



Impact of Iron Deficiency on Peripheral Artery Disease After Endovascular Therapy

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Background: Despite advances in endovascular therapy (EVT), peripheral artery disease (PAD) is a public health problem associated with high cardiovascular mortality. Iron deficiency (ID) is associated with poor clinical outcome in patients with heart disease, but whether ID is associated with the severity and clinical outcome of PAD remains unclear.

Methods and Results: A total of 449 patients with PAD who received EVT and who had iron and red blood cell measurement were enrolled. ID was defined as transferrin saturation (TSAT) <20%, based on a previous report. TSAT and hemoglobin decreased with deteriorating Fontaine class. During a median follow-up period of 1,064 days, 71 major adverse cardiovascular and leg events (MACLE) and 47 major adverse cardiovascular events (MACE) were noted. All patients were divided into 2 groups based on the presence of ID. On Kaplan-Meier analysis, patients with ID had higher rates of MACE and MACLE than those without. On multivariate Cox proportional hazard regression analysis, TSAT and hemoglobin were independently associated with MACLE. Addition of TSAT to the known risk factors significantly improved the net reclassification index and integrated discrimination improvement.

Conclusions: ID, as assessed by TSAT, was associated with the severity and clinical outcome of PAD, indicating that it could be a therapeutic target.

Key Words: Clinical outcome; Iron deficiency; Peripheral artery disease

Peripheral artery disease (PAD) is an arterial occlusive disease of the lower limb arteries associated with increased morbidity. Despite advances in endovascular therapy (EVT), PAD remains an important medical issue with an increasing prevalence and high all-cause and cardiovascular mortality rates.¹⁻⁴ Therefore, identifying patients with PAD at an early stage is increasingly important. Further, high-risk patients with PAD should be stratified according to risk.

Iron deficiency (ID) is the most common nutritional deficiency worldwide.⁵ Iron is a metabolically active and multifunctional micronutrient, which plays a pivotal role in oxygen transport, oxygen storage, cardiac and skeletal muscle metabolism, protein and ribonucleic acid synthesis, and mitochondrial function.⁶ Iron balance is related to cardiovascular disease pathophysiology.⁷ An excessive accumulation of iron has been suggested to be a risk factor for cardiovascular disease, and a large increase in iron content has been observed in the arterial plaque.⁸ The iron and atherosclerosis study (FeAST) demonstrated that reduction of stored iron using phlebotomy failed to improve

clinical outcome in patients with PAD, despite the beneficial effect of phlebotomy to reduce all-cause mortality, in particular cancer deaths, in patients aged between 43 and 61.^{9,10} In contrast, a cross-sectional study showed that the prevalence of anemia and ID was higher in patients with critical limb ischemia (CLI) than in those with claudication.¹¹ ID has been associated with sympathetic nervous activation, anemia, and left ventricular remodeling with resultant development of heart failure (HF).¹²⁻¹⁴ Similarly, the importance of iron status in the skeletal muscle has been documented in sports medicine.^{15,16} The effects of ID on the clinical outcomes in PAD, however, remains to be determined.

The aim of this study was therefore to examine whether ID, as assessed on transferrin saturation (TSAT), is associated with the severity and clinical outcome of PAD.

Methods

Subjects

This was a prospective observational study of 449 patients

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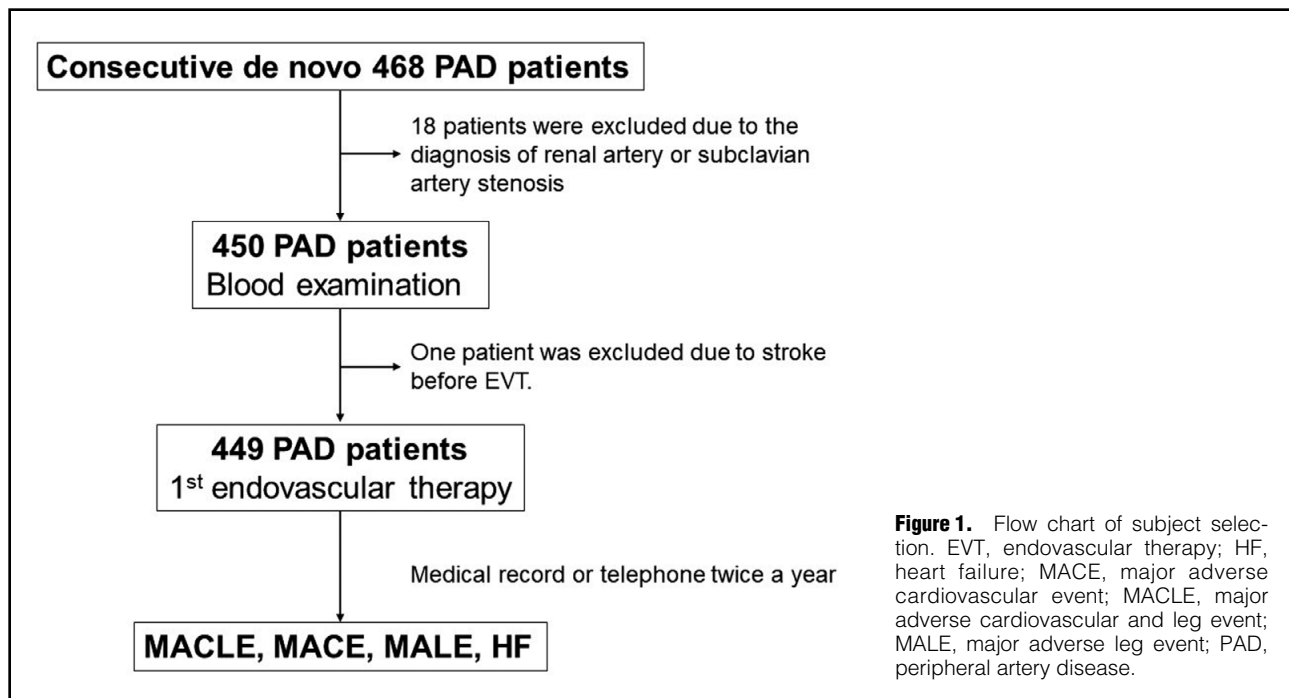


