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Evaluation of Ferroptosis Related Oxidative Stress and Antioxidant Defense Biomarkers for Early Detection of Sepsis Associated Acute Kidney Injury

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Abstract

Background: Sepsis associated acute kidney injury (SA AKI) is frequent, yet creatinine rises only after injury is established. Ferroptosis, an iron dependent cell death driven by lipid peroxidation, is a plausible early mechanism. We evaluated ferroptosis related biomarkers for early detection of SA AKI.

Methods: In a prospective cohort at three Baghdad hospitals, 360 adults with sepsis were sampled at sepsis recognition (T0) for malondialdehyde (MDA), 4 hydroxynonenal adducts, glutathione peroxidase 4 (GPX4), glutathione, superoxide dismutase and iron indices. The primary outcome was KDIGO AKI within 48 hours.

Results: AKI developed in 160 patients (44.4%). Creatinine at T0 did not differ, whereas all lipid peroxidation and glutathione markers did (all $p < 0.01$). MDA was the strongest marker (AUC 0.637). A composite ferroptosis redox index was highest in KDIGO stage 3; AKI incidence increased from 31.7% to 60.8% and mortality from 4.2% to 21.7% across index tertiles (both $p < 0.001$). The association had the same direction at all hospitals and was closely coupled with nonrenal SOFA score; adding the panel to clinical covariates raised the AUC from 0.715 to 0.722, a nonsignificant gain.

Conclusion: A ferroptotic redox signature is detectable at sepsis onset, before creatinine changes, and stratifies kidney and survival risk.

Keywords: *Sepsis; acute kidney injury; ferroptosis; lipid peroxidation; glutathione peroxidase 4; malondialdehyde; oxidative stress; iron metabolism*

1. Introduction

Sepsis remains a major global health problem. The most recent Global Burden of Disease analysis estimated 166 million sepsis cases and 21.4 million sepsis related deaths worldwide in 2021, close to one third of all deaths [1]. Despite guideline based early recognition and resuscitation [2], outcome is driven by the ensuing organ dysfunction, in which the kidney is frequently involved. The 28th Acute Disease Quality Initiative (ADQI) defined sepsis associated acute kidney injury (SA AKI) as AKI fulfilling the Kidney Disease: Improving Global Outcomes (KDIGO) criteria within 7 days of sepsis diagnosed by the Sepsis 3 criteria [3]. Applying this definition in a large multicenter intensive care cohort, SA AKI affected roughly one in six admissions, was usually diagnosed on the first day, and was independently associated with death [4]. Survivors also face a lasting risk of chronic kidney disease [3], [5].

A central obstacle is that the current diagnostic criteria rely on serum creatinine and urine output, which are markers of kidney function rather than kidney injury and change only after substantial damage has occurred [5], [6], [7].

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Biomarkers of tubular stress and damage, such as the product of tissue inhibitor of metalloproteinases 2 and insulin like growth factor binding protein 7 and neutrophil gelatinase associated lipocalin, have improved early recognition [8], [9], but they report injury already under way rather than a targetable mechanism. ADQI and recent trial methodology reviews have therefore called for mechanism based biomarkers that support early identification and enrichment for targeted therapy [3], [10].

The pathophysiology of SA AKI extends well beyond renal hypoperfusion and involves dysregulated inflammation, microcirculatory dysfunction, metabolic reprogramming and regulated cell death of the tubular epithelium [11], [12], [13], [14]. Ferroptosis is an iron dependent form of regulated necrosis caused by the uncontrolled peroxidation of polyunsaturated phospholipids in cellular membranes [15], [16]. It is restrained mainly by the cystine glutamate antiporter, reduced glutathione (GSH) and the selenoenzyme glutathione peroxidase 4 (GPX4), which reduces lipid hydroperoxides to nontoxic alcohols, and it is promoted by a labile iron pool that catalyzes Fenton chemistry [17], [18]. Ferroptosis thus reflects the convergence of iron, thiol and lipid metabolism [19], and it leaves a footprint of reactive aldehydes such as malondialdehyde (MDA) and 4 hydroxynonenal (4 HNE).

Proximal tubular cells are especially susceptible because they combine high oxidative metabolism, polyunsaturated membranes and intense iron handling [19], [20], [21], [22]. In experimental SA AKI induced by cecal ligation and puncture or endotoxin, renal GPX4 expression falls, the GSH/GSSG ratio declines, MDA and iron accumulate, and ferroptosis inhibitors or iron chelation attenuate tubular injury and improve survival [23], [24], [25], [26]. In critically ill patients, plasma catalytic iron and MDA have been associated with the severity of multiorgan dysfunction and the probability of death, and blocking lipid peroxidation protected against iron driven organ failure in experimental models [27]. Pharmacological targeting of ferroptosis is now regarded as a promising strategy for sepsis related organ injury [28].

Clinical translation remains limited. Serum ferroptosis related proteins, including GPX4 and acyl coenzyme A synthetase long chain family member 4, have recently been shown to discriminate sepsis and predict short term mortality in critically ill patients [29], but kidney specific outcomes were not addressed. To our knowledge, no prospective multicenter study has evaluated an integrated panel covering the lipid peroxidation, glutathione and iron arms of ferroptosis at the moment of sepsis recognition, with incident SA AKI defined by consensus criteria as the outcome.

The present study addressed this gap. In a prospective cohort of adults with sepsis from three tertiary hospitals in Baghdad, we measured these biomarkers at sepsis recognition and determined whether they differ before creatinine rises, track the severity of subsequent kidney injury, and, combined into a composite ferroptosis redox index, stratify kidney and survival risk in relation to established clinical indicators of sepsis severity.

2. Methodology

2.1. Study design and setting

This multicenter prospective observational cohort study was conducted in three tertiary referral hospitals in Baghdad, Iraq: Baghdad Teaching Hospital, Al Kindi Teaching Hospital and Al Yarmouk Teaching Hospital. Adult patients with sepsis were enrolled consecutively between January and December 2025 until a prespecified target of 120 patients per site was reached. The study was designed and reported in accordance with the Standards for Reporting of Diagnostic Accuracy Studies (STARD 2015) and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

The study protocol was approved by the research ethics committee of each participating hospital: Baghdad Teaching Hospital (approval number MCH-IRB-2025-016, dated January 4, 2025), Al Kindi Teaching Hospital (approval number MOH-11, dated January 8, 2025) and Al Yarmouk Teaching Hospital (approval number MOH-033, dated January 6, 2025). The study conformed to the principles of the Declaration of Helsinki. Written informed consent was obtained from each patient or, when the patient lacked decisional capacity, from the legally authorized next of kin before any study procedure.

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2.2. Participants and eligibility criteria

Patients aged 18 years or older were eligible when they fulfilled the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis 3), namely suspected or documented infection accompanied by an acute increase of two or more points in the Sequential Organ Failure Assessment (SOFA) score. Septic shock was defined as sepsis requiring vasopressor therapy to maintain a mean arterial pressure of at least 65 mmHg together with a serum lactate above 2 mmol/L despite adequate volume resuscitation. Patients were excluded if they met the KDIGO serum creatinine criterion for AKI at enrollment, had preexisting end stage kidney disease or chronic maintenance hemodialysis, had a history of kidney transplantation, were pregnant, had been exposed to nephrotoxic contrast media within 7 days before admission, or had received intravenous iron supplementation or blood transfusion within the preceding 4 weeks.

The index time point (T0) was defined as the time of sepsis recognition and enrollment, at which all baseline clinical data were recorded and the biomarker blood sample was drawn. To ensure that the study captured incident rather than prevalent kidney injury, no patient fulfilled the KDIGO creatinine criterion for AKI at T0: in every participant, creatinine at T0 was below 1.5 times the reference value and less than 0.3 mg/dL above it. The reference creatinine was the most recent stable value documented in the medical record within the 3 to 12 months before enrollment. The urine output criterion was not applied at enrollment; patients with urine output below 0.5 mL/kg/h during the 6 hours before T0 were retained and examined in a sensitivity analysis (Section 2.8). A total of 360 patients formed the final analytic cohort.

2.3. Clinical data collection

Trained investigators recorded demographic characteristics (age, sex and body mass index), comorbidities (diabetes mellitus, hypertension and chronic kidney disease), the anatomical source of infection (pulmonary, urinary, abdominal, bloodstream, skin and soft tissue, or other), and the severity of illness at T0. Severity was summarized with the nonrenal SOFA score, calculated as the total SOFA score minus its renal component so that the predictor was independent of the outcome definition, together with the presence of septic shock and vasopressor use. Hourly urine output and cumulative fluid balance during the 6 hours preceding T0 were extracted from the nursing charts. Renal ultrasonography performed before T0 as part of routine care was recorded and categorized as no hydronephrosis, chronic parenchymal changes or hydronephrosis, with the category not performed retained as a distinct level.

