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Time-Varying Associations of Iron Homeostasis Markers with 365-Day Mortality in Critically Ill Patients with Acute Myocardial Infarction: A Retrospective MIMIC-IV Cohort Study

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Abstract

Background: Iron-homeostasis markers may reflect both iron status and acute inflammatory stress after acute myocardial infarction (AMI), but their prognostic associations may vary over time.

Methods: We retrospectively studied adults with AMI admitted to an ICU in MIMIC-IV. Ferritin, transferrin, and transferrin saturation (TSAT) were assessed within 24 h of ICU admission. The primary outcome was 365-day all-cause mortality. Multivariable Cox models adjusted for age, sex, SOFA score, hemoglobin, and chronic kidney disease. Proportional-hazards diagnostics, 0–30- and 31–365-day interval-specific analyses, restricted cubic splines, and sensitivity analyses were performed.

Results: Among 452 patients, 202 died within 365 days. Ferritin was associated with higher mortality (HR 1.107 per doubling, 95% CI 1.035–1.184; $p = 0.003$), whereas transferrin was inversely associated (HR 0.960 per 10 mg/dL, 95% CI 0.935–0.985; $p = 0.002$); TSAT was not significant after adjustment ($p = 0.111$). Ferritin and transferrin showed stronger associations during days 0–30 than days 31–365 (interaction $p = 0.015$ and 0.045 , respectively), whereas TSAT did not ($p = 0.116$).

Conclusions: Ferritin and transferrin showed early-predominant prognostic associations after AMI, supporting time-aware interpretation of iron-homeostasis markers.

Keywords: ferritin; transferrin; transferrin saturation; acute myocardial infarction; intensive care; mortality; time-varying association; MIMIC-IV

Background

Acute myocardial infarction (AMI) remains a major cause of death and disability worldwide despite advances in reperfusion therapy, intensive care, and secondary prevention [1–3]. Patients with AMI who require intensive care constitute a particularly high-risk population in whom ischemia-reperfusion injury, systemic inflammation, organ dysfunction, anemia, and metabolic disturbances frequently coexist. Biomarkers reflecting these acute pathophysiological processes may provide prognostic information, but their interpretation in critical illness may differ substantially from that in stable cardiovascular disease.

Iron is essential for oxygen transport, mitochondrial respiration, and cellular energy metabolism [4,5]. Conversely, redox-active iron can promote oxidative stress, lipid peroxidation, and cellular injury when iron handling becomes dysregulated [6–8]. Interest in cardiovascular iron homeostasis has increased further with recognition of ferroptosis and related iron-dependent pathways, although direct clinical evidence linking these mechanisms to outcomes after AMI remains limited [7,8].

Ferritin, transferrin, and transferrin saturation (TSAT) capture different aspects of iron homeostasis. Ferritin is the principal intracellular iron-storage protein but is also an acute-phase reactant and can rise substantially during systemic inflammation [9,10]. Transferrin is the major circulating iron-transport protein and a negative acute-phase reactant; low concentrations may reflect inflammation,

46 impaired hepatic protein synthesis, nutritional compromise, or altered intravascular distribution [11].

47 TSAT, calculated from serum iron and total iron-binding capacity (TIBC), represents the proportion

48 of circulating iron-binding capacity occupied by iron. In chronic heart failure and other stable

49 cardiovascular populations, low TSAT and iron deficiency have been associated with adverse

50 outcomes [12–18]. In acute critical illness, however, the same markers may be strongly influenced

51 by the acute-phase response, making their prognostic interpretation less straightforward.

52 Previous studies have linked ferritin and other iron-related biomarkers to cardiovascular risk, acute

53 coronary syndromes, and mortality in critical illness, but findings have been heterogeneous [19–32].

54 A recent MIMIC-IV study of 992 ICU-admitted patients with AMI reported that higher ferritin and

55 TSAT were associated with increased short-term all-cause mortality, with analyses extending to 120

56 days [33]. That study primarily examined hemoglobin and iron-deficiency phenotypes and did not

57 directly evaluate transferrin or formally characterize whether marker-outcome associations changed

58 over longer follow-up. Thus, the temporal behavior of ferritin, transferrin, and TSAT across early

59 and later periods after ICU admission remains insufficiently characterized. For biomarkers measured

60 during an acute inflammatory and ischemic episode, assuming a constant relative association

61 throughout follow-up may obscure clinically relevant temporal heterogeneity.

62 Accordingly, we evaluated the associations of ferritin, transferrin, and TSAT measured within 24

63 hours of ICU admission with 365-day all-cause mortality in critically ill patients with AMI. In

64 addition to overall Cox estimates, we formally assessed the proportional-hazards assumption and

65 compared associations during early (0-30 days) and later (31-365 days) follow-up. We also examined

66 nonlinear exposure-response patterns and the robustness of findings across sensitivity analyses,

67 including an independently constructed principal-diagnosis AMI cohort.

Methods

Data source and study design

This retrospective cohort study used the Medical Information Mart for Intensive Care IV (MIMIC-IV, version 3.0) database, which contains de-identified electronic health record data from Beth Israel Deaconess Medical Center in Boston, Massachusetts, from 2008 to 2022 [34,35]. MIMIC-IV includes demographics, ICU encounters, laboratory measurements, diagnostic codes, severity scores, and mortality information. The present study used only de-identified data in accordance with PhysioNet data-use requirements [34,36,37].

