

Supplemental Material

Leaf DE *et al.* Iron, Heparin, and Death in Human Acute Kidney Injury

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Characteristic	All Patients in the ATN Study (n = 1124)	Biorepository Subcohort (n = 817)	P Value
Demographics			
Age, yr, median (IQR)	61 (50–71)	62 (51–72)	0.30
Male sex – no. (%)	793 (71)	568 (70)	0.60
White race – no. (%)	835 (74)	628 (77)	0.19
Comorbidities – no. (%)			
Diabetes mellitus	275 (25)	199 (25)	0.90
Congestive heart failure	260 (24)	198 (25)	0.63
Chronic liver disease	131 (12)	95 (12)	0.95
Chronic lung disease ^a	135 (12)	103 (13)	0.74
Chronic kidney disease ^b	390 (37)	293 (38)	0.67
Malignancy	224 (20)	158 (19)	0.74
Baseline renal function			
Creatinine, mg/dl, median (IQR) ^c	1.1 (0.9–1.4)	1.1 (0.9–1.4)	0.94
eGFR, median (IQR) ^d	71 (52–94)	71 (50–94)	0.62
Medical ICU – no. (%)			
531 (47)	361 (44)	0.18	
Severity of illness			
Sepsis – no. (%)	708 (63)	527 (65)	0.49
APACHE II score, median (IQR) ^e	26 (21–32)	26 (21–31)	0.64
Mechanical ventilation – no. (%)	905 (81)	661 (81)	0.86
Shock – no. (%)	695 (62)	515 (63)	0.59
Enrollment labs, median (IQR)			
White cell count, per mm ³	13 (8–19)	13 (9–19)	0.68
Hemoglobin, g/dl	10 (9–11)	10 (9–11)	0.83
Creatinine, mg/dl	3.9 (2.9–5.1)	3.9 (2.9–5.1)	0.67
Albumin, g/dl	2.3 (1.9–2.9)	2.3 (1.9–2.8)	0.70
60-day mortality – no. (%)			
591 (53)	415 (51)	0.41	

Supplemental Table 1. Enrollment characteristics in the ATN study, all patients versus biorepository subcohort. Percentages are based on the number of patients without missing data.

^aDefined as chronic hypoxemia, hypercapnea, pulmonary hypertension, or ventilator dependence.

^bDefined as baseline eGFR < 60 ml/min/1.73m².

^cBaseline serum creatinine (SCr) was defined as the pre-morbid SCr at the time of screening or, if unavailable, the lowest SCr within 4 days prior to screening.

^deGFR is reported in ml/min per 1.73m² and was determined using the CKD-EPI equation.

^eAPACHE II score is an ICU severity of illness scoring system ranging from 0 to 71, with higher scores indicating more severe disease.

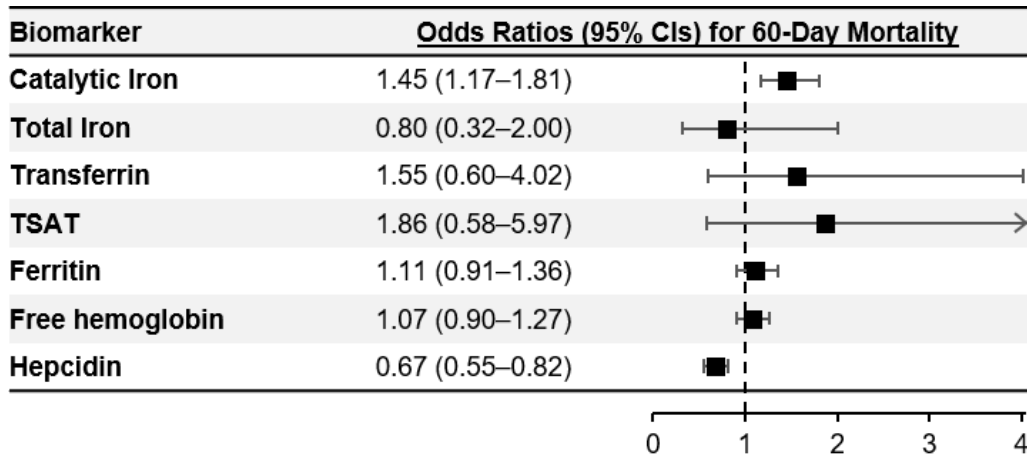
Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, interquartile range; SCr, serum creatinine.

Biomarker	Assay Type	Manufacturer	Lower Limit of detection	Interassay CV (%)
Catalytic Iron	Colorimetry	N/A	0.03 µmol/L	14.1
Total Iron	Ferene Colorimetry	Teco Diagnostics (Anaheim, CA)	2 µg/dl	12.2
Transferrin	Immuno- turbidimetry	Beckman Coulter (USA)	1mg/dl	7.7
TSAT	N/A	N/A	N/A	N/A
Ferritin	CLIA	Maglumi, Snibe	1.3 ng/ml	7.1
Free Hemoglobin	ELISA	My Biosource (USA)	0.625 µg/dl	17.2
Hepcidin	ELISA	Intrinsic LifeSciences (La Jolla, CA)	0.55 ng/ml	8.0

Supplemental Table 2. Assay characteristics. Abbreviations: CLIA, chemiluminescence immunoassay; CV, coefficient of variation (calculated from blinded replicate samples); ELISA, enzyme-linked immunosorbent assay; N/A, not applicable.