2.4. Blood sampling and laboratory analysis

Venous blood was collected at T0 into plain and EDTA tubes. Serum and plasma were separated by centrifugation at 2,000 × g for 15 minutes within 2 hours of collection, divided into aliquots to avoid repeated freeze and thaw cycles, and stored at minus 20 °C until batch analysis. Specialized oxidative stress and ferroptosis assays that required instruments not available within the participating hospitals were performed at accredited private clinical laboratories outside the hospitals; all remaining tests were performed in the hospital laboratories.

Biomarkers were selected to represent the three interlinked arms of the ferroptotic cascade. Lipid peroxidation was assessed by malondialdehyde (MDA), measured as thiobarbituric acid reactive substances on a UV visible spectrophotometer, and by 4 hydroxynonenal (4 HNE) protein adducts, measured by enzyme linked immunosorbent assay (ELISA) (Human 4 HNE ELISA Kit, Elabscience, E-EL-0080). The glutathione dependent defense axis was evaluated by glutathione peroxidase 4 (GPX4) concentration using a sandwich ELISA (Human GPX4 ELISA Kit, Cusabio, CSB-E16345h), by reduced glutathione (GSH) on a UV visible spectrophotometer using the Ellman method, and by oxidized glutathione (GSSG) using an enzymatic recycling colorimetric method (Glutathione Assay Kit, Cayman Chemical, Item No. 703002); the GSH/GSSG ratio was calculated as an integrated index of the thiol redox state. Superoxide dismutase (SOD) activity was measured on an automated chemistry analyzer. Iron status was characterized by serum ferritin (Roche Cobas e 411 electrochemiluminescence immunoassay), serum iron (Beckman

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Coulter AU480) and total iron binding capacity (TIBC) on an automated chemistry analyzer, with transferrin saturation (TSAT) calculated as serum iron divided by TIBC multiplied by 100.

Routine markers comprised serum creatinine (Roche Cobas c 702, enzymatic method), C reactive protein (CRP), serum albumin and arterial lactate (Radiometer ABL90 FLEX blood gas analyzer). All assays were run in duplicate according to the manufacturers' instructions; the mean of duplicate values was used, and intra assay and inter assay coefficients of variation were below 5.0% and 8.0%, respectively.

2.5. Outcome definitions

The primary outcome was incident AKI within 48 hours of T0, defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria as an increase in serum creatinine of at least 0.3 mg/dL, an increase to at least 1.5 times the reference value, or urine output below 0.5 mL/kg/h for 6 hours or longer. Serum creatinine was measured at 24 and 48 hours, and urine output was monitored hourly throughout the first 48 hours. Outcome adjudication was performed by investigators who had no access to the biomarker results.

Secondary outcomes were AKI within 7 days, the maximum KDIGO stage reached within 7 days (stages 1 to 3, with stage 0 denoting no AKI), receipt of renal replacement therapy (RRT) within 7 days, and in hospital mortality.

2.6. Temporal definition of baseline variables

Because the clinical question concerns risk at the moment of sepsis recognition, a strict temporal boundary was applied. Only information available at or before T0 was analyzed as an exposure or covariate. All measurements obtained after T0, including creatinine at 24 hours, 48 hours and day 7, urine output and fluid balance after T0, and imaging performed after T0, were used exclusively for outcome ascertainment and secondary analyses.

2.7. Statistical analysis

Continuous variables are presented as median and interquartile range (IQR) and were compared between patients with and without AKI using the Mann Whitney U test. Categorical variables are presented as counts and percentages and were compared using the chi square test. Associations between each biomarker and the maximum KDIGO stage within 7 days were assessed with the Spearman rank correlation coefficient (ρ), and differences across KDIGO stages with the Kruskal Wallis test.

The discriminative ability of each biomarker for the primary outcome was quantified with the area under the receiver operating characteristic curve (AUC). Confidence intervals for the AUC were obtained from 2000 nonparametric bootstrap resamples, and AUC values were compared using the DeLong test for correlated curves. The optimal cut off was determined by the maximum Youden index, and the corresponding sensitivity and specificity were reported. Unadjusted odds ratios (OR) were estimated per one standard deviation (SD) increase in each biomarker to allow direct comparison across markers measured in different units.

To summarize the overall ferroptotic burden in a single measure, a composite ferroptosis redox index (FRI) was constructed from six markers. Each marker was first converted to a z score (the value minus the cohort mean, divided by the cohort SD). The FRI was then calculated as the sum of the z scores of the three oxidative injury markers (MDA, 4 HNE adducts and GSSG), minus the sum of the z scores of the three antioxidant defense markers (GPX4, GSH and SOD), with the result divided by six. Iron indices were not included in the FRI. Higher FRI values therefore indicate a greater imbalance between lipid peroxidation and antioxidant capacity. The FRI was analyzed as a continuous variable and in cohort tertiles.

Multivariable logistic regression was used to evaluate the independent association of ferroptosis biomarkers with AKI after adjustment for an a priori clinical set comprising age, nonrenal SOFA score, septic shock, chronic kidney disease, lactate and creatinine at T0. Continuous predictors were standardized so that adjusted OR are expressed per one SD,

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whereas binary predictors (septic shock and chronic kidney disease) were entered as presence versus absence. Confidence intervals for OR were Wald intervals. Multicollinearity was assessed with the variance inflation factor (VIF), and goodness of fit with the Hosmer Lemeshow test. The incremental value of the ferroptosis panel was assessed by comparing the AUC of the clinical model with that of the clinical model plus the ferroptosis panel using the DeLong test, and by the category free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI), with bootstrap confidence intervals.

Consistency of the ferroptotic signature across the three participating hospitals was examined by estimating the AUC of the principal markers and of the FRI separately at each site and by testing the FRI by hospital interaction term in logistic regression with a likelihood ratio test.

2.8. Sensitivity analyses

Sensitivity analyses were performed. First, because urine output below 0.5 mL/kg/h in the 6 hours before T0 might indicate evolving kidney injury already present at enrollment, the main analyses were repeated after excluding the 25 patients with pre T0 urine output below this threshold. Second, the main comparisons were repeated after excluding patients with chronic kidney disease. Third, because obstructive causes of AKI were not otherwise excluded, the comparisons were repeated after excluding patients with hydronephrosis on ultrasonography before T0. The relationship of the FRI with AKI within 7 days and with in hospital mortality was also assessed.

Missing data were limited to fluid balance during the 6 hours before T0, which was not recorded in 42 patients (11.7%). This variable was summarized and compared using the available cases only and was not included in any multivariable model; no imputation was performed.

All tests were two sided, and a p value below 0.05 was considered statistically significant. Statistical analyses were performed in Python 3.13 using pandas (version 3.0.5) and NumPy (version 2.5.3) for data handling, SciPy (version 1.18.1) for nonparametric tests and correlation analyses, and scikit learn (version 1.9.1) for fitting unpenalized logistic regression models. Wald confidence intervals for OR, bootstrap confidence intervals, the DeLong test, NRI, IDI, the Hosmer Lemeshow test, VIF and the likelihood ratio test for interaction were computed with custom code implementing their published formulas. Figures were produced with Matplotlib (version 3.11.2).

3. Results

3.1. Cohort characteristics

A total of 428 adults with sepsis were screened at the three sites (145 at Baghdad Teaching Hospital, 138 at Al Kindi Teaching Hospital and 145 at Al Yarmouk Teaching Hospital). Of these, 68 were excluded. Each excluded patient was counted once, under a single primary reason for ineligibility. Listed in the order of the eligibility criteria (Section 2.2), these were AKI fulfilling the KDIGO creatinine criterion at T0 (n = 28), preexisting end stage kidney disease or maintenance dialysis (n = 14), history of kidney transplantation (n = 0), active pregnancy (n = 4), exposure to nephrotoxic contrast media within 7 days (n = 9), and blood transfusion or intravenous iron therapy within the preceding 4 weeks (n = 13). No eligible patient or next of kin declined consent. The remaining 360 patients (120 per site) were enrolled and analyzed, and no patient was lost to follow up (Figure 1). Fluid balance during the 6 hours before T0 was not recorded in 42 patients; no other baseline variable had missing values.

