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RESEARCH ARTICLE

High soluble transferrin receptor in patients with heart failure: a measure of iron deficiency and a strong predictor of mortality

Radoslaw Sierpinski[1,2], Krystian Josiak[3,4], Tomasz Suchocki[5], Katarzyna Wojtas-Polc[3,6], Grzegorz Mazur[7], Aleksandra Butrym[7], Piotr Rozentryt[8], Peter van der Meer[9], Josep Comin-Colet[10,11], Stephan von Haehling[12], Wojciech Kosmala[4,13], Monika Przewlocka-Kosmala[4,13], Waldemar Banasiak[6], Jolanta Nowak[8], Adriaan A. Voors[9], Stefan D. Anker[12], John G.F. Cleland[14], Piotr Ponikowski[3,4], and Ewa A. Jankowska[3,4]*

[1]Medical Research Agency, Warsaw, Poland; [2]Collegium Medicum, Cardinal Wyszyński University in Warsaw, Warsaw, Poland; [3]Department of Heart Diseases, Wroclaw Medical University, Wroclaw, Poland; [4]Centre for Heart Diseases, University Hospital, Wroclaw, Poland; [5]Biostatistics Group, Department of Genetics, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland; [6]Department of Cardiology, Military Hospital, Wroclaw, Poland; [7]Department of Internal Diseases, Occupational Medicine and Hypertension, Wroclaw Medical University, Wroclaw, Poland; [8]Third Department of Cardiology, Silesian Center for Heart Disease, Zabrze, Poland; [9]Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [10]Department of Cardiology, Heart Failure Program, Hospital del Mar, Barcelona, Spain; [11]Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain; [12]Division of Applied Cachexia Research, Department of Cardiology, Charité Medical School, Berlin, Germany; [13]Department of Cardiology, Wroclaw Medical University, Wroclaw, Poland; and [14]Department of Cardiology, Hull York Medical School, University of Hull, Castle Hill Hospital, Kingston-upon-Hull, UK

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Aims Iron deficiency (ID) is frequent in heart failure (HF), linked with exercise intolerance and poor prognosis. Intravenous iron repletion improves clinical status in HF patients with left ventricular ejection fraction (LVEF) ≤45%. However, uncertainty exists about the accuracy of serum biomarkers in diagnosing ID. The aims of this study were (i) to identify the iron biomarker with the greatest accuracy for the diagnosis of ID in bone marrow in patients with ischaemic HF, and (ii) to establish the prevalence of ID using this biomarker and its prognostic value in HF patients.

Methods and results Bone marrow was stained for iron in 30 patients with ischaemic HF with LVEF ≤45% and 10 healthy controls, and ID was diagnosed for 0–1 grades (Gale scale). A total of 791 patients with HF with LVEF ≤45% were prospectively followed up for 3 years. Serum ferritin, transferrin saturation, soluble transferrin receptor (sTfR) were assessed as iron biomarkers. Most patients with HF ($n = 25$, 83%) had ID in bone marrow, but none of the controls ($P < 0.001$). Serum sTfR had the best accuracy in predicting ID in bone marrow (area under the curve 0.920, 95% confidence interval 0.761–0.987, for cut-off 1.25 mg/L sensitivity 84%, specificity 100%). Serum sTfR was ≥1.25 mg/L in 47% of HF patients, in 56% and 46% of anaemics and non-anaemics, respectively ($P < 0.05$). The reclassification methods revealed that serum sTfR significantly added the prognostic value to the baseline prognostic model, and to the greater extent than plasma N-terminal pro B-type natriuretic peptide. Based on internal derivation and validation procedures, serum sTfR ≥1.41 mg/L was the optimal threshold for predicting 3-year mortality, independent of other established variables.

Conclusions High serum sTfR accurately reflects depleted iron stores in bone marrow in patients with HF, and identifies those with a high 3-year mortality.

Keywords Heart failure • Iron deficiency • Bone marrow • Soluble transferrin receptor • Prognosis • Mortality

*Corresponding author. Department of Heart Diseases, Wroclaw Medical University, Centre for Heart Diseases, University Hospital, ul. Borowska 213, 50-556 Wrocław, Poland. Tel: +48 71 7331112, Email: ewa.jankowska@umed.wroc.pl

Introduction

Iron deficiency (ID) is the most prevalent nutritional disorder worldwide,[1] and is also common is patients with heart failure (HF).[2–8] ID, regardless of concomitant anaemia, is associated with exercise intolerance,[5,9] poor quality of life and fatal prognosis in patients with HF and a reduced left ventricular ejection fraction (LVEF).[3–5,10–12] Intravenous iron supplementation in these patients safely lessens the symptoms, improves exercise capacity and quality of life.[13–19] Meta-analyses suggest that this therapy may bring survival benefits in patients with HF,[20,21] however it still needs to be prospectively confirmed in clinical trials.[22]

Accurate diagnosis of ID in patients with HF remains uncertain.[23] The gold standard for evaluating iron status is the assessment of iron stores directly in bone marrow aspirate, but its invasiveness limits clinical applicability. In clinical practice, ID is assessed by measuring iron biomarkers in circulating blood.[24–26] Among them, the soluble transferrin receptor (sTfR) might be the most accurate,[27,28] but until now in patients with HF ID has been diagnosed based on the assessment of serum ferritin and low transferrin saturation (Tsat).[3–5,8,9,13–15,23] However, HF is increasingly recognized to have an inflammatory component that may modify the serum concentrations of iron biomarkers,[24–26,29,30] confounding their interpretation.

Therefore, we investigated the prevalence of ID in bone marrow in patients with ischaemic HF with LVEF ≤45%, identified the most accurate serum biomarker of ID and applied this to a larger cohort of patients with HF and LVEF ≤45% to assess its prognostic value.

Methods

Subjects examined in the study on iron assessment in bone marrow

Bone marrow samples were obtained from stable patients with ischaemic HF who underwent elective cardiac surgery requiring a sternotomy at the Centre for Heart Diseases, Military Hospital (Wroclaw, Poland). They had to present a documented history of stable ischaemic HF of ≥6 month duration and LVEF ≤45% (assessed by echocardiography at the time of the study, using the biplane Simpson method). Exclusion criteria included: (i) acute coronary syndrome and/or coronary revascularization within 3 months prior to the study; (ii) unplanned hospitalization due to any cardiovascular reason within 1 month prior to the study; (iii) any acute or chronic illness that might influence iron metabolism (including cancer, infection, severe chronic kidney disease requiring dialysis, and haematological diseases); (iv) any treatment for anaemia and/or ID during the previous 12 months.

Bone marrow samples were obtained also from healthy subjects with no history of chronic disease recruited among volunteers and bone marrow donors in the Department of Haematology, Blood Neoplasms, and Bone Marrow Transplantation, Wroclaw Medical University (Wroclaw, Poland).

Patients participating in the observational study

Patients with HF attending outpatient clinics or admitted electively in three tertiary referral cardiology centres (Wroclaw and Zabrze, Poland; Groningen, The Netherlands) were enrolled.

The criteria for study inclusion were: (i) a documented history of HF of ≥6 months; (ii) LVEF ≤45% as assessed by echocardiography (performed at the time of screening using the biplane Simpson method to determine LVEF); (iii) clinical stability and unchanged medications for ≥1 month preceding the study. Exclusion criteria included: (i) acute coronary syndrome and/or coronary revascularization within 1 month prior to the study; (ii) unplanned hospitalization due to any cardiovascular reason within 1 month prior to the study; (iii) any acute or chronic illness that might influence iron metabolism (including known malignancy, infection, severe chronic kidney disease requiring dialysis, and haematological diseases); (iv) any anaemia and/or ID treatment either at the time of the study or during the previous 12 months.

When subjects were screened for this project (both studies), they were asked in details about blood transfusions, erythropoietin therapy, intravenous iron infusions, and also any nutritional supplements potentially containing iron. None of the subjects included in the study received such a therapy. Additionally, all anaemic subjects included into the study underwent a routine clinical evaluation in order to detect any potential secondary causes of anaemia, and subsequently subjects with an evidence of active bleeding were not included in the study. In case of clinical suspicion of gastrointestinal pathologies, endoscopy was ordered at the physician's discretion. No routine endoscopy was required for inclusion in the present study.