Study population

Adults aged 18 years or older with AMI were identified using ICD-9 codes beginning with 410 or ICD-10 codes beginning with I21. For the primary cohort, an AMI diagnosis in any diagnosis position was accepted. To avoid repeated observations, only the first AMI-associated ICU stay for each patient was retained. ICU length of stay was not used as an exclusion criterion; therefore, ICU stays shorter than 24 hours remained eligible.

Inclusion required a complete early iron-homeostasis assessment within 24 hours of ICU admission. Ferritin and transferrin were defined using the earliest valid measurement within this window. Serum iron and TIBC were required to share the same charted measurement time, and the earliest valid exact-time iron/TIBC pair was used to calculate TSAT. The final primary cohort comprised 452 patients. An independent sensitivity cohort was constructed separately by requiring AMI to be the principal hospital diagnosis and applying the same iron-measurement criteria.

Exposure definitions

The exposures of interest were ferritin, transferrin, and TSAT. TSAT was calculated as serum iron divided by TIBC and multiplied by 100. The iron-homeostasis markers were primarily modeled as

91 continuous variables to preserve information and avoid reliance on arbitrary categorization thresholds.
92 Because ferritin concentrations were markedly right-skewed over a wide concentration range, ferritin
93 was log2-transformed so that the hazard ratio represented the association with each doubling in
94 ferritin concentration. Transferrin and TSAT were retained on their original scales and rescaled to
95 clinically interpretable increments of 10 mg/dL and 10 percentage points, respectively. These
96 continuous estimates were intended as parsimonious summary associations rather than assumptions
97 of a strictly linear exposure-response relationship across the entire observed range. Accordingly,
98 tertile analyses and restricted cubic splines were additionally used to assess the robustness and
99 functional form of the associations. Each marker was categorized into empirical tertiles (T1-T3), with
100 T1 as the reference group. Identical values at a tertile boundary were kept in the same category rather
101 than artificially split; consequently, tertile group sizes were not necessarily equal.

102 **Baseline covariates**

103 Baseline variables available in the final primary dataset included age, sex, height, weight, body mass
104 index (BMI), Sequential Organ Failure Assessment (SOFA) score, Simplified Acute Physiology
105 Score II (SAPS II), Acute Physiology Score III (APS III), hemoglobin, chronic kidney disease (CKD),
106 diabetes mellitus, atrial fibrillation, and chronic obstructive pulmonary disease. The primary
107 adjustment set was selected on clinical grounds before interpretation of the final exposure-outcome
108 associations and was not determined by statistical significance in baseline comparisons.

109 **Outcome and follow-up**

110 The primary outcome was all-cause mortality within 365 days after ICU admission. Follow-up was
111 measured from ICU admission to death or administrative censoring at 365 days. When an exact in-
112 hospital death timestamp was available, follow-up was calculated directly to that timestamp. When

113 only a recorded date of death was available, the recorded calendar date was used to determine death
114 status and date-based follow-up; no unrecorded time of day was imputed. Patients without a recorded
115 death within 365 days were censored at day 365. No death event had zero or negative follow-up time
116 in the final dataset.

117 **Statistical analysis**

118 Continuous variables are summarized as median (interquartile range), and categorical variables as
119 number (percentage). Baseline characteristics were compared descriptively according to 365-day
120 survival status using the Mann-Whitney U test for continuous variables and the chi-square test or
121 Fisher's exact test for categorical variables, as appropriate. These comparisons were descriptive and
122 were not used for covariate selection.

123 Associations between each iron-homeostasis marker and 365-day mortality were estimated using Cox
124 regression. Model 1 was unadjusted; Model 2 adjusted for age and sex; and Model 3, the primary
125 adjusted model, included age, sex, SOFA score, hemoglobin, and CKD. Because hemoglobin was
126 missing in two patients, the Model 3 complete-case analytic population comprised 450 patients.
127 Ferritin, transferrin, and TSAT were evaluated separately as continuous exposures and as tertiles.

128 Kaplan-Meier curves were generated across tertiles of each marker and compared using the log-rank
129 test. Restricted cubic spline models with three knots at the 10th, 50th, and 90th percentiles were used
130 to assess potential nonlinearity. The median exposure value served as the reference, and spline
131 models used the Model 3 adjustment set. Because proportional-hazards violations were subsequently
132 identified, these spline models were interpreted as time-averaged exposure-response associations
133 over the 365-day follow-up rather than as constant hazard-ratio functions.

134 The proportional-hazards assumption was assessed using scaled Schoenfeld residuals. Exposure-
135 specific tests indicated evidence of non-proportionality for ferritin ($P = 0.0039$), transferrin ($P =$
136 0.0029), and TSAT ($P = 0.040$). For the time-stratified analyses, follow-up was split at day 30 using
137 a start-stop representation, and separate exposure coefficients were estimated for days 0-30 and 31-
138 365 while retaining the Model 3 covariates. Differences between the early and later exposure
139 coefficients were formally tested using Wald interaction tests. Overall 365-day Cox estimates were
140 retained as time-averaged summaries and interpreted together with the interval-specific estimates.