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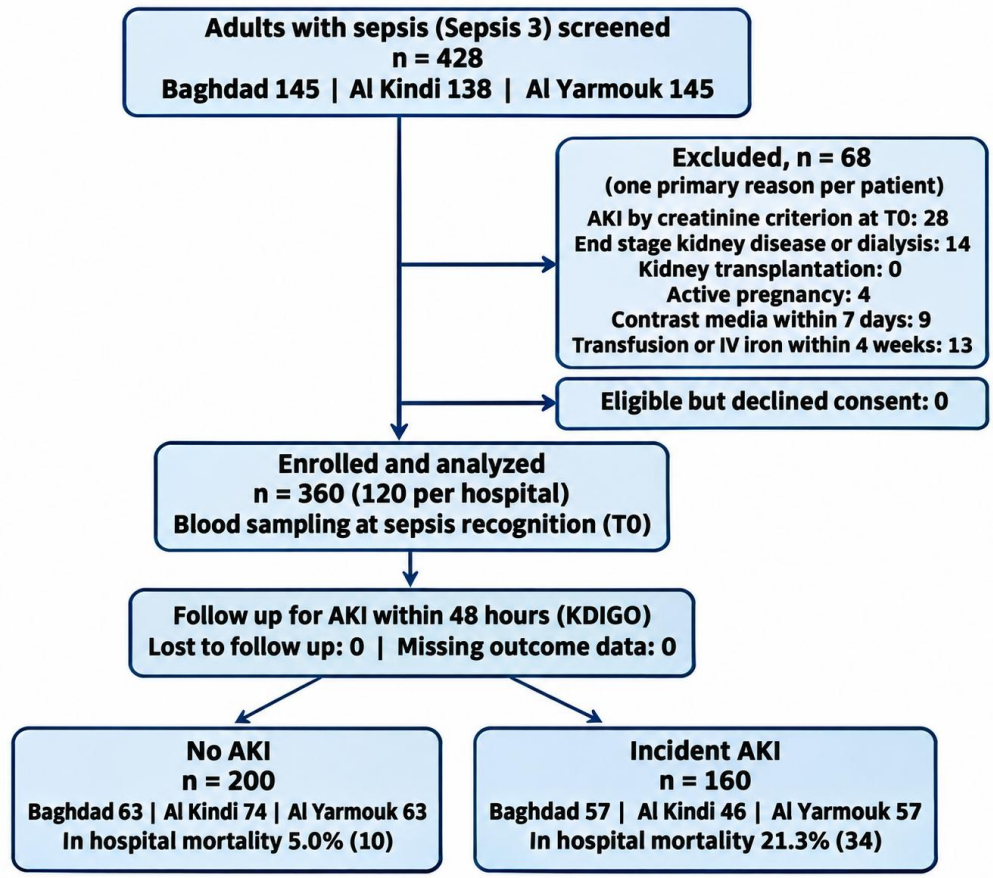


Figure 1. STARD flow diagram of patient screening, exclusion, enrollment at the three participating hospitals and primary outcome groups.

The median age was 52 years (IQR 34 to 67), and 182 patients (50.6%) were male. The lungs were the most frequent source of infection (34.2%), followed by the urinary tract (24.2%) and the abdomen (18.6%). Septic shock was present at T0 in 106 patients (29.4%).

Incident AKI within 48 hours developed in 160 patients (44.4%). The incidence was consistent across centers (57 of 120 at Baghdad Teaching Hospital, 46 of 120 at Al Kindi Teaching Hospital and 57 of 120 at Al Yarmouk Teaching Hospital). Among patients with AKI, the maximum KDIGO stage within 7 days was stage 1 in 97 (60.6%), stage 2 in 47 (29.4%) and stage 3 in 16 (10.0%), and all five patients who required RRT belonged to this group. By day 7, 179 patients (49.7%) had developed AKI. In hospital mortality was more than four times higher in patients with AKI than in those without (21.3% [34 of 160] versus 5.0%, $p < 0.001$), confirming the prognostic relevance of the primary outcome in this cohort.

Patients who developed AKI had a higher nonrenal SOFA score and were more often in septic shock and receiving vasopressors at T0. They also had higher lactate and lower albumin concentrations. Age, sex, body mass index, comorbidities, source of infection, CRP and creatinine at T0 did not differ significantly between groups (Table 1). The similarity of creatinine at T0 between groups (0.90 versus 0.87 mg/dL, $p = 0.134$) shows that conventional renal function testing could not identify the patients who would progress to AKI at the time of sepsis recognition, which defines the diagnostic gap the study set out to address.

Table 1. Baseline characteristics at T0 according to the development of AKI within 48 hours.

Characteristic	All (n = 360)	No AKI (n = 200)	AKI (n = 160)	p value
Age (years)	52.00 (34.00 to 67.00)	52.00 (35.00 to 66.00)	52.00 (34.00 to 68.00)	0.757
Male sex, n (%)	182 (50.6)	103 (51.5)	79 (49.4)	0.768
BMI (kg/m²)	27.20 (23.10 to 30.52)	27.20 (23.20 to 30.62)	27.15 (22.77 to 30.35)	0.436
Diabetes mellitus, n (%)	112 (31.1)	56 (28.0)	56 (35.0)	0.190
Hypertension, n (%)	116 (32.2)	58 (29.0)	58 (36.2)	0.177
Chronic kidney disease, n (%)	42 (11.7)	17 (8.5)	25 (15.6)	0.054
Nonrenal SOFA score	6.00 (4.00 to 8.00)	5.00 (3.00 to 7.00)	7.00 (5.00 to 9.00)	< 0.001
Septic shock at T0, n (%)	106 (29.4)	37 (18.5)	69 (43.1)	< 0.001
Vasopressor at T0, n (%)	117 (32.5)	44 (22.0)	73 (45.6)	< 0.001
Lactate (mmol/L)	2.38 (1.90 to 3.11)	2.21 (1.73 to 2.81)	2.68 (2.14 to 3.49)	< 0.001
CRP (mg/L)	98.95 (66.05 to 149.57)	93.25 (66.65 to 146.78)	109.25 (65.47 to 153.25)	0.407
Albumin (g/dL)	3.16 (2.90 to 3.45)	3.25 (2.99 to 3.52)	3.08 (2.81 to 3.28)	< 0.001
Reference creatinine (mg/dL)	0.83 (0.75 to 0.94)	0.82 (0.74 to 0.93)	0.84 (0.77 to 0.99)	0.083
Creatinine at T0 (mg/dL)	0.89 (0.76 to 1.01)	0.87 (0.76 to 0.99)	0.90 (0.78 to 1.06)	0.134
Urine output, 6 h before T0 (mL/kg/h)	1.04 (0.79 to 1.28)	1.05 (0.78 to 1.27)	1.01 (0.79 to 1.28)	0.693
Fluid balance, 6 h before T0 (mL)[a]	347.50 (−136.75 to 821.75)	324.50 (−197.75 to 755.75)	363.00 (−58.00 to 933.75)	0.138
Source of infection, n (%)				0.527
Abdominal	67 (18.6)	43 (21.5)	24 (15.0)	
Bloodstream	36 (10.0)	16 (8.0)	20 (12.5)	
Other	20 (5.6)	11 (5.5)	9 (5.6)	
Pulmonary	123 (34.2)	66 (33.0)	57 (35.6)	
Skin and soft tissue	27 (7.5)	16 (8.0)	11 (6.9)	
Urinary	87 (24.2)	48 (24.0)	39 (24.4)	
Renal ultrasound before T0, n (%)				0.281
Chronic parenchymal changes	40 (11.1)	27 (13.5)	13 (8.1)	
Hydronephrosis	12 (3.3)	8 (4.0)	4 (2.5)	
No hydronephrosis	119 (33.1)	61 (30.5)	58 (36.2)	
Not performed	189 (52.5)	104 (52.0)	85 (53.1)	

Continuous variables are median (interquartile range); categorical variables are n (%). [a] Available for 318 patients (174 without AKI and 144 with AKI); not recorded in 42 patients. p values from the Mann Whitney U test or chi square test. BMI, body mass index; CRP, C reactive protein; SOFA, Sequential Organ Failure Assessment; T0, time of sepsis recognition and enrollment.

3.2. Ferroptosis related biomarker profile

Every marker of the glutathione and lipid peroxidation axis differed significantly between patients who did and did not develop AKI, and all differences were in the biologically expected direction (Table 2, Figure 2). Patients with AKI had higher concentrations of MDA (median 3.69 versus 3.09 µmol/L, $p < 0.001$), 4 HNE adducts ($p = 0.002$) and GSSG ($p = 0.003$), indicating enhanced lipid peroxidation and thiol oxidation. In parallel, antioxidant defense was depleted, with lower GPX4 (7.88 versus 8.41 ng/mL, $p = 0.001$), GSH ($p < 0.001$) and SOD activity ($p = 0.002$). The GSH/GSSG ratio, an integrated readout of the thiol redox state, was markedly reduced in the AKI group (4.76 versus 5.91, $p < 0.001$).