The protocol of these two studies was approved by the local ethics committees, and all subjects gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Iron assessment in bone marrow

Bone marrow samples were taken from the sternum in patients with ischaemic HF during cardiac surgery (all patients were qualified for coronary artery bypass grafting procedure), and from the iliac crest in healthy subjects. Bone marrow smears were iron stained using potassium ferrocyanide (Prussian blue). Iron smears with ≥7 fragments were assessed according to Gale's histological grading method, where ID was diagnosed when iron was absent or present in only very small amounts (grades 0–1).[31,32]

Haematological variables, indices of iron status and other laboratory measurements assessed in peripheral blood

In all patients, venous blood samples were taken in the morning following an overnight fast and after a supine rest of at least 15 min. Haematological variables were assessed from fresh venous blood with EDTA. After centrifuging of heparinized and clotted venous blood, the plasma and serum, respectively, were collected and frozen at −70°C until being analysed.

Haemoglobin (g/dL) was measured using the ADVIA 120 automated system (Siemens, Healthcare Diagnostics, Deerfield, IL, USA). Anaemia was defined as haemoglobin <12 g/dL in women and <13 g/dL in men.[33]

The following biomarkers of iron status were assessed in peripheral blood:

(a) serum ferritin (μg/L) measured using electrochemiluminescence with the Elecsys 2010 System (Roche Diagnostics GmbH, Mannheim, Germany);

(b) Tsat calculated as a ratio of 0.7217 × serum iron (μg/dL) and serum transferrin (mg/dL), multiplied by 100 and expressed in %, or when serum transferrin was not available as a ratio serum iron (μg/dL) and total iron-binding capacity (TIBC, μg/dL), also multiplied by 100 and expressed in %; for these calculations serum iron (μg/dL) and TIBC (μg/dL) were assessed using a substrate method with Feren S (Thermo Fisher Scientific, Waltham, MA, USA);
(c) serum sTfR (mg/L) measured using immunonephelometry (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA).

Plasma N-terminal pro B-type natriuretic peptide (NT-proBNP, pg/mL) was measured using immunoassay based on electrochemiluminescence on the Elecsys 1010/2010 System (Roche Diagnostics GmbH, Mannheim, Germany).

Renal function was assessed using the estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2), calculated from the Modification of Diet in Renal Disease equation.[34]

Clinical follow-up

Patients from the observational study were seen regularly by the study investigators in outpatient HF clinics. Information regarding survival was obtained directly from patients or their relatives, from the HF clinic database or from the hospital system. The primary endpoint was all-cause death. The length of follow-up of survivors and patients in whom events occurred after 3 years was censored at 1095 days.

Statistical analyses

Continuous variables with a normal distribution [age, body mass index (BMI), LVEF, serum sodium, haemoglobin, serum iron] were expressed as mean [with standard deviation (SD)]. The remaining continuous variables had a skewed distribution (plasma NT-proBNP, eGFR, serum ferritin, Tsat, serum sTfR) and were expressed as median [with interquartile range (IQR)]. These variables were ln-transformed in order to normalize their distribution, and ln-transformed values were used for further statistical analyses. Intergroup differences in continuous variables were tested using the Student's *t*-test. Categorical variables were expressed as number of patients in given categories (with percentage). Intergroup differences in categorical variables were tested using the χ^2 test.

In order to estimate the accuracy of circulating iron biomarkers for predicting ID in bone marrow, the receiver operating characteristic curve (ROC) analysis was performed with estimation of the area under the curve (AUC). For the most accurate cut-off values of subsequent iron biomarkers, sensitivity (true positive/true positive + false negative) and specificity (true negative/true negative + false positive) were calculated (and expressed in %). The iron status biomarker having the highest accuracy in predicting ID in bone marrow was selected for the definition of ID in the observational study.

Clinical variables, applied treatment and laboratory variables (including haematological variables and all assessed indices of iron status) were compared among patients with HF divided into two groups, i.e. in those with vs. without ID (defined based on the most accurate biomarker of iron status and its cut-off value, established in the ROC analysis). The relationships between the presence of ID (defined as described above) and its potential associates were established using the logistic regression analyses (both univariable and multivariable models). In univariable analyses, the following variables were assessed as potential risk factors of the presence of ID in patients with HF: clinical and laboratory variables, applied treatment, and haematological variables and indices of iron status (haemoglobin, serum ferritin, serum iron, Tsat, serum sTfR). In the multivariable regression model, all aforementioned potential associates were included. For both univariable and multivariable models, the odds ratios (OR) [with 95% confidence interval (CI)] with corresponding χ^2 and *P*-values were estimated for all potential associates incorporated into the models.

The associations between circulating biomarkers of iron status and other analysed variables, and survival during the 3-year follow-up in patients with HF were established using Cox proportional hazard regression analyses (both univariable and multivariable models). In univariable analyses, the following potential prognosticators were included: age, gender, BMI, HF aetiology, New York Heart Association (NYHA) class, LVEF, presence of diabetes, plasma NT-proBNP, serum sodium, eGFR, haemoglobin, serum ferritin, Tsat, serum sTfR, as well as the presence of ID (defined as described above). In multivariable models, all aforementioned potential prognosticators were included, and the circulating biomarker of iron status reflecting most accurately ID in bone marrow (in ROC analysis) was considered as a continuous variable in the first model, and as a dichotomized variable (with the application of the established cut-off value) in the second model. For both univariable and multivariable models, hazard ratios (HR) (with 95% CI) with corresponding χ^2 and *P*-values were estimated for all potential prognosticators incorporated into the models. The assumption of the proportional hazard was tested for each derived model.

The prognostic value of circulating biomarker of iron status reflecting most accurately ID in bone marrow (in ROC analysis) and plasma NT-proBNP in patients with HF was compared using the following statistical approaches. ROC curves along with the AUC[35] were calculated for four sets of prognosticators for the 3-year follow-up: (i) the baseline set of variables (age, gender, BMI, HF aetiology, NYHA class, presence of diabetes, serum sodium, eGFR, haemoglobin, serum ferritin, Tsat, serum sTfR – but excluding the most accurate biomarker of iron status in bone marrow); (ii) the baseline set of variables with plasma NT-proBNP; (iii) the baseline set of variables with the most accurate biomarker of iron status in bone marrow; (iv) the baseline set of variables with plasma NT-proBNP and the most accurate biomarker of iron status in bone marrow. In order to test the significance of adding to the prognostic model one of tested biomarkers (plasma NT-proBNP, the most accurate biomarker of iron status in bone marrow), the four-model comparisons were performed [model (i) with model (ii) and (iii), and model (iv) with model (ii) and (iii)], and the following statistics were calculated: c-statistics,[35,36] Akaike information criterion (AIC)[37] and likelihood ratio test (LRT).[38]

The risk estimates for 3-year all-cause mortality were categorized as: 0% to <10%, ≥10% to <20%, and ≥20%, which corresponded to the low, intermediate, and high-risk groups of patients with HF, respectively. The cross-tabulation of risk categories was performed to describe the number of participants who were reclassified appropriately (i.e. to the lower risk group for non-events or to the higher risk group with events) and inappropriately (i.e. to the higher risk group for non-events or to the lower risk group with events). Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were calculated for each comparison.[39]

The following statistical procedure was applied to derive and validate the best cut-off point for the circulating biomarker of iron status for the prediction of 3-year mortality in patients with HF. The whole analysed cohort was randomly distributed into two separate data sets (the derivation and the validation cohort), with the same number of events in both cohorts. Within the derivation cohort, the best cut-off of this circulating biomarker of iron status was determined based on the sensitivity and specificity from the ROC curves for the prediction of 3-year mortality. Within the validation cohort, the derived cut-off value was validated in both non-adjusted and adjusted models (adjusted for: age, gender, BMI, HF aetiology, NYHA class, LVEF, presence of diabetes, plasma NT-proBNP, serum sodium, eGFR, haemoglobin, serum ferritin, Tsat, serum sTfR – but excluding the tested circulating biomarker of iron status). All variables included in the adjusted model had to be dichotomized, i.e. the continuous variables were divided into $<$ and $\geq$ the median, and the NYHA class was analysed as I + II and III + IV classes. There was the following procedure of validation of the derived cut-off value. The tested circulation biomarker of iron status was dichotomized into $<$ and $\geq$ the derived cut-off value, for these two groups the Kaplan–Meier estimator with the corresponding *P*-value was calculated both for non-adjusted and adjusted models (as described above), and if the obtained *P*-value was <0.05, the cut-off point was approved. Differences in survival rates were tested using the Cox–Mantel log-rank test (non-adjusted models) or the Z statistic (adjusted models).[40] The aforementioned procedure was repeated 1000 times. The optimal cut-off point is an average from all approved cut-off points.

