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ORIGINAL RESEARCH

Iron Metabolism Contributes to Prognosis in Coronary Artery Disease: Prognostic Value of the Soluble Transferrin Receptor Within the AtheroGene Study

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BACKGROUND: Coronary heart disease is a leading cause of mortality worldwide. Iron deficiency, a frequent comorbidity of coronary heart disease, causes an increased expression of transferrin receptor and soluble transferrin receptor levels (sTfR) levels, while iron repletion returns sTfR levels to the normal physiological range. Recently, sTfR levels were proposed as a potential new marker of iron metabolism in cardiovascular diseases. Therefore, we aimed to evaluate the prognostic value of circulating sTfR levels in a large cohort of patients with coronary heart disease.

METHODS AND RESULTS: The disease cohort comprised 3423 subjects who had angiographically documented coronary heart disease and who participated in the AtheroGene study. Serum levels of sTfR were determined at baseline using an automated immunoassay (Roche Cobas Integra 400). Two main outcomes were considered: a combined end point of myocardial infarction and cardiovascular death and cardiovascular death alone. During a median follow-up of 4.0 years, 10.3% of the patients experienced an end point. In Cox regression analyses for sTfR levels, the hazard ratio (HR) for future cardiovascular death and/or myocardial infarction was 1.27 (95% CI, 1.11–1.44, $P<0.001$) after adjustment for sex and age. This association remained significant (HR, 1.23; 95% CI, 1.03–1.46, $P=0.02$) after additional adjustment for body mass index, smoking status, hypertension, diabetes mellitus, dyslipidemia, C-reactive protein, and surrogates of cardiac function, size of myocardial necrosis (hs-TnI), and hemoglobin levels.

CONCLUSIONS: In this large cohort study, sTfR levels were strongly associated with future myocardial infarction and cardiovascular death. This implicates a role for sTfR in secondary cardiovascular risk prediction.

Key Words: biomarker ■ coronary artery disease ■ iron ■ prognosis ■ soluble transferrin receptor

Iron is the most prevalent inorganic electron acceptor in the body and thus is central for oxygen use and transport, which are required to maintain mitochondrial functions.[1] Because the cardiac tissue has a high energy consumption and requires high mitochondrial activity, maintaining iron metabolism is a major issue for cardiovascular health. Previously, it has been demonstrated that patients with heart failure, or acute myocardial infarction (MI), and concomitant iron deficiency have an increased risk of future cardiovascular events. Treating those patients with intravenous iron, which has now emerged as a guideline-endorsed

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CLINICAL PERSPECTIVE

What Is New

- Soluble transferrin receptor levels are strongly associated with future myocardial infarction or cardiovascular death in a large cohort study.

What Are the Clinical Implications?

- These results strengthen the available evidence showing an association between soluble transferrin receptor levels and cardiovascular death and myocardial infarction.
- This further emphasizes the important role of iron metabolism in cardiovascular disease.

Nonstandard Abbreviations and Acronyms

BMI	body mass index
CHD	coronary heart disease
CRP	C-reactive protein
CVD	cardiovascular disease
HR	hazard ratio
hsTnI	high-sensitivity troponin assays
MI	myocardial infarction
NT-proBNP	N-terminal pro B-type natriuretic peptide
sTfR	soluble transferrin receptor

therapy, improves their exercise capabilities while reducing inflammation and biomarkers of heart dysfunction.[2–6] Ferritin and transferrin saturation are routinely measured to assess iron metabolism and have already demonstrated a predictive value for inflammatory responses, cardiovascular disease (CVD), and cardiovascular mortality.

Intracellular iron intake occurs via the transferrin receptor (TfR) that allows the internalization of iron-bound transferrin. Soluble transferrin receptor (sTfR) is a truncated form of the transferrin receptor 1 (TfR)[7] that is released into the circulation. When iron requirements increase, as is the case in iron deficiency, TfR receptors are overexpressed at the cells surface and an increasing amount of sTfR is shed into the circulation. Iron repletion then brings sTfR levels back to normal physiological levels.[8,9] Therefore, increased level of sTfR reflects iron demands throughout the body, rather than iron storage. While sTfR has already been shown to be a more sensitive biomarker than ferritin or transferrin saturation regarding iron deficiency, its role in CVD remains uncertain. This study aimed to evaluate whether circulating sTfR levels, as biomarker of intrinsically available iron, can also be an efficient marker for subsequent coronary events in patients with established coronary heart disease (CHD).

