

Association of Circulating Inflammatory Cytokines IL-6, TNF- α , and Fibroblast Growth Factor 2 with Disease Progression and Clinical Outcomes in Acute Kidney Injury

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ABSTRACT

Acute kidney injury (AKI) presents challenges for early risk stratification due to its rapid progression and variable outcomes. This study investigated the predictive value of serum interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and fibroblast growth factor 2 (FGF-2) for disease progression and clinical outcomes in 104 AKI patients.

Serum biomarkers were measured at diagnosis. Disease progression (worsening of AKI stage) and 28-day major adverse kidney events (MAKE) were assessed.

IL-6 and TNF- α positively correlated with progression and MAKE, while FGF-2 showed negative correlations. Multivariate logistic regression identified IL-6 (aOR=1.034) and TNF- α (aOR=1.082) as independent predictors of progression, but not FGF-2. Cox regression showed TNF- α independently predicted earlier MAKE (aHR=1.031), whereas FGF-2 predicted delayed MAKE (aHR=0.832). IL-6 did not independently affect time to MAKE. The combined biomarker model achieved an AUC of 0.962 for predicting MAKE. Day-of-diagnosis measurement of IL-6, TNF- α , and FGF-2 is significantly associated with AKI progression and adverse outcomes, independent of traditional risk factors.

Evaluating the balance between inflammation and repair may improve risk stratification. The combination of elevated IL-6 and TNF- α with decreased FGF-2 could identify a high-risk phenotype.

Keywords: Acute kidney injury; Biomarkers; Disease progression; Fibroblast growth factor 2; Interleukin-6; Prognosis; Tumor necrosis factor- α

INTRODUCTION

Acute kidney injury (AKI) represents a common critical condition among hospitalized patients, with an

incidence rate exceeding 50% in intensive care units that continues to rise annually.¹⁻³ Beyond prolonging hospital stays and increasing healthcare resource utilization, AKI independently predicts chronic kidney disease (CKD) progression, end-stage renal disease (ESRD), and long-term all-cause mortality.^{4,5} Despite recent advancements in continuous renal replacement therapy techniques and organ support modalities, short-term mortality among AKI patients remains persistently high (20% to 40%), with approximately 30% of

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survivors experiencing varying degrees of persistent renal function impairment.^{6,7} This challenging clinical landscape suggests that sole reliance on post-injury supportive care may no longer suffice to overcome current prognostic improvement bottlenecks. There is an urgent need to establish early risk stratification systems grounded in pathophysiological mechanisms to guide precise interventions and ultimately enhance long-term patient outcomes.⁸

The pathophysiological mechanisms underlying AKI are complex, involving multiple interconnected processes including renal tubular epithelial cell injury, microcirculatory disturbances, energy metabolic failure, and inflammatory cascades.^{9,10} Among these, dysregulated inflammatory responses coupled with exhausted endogenous repair capacity are recognized as core dual mechanisms driving AKI development, progression, and adverse prognosis.^{11,12} Interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), as classical pro-inflammatory agents, are significantly elevated during the acute phase of AKI. These mediators not only directly induce renal tubular epithelial cell apoptosis, necroptosis, and pyroptosis but also amplify inflammatory cascades through nuclear factor- κ B (NF- κ B)-mediated signaling, thereby contributing to “cytokine storms” and mediating distant organ injury.^{13–15} Accumulating clinical evidence indicates that IL-6 and TNF- α levels are significantly correlated with AKI severity, renal non-recovery rates, and in-hospital mortality, positioning them as promising candidate biomarkers for AKI prognostication.^{16,17} However, focusing exclusively on injurious inflammatory signals may incompletely capture the full spectrum of AKI clinical trajectories—clinically, some patients experiencing severe AKI ultimately achieve complete renal function recovery due to robust endogenous repair capacity. Conversely, patients with mild-to-moderate AKI may still progress to persistent renal insufficiency or even dialysis dependence when repair mechanisms are exhausted. This phenomenon suggests that renal “repair potential” may be equally important, or even more determinative, than “inflammatory burden” as a prognostic factor.¹⁸

Several biomarkers have been established for early AKI detection or risk stratification, including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and the cell cycle arrest markers insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2).

