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Item Type	Article
Authors	Stack, Austin G.;Mutwali, A.I;Nguyen, H.T;Cronin, Cornelius J.;Casserly, Liam F.;Ferguson, John P.
Citation	QJM: An International Journal of Medicine;107, pp. 623-633
Publisher	Oxford University Press
Download date	2026-06-08 02:36:27
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Link to Item	https://hdl.handle.net/10344/4861

Transferrin saturation ratio and risk of total and cardiovascular mortality in the general population

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Received 21 November 2013 and in revised form 18 February 2014

Summary

Background: The transferrin saturation (TSAT) ratio is a commonly used indicator of iron deficiency and iron overload in clinical practice but precise relationships with total and cardiovascular mortality are unclear.

Purpose: To better understand this relationship, we explored the association of TSAT ratio (serum iron/total iron binding capacity) with mortality in the general population.

Methods: The relationships of TSAT ratio with total and cardiovascular mortality were explored in 15 823 subjects age 20 and older from the Third National Health and Nutrition Examination Survey (1988–94). All subjects had vital status assessed through to 2006.

Results: During follow-up, 9.7% died of which 4.4% were from cardiovascular disease. In unadjusted analysis, increasing TSAT ratio was inversely associated with mortality. With adjustment for baseline demographic and clinical characteristics, the

TSAT–mortality relationship followed a j-shaped pattern. Compared with the referent group [ratio 23.7–31.3%: hazard ratio (HR) =1.00], subjects in the lowest two quartiles, <17.5 % and 17.5–23.7 %, experienced significantly higher mortality risks of 1.45 (1.19–1.77) and 1.27 (1.06–1.53), respectively, whereas subjects in the highest quartile, >31.3 %, experienced significantly higher mortality risks of 1.23 (1.01–1.49). The pattern of association was more pronounced for cardiovascular mortality with significantly higher mortality risks for the lowest two quartiles [HR = 2.09 (1.43–3.05) and 1.90 (1.33–2.72), respectively] and highest quartile HR = 1.59 (1.05–2.40).

Conclusions: Both low and high TSAT ratios are significantly and independently associated with increased total and cardiovascular mortality. The optimal TSAT ratio associated with the greatest survival is between 24% and 40%.

Introduction

Serum transferrin saturation (TSAT) ratio is a commonly used laboratory measure of iron deficiency and iron overload in clinical practice.^{1,2} It has become a first step in the routine screening of iron deficiency anaemia in patients with chronic kidney disease and for the detection of pathological iron

overload in assessment for hemochromatosis.^{3–6} Used alone or in combination with other measures of iron metabolism, low levels of TSAT (typically <20%) reflect a state of iron deficiency whereas levels in excess of 50% indicate an excess of total body iron. Despite its increasing use in clinical practice, few studies have addressed the association of

TSAT with mortality in the general population.^{7,8} Such studies are important in determining the optimal range for TSAT in clinical practice.

Two previous epidemiological studies have examined the relationship of TSAT ratio with mortality. An earlier report based on analysis of data from the First National Health and Nutrition Examination Survey (NHANES 1) Epidemiologic Follow-up Study found a significant inverse association of TSAT with overall and cardiovascular mortality but only for white men and women.⁷ A more recent study by Mainous *et al.*⁸ demonstrated elevated mortality risks for TSAT levels >55%. These seemingly discordant findings leave several unanswered questions as to the true association between TSAT ratio and the risk of death. On one hand, there is a strong biological argument to suggest that iron deficiency may theoretically increase mortality risk,^{1,9} whereas on the other hand excess iron stores contribute to pathological iron overload resulting in parenchymal liver damage, cardiac abnormalities and diabetes mellitus.^{10–13}

The purpose of this study was to (i) re-examine the nature of the relationship between TSAT ratio and mortality in a nationally representative cohort of the US population, (ii) identify threshold values for mortality risks and (iii) explore interactions with other disease states that may have significant clinical implications.

Methods

The NHANES III was a national survey conducted by the National Center for Health Statistics that assessed the health status of a representative sample of non-institutionalized persons living in the USA from 1988 to 1994.^{14,15} The NHANES III used a complex stratified multistage sampling design with over-sampled vulnerable groups including the elderly and minority populations. The data collection instrument consisted of a standardized questionnaire followed by a detailed physical examination that included collection of blood specimens. Almost all NHANES participants were linked to records in the National Death Index through 31 December 2006.¹⁵ Only 26 of the 20 024 eligible participants (0.1%) did not have follow-up status. Ethical approval for the study was obtained from the Ethics Review Board of the National Centre for Health Statistics and further ethical approval for use of NHANES data that is freely available on the web is not required as it is anonymized.