In order to illustrate the effect of the presence of ID on 3-year survival rates, the Kaplan–Meier curves for cumulative survival were constructed for patients with HF varying of iron status: (i) divided into two groups, i.e. in those with vs. without ID defined based on the most accurate biomarker of iron status biomarker and its cut-off value, established in the ROC analysis; (ii) divided into two groups, i.e. in those with this circulating biomarker of iron status $\geq$ vs. $<$ the derived and validated cut-off value with the best discriminative power regarding the 3-year survival rate. Differences in survival rates were tested using the Cox–Mantel log-rank test (non-adjusted models) or the Z statistic (adjusted models).[40]

All statistical analyses were performed using R software version 2.13.1,[41] SAS software version 9.2 (http://www.sas.com) and Statistica 9.1 (StatSoft. Inc., Tulsa, OK, USA).

A *P*-value <0.05 was considered statistically significant.

Table 1 Clinical characteristics of patients with ischaemic heart failure recruited for the study on iron assessment in bone marrow

Variables	Patients with ischaemic HF ($n = 30$)
Clinical and laboratory variables	
Age, years	63 (9)*
Gender, men	28 (93)*
BMI, kg/m^2	27.6 (3.9)
HF aetiology, CAD	30 (100)***
NYHA class III–IV	6 (20)***
LVEF, %	37 (7)***
NT-proBNP, pg/mL	1311 [490–4032]***
Sodium, mmol/L	140 (2)
Diabetes mellitus	14 (47)***
eGFR, mL/min/1.73 m^2	84.5 (25.6)
Treatment	
ACE-I/ARB	23 (77)***
β-blocker	28 (93)
Aldosterone antagonist	10 (33)***
Digoxin	4 (13)***
Loop diuretic	12 (40)***
Statin	30 (100)***
Antiplatelet/anticoagulant	28 (93)*
Haematological parameters and indices of iron status assessed in peripheral blood	
Haemoglobin, g/dL	13.6 (1.5)
Anaemia[a]	8 (27)***
Ferritin, μg/L	174 [104–277]
Iron, μg/dL	107 (37)
Tsat, %	34 [29–48]*
STfR, mg/L	1.53 [1.24–1.70]

Data are presented as mean (± standard deviation), *n* (%), or median [interquartile range expressed as lower and upper quartiles).
ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HF, heart failure; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; sTfR, soluble transferrin receptor; Tsat, transferrin saturation.
[a]Anaemia was defined as haemoglobin <12 g/dL in women and <13 g/dL in men.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ – comparisons with a cohort of patients recruited for the prospective study (described in *Table 3*).

Results

Prevalence of bone marrow deficiency in patients with ischaemic heart failure and healthy controls

Iron status was quantified in bone marrow in 30 patients with ischaemic HF (*Table 1*) and 10 healthy subjects (five men, age: 38 ± 16 years).

Based on bone marrow examinations, ID (depleted iron stores in bone marrow) was found in 25 (83%) of patients with ischaemic HF, and in none of controls ($P < 0.001$). The prevalence of ID in bone marrow did not differ between those with vs. without anaemia [7 (85%) and 18 (82%), respectively; $P > 0.2$].

Accuracy of indices of iron status measured in peripheral blood for predicting iron deficiency in bone marrow in patients with ischaemic heart failure

Patients with HF and depleted bone marrow iron had higher serum concentrations of sTfR [median and 1st and 3rd quartiles: 1.45 (1.28–1.74)] compared to those who had more iron in bone marrow aspirates [1.14 (0.97–1.24) mg/L; $P < 0.05$]. There were no significant differences in other haematological variables or serum biomarkers of ID (all $P > 0.2$).

Table 2 Accuracy of indices of iron status measured in peripheral blood for predicting of absolute iron deficiency in bone marrow (grades 0–1 according to gale scale) in patients with ischaemic heart failure

Variable	AUC	95% CI	Cut-off	Sensitivity	Specificity
Ferritin, μg/L	0.600	0.406–0.773	≤135.9	48%	100%
Iron, μg/dL	0.568	0.376–0.747	≤111	72%	60%
Tsat, %	0.640	0.445–0.806	≤33	52%	80%
sTfR, mg/L	0.920	0.761–0.987	≥1.25	84%	100%

AUC, area under the curve; CI, confidence interval; sTfR, soluble transferrin receptor; Tsat, transferrin saturation.

Among circulating iron biomarkers, serum sTfR had the best accuracy in predicting ID in bone marrow in this cohort (the best cut-off ≥1.25 mg/L) (*Table 2*).

Prevalence of iron deficiency (defined as serum soluble transferrin receptor ≥1.25 mg/L) in patients with heart failure

The baseline clinical characteristics of 791 patients with HF (328, 368 and 95 subjects recruited in Wroclaw, Zabrze and Groningen, respectively) are shown in *Table 3*.

Iron deficiency (serum sTfR ≥1.25 mg/L) was found in 374 of all patients with HF, which corresponded to a prevalence of 47%. ID was found in 41%, 39%, 55% and 72% of subjects in subsequent NYHA classes ($P < 0.001$), and in 56% vs. 46% of anaemics vs. non-anaemics, respectively ($P < 0.05$).

Risk factors associated with the higher prevalence of iron deficiency (defined as serum soluble transferrin receptor ≥1.25 mg/L) in patients with heart failure

In univariable logistic regression models, the following variables were associated with the higher prevalence of ID (serum sTfR ≥1.25 mg/L) in patients with HF: advanced NYHA class, low LVEF, high plasma NT-proBNP, presence of diabetes, reduced eGFR, therapy with digoxin, therapy with loop diuretic, presence of anaemia, low serum ferritin, low serum iron, and low Tsat (all $P < 0.05$) (*Table 3*). In a multivariable logistic regression model, the following variables remained statistically significantly associated with the prevalence of ID in this group of patients: plasma NT-proBNP, eGFR, haemoglobin, serum ferritin, and Tsat (all $P < 0.05$) (*Table 3*).

Circulating biomarkers of iron status and survival in patients with heart failure

The mean follow-up was 786 ± 330 days (median 931 days, range: 1–1095 days). The proportion of surviving the 3-year follow-up was 77% (± 95% CI 74–80%).

The proportionality assumption and the assumption of a log-linear relationship between the prognosticators and the hazard function were fulfilled for all tested variables.

In univariable Cox proportional hazard regression models, the following variables were shown to predict increased 3-year mortality in patients with HF: low BMI, high NYHA class, low LVEF, presence of diabetes, high plasma NT-proBNP, reduced serum sodium, reduced eGFR, low haemoglobin and low Tsat (all $P < 0.05$, *Table 4*). High serum sTfR, both when analysed as a continuous ln-transformed variable and when dichotomized at ≥ vs. <1.25 mg/L was associated with increased 3-year mortality in patients with HF (both $P < 0.001$, *Table 4*).

Serum sTfR remained a significant predictor of death in these patients also in multivariable models, when adjusted for all other prognosticators, including plasma NT-proBNP, haemoglobin, serum ferritin and Tsat (*Table 4*). The adjusted 3-year survival rates were 66 ± 5% (± 95% CI) vs. 80 ± 5% (±95% CI) for patients with HF with serum sTfR ≥ vs. <1.25 mg/L ($Z = 4.03$, $P < 0.001$) (*Figure 1*).