Figure 1. Flow chart of subject selection. EVT, endovascular therapy; HF, heart failure; MACE, major adverse cardiovascular event; MACLE, major adverse cardiovascular and leg event; MALE, major adverse leg event; PAD, peripheral artery disease.

who were admitted to hospital for their first PAD treatment. A flow chart of subject selection is shown in **Figure 1**. PAD was diagnosed based on ankle brachial index (ABI) <0.9 and peripheral artery stenosis or occlusion on computed tomography angiography. Experienced cardiologists performed EVT according to the recommendations of the Trans-Atlantic Inter-Society Consensus II (TASC II) guidelines.¹⁷ Optimized medical therapy was given independently by physicians based on symptom improvement. The exclusion criteria were acute coronary syndrome (ACS) in the 3 months prior to admission and malignant disease, such as hematological malignancy. Demographic and clinical data, including age, sex, smoking history, cardiovascular risk factors, ABI, and medication, were collected from the patients' medical records and through interviews.

Ethics

The study protocol was approved by the institutional ethics committee of Yamagata University School of Medicine (no. 395), and all participants provided written informed consent. All procedures were performed in accordance with the Helsinki Declaration principles.

Definitions

Hypertension was defined as systolic blood pressure (SBP) ≥ 130 mmHg, diastolic blood pressure (DBP) ≥ 80 mmHg, or use of antihypertensive medication.¹⁸ Hyperlipidemia was defined as total cholesterol ≥ 220 mg/dL, triglyceride ≥ 150 mg/dL, or use of anti-hyperlipidemic medication. Diabetes mellitus (DM) was defined as fasting blood sugar (FBS) ≥ 126 mg/dL and glycosylated hemoglobin A1c (HbA1c) $\geq 6.5\%$ (National Glycohemoglobin Standardization Program).

Biochemistry Markers

Blood samples were obtained early in the morning before

the first EVT. Iron parameters, including serum iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), serum ferritin, and TSAT, were measured. TSAT was calculated using the following formula: $TSAT = \text{serum iron} \times 100 / TIBC$. ID was defined as $TSAT < 20\%$, based on the previous report.¹⁹

Moreover, red blood cell (RBC) parameters, including RBC count, hemoglobin (Hb), hematocrit (Hct), mean cell Hb (MCH), mean cell Hb concentration (MCHC), mean cell volume (MCV), and red cell distribution width (RDW), were measured.

Severity of PAD

The severity of PAD was determined using Fontaine class according to the TASC II guidelines.¹⁷ Briefly, Fontaine classes II, III, and IV were defined as intermittent claudication, rest pain, and CLI, respectively.