Sample

The current analysis was restricted to adult NHANES III participants, 20 years of age or older ($N=17\,030$) who had valid serum creatinine measurements ($N=15\,823$). Serum creatinine values were used to determine the estimated glomerular filtration rate (eGFR) in ml/min per 1.73 m², for all participants.¹⁶

Baseline measurements

The NHANES III captured data on demographic factors, self-reported clinical conditions, lifestyle factors, socioeconomic indicators, physical attributes and an extensive range of laboratory biomarkers. Blood samples were obtained from non-fasting persons and frozen serum sent to the Centers for Disease Control and Prevention for analysis. Serum iron and total iron binding capacity (TIBC) were measured colorimetrically (Alpkem RFA analyzer, Clackamas, OR), and 1% thiourea was added to complex copper to prevent copper interference.^{17,18} TSAT saturation was computed from the serum iron (Fe) divided by the TIBC. Serum ferritin was measured with the BioRad Quantimmune IRMA kit (BioRad Laboratories, Hercules, CA). Haemoglobin was measured using a Coulter S-Plus Jr electronic counter (Coulter Electronics, Hialeah, FL). Serum creatinine concentrations were measured by the modified kinetic Jaffe reaction using a Hitachi 737 analyzer (Boehringer Mannheim Corp., Indianapolis, IN) and glomerular filtration rate was estimated from the abbreviated Modification of Diet in Renal Disease (MDRD) Study formula.^{19,20}

Assessment of all-cause and cardiovascular mortality

Deaths were analysed for all causes and cardiovascular causes. Cardiovascular causes of death were identified from the International Classification of Diseases (ICD 10) diagnosis codes in the NHANES-linked mortality files and included: deaths from acute myocardial infarction (121–122), other acute ischaemic heart disease (124), atherosclerotic cardiovascular disease (125.0), all other forms of chronic ischaemic heart disease (120, 125.1, 125.9) and cerebrovascular disease (160–169).

Statistical analysis

All subjects were stratified into quartiles of TSAT ratio and characteristics were compared across quartile groups. For continuous variables, differences across quartiles were tested with analysis of variance. For dichotomous variables, comparisons across quartiles were conducted using the chi-square.

For the principal analyses, years of follow-up for each individual were calculated from baseline to the date of death for decedents and to 31 December 2000 for those still alive. To assess the mortality impact of TSAT levels over longer periods, follow-up was extended to 31 December 2006. Total and cardiovascular mortality rates were calculated for the entire cohort and quartile groups expressed as deaths per 1000 person-years. Cox proportional hazard regression models examined the relationships of TSAT ratio to all-cause and cardiovascular mortality adjusting for baseline characteristics with the third quartile was set as the referent. Adjustments were made for baseline comorbid conditions, lifestyle factors, nutritional and socioeconomic indicators. The poverty income ratio (PIR) was used as an indicator of socioeconomic status and represents the annual family income divided by the federal poverty line. This line is adjusted each calendar year for inflation and varies with the size of the household. To determine whether the presence of anaemia, impaired kidney function or inflammation altered the relationship between TSAT and mortality, we created a number of interaction terms in the Cox model to test for effect modification. For each covariate, the unadjusted hazard ratio (HR) of death and adjusted HR of death were calculated with corresponding 95% confidence intervals. Weighted multivariable Cox regression was used to account for the complex survey design, the unequal probability of subject selection and non-response rates. Model fit was assessed using the Taylor method and $-2 \log$ likelihood ratio (SAS-callable SUDAAN statistical software from Research Triangle Institute).

Sensitivity analyses

Sensitivity analyses were conducted to explore the robustness of our observations. First, we determined whether the pattern of association between TSAT and mortality was consistent across multiple high-risk groups. Second, we re-classified the study population according to deciles of TSAT. With this classification, we were able to identify with greater precision threshold values above or below which mortality risks varied. Third, we evaluated whether the mortality impact of high and low TSAT values varied over time by evaluating risks in the first 5 years of follow-up (early impact).

Results

Baseline characteristics of the population

The mean characteristics of all study participants age 20 and over are shown in Table 1. The weighted

mean age of subjects was 44.7 years (± 0.5), 77% were White, 10.4% were Black and 5.1% were Mexican-American. The mean values for TSAT (\pm SE %) were 26.3 (± 0.22); haemoglobin 14.2 g/dl (± 0.03) and serum ferritin level 129 (± 2.0) ng/ml.

Characteristics of population by quartile of serum transferrin

The mean TSAT ratio increased from 12.9% to 40.6% from the lowest to the highest quartile group. In general, lower quartiles were associated with an increasing prevalence of cardiovascular and non-cardiovascular conditions.

All-cause mortality

During 8.7 years of follow-up (9.1 years, survivors; 5.2 years, decedents), 2506 (9.7%) in the study group died. Of the total deaths, 1182 (47.2%) were ascribed to cardiovascular disease. The crude all-cause mortality rate decreased significantly from 13.3 per 1000 person-years to 9.1 per 1000 person-years with increasing quartile of TSAT (Table 2). The corresponding unadjusted HRs were 1.40 (1.15–1.71) and 1.39 (1.17–1.65) in the lowest two quartiles to 0.95 (0.79–1.14) in the highest quartile compared with the referent group (Q3, 23.7–31.3%). With adjustment for age only, the pattern of association between TSAT and mortality followed a j-shaped curve. Compared with the referent (23.7–31.3%, HR = 1.00), participants in the lowest two quartiles experienced significantly higher mortality risks, HR = 1.52 (1.28–1.81) and HR = 1.27 (1.09–1.49) whereas those in the highest quartile HR = 1.17 (0.97–1.40) also tended to have higher mortality risk. With adjustment for demographic and clinical variables, the pattern of association was more pronounced and when we adjusted additionally for haemoglobin and serum ferritin the pattern was accentuated even further with significantly higher mortality for subjects in the highest quartile group (HR = 1.23, 1.01–1.49).