141 **Sensitivity analyses**

142 Sensitivity analyses assessed the robustness of the primary Model 3 results. These analyses replaced
143 SOFA with SAPS II or APS III; additionally adjusted for diabetes mellitus; restricted the cohort to
144 ICU stays of at least 24 hours; excluded ferritin values above the 99th percentile; excluded patients
145 with TSAT $>100\%$; and evaluated BMI in an expanded model after multiple imputation. BMI was
146 missing in 194 of 452 patients (42.9%) and was therefore not included in the primary model. For the
147 BMI sensitivity analysis, the imputation model incorporated the mortality event indicator and a
148 Nelson-Aalen estimate of cumulative hazard; 50 imputed datasets were generated using predictive
149 mean matching and pooled according to Rubin's rules.

150 Finally, the primary continuous Model 3 analyses were repeated in an independently constructed
151 principal-diagnosis AMI cohort. This sensitivity cohort included 148 patients, with 54 deaths within
152 365 days. One patient had missing hemoglobin, resulting in an adjusted analytic sample of 147
153 patients.

154 All tests were two-sided, and $P < 0.05$ was considered statistically significant. No adjustment was
155 made for multiple comparisons; secondary analyses, including tertile, spline, interaction, and

sensitivity analyses, were interpreted in conjunction with effect estimates and confidence intervals rather than as standalone confirmatory tests. Analyses were performed using R (version 4.5.0).

Results

Study population and baseline characteristics

The primary cohort was derived from 9,536 AMI-associated ICU stays. After retaining the first AMI-associated ICU stay per patient, 7,589 patients remained; 452 had a complete early iron assessment within 24 hours, including an exact-time serum iron/TIBC pair, and constituted the primary cohort (Figure 1). During 365 days of follow-up, 202 patients (44.7%) died and 250 survived.

Compared with 365-day survivors, patients who died were older, had higher SOFA, SAPS II, and APS III scores, lower hemoglobin, and higher prevalences of CKD and atrial fibrillation. Non-survivors also had higher ferritin and lower transferrin concentrations. Detailed baseline characteristics are presented in Table 1.

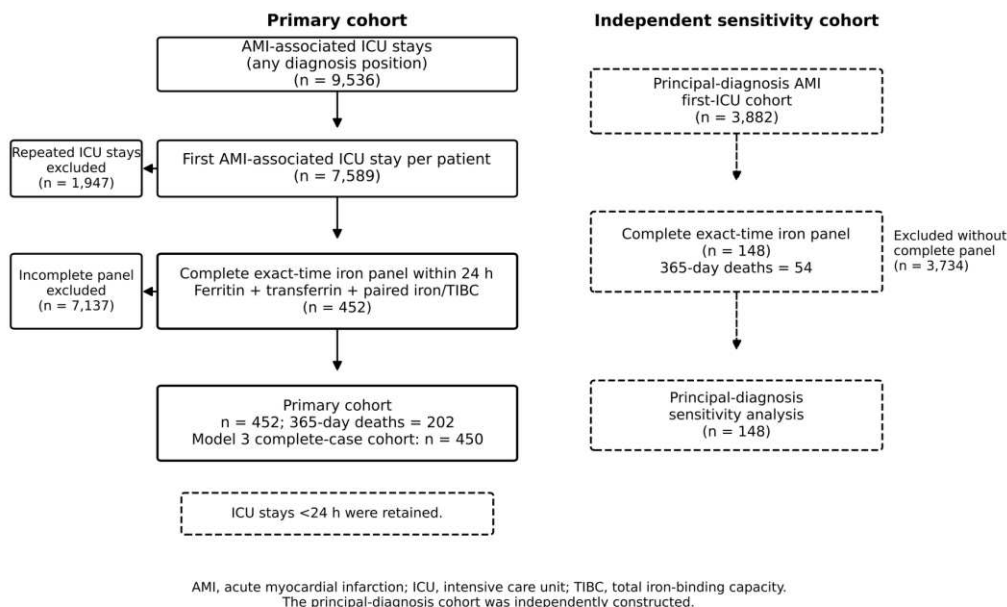


Figure 1. Study flowchart. The primary cohort included adults with an AMI diagnosis in any diagnosis position. The first AMI-associated ICU stay per patient was retained, and inclusion required ferritin and transferrin measurements plus a serum iron/TIBC pair sharing the same charted time within 24 hours of ICU admission. The

principal-diagnosis AMI sensitivity cohort was constructed independently. ICU stays shorter than 24 hours were retained. AMI, acute myocardial infarction; ICU, intensive care unit; TIBC, total iron-binding capacity.

Table 1. Baseline characteristics of the primary cohort according to 365-day survival status.