Endpoints and Follow-up

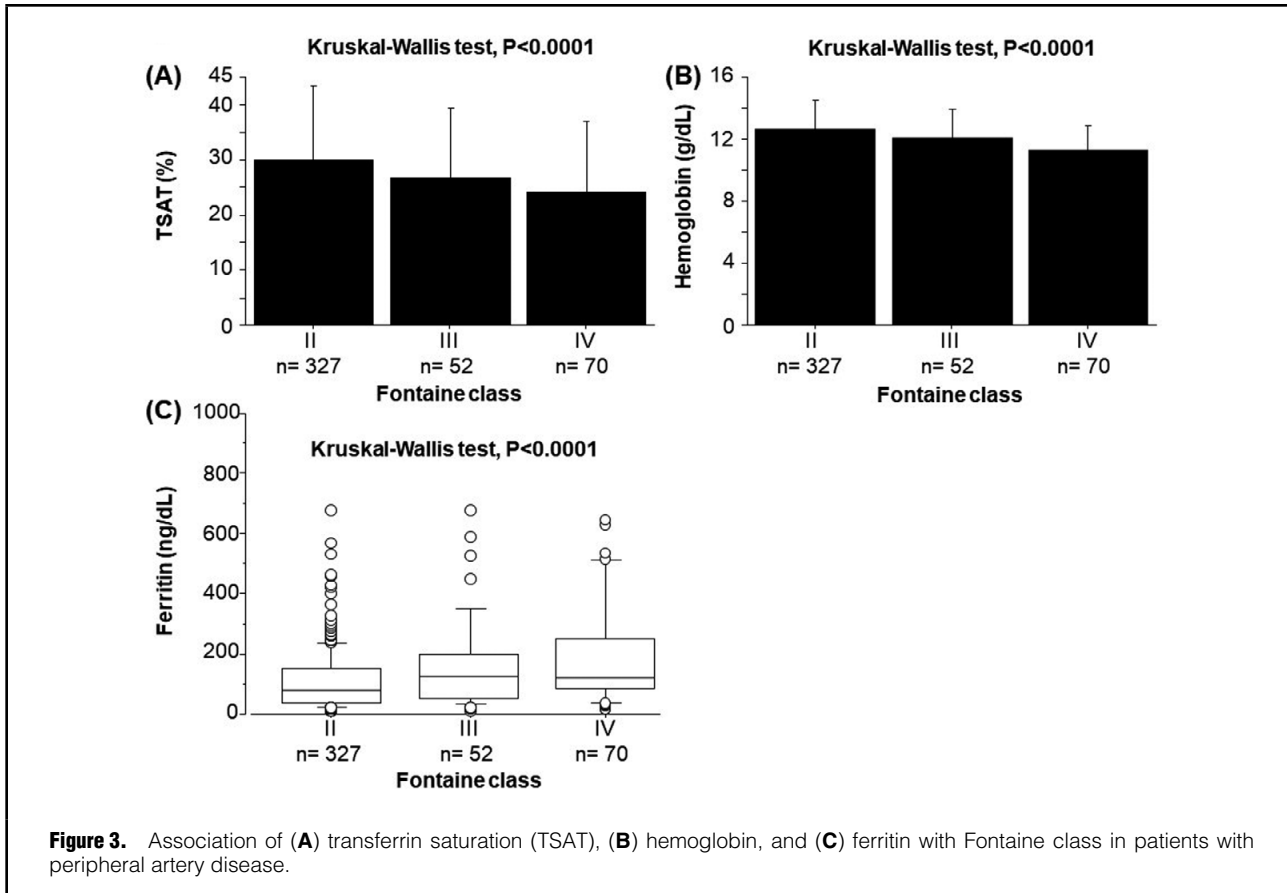
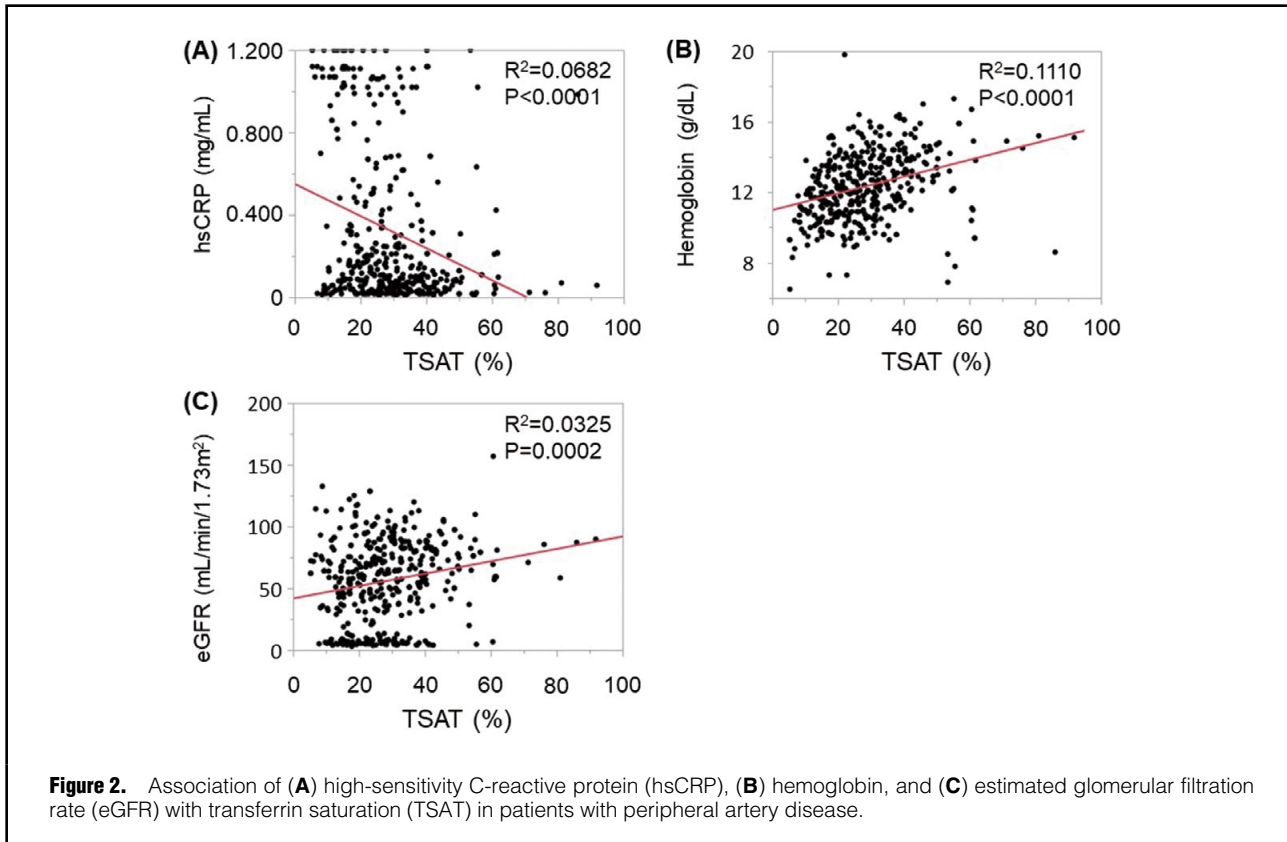
All participants were prospectively followed up via telephone interview or medical records, twice a year for a median period of 1,064 days (IQR, 452–1,490 days; longest follow-up, 1,825 days). The primary endpoints were major adverse cardiovascular and leg events (MACLE), including cardiovascular mortality; rehospitalization due to cardiovascular disease, such as ACS and HF; and major leg events, such as major and minor amputation. The secondary endpoint was major adverse cardiovascular events (MACE), including cardiovascular death and rehospitalization due to cardiovascular disease such as ACS and HF.