	All (n = 556)	Survivors (n = 340)	Non-Survivors (n = 216)	P Value*
Catalytic Iron (µmol/L)	0.40 (0.28–0.62)	0.39 (0.27–0.56)	0.43 (0.30–0.71)	0.012
Total Iron (µg/dl)	64 (56–72)	65 (58–72)	63 (55–73)	0.31
Transferrin (mg/dl)	156 (130–182)	158 (134–182)	149 (123–178)	0.028
TSAT (%)	23 (20–27)	23 (20–27)	23 (20–27)	0.26
Ferritin (µg/L)	582 (302–1143)	550 (295–967)	659 (304–1449)	0.047
Free Hgb (mg/dl)	11 (6–20)	11 (6–19)	14 (9–21)	<0.001

Supplemental Table 3. Iron marker levels on day 8 according to 60-day survival status. Data are shown as median and interquartile range (25th–75th percentiles). *P values refer to the comparison between iron parameters in patients alive versus dead at 60 days. For these analyses, only P values <0.01 are considered significant to account for multiple comparisons.



Supplemental Table 4. Odds ratios (and 95% CIs) for death according to iron marker levels – exploratory analysis adjusted for all model 2 and model 3 covariates and all iron parameters. These analyses include all model 2 covariates (age, gender, race, baseline eGFR, diabetes mellitus, congestive heart failure, chronic liver disease, and chronic lung disease) and all model 3 covariates (ICU type, mechanical ventilation, APACHE II score, RRT prior to randomization, treatment randomization group, oliguria, sepsis, shock, and serum/plasma levels of albumin, creatinine, and interleukin-6). Additionally, these analyses are adjusted for all iron parameters (catalytic iron, total iron, transferrin, TSAT, ferritin, free hemoglobin, and hepcidin). Each iron parameter is natural log-transformed and standardized to one SD.

Characteristic	Catalytic iron quintiles [range (μmol/L)]						P _{trend}
	All (n = 807)	Q1 (n = 160) [0.18–0.33]	Q2 (n = 164) [0.34–0.46]	Q3 (n = 160) [0.47–0.80]	Q4 (n = 161) [0.81–2.12]	Q5 (n = 162) [2.16–47.01]	
Demographics							
Age, yr, median (IQR)	62 (51–72)	66 (55–76)	63 (54–73)	63 (50–75)	59 (51–67)	58 (48–69)	<0.001
Male sex – no. (%)	561 (70)	105 (66)	127 (77)	113 (71)	115 (71)	101 (62)	0.04
White race – no. (%)	621 (77)	121 (76)	132 (80)	112 (70)	131 (81)	125 (77)	0.11
Comorbidities – no. (%)							
Diabetes mellitus	198 (25)	49 (32)	40 (26)	36 (23)	47 (30)	26 (17)	0.02
Congestive heart failure	197 (25)	50 (33)	51 (32)	41 (26)	35 (22)	20 (13)	<0.001
Chronic liver disease	94 (12)	13 (8)	8 (5)	12 (8)	24 (15)	37 (24)	<0.001
Chronic lung disease ^a	102 (13)	31 (20)	19 (12)	22 (14)	14 (9)	16 (10)	0.03
Chronic kidney disease ^b	293 (39)	63 (41)	69 (45)	64 (43)	57 (38)	40 (26)	0.01
Malignancy	156 (19)	26 (16)	27 (16)	30 (19)	33 (21)	40 (25)	0.29
Baseline renal function							
Creatinine, mg/dl, median (IQR) ^c	1.1 (0.9–1.4)	1.1 (0.9–1.4)	1.2 (0.9–1.4)	1.1 (0.9–1.4)	1.1 (0.9–1.4)	0.9 (0.8–1.2)	<0.001
eGFR, median (IQR) ^d	70 (50–93)	68 (47–85)	66 (48–89)	69 (49–97)	68 (50–94)	82 (58–102)	<0.001
Medical ICU – no. (%)	356 (44)	64 (40)	72 (44)	67 (42)	73 (45)	80 (49)	0.50
Severity of illness							
Sepsis – no. (%)	518 (64)	87 (54)	97 (59)	102 (64)	109 (68)	123 (76)	<0.001
APACHE II score, median (IQR) ^e	26 (21–31)	24 (19–29)	26 (20–31)	27 (21–31)	26 (22–31)	27 (22–33)	0.009
Mechanical ventilation – no. (%)	652 (81)	124 (78)	129 (79)	136 (85)	124 (77)	139 (86)	0.11
Shock – no. (%) ^f	508 (63)	93 (58)	93 (57)	94 (59)	101 (63)	127 (78)	<0.001
Oliguria – no. (%) ^g	645 (80)	121 (76)	126 (77)	120 (75)	132 (82)	146 (90)	0.003
RRT prior to randomization – no. (%)	558 (69)	119 (74)	113 (69)	103 (64)	108 (67)	115 (71)	0.36
Days in ICU, median (IQR) ^h	4 (3–8)	5 (3–8)	4 (3–8)	5 (3–8)	4 (3–7)	4 (2–8)	0.14
Enrollment labs, median (IQR)							
White cell count, per mm ³	13 (9–19)	13 (9–17)	14 (10–19)	13 (8–20)	14 (9–21)	13 (7–22)	0.58
Hemoglobin, g/dl	9.8 (8.9–10.8)	9.7 (9.0–10.4)	9.8 (9.1–10.8)	10.0 (9.0–10.8)	9.7 (8.9–11.0)	9.6 (8.6–10.8)	0.53
Creatinine, mg/dl	3.9 (2.9–5.1)	3.8 (3.0–4.7)	4.0 (2.9–5.3)	4.2 (3.3–5.4)	4.0 (3.2–5.4)	3.4 (2.4–4.7)	<0.001
Albumin, g/dl	2.3 (1.9–2.8)	2.5 (2.0–3.0)	2.3 (2.0–2.8)	2.2 (1.7–2.7)	2.3 (1.9–2.9)	2.3 (1.7–2.8)	0.01
IL-6, pg/ml	166 (74–540)	132 (63–467)	165 (62–412)	176 (83–709)	145 (63–432)	252 (114–1053)	<0.001
60-day mortality – no. (%)	409 (51)	65 (41)	67 (41)	79 (49)	80 (50)	118 (73)	<0.001