However, these markers predominantly reflect acute tubular injury, inflammation, or G1 cell cycle arrest, rather than the capacity for tubular regeneration or vascular repair. In contrast, fibroblast growth factor 2 (FGF-2) is a multifunctional growth regulator primarily secreted by fibroblasts, endothelial cells, and injured renal tubular epithelial cells within the tubulointerstitium.^{19,20} Unlike NGAL and KIM-1, which correlate with the severity of tubular damage, FGF-2 exerts multiple protective effects encompassing angiogenesis promotion, apoptosis inhibition, endogenous stem cell homing induction, and accelerated renal tubular epithelial regeneration.²¹ Thus, FGF-2 was selected as a candidate biomarker specifically representing the “repair” dimension of the injury-repair balance in AKI. Animal studies have demonstrated that exogenous FGF-2 administration significantly attenuates renal function impairment in ischemia-reperfusion-induced AKI models and promotes tubular proliferation and repair. Conversely, FGF-2 knockout mice exhibit delayed repair, aggravated renal fibrosis, and decreased survival rates. These lines of evidence suggest that FGF-2-mediated tissue repair capacity constitutes a critical determinant of whether AKI patients can surmount the “injury threshold” and achieve renal function recovery.²²

Based on these considerations, this retrospective single-center analysis systematically evaluates: the distribution differences of serum IL-6, TNF- α , and FGF-2 levels measured on the day of diagnosis between AKI stage I versus stage II–III patients and between major adverse kidney events (MAKE) versus non-MAKE patients; the strength of correlations between each biomarker and disease progression as well as MAKE; the independent predictive value of each biomarker for disease progression; the independent predictive value of each biomarker for 28-day MAKE risk and time-to-event; and the predictive performance of individual biomarkers and their combination for disease progression and MAKE. This investigation aims to elucidate the differential roles of inflammatory versus reparative factors across various AKI phases, providing clinical evidence to support the development of individualized risk stratification models based on “injury-repair balance” assessment, while establishing a theoretical foundation for exploring intervention strategies targeting repair pathways.

MATERIALS AND METHODS

Study Population

Patients meeting the Kidney Disease: Improving Global Outcomes (KDIGO) diagnostic criteria for AKI who were admitted to our hospital between January 2023 and June 2025 were identified from the hospital database. Inclusion criteria: (1) age ≥ 18 years; (2) fulfillment of the KDIGO 2012 AKI diagnostic criteria: serum creatinine increase by ≥ 26.5 $\mu\text{mol/L}$ within 48 hours, or increase to ≥ 1.5 times baseline value within 7 days, or urine output < 0.5 mL/kg/h for ≥ 6 hours. Baseline serum creatinine (SCr) was defined as follows: if 2 or more SCr measurements were available within 3 months prior to admission, the most recent value was used as baseline; if no recent pre-admission SCr data were available, the lowest SCr value recorded during the current hospitalization prior to AKI diagnosis was used as baseline; in patients without any prior SCr

measurement, baseline SCr was back-calculated using the Modified Diet in Renal Disease (MDRD) equation assuming a normal baseline eGFR of 75 mL/min/1.73 m^2 , consistent with KDIGO guideline recommendations. (3) availability of serum samples collected on the day of AKI diagnosis for cytokine measurement; (4) complete clinical data. Exclusion criteria: (1) age < 18 years; (2) chronic kidney disease stage 5 (eGFR < 15 mL/min/1.73m^2) or prior receipt of renal replacement therapy; (3) post-kidney transplantation status; (4) pregnancy or lactation; (5) presence of severe cardiac, cerebral, or pulmonary insufficiency or other severe systemic diseases; (6) active autoimmune disease or receipt of immunosuppressive therapy within 4 weeks prior to AKI diagnosis. After screening, 104 patients were included. Based on the initial AKI stage at admission, patients were categorized into the AKI stage I group ($n=46$) and the AKI stage II–III group ($n=58$). The study flowchart is presented in Figure 1.