MATERIALS AND METHODS

Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to the corresponding author (m.Karakas@uke.de).

Study Population

Three thousand eight hundred patients, who underwent coronary angiography, were recruited in the AtheroGene Study at the Department of Medicine II of the Johannes Gutenberg-University Mainz or the Bundeswehr-Zentralkrankenhaus Koblenz, from June 1999 to March 2004.[10] Exclusion criteria included known cardiomyopathy, hemodynamically significant valvular heart disease, surgery, or trauma within the previous month, cancer, febrile conditions, or use of oral anticoagulant therapy within a 4-week time period before recruitment. Missing information on the clinical presentation, or on the cause of death were also among the exclusion criteria, resulting in a final number of 3423 patients with CHD selected for measurements. After removing subjects with low sample volume or missing samples, sTfR levels were measured in 2333 patients. The subcohort (Table 1) and the overall CHD cohort had no relevant differences in baseline characteristics.

All patients gave written informed consent. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Board of the Johannes Gutenberg-University Mainz (approval number 837.057.99) and of the Physicians' chamber of the State Rhineland-Palatinate (Germany).

Data Collection

All participants were subjected at baseline to a standardized questionnaire including medical history and sociodemographic information. Information was also taken from the patients' hospital charts. Coronary angiogram showing at least 1 stenosis of >30% in a major coronary artery was used to diagnose coronary artery disease. Acute coronary syndrome included unstable angina pectoris and acute MI. Unstable angina pectoris was diagnosed in accordance with Braunwald criteria.[11] Acute myocardial infarction was stratified between ST-segment–elevation myocardial infarction with significant elevation in at least 2 contiguous leads, and

Table 1. Baseline Characteristics of the Study Patients

Variable	n=2333
Age, y*	63.0 (56.0, 70.0)
Male sex [%]	1789 [76.7]
BMI, kg/m²*	27.1 (25.0, 30.0)
Current smoker [%]	550 [23.6]
Diabetes mellitus [%]	511 [21.9]
Hypertension [%]	1779 [76.3]
Hyperlipidemia [%]	1668 [71.5]
History of MI [%]	964 [41.3]
eGFR, mL/min for 1.73 m²*	84.5 (70.3, 95.4)
Total cholesterol, mg/dL*	198.0 (168.2, 227.0)
HDL-C, mg/dL*	47.0 (40.0, 57.0)
LDL-C, mg/dL*	123 (97.0, 149.0)
Triglycerides, mg/dL*	128.0 (94.0, 180)
Creatinine, mg/dL*	1.0 (0.8, 1.1)
NT-proBNP, pg/mL*	215 (94, 709.9)
CRP, mg/dL*	3.5 (1.5, 9.4)
Hemoglobin, g/dL*	14.3 (13.3, 15.1)
hs-TnI, ng/L*	8.9 (4.0, 65.5)
sTfR, mg/L*	2.2 (1.7, 2.9)

BMI indicates body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein-cholesterol; hs-TnI, high-sensitivity troponin assays; LDL-C, low-density lipoprotein-cholesterol; MI, myocardial infarction; NT-proBNP, N-terminal pro B-type natriuretic peptide; and sTfR, soluble transferrin receptor.

*Median (25th, 75th quartile cut point).

non-ST-segment–elevation myocardial infarction based on clinic and positive in-house hs-TnI levels.

Active follow-up was performed for all patients until a median time of 4.0 years after discharge. Data regarding adverse CVD events and treatment since discharge from the in-hospital rehabilitation clinic were provided by the primary care physicians in addition to standardized questionnaire. In case of patient death during follow-up, the death certificate was collected from the local Public Health Department. The main cause of death was then coded according to the *International Classification of Diseases, Ninth and Tenth Revisions* (*ICD-9* pos. 390–459: *ICD-10* pos. I0-I99 and R57.0).