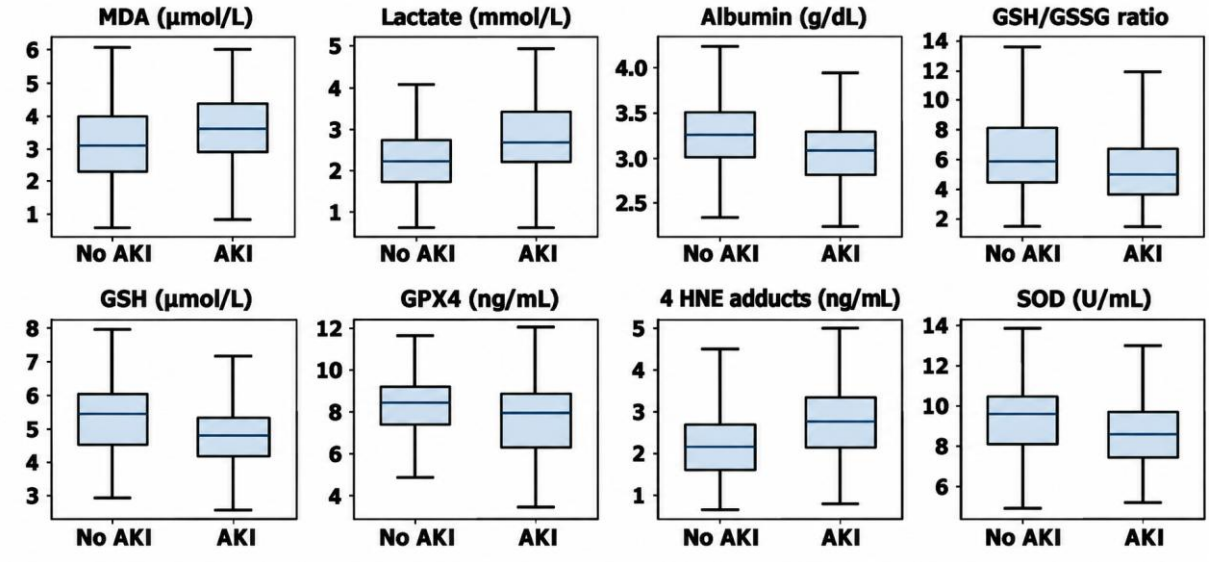
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Within the iron axis, patients with AKI had lower serum iron (p = 0.031) and lower TSAT (20.85% versus 23.30%, p = 0.005), consistent with inflammation driven redistribution of iron from the circulation into tissue stores. Serum ferritin was elevated across the whole cohort (median 375.95 ng/mL) without a significant difference between groups (p = 0.571), suggesting that it reflected the systemic acute phase response to sepsis rather than kidney specific risk.

Table 2. Ferroptosis related biomarkers at T0 according to AKI within 48 hours and their correlation with maximum KDIGO stage within 7 days.

Biomarker	No AKI (n = 200)	AKI (n = 160)	p value	Spearman ρ with KDIGO stage (p)
Lipid peroxidation				
MDA (µmol/L)	3.09 (2.32 to 3.99)	3.69 (3.00 to 4.38)	< 0.001	0.226 (< 0.001)
4 HNE adducts (ng/mL)	2.35 (1.58 to 2.96)	2.62 (1.93 to 3.49)	0.002	0.157 (0.003)
GSSG (µmol/L)	0.90 (0.68 to 1.10)	1.03 (0.79 to 1.21)	0.003	0.165 (0.002)
Glutathione and antioxidant defense				
GPX4 (ng/mL)	8.41 (7.31 to 9.17)	7.88 (6.54 to 8.82)	0.001	−0.178 (< 0.001)
GSH (µmol/L)	5.41 (4.70 to 5.99)	4.99 (4.35 to 5.60)	< 0.001	−0.178 (< 0.001)
GSH/GSSG ratio	5.91 (4.63 to 8.43)	4.76 (3.69 to 7.09)	< 0.001	−0.200 (< 0.001)
SOD (U/mL)	9.43 (8.23 to 10.75)	8.96 (7.67 to 9.89)	0.002	−0.161 (0.002)
Iron metabolism				
Ferritin (ng/mL)	364.70 (253.25 to 572.08)	404.45 (264.32 to 560.15)	0.571	0.002 (0.974)
Serum iron (µg/dL)	62.60 (51.42 to 72.48)	57.50 (44.95 to 72.93)	0.031	−0.091 (0.084)
TIBC (µg/dL)	266.00 (239.60 to 296.12)	263.85 (239.60 to 298.05)	0.501	0.019 (0.719)
TSAT (%)	23.30 (19.08 to 28.70)	20.85 (16.70 to 27.07)	0.005	−0.122 (0.020)

Values are median (interquartile range). 4 HNE, 4 hydroxynonenal; GPX4, glutathione peroxidase 4; GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; TIBC, total iron binding capacity; TSAT, transferrin saturation.



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Figure 2. Distribution of the eight laboratory variables with the highest univariate discrimination at T0 in patients with and without AKI within 48 hours. Boxes show the median and interquartile range; whiskers extend to 1.5 times the interquartile range.

3.3. Association with the severity of kidney injury

The ferroptotic signature showed a graded relationship with the severity of subsequent kidney injury. MDA showed the strongest positive correlation with the maximum KDIGO stage within 7 days ($\rho = 0.226$, $p < 0.001$), followed by GSSG and 4 HNE adducts, whereas the GSH/GSSG ratio ($\rho = -0.200$, $p < 0.001$), GSH, GPX4 and SOD correlated inversely with stage (all $p < 0.01$). TSAT also correlated inversely with stage ($\rho = -0.122$, $p = 0.020$), while ferritin and TIBC showed no relationship with stage (Table 2), and neither did CRP ($\rho = 0.020$, $p = 0.700$).

The composite FRI captured this gradient in a single measure. Its median was higher in every AKI stage than in patients without AKI and was highest in stage 3 (median −0.19 in patients with no AKI within 7 days [n = 181], 0.13 in stage 1 [n = 116], 0.14 in stage 2 [n = 47] and 0.43 in stage 3 [n = 16]; Kruskal Wallis $p < 0.001$; $\rho = 0.244$, $p < 0.001$). When patients were divided into FRI tertiles, the incidence of AKI within 48 hours increased stepwise from 31.7% in the lowest tertile to 40.8% in the middle tertile and 60.8% in the highest tertile ($p < 0.001$), and in hospital mortality increased in parallel from 4.2% to 10.8% and 21.7% ($p < 0.001$) (Table 3, Figure 4). Each one SD increase in FRI was associated with a 67% higher odds of AKI (unadjusted OR 1.67, 95% CI 1.33 to 2.09, $p < 0.001$), and the FRI discriminated AKI with an AUC of 0.644 (95% CI 0.584 to 0.703).

Table 3. Incidence of AKI within 48 hours and in hospital mortality across tertiles of the ferroptosis redox index (FRI).

FRI tertile	Patients, n	AKI within 48 h, n (%)	In hospital mortality, %
Tertile 1 (lowest)	120	38 (31.7)	4.2
Tertile 2	120	49 (40.8)	10.8
Tertile 3 (highest)	120	73 (60.8)	21.7
p for difference		< 0.001	< 0.001

FRI = (sum of z scores of MDA, 4 HNE adducts and GSSG, minus sum of z scores of GPX4, GSH and SOD) divided by 6, where each z score is the value minus the cohort mean, divided by the cohort SD. p values from the chi square test.

3.4. Univariate diagnostic performance

The diagnostic performance of each biomarker is shown in Table 4. MDA was the best single ferroptosis marker, with an AUC of 0.637 (95% CI 0.577 to 0.693); at a cut off of 3.23 µmol/L it identified 68.8% of patients who went on to develop AKI. The GSH/GSSG ratio, GSH, GPX4, 4 HNE adducts, SOD, GSSG and TSAT all discriminated significantly better than chance, with confidence intervals excluding 0.5. 4 HNE adducts above 3.31 ng/mL had a specificity of 85.5% with a sensitivity of 30.6% (positive likelihood ratio 2.1), and GPX4 below 7.45 ng/mL had a specificity of 72.5% with a sensitivity of 45.6% (positive likelihood ratio 1.7); neither threshold is sufficient on its own to confirm risk. By contrast, creatinine at T0 (AUC 0.546), CRP and ferritin offered no meaningful discrimination.