Supplemental Table 5. Enrollment characteristics according to quintiles of catalytic iron. Percentages are based on the number of patients without missing data. Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, interquartile range; RRT, renal replacement therapy. P values were calculated using Kruskal Wallis and chi-squared tests for continuous and categorical variables, respectively, across the five quintiles of catalytic iron.

^aDefined as chronic hypoxemia, hypercapnea, pulmonary hypertension, or ventilator dependence.

^bDefined as baseline eGFR<60 ml/min/1.73m².

^cBaseline serum creatinine (SCr) was defined as the pre-morbid SCr at the time of screening or, if unavailable, the lowest SCr within 4 days prior to screening.

^deGFR is reported in ml/min per 1.73m² and was determined using the CKD-EPI equation³⁶.

^eAPACHE II score is an ICU severity of illness scoring system ranging from 0 to 71, with higher scores indicating more severe disease.

^fShock was defined as requirement for vasopressor support for greater than 1 hour.

^gOliguria was defined as an average urine output <20 ml/hour for >24 hours.

^hPrior to randomization.

Characteristic	Univariate		Multivariable	
	β	P value	β	P value
Demographics				
Age, yr	-0.01	<0.001	-0.006	0.06
Male sex	-0.09	0.25		
White race	-0.02	0.75		
Comorbidities				
Diabetes mellitus	-0.19	0.02	-0.06	0.45
Congestive heart failure	-0.39	<0.001	-0.27	0.002
Chronic liver disease	0.64	<0.001	0.45	<0.001
Chronic lung disease ^a	-0.27	0.009	-0.26	0.02
Chronic kidney disease ^b	-0.23	0.002	0.09	0.46
Malignancy	0.16	0.08		
Baseline renal function				
Creatinine, mg/dl ^c	-0.42	<0.001	-0.33	0.17
eGFR ^d	0.006	<0.001	-0.001	0.71
Medical ICU				
Severity of illness				
Sepsis	0.28	<0.001	0.19	0.01
APACHE II score ^e	0.02	<0.001	0.01	0.02
Mechanical ventilation	0.17	0.052		
Shock ^f	0.34	<0.001	0.24	0.004
Oliguria ^g	0.34	<0.001	0.15	0.12
RRT prior to randomization	-0.005	0.95		
Days in ICU ^h	-0.0009	0.79		
Enrollment labs				
White cell count, per mm ³	0.0003	0.36		
Hemoglobin, g/dl	-0.02	0.34		
Creatinine, mg/dl	-0.05	0.008	-0.01	0.62
Albumin, g/dl	-0.09	0.10		
IL-6, ln (pg/ml)	0.16	<0.001	0.10	0.01

Supplemental Table 6. Univariate and multivariable associations between enrollment characteristics and catalytic iron concentrations. Catalytic iron concentrations were natural log-transformed. Characteristics that were significant ($P < 0.05$) in univariate analyses were included in multivariable analyses.

^aDefined as chronic hypoxemia, hypercapnea, pulmonary hypertension, or ventilator dependence.

^bDefined as baseline eGFR < 60 ml/min/1.73m².

^cBaseline serum creatinine (SCr) was defined as the pre-morbid SCr at the time of screening or, if unavailable, the lowest SCr within 4 days prior to screening.

^deGFR is reported in ml/min per 1.73m² and was determined using the CKD-EPI equation ³⁶.

^eAPACHE II score is an ICU severity of illness scoring system ranging from 0 to 71, with higher scores indicating more severe disease.

^fShock was defined as requirement for vasopressor support for greater than 1 hour.

^gOliguria was defined as an average urine output < 20 ml/hour for > 24 hours.

^hPrior to randomization.