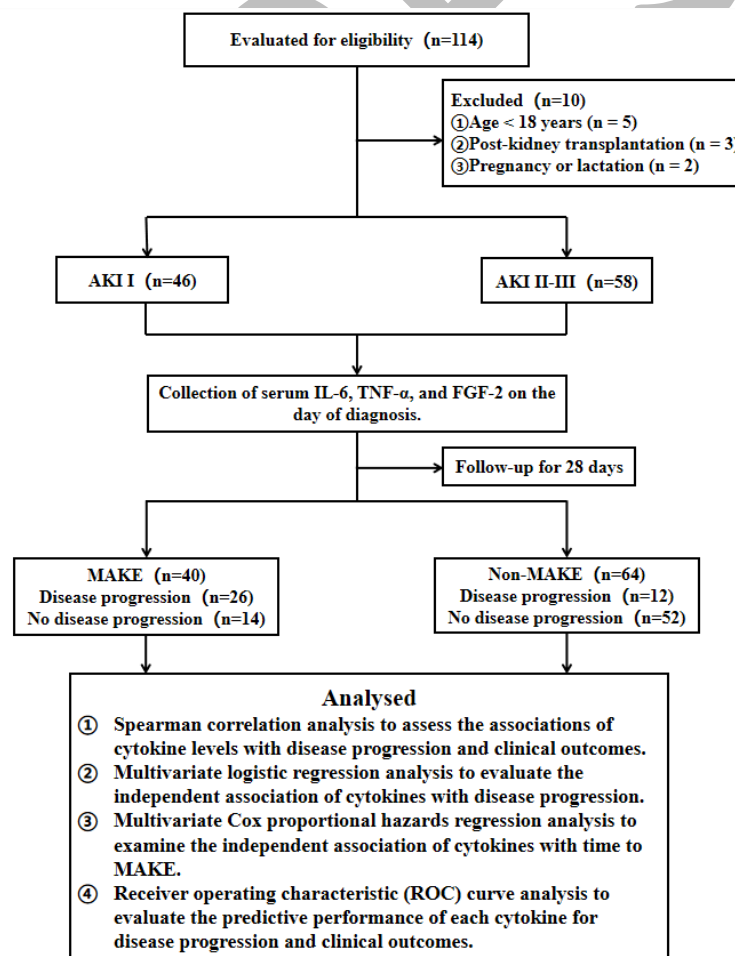


Figure 1. Research flowchart

Ethical Considerations

Approval was obtained from the hospital ethics committee, and the study adhered to the Declaration of Helsinki and international ethical guidelines. Given the retrospective design using archived clinical data, the ethics committee waived the requirement for informed consent. All data were anonymized to protect participant privacy throughout the study.

Sample Size Calculation

This study was designed as a retrospective clinical comparison. Based on previous studies and published literature, the incidence of MAKE was estimated to be approximately 35% to 40%.²³ Multivariate Cox regression analysis incorporated 3 to 5 independent variables. According to the events per variable (EPV) principle (requiring at least 10 outcome events per variable), a minimum of 30 to 50 MAKE events was required. The final analysis included 104 AKI patients, among whom 40 (38.5%) experienced MAKE, satisfying the statistical requirements for multivariate regression analysis.

Observational Parameters

Data for the following observational parameters were retrospectively extracted from the hospital electronic medical record system, communication systems, and nursing records.

Baseline Characteristics

The following baseline data were collected: demographic characteristics including age, sex, and body mass index (BMI); comorbidities including hypertension, diabetes mellitus, chronic kidney disease, heart failure, and coronary heart disease; AKI characteristics including SCr on the day of diagnosis, estimated glomerular filtration rate (eGFR), and 24-hour urine output; AKI etiologies including infection/sepsis, cardiogenic causes/hypoperfusion, contrast media exposure, and nephrotoxic medication use, with independent assessment by 2 attending nephrologists and consensus reached through discussion in cases of disagreement; disease severity assessed by Sequential Organ Failure Assessment (SOFA) score, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, requirement for vasoactive drug support, and requirement for mechanical ventilation.

Study Endpoints

The following endpoints were extracted and analyzed from the medical records system:

(1) Disease progression: Defined as worsening of AKI stage during hospitalization compared to baseline (e.g., progression from stage I to stage II or III, or from stage II to stage III).