Additive prognostic value of serum soluble transferrin receptor in comparison to plasma NT-proBNP in patients with heart failure

Several metrics were used to quantify the prognostic utility of adding serum sTfR or/and plasma NT-proBNP to the set of prognosticators included in the baseline model in patients with HF (*Tables 5–7*).

The inclusion of plasma NT-proBNP as an additional prognosticator to multivariable models (both with and without serum sTfR) resulted in a significant increase in the χ^2 values and c-statistics of all these models. Importantly, the inclusion of serum sTfR as an additional prognosticator to multivariable models (both with and without plasma NT-proBNP) also resulted in a significant additional increase in the χ^2 values and c-statistics of all these models. The increase in the χ^2 value due to the inclusion of serum sTfR was greater than the increase in the χ^2 value due to the inclusion of plasma NT-proBNP (*Tables 5* and *6*).

Similar results were obtained when the statistical approach using AIC and LRT was applied for these comparisons (*Tables 5* and *6*). Also, the inclusion of both plasma NT-proBNP and serum sTfR to the prognosticator models independently improved the reclassification of patients with HF regarding the three risk categories for 3-year mortality (for all model comparisons, for both NRI and

Table 3 **Clinical characteristics of patients with heart failure recruited for the observational study, also separately in those with vs. without iron deficiency (serum soluble transferrin receptor ≥1.25 mg/L), along with the risk factors of iron deficiency (univariable and multivariable logistic regression models)**

Variables	All patients ($n = 791$)	Serum sTfR		Risk factors of serum sTfR ≥ 1.25 mg/L (vs. < 1.25 mg/L) – logistic regression models				
		< 1.25 mg/L ($n = 417$)	≥ 1.25 mg/L ($n = 374$)	Units of risk factors	Univariable models		Multivariable model[b]	
					OR (95% CI)	χ^2 (*P*-value)	OR (95% CI)	χ^2 (*P*-value)
Clinical and laboratory variables								
Age, years	58 (11)	57 (11)	58 (11)	1 year	1.01 (1.00–1.02)	1.69 (0.19)	0.98 (0.96–1.00)	2.64 (0.10)
Gender, men	665 (84)	356 (85)	309 (83)	Men vs. women	0.81 (0.56–1.19)	1.12 (0.29)	1.14 (0.68–1.89)	0.24 (0.62)
BMI, kg/m^2	27.2 (4.3)	27.3 (4.0)	27.0 (4.5)	1 kg/m^2	0.99 (0.96–1.02)	0.52 (0.47)	0.99 (0.95–1.04)	0.13 (0.71)
HF aetiology, CAD	556 (70)	286 (69)	270 (72)	CAD vs. non-CAD	1.19 (0.88–1.62)	1.23 (0.27)	1.21 (0.78–1.87)	0.72 (0.40)
NYHA class III–IV	337 (43)	143 (34)	194 (52)***	III–IV vs. I–II	2.07 (1.55–2.75)	24.63 (<0.001)	1.41 (0.94–2.12)	2.75 (0.10)
LVEF, %	28 (8)	29 (8)	27 (9)**	LVEF, %	0.98 (0.96–0.99)	8.99 (0.003)	1.01 (0.98–1.03)	0.16 (0.69)
NT-proBNP, pg/mL	1189 [479–3052]	857 [378–1898]	1834 [651–4408]***	1 ln pg/mL	1.51 (1.35–1.70)	47.74 (<0.001)	1.44 (1.21–1.73)	15.85 (<0.001)
Sodium, mmol/L	139 (4)	139 (4)	139 (5)	1 mmol/L	0.98 (0.95–1.02)	1.07 (0.30)	1.02 (0.97–1.07)	0.32 (0.57)
Diabetes	221 (28)	99 (24)	122 (33)**	Yes vs. no	1.56 (1.14–2.13)	7.67 (0.006)	1.33 (0.89–2.00)	1.93 (0.16)
eGFR, mL/min/1.73 m^2	77.3 [62.3–92.3]	81.0 [67.7–96.6]	71.6 [57.4–87.9]***	1 ln mL/min/1.73m^2	0.28 (0.18–0.44)	30.57 (<0.001)	0.25 (0.13–0.46)	19.75 (<0.001)
Treatment								
ACE-I and/or ARB	747 (94)	400 (96)	347 (93)	Yes vs. no	0.55 (0.29–1.02)	3.61 (0.06)	0.60 (0.27–1.32)	1.61 (0.20)
β-blocker	769 (97)	408 (98)	361 (97)	Yes vs. no	0.61 (0.26–1.45)	1.24 (0.26)	0.92 (0.28–2.99)	0.02 (0.89)
Aldosterone antagonist	473 (60)	247 (59)	226 (60)	Yes vs. no	1.05 (0.79–1.40)	0.12 (0.73)	0.67 (0.42–1.09)	2.64 (0.10)
Digoxin[b]	263 (38)	123 (34)	140 (42)*	Yes vs. no	1.46 (1.07–1.98)	5.72 (0.02)	1.27 (0.84–1.90)	1.28 (0.26)
Loop diuretic	546 (69)	268 (64)	278 (74)**	Yes vs. no	1.61 (1.19–2.19)	9.27 (0.002)	1.03 (0.63–1.67)	0.01 (0.91)
Statin	602 (76)	319 (76)	283 (76)	Yes vs. no	0.96 (0.69–1.33)	0.07 (0.78)	1.03 (0.67–1.60)	0.02 (0.88)
Anticoagulant and/or antiplatelet[b]	581 (83)	305 (83)	276 (84)	Yes vs. no	1.02 (0.69–1.53)	0.01 (0.91)	1.33 (0.82–2.16)	1.31 (0.25)
Haematological parameters and indices of iron status assessed in peripheral blood								
Haemoglobin, g/dL	14.1 (1.5)	14.2 (1.4)	14.0 (1.7)	1 g/dL	0.93 (0.85–1.02)	2.35 (0.13)	1.17 (1.03–1.33)	5.82 (0.02)
Anaemia[a]	132 (17)	58 (14)	74 (20)*	Yes vs. no	1.53 (1.05–2.22)	4.86 (0.03)	–	–
Ferritin, μg/L	165 [93–292]	182 [120–314]	138 [75–262]***	1 log μg/L	0.63 (0.53–0.74)	28.52 (<0.001)	0.63 (0.50–0.79)	16.14 (<0.001)
Iron, μg/L	98 (46)	105 (46)	89 (45)***	–	0.99 (0.99–1.00)	23.41 (<0.001)	–	–
Tsat, %	28 [19–40]	33 [23–43]	23 [16–33]***	1 log %	0.28 (0.21–0.39)	61.08 (<0.001)	0.26 (0.17–0.41)	36.12 (<0.001)
sTfR, mg/L	1.2 [1.0–1.7]	1.0 [0.9–1.1]	1.7 [1.4–2.1]***	–	–	–	–	–

Data are presented as mean (standard deviation), *n* (%), or median [interquartile range expressed as lower and upper quartiles].

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; OR, odds ratio; sTfR, soluble transferrin receptor; sTfR, soluble transferrin receptor; Tsat, transferrin saturation.

χ^2 of the multivariable logistic regression model is 123.94 ($P < 0.001$).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ – comparisons of patients with HF with serum sTfR ≥ vs. < 1.25 mg/L.

[a] Anaemia was defined as haemoglobin <12 g/dL in women and <13 g/dL in men.

[b] $n = 696$.