Characteristic	Hepcidin quintiles [range (ng/ml)]						P _{trend}
	All (n = 802)	Q1 (n = 160) [4–33]	Q2 (n = 160) [33–86]	Q3 (n = 160) [86–163]	Q4 (n = 161) [163–266]	Q5 (n = 161) [266–2415]	
Demographics							
Age, yr, median (IQR)	62 (51–72)	59 (51–69)	64 (53–75)	65 (53–74)	63 (54–74)	61 (47–69)	0.002
Male sex – no. (%)	560 (70)	100 (63)	105 (66)	114 (71)	120 (75)	121 (75)	0.05
White race – no. (%)	615 (77)	134 (84)	122 (76)	122 (76)	123 (76)	114 (71)	0.11
Comorbidities – no. (%)							
Diabetes mellitus	196 (25)	32 (20)	45 (28)	47 (31)	36 (23)	36 (24)	0.18
Congestive heart failure	193 (25)	53 (34)	42 (27)	49 (32)	29 (19)	20 (13)	<0.001
Chronic liver disease	95 (12)	43 (28)	26 (17)	14 (9)	8 (5)	4 (3)	<0.001
Chronic lung disease ^a	102 (13)	19 (12)	17 (11)	22 (14)	20 (13)	24 (15)	0.75
Chronic kidney disease ^b	288 (38)	52 (34)	62 (40)	58 (40)	63 (42)	53 (35)	0.61
Malignancy	115 (19)	22 (14)	33 (21)	24 (15)	33 (21)	43 (27)	0.03
Baseline renal function							
Creatinine, mg/dl, median (IQR) ^c	1.1 (0.9–1.4)	1.0 (0.9–1.4)	1.0 (0.8–1.4)	1.1 (0.9–1.4)	1.1 (0.9–1.3)	1.1 (0.9–1.4)	0.76
eGFR, median (IQR) ^d	71 (50–94)	71 (52–88)	71 (49–94)	69 (49–93)	67 (51–93)	75 (53–97)	0.59
Medical ICU – no. (%)	356 (44)	71 (44)	74 (46)	63 (39)	70 (43)	78 (48)	0.56
Severity of illness							
Sepsis – no. (%)	520 (65)	91 (57)	113 (71)	105 (66)	101 (63)	110 (68)	0.09
APACHE II score, median (IQR) ^e	26 (21–31)	27 (22–31)	28 (22–32)	25 (21–30)	24 (20–29)	27 (21–32)	0.002
Mechanical ventilation – no. (%)	649 (81)	128 (80)	135 (84)	132 (83)	123 (76)	131 (81)	0.44
Shock – no. (%) ^f	507 (63)	111 (69)	117 (73)	104 (65)	98 (61)	77 (48)	<0.001
Oliguria – no. (%) ^g	642 (80)	135 (84)	140 (88)	127 (79)	120 (75)	120 (75)	0.009
RRT prior to randomization – no. (%)	550 (69)	123 (77)	113 (71)	115 (72)	104 (65)	95 (59)	0.007
Days in ICU, median (IQR) ^h	4 (3–8)	4 (3–8)	4 (3–8)	4 (3–8)	4 (3–8)	5 (3–9)	0.94
Enrollment labs, median (IQR)							
White cell count, per mm ³	13 (9–19)	14 (5–19)	15 (11–22)	13 (8–18)	13 (10–18)	12 (7–18)	0.002
Hemoglobin, g/dl	9.8 (8.9–10.8)	9.5 (8.5–10.4)	9.9 (9.1–11.0)	9.8 (8.9–10.9)	10.0 (9.1–10.8)	9.7 (8.9–10.7)	0.009
Creatinine, mg/dl	3.9 (2.9–5.1)	3.5 (2.6–4.7)	3.6 (2.8–4.8)	3.6 (2.7–4.7)	4.1 (3.2–5.3)	4.8 (3.6–6.3)	<0.001
Albumin, g/dl	2.3 (1.9–2.8)	2.6 (2.1–3.1)	2.4 (1.8–2.9)	2.2 (1.8–2.8)	2.3 (1.9–2.7)	2.2 (1.8–2.7)	<0.001
IL-6, pg/ml	166 (74–523)	124 (58–328)	145 (61–575)	145 (59–466)	179 (101–467)	235 (103–1003)	<0.001
60-day mortality – no. (%)	409 (51)	101 (63)	92 (58)	84 (53)	70 (43)	62 (39)	<0.001

Supplemental Table 7. Enrollment characteristics according to quintiles of hepcidin. Percentages are based on the number of patients without missing data. Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, interquartile range; RRT, renal replacement therapy. P values were calculated using Kruskal Wallis and chi-squared tests for continuous and categorical variables, respectively, across the five quintiles of hepcidin.

^aDefined as chronic hypoxemia, hypercapnea, pulmonary hypertension, or ventilator dependence.

^bDefined as baseline eGFR < 60 ml/min/1.73m².

^cBaseline serum creatinine (SCr) was defined as the pre-morbid SCr at the time of screening or, if unavailable, the lowest SCr within 4 days prior to screening.

^deGFR is reported in ml/min per 1.73m² and was determined using the CKD-EPI equation³⁶.

^eAPACHE II score is an ICU severity of illness scoring system ranging from 0 to 71, with higher scores indicating more severe disease.

^fShock was defined as requirement for vasopressor support for greater than 1 hour.

^gOliguria was defined as an average urine output < 20 ml/hour for > 24 hours.

^hPrior to randomization.