(2) Clinical outcome: 28-day major adverse kidney events (MAKE), defined as a composite endpoint encompassing all-cause mortality, initiation of new renal replacement therapy, or persistent serum creatinine ≥ 2 times baseline value at hospital discharge. The 28-day follow-up period was selected based on the following considerations: (i) the primary objective of this study was to evaluate the predictive value of biomarkers measured on the day of AKI diagnosis for short-term disease progression and early clinical outcomes, rather than long-term renal fibrosis or transition to chronic kidney disease; (ii) a 28-day window minimizes confounding effects from post-discharge interventions, intercurrent illnesses, and variability in outpatient management, which is particularly important in a retrospective study design; (iii) this timeframe aligns with the acute pathophysiological phase of AKI, during which the balance between inflammatory injury and reparative capacity most directly determines early clinical trajectory. While we acknowledge that contemporary AKI studies often employ 60-day or 90-day endpoints to capture longer-term kidney recovery, the 28-day endpoint is appropriate for the current biomarker-focused, early-phase prognostic investigation.

Serum Biomarker Measurement

Peripheral venous blood samples (5 mL) were collected from all patients on the day of AKI diagnosis. Blood samples were allowed to clot (30 minutes, room temperature), centrifuged at 3000 rpm for 10 minutes, and stored at -80°C until use, with no repeated freeze-thaw cycles. Serum IL-6, TNF- α , and FGF-2 were quantified using human enzyme-linked immunosorbent assay (ELISA) kits (IL-6: R&D Systems, USA, 0.7 pg/mL sensitivity, 3.12–300 pg/mL range; TNF- α : eBioscience, USA, 1.6 pg/mL sensitivity, 7.8–500 pg/mL range; FGF-2: Abcam, UK, 1.0 pg/mL sensitivity, 4.0–500 pg/mL range), with all assays performed in duplicate according to the manufacturers' protocols.

Statistical Analysis

Statistical analyses were conducted using SPSS 26.0, with ROC curves generated via MedCalc 15.2.2. Shapiro-Wilk and Levene tests were used to check for normality and homogeneity of variance. Normally distributed continuous variables (e.g., age, BMI) were expressed as mean $\pm$ SD and compared using the independent samples *t*-test, while categorical variables were summarized as frequencies (percentages) and compared using the chi-square or Fisher exact test. Spearman rank correlation was used to evaluate relationships among IL-6, TNF- α , and FGF-2, as well as their associations with disease progression and MAKE.

For both logistic regression (predicting disease progression) and Cox proportional hazards regression (predicting time to MAKE), a 2-step variable selection process was implemented. First, a comprehensive set of candidate variables was considered for entry into the univariate analyses, including: demographic characteristics (age, sex, BMI), comorbidities (hypertension, diabetes, chronic kidney disease, heart failure, coronary heart disease), AKI etiology (infection/sepsis, cardiogenic/hypoperfusion, contrast exposure, nephrotoxic medications), baseline renal function (SCr, eGFR, 24-hour urine output), disease severity scores (SOFA, APACHE II), and the 3 biomarkers (IL-6, TNF- α , FGF-2). Second, variables with a *p* value < 0.10 in univariate analysis were retained as candidates for multivariate adjustment. The final multivariate models were built using forward stepwise selection based on the likelihood ratio test, with entry and removal thresholds set at *p* < 0.05 and *p* > 0.10 , respectively. In the final models, only IL-6, TNF- α , and FGF-2 met the pre-specified entry criteria and were retained; other clinical covariates did not achieve statistical significance and were not included.

For disease progression, multivariate logistic regression using the above forward stepwise selection was performed. Similarly, Cox proportional hazards regression was employed for time-to-MAKE analysis using the same variable selection strategy; the proportional hazards assumption was verified by Schoenfeld residuals and found to be satisfied (all *p* > 0.05). The predictive performance of individual biomarkers and their combination for disease progression and 28-day MAKE was assessed using ROC curve analysis. Combined predictive probabilities were derived from the linear predictors of multivariate logistic regression (for progression prediction) and multivariate

Cox regression (for MAKE prediction). Comparisons of AUC values were performed using the DeLong test. To assess potential multicollinearity among IL-6, TNF- α , and FGF-2 in the multivariate regression models, variance inflation factors (VIFs) were calculated for each independent variable in both the logistic regression (disease progression) and Cox regression (time to MAKE) models. A VIF > 5 (or > 10) was considered indicative of problematic collinearity.