On a per SD basis, MDA showed the strongest unadjusted association with AKI (OR 1.66, 95% CI 1.32 to 2.08), followed by 4 HNE adducts (OR 1.43, 95% CI 1.15 to 1.77) and GSSG (OR 1.39, 95% CI 1.12 to 1.73), whereas each one SD increase in GPX4 (OR 0.70, 95% CI 0.56 to 0.86), GSH (OR 0.70, 95% CI 0.56 to 0.87), SOD (OR 0.70, 95% CI 0.56 to 0.87), GSH/GSSG ratio (OR 0.68, 95% CI 0.52 to 0.88) and TSAT (OR 0.74, 95% CI 0.60 to 0.93) was protective (all $p < 0.01$).

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Table 4. Diagnostic performance of individual T0 biomarkers for AKI within 48 hours.

Biomarker	AUC (95% CI)	Optimal cut off	Sensitivity, %	Specificity, %	Unadjusted OR per SD (95% CI)
MDA (µmol/L)	0.637 (0.577 to 0.693)	≥ 3.23	68.8	57.5	1.66 (1.32 to 2.08)
Lactate (mmol/L)	0.634 (0.576 to 0.690)	≥ 2.20	72.5	49.5	
Albumin (g/dL)	0.626 (0.567 to 0.685)	≤ 3.17	65.6	59.0	
GSH/GSSG ratio	0.616 (0.552 to 0.674)	≤ 4.97	55.0	69.5	0.68 (0.52 to 0.88)
GSH (µmol/L)	0.605 (0.548 to 0.663)	≤ 5.42	68.8	49.5	0.70 (0.56 to 0.87)
GPX4 (ng/mL)	0.597 (0.535 to 0.658)	≤ 7.45	45.6	72.5	0.70 (0.56 to 0.86)
4 HNE adducts (ng/mL)	0.597 (0.540 to 0.655)	≥ 3.31	30.6	85.5	1.43 (1.15 to 1.77)
SOD (U/mL)	0.594 (0.536 to 0.649)	≤ 9.95	76.2	42.0	0.70 (0.56 to 0.87)
GSSG (µmol/L)	0.592 (0.534 to 0.653)	≥ 1.03	50.6	68.5	1.39 (1.12 to 1.73)
TSAT (%)	0.586 (0.528 to 0.644)	≤ 21.00	52.5	66.0	0.74 (0.60 to 0.93)
Serum iron (µg/dL)	0.566 (0.506 to 0.624)	≤ 59.30	55.0	60.0	
Creatinine at T0 (mg/dL)	0.546 (0.483 to 0.606)	≥ 0.88	58.1	51.5	
CRP (mg/L)	0.525 (0.465 to 0.586)	≥ 102.50	53.8	57.5	
TIBC (µg/dL)	0.521 (0.457 to 0.582)	≥ 311.50	20.0	88.0	
Ferritin (ng/mL)	0.517 (0.457 to 0.575)	≥ 320.10	66.2	44.0	

Cut offs were selected by the Youden index. For markers with an inverse association, lower values indicate higher risk. AUC confidence intervals from 2000 bootstrap resamples. OR, odds ratio; SD, standard deviation.

3.5. Multivariable analysis and relation to illness severity

In the multivariable logistic regression model (Table 5), the nonrenal SOFA score (OR 1.63, 95% CI 1.24 to 2.15, p < 0.001), and septic shock (OR 2.71, 95% CI 1.35 to 5.42, p = 0.005) were independently associated with AKI, whereas chronic kidney disease showed a borderline association that did not reach significance (OR 2.46, 95% CI 1.00 to 6.07, p = 0.0503). Multicollinearity was low, with all VIF values below 2.5. After adjustment for these severity indicators, MDA (OR 1.22, 95% CI 0.87 to 1.70, p = 0.252), GPX4 (OR 0.93, 95% CI 0.71 to 1.21, p = 0.579) and TSAT (OR 0.86, 95% CI 0.68 to 1.10, p = 0.240) retained their expected directions of effect, whereas the adjusted OR for 4 HNE adducts, the GSH/GSSG ratio and SOD were close to 1 (Table 5). No ferroptosis marker reached independent statistical significance, and the composite FRI was likewise not independently associated with AKI (adjusted OR 1.15, 95% CI 0.85 to 1.55, p = 0.368).

This attenuation is consistent with the close coupling between ferroptotic stress and the systemic severity of sepsis. The FRI correlated strongly with the nonrenal SOFA score (ρ = 0.545, p < 0.001) and with lactate (ρ = 0.568, p <

0.001), and MDA alone correlated with the nonrenal SOFA score (ρ = 0.488, p < 0.001). These findings suggest that lipid peroxidation and antioxidant depletion rise in step with multiorgan dysfunction and tissue hypoperfusion, so that the ferroptosis panel and the clinical severity indicators carry overlapping information on the same pathophysiological process.

Table 5. Multivariable logistic regression for AKI within 48 hours in the full cohort and in the sensitivity cohort.

Predictor	Full cohort (n = 360) adjusted OR (95% CI)	p value	Sensitivity cohort (n = 335) adjusted OR (95% CI)	p value
Age	0.96 (0.77 to 1.21)	0.748	0.96 (0.76 to 1.22)	0.766
Nonrenal SOFA score	1.63 (1.24 to 2.15)	< 0.001	1.57 (1.17 to 2.10)	0.003
Septic shock at T0	2.71 (1.35 to 5.42)	0.005	2.77 (1.35 to 5.68)	0.005
Chronic kidney disease	2.46 (1.00 to 6.07)	0.0503	2.25 (0.89 to 5.65)	0.086
Lactate	0.87 (0.61 to 1.25)	0.467	0.92 (0.64 to 1.34)	0.669
Creatinine at T0	1.03 (0.77 to 1.38)	0.848	1.09 (0.81 to 1.48)	0.560
MDA	1.22 (0.87 to 1.70)	0.252	1.18 (0.84 to 1.67)	0.340
4 HNE adducts	0.96 (0.72 to 1.27)	0.762	0.97 (0.72 to 1.30)	0.815
GPX4	0.93 (0.71 to 1.21)	0.579	0.97 (0.74 to 1.29)	0.856
GSH/GSSG ratio	1.05 (0.77 to 1.43)	0.769	1.02 (0.73 to 1.43)	0.894
SOD	1.01 (0.77 to 1.34)	0.925	0.98 (0.73 to 1.30)	0.874
TSAT	0.86 (0.68 to 1.10)	0.240	0.90 (0.70 to 1.15)	0.395

Odds ratios are per one SD for continuous predictors and for presence versus absence for binary predictors. The sensitivity cohort excludes 25 patients with urine output below 0.5 mL/kg/h in the 6 hours before T0.

3.6. Incremental value of the ferroptosis panel

The clinical model discriminated AKI with an AUC of 0.715 (95% CI 0.662 to 0.769), and the ferroptosis panel alone with an AUC of 0.657 (95% CI 0.600 to 0.713). Adding the ferroptosis panel to the clinical model increased the AUC to 0.722 (95% CI 0.670 to 0.775) (Figure 3B). This increment did not reach statistical significance (DeLong p = 0.350). The category free NRI was 0.203 (95% CI −0.005 to 0.409), with correct upward reclassification of 11.3% of patients with AKI and correct downward reclassification of 9.0% of patients without AKI. The IDI was 0.0097 (95% CI −0.0002 to 0.0201). Both the clinical model and the combined model showed good fit (Hosmer Lemeshow p = 0.776 and 0.818, respectively). The direction of these reclassification measures favors the panel, but their confidence intervals include zero, so its added value beyond routine severity indicators was not statistically confirmed in this cohort.

Among the single measures, the FRI (AUC 0.644) and MDA (AUC 0.637) performed similarly (DeLong p = 0.703). Both discriminated AKI significantly better than creatinine at T0 (AUC 0.546; DeLong p = 0.016 for the FRI and p = 0.028 for MDA) (Figure 3A).