Laboratory Methods

Blood was drawn before angiography at baseline under standardized conditions, including fasting state, and stored at −80°C until the time of analysis. Levels of sTfR were determined by an automated immunoassay (Roche Cobas Integra 400). The coefficient of variations for the inter-assay and intra-assay were 8.8% and 2.4%, respectively. Levels of CRP (C-reactive protein), high-sensitivity troponin assays (hsTnI), NT-proBNP (N-terminal pro B-type natriuretic peptide), total cholesterol, high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol were measured using routine methods in the participating hospitals.[12,13] All measurements were performed in a blinded fashion.

Statistical Analysis

The population study was described using a wide range of sociodemographic and medical characteristics using absolute and relative frequencies for binary variables and quartiles for continuous variables. Spearman correlations between sTfR and selected variables were computed. The 2 considered end points were (1) cardiovascular death or MI, and (2) cardiovascular death. Their association with sTfR was examined first by means of survival curves according to sTfR categories defined using tertiles. The curves were produced using the Kaplan–Meier method and the equality of the curves was tested via the logrank test. Then adjusted associations were examined via Cox regression analyses considering different models: model 1 was adjusted for age and sex, model 2 was additionally adjusted for body mass index (BMI), diabetes mellitus, hypertension, smoking status, and dyslipidemia. Model 3 was additionally adjusted for log (NT-proBNP), log (hs-TnI), and hemoglobin. Model 4 was additionally adjusted for log(CRP). Finally, model 5 was additionally adjusted for iron levels and ferritin. The models were expanded by including interaction terms between sTfR and the following variables (if already present in the model): age, sex, hypertension, diabetes mellitus, BMI, and hemoglobin. A single global test of interaction was performed for each model. The proportional hazards assumption of the Cox models was examined graphically using the methods described by Grambsch and Thernau. No evidence of violation was found.[14] Harell's C-indices[15] for models 1 to 5 were computed using the 5-year predicted probability of an event and compared with the C-index of the corresponding model after removing sTfR from the predictors. Fivefold cross-validation was used for these computations. The computations for models 1 to 4 were repeated substituting sTfR by the sTfR/ferritin, which was also used after log-transformation in the models.

Statistical procedures were carried out using R 3.6.1 (http://www.r-project.org/). Survival analyses, including the C-indices computations, were performed using the *survival* R package. Statistical significance was set to a $P<0.05$.

RESULTS

Baseline characteristics of the patients displayed a typical CVD cohort pattern (Table 1). They had a median age of 63.0 years and predominant male sex (76.7%).

The median sTfR level was 2.2 mg/L (25/75 percentiles: 1.7; 2.9) with a right-skewed distribution (Figure 1). Missing values for each variable are illustrated in Figure S1. Correlations between sTfR levels and other variables were negligible (<0.2), although correlation with age, male sex, diabetes mellitus, BMI, high-sensitivity CRP, hs-TnI, and NT-proBNP formally reached statistical significance (Table S1).

During a median follow-up of 4.0 years, 10.3% of the subjects had a nonfatal MI and/or cardiovascular death.

Survival curves for cardiovascular death or MI according to tertiles of sTfR levels at baseline supported the prognostic value of sTfR levels (P<0.001), with high levels being associated with worst prognosis (Figure 2).

In Cox regression analyses, the hazard ratio (HR) for future cardiovascular death or MI per 1 SD increase of sTfR levels was 1.27 after adjustment for sex and age (Model 1: HR, 1.27 [95% CI, 1.11–1.44], P<0.001), Table 2. This association did not change after additional adjustment for BMI, smoking status, hypertension, diabetes mellitus, and dyslipidemia (Model 2: HR, 1.26 [95% CI, 1.11–1.43], P<0.001). The association was only slightly attenuated after further adjustment for hemoglobin, high-sensitivity CRP, NT-proBNP, and hs-TnI (Model 4: HR, 1.23 [95% CI, 1.03–1.46], P=0.021). Finally, adjustment for ferritin and iron levels did not affect the values further (model 5: HR, 1.23, 95% CI, 1.01–1.50).