Table 4 Prognosticators of 3-year all-cause mortality in patients with heart failure (Cox proportional hazard univariable and multivariable regression models)

Variables, units	Univariable models			Multivariable model with serum sTfR as a continuous (log) variable			Multivariable model with serum sTfR as a dichotomized variable		
	HR (95% CI)	χ^2	*P*-value	HR (95% CI)	χ^2	*P*-value	HR (95% CI)	χ^2	*P*-value
Age, 1 year	1.00 (0.99–1.01)	0.0006	0.98	1.01 (0.99–1.02)	0.46	0.50	1.01 (0.99–1.02)	0.46	0.50
Gender, men vs. women	1.43 (0.92–2.24)	2.49	0.12	1.78 (1.10–2.87)	5.59	0.02	1.77 (1.10–2.86)	5.52	0.02
BMI, 1 kg/m^2	0.95 (0.92–0.99)	6.59	0.01	1.01 (0.97–1.05)	0.30	0.59	1.02 (0.98–1.06)	0.70	0.40
HF aetiology, CAD vs. non-CAD	0.93 (0.68–1.29)	0.18	0.68	1.01 (0.71–1.44)	0.01	0.94	0.99 (0.69–1.40)	0.01	0.94
NYHA class		46.80	<0.001		2.86	0.41		4.29	0.23
II vs. I	1.13 (0.61–2.09)	0.14	0.71	1.04 (0.55–1.97)	0.02	0.89	0.97 (0.51–1.82)	0.01	0.92
III vs. I	2.28 (1.24–4.18)	7.11	0.01	1.14 (0.60–2.19)	0.16	0.68	1.13 (0.59–2.15)	0.13	0.72
IV vs. I	5.13 (2.55–10.31)	21.01	<0.001	1.69 (0.76–3.77)	1.65	0.20	1.77 (0.80–3.92)	1.95	0.16
LVEF, %	0.94 (0.92–0.95)	45.83	<0.001	0.97 (0.94–0.99)	9.05	0.003	0.97 (0.95–0.99)	8.12	0.004
Diabetes, yes vs. no	1.61 (1.19–2.18)	9.62	0.002	1.27 (0.90–1.80)	1.83	0.18	1.27 (0.90–1.79)	1.85	0.17
NT-proBNP, 1 ln pg/mL	1.80 (1.59–2.03)	86.00	<0.001	1.44 (1.23–1.70)	19.52	<0.001	1.52 (1.29–1.78)	25.43	<0.001
Sodium, 1 mmol/L	0.91 (0.88–0.94)	33.49	<0.001	0.97 (0.94–1.01)	1.73	0.19	0.96 (0.93–1.00)	3.96	0.047
eGFR, 1 ln mL/min/1.73 m^2	0.57 (0.38–0.84)	7.93	0.005	1.31 (0.82–2.09)	1.30	0.25	1.18 (0.73–1.89)	0.46	0.50
Haemoglobin, 1 g/dL	0.87 (0.79–0.96)	7.53	0.01	0.91 (0.82–1.01)	2.91	0.09	0.92 (0.83–1.03)	2.19	0.14
Ferritin, 1 log µg/L	0.89 (0.75–1.05)	1.86	0.17	0.95 (0.78–1.16)	0.23	0.63	0.86 (0.71–1.05)	2.19	0.14
Tsat, 1 log %	0.58 (0.44–0.77)	14.78	0.001	1.19 (0.84–1.69)	0.97	0.32	0.95 (0.68–1.34)	0.07	0.78
sTfR, 1 log mg/L	19.21 (10.99–33.58)	107.63	<0.001	7.93 (3.91–16.09)	32.88	<0.001	–	–	–
sTfR, ≥ vs. < 1.25 mg/L	2.68 (1.96–3.67)	37.80	<0.001	–	–	–	1.74 (1.22–2.47)	9.53	0.002
χ^2 of the multivariable models					181.24	<0.001		148.82	<0.001

BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, hazard ratio; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; sTfR, soluble transferrin receptor; Tsat, transferrin saturation.

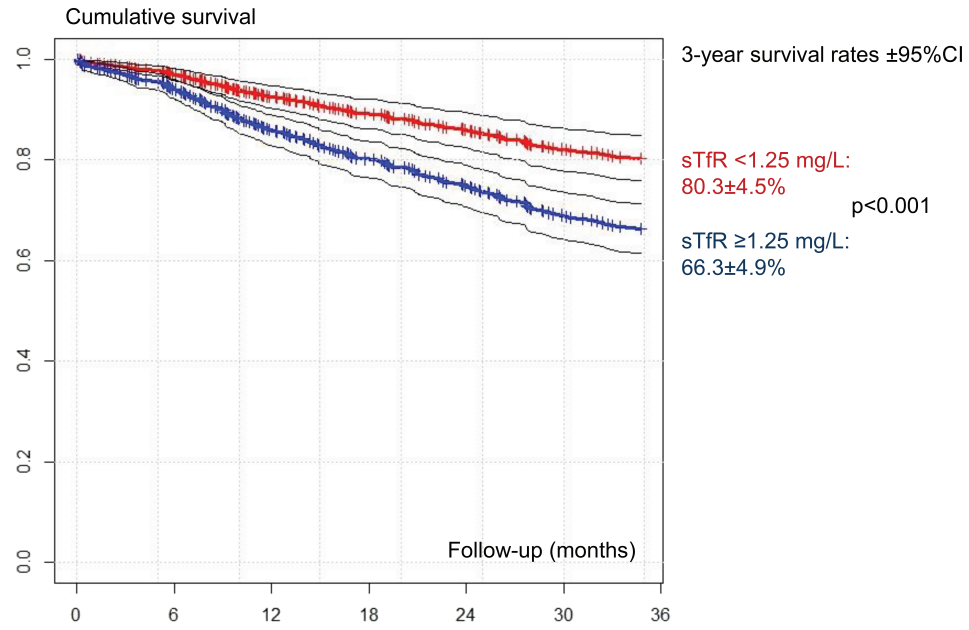

Figure 1 Kaplan–Meier curves reflecting adjusted 3-year cumulative survival rates in patients with heart failure with vs. without serum soluble transferrin receptor (sTfR) ≥1.25 mg/L. CI, confidence interval.

IDI – all $P < 0.05$). Approximately 9% of patients with HF were reclassified to the correct risk group when plasma NT-proBNP was added to the baseline model, whereas around 11% of patients with HF were reclassified to the correct risk group when serum sTfR was added to the baseline model. Moreover, additional 11% of subjects were reclassified when plasma NT-proBNP was added to the prognostic model (including serum sTfR), whereas additional 12% of subjects were reclassified when serum sTfR was added to the prognostic model (including plasma NT-proBNP) (*Tables 6* and *7*).

Table 5 Fitness of the subsequent Cox proportional hazard multivariable regression models including baseline prognosticators of 3-year all-cause mortality with and without N-terminal pro B-type natriuretic peptide, and with and without soluble transferrin receptor (both considered as potential prognosticators) in patients with heart failure

Multivariable models	χ^2 for the assumption of proportional hazard (*P*-value)	χ^2 of the model (*P*-value)	AUC (± SE)	Log likelihood	AIC
Baseline variables[a]	21.70 (0.09)	114.87 (<0.001)	0.710 (0.02)	−1093.32	2214.65
Baseline variables[a] + NT-proBNP (1 log pg/mL)	22.10 (0.11)	141.18 (<0.001)	0.747 (0.02)	−1076.11	2182.22
Baseline variables[a] + sTfR (1 log mg/L)	22.16 (0.10)	175.70 (<0.001)	0.750 (0.02)	−1070.42	2170.84
Baseline variables[a] + NT-proBNP (1 log pg/mL) + sTfR (1 log mg/L)	23.82 (0.09)	181.24 (<0.001)	0.770 (0.02)	−1060.23	2152.46

AIC, Akaike information criterion; AUC, area under the curve; NT-proBNP, N-terminal pro B-type natriuretic peptide; SE, standard error; sTfR, soluble transferrin receptor.
[a]The set of baseline variables included the following prognosticators: age, gender, body mass index, heart failure aetiology, New York Heart Association class, diabetes, sodium, estimated glomerular filtration rate, haemoglobin, ferritin, and transferrin saturation.