Statistical Analysis

Continuous data are expressed as mean \pm SD or median. Continuous and categorical variables were compared using the t-test or chi-squared test, respectively. Data that were not normally distributed were compared using the Mann-Whitney U-test. Survival curves were constructed using the

Table 1. PAD Patient Clinical Characteristics vs. Presence of ID (n=449)				
Variables	All patients (n=449)	ID (-) (n=322)	ID (+) (n=127)	P value
Age (years)	74±9	74±9	73±9	0.3667
Men	359 (80)	267 (83)	92 (72)	0.0146
Hypertension	353 (79)	257 (80)	96 (75)	0.3302
SBP (mmHg)	124±13	125±14	124±13	0.3407
DBP (mmHg)	68±11	67±12	69±11	0.3661
Diabetes mellitus	209 (47)	142 (44)	67 (53)	0.0979
Hyperlipidemia	242 (54)	176 (55)	66 (52)	0.6067
Previous IHD	148 (33)	95 (30)	53 (42)	0.0140
Hemodialysis	94 (21)	60 (19)	34 (27)	0.0607
CKD	220 (49)	146 (45)	74 (58)	0.0135
Fontaine II/III/IV	327/52/70	250/33/39	77/19/31	0.0012
Endovascular therapy				
Iliac artery	269 (60)	199 (62)	70 (55)	0.1946
Femoropopliteal artery	259 (58)	185 (57)	74 (58)	0.8750
Tibial or peroneal artery	82 (18)	49 (15)	33 (26)	0.0096
Stent	392 (87)	288 (89)	104 (82)	0.0356
Pre-treatment ABI	0.58±0.18	0.59±0.17	0.55±0.21	0.1037
Post-treatment ABI	0.87±0.19	0.88±0.19	0.85±0.20	0.1972
Biochemistry				
RBC (10 ⁴ /μL)	4.1±0.6	4.1±0.6	3.9±0.6	0.0012
Hb (g/dL)	12.3±1.8	12.7±1.9	11.4±1.6	<0.0001
Hct (mg/dL)	38±5	39±6	36±5	<0.0001
MCV (fL)	94±5	94±5	92±6	<0.0001
MCH (pg)	30±2	31±2	29±2	<0.0001
MCHC (%)	33±1	33±1	32±1	<0.0001
RDW (%)	14.1±1.4	13.9±1.2	14.6±1.9	<0.0001
Fe (μg/mL)	78±30	93±35	40±15	<0.0001
TIBC (μg/mL)	276±60	272±53	284±76	0.0728
UIBC (μg/mL)	197±54	179±48	243±67	0.0002
Ferritin (ng/mL)	91 (44–165)	105 (56–167)	59 (24–143)	0.1348
TSAT (%)	28.7±13.3			
hsCRP (mg/dL)	0.124 (0.046–0.502)	0.101 (0.042–0.313)	0.256 (0.064–1.063)	<0.0001
eGFR (mL/min/1.73 m ²)	56±36	59±38	50±35	0.0227
FBS (mg/dL)	118±39	116±35	125±46	0.0270
Medication				
Aspirin	314 (70)	221 (69)	93 (73)	0.3356
Clopidogrel	312 (69)	229 (71)	83 (65)	0.2355
Cilostazol	132 (29)	102 (32)	30 (24)	0.0872
Vitamin K antagonist	77 (17)	51 (16)	26 (21)	0.2473
ACEI and/or ARB	267 (59)	198 (62)	69 (54)	0.1654
Statins	237 (53)	170 (53)	67 (53)	0.9940
Iron supplementation	28 (6)	12 (4)	16 (13)	0.0009
ESA	73 (26)	47 (15)	26 (20)	0.1356
Aspirin+Clopidogrel	216 (48)	155 (48)	61 (48)	0.9840
Clopidogrel+Cilostazol	63 (14)	52 (16)	11 (9)	0.0396
Aspirin+Cilostazol	54 (12)	40 (12)	14 (11)	0.6815