We also performed Cox regression analyses of HR for future cardiovascular death per 1 SD increase of sTfR levels. The HR was 1.31 after adjustment for sex and age ([95% CI, 1.10–1.56], P=0.002) (Table 3). This association was slightly attenuated after additional adjustment for BMI, smoking status, hypertension, diabetes mellitus, and dyslipidemia (HR, 1.29 [95% CI, 1.09–1.54], P=0.003). The association was not significant upon further adjustment for surrogates of inflammation (high-sensitivity CRP), cardiac function NT-proBNP, size of myocardial necrosis (hsTnI), and hemoglobin levels (anemia), although an increased HR trend was still observed (HR, 1.18 [95% CI, 0.93–1.50], P=0.16). Finally, adjustment for ferritin and iron levels did not relevantly affect the values (model 5: HR, 1.20, 95% CI, 0.91–1.57). The C-indexes were also calculated, with only marginal differences and most P values for the corresponding comparison coming up as nonsignificant (Table S2).

Both Cox regression for cardiovascular death alone or cardiovascular death or MI were also performed for log(sTfR/ferritin) ratio (Tables S3 and S4). The HR for future cardiovascular death or MI per 1 SD increase of sTfR levels was 1.16 after adjustment for sex and age (Model 1: HR, 1.16 [95% CI, 1.03–1.31], P<0.017)

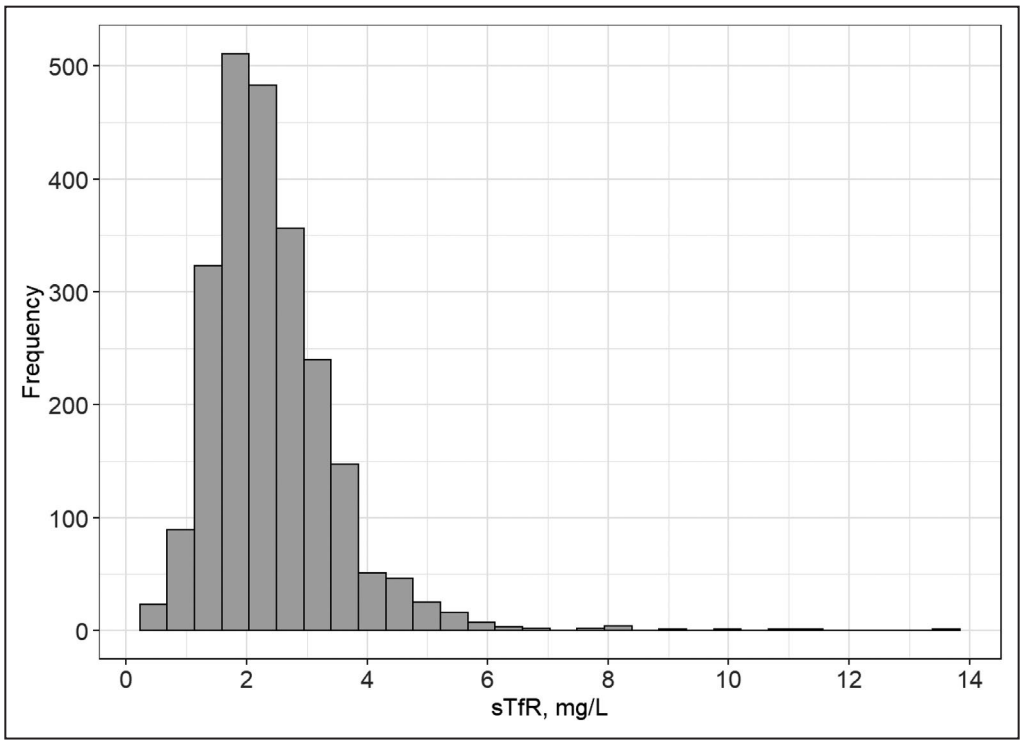

Figure 1. Distribution of levels of the sTfR in the AtheroGene Study (n=2333).
Right-skewed distribution of sTfR levels. Median sTfR level=2.2 mg/L (25/75 percentiles: 1.7; 2.9). sTfR indicates soluble transferrin receptor.