Table 6 Additive prognostic value for the prediction of 3-year all-cause mortality of N-terminal pro B-type natriuretic peptide and soluble transferrin receptor in patients with heart failure

Baseline model	Added prognosticator	Δc (95% CI)	χ^2 for Δc (*P*-value)	χ^2 for LRT (*P*-value)	HR (*P*-value)	NRI (*P*-value)	IDI (*P*-value)
Baseline variables[a]	+ NT-proBNP (1 log pg/mL)	0.037 (0.011–0.064)	7.71 (0.006)	34.43 (<0.001)	1.60 (<0.001)	0.0944 (0.03)	0.0419 (<0.001)
Baseline variables[a]	+ sTfR (1 log mg/L)	0.040 (0.016–0.065)	10.43 (0.001)	45.80 (<0.001)	11.25 (<0.001)	0.1091 (0.009)	0.0501 (<0.001)
Baseline variables[a] + sTfR (1 log mg/L)	+ NT-proBNP (1 log pg/mL)	0.020 (0.002–0.038)	4.82 (0.028)	20.38 (<0.001)	1.44 (<0.001)	0.1103 (0.003)	0.0225 (<0.001)
Baseline variables[a] + NT-proBNP (1 log pg/mL)	+ sTfR (1 log mg/L)	0.023 (0.006–0.041)	6.83 (0.009)	31.76 (<0.001)	7.93 (<0.001)	0.1227 (<0.001)	0.0307 (<0.001)

CI, confidence interval; HR, hazard ratio; IDI, integrated discrimination improvement; LRT, likelihood ratio test; NRI, net reclassification improvement; NT-proBNP, N-terminal pro B-type natriuretic peptide; sTfR, soluble transferrin receptor.
NRI and IDI were calculated for pre-defined risk groups: (1) low risk <10%; (2) 10% ≤ medium risk <20%; (3) high risk ≥20%.
[a]The set of baseline variables included the following prognosticators: age, gender, body mass index, heart failure aetiology, New York Heart Association class, diabetes, sodium, estimated glomerular filtration rate, haemoglobin, ferritin, and transferrin saturation.

Derivation and validation of the cut-off of serum soluble transferrin receptor with the best prognostic accuracy for 3-year mortality in patients with heart failure

Based on the previously described derivation procedure, serum sTfR ≥1.41 ± 0.13 mg/L was established as the cut-off with the best accuracy in predicting death at 3-year follow-up. Its prognostic value was confirmed during the validation procedure in both non-adjusted and adjusted models. In the validation procedure of non-adjusted models, mean *P*-value from the long-rank test of Kaplan–Meier estimators was <0.001 (100% of *P*-values were <0.05). In the validation procedure of adjusted models, mean *P*-value from the Z test of Kaplan–Meier estimators was 0.01 (95% of *P*-values were <0.05).

The adjusted 3-year survival rates were 63 ± 6% (± 95% CI) vs. 80 ± 4% (± 95% CI) for patients with HF with serum sTfR ≥ vs. <1.41 mg/L ($Z = 4.76$, $P < 0.001$) (*Figure 2*).

We identified the optimal cut-off point of serum sTfR for prognostic purposes (1.41 mg/L) in the aforementioned derivation and validation procedure, and also identified the cut-off point for serum sTfR based on data taken from bone marrow (1.25 mg/L), which reflects depleted iron stores in bone marrow. Patients with sTfR ≥1.41 mg/L are those with the highest mortality and at the same time those with depleted iron (all subjects with sTfR ≥1.41 mg/L fulfil the condition of sTfR ≥1.25 mg/L).

Table 7 Reclassification rates of alive, dead and all patients with heart failure at 3-year follow-up between low (<10%), medium (10%≤ and <20%) and high-risk (≥20%) categories established using different prognostic models (including baseline variables, with and without N-terminal pro B-type natriuretic peptide and soluble transferrin receptor)

Analysed subgroups	Risk categories for model no. 1	Risk categories for model no. 2			% of reclassified patients
		[0.00;0.10)	[0.10,0.20)	[0.20;1.00]	
Alive patients	[0.00;0.10)	87	21	3	22
	[0.10,0.20)	78	132	53	50
	[0.20;1.00]	7	60	171	28
Dead patients	[0.00;0.10)	7	3	0	30
	[0.10,0.20)	8	18	16	57
	[0.20;1.00]	0	14	113	11
All patients	[0.00;0.10)	94	24	3	22
	[0.10,0.20)	86	150	69	51
	[0.20;1.00]	7	74	284	22
	Risk categories for model no. 1	**Risk categories for model no. 3**			
		[0.00;0.10)	**[0.10,0.20)**	**[0.20;1.00]**	
Alive patients	[0.00;0.10)	92	19	0	17
	[0.10,0.20)	65	150	48	43
	[0.20;1.00]	4	75	159	33
Dead patients	[0.00;0.10)	6	4	0	40
	[0.10,0.20)	6	23	13	45
	[0.20;1.00]	1	13	113	11
All patients	[0.00;0.10)	98	23	0	19
	[0.10,0.20)	71	173	61	43
	[0.20;1.00]	5	88	272	25
	Risk categories for model no. 2	**Risk categories for model no. 4**			
		[0.00;0.10)	**[0.10,0.20)**	**[0.20;1.00]**	
Alive patients	[0.00;0.10)	154	16	2	10
	[0.10,0.20)	42	149	22	30
	[0.20;1.00]	0	56	171	25
Dead patients	[0.00;0.10)	9	6	0	40
	[0.10,0.20)	3	23	9	34
	[0.20;1.00]	0	7	122	5
All patients	[0.00;0.10)	163	22	2	13
	[0.10,0.20)	45	172	31	31
	[0.20;1.00]	0	63	293	18
	Risk categories for model no. 3	**Risk categories for model no. 4**			
		[0.00;0.10)	**[0.10,0.20)**	**[0.20;1.00]**	
Alive patients	[0.00;0.10)	125	35	1	22
	[0.10,0.20)	70	144	30	41
	[0.20;1.00]	1	42	164	21
Dead patients	[0.00;0.10)	10	3	0	23
	[0.10,0.20)	2	23	15	42
	[0.20;1.00]	0	10	116	8
All patients	[0.00;0.10)	135	38	1	22
	[0.10,0.20)	72	167	45	41
	[0.20;1.00]	1	52	280	16

NT-proBNP, N-terminal pro B-type natriuretic peptide; sTfR, soluble transferrin receptor.
Prognosticators in model no. 1 = Baseline variables[a].
Prognosticators in model no. 2 = Baseline variables[a] + NT-proBNP.
Prognosticators in model no. 3 = Baseline variables[a] + sTfR.
Prognosticators in model no. 4 = Baseline variables[a] + NT-proBNP + sTfR.
[a]The set of baseline variables included the following prognosticators: age, gender, body mass index, heart failure aetiology, New York Heart Association class, diabetes, sodium, estimated glomerular filtration rate, haemoglobin, ferritin, and transferrin saturation.

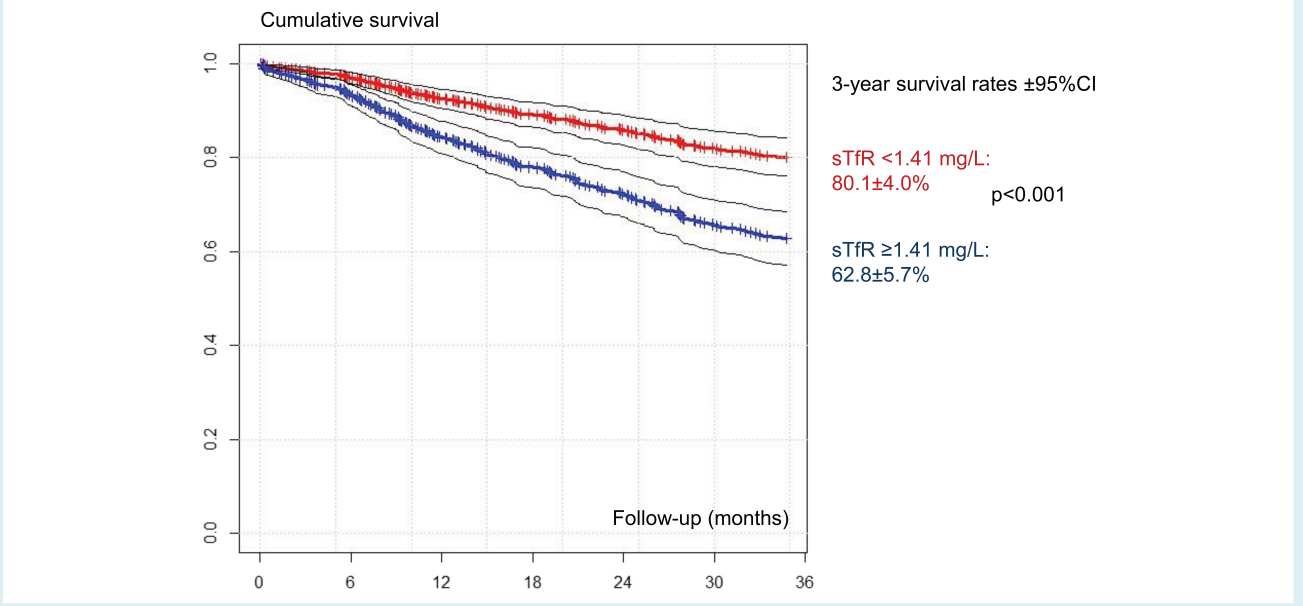

Figure 2 Kaplan–Meier curves reflecting adjusted 3-year cumulative survival rates in patients with heart failure with vs. without serum soluble transferrin receptor (sTfR) ≥1.41 mg/L. CI, confidence interval.