Data given as mean±SD, n (%) or median (IQR). ABI, ankle brachial index; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, glomerular filtration rate; ESA, erythropoietin-stimulating agents; FBS, fasting blood sugar; Fe, iron; Hb, hemoglobin; Hct, hematocrit; hsCRP, high-sensitivity C-reactive protein; ID, iron deficiency; IHD, ischemic heart disease; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; PAD, peripheral artery disease; RBC, red blood cells; RDW, red cell distribution width; SBP, systolic blood pressure; TIBC, total iron binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron binding capacity.



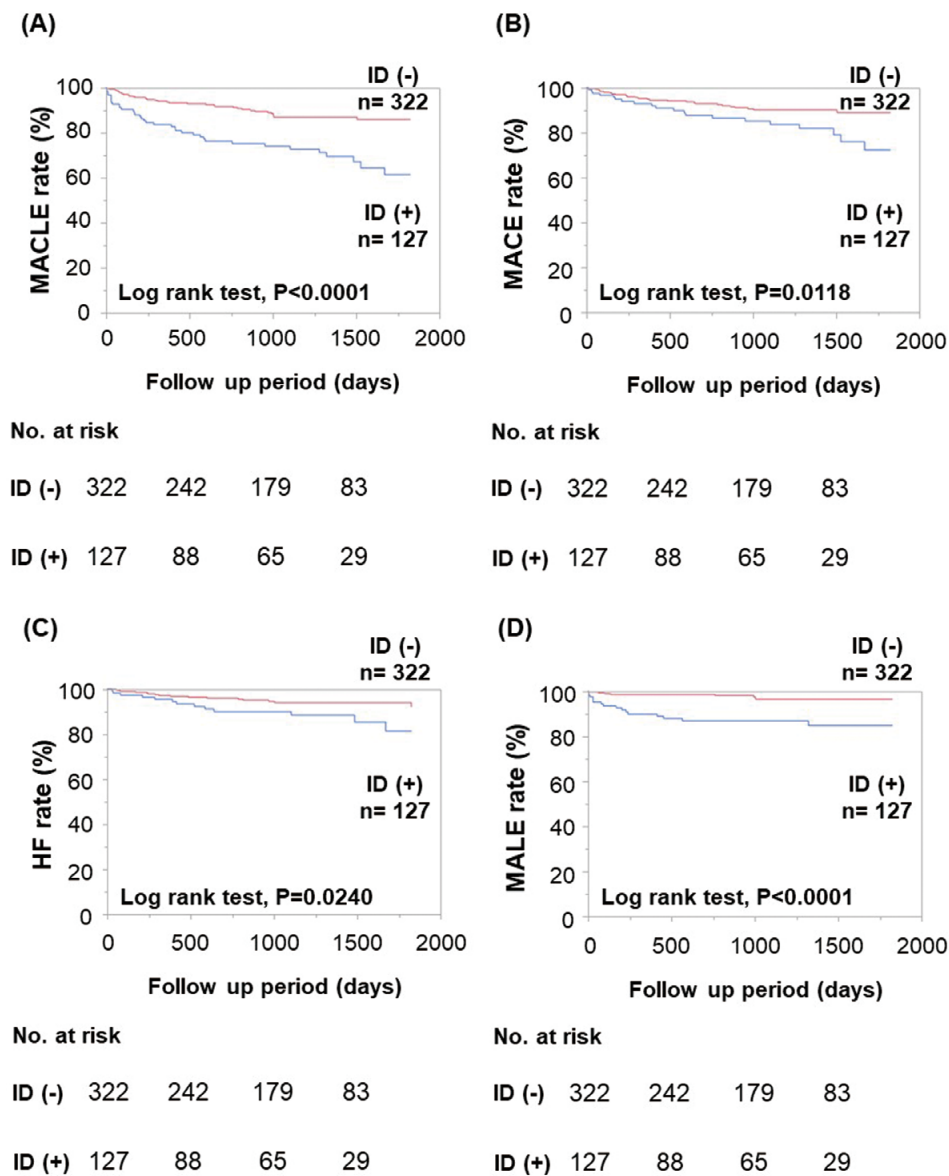


Figure 4. Kaplan-Meier analysis for (A) major adverse cardiovascular and leg events (MACLE), (B) major adverse cardiovascular events (MACE), (C) heart failure (HF), and (D) major adverse leg events (MALE) in peripheral artery disease patients with and without iron deficiency (ID).