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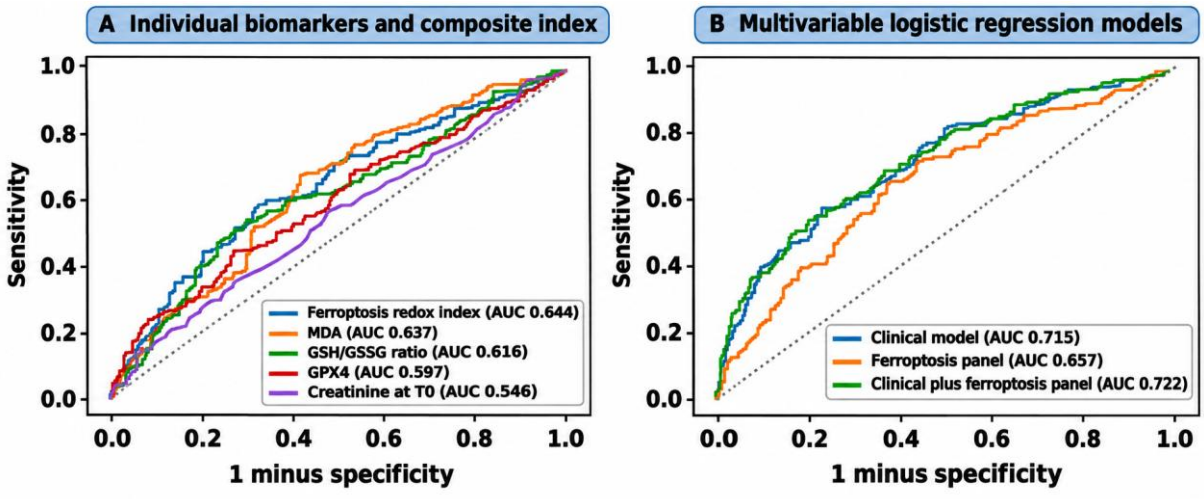


Figure 3. Receiver operating characteristic curves for AKI within 48 hours. (A) Ferroptosis redox index, MDA, GSH/GSSG ratio, GPX4 and creatinine at T0. For GSH/GSSG ratio and GPX4, lower values indicate higher risk. (B) Multivariable logistic regression models including the clinical covariates, the ferroptosis panel, and both combined.

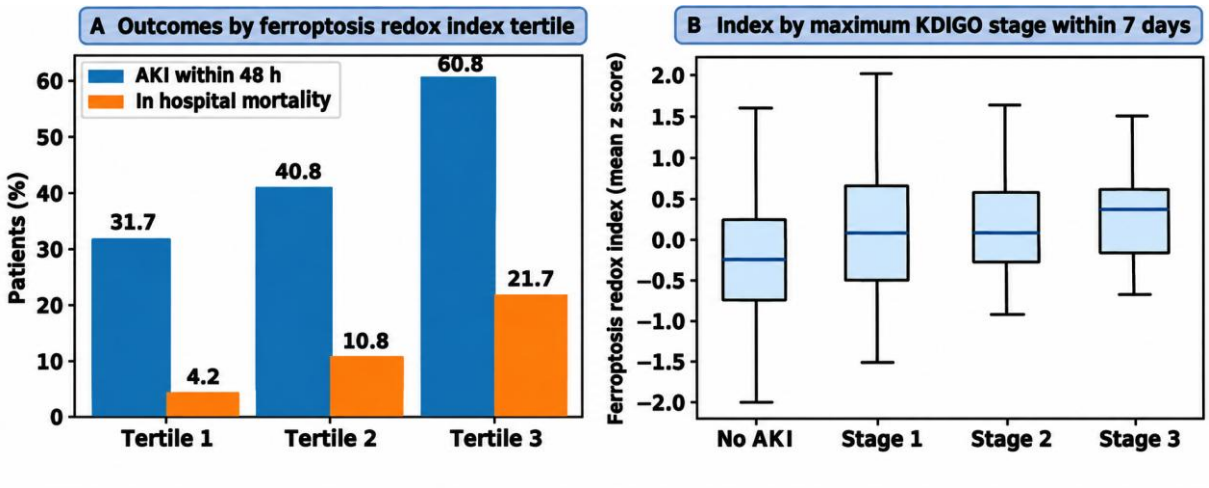


Figure 4. (A) Incidence of AKI within 48 hours and in hospital mortality across tertiles of the ferroptosis redox index. (B) Distribution of the ferroptosis redox index according to the maximum KDIGO stage reached within 7 days. Boxes show the median and interquartile range.

3.7. Consistency across participating hospitals

The direction of the ferroptotic signature was the same at all three hospitals, but its strength varied (Table 6). Patients with AKI had higher FRI values at every site, with AUC values of 0.670 (95% CI 0.571 to 0.769) at Baghdad Teaching Hospital, 0.662 (95% CI 0.564 to 0.761) at Al Yarmouk Teaching Hospital and 0.599 (95% CI 0.495 to 0.703) at Al Kindi Teaching Hospital. The difference reached significance at the first two sites; at Al Kindi Teaching Hospital, which had the fewest events, the FRI did not reach significance ($p = 0.070$) and the GSH/GSSG ratio did not discriminate (AUC 0.545, $p = 0.410$). The interaction test did not detect a difference in the FRI association between hospitals (p for interaction = 0.502), although this test has limited power with 120 patients per site, so it does not by itself demonstrate reproducibility.

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Table 6. Discrimination of AKI within 48 hours by the principal ferroptosis markers at each participating hospital.

Hospital	Patients (AKI events), n	Marker	AUC (95% CI)	p value
Baghdad Teaching Hospital	120 (57)	MDA	0.633 (0.533 to 0.733)	0.012
		GSH/GSSG ratio	0.691 (0.593 to 0.790)	< 0.001
		GPX4	0.603 (0.500 to 0.705)	0.053
		Ferroptosis redox index	0.670 (0.571 to 0.769)	0.001
Al Kindi Teaching Hospital	120 (46)	MDA	0.579 (0.475 to 0.683)	0.147
		GSH/GSSG ratio	0.545 (0.439 to 0.651)	0.410
		GPX4	0.591 (0.484 to 0.698)	0.095
		Ferroptosis redox index	0.599 (0.495 to 0.703)	0.070
Al Yarmouk Teaching Hospital	120 (57)	MDA	0.690 (0.594 to 0.786)	< 0.001
		GSH/GSSG ratio	0.607 (0.505 to 0.710)	0.043
		GPX4	0.594 (0.490 to 0.698)	0.077
		Ferroptosis redox index	0.662 (0.564 to 0.761)	0.002

p values from the Mann Whitney U test comparing patients with and without AKI. For GSH/GSSG ratio and GPX4, lower values indicate higher risk. p for FRI by hospital interaction = 0.502.

3.8. Sensitivity and secondary analyses

Excluding the 25 patients with urine output below 0.5 mL/kg/h in the 6 hours before T0 (9 of whom developed AKI) did not change the conclusions. In the remaining 335 patients (151 with AKI), all seven redox markers (MDA, 4 HNE adducts, GSSG, GPX4, GSH, GSH/GSSG ratio and SOD) and TSAT still differed significantly between patients with and without AKI (all $p \leq 0.011$). MDA remained the strongest single marker (AUC 0.640, $p < 0.001$), and the FRI discriminated AKI with an AUC of 0.650 (95% CI 0.591 to 0.710). AKI incidence across FRI tertiles rose from 31.2% to 42.3% and 61.6% ($p < 0.001$). The adjusted associations of the nonrenal SOFA score and septic shock with AKI were virtually unchanged (Table 5), and the AUC of the combined model (0.726) again exceeded that of the clinical model (0.719), without reaching significance (DeLong $p = 0.263$). These results indicate that the findings were not driven by patients with evolving kidney injury at enrollment.

After excluding patients with chronic kidney disease (318 patients, 135 with AKI), all seven redox markers and TSAT remained significantly different between groups (all $p \leq 0.016$), and the FRI retained an AUC of 0.630 (95% CI 0.568 to 0.693). This indicates that the ferroptotic signature was not explained by preexisting kidney disease.

After excluding the 12 patients with hydronephrosis on ultrasonography before T0 (4 of whom developed AKI), all seven redox markers and TSAT again differed significantly between groups (all $p \leq 0.010$), and the FRI discriminated AKI with an AUC of 0.640 (95% CI 0.582 to 0.699). The findings were therefore not driven by patients with known hydronephrosis; however, only 171 patients underwent ultrasonography before T0, so obstructive causes could not be excluded in the remainder.

The FRI measured at T0 was also related to longer term outcomes. It discriminated AKI within 7 days with an AUC of 0.634 (95% CI 0.577 to 0.691) and in hospital mortality with an AUC of 0.682 (95% CI 0.602 to 0.763). Together with the stepwise rise in mortality across FRI tertiles, this supports the prognostic relevance of ferroptotic stress beyond the 48 hour window.

