Loic F, Rohling R, et al: The effect of intravenous iron on the reticulocyte response to recombinant human erythropoietin. Br J Haematol 1997;98:292–294.
25 Wish JB: Assessing iron status: beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol 2006;1(suppl 1):S4–S8.
26 Goodnough LT, Nemeth E, Ganz T: Detection, evaluation, and management of iron-restricted erythropoiesis. Blood 2010;116: 4754–4761.
27 Pasricha SR, Flecknoe-Brown SC, Allen KJ, et al: Diagnosis and management of iron deficiency anaemia: a clinical update. Med J Aust 2010;193:525–532.
28 Koo HM, Kim CH, Doh FM, et al: The relationship of initial transferrin saturation to cardiovascular parameters and outcomes in patients initiating dialysis. PLoS One 2014;9:e87231.
29 Worwood M: Ferritin. Blood Rev 1990;4:259–269.
30 Jankowska EA, von Haehling S, Anker SD, et al: Iron deficiency and heart failure: diagnostic dilemmas and therapeutic perspectives. Eur Heart J 2013;34:816–829.
31 Pagani A, Nai A, Corna G, et al: Low hepcidin accounts for the proinflammatory status associated with iron deficiency. Blood 2011;118: 736–746.
32 Noto JM, Gaddy JA, Lee JY, et al: Iron deficiency accelerates Helicobacter pylori-induced carcinogenesis in rodents and humans. J Clin Invest 2013;123:479–492.
33 Jian J, Yang Q, Shao Y, et al: A link between premenopausal iron deficiency and breast cancer malignancy. BMC Cancer 2013;13:307.
34 Bailie GR, Larkina M, Goodkin DA, et al: Variation in intravenous iron use internationally and over time: the Dialysis Outcomes and Practice Patterns Study (DOPPS). Nephrol Dial Transplant 2013;28:2570–2579.
35 Canavese C, Bergamo D, Ciccone G, et al: Validation of serum ferritin values by magnetic susceptometory in predicting iron overload in dialysis patients. Kidney Int 2004;65:1091–1098.
36 Rostoker G, Griuncelli M, Loridon C, et al: Hemodialysis-associated hemosiderosis in the era of erythropoiesis-stimulating agents: a MRI study. Am J Med 2012;125:991–999.
37 Van Buren P, Velez RL, Vaziri ND, et al: Iron overdose: a contributor to adverse outcomes in randomized trials of anemia correction in CKD. Int Urol Nephrol 2012;44:499–507.
38 Hasuike Y, Nonoguchi H, Tokuyama M, et al: Serum ferritin predicts prognosis in hemodialysis patients: the Nishinomiya study. Clin Exp Nephrol 2010;14:349–355.
39 Kuragano T, Matsumura O, Matsuda A, et al: Association between hemoglobin variability, serum ferritin levels, and adverse events/mortality in maintenance hemodialysis patients. Kidney Int 2014;86:845–854.
40 Akizawa T, Pisoni RL, Akiba T, et al: Japanese haemodialysis anaemia management practices and outcomes (1999–2006): results from the DOPPS. Nephrol Dial Transplant 2008; 23:3643–3653.
41 Tomosugi N, Kawabata H, Wakatabe R, et al: Detection of serum hepcidin in renal failure and inflammation by using ProteinChip System. Blood 2006;108:1381–1387.
42 Gejyo F, Saito A, Akizawa T, et al; Japanese Society for Dialysis Therapy: 2004 Japanese Society for Dialysis Therapy guidelines for renal anemia in chronic hemodialysis patients. Ther Apher Dial 2004;8:443–459.