Among the 104 included patients, no missing data were present for serum IL-6, TNF- α , or FGF-2 levels, nor for the primary endpoints (disease progression and 28-day MAKE). For baseline covariates, the proportion of missing values was less than 5% for any single variable. Given the low rate of missingness, a complete-case analysis was performed without imputation. All statistical tests were 2-sided, and *p* < 0.05 was considered to indicate statistical significance.

To explore potential non-linear associations between the primary biomarkers (IL-6, TNF- α) and clinical outcomes (disease progression and 28-day MAKE), we additionally performed restricted cubic spline (RCS) analyses with 3 knots placed at the 10th, 50th, and 90th percentiles of the log-transformed biomarker distributions. Non-linearity was tested using the likelihood ratio test comparing the model with only the linear term to the model with both linear and spline terms. As no significant non-linear relationships were identified (all *p* for non-linearity > 0.05), the linear assumption was retained for the primary analyses.

RESULTS

Comparison of Baseline Characteristics

As shown in Table 1, patients in the AKI stage II–III group exhibited significantly higher serum SCr levels on the day of diagnosis compared to those in the AKI stage I group (248.64 ± 74.48 vs 137.11 ± 28.94 $\mu\text{mol/L}$, *p* < 0.001), significantly lower eGFR (22.61 ± 9.23 vs 43.28 ± 11.84 mL/min/1.73m^2 , *p* < 0.001), and significantly reduced 24-hour urine output (673.47 ± 251.87 vs 1280.52 ± 375.92 mL , *p* < 0.001). Regarding disease severity, patients in the AKI stage II–III group demonstrated significantly higher SOFA scores (8.38 ± 2.64 vs 4.26 ± 1.08 , *p* < 0.001) and APACHE II scores (20.34 ± 6.84 vs 14.74 ± 5.20 , *p* < 0.001) compared to those in the AKI stage I group. Correspondingly, the proportions of patients requiring vasoactive drug support (55.17% vs 21.74% , *p* < 0.001)

and mechanical ventilation (37.93% vs 13.04%, $p < 0.001$) were also higher in the AKI stage II–III group. Prevalence of comorbidities—hypertension, diabetes, CKD, heart failure, and coronary heart disease—did not differ significantly between groups (all $p > 0.05$). Additionally, the distribution of AKI etiologies did not differ significantly between the 2 groups (all $p > 0.05$).

Comparison of IL-6, TNF- α , and FGF-2 Levels

Regarding the primary study endpoint, 40 patients (38.5%) experienced MAKE, while 64 patients (61.5%) were classified into the non-MAKE group. As shown in Table 2, IL-6 (130.73 ± 38.36 vs. 75.16 ± 22.34 pg/mL) and TNF- α (36.94 ± 14.00 vs. 18.45 ± 5.61 pg/mL) were

higher in the MAKE group (both $p < 0.001$). Conversely, FGF-2 levels were markedly lower in the MAKE group (11.16 ± 3.91 vs. 19.05 ± 4.69 pg/mL, $p < 0.001$).

Disease Progression Distribution and Temporal Characteristics

As shown in Table 3, the proportion of patients experiencing disease progression (worsening of AKI stage) during hospitalization was higher in the MAKE group, at 65.00% (26 of 40) versus 18.75% (12 of 64) ($\chi^2 = 22.708$, $p < 0.001$). Regarding temporal characteristics, the time to MAKE occurrence or censoring was 26.45 ± 4.45 days in the non-MAKE group, compared to 16.70 ± 8.64 days in the MAKE group ($t = -7.573$, $p < 0.001$).