Concordance between iron deficiency assessed using serum soluble transferrin receptor and based on serum ferritin and transferrin saturation

In the study cohort, 159 (20%) patients had ID defined as sTfR ≥1.41 mg/L and defined based on serum ferritin and Tsat, 347 (44%) had neither ID defined as sTfR ≥1.41 mg/L nor defined based on serum ferritin and Tsat, 132 (17%) patients had ID defined only as sTfR ≥1.41 mg/L (with normal values of ferritin and Tsat), whereas 153 (19%) patients had ID defined based on serum ferritin and Tsat (with normal values of sTfR). Therefore, the concordance between these two definitions of ID was found in 64% of cases. There were few clinical differences between these four groups. Patients with ID defined as sTfR ≥1.41 mg/L and defined based on serum ferritin and Tsat had the highest plasma NT-proBNP, the lowest LVEF and the most severe HF symptoms assessed using the NYHA class (*Table 8*). Patients with ID defined as sTfR ≥1.41 mg/L (regardless of serum ferritin and Tsat) had lower eGFR as compared to those with sTfR <1.41 mg/L (*Table 8*). There were significant differences in 3-year all-rate survival between these four groups, with the worst outcome observed in patients with ID defined as sTfR ≥1.41 mg/L and defined based on serum ferritin and Tsat (*Figure 3*).

Discussion

There are two major findings arising from our study. Firstly, depleted iron stores in bone marrow have been found in the vast majority of patients with ischaemic HF with LVEF ≤45% (80%), regardless of concomitant anaemia. Secondly, high serum sTfR reflecting depleted iron stores in bone marrow has been demonstrated to be the most accurate biomarker measured in peripheral blood, which strongly predicted increased mortality in this group of patients.

Although the assessment of iron status directly in bone marrow is the accepted worldwide gold standard,[24–26,42] this invasive method for the diagnosis of ID is rarely applied in clinical practice, also in patients with cardiovascular disease. So far, Nanas *et al.*[7] investigated iron stores in bone marrow in a special subset of patients with HF, and confirmed the diagnosis of ID in bone marrow in 73% of anaemic patients with decompensated advanced HF. On the other hand, Grote Beverborg *et al.*[43] identified ID in bone marrow in 40% of patients with mild to moderate HF with LVEF ≤45%, and low Tsat and low serum iron (but not circulating ferritin) predicted depleted iron stores in bone marrow. In our study, ID diagnosed using the same technique appeared to be very prevalent also in stable patients with HF with LVEF ≤45%, who were scheduled for cardiac surgery procedures and therefore *a priori* did not have severe anaemia. Indeed, we found mild anaemia in 8 (27%) of examined patients, and the prevalence of ID was high in both anaemics (85%) and non-anaemics (82%).

In order to validate the circulating biomarkers of iron status, which are generally used for the diagnosis of ID in different clinical settings,[24–26,42] we compared them with iron stained in bone marrow in patients with ischaemic HF. Circulating ferritin is considered as a reliable surrogate for the quantity of stored iron, whereas circulating iron bound to transferrin (expressed as Tsat) reflects the amount of iron available to metabolizing cells.[24–26,42] Importantly, these statements are based mainly on evidence concerning the diagnosis of ID-associated anaemia, but not ID itself. Moreover, inflammatory response contributing to HF

Table 8 Clinical characteristics of patients with heart failure recruited for the observational study in four subgroups according to the presence of iron deficiency defined based on soluble transferrin receptor and/or ferritin and trasferrin saturation

Variables	All patients ($n = 791$)	No ID based on sTfR and no ID based on ferritin/Tsat ($n = 347$) (1)	ID based on sTfR and no ID based on ferritin/ Tsat ($n = 132$) (2)	No ID based on sTfR and ID based on ferritin/Tsat ($n = 153$) (3)	ID based on sTfR and ID based on ferritin/ Tsat ($n = 159$) (4)
Clinical and laboratory variables					
Age, years	58 (11)	57 (11)	58 (11)	59 (11)	60 (12)*
Gender, men	665 (84)	303 (87)	112 (85%)	120 (78)*	130 (82)
BMI, kg/m^2	27.2 (4.3)	27.4 (4.2)	27.3 (4.4)	27 (3.7)	26.7 (4.7)
HF aetiology, CAD	556 (70)	231 (67)	93 (70)	116 (76)*	116 (73)
NYHA class III–IV	337 (43)	115 (33)	66 (50)***	61 (40)	95 (60)***###
LVEF, %	28 (8)	29 (8)	27 (9)	29 (8)$^{\$}$	26 (9) **###
NT-proBNP, pg/mL	1189 [479–3052]	966 [404–2082]	1684 [844–4425]***	747 [300–1623]$^{\$\$\$}$	2828 [860–4960]***###
Sodium, mmol/L	139 (4)	139 (4)	138 (5)	139 (3)$^{*\$}$	138 (4)$^{\#\#}$
Diabetes	221 (28)	87 (25)	49 (37)*	36 (24)$^{\$}$	49 (31)
eGFR, mL/min/1.73 m^2	77.3 [62.3–92.3]	79.7 [66.2–96.4]	69.8 [54.5–82.7]***	81.2 [69–94.1]$^{\$\$\$}$	71 [55.5–87.8]***###
Treatment					
ACE-I and/or ARB	747 (94)	333 (96)	122 (92)	147 (96)	145 (91)*
β-blocker	769 (97)	341 (98)	127 (96)	147 (96)	154 (97)
Aldosterone antagonist	473 (60)	206 (59%)	84 (64)	84 (55)	99 (62)
Digoxin$^{\$}$	263 (38)	112 (32)	60 (45)*	34 (22)$^{\$}$	57 (36)
Loop diuretic	546 (69)	231 (67)	91 (69)	100 (65)	124 (78)$^{*\#}$
Statin	602 (76)	257 (74)	101 (77)	125 (82)	119 (75)
Anticoagulant and/or antiplatelet$^{\$}$	581 (83)	261 (75)	113 (86)*	99 (65)*	108 (68)$^{\$\#\#}$
Haematological parameters and indices of iron status assessed in peripheral blood					
Haemoglobin, g/dL	14.1 (1.5)	14.3 (1.4)	14.4 (1.8)	14.0 (1.3)$^{**\$}$	13.7 (1.6)$^{***\$\$\$}$
Anaemia§	132 (17)	47 (14)	19 (14)	23(15)	43(27)$^{***\$\#}$
Ferritin, μg/L	165 [93–292]	241 [157–376]	256 [147–413]	84 [57–134]$^{***\$\$\$}$	75 [49–115]$^{***\$\$\$\#}$
Iron, μg/L	98 (46)	115 (42)	110 (46)	75 (39)$^{***\$\$\$}$	72 (39)$^{***\$\$\$}$
Tsat, %	28 [19–40]	36 [28–45]	29 [23–39]***	19 [16–32]$^{***\$\$\$}$	17 [12–22]$^{***\$\$\$\#\#\#}$
STfR, mg/L	1.2 [1.0–1.7]	1.1 [0.9–1.2]	1.7 [1.6–2.0]***	1.1 [1.0–1.2]$^{***\$\$\$}$	2.0 [1.6–2.6]$^{***\$\$\$\#\#\#}$