Kaplan-Meier method and compared using the log-rank test. A Cox proportional hazard analysis was performed to identify the independent predictors of MACLE. Significant predictors ($P < 0.05$) on univariate Cox proportional hazard regression analysis were screened using the Bayesian method. The selected predictors were entered into multivariate analysis. Multicollinearity was checked using the variance inflation factor. Multivariate analysis was performed to evaluate the independent predictors of MACLE. $P < 0.05$ was considered statistically significant.

The receiver operating characteristics curve for MACLE was constructed and C index was measured. We calculated the net reclassification index (NRI) and integrated discrimination improvement (IDI) to measure the quality of improvement for correct reclassification after adding TSAT

to the current model. All statistical analyses were performed using JMP version 12 (SAS Institute, Cary, NC, USA) and R 3.2.4 with additional packages, including Rcmdr, Epi, pROC, and PredictABEL.

Results

Baseline PAD Patient Characteristics

Patient baseline characteristics are listed in **Table 1**. A total of 359 patients (80%) were men. Hypertension, DM, and hyperlipidemia were identified in 353 (79%), 209 (47%), and 242 patients (54%), respectively. A total of 148 patients (33%) had previous ischemic heart disease (IHD), and 94 (21%) underwent hemodialysis. There were 327 and 52 patients in Fontaine classes II and III, respectively, and the

Table 2. Univariate and Multivariate Predictors of MACLE in PAD Patients						
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Age (1-year increase)	1.026	0.999–1.056	0.0528	1.022	0.993–1.053	0.1292
Gender	1.680	0.979–2.774	0.0591	1.535	0.882–2.577	0.1148
Hypertension	1.548	0.893–2.572	0.1161			
SBP (1-SD increase)	0.946	0.716–1.248	0.6901			
DBP (1-SD increase)	0.928	0.683–1.252	0.6282			
Diabetes mellitus	2.138	1.326–3.529	0.0017			
Previous IHD	1.887	1.178–3.009	0.0086	1.812	1.093–2.993	0.0201
Hemodialysis	2.205	1.326–3.572	0.0028	1.233	0.675–2.191	0.4836
CLI	5.387	3.329–8.618	<0.0001	4.074	2.358–6.955	<0.0001
Pre-treatment ABI (1-SD increase)	0.714	0.564–0.912	0.0072			
Post-treatment ABI (1-SD increase)	0.688	0.542–0.884	0.0040			
RBC (1-SD increase)	0.557	0.453–0.734	<0.0001			
Hb (1-SD increase)	0.549	0.441–0.685	<0.0001	0.712	0.534–0.948	0.0204
Hct (1-SD increase)	0.568	0.451–0.713	<0.0001			
MCV (1-SD increase)	0.952	0.755–1.213	0.6868			
MCH (1-SD increase)	0.801	0.671–0.993	0.0432			
MCHC (1-SD increase)	0.757	0.658–0.899	0.0025			
RDW (1-SD increase)	1.362	1.161–1.555	0.0004			
Fe (1-SD increase)	0.483	0.373–0.612	<0.0001			
TIBC (1-SD increase)	0.700	0.550–0.889	0.0034			
UIBC (1-SD increase)	1.120	0.886–1.401	0.3306			
Ferritin (1-SD increase)	1.135	0.909–1.338	0.2351			
TSAT (1-SD increase)	0.470	0.344–0.629	<0.0001	0.606	0.437–0.843	0.0029
hsCRP (1-SD increase)	1.550	1.282–1.863	<0.0001	1.007	0.814–1.247	0.9430
eGFR (1-SD increase)	0.731	0.562–0.947	0.0187			
FBS (1-SD increase)	1.365	1.108–1.652	0.0043	1.203	0.963–1.504	0.1033

CLI, critical limb ischemia; MACLE, major adverse cardiovascular and leg event. Other abbreviations as in Table 1.