Table 1. Comparison of baseline characteristics

Characteristic	AKI I (n=46)	AKI II–III (n=58)	t/χ^2	p
Demographic Characteristics				
Age, mean $\pm$ SD, y	66.41 $\pm$ 11.31	68.86 $\pm$ 10.64	-1.134	0.259
Male, n (%)	27 (58.70)	38 (65.52)	0.509	0.475
BMI, mean $\pm$ SD, kg/m ²	23.96 $\pm$ 3.56	24.78 $\pm$ 4.53	-1.004	0.318
Comorbidities				
Hypertension, n (%)	31 (67.39)	41 (70.69)	0.131	0.717
Diabetes mellitus, n (%)	20 (43.48)	24 (41.38)	0.046	0.830
Chronic kidney disease, n (%)	10 (21.74)	18 (31.03)	1.127	0.288
Heart failure, n (%)	12 (26.09)	19 (32.76)	0.546	0.460
Coronary heart disease, n (%)	17 (36.96)	27 (46.55)	0.968	0.325
Renal Function Parameters				
SCr at diagnosis, mean $\pm$ SD, μ mol/L	137.11 $\pm$ 28.94	248.64 $\pm$ 74.48	-9.590	<0.001
eGFR, mean $\pm$ SD, mL/min/1.73 m ²	43.28 $\pm$ 11.84	22.61 $\pm$ 9.23	-10.000	<0.001
24-Hour urine output, mean $\pm$ SD, mL	1280.52 $\pm$ 375.92	673.47 $\pm$ 251.87	-9.832	<0.001
AKI Etiologies				
Infection/sepsis, n (%)	16 (34.78)	29 (50.00)	2.420	0.120
Cardiogenic/hypoperfusion, n (%)	14 (30.43)	16 (27.59)	0.101	0.750
Contrast media exposure, n (%)	13 (28.26)	15 (25.86)	0.075	0.784
Nephrotoxic medication, n (%)	12 (26.09)	23 (39.66)	2.115	0.146
Disease Severity				
SOFA score, mean $\pm$ SD, points	4.26 $\pm$ 1.08	8.38 $\pm$ 2.64	-9.925	<0.001
APACHE II score, mean $\pm$ SD, points	14.74 $\pm$ 5.20	20.34 $\pm$ 6.84	-4.604	<0.001
Vasoactive drug requirement, n (%)	10 (21.74)	32 (55.17)	11.911	0.001
Mechanical ventilation requirement, n (%)	6 (13.04)	22 (37.93)	8.076	0.004

AKI: acute kidney injury; APACHE II: Acute Physiology and Chronic Health Evaluation II; BMI: body mass index; eGFR: estimated glomerular filtration rate; SCr: serum creatinine; SD: standard deviation; SOFA: Sequential Organ Failure Assessment.

Table 2. Comparison of IL-6, TNF- α , and FGF-2 levels

Biomarker	Non-MAKE (n=64)	MAKE (n=40)	t	p
IL-6, pg/mL	75.16 $\pm$ 22.34	130.73 $\pm$ 38.36	-9.342	<0.001
TNF- α , pg/mL	18.45 $\pm$ 5.61	36.94 $\pm$ 14.00	-9.441	<0.001
FGF-2, pg/mL	19.05 $\pm$ 4.69	11.16 $\pm$ 3.91	8.878	<0.001

FGF-2: fibroblast growth factor 2; IL-6: interleukin-6; MAKE: major adverse kidney events; TNF- α : tumor necrosis factor- α .

Table 3. Disease progression distribution and temporal characteristics

Variable	Non-MAKE (n=64)	MAKE (n=40)	χ^2/t	p
Disease progression				
No progression, n (%)	52 (81.25)	14 (35.00)	22.708	<0.001
Progression, n (%)	12 (18.75)	26 (65.00)		
Time to MAKE occurrence/censoring, d	26.45 $\pm$ 4.45	16.70 $\pm$ 8.64	-7.573	<0.001

MAKE: major adverse kidney events.

Correlation Analysis of IL-6, TNF- α , and FGF-2 with Disease Progression and Clinical Outcomes

Spearman correlation analysis (Table 4) revealed that IL-6 was strongly positively correlated with disease progression ($r=0.662$) and significantly positively correlated with MAKE ($r=0.637$). TNF- α exhibited a similar pattern, demonstrating positive correlations with both disease progression ($r=0.606$) and MAKE ($r=0.677$), with the strongest correlation with MAKE among the 3 biomarkers. Conversely, FGF-2 showed significant negative correlations with disease progression ($r=-0.504$) and MAKE ($r=-0.658$), suggesting a potential protective role. Additionally, IL-6 and TNF- α showed a moderate positive correlation ($r=0.575$), while both inflammatory cytokines were negatively correlated with FGF-2 ($r=-0.493$; $r=-0.464$; all $p<0.001$). These findings suggest an intrinsic balance between inflammatory responses and reparative processes.