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4. Discussion

This prospective multicenter study shows that a coherent ferroptotic redox signature is present at sepsis recognition, before any change in serum creatinine, and that this signature distinguishes patients who go on to develop AKI within 48 hours. Every lipid peroxidation and glutathione marker differed between groups in the expected direction, the signature was stronger in patients who developed AKI and strongest in those reaching stage 3, and a composite ferroptosis redox index separated patients into risk strata in which AKI incidence nearly doubled and in hospital mortality increased fivefold. The direction of association was the same at all three hospitals, although its strength varied, and the findings were unchanged after exclusion of patients with low urine output at enrollment, with chronic kidney disease or with hydronephrosis.

The clinical profile of the cohort agrees with contemporary epidemiology. In a 12 unit cohort using the ADQI definition, SA AKI was usually diagnosed early, was mostly stage 1 and carried a hospital mortality of 18% [4]; in our cohort, 60.6% of AKI cases reached a maximum of stage 1 and mortality was 21.3% with AKI versus 5.0% without.

The lipid peroxidation arm provided the clearest signal. MDA was the strongest single discriminator and the marker most closely correlated with the maximum KDIGO stage, and 4 HNE adducts were also elevated. These aldehydes are terminal products of the oxidative fragmentation of polyunsaturated phospholipids and are the expected biochemical footprint of ferroptotic membrane damage [15], [19]. Our observations in patients mirror experimental SA AKI, in which cecal ligation and puncture increases renal MDA and lipid peroxides in parallel with tubular injury, while ferroptosis inhibition with liproxstatin 1, ferrostatin 1 or deferoxamine reverses both changes [23], [25]. They are also consistent with mechanistic evidence that ferroptotic phospholipid hydroperoxides in urine are associated with failure of kidney recovery in patients with established AKI [19].

The antioxidant arm was depleted in a pattern that closely reproduces the canonical ferroptosis pathway. Patients who developed AKI had lower GPX4, lower GSH and SOD, higher GSSG and a markedly reduced GSH/GSSG ratio. In animal models, GPX4 suppression and a falling GSH/GSSG ratio precede tubular cell death, and restoration of the nuclear factor erythroid 2 related factor 2 and GPX4 axis protects the kidney [23], [24], [25]. Zeng and colleagues recently reported that serum GPX4 has diagnostic and prognostic value in sepsis [29]. In their cohort, GPX4 correlated positively with SOFA and APACHE II scores, whereas in ours lower GPX4 accompanied subsequent kidney injury. This difference may reflect the kidney outcome, the timing of sampling and release of GPX4 from injured cells, and it suggests that the integrated glutathione system, rather than any single enzyme, provides the most informative readout [17], [18].

The iron profile adds biological coherence to these findings. Patients who developed AKI had lower serum iron and transferrin saturation, while ferritin was elevated throughout the cohort without discriminating between groups. Inflammation driven hypoferremia redistributes iron from the circulation into tissue stores, which expands the intracellular labile iron pool in which ferroptosis is initiated [19], [22]. Large critical care cohorts have linked high ferritin to mortality in patients with AKI and with sepsis [30], [31]. In our study, ferritin behaved as a marker of the systemic acute phase response rather than of kidney specific risk, whereas transferrin saturation tracked kidney outcome. This distinction suggests that indices of iron redistribution are more informative than iron stores for early renal risk assessment.

A key strength of the present work is the demonstration of a graded relationship. The ferroptosis redox index was higher in every AKI stage than in patients without AKI and highest in stage 3, and AKI incidence and mortality rose across its tertiles. This biological gradient, together with the sensitivity analyses, is consistent with a biological role for ferroptotic stress in SA AKI, although an observational design cannot establish causality. The index also discriminated in hospital mortality, which agrees with the observation in critically ill patients that plasma catalytic iron and MDA rise with the severity of multiorgan dysfunction and the probability of death [27], and with the proposed contribution of ferroptosis to sepsis related organ injury [28].

After adjustment for nonrenal SOFA score, septic shock and other clinical covariates, MDA, GPX4 and transferrin saturation retained their expected directions of effect, whereas the odds ratios for 4 HNE adducts, the GSH/GSSG ratio and SOD were close to 1, and no marker remained independently significant, and adding the panel to the clinical

model produced a numerically higher AUC and a positive reclassification trend without a significant gain. The strong correlations of the index with nonrenal SOFA score and lactate are consistent with this pattern, and they reproduce the association between lipid peroxidation, catalytic iron and SOFA score described in a mixed intensive care cohort [27]. This fits the view of SA AKI as one manifestation of a systemic process involving inflammation, metabolic reprogramming and regulated cell death [11], [13], [14]. In this framework, the ferroptosis panel and the severity indicators capture overlapping information on the same pathophysiological cascade, and the panel may help explain why sicker patients are more likely to develop AKI. The clinical predictors identified here also agree with previous cohorts, in which lactate, the lactate to albumin ratio and hypoalbuminemia were associated with SA AKI and with mortality [32], [33], [34], [35].

Urinary tubular stress biomarkers achieve higher pooled accuracy for AKI [9], but they detect injury that has already begun; circulating ferroptosis markers are complementary because they indicate an upstream and potentially druggable process. Recent consensus work has emphasized prognostic and predictive enrichment based on biological sub phenotypes [3], [10], and a ferroptosis redox profile could identify patients most likely to benefit from ferroptosis inhibitors, iron chelation or glutathione restoring strategies that are effective in experimental models [26], [28], [21].

The study has several strengths: a prospective multicenter design, consensus definitions of sepsis and SA AKI, a strict temporal boundary between baseline measurements and outcomes, exclusion of patients who met the creatinine criterion for AKI at enrollment, blinded outcome adjudication and several sensitivity analyses.

Several limitations should also be considered. Biomarkers were measured at a single time point, so their trajectory over the first days of sepsis is unknown. Samples were stored at minus 20 °C rather than the usual minus 80 °C, and because GSH can oxidize during storage, absolute GSH and GSSG concentrations may have been affected; because outcome status was not known when samples were stored and all samples were analyzed in batches, any such effect would be expected to be nondifferential between outcome groups. The discriminative performance of individual markers was moderate, and the panel did not significantly improve discrimination beyond clinical severity indicators. The observational design does not allow causal conclusions about the role of ferroptosis in kidney injury. The urine output criterion was not applied at enrollment, so some patients with low urine output before T0 may already have been developing AKI, although excluding them gave similar results. Obstructive causes of AKI were not systematically excluded, because only 171 patients had ultrasonography before T0, although excluding the 12 patients with hydronephrosis did not change the findings. Finally, all three hospitals were in one city, and external validation is required. Future work should include serial sampling, paired urinary measurements of lipid peroxidation products, combination with tubular stress biomarkers and validation in independent populations.

5. Conclusion

In adults with sepsis, a ferroptotic redox signature, characterized by increased lipid peroxidation and depletion of the GPX4 and glutathione defense system, was present at sepsis recognition, before serum creatinine changed, and was more pronounced in patients who developed AKI within 48 hours. These patients also had lower serum iron and transferrin saturation. MDA was the strongest single marker, and a composite ferroptosis redox index stratified the incidence of AKI and in hospital mortality in a graded manner, with the same direction of association at all three hospitals and in sensitivity analyses. The signature was closely coupled with multiorgan dysfunction and did not add significant discrimination beyond clinical severity indicators, which suggests that ferroptotic stress accompanies the systemic processes that lead to kidney injury. Validation in external cohorts with serial sampling is needed before clinical use, and these markers may help enrich future trials of ferroptosis targeted therapies in sepsis associated acute kidney injury.

References

[1] GBD 2021 Global Sepsis Collaborators, “Global, regional, and national sepsis incidence and mortality, 1990 to 2021: a systematic analysis,” Lancet Glob. Health, vol. 13, no. 12, pp. e2013 to e2026, Dec. 2025.

[2] L. Evans, A. Rhodes, W. Alhazzani, M. Antonelli, C. M. Coopersmith, C. French, et al., "Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021," Crit. Care Med., vol. 49, no. 11, pp. e1063 to e1143, Nov. 2021.

[3] A. Zarbock, M. K. Nadim, P. Pickkers, H. Gomez, S. Bell, M. Joannidis, et al., "Sepsis associated acute kidney injury: consensus report of the 28th Acute Disease Quality Initiative workgroup," Nat. Rev. Nephrol., vol. 19, no. 6, pp. 401 to 417, Jun. 2023.

[4] K. C. White, A. Serpa Neto, R. Hurford, P. Clement, K. B. Laupland, E. See, et al., "Sepsis associated acute kidney injury in the intensive care unit: incidence, patient characteristics, timing, trajectory, treatment, and associated outcomes. A multicenter, observational study," Intensive Care Med., vol. 49, no. 9, pp. 1079 to 1089, Sep. 2023.