Characteristic	Overall (N=452)	365-day survivors (n=250)	365-day deaths (n=202)	P value
Age, years	72.0 (61.8–81.0)	68.0 (59.0–77.0)	76.0 (67.0–84.0)	<0.001
BMI, kg/m ²	27.6 (24.8–31.5)	28.0 (24.7–32.1)	27.3 (24.8–31.1)	0.484
SOFA score	5.0 (3.0–8.0)	4.0 (2.0–7.0)	6.0 (4.0–9.0)	<0.001
SAPS II	39.0 (31.0–48.0)	35.0 (28.2–42.0)	44.5 (37.0–54.0)	<0.001
APS III	47.0 (38.0–60.0)	44.0 (34.0–54.0)	52.0 (41.0–68.8)	<0.001
Hemoglobin, g/dL	9.2 (7.8–10.9)	9.6 (7.9–11.2)	8.9 (7.7–10.5)	0.009
ICU length of stay, days	2.6 (1.4–4.8)	2.3 (1.2–4.1)	3.0 (1.6–5.9)	0.021
Ferritin, ng/mL	295.5 (127.5–768.5)	213.5 (104.0–535.8)	399.5 (175.2–1124.2)	<0.001
Transferrin, mg/dL	179.0 (134.0–222.0)	184.5 (150.0–227.8)	169.0 (120.2–213.0)	<0.001
Serum iron, µg/dL	36.5 (20.0–69.0)	37.0 (19.0–66.8)	35.5 (22.0–71.8)	0.658
TIBC, µg/dL	233.0 (174.0–289.0)	240.0 (195.0–295.8)	220.0 (156.2–277.0)	<0.001
TSAT, %	15.4 (9.1–31.0)	15.0 (8.4–25.8)	15.9 (9.4–39.2)	0.051
Male sex	286 (63.3%)	159 (63.6%)	127 (62.9%)	0.873
CKD	198 (43.8%)	93 (37.2%)	105 (52.0%)	0.002
Diabetes mellitus	199 (44.0%)	101 (40.4%)	98 (48.5%)	0.084
Atrial fibrillation	193 (42.7%)	94 (37.6%)	99 (49.0%)	0.015
COPD	96 (21.2%)	48 (19.2%)	48 (23.8%)	0.238
Principal-diagnosis				
AMI within primary cohort	146 (32.3%)	93 (37.2%)	53 (26.2%)	0.013

Continuous variables are presented as median (interquartile range) and categorical variables as number (percentage). P values are descriptive and were not used for covariate selection. BMI is descriptive because of substantial missingness and is not included in the primary Model 3. The principal-diagnosis AMI row refers to principal-diagnosis status within the 452-patient primary cohort (n=146); the independently constructed principal-diagnosis sensitivity cohort (n=148) was derived separately.

Abbreviations: APS III, Acute Physiology Score III; BMI, body mass index; CKD, chronic kidney disease; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; TSAT, transferrin saturation.

Overall associations with 365-day mortality

In the primary Model 3 analytic population (n = 450), higher ferritin was associated with higher 365-day mortality when modeled continuously. Each doubling of ferritin corresponded to an adjusted HR of 1.107 (95% CI 1.035-1.184; P = 0.003). Higher transferrin was inversely associated with mortality, with an adjusted HR of 0.960 per 10 mg/dL increase (95% CI 0.935-0.985; P = 0.002). In contrast, the primary linear association for TSAT was not statistically significant (HR 1.032 per 10-percentage-point increase, 95% CI 0.993-1.073; P = 0.111) (Table 2).

Tertile analyses were directionally consistent while indicating differences in association strength and functional form. Compared with the lowest ferritin tertile, the middle tertile was associated with higher mortality (HR 1.481, 95% CI 1.019-2.154; P = 0.040), and the highest tertile showed a stronger association (HR 1.968, 95% CI 1.366-2.836; P < 0.001). For transferrin, the middle tertile was associated with lower mortality (HR 0.651, 95% CI 0.464-0.914; P = 0.013), whereas the highest tertile showed a similar direction but did not meet the conventional significance threshold (HR 0.706, 95% CI 0.497-1.003; P = 0.052). For TSAT, the middle tertile was not associated with mortality (HR 1.284, 95% CI 0.898-1.835; P = 0.171), whereas the highest tertile was associated with higher mortality (HR 1.451, 95% CI 1.023-2.058; P = 0.037). Baseline characteristics across ferritin, transferrin, and TSAT tertiles and the corresponding Model 3 tertile estimates are provided in Additional file 1 (Tables S1–S3). Full Model 1-3 Cox results for 365-day mortality are provided in Additional file 1 (Table S4).

Table 2. Overall and time-stratified associations of ferritin, transferrin, and TSAT with 365-day all-cause mortality.

Exposure	Overall 365-day HR (95% CI)	P value	0–30 days HR (95% CI)	31–365 days HR (95% CI)	P for early-vs- late interaction	Interpretation
Ferritin, per doubling	1.107 (1.035– 1.184)	0.003	1.183 (1.087– 1.286)	1.023 (0.931– 1.125)	0.015	Association concentrated in the early period
Transferrin, per 10 mg/dL	0.960 (0.935– 0.985)	0.002	0.936 (0.902– 0.971)	0.984 (0.951– 1.019)	0.045	Stronger early inverse association
TSAT, per 10 percentage points	1.032 (0.993– 1.073)	0.111	1.058 (1.011– 1.108)	1.000 (0.941– 1.063)	0.116	No significant early-vs-late heterogeneity

All estimates are from Model 3 adjusted for age, sex, SOFA score, hemoglobin, and CKD. Ferritin is modeled per doubling, transferrin per 10 mg/dL increase, and TSAT per 10-percentage-point increase. Because proportional-hazards diagnostics indicated time-varying effects, the overall 365-day Cox estimate is interpreted as a time-averaged summary association. The 0–30-day and 31–365-day estimates are interval-specific, and P for interaction tests the difference between the two periods. Abbreviations: CI, confidence interval; CKD, chronic kidney disease; HR, hazard ratio; SOFA, Sequential Organ Failure Assessment; TSAT, transferrin saturation.