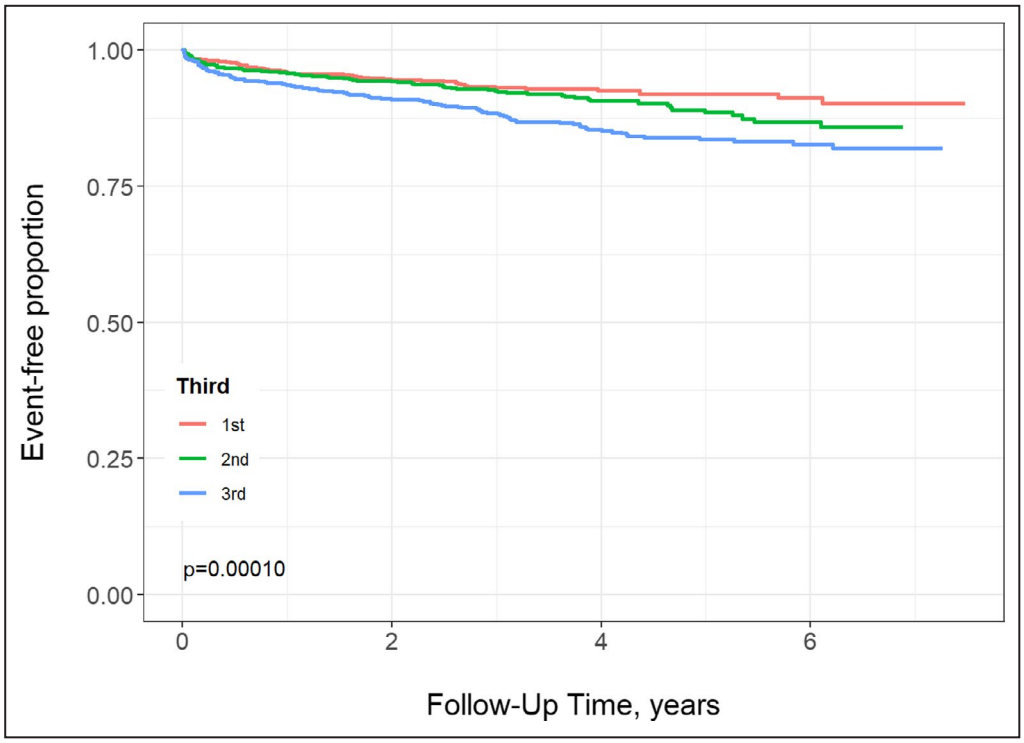

Figure 2. Kaplan–Meier analysis according to baseline sTfR levels.
Survival curves for cardiovascular death or MI according to tertiles of sTfR levels at baseline. Elevated sTfR levels are associated with worst prognosis (P<0.001). MI indicates myocardial infarction; and sTfR, soluble transferrin receptor.

and was only mildly increased upon further adjustment (Table S3). The HR for future cardiovascular death alone was 1.19 after adjustment for sex and age (Model 1: HR, 1.19 [95% CI, 1.01–1.41]). HR value did not vary significantly upon further adjustment (Table S4).

DISCUSSION

Because of the high energy consumption of the heart, it is important to maintain an efficient iron metabolism in cardiovascular medicine. Recent studies highlighted sTfR as a most auspicious new marker of iron metabolism, and its dysfunction, rising before the development of anemia, as a potential early indicator of CVD.[16]

In this study, we sought to elucidate the value of circulating sTfR levels to predict cardiovascular death and nonfatal MI in a large prospective cohort of patients with manifest CHD. We found a significant and independent association between sTfR levels and risk of incident MI or cardiovascular death. This association was consistent throughout all the

Table 2. Association of sTfR Levels (log [sTfR] Per 1 SD Increase) With Cardiovascular Death or Nonfatal MI During 4 Years of Follow-Up

Model	HR	95% CI	*P* Value	Global Interaction *P* Value	N	Events	EPV
#1	1.27	1.11–1.44	<0.001	0.063	2333	240 (10.2%)	80
#2	1.26	1.11–1.43	<0.001	0.33	2332	240 (10.2%)	30
#3	1.24	1.04–1.48	0.014	0.69	1693	132 (7.8%)	12
#4	1.23	1.03–1.46	0.021	0.69	1669	132 (7.9%)	11
#5	1.23	1.01–1.50	0.041	0.28	1388	113 (8.1%)	8.1

Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for hypertension, smoking status, diabetes mellitus, hyperlipidemia, BMI. Model 3: Model 2 additionally adjusted for hemoglobin, log (NT-proBNP), log (hs-TnI). Model 4: Model 3 additionally adjusted for log (C-reactive protein). Model 5: Model 4 additionally adjusted for iron levels and ferritin. For the variables age, sex, hypertension, diabetes mellitus, BMI, and hemoglobin (if present in a particular model), a single global test of interaction with sTfR was performed. BMI indicates body-mass index; EPV, number of events per variable; Events, number of events (% of subjects affected by an event); HR, hazard ratio; hs-TnI, high-sensitivity troponin assays; MI, myocardial infarction; N, number of subjects; N-terminal pro B type natriuretic peptide; and sTfR, soluble transferrin receptor.