Logistic Regression Analysis

IL-6, TNF- α , and FGF-2 were each significantly associated with disease progression, as shown in Table 5. For each 1 pg/mL increase in IL-6, the risk of disease progression increased by 5.7% (OR = 1.057). TNF- α exhibited a stronger effect, with each 1 pg/mL increment associated with a 17.3% increase in progression risk (OR = 1.173). Conversely, FGF-2 emerged as a protective factor, with each 1 pg/mL increase

corresponding to a 20.6% reduction in disease progression risk (OR = 0.794; all $p<0.001$).

Multivariate logistic regression analysis, with simultaneous adjustment for all three biomarkers, revealed that both IL-6 (aOR = 1.034, $p=0.005$) and TNF- α (aOR = 1.082, $p=0.039$) remained independent predictors of disease progression. However, FGF-2 did not demonstrate independent predictive value in the multivariate model (aOR = 0.916, $p=0.137$). These findings indicate that both IL-6 and TNF- α are independently associated with AKI progression, with partially overlapping yet distinct contributions.

Cox Regression Analysis

Univariate Cox regression analysis (Table 6) demonstrated that both IL-6 (HR = 1.024, $p<0.001$) and TNF- α (HR = 1.058, $p<0.001$) were significant risk factors for MAKE occurrence, whereas FGF-2 emerged as a significant protective factor (HR = 0.806, $p<0.001$). Multivariate Cox analysis revealed that TNF- α was the only risk factor independently associated with earlier MAKE occurrence (aHR = 1.031, $p=0.017$). FGF-2 demonstrated a robust protective effect in delaying event occurrence (aHR = 0.832, $p<0.001$). Notably, IL-6 did not show a significant independent effect on time to event in the multivariate model (aHR = 1.009, $p=0.093$). These findings suggest distinct prognostic patterns among the three biomarkers in the multivariate Cox model: TNF- α was associated with

earlier MAKE occurrence, whereas FGF-2 was independently associated with delayed event timing. Although IL-6 showed a strong univariate association with MAKE, its effect did not remain significant for time-to-event after adjustment for TNF- α and FGF-2. This differential pattern indicates that these biomarkers may impact the final outcomes of AKI through distinct mechanisms.

Collinearity Diagnostics

In the multivariate logistic regression model containing IL-6, TNF- α , and FGF-2, the VIFs were 1.62, 1.58, and 1.35, respectively. In the multivariate Cox regression model, the VIFs were 1.60, 1.57, and 1.34, respectively. All VIF values were substantially below the conventional threshold of 5, indicating that multicollinearity did not materially affect the stability or interpretation of the regression coefficients.

ROC Curve Analysis

As presented in Table 7 and Figure 2, for predicting

disease progression, IL-6 demonstrated the best discriminatory ability (AUC = 0.897), outperforming TNF- α (AUC = 0.863) and FGF-2 (AUC = 0.802). The combined model incorporating all 3 biomarkers improved the AUC to 0.919. For predicting 28-day MAKE, both TNF- α (AUC = 0.902) and FGF-2 (AUC = 0.891) exhibited excellent predictive performance, surpassing IL-6 (AUC = 0.878). The combination of IL-6, TNF- α , and FGF-2 achieved an AUC of 0.962 for predicting MAKE, which was significantly higher than that of IL-6 (AUC = 0.878, $p=0.008$ by DeLong test), TNF- α (AUC = 0.902, $p=0.041$), and FGF-2 (AUC = 0.891, $p=0.023$), respectively. For disease progression, the combined model (AUC = 0.919) also showed numerically superior performance, although the difference did not reach statistical significance compared to IL-6 alone (AUC = 0.897, $p=0.312$). These findings support that integrating inflammatory and reparative biomarkers enhances the prediction of adverse outcomes in AKI.