Stratification by disease group

The stratified analysis explored the association of TSAT and mortality across age, race and gender groups as well as across disease categories (Table 3). There were significant age-gender interactions as illustrated in Figure 1a and b ($P < 0.05$). For men, the j-shaped association of TSAT with mortality was present only for older age (> 65 years) subjects as illustrated in Figure 1a. For women, the opposite was true, with the j-shaped mortality pattern seen only in subjects' age < 45 years and to a lesser degree in subjects age 45–65 years as shown

Table 1 Characteristics of study participants by quartiles of TSAT ratio

Patient characteristics ^a	Entire cohort	TSAT Ratio % (quartiles)			
		First (<17.5)	Second (17.5–23.7)	Third (23.7–31.3)	Fourth (>31.3)
Demographics					
Age at interview (years)	44.69 (0.45)	44.92 (0.48)	46.95 (0.58)	44.99 (0.59)	42.33 (0.56)
Gender					
% Men	47.92 (0.42)	28.80 (1.21)	43.85 (1.07)	53.29 (0.99)	61.15 (0.92)
% Women	52.08 (0.42)	71.20 (1.21)	56.15 (1.07)	46.71 (0.99)	38.85 (0.92)
Race/ethnicity					
% White-non Hispanic	76.97 (1.21)	72.63 (1.66)	76.32 (1.53)	78.36 (1.41)	79.58 (1.40)
% Black non-Hispanic	10.35 (0.60)	13.70 (0.99)	11.40 (0.78)	9.42 (0.69)	7.74 (0.51)
% Mexican American	5.10 (0.41)	5.76 (0.58)	5.06 (0.49)	4.78 (0.39)	4.92 (0.42)
% Other	7.58 (0.77)	7.90 (1.11)	7.23 (0.99)	7.44 (0.93)	7.77 (1.05)
Clinical condition (%)					
Coronary artery disease	6.00 (0.33)	6.27 (0.53)	7.36 (0.65)	5.86 (0.54)	4.77 (0.58)
History of myocardial infarction	3.49 (0.26)	3.61 (0.39)	4.56 (0.59)	3.16 (0.44)	2.78 (0.30)
Angina by Rose questionnaire	4.36 (0.33)	4.61 (0.48)	5.47 (0.72)	4.52 (0.44)	3.14 (0.61)
Chronic bronchitis	6.34 (0.27)	6.38 (0.52)	7.56 (0.62)	5.91 (0.57)	5.67 (0.52)
Gout	2.73 (0.20)	2.89 (0.34)	2.99 (0.47)	2.94 (0.42)	2.20 (0.26)
Congestive heart failure	2.19 (0.16)	2.67 (0.34)	2.80 (0.38)	1.97 (0.30)	1.51 (0.24)
Stroke	2.01 (0.17)	2.46 (0.35)	2.50 (0.31)	1.75 (0.26)	1.49 (0.25)
Arthritis	18.55 (0.60)	20.93 (1.05)	21.66 (0.97)	18.23 (0.90)	14.41 (1.03)
Diabetes (by history)	5.02 (0.26)	5.72 (0.46)	6.18 (0.46)	4.78 (0.41)	3.73 (0.42)
Hypertension (by history)	23.74 (0.67)	27.18 (1.39)	27.78 (1.11)	22.81 (0.91)	18.56 (0.89)
JNC VII staging of hypertension^b					
Normal < 120/80	76.10 (0.62)	76.69 (1.24)	74.30 (0.84)	75.19 (0.88)	77.98 (1.06)
Pre-hypertension 120/80	20.07 (0.55)	19.60 (1.13)	20.18 (0.75)	21.71 (0.84)	18.82 (1.03)
Stage I 140/90	3.36 (0.21)	3.15 (0.40)	4.87 (0.55)	2.59 (0.34)	2.97 (0.34)
Stage II 160/100	0.47 (0.07)	0.56 (0.15)	0.65 (0.16)	0.50 (0.12)	0.23 (0.06)
Family history of MI < 50 years ^b	17.10 (0.51)	17.43 (1.08)	18.55 (0.93)	16.50 (0.97)	16.19 (0.97)
Lifestyle and socioeconomic indicators (%)					
Tobacco use					
Current smokers	28.40 (0.85)	27.79 (1.49)	26.72 (1.27)	25.17 (1.24)	33.28 (1.32)
Former smokers	25.94 (0.61)	23.52 (1.24)	26.92 (1.13)	28.22 (1.16)	24.89 (0.93)
Never smokers	45.65 (0.79)	48.69 (1.36)	46.36 (1.12)	46.61 (1.70)	41.83 (1.50)
Physically inactive	15.06 (0.77)	18.41 (1.16)	15.66 (1.03)	13.49 (1.23)	13.40 (1.01)
Body mass index (kg/m ²)	26.5 (0.11)	27.5 (0.23)	27.2 (0.17)	26.5 (0.15)	25.3 (0.13)
Poverty income ratio (PIR) ^c	0.90 (0.02)	0.79 (0.03)	0.89 (0.03)	0.92 (0.03)	0.96 (0.03)
Specific haematology variables					
Serum TSAT (%)	26.29 (0.22)	12.85 (0.11)	20.62 (0.05)	27.25 (0.05)	40.57 (0.23)
Haemoglobin (g/dl)	14.15 (0.03)	13.41 (0.05)	14.06 (0.05)	14.31 (0.05)	14.66 (0.03)
Anaemia males (%)	3.46 (0.28)	8.32 (0.81)	5.00 (0.81)	2.34 (0.43)	1.65 (0.27)
Anaemia females (%)	10.73 (0.73)	20.86 (1.29)	7.01 (0.82)	6.80 (0.78)	5.28 (0.92)
Serum ferritin (ng/ml)	128.97 (2.02)	86.59 (2.44)	121.36 (3.10)	136.41 (3.69)	161.30 (4.45)
Serum ferritin < 12 ng/ml (%)	4.80 (0.25)	15.77 (0.94)	2.12 (0.35)	1.73 (0.32)	1.37 (0.36)
Other laboratory variables					
Serum albumin (g/dl)	4.18 (0.02)	4.07 (0.02)	4.18 (0.02)	4.21 (0.02)	4.24 (0.03)
Serum creatinine (mg/dl)	1.07 (0.00)	1.04 (0.01)	1.09 (0.01)	1.08 (0.00)	1.08 (0.00)
GFR MDRD (ml/min/1.73 m ²) ^d	99.62 (0.56)	100.28 (0.63)	96.90 (0.83)	99.55 (0.70)	101.45 (0.62)