Table 3. Association of sTfR Levels (Log [sTfR] Per 1 SD Increase) With Cardiovascular Death During 4 Years of Follow-Up

Model	HR	95% CI	*P* Value	Global Interaction *P* Value	N	Events	EPV
#1	1.31	1.10–1.56	0.002	0.89	2333	128 (5.5%)	42.7
#2	1.29	1.09–1.54	0.003	0.24	2332	128 (5.5%)	16
#3	1.22	0.97–1.54	0.087	0.32	1693	71 (4.2%)	6.5
#4	1.18	0.93–1.50	0.16	0.27	1669	71 (4.3%)	5.9
#5	1.20	0.91–1.57	0.19	0.20	1388	58.5 (4.1%)	4.1

Model 1: adjusted for age and sex. Model 2: Model 1 additionally adjusted for hypertension, smoking status, diabetes mellitus, hyperlipidemia, BMI. Model 3: Model 2 additionally adjusted for hemoglobin, log (NT-proBNP), log (hs-TnI). Model 4: Model 3 additionally adjusted for log (C-reactive protein). Model 5: Model 4 additionally adjusted for iron levels and ferritin. For the variables age, sex, hypertension, diabetes mellitus, BMI, and hemoglobin (if present in a particular model) a single global test of interaction with sTfR was performed. BMI indicates body-mass index; EPV, number of events per variable; Events, number of events (% of subjects affected by an event); HR, hazard ratio; N, number of subjects; and sTfR, soluble transferrin receptor.

used models of adjustments. In order to focus the exploration of association with clinically relevant end points to a more severe setting, we performed the same independent association analysis for the single end point cardiovascular death. Despite the fact that the event rate was consequently decreased by 50%, multiple adjustments in our models still returned significant associations. Taken together, our data add to previous reports that showed a prognostic value of sTfR levels even in the absence of anemia (eg, at physiological hemoglobin levels),[17] and implicate a potential role for sTfR in secondary cardiovascular risk prediction. In addition, consistent with previous results showing sTfR levels to be unaffected by inflammation, our Cox regression results did not show a major attenuation of the association after adjustment for CRP.[16] Those results are also in line with the results found in the LURIC (Ludwigshafen Risk and Cardiovascular Health) Study where sTfR was found to have a j-shaped association with cardiovascular and total mortality. However, the authors only found a marginal association between sTfR and angiographic coronary artery disease within the LURIC study.[17,18] Models used were comparable with the exception of the log (hs-TnI) adjustment in our models.

sTfR: A Sensitive and Easy-to-Access Marker of Iron Deficiency

The transferrin receptor (TfR) is crucial for cellular iron influx. As a transmembrane protein, TfR binds with high affinity to iron-bound transferrin, allowing its internalization, and iron storage via ferritin and energy production via the mitochondria.[19] Therefore, the levels of TfR receptor at the cell surface reflect the intracellular iron needs. Furthermore, the level of sTfR, a truncated 85-kDa version of the receptor that circulates in the blood, is directly associated with the levels of membrane TfR and therefore reflects the overall body TfR expression.[20] Thus, sTfR levels are an easy-to-access indicator of the whole-body iron storage, most of which, along with TfR expression, is located in the bone marrow erythroid precursors (to sustain erythropoiesis).[21] Not surprisingly, sTfR levels have also been previously demonstrated to be positively correlated with erythroid precursor mass.[22] Thus, circulating sTfR levels quantitatively reflect both iron demand of the body and the erythroid proliferation rate.