Characteristic	Univariate		Multivariable	
	β	P value	β	P value
Demographics				
Age, yr	-0.0004	0.87		
Male sex	0.25	0.001	0.08	0.34
White race	0.11	0.046	0.06	0.37
Comorbidities				
Diabetes mellitus	0.03	0.76		
Congestive heart failure	-0.37	<0.001	-0.21	0.02
Chronic liver disease	-0.82	<0.001	-0.71	<0.001
Chronic lung disease ^a	0.07	0.53		
Chronic kidney disease ^b	0.02	0.80		
Malignancy	0.25	0.005	0.19	0.04
Baseline renal function				
Creatinine, mg/dl ^c	0.10	0.32		
eGFR ^d	0.002	0.27		
Medical ICU	-0.03	0.65		
Severity of illness				
Sepsis	0.15	0.046	0.13	0.08
APACHE II score ^e	-0.007	0.15		
Mechanical ventilation	-0.03	0.74		
Shock ^f	-0.33	<0.001	-0.28	<0.001
Oliguria ^g	-0.25	0.004	-0.14	0.13
RRT prior to randomization	-0.24	0.001	-0.16	0.045
Days in ICU ^h	0.004	0.25		
Enrollment labs				
White cell count, per mm ³	-0.0006	0.08		
Hemoglobin, g/dl	0.02	0.29		
Creatinine, mg/dl	0.08	<0.001	0.08	<0.001
Albumin, g/dl	-0.24	<0.001	-0.10	0.053
IL-6, pg/ml	0.14	<0.001	0.19	<0.001

Supplemental Table 8. Univariate and multivariable associations between enrollment characteristics and hepcidin concentrations. Heparin concentrations were natural log-transformed. Characteristics that were significant ($P < 0.05$) in univariate analyses were included in multivariable analyses.

^aDefined as chronic hypoxemia, hypercapnea, pulmonary hypertension, or ventilator dependence.

^bDefined as baseline eGFR < 60 ml/min/1.73m².

^cBaseline serum creatinine (SCr) was defined as the pre-morbid SCr at the time of screening or, if unavailable, the lowest SCr within 4 days prior to screening.

^deGFR is reported in ml/min per 1.73m² and was determined using the CKD-EPI equation ³⁶.

^eAPACHE II score is an ICU severity of illness scoring system ranging from 0 to 71, with higher scores indicating more severe disease.

^fShock was defined as requirement for vasopressor support for greater than 1 hour.

^gOliguria was defined as an average urine output < 20 ml/hour for > 24 hours.

^hPrior to randomization.

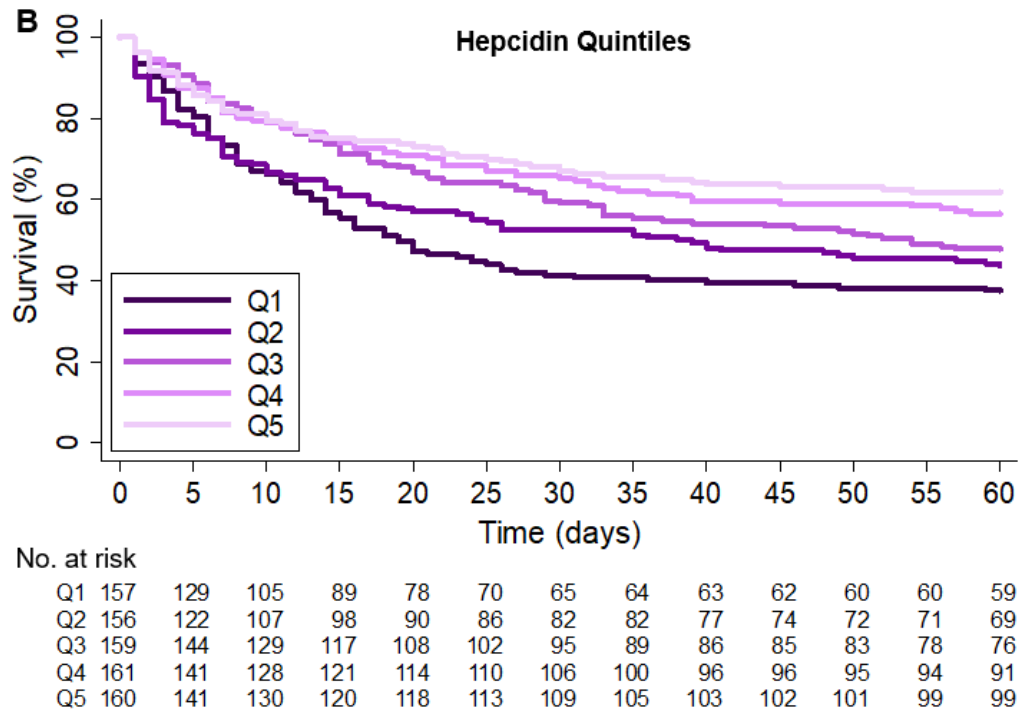
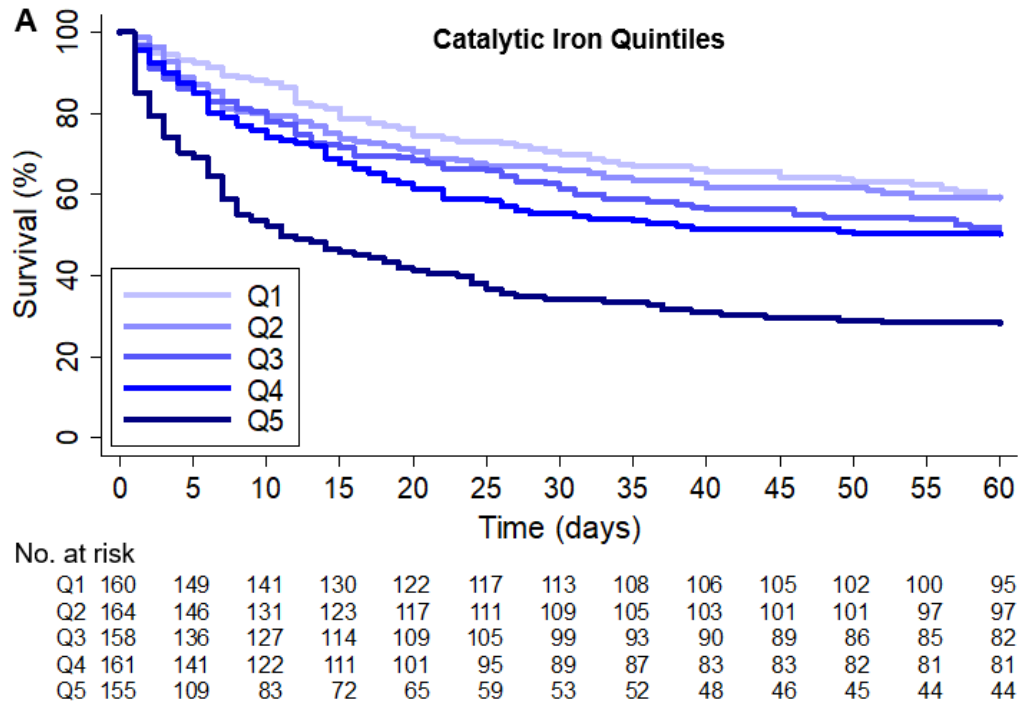
	Total Iron	Transferrin	TSAT	Ferritin	Free Hgb	Hepcidin	IL-6
Catalytic Iron	0.11**	-0.19***	0.23***	0.46***	0.23***	-0.20***	0.12***
Total Iron	---	0.20***	0.60***	0.06	0.06	-0.06	-0.10**
Transferrin	---	---	-0.61***	-0.17***	0.01	-0.21***	-0.16***
TSAT	---	---	---	0.17***	0.02	0.11**	0.06
Ferritin	---	---	---	---	0.11**	0.06	0.18***
Free Hgb	---	---	---	---	---	-0.18***	0.13***
Hepcidin	---	---	---	---	---	---	0.16***