[5] J. A. Kellum, P. Romagnani, G. Ashuntantang, C. Ronco, A. Zarbock, and H. J. Anders, "Acute kidney injury," Nat. Rev. Dis. Primers, vol. 7, no. 1, Art. no. 52, Jul. 2021.

[6] T. Pais, S. Jorge, and J. A. Lopes, "Acute kidney injury in sepsis," Int. J. Mol. Sci., vol. 25, no. 11, Art. no. 5924, May 2024.

[7] J. A. Kellum, C. Ronco, and R. Bellomo, "Conceptual advances and evolving terminology in acute kidney disease," Nat. Rev. Nephrol., vol. 17, no. 7, pp. 493 to 502, Jul. 2021.

[8] D. Kounatidis, N. G. Vallianou, S. Psallida, F. Panagopoulos, E. Margellou, D. Tsilingiris, et al., "Sepsis associated acute kidney injury: where are we now?," Medicina, vol. 60, no. 3, Art. no. 434, Mar. 2024.

[9] F. Huang, Y. Zeng, L. Lv, Y. Chen, Y. Yan, L. Luo, et al., "Predictive value of urinary cell cycle arrest biomarkers for all cause acute kidney injury: a meta analysis," Sci. Rep., vol. 13, Art. no. 6037, Apr. 2023.

[10] M. Legrand, S. M. Bagshaw, P. K. Bhatraju, A. Bihorac, E. Caniglia, A. K. Khanna, et al., "Sepsis associated acute kidney injury: recent advances in enrichment strategies, sub phenotyping and clinical trials," Crit. Care, vol. 28, no. 1, Art. no. 92, Mar. 2024.

[11] C. L. Manrique Caballero, G. Del Rio Pertuz, and H. Gomez, "Sepsis associated acute kidney injury," Crit. Care Clin., vol. 37, no. 2, pp. 279 to 301, Apr. 2021.

[12] Z. Wu, J. Deng, H. Zhou, W. Tan, L. Lin, and J. Yang, "Programmed cell death in sepsis associated acute kidney injury," Front. Med., vol. 9, Art. no. 883028, May 2022.

[13] A. B. Liu, B. Tan, P. Yang, N. Tian, J. K. Li, S. C. Wang, et al., "The role of inflammatory response and metabolic reprogramming in sepsis associated acute kidney injury: mechanistic insights and therapeutic potential," Front. Immunol., vol. 15, Art. no. 1487576, Oct. 2024.

[14] J. Toro, C. L. Manrique Caballero, and H. Gomez, "Metabolic reprogramming and host tolerance: a novel concept to understand sepsis associated AKI," J. Clin. Med., vol. 10, no. 18, Art. no. 4184, Sep. 2021.

[15] B. R. Stockwell, "Ferroptosis turns 10: emerging mechanisms, physiological functions, and therapeutic applications," Cell, vol. 185, no. 14, pp. 2401 to 2421, Jul. 2022.

[16] X. Jiang, B. R. Stockwell, and M. Conrad, "Ferroptosis: mechanisms, biology and role in disease," Nat. Rev. Mol. Cell Biol., vol. 22, no. 4, pp. 266 to 282, Apr. 2021.

[17] D. Tang, X. Chen, R. Kang, and G. Kroemer, "Ferroptosis: molecular mechanisms and health implications," Cell Res., vol. 31, no. 2, pp. 107 to 125, Feb. 2021.

[18] S. J. Dixon and J. A. Olzmann, "The cell biology of ferroptosis," Nat. Rev. Mol. Cell Biol., vol. 25, no. 6, pp. 424 to 442, Jun. 2024.

[19] H. Bayır, S. J. Dixon, Y. Y. Tyurina, J. A. Kellum, and V. E. Kagan, "Ferroptotic mechanisms and therapeutic targeting of iron metabolism and lipid peroxidation in the kidney," Nat. Rev. Nephrol., vol. 19, no. 5, pp. 315 to 336, May 2023.

[20] J. Zhang, B. Wang, S. Yuan, Q. He, and J. Jin, "The role of ferroptosis in acute kidney injury," Front. Mol. Biosci., vol. 9, Art. no. 951275, Jun. 2022.

[21] Z. Long, Y. Luo, M. Yu, X. Wang, L. Zeng, and K. Yang, "Targeting ferroptosis: a new therapeutic opportunity for kidney diseases," Front. Immunol., vol. 15, Art. no. 1435139, Jul. 2024.

[22] X. Zhang and X. Li, "Abnormal iron and lipid metabolism mediated ferroptosis in kidney diseases and its therapeutic potential," Metabolites, vol. 12, no. 1, Art. no. 58, Jan. 2022.

[23] J. Xiao, Q. Yang, Y. Zhang, H. Xu, Y. Ye, L. Li, et al., "Maresin conjugates in tissue regeneration 1 suppresses ferroptosis in septic acute kidney injury," Cell Biosci., vol. 11, no. 1, Art. no. 221, Dec. 2021.

[24] Q. Zheng, J. Xing, X. Li, X. Tang, and D. Zhang, "PRDM16 suppresses ferroptosis to protect against sepsis associated acute kidney injury by targeting the NRF2/GPX4 axis," Redox Biol., vol. 78, Art. no. 103417, Dec. 2024.

[25] Y. Zhao, B. Jiang, D. Huang, J. Lou, et al., "Ferrostatin 1 post treatment attenuates acute kidney injury in mice by inhibiting ferritin production and regulating iron uptake related proteins," PeerJ, vol. 11, Art. no. e15786, Sep. 2023.

[26] L. Zhang, F. Luo, N. Yuan, J. Yin, B. Shen, Y. Chai, et al., "Research progress of ferroptosis in acute kidney injury," Front. Cell Dev. Biol., vol. 13, Art. no. 1614156, Jun. 2025.

[27] S. Van Coillie, E. Van San, I. Goetschalckx, B. Wiernicki, B. Mukhopadhyay, W. Tonnus, et al., "Targeting ferroptosis protects against experimental (multi)organ dysfunction and death," Nat. Commun., vol. 13, Art. no. 1046, Feb. 2022.

[28] P. Zhang, W. Liu, S. Wang, Y. Wang, and H. Han, "Ferroptosis and its role in the treatment of sepsis related organ injury: mechanisms and potential therapeutic approaches," Infect. Drug Resist., vol. 17, pp. 5715 to 5727, Dec. 2024.

[29] Z. Zeng, J. Deng, G. Wang, Z. Luo, W. Xiao, W. Xie, et al., "Ferroptosis related protein biomarkers for diagnosis, differential diagnosis, and short term mortality in patients with sepsis in the intensive care unit," Front. Immunol., vol. 16, Art. no. 1528986, 2025.

[30] X. Ren, Z. Jiang, F. Liu, Q. Wang, H. Chen, L. Yu, et al., "Association of serum ferritin and all cause mortality in AKI patients: a retrospective cohort study," Front. Med., vol. 11, Art. no. 1368719, Jun. 2024.

[31] Y. P. Fang, H. J. Zhang, Z. Guo, C. H. Ren, Y. F. Zhang, Q. Liu, et al., "Effect of serum ferritin on the prognosis of patients with sepsis: data from the MIMIC IV database," Emerg. Med. Int., vol. 2022, Art. no. 2104755, 2022.

[32] S. Sheng, Y. H. Zhang, H. K. Ma, and Y. Huang, "Albumin levels predict mortality in sepsis patients with acute kidney injury undergoing continuous renal replacement therapy: a secondary analysis based on a retrospective cohort study," BMC Nephrol., vol. 23, Art. no. 52, Feb. 2022.

[33] W. Lin, C. Fu, J. Miao, W. Hong, X. Chen, S. Yan, et al., "Association between the serum albumin creatinine ratio and 28 day intensive care unit mortality among patients with sepsis: a multicenter retrospective cohort study," Front. Med., vol. 11, Art. no. 1484370, Nov. 2024.

[34] S. Zhang, M. Luo, Z. Lu, and Q. Shi, "Lactate and lactylation in sepsis associated acute kidney injury: clinical evidence from the MIMIC IV database and mechanistic insights," Front. Med., vol. 12, Art. no. 1708145, Nov. 2025.

[35] S. He, Y. Wang, S. Wei, and S. Yang, "Correlation between lactate/albumin ratio and 28 day mortality in sepsis associated acute kidney injury patients," Front. Med., vol. 12, Art. no. 1546112, 2025.