Kaplan–Meier analyses

Kaplan-Meier curves differed across ferritin and transferrin tertiles (both log-rank $P < 0.001$), whereas survival differences across TSAT tertiles were not statistically significant (log-rank $P = 0.173$) (Figure 2). Because identical exposure values at tertile boundaries were kept together, group sizes were 155/146/151 for ferritin, 152/151/149 for transferrin, and 151/150/151 for TSAT. Corresponding Kaplan-Meier log-rank results are summarized in Additional file 1 (Table S5).

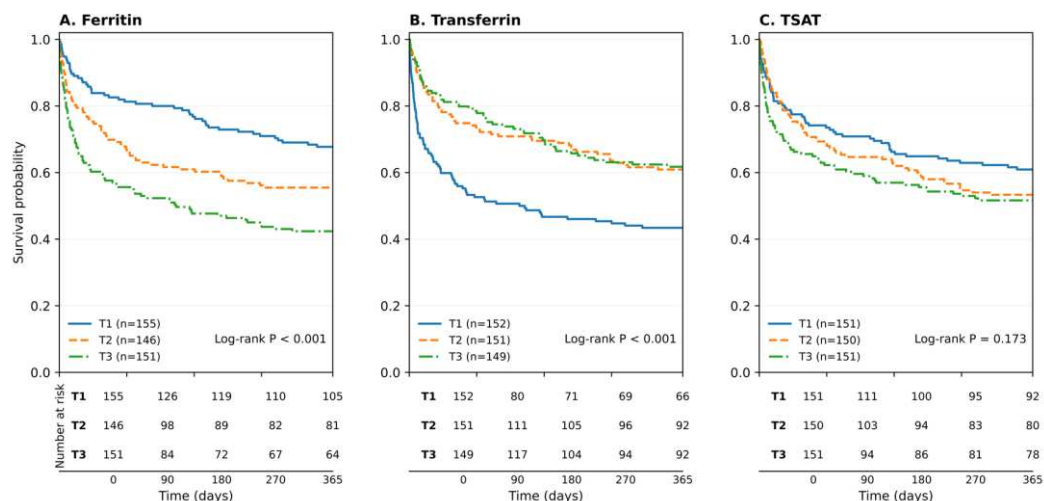


Figure 2. Kaplan-Meier survival curves by tertiles of ferritin, transferrin, and TSAT. Tertiles were defined using empirical exposure quantiles while retaining identical values within the same category. Numbers at risk are shown below each panel. Between-group differences were assessed using the log-rank test. TSAT, transferrin saturation.

Proportional-hazards diagnostics and time-stratified associations

Schoenfeld residual diagnostics indicated departures from the proportional-hazards assumption for ferritin, transferrin, and TSAT. Time-stratified analyses showed that the associations of ferritin and transferrin were concentrated mainly during the first 30 days of follow-up (Table 2 and Figure 3).

Detailed proportional-hazards diagnostics and interval-specific estimates are provided in Additional file 1 (Table S6).

For ferritin, each doubling was associated with higher mortality during days 0-30 (HR 1.183, 95% CI 1.087-1.286), whereas the association was attenuated during days 31-365 (HR 1.023, 95% CI 0.931-1.125). The difference between interval-specific coefficients was statistically significant (P for interaction = 0.015).

For transferrin, each 10 mg/dL increase was inversely associated with mortality during days 0-30 (HR 0.936, 95% CI 0.902-0.971), whereas the later association was attenuated (HR 0.984, 95% CI 0.951-1.019; P for interaction = 0.045). For TSAT, the estimate was higher during days 0-30 (HR

1.058 per 10-percentage-point increase, 95% CI 1.011-1.108) than during days 31-365 (HR 1.000, 95% CI 0.941-1.063), but the early-late difference was not statistically significant (P for interaction = 0.116).