^aValues are reported as % or mean with standard errors (SE). ^bThe Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. ^cThis is a calculated variable based on family income and family size using tables published each year by the Bureau of the Census in a series 'Current Population Reports' on poverty in the USA. ^dGlomerular filtration rate (ml/min per 1.73 m²) was based on the MDRD Study equation (20).

Table 2 Relationship between TSAT ratio (%) and all-cause mortality in the US population

		TSAT Ratio (%) Quartiles			
		First (<17.5) (N= 3905)	Second (17.5–23.7) (N= 3933)	Third (23.7–31.3) (N= 3936)	Fourth (>31.3) (N= 3924)
All participants					
Total alive (N, %)	13 192	3241 (88.64)	3235 (88.53)	3357 (91.65)	3359 (91.94)
Total deaths (N, %)	2506	664 (11.36)	698 (11.47)	579 (8.35)	565 (8.06)
Person-years at risk		308 831 767	338 966 626	379 433 911	414 233 020
Unadjusted death rate per 1000 person-years		13.34	13.25	9.56	9.11
Age-adjusted death rate per 1000 person-years		11.41	10.09	8.14	9.33
HR for death ^a					
Unadjusted		1.40 (1.15–1.71)	1.39 (1.17–1.65)	Referent group 1.00	0.95 (0.79–1.14)
Adjusted for age		1.52 (1.28–1.81)	1.27 (1.09–1.49)	1.00	1.17 (0.97–1.40)
Adjusted demographics only		1.63 (1.38–1.94)	1.32 (1.12–1.56)	1.00	1.14 (0.95–1.37)
Plus comorbid conditions and serum albumin		1.53 (1.25–1.86)	1.30 (1.08–1.56)	1.00	1.20 (0.99–1.44)
Plus haemoglobin		1.41 (1.16–1.72)	1.26 (1.05–1.52)	1.00	1.25 (1.03–1.51)
Plus serum ferritin		1.45 (1.19–1.77)	1.27 (1.06–1.53)	1.00	1.23 (1.01–1.49)
Plus poverty income ratio ^b		1.43 (1.14–1.80)	1.28 (1.07–1.54)	1.00	1.21 (1.00–1.48)

^aHRs and 95% confidence intervals. Multivariate models sequentially adjusted for demographic characteristics, comorbid conditions, laboratory variables and socioeconomic indicators. There were missing data on 125 subjects leaving 15, 698 for the multivariable analysis. ^bPoverty income ratio: this is a calculated variable based on family income and family size using tables published each year by the Bureau of the Census in a series 'Current Population Reports' on poverty in the USA.

in Figure 1b. The association of TSAT with mortality varied by race with the j-shaped mortality pattern observed in Whites and Blacks but not in Mexican-Americans. The j-shaped pattern of association of TSAT with mortality was generally consistent across most represented categories with the exception of subjects with coronary disease or a haemoglobin <11.5 g/dl, where mortality risks did not increase for the highest transferrin quartile.

Cardiovascular mortality

Cardiovascular mortality rates decreased significantly from 6.45 to 4.04 per 1000 person-years with increasing quartile of TSAT (Table 4). Without adjustment, the mortality risks for subjects in the lowest two quartiles were significantly higher than the referent group (HR = 1.74 (1.46–2.08) and HR = 1.72 (1.36–2.17), respectively) and the mortality risks for those in the highest quartile tended also to be higher, although non-significant. With adjustment for baseline differences in comorbid conditions and serum albumin, the mortality risks remained significantly higher for subjects in the lowest quartiles, but became significant for subjects in the highest quartile (HR = 1.53, 1.03–2.26) compared with the referent. Additional adjustment for haemoglobin and serum ferritin accentuated this

relationship and the pattern of association was similar for most subgroups as demonstrated in Table 4.