Supplemental Table 9. Associations between iron and inflammatory parameters.

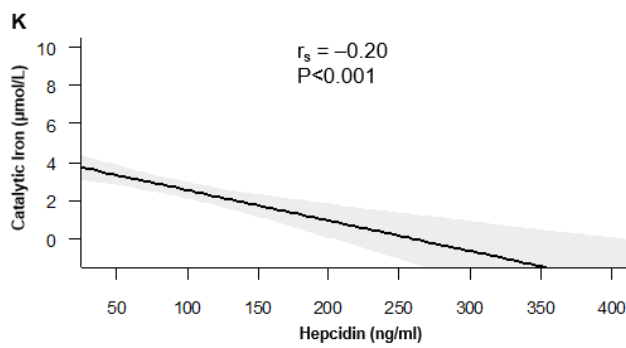
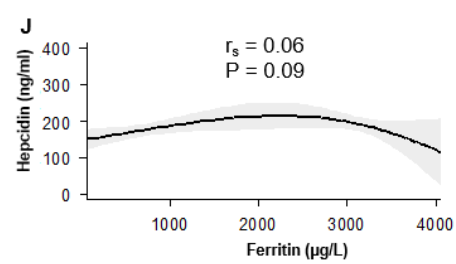
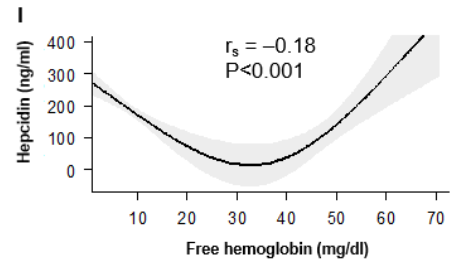
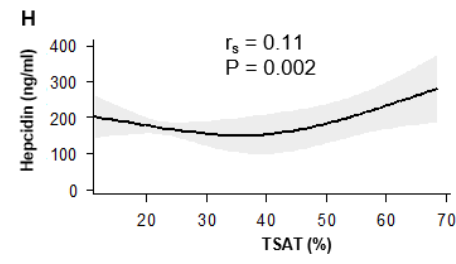
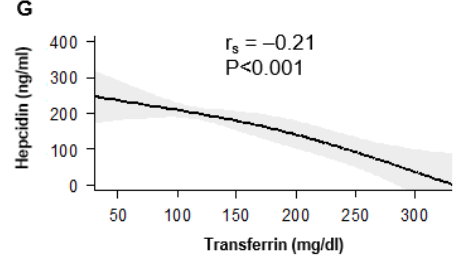
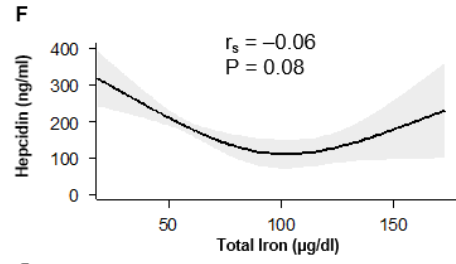
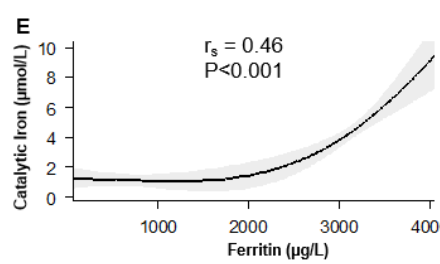
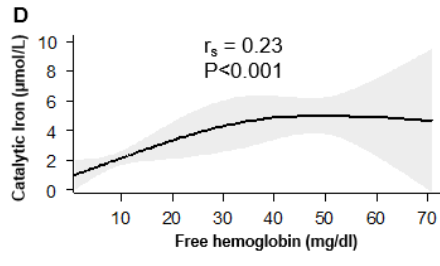
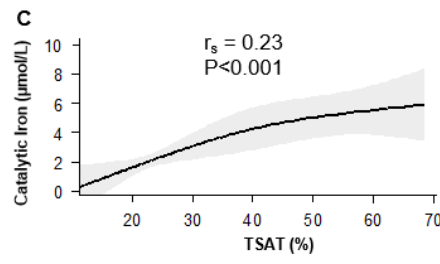
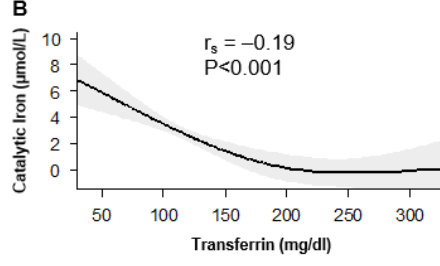
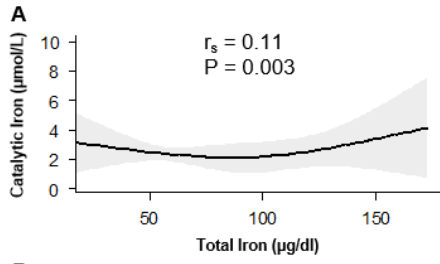
Spearman correlation coefficients are shown for each comparison. *P<0.05; **P<0.01; ***P<0.001. Abbreviations: Hgb, hemoglobin; TSAT, transferrin saturation.