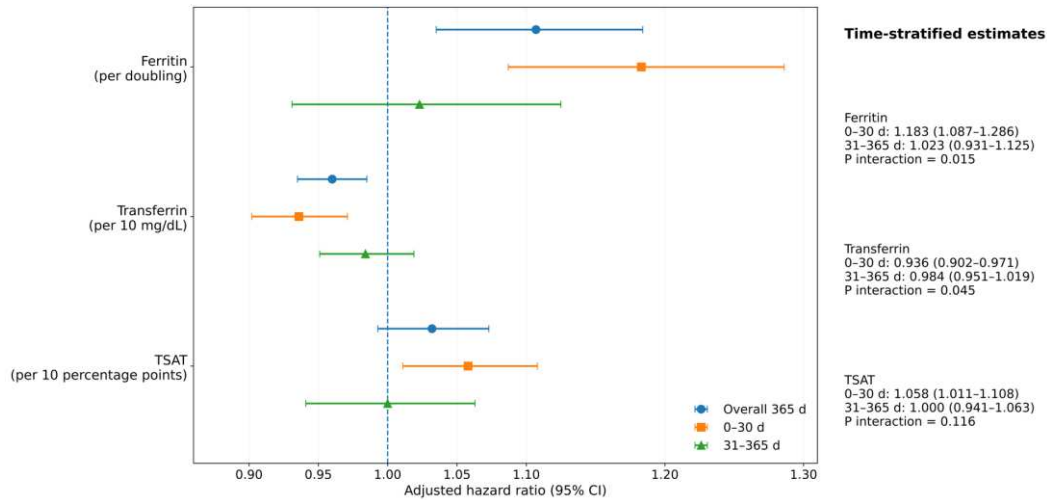


Figure 3. Overall and time-stratified associations of iron-homeostasis markers with 365-day mortality. Hazard ratios are shown for the overall 365-day follow-up and separately for days 0-30 and 31-365. All adjusted models included age, sex, SOFA score, hemoglobin, and CKD. P for interaction tests the difference between the early and later exposure coefficients. Because proportional-hazards violations were detected, the overall 365-day estimates should be interpreted as time-averaged summary associations. Ferritin is modeled per doubling, transferrin per 10 mg/dL increase, and TSAT per 10-percentage-point increase. CI, confidence interval; CKD, chronic kidney disease; HR, hazard ratio; SOFA, Sequential Organ Failure Assessment; TSAT, transferrin saturation.

Restricted cubic spline analyses

Restricted cubic spline analyses showed significant overall and nonlinear associations for ferritin (P overall < 0.001; P nonlinear < 0.001) and transferrin (P overall < 0.001; P nonlinear = 0.001). For TSAT, the overall spline test was significant (P = 0.042), whereas evidence for nonlinearity was borderline (P = 0.051) (Figure 4). Given the proportional-hazards violations, these curves are interpreted as time-averaged exposure-response patterns over the full follow-up. Restricted cubic spline test results are also summarized in Additional file 1 (Table S5).

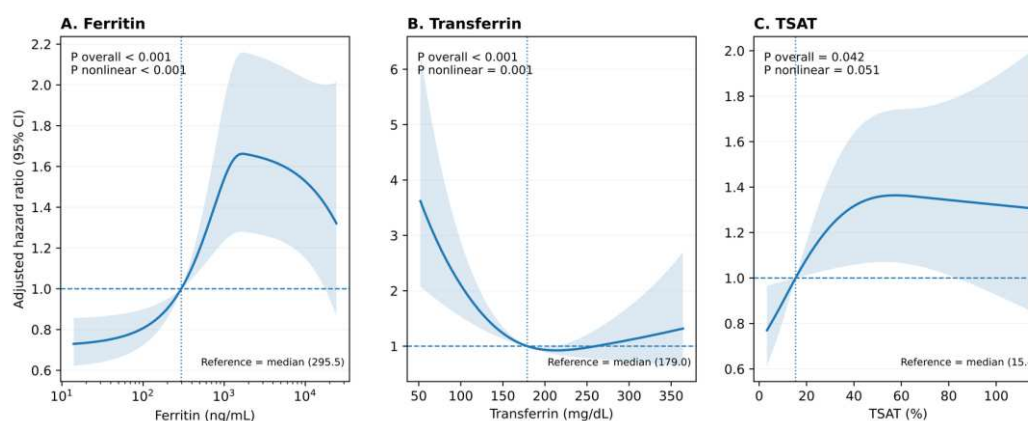


Figure 4. Restricted cubic spline associations of ferritin, transferrin, and TSAT with 365-day mortality.

Models used three knots at the 10th, 50th, and 90th percentiles and were adjusted for age, sex, SOFA score, hemoglobin, and CKD. The median exposure value served as the reference. Solid lines indicate adjusted hazard ratios and shaded areas indicate 95% confidence intervals. Curves are displayed over the central 1st-99th percentile of each exposure for readability, while all observations were used for model fitting. Because proportional-hazards violations were identified, the curves are interpreted as time-averaged exposure-response associations over the 365-day follow-up.

Sensitivity analyses

The ferritin association remained directionally stable and statistically significant across sensitivity analyses, including replacement of SOFA with SAPS II or APS III, additional adjustment for diabetes, restriction to ICU stays of at least 24 hours, and exclusion of ferritin values above the 99th percentile. The inverse transferrin association was also generally stable across alternative model specifications. Detailed estimates from these model and extreme-value sensitivity analyses are reported in Additional file 1 (Table S7).

The BMI multiple-imputation sensitivity analysis yielded estimates nearly identical to those from the primary model: ferritin HR 1.106 per doubling (95% CI 1.034-1.184; $P = 0.004$), transferrin HR 0.960 per 10 mg/dL (95% CI 0.936-0.985; $P = 0.002$), and TSAT HR 1.032 per 10-percentage-point increase (95% CI 0.992-1.073; $P = 0.114$). The corresponding multiple-imputation results are presented in Additional file 1 (Table S8).

TSAT was more sensitive to functional form and extreme observations. After excluding 13 patients with TSAT >100%, the continuous association became stronger (HR 1.102 per 10-percentage-point increase, 95% CI 1.037-1.171; P = 0.002), indicating that the primary linear TSAT estimate should be interpreted cautiously. This extreme-value sensitivity analysis is also reported in Additional file 1 (Table S7).