Sensitivity analysis

When the study population was reclassified according to deciles of TSAT, subjects in the lowest five deciles as well as those in the highest decile (TSAT > 39.7%) experienced significantly higher mortality risks than that of the referent group (subjects in the sixth decile; TSAT ratio 23.7–26.4%), as shown in Figure 2. Furthermore, the pattern of association between TSAT and mortality at 5 years (early impact analysis) of follow-up was virtually identical to those of the original analyses (Supplementary Table 1).

Discussion

In this population-based cohort, we demonstrated a significant and independent relationship between TSAT with total and cardiovascular mortality. The pattern of association was j-shaped with significantly higher mortality for subjects with TSAT values <23.7% and higher than 31.3%. The association of TSAT with mortality differed by race and sex. Among Whites and Blacks, low and high

Table 3 Relationship between TSAT (%) and all-cause mortality in sub-populations of interest^a

	Subjects (N)	TSAT Ratio (%) Quartiles			
		First (<17.5)	Second (17.5–23.7)	Third (23.7–31.3)	Fourth (>31.3)
Subject groups				Referent group	
By age					
<45 years	7991	1.35 (0.74–2.47)	1.09 (0.61–1.95)	1.00	1.24 (0.67–2.29)
45–65 years	3896	1.62 (1.02–2.60)	1.18 (0.79–1.76)	1.00	0.97 (0.65–1.44)
>65 years	3811	1.55 (1.35–1.85)	1.24 (1.01–1.52)	1.00	1.12 (0.94–1.32)
By sex					
Males	7347	1.52 (1.19–1.94)	1.42 (1.11–1.80)	1.00	1.37 (1.12–1.68)
Females	8351	1.33 (0.98–1.80)	1.12 (0.86–1.46)	1.00	1.05 (0.76–1.45)
By race					
White	6644	1.56 (1.27–1.92)	1.30 (1.06–1.61)	1.00	1.11 (0.91–1.34)
Black	4177	1.70 (1.33–2.18)	1.20 (0.96–1.51)	1.00	1.27 (0.91–1.79)
Mexican	4258	1.13 (0.77–1.65)	1.12 (0.68–1.64)	1.00	0.87 (0.55–1.37)
By haemoglobin (g/dl)					
<11.5	796	1.59 (0.80–3.14)	1.47 (0.81–2.65)	1.00	0.86 (0.33–2.24)
11.5–12.5	1708	1.51 (0.93–2.45)	0.99 (0.62–1.56)	1.00	1.48 (0.82–2.69)
>12.5/dl	12 963	1.44 (1.19–1.74)	1.24 (1.03–1.50)	1.00	1.17 (0.95–1.45)
By level of GFR ^b					
<60	1002	2.10 (1.36–3.23)	1.80 (1.23–2.61)	1.00	1.15 (0.73–1.81)
60–89	4284	1.29 (1.00–1.66)	1.07 (0.81–1.42)	1.00	1.14 (0.84–1.53)
>90	10 412	1.42 (1.05–1.93)	1.25 (0.93–1.67)	1.00	1.38 (0.98–1.95)
By hypertension history					
Hypertension present	4353	1.55 (1.22–1.96)	1.21 (0.94–1.55)	1.00	1.19 (0.86–1.66)
Hypertension absent	11 216	1.32 (0.99–1.77)	1.26 (0.99–1.61)	1.00	1.23 (0.99–1.53)
By coronary disease					
Coronary disease present	742	1.47 (0.82–2.65)	1.22 (0.77–1.93)	1.00	0.92 (0.61–1.41)
Coronary disease absent	14 760	1.41 (1.15–1.71)	1.20 (0.98–1.47)	1.00	1.22 (0.98–1.52)
By heart failure					
Heart failure present	595	1.80 (1.12–2.90)	1.73 (1.09–2.75)	1.00	1.20 (0.71–2.02)
Heart failure absent	15 080	1.38 (1.15–1.64)	1.17 (0.96–1.41)	1.00	1.17 (0.97–1.42)

^aRelationships are expressed as HRs and 95% confidence intervals. ^bGlomerular filtration rate (ml/min per 1.73 m²) was based on the abbreviated MDRD Study equation.

TSAT levels were associated with higher mortality risk whereas no association was manifested in Mexican-Americans. For men, the impact on mortality was demonstrated only in older males, whereas for women the j-shaped mortality association was confined to younger females. The patterns of association between TSAT and cardiovascular mortality were similar in direction although greater in magnitude and strength. Our findings demonstrate that the TSAT saturation is a useful prognostic tool in clinical medicine and that low levels and very high levels are independent markers of elevated mortality risk. We suggest that the optimal TSAT range for patient survival should be between

23% and 40% and that careful clinical assessment is warranted for patients with low and high levels in order to identify states of iron deficiency or iron excess.