Pathophysiological Rationale Behind sTfR Predicting Secondary Events

In epidemiological studies, altered sTfR levels have been linked with anemia, insulin resistance, diabetes mellitus, and poor outcome in diabetic patients with CVD.[8,23,24] Likewise, various experimental studies reported strongly raised sTfR levels during hypoxia and ischemia.[25] Experimental studies have suggested that sTfR might mirror hypoxia and that sTfR levels increase at very early stages of iron deficiency.[25] Iron deficiency, classically diagnosed using ferritin levels—and also transferrin saturation in cardiovascular settings—has long been known to result in impaired prognosis.[5] Our findings are in line with epidemiological studies, which were mostly performed in primary-prevention or in community-based settings, and which also reported sTfR to be associated with predictors of CVD and very early stage of the disease, such as arterial hypertension, metabolic syndrome, aortic stiffness, or other measures of subclinical atherosclerosis.[7]

Based on the data currently available in medical literature, we hypothesize that iron deficiency, indicated by elevated sTfR levels, impairs 3 major pathophysiologic pathways of CVD: (1) cardiomyocyte survival and apoptosis, (2) inflammation, and (3) remodeling. Merle and co-workers indeed have shown in a rat model of hypoxia, but also in an acute MI model, that myocardial hypoxia and ischemia cause protective upregulation of myocardial iron.[26,27] This in turn decreases apoptosis and enhances cardiomyocyte survival.[28] Moreover, it is known that upon intravenous iron administration in

acute MI, macrophages in infarcted heart shift their immunological profile towards anti-inflammatory phenotypes and thereby exert beneficial effects in these patients.[2] Lastly, because it is known that iron is essential in terms of mitochondrial function, this becomes even more evident in CVD and acute MI: mice with cardiomyocyte-targeted deletion of iron-regulatory proteins are unable to increase heart function in response to catecholamine and develop more severe left-ventricular dysfunction with increased mortality after acute MI than wild-type mice.[29] Importantly, intravenous injection of iron in these mice replenished cardiac iron stores, restored mitochondrial respiratory capacity and inotropic reserve, and attenuated adverse remodeling.[29] With sTfR levels being elevated in iron-deficient subjects, these beneficial mechanisms might fall short in acute MI and thereby impair short- and mid-term prognosis of affected patients. Of note, iron-laden macrophages are hypothesized to destabilize atherosclerotic plaques,[30] thus increasing the risk for plaque rupture, the main cause of MI. Iron deficiency could with this regard contribute to plaque stability. However, our current results would suggest this potential protective effect towards MI is dwarfed by deleterious mechanisms such as those described above, because in this cohort, high levels of sTfR are associated with future MI.

Strengths and Limitations of the Study

Previous studies assessing the association between sTfR levels and CVD were limited either by small sample size, cross-sectional design, testing in primary-prevention settings and interpretation of results in manifest disease settings, or by missing adjustment for potential confounders.[8,23,25]

The strengths of our study include the diagnosis of coronary artery disease that was exclusively based on coronary angiography, a large cohort of 2333 individuals, and a long-term follow-up of 4 years. Therefore, association between sTfR levels and the combined end point of MI and cardiovascular death could be found statistically significant, even in our strictest models. This association was also confirmed for cardiovascular death alone.

Important limitations are (1) the use of NT-proBNP as a surrogate for heart function, since routine echocardiography measures were not applied within the protocol of this study; (2) that, as in most coronary artery disease populations, women were clearly underrepresented; and (3) that Cox regression analyses should be interpreted with caution, because of a possible overadjustment, as discussed by Schisterman et al.[31] Hence, the association of sTfR levels in some of the models used adjusted for up to 11 variables (models 3 and 4), respectively, and did not reach statistical significance. This can be explained by the sharp decrease of number of events in these models combined with more strict variables adjustment.

CONCLUSIONS

In this study, to date the largest to evaluate the predictive value of sTfR levels in patients with manifest CHD, levels of sTfR were strongly associated with future MI and/or cardiovascular death. This implicates a potential role for iron metabolism in secondary cardiovascular risk prediction. Based on these findings, we strongly emphasize further evaluation of circulating sTfR as a marker of incident CHD in primary and secondary prevention settings.

ARTICLE INFORMATION

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Disclosures

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Supplementary Materials

Tables S1–S4
Figure S1

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Supplemental Material

Table S1. Spearman correlation coefficients between sTfR and variables of interest.