In the independently constructed principal-diagnosis AMI cohort, ferritin remained associated with mortality (HR 1.294 per doubling, 95% CI 1.093-1.533; P = 0.003). Transferrin (HR 0.993 per 10 mg/dL, 95% CI 0.946-1.043; P = 0.791) and TSAT (HR 1.082 per 10-percentage-point increase, 95% CI 0.967-1.210; P = 0.169) were not statistically significant. Thus, ferritin showed the most consistent association across alternative AMI cohort definitions. Full results for the independently constructed principal-diagnosis AMI cohort are shown in Additional file 1 (Table S9).

Discussion

Principal findings

In this cohort of critically ill patients with AMI, ferritin and transferrin measured within 24 hours of ICU admission were associated with 365-day mortality after adjustment for age, sex, SOFA score, hemoglobin, and CKD. The principal finding was temporal heterogeneity: associations for both markers were substantially stronger during the first 30 days and attenuated thereafter, with statistically significant early-versus-later interaction tests. Ferritin showed the most consistent evidence across continuous, tertile, spline, sensitivity, and principal-diagnosis analyses. Transferrin showed a reproducible inverse association in the primary cohort but was not replicated in the stricter principal-diagnosis cohort. TSAT showed weaker and less consistent evidence, with a non-significant

primary linear estimate, a significant highest-tertile association, borderline spline nonlinearity, and sensitivity to extreme observations.

These findings suggest that admission iron-homeostasis markers may provide predominantly acute-phase prognostic information rather than act as fixed long-term risk markers. The overall 365-day Cox estimates remain useful as time-averaged summaries, but the time-stratified analyses show that a constant hazard ratio over the full year would incompletely characterize the observed associations.

Ferritin and mortality after AMI

Ferritin is an iron-storage protein and a well-established acute-phase reactant [10]. Elevated ferritin has been associated with adverse outcomes in ischemic heart disease and several critical-illness settings [9,27–32]. More recently, Luo et al. used MIMIC-IV to study 992 ICU-admitted patients with AMI and found that elevated ferritin and TSAT were associated with increased short-term all-cause mortality, with follow-up analyses extending to 120 days [33]. Our findings therefore do not seek to establish ferritin as a novel prognostic marker in ICU-treated AMI; rather, they extend the existing evidence by showing that the ferritin-mortality association is non-proportional over 365 days, is concentrated mainly during the first 30 days, remains evident after parsimonious clinical adjustment, and persists in a separately constructed principal-diagnosis AMI cohort. The nonlinear spline pattern further indicates that the association is not adequately represented by a simple untransformed linear term.

The stronger ferritin association during the first 30 days is biologically plausible. In critically ill patients with AMI, ferritin may reflect the combined burden of acute inflammation, tissue injury, oxidative stress, and disturbed iron handling rather than total body iron stores alone. Iron dysregulation during ischemic and reperfusion injury can promote oxidative stress, mitochondrial

dysfunction, and lipid peroxidation [6–8,38]. Ferroptosis provides a plausible mechanistic framework linking iron dysregulation to cardiomyocyte injury [8,39–42]. Experimental data also suggest that ferritin heavy-chain biology can modulate vascular oxidative and inflammatory processes, underscoring the context-dependent biology of ferritin itself [43]. However, the present observational data do not measure ferroptosis and cannot establish it as the mechanism underlying the mortality association. Ferritin should therefore be interpreted as a prognostic correlate of the acute pathophysiological state.

Transferrin as an inverse acute-phase marker

Transferrin showed an inverse association with mortality in the primary cohort, again concentrated in the first 30 days. As the principal circulating iron-transport protein and a negative acute-phase reactant [11,44], low transferrin in critical illness may reflect inflammation-related suppression of hepatic synthesis, nutritional compromise, capillary leak, and altered iron transport [45–49]. Accordingly, low transferrin may integrate several dimensions of acute illness severity rather than represent an isolated defect in iron transport.

The attenuation of the transferrin association after day 30 is consistent with an acute-phase interpretation. As the acute episode resolves, later outcomes may increasingly reflect comorbidity, recurrent cardiovascular events, rehabilitation, and post-discharge care. Importantly, the absence of a significant association in the independently constructed principal-diagnosis cohort argues against describing higher transferrin as protective. The more appropriate interpretation is an inverse prognostic association in the primary ICU-AMI cohort that requires external validation.

TSAT requires cautious interpretation

TSAT produced the least consistent findings. The primary continuous linear association was not statistically significant, whereas the highest tertile was associated with higher mortality and the overall spline test was significant. Evidence for spline nonlinearity was borderline, and exclusion of values >100% materially strengthened the continuous estimate. Collectively, these findings indicate sensitivity to functional form and extreme observations and do not support TSAT as a stable linear prognostic marker in this cohort.

This complexity is clinically plausible because TSAT is a ratio derived from serum iron and TIBC. During acute inflammation, changes in transferrin and TIBC can substantially alter TSAT even in the absence of classical systemic iron overload. This differs from chronic heart failure, in which low TSAT and iron deficiency are commonly associated with worse outcomes [12–18]. Thresholds and treatment concepts derived from chronic heart failure should therefore not be extrapolated directly to critically ill patients with AMI on the basis of the present data.