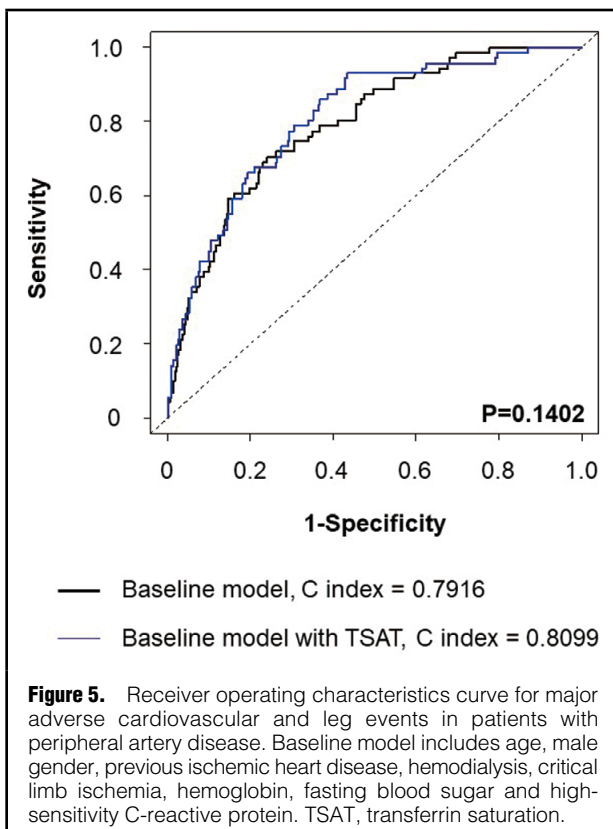


Figure 5. Receiver operating characteristics curve for major adverse cardiovascular and leg events in patients with peripheral artery disease. Baseline model includes age, male gender, previous ischemic heart disease, hemodialysis, critical limb ischemia, hemoglobin, fasting blood sugar and high-sensitivity C-reactive protein. TSAT, transferrin saturation.

remaining 70 patients belonged to Fontaine class IV. Mean iron, Hb, and TSAT were $78 \mu\text{g/mL}$, 12.3 g/dL , and 28.7% , respectively. The overall success rate of the first EVT was 95% , and in the subgroup of CLI patients it was 91% . On simple linear analysis TSAT was inversely correlated with high-sensitivity C-reactive protein (hsCRP) and proportionally correlated with Hb and estimated glomerular filtration rate (eGFR; **Figure 2**). TSAT and Hb were decreased with worsening Fontaine class (**Figure 3A,B**). In contrast, serum ferritin was increased with worsening Fontaine class (**Figure 3C**).

Clinical Patient Characteristics and ID Status

ID was identified in 127 patients (28%) with PAD. Patients with ID had more severe disease according to Fontaine class and had a higher prevalence of male gender, previous IHD, tibial or peroneal artery stenosis, or occlusion compared with those without ID (**Table 1**). Patients with ID also had lower RBC, Hb, Hct, MCV, MCH, MCHC, iron, TIBC, and eGFR, and higher RDW, hsCRP and FBS than those without ID. Differences in age; prevalence of hypertension, hyperlipidemia, and hemodialysis; SBP and DBP; pre- and post-treatment ABI; UIBC and serum ferritin; and medication use excluding iron supplementation and combination of clopidogrel and cilostazol were not significant between patients with and without ID.

TSAT and Clinical Outcome

During the follow-up period, 47 MACE, 24 major adverse leg events (MALE), and 71 MACLE were observed. On

Table 3. Model Improvement on Addition of TSAT to Prediction of MACLE in PAD Patients			
	C index	NRI (95% CI, P-value)	IDI (95% CI, P-value)
Baseline model	0.7916	Ref.	Ref.
+TSAT	0.8099 P=0.1402	0.3383 (0.0899–0.5866, 0.0076)	0.025 (0.0061–0.0433, 0.0093)

Baseline model includes age, gender, previous IHD, CLI, hemodialysis, Hb, FBS and hsCRP. CI, confidence interval; IDI, integrated discrimination improvement; NRI, net reclassification index. Other abbreviations as in Tables 1,2.

Kaplan-Meier analysis, patients with ID had higher rates of MACLE, MACE, HF, and MALE compared with those without ID (**Figure 4**).

We performed univariate and multivariate Cox proportional hazard regression analyses to examine the effects of TSAT on MACLE in patients with PAD. On univariate Cox proportional hazard regression analysis TSAT was significantly associated with MACLE in patients with PAD. Moreover, DM, previous IHD, hemodialysis, CLI, pre- and post-treatment ABI, RBC, Hb, Hct, MCH, MCHC, RDW, iron, TIBC, hsCRP, eGFR and FBS were also significantly related with MACLE (**Table 2**). On multivariate Cox proportional hazard regression analysis TSAT was an independent predictor of MACLE after adjusting for age, male gender, previous IHD, hemodialysis, CLI, Hb, hsCRP and FBS (**Table 2**).

Addition of Serum Iron to Predict MACLE: Improvement of Reclassification

We evaluated C index, NRI and IDI to examine whether prediction capacity was improved by adding TSAT to the basic predictors, such as age, male gender, previous IHD, hemodialysis, CLI, Hb, FBS and hsCRP. There was no significant difference in C index between the baseline model with and without TSAT (**Figure 5**), but NRI and IDI significantly improved following the addition of TSAT to the basic predictors (NRI, 0.31383; 95% CI: 0.0899–0.5866; P=0.0076; IDI, 0.025; 95% CI: 0.0061–0.0433; P=0.0093; **Table 3**).