Variable	Correlation with sTfR	p-Value
Age	0.11	*<0.001*
Male sex	-0.06	*0.0072*
Smoking	-0.04	*0.039*
Diabetes	0.06	*0.0021*
Hypertension	0.01	0.65
History of MI	0.00	0.90
Dyslipidemia	-0.04	0.065
Body-mass-index	0.06	*0.0028*
Hemoglobin	-0.01	0.53
C-reactive protein	0.15	*<0.001*
hs-TnI	0.10	*<0.001*
NT-proBNP	0.16	*<0.001*

MI = myocardial infarction, NT-proBNP = N-terminal pro brain natriuretic peptide, sTfR = soluble transferrin receptor, hs-Tnl = high sensitivity troponin.

Table S2. C-indices for all considered models for CV death or nonfatal MI.

Model	C-index (95% CI) Base model	C-index (95% CI) Base model + log(sTfR)	C-index difference (95% CI)	p-value	N
Age+Male	0.55 (0.52, 0.59)	0.59 (0.54, 0.63)	0.031 (0.002, 0.059)	0.025	2333
Age+Male+Hypertension+Current smoker+Diabetes+Dyslipidemia+BMI	0.58 (0.54, 0.62)	0.61 (0.57, 0.65)	0.025 (-0.004, 0.047)	0.022	2332
Age+Male+Hypertension+Current smoker+Diabetes+Dyslipidemia+BMI+Hb+log(NT-proBNP)+log(hs-TnI)	0.63 (0.58, 0.68)	0.64 (0.59, 0.69)	0.010 (-0.008, 0.028)	0.28	1693
Age+Male+Hypertension+Current smoker+Diabetes+Dyslipidemia+BMI+Hb+log(NT-proBNP)+log(hs-TnI)+log(CRP)	0.63 (0.58, 0.68)	0.64 (0.59, 0.69)	0.008 (-0.009, 0.025)	0.33	1669
Age+Male+Hypertension+Current smoker+Diabetes+Dyslipidemia+BMI+Hb+log(NT-proBNP)+log(hs-TnI)+log(CRP)+Iron+Ferritin	0.62 (0.57, 0.68)	0.63 (0.57, 0.68)	0.003 (-0.016, 0.022)	0.76	1388

Table S3. Association of log(sTfR/ferritin) ratio with CV death and/ or nonfatal MI during 4 years of follow-up.

Model	HR	95% CI	P-value	Global interaction P-value	N	events	EPV
#1	1.16	1.03-1.31	*0.017*	0.19	2320	239	79.7
#2	1.16	1.02-1.31	*0.019*	0.36	2319	239	29.9
#3	1.21	1.03-1.43	0.018	0.33	1683	131	11.9
#4	1.23	1.05-1.45	0.012	0.33	1659	131	10.9

SD = standard deviation, CV = cardiovascular, HR = hazard ratio, CI = confidence interval, BMI = body-mass-index, sTfR = soluble transferrin receptor, N= number of subjects. Events: number of events (% of subjects affected by an event), EPV = number of Events Per Variable

Model 1: adjusted for age and sex

Model 2: Model 1 additionally adjusted for hypertension, smoking status, diabetes, hyperlipidemia, BMI

Model 3: Model 2 additionally adjusted for hemoglobin, log (NT-proBNP), log (hs-TnI)

Model 4: Model 3 additionally adjusted for log (C-reactive protein)

Table S4. Association of log(sTfR/ferritin) ratio with CV death during 4 years of follow-up.

Model	HR	95% CI	P-value	Global interaction P-value	N	events	EPV
#1	1.19	1.01-1.41	*0.041*	0.12	2320	127	42.3
#2	1.18	1.00-1.40	*0.045*	0.11	2319	127	15.9
#3	1.14	0.91-1.42	0.24	0.028	1683	70	6.4
#4	1.18	0.94-1.48	0.15	0.035	1659	70	5.8

SD = standard deviation, CV = cardiovascular, HR = hazard ratio, CI = confidence interval, BMI = body-mass-index, sTfR = soluble transferrin receptor, N= number of subjects. Events: number of events (% of subjects affected by an event), EPV = number of Events Per Variable

Model 1: adjusted for age and sex

Model 2: Model 1 additionally adjusted for hypertension, smoking status, diabetes, hyperlipidemia, BMI

Model 3: Model 2 additionally adjusted for hemoglobin, log (NT-proBNP), log (hs-TnI)

Model 4: Model 3 additionally adjusted for log (C-reactive protein)

Figure S1. Proportion of missing values in each variable.