Data are presented as mean (standard deviation), *n* (%), or median [interquartile range expressed as lower and upper quartiles].
ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; ID, iron deficiency; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; OR, odds ratio; sTfR, soluble transferrin receptor; Tsat, transferrin saturation.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ – group (1) vs. groups (2), (3), (4).
$^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ – group (2) vs. groups (3), (4).
$^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ – group (3) vs. group (4).

progression also interferes with iron metabolism,[29,30] which may limit the diagnostic accuracy of standard circulating biomarkers of iron status. In the study of Nanas *et al.*,[7] ID diagnosed in bone marrow of anaemic patients with decompensated advanced HF was not associated with reduced serum ferritin, which excluded it as a reliable marker of ID in these patients. Also Grote Beverborg *et al.*[43] questioned the value of serum ferritin for diagnosis of ID in patients with HF. We demonstrated that in patients with ischaemic HF, either serum ferritin, serum iron or Tsat had a limited accuracy for identifying depleted iron stores in bone marrow, regardless of the current status of erythropoiesis. Instead, circulating sTfR, a relatively novel emerging biomarker of iron status,[27,28] accurately predicted depleted iron stores in bone marrow in patients with ischaemic HF, even though iron status was tracked at the early stage without laboratory features of iron-restricted erythropoiesis.

Our study provides evidence that ID diagnosed based on high serum sTfR has an unfavourable impact on long-term survival in a cohort of patients with HF with LVEF ≤45%. Patients with sTfR ≥1.41 mg/L are those with the highest mortality and at the same time those with depleted iron in bone marrow. Serum sTfR not only abolished the prognostic value of haemoglobin, but also of other standard circulating biomarkers of iron status (serum ferritin, Tsat). Serum sTfR when added to the multivariable models markedly improved the 3-year survival prediction beyond established prognosticators, and its additive prognostic value was at least as good or even better than that of plasma NT-proBNP (even when the model included serum ferritin and Tsat). Importantly,

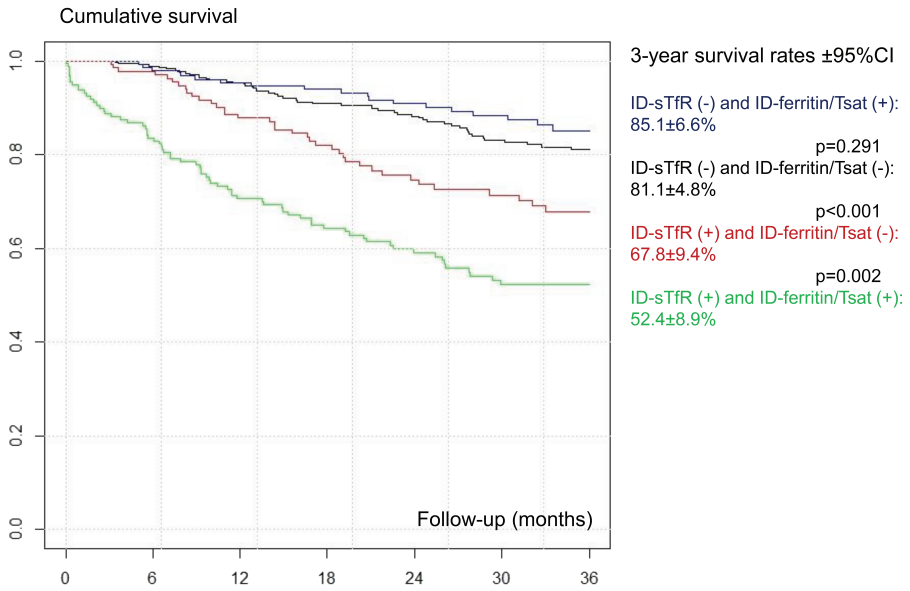

Figure 3 Kaplan–Meier curves reflecting adjusted 3-year cumulative survival rates in patients with heart failure, stratified according to presence or absence of iron deficiency (ID) defined using serum ferritin and transferin saturation (Tsat) and presence or absence of serum soluble transferrin receptor (sTfR) ≥1.41 mg/L. CI, confidence interval.

patients with both sTfR ≥1.41 mg/L and ID defined based on serum ferritin and Tsat had the highest 3-year mortality in the investigated cohort of patients with HF (*Figure 3*).

We need to acknowledge that sTfR has already been identified as a predictor of poor clinical outcomes in patients with acute HF (in the clinical scenario when inflammatory drive and oxidative stress predominate)[44,45] as well as a predictor of impaired exercise capacity in patients with HF,[46,47] but most likely we are tackling here the phenomenon which is valid across the whole spectrum of cardiovascular disease. High circulating sTfR has been identified as a strong independent predictor of long-term mortality in diabetic patients with coronary artery disease[48] and also a risk factor for myocardial infarction and cardiovascular death in patients with stable coronary artery disease.[49]

Therefore, we presume that we have been able to identify not just another biomarker whose prognostic value in patients with HF with LVEF ≤45% is at least similar (or even better) compared to plasma NT-proBNP, but we have distinguished the abnormality, which is at least as detrimental as the neurohormonal activation for survival in these patients. Traditionally, high circulating sTfR was a measure of ineffective erythropoiesis due to depleted iron within the erythron, later it was considered as a measure of depleted intracellular iron which is required for metabolic needs. Taking into consideration close links between intracellular iron depletion and defected energy metabolism, one may hypothesize that high circulating sTfR could depict disturbed cellular metabolic homeostasis (related or not with depleted iron). Importantly, there is a direct association between an increased sympathetic activation reflected by high circulating norepinephrine and increased iron demand for cellular metabolism identified by high circulating sTfR.[50] In this context, it should be emphasized that, beyond erythropoiesis, iron is involved in numerous biological processes critical for maintenance of homeostasis, and is critical for functioning and survival across all levels of complexity of living structures.[23,42,51–57] Iron plays a crucial role in oxygen transport (haemoglobin component), oxygen storage (myoglobin component), cardiac and skeletal muscle metabolism (component of oxidative enzymes and respiratory chain proteins), synthesis, and degradation of proteins, lipids, ribonucleic acids (enzyme component), and mitochondrial function.[51–57] Normal iron metabolism is particularly critical for the optimal cellular energy generation and utilization, and ID impairs primarily the functioning of cells of high energy demand (such as cardiomyocytes).[23,52–55] This seems to be particularly important in the context of HF, as abnormal energy generation and utilization in the myocardium and the peripheral tissues (e.g. skeletal muscles) contribute to HF pathophysiology.[56,58–63]

Clinical implications

Current guidelines for the management of HF provide only rough recommendations for the evaluation of iron status and for potential repletion of ID in iron-deficient patients with HF.[64] Our study clearly demonstrates that the vast majority of patients with ischaemic HF with LVEF ≤45% have significantly depleted iron stores in bone marrow. There is a need for accurate non-invasive testing of iron status in these patients, as ID likely may negatively affect clinical course,[3–5,9] and iron supplementation may be an attractive therapeutic option.[13–17,22] The best candidate as a screening tool for ID in these patients is serum sTfR.

Conclusions

High serum sTfR accurately reflects depleted iron stores in bone marrow in patients with ischaemic HF with LVEF ≤45%, and allows to identify those with high mortality during 3-year follow-up. Importantly, prognostic effects of high sTfR are independent of other prognosticators reflecting neurohormonal activation (NT-proBNP) and iron status assessed in a traditional way (ferritin, Tsat).

The presented results constitute premises that high serum sTfR could serve as an inclusion criterion indicating ID and indication for iron supplementation in future clinical trials. The assessment of sTfR might serve as a tool for monitoring this therapy, which, given the association of abnormal iron markers with mortality, might be expected to improve patient prognosis. However, all these presumptions need to be prospectively verified.

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