Temporal heterogeneity and clinical interpretation

Temporal heterogeneity is central to the interpretation of this study. Ferritin and transferrin were most strongly associated with mortality during the first 30 days, whereas their associations were markedly attenuated during days 31–365. This pattern is consistent with admission iron-homeostasis markers reflecting acute inflammatory, metabolic, and organ-dysfunction burden more strongly than fixed long-term risk. It also illustrates the importance of proportional-hazards diagnostics in biomarker studies: a single 365-day hazard ratio would obscure clinically relevant changes in association over time.

These findings do not establish that modifying ferritin, transferrin, or TSAT would improve outcomes. Rather, abnormal iron-homeostasis profiles may identify patients with a more severe acute biological

response. If externally validated, these markers may complement clinical characterization of high-risk ICU patients, but the present data do not support their use as standalone therapeutic targets, screening thresholds, or treatment-selection biomarkers.

Strengths and limitations

Several features strengthen this study. The cohort used a reproducible first-stay design, retained short ICU stays, and required iron and TIBC measurements recorded at the same charted time for TSAT calculation. The primary covariate set was clinically defined before interpretation of the final associations and was parsimonious, avoiding simultaneous adjustment for multiple overlapping severity scores. We formally evaluated the proportional-hazards assumption and reported interval-specific estimates when violations were detected. Findings were further examined using nonlinear models and multiple sensitivity analyses, including an independently constructed principal-diagnosis cohort.

Several limitations should be considered. First, the retrospective, single-center design precludes causal inference and leaves the possibility of residual confounding. The final primary dataset lacked several potentially relevant AMI-specific and treatment variables, including STEMI/NSTEMI classification, revascularization status, cardiogenic shock, vasopressor use, left ventricular function, and detailed admission renal function; unmeasured cardiovascular severity may therefore partly account for the observed associations. Second, a complete iron panel is not routinely measured in all patients with AMI. Requiring ferritin, transferrin, and paired iron/TIBC measurements within 24 hours may preferentially select patients with anemia, CKD, nutritional concerns, suspected iron disorders, or greater illness complexity, limiting generalizability. Third, exposures were based on single early measurements and did not capture subsequent trajectories of iron metabolism. Fourth,

the cohort was restricted to ICU-treated AMI and should not be generalized to lower-risk AMI populations. Fifth, the independent principal-diagnosis cohort was relatively small, reducing precision, particularly for transferrin and TSAT. Finally, although time-stratified analyses addressed the major proportional-hazards violations, the 30-day cut point is a clinically interpretable simplification of potentially continuous changes in association over time.

Conclusions

Among critically ill patients with AMI, higher ferritin and lower transferrin measured within 24 hours of ICU admission were associated with higher 365-day mortality, with associations concentrated mainly during the first 30 days. Ferritin was the most consistent marker across model forms and sensitivity analyses, whereas TSAT showed less consistent and more model-sensitive evidence. These findings support interpreting early iron-homeostasis markers as prognostic correlates whose associations vary over time rather than as fixed long-term risk factors or direct therapeutic targets. Prospective multicenter studies with serial iron measurements and richer AMI-specific clinical data are needed for validation.

List of abbreviations

AMI, acute myocardial infarction; APS III, Acute Physiology Score III; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; ICD, International Classification of Diseases; ICU, intensive care unit; MIMIC-IV, Medical Information Mart for Intensive Care IV; RCS, restricted cubic spline; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

Additional file

File name: Additional file 1

401 File format: .xlsx

402 Title: Supplementary Tables S1–S9

403 Description: Tables S1–S9 provide baseline characteristics across ferritin, transferrin, and TSAT
404 tertiles; full 365-day Cox regression analyses; Kaplan–Meier and restricted cubic spline analyses;
405 proportional-hazards diagnostics and time-stratified analyses; predefined model and extreme-value
406 sensitivity analyses; BMI multiple-imputation analyses; and the independent principal-diagnosis
407 AMI cohort sensitivity analysis.

408 **Declarations**

409 **Ethics approval and consent to participate**

410 This study was a secondary analysis of de-identified data from the MIMIC-IV database. The
411 MIMIC-IV database was approved by the Institutional Review Boards of Beth Israel Deaconess
412 Medical Center (protocol No. 2001-P-001699/14) and the Massachusetts Institute of Technology
413 (protocol No. 0403000206). Database access was obtained after completion of the required training
414 and authorization procedures. No additional patient contact or collection of identifiable information
415 was performed in the present study. The requirement for individual informed consent was waived
416 for the MIMIC-IV data resource because the data were de-identified and the project did not affect
417 clinical care.

418 **Consent for publication**

419 Not applicable.

420 **Availability of data and materials**

421 The dataset analysed during the current study is available in the PhysioNet MIMIC-IV repository
422 (version 3.0), <https://doi.org/10.13026/hxp0-hg59>, subject to credentialing, completion of the

423 required training, and the PhysioNet Data Use Agreement. The authors are not permitted to
424 redistribute the underlying patient-level data.

425 **Competing interests**

426 The authors declare that they have no competing interests.

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430 **Authors' contributions**

431 YS: conceptualization, methodology, formal analysis, and writing—original draft preparation. CW
432 and TY: investigation and resources. YS, YD, CW, and TY: writing—review and editing. YD:
433 supervision. All authors read and approved the final manuscript.

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436 **References**

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