	N	Day1	Day8	ΔDay8-Day1	P value ^a	P value ^b
Catalytic Iron (μmol/L)						
Overall	556	0.55 (0.34, 1.21)	0.40 (0.28, 0.62)	-0.12 (-0.69, 0.05)	<0.001	
Intensive RRT	267	0.54 (0.34, 1.20)	0.37 (0.27, 0.59)	-0.14 (-0.74, 0.02)	<0.001	
Less-Intensive RRT	289	0.55 (0.35, 1.22)	0.43 (0.30, 0.68)	-0.10 (-0.65, 0.11)	<0.001	0.03
Total Iron (μg/dL)						
Overall	556	65 (54, 81)	64 (56, 72)	-1 (-20, 13)	<0.001	
Intensive RRT	267	64 (54, 82)	64 (56, 72)	-2 (-20, 12)	0.04	
Less-Intensive RRT	289	65 (55, 80)	64 (57, 73)	-1 (-19, 13)	0.08	0.84
Transferrin (mg/dL)						
Overall	556	134 (110, 161)	156 (130, 182)	19 (-6, 44)	<0.001	
Intensive RRT	267	132 (108, 163)	156 (129, 182)	19 (-4, 44)	<0.001	
Less-Intensive RRT	289	135 (113, 160)	156 (130, 179)	10 (-7, 44)	<0.001	0.79
TSAT (%)						
Overall	556	26 (22, 32)	23 (20, 27)	-3 (-8, 1)	<0.001	
Intensive RRT	267	26 (22, 32)	23 (20, 27)	-3 (-8, 2)	<0.001	
Less-Intensive RRT	289	26 (22, 32)	23 (20, 27)	-3 (-8, 1)	<0.001	0.89
Ferritin (μg/L)						
Overall	556	744 (356, 1975)	582 (302, 1143)	-89 (-845, 113)	<0.001	
Intensive RRT	267	735 (338, 1916)	603 (296, 1120)	-86 (-756, 127)	<0.001	
Less-Intensive RRT	289	756 (364, 2013)	566 (307, 1190)	-91 (-926, 100)	<0.001	0.73
Free hemoglobin (mg/dL)						
Overall	556	11.3 (6.4, 20.4)	11.3 (6.4, 19.6)	-0.2 (-7.1, 6.4)	0.89	
Intensive RRT	267	12.0 (6.7, 21.3)	11.2 (6.2, 18.7)	-1.1 (-9.5, 5.1)	0.06	
Less-Intensive RRT	289	10.6 (6.3, 19.9)	11.8 (7.1, 21.2)	1.0 (-5.5, 8.3)	0.11	0.01

Supplemental Table 10. Changes in iron markers from day 1 to 8, overall and according to treatment assignment. Data are shown as median (interquartile range), and are restricted to patients with iron marker levels available on both days 1 and 8 (n=556). ^aComparison of change in iron marker levels from day 1 to day 8. ^bComparison of change in iron marker levels from day 1 to day 8 in patients assigned to intensive versus less-intensive RRT. Patients assigned to intensive RRT received intermittent hemodialysis or sustained low-efficiency dialysis six times per week (every day except Sunday) or continuous venovenous hemodiafiltration at a total effluent flow rate of 35 ml per kilogram of body weight per hour. Patients assigned to less-intensive RRT received intermittent hemodialysis or sustained low-efficiency dialysis three times per week (on alternate days except Sunday) or continuous venovenous hemodiafiltration at a total effluent flow rate of 20 ml per kilogram per hour. Abbreviations: RRT, renal replacement therapy; TSAT, transferrin saturation.