Our findings illustrate that very low levels of TSAT, a marker of iron deficiency, and very high levels of TSAT, a marker of iron overload, contribute to increased all-cause and cardiovascular mortality. Subjects with the lowest TSAT levels experienced the highest mortality across most age, race and disease categories. We had speculated that the greater burden of illness in these subjects could explain the higher mortality risks; however, when we accounted for these differences, the relationships between low

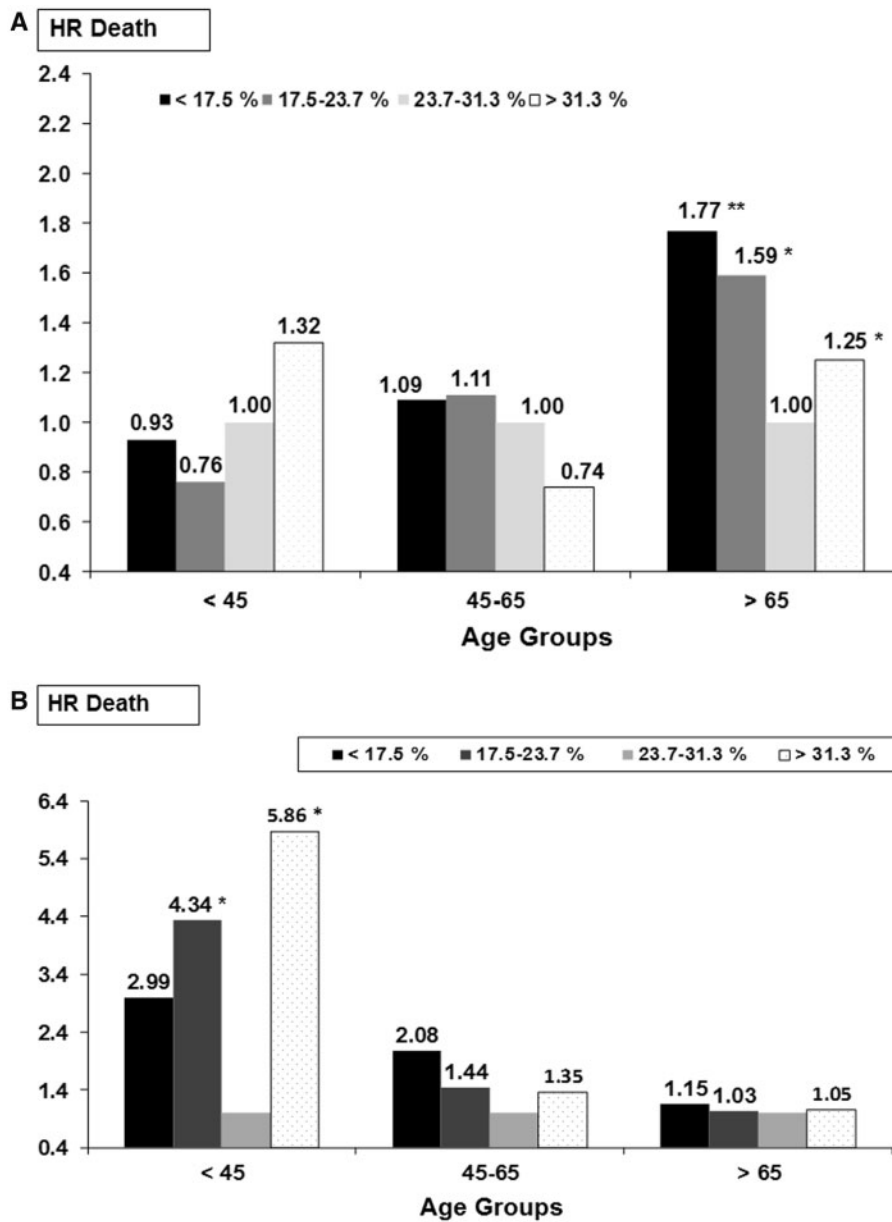


Figure 1. Association of TSAT ratio (%) in quartile groups with all-cause mortality in (a) men and (b) women. In (a), * $P < 0.05$, ** $P < 0.01$ vs. referent quartile 23.7–31.3% ($N = 7405$); in (b), * $P < 0.05$ vs. referent quartile 23.7–31.3% ($N = 8418$). Adjusted for race, comorbid conditions, serum albumin, ferritin, haemoglobin level and poverty income ratio.

TSAT levels and mortality remained significant. To exclude the possibility that our findings were due to low haemoglobin levels, poor nutrition and low socioeconomic status, we accounted for these potential confounders in multivariable models and our results remained steadfast. Indeed when we adjusted for baseline differences in haemoglobin and serum ferritin, the associations between TSAT and mortality become even stronger. Our results also demonstrate that elevated TSAT levels predict all-cause mortality in the general population. Unlike Sempos *et al.*,⁷ we found that TSAT > 39.7% was

significantly associated with increased mortality. Our findings extend the observations of Mainous *et al.*⁸ in several ways. First, we provide greater specification of the gradient of risk across a wide range of serum TSAT. Second, we demonstrate that the upper threshold values for elevated mortality are appreciably lower than previously reported (40% vs. 55%).⁸

This study also provides important new insights into relationships between TSAT ratio and mortality among men and women. For men, the j-shaped mortality association was only observed among

Table 4 Relationship between TSAT (%) and cardiovascular mortality in the US population