Discussion

The main findings of this study are as follows: (1) TSAT was correlated with hsCRP, Hb, and eGFR; (2) TSAT decreased with advancing Fontaine class; (3) ID, as assessed by TSAT, was identified in 127 patients (28%) with PAD; (4) on Kaplan-Meier analysis, patients with ID had higher rates of MACLE, MACE, HF, and MALE compared with those without ID; (5) on multivariate analysis TSAT was significantly associated with MACLE after adjusting for confounding risk factors; and (6) NRI and IDI were significantly improved by adding TSAT to the established risk factors.

The goal of PAD treatment includes 2 important issues: limb salvage and cardiovascular disease prevention. Therefore, skeletal and cardiac muscle functions are a potential therapeutic target to achieve these goals. The iron level inside the body is distributed in the Hb (66%), tissue ferritin (27%), and circulation.²⁰ The skeletal and cardiac muscles are sensitive to ID due to their high energy demand.²¹ ID is classically defined as low ferritin (<100ng/mL) or normal ferritin and low TSAT (<20%) in chronic disease.¹⁴ This criterion, however, has never been

validated to date. Recently, ID in HF was redefined, and the usefulness of TSAT, but not serum ferritin, was shown.¹⁹ Given that serum ferritin level increases in response to inflammation,²² assessing tissue ferritin in patients with PAD would be inappropriate.

PAD Severity and TSAT

Serum iron is affected by several factors, such as anemia, kidney function, chronic disease, malnutrition, and inflammation.^{22,23} Similarly, TSAT is also related to inflammation, anemia, and kidney function in patients with PAD. Notably, TSAT is closely associated with PAD severity. TSAT is a feasible marker for the amount of iron available for high energy-demanding tissues, such as skeletal and cardiac muscles.²⁴ TSAT <20% was identified in 28% of patients with PAD, suggesting that ID was common in these patients. PAD is considered a malnutrition inflammation syndrome accompanied with malnutrition and sarcopenia.^{25,26} Given that iron micronutrient insufficiency can occur easily due to low dietary intake and that inflammation inhibits iron absorption through hepcidin induction,²⁷ patients with PAD are presumably at high risk for ID. Considering the role of iron in the skeletal muscle, ID may result in decreased physical activity in patients with PAD.

Clinical Outcome and TSAT

PAD increases the risk for cardiovascular disease and mortality. The American College of Cardiology/American Heart Association guidelines regard PAD as stage A HF. Abnormal iron status, notably TSAT, was related to exercise capacity and survival independently of anemia in patients with HF.²⁸ In accordance with a previous report, we showed that TSAT was significantly associated with MACLE independent of Hb, indicating the possibility that tissue iron metabolism contributes to poor prognosis in patients with PAD. In the present study TSAT was also closely associated with MACE and HF in patients with PAD and served as a means of identifying high-risk patients.

Given that this was a prospective observational study, we could not determine the causal relationship between ID and PAD severity and clinical outcome. One possibility may be the mitochondria dysfunction caused by ID in cardiac and skeletal muscles.^{29,30} Another explanation is that comorbidities or the underlying pathophysiology of ID in patients with PAD may worsen the PAD severity and clinical outcome. In this study, patients with ID had inflammation, anemia, and kidney dysfunction, which are established risks for poor clinical outcome in patients with PAD.^{31–34} Importantly, we showed that NRI and IDI were improved by adding TSAT, indicating that TSAT could provide additional information to the existing confounding

risk factors. Therefore, TSAT is a feasible marker for MACLE and MACE in patients with PAD.

Niels et al showed that TSAT, but not serum ferritin, is the most accurate surrogate marker for tissue ID using bone marrow iron staining, and TSAT-guided intra venous iron supplementation improved the clinical outcomes in patients with HF.^{19,35} Future studies are required to clarify the beneficial effect of TSAT-guided i.v. iron supplementation on the clinical outcome in patients with PAD.

Study Limitations

This study had several limitations. First, the precise mechanism by which ID initiates or accelerates atherosclerosis was not identified because this was a prospective, observational study. Second, bone marrow biopsy was not performed to diagnose ID because it is invasive and painful. Finally, the subject group was small; thus, further studies with larger populations are needed to determine the abnormal cut-off of TSAT in patients with PAD.

Conclusions

We have shown for the first time that TSAT, a useful marker for ID in chronic disease, is associated with the severity and clinical outcome of PAD. ID could potentially be a therapeutic target and useful marker for clinical outcome, specifically in tracking the cardiovascular health status in patients with PAD. A prospective randomized controlled trial should be conducted to determine whether iron supplementation improves clinical outcome in patients with PAD and ID.

Disclosures

The authors declare no conflicts of interest.

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