Supplemental Figure 1. Kaplan-Meier survival curves according to quintiles of catalytic iron and hepcidin.



Supplemental Figure 2. Associations between catalytic iron and hepcidin with other iron parameters. Restricted cubic spline graphs depict the relationships between catalytic iron and hepcidin with total iron (panels A and F), transferrin (panels B and G), TSAT (panels C and H), free hemoglobin (panels D and I), and ferritin (panels E and J). The relationship between catalytic iron and hepcidin is shown in panel K. For each graph, three knots were specified at the 10th, 50th, and 90th percentiles. The 95% confidence intervals are indicated by the shaded areas. Abbreviations: r_s , spearman correlation coefficient; TSAT, transferrin saturation.

Supplemental Methods

Plasma Catalytic Iron Measurement. Plasma catalytic iron ($\mu\text{mol/l}$) was measured using the modified bleomycin assay as described by us previously.¹ The measurement of catalytic iron in plasma samples is based on the reaction of the antitumor antibiotic bleomycin with DNA in the presence of iron and suitable reducing agent to bind and degrade it. The DNA degradation products react with thiobarbituric acid to form a chromogen to be measured at 532 nm spectrophotometrically. The assay detects iron capable of catalyzing free radical reactions in plasma. A Beckman (DU800) spectrophotometer was used to measure the intensity of the colored assay product.

Plasma Free Hemoglobin Measurement. Plasma free hemoglobin (mg/dl) was measured using an ELISA kit (catalogue # MBS 564144, "My Biosource" company). Free hemoglobin present in the samples was sandwiched between two anti-hemoglobin antibodies, one bound to the wall of the microtitre well while the other was conjugated to horseradish peroxidase enzyme. The quantity of bound enzyme varies directly with the concentration of the free hemoglobin in the sample. The intensity of the colored product developed by the enzyme from specific chromogenic substrate added to the assay was measured quantitatively at 450 nm using a Bio-Rad microplate reader (model #PR4100).

Plasma Total Iron Measurement. Plasma total iron ($\mu\text{g/dl}$) was measured using the Ferene colorimetric assay using a commercial kit (Teco Diagnostics, Anaheim, CA) following the modification used by Walmsley et al, 1992.² The iron in EDTA plasma was dissociated from its Fe (III)-Transferrin complex by the addition of an acidic buffer containing hydroxylamine and 7.7mmol/l zinc sulphate heptahydrate (AR grade, BDH Laboratory Chemicals). The colored Fe (II) complex with the chromogenic reagent Ferene was measured at 560 nm in a semi automated biochemistry analyzer, (Chem 7, ERBA Mannheim, Transasia).

Plasma Transferrin Measurement. Plasma transferrin (mg/dl) was measured in an automated clinical chemistry analyser (AU480, Beckman Coulter, USA) using a commercial immunoturbidimetry kit (OSR 6152, Beckman Coulter, USA).

Plasma Total Iron Binding Capacity (TIBC) Estimation. Plasma TIBC ($\mu\text{g/dL}$) was estimated from the plasma transferrin concentration, since TIBC cannot be measured directly in EDTA plasma. The estimation is based on the molecular weight of transferrin (80kD) and the known ratio of TIBC to transferrin.³

Plasma Ferritin Measurement. Plasma ferritin (ng/ml) was measured by a two site immunoenzymometric assay kit (Tosoh Corporation, Japan) using the automate Tosoh AIA 360 analyzer. Ferritin present in the test sample was bound with a monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA Pack test cups. The magnetic beads were washed to remove the unbound enzyme-labeled monoclonal antibody and were incubated with a fluorogenic substrate. The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the ferritin level in the plasma sample.

Plasma Hepcidin Measurement. Plasma hepcidin was measured using a commercial, solid-phase, competitive ELISA (Intrinsic LifeSciences, La Jolla, CA) that utilizes a monoclonal anti-hepcidin antibody. The assay is designed specifically for the quantification of hepcidin-25, the bioactive 25-amino acid peptide, in human EDTA plasma samples.

Supplemental References

1. Lele S, McCullough PA, Rajapurkar M. Serum catalytic iron as a novel biomarker of vascular injury in acute coronary syndromes. *EuroIntervention* 2009; 5:336-342.
2. Walmsley TA, George PM, Fowler RT. Colorimetric measurement of iron in plasma samples anticoagulated with EDTA. *J Clin Pathol* 1992; 45:151-154.
3. Gambino R, Desvarieux E, Orth M, Matan H, Ackattupathil T, Lijoi E, Wimmer C, Bower J, Gunter E. The relation between chemically measured total iron-binding capacity concentrations and immunologically measured transferrin concentrations in human serum. *Clin Chem* 1997; 43(12):2408-2412.