		First (<17.5) ($N=3905$)	Second ($17.5-23.7$) ($N=3933$)	Third ($23.7-31.3$) ($N=3936$)	Fourth (>31.3) ($N=3924$)
All participants	Subjects (N)				
Cardiovascular deaths (N)	1182	341 (5.49)	338 (5.55)	245 (3.26)	258 (3.57)
Non-cardiovascular deaths (N)	1324	323 (5.86)	360 (5.92)	334 (5.09)	307 (4.49)
Person-years		308 831 767	338 966 626	379 433 911	414 233 020
Cardiovascular death rate per 1000 person-years		6.45	6.41	3.73	4.04
Age-adjusted cardiovascular death rate per 1000 person-years		5.54	4.80	3.17	4.21
Relative risk death					
Unadjusted		1.74 (1.46–2.08)	1.72 (1.36–2.17)	1.00	1.08 (0.83–1.40)
Adjusted for demographic factors (age, sex and race)		2.10 (1.79–2.46)	1.66 (1.31–2.10)	1.00	1.30 (0.99–1.70)
Plus comorbid conditions and serum albumin		2.10 (1.44–3.04)	1.88 (1.34–2.65)	1.00	1.53 (1.03–2.26)
Plus haemoglobin		1.94 (1.33–2.81)	1.82 (1.28–2.57)	1.00	1.58 (1.06–2.35)
Plus ferritin		2.09 (1.43–3.05)	1.90 (1.33–2.72)	1.00	1.59 (1.05–2.40)
Plus poverty income ratio ^a		2.01 (1.58–2.54)	1.60 (1.19–2.16)	1.00	1.31 (0.99–1.77)
Stratified analysis					
By age (years)					
<65	11 653	2.52 (0.88–7.24)	1.52 (0.56–4.16)	1.00	1.45 (0.68–3.08)
≥65	4045	2.30 (1.69–3.11)	2.07 (1.45–2.95)	1.00	1.59 (1.03–2.47)
By sex					
Males	7347	2.34 (1.35–4.06)	1.85 (1.11–3.07)	1.00	1.61 (1.07–2.44)
Females	8351	1.88 (1.19–2.96)	1.82 (1.11–2.98)	1.00	1.50 (0.79–2.86)
By haemoglobin (g/dl)					
<11.5	796	2.62 (0.82–8.38)	3.32 (0.97–11.35)	1.00	0.74 (0.08–6.50)
11.5–12.5	1708	3.21 (1.10–9.31)	1.24 (0.48–3.18)	1.00	3.86 (1.42–10.47)
>12.5	12 963	2.01 (1.39–2.91)	1.79 (1.28–2.50)	1.00	1.40 (0.94–2.10)
By level of GFR ^b					
<60	1002	2.27 (1.20–4.32)	2.47 (1.16–5.22)	1.00	0.93 (0.37–2.31)
60–90	4284	2.13 (1.15–3.94)	2.20 (1.38–3.50)	1.00	2.18 (1.22–3.88)
>90	10 412	1.97 (1.04–3.76)	1.03 (0.56–1.88)	1.00	1.31 (0.65–2.66)
By hypertension history					
Hypertension present	4353	2.16 (1.39–3.37)	1.48 (0.93–2.35)	1.00	0.99 (0.61–1.61)
Hypertension absent	11 216	1.90 (1.29–2.81)	1.99 (1.31–3.04)	1.00	1.98 (1.27–3.08)
By coronary disease					
Coronary disease present	742	4.41 (1.69–11.56)	3.62 (1.61–8.13)	1.00	2.41 (0.99–5.83)
Coronary disease absent	14 760	1.81 (1.32–2.46)	1.56 (1.15–2.13)	1.00	1.38 (0.97–1.96)
By heart failure					
Heart failure present	595	1.40 (0.64–3.06)	1.74 (0.72–4.21)	1.00	1.41 (0.58–3.42)
Heart failure absent	15 080	2.15 (1.52–3.04)	1.74 (1.27–2.37)	1.00	1.48 (1.01–2.17)

^aGlomerular filtration rate (ml/min per 1.73 m²) was based on the abbreviated MDRD Study equation. ^bPoverty income ratio: this is a calculated variable based on family income and family size using tables published each year by the Bureau of the Census in a series 'Current Population Reports' on poverty in the USA.

those >65 years with no significant relationship for those <65 years. In contrast, for women, the j-shaped TSAT–mortality pattern was only observed for those age <45 years. We had speculated that a higher prevalence of anaemia in younger females might in part be responsible for the surprisingly

elevated mortality risk. However, adjusting for haemoglobin levels and serum ferritin did not materially alter this risk. Similarly, the association between TSAT levels and mortality was confined to men >65 years and did not attenuate following adjustment for haemoglobin, nutritional or

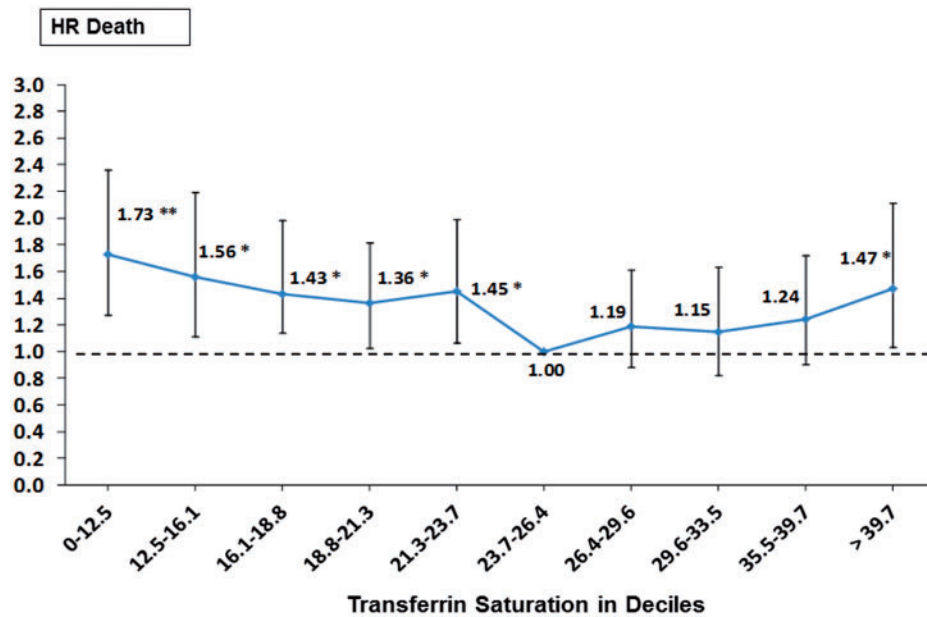


Figure 2. Association of TSAT ratio (%) in decile groups with all-cause mortality. * $P < 0.05$, ** $P < 0.01$ vs. referent quartile 23.7–31.3%. Multivariable model adjusted for age, sex, race, comorbid conditions, serum albumin, ferritin, haemoglobin level and poverty income ratio.

socioeconomic markers. It is quite possible that the TSAT ratio is a strong indicator of iron deficient states and/or iron overload states in these patient subgroups and therefore the ratio has the strongest association with mortality in these subgroups.

An important aspect of this study was the exploration of potential interactions of TSAT with mortality in patients with and without chronic disease. In general, we found that the pattern of association of TSAT with mortality was generally consistent across most disease groups with some exceptions. In particular, the j-shaped mortality relationship was more pronounced and significant among subjects with heart failure and hypertension. The significantly higher mortality for subjects with low TSAT and heart failure would support the rationale for correction of iron deficient states in this category of disease. However, the higher than expected mortality for TSAT levels $>39.7\%$, although not significant, should merit caution. We did not find evidence that the relationship between transferrin and mortality differed by level of kidney function either for all-cause mortality or cardiovascular mortality, although the magnitude of the HRs for low transferrin levels was greater for subjects with $eGFR < 60$ ml/min than above. This would suggest that iron deficiency is an equally important predictor of mortality across all categories of kidney impairment.

There are several possible pathological mechanisms that might explain the j-shaped association of TSAT with mortality.^{9–13,21} The development of iron

deficiency anaemia as a consequence of iron deficiency is a very plausible pathway; however, the adjustment for haemoglobin in the analysis suggests that other mechanisms are important. Adequate iron stores are also necessary for several non-erythropoiesis biologic processes such as preservation of immune function, thermoregulation and cognition.^{1,9} Moreover, chronic iron deficiency has been reported to cause structural alterations in cardiomyocytes and impair cardiac performance, establishing a theoretical link with increased cardiovascular mortality.²¹ At the other end of the spectrum, there is evidence to suggest that excess iron stores may confer increased mortality risk through accumulation of iron in several vital organs such as the heart, liver and pancreas.^{10–13} Equally important are the observations that large doses of parenteral iron used in the treatment of iron deficient states contribute to oxidative stress and increased susceptibility to infection.^{22–25} Although the precise mechanisms of elevated mortality are unknown, there are compelling biologic arguments to support associations between pathological states of iron deficiency and iron overload.

This study has some limitations including, loss to follow-up although minimal, lack of information on other potentially important explanatory variables, errors in measurement of baseline variables and misclassification of cardiovascular causes of death. Nevertheless, this study has several strengths which enhance its internal and external validity;

the sample large size and the ability to test the hypothesis in several interest groups; the standardized methods of data collection; the relatively long follow-up, the large number of events; the outcome of mortality as the end point; and the ability to adjust sequentially for a large number of known mortality predictors that were captured in the baseline questionnaire.

In conclusion, this study demonstrates a strong independent j-shaped association of TSAT with all-cause and cardiovascular mortality. This association was present for older men and younger women, for Black and White populations but not Mexican Americans; and remained strong regardless of comorbid conditions, nutritional markers and socio-economic indicators. Threshold analyses found that the optimal cut-points for TSAT, at which the lowest mortality was observed, were between 23% and 40%, above and below which mortality risks were significantly increased. Our results suggest that the optimal target range for TSAT should be between 24% and 40% and provide support for the correction of low TSAT levels in the general population while at the same time advising caution against excessive iron loading to levels beyond 40%.

Supplementary material

Supplementary material is available at *QJMED* online

Acknowledgements

Professor Stack had full access to all of the data in the study and takes responsibility for the integrity of the data and data analysis. All authors collaborated and contributed to the study design, analysis, editing and final manuscript. Professor Stack and Dr Ferguson are supported by the Irish Health Research Board (HRB) and the Irish Heart Foundation.

Funding

Funding for this project was provided by the University of Limerick Strategic Research Fund, Limerick, Ireland: the funder did not contribute to the design, performance or interpretation of the results of this study.

Conflict of interest: None declared.

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