Table 4. Correlations among IL-6, TNF- α , FGF-2, disease progression, and clinical outcomes

Variable	MAKE	IL-6	TNF- α	FGF-2	Disease progression
MAKE	1	0.637 ^a	0.677 ^a	-0.658 ^a	0.467 ^a
IL-6		1	0.575 ^a	-0.493 ^a	0.662 ^a
TNF- α			1	-0.464 ^a	0.606 ^a
FGF-2				1	-0.504 ^a
Disease progression					1

$p < 0.001$. FGF-2: fibroblast growth factor 2; IL-6: interleukin-6; MAKE: major adverse kidney events; TNF- α : tumor necrosis factor- α .

Table 5. Logistic regression analysis of IL-6, TNF- α , and FGF-2 for disease progression

Variable	Univariate OR (95% CI)	p	Multivariate aOR (95% CI)	p
IL-6	1.057 (1.035–1.079)	<0.001	1.034 (1.010–1.059)	0.005
TNF- α	1.173 (1.099–1.251)	<0.001	1.082 (1.004–1.165)	0.039
FGF-2	0.794 (0.720–0.876)	<0.001	0.916 (0.816–1.028)	0.137

aOR: adjusted odds ratio; CI: confidence interval; FGF-2: fibroblast growth factor 2; IL-6: interleukin-6; OR: odds ratio; TNF- α : tumor necrosis factor- α .

Table 6. Cox regression analysis of IL-6, TNF- α , and FGF-2 for clinical outcomes

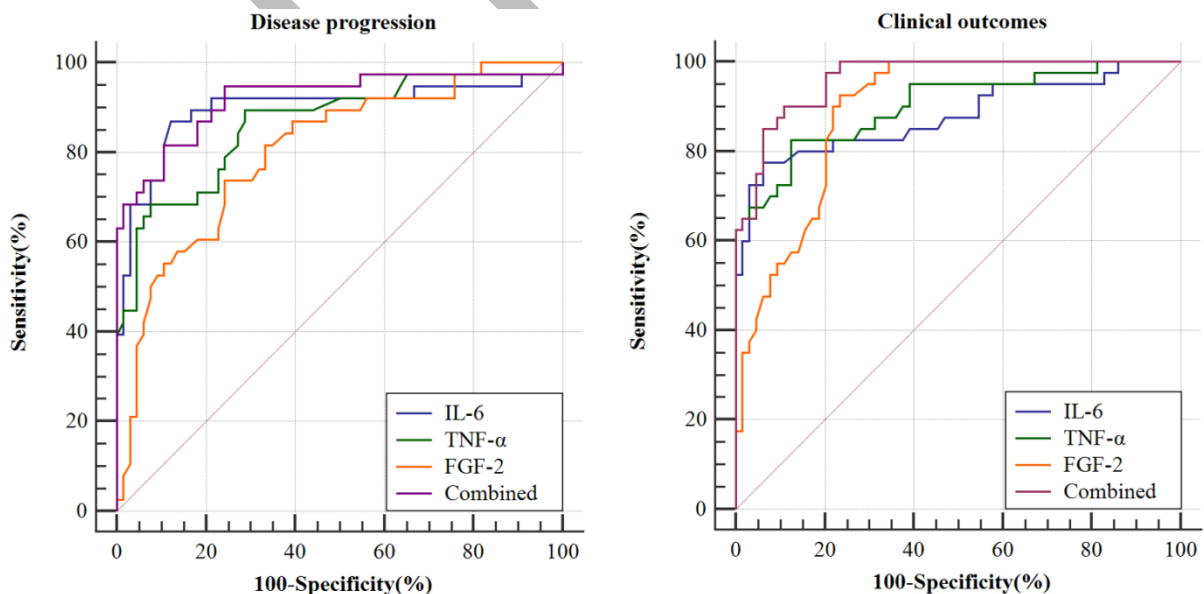
Variable	Univariate HR (95% CI)	<i>p</i>	Multivariate aHR (95% CI)	<i>p</i>
IL-6	1.024 (1.017–1.032)	<0.001	1.009 (0.999–1.019)	0.093
TNF- α	1.058 (1.041–1.076)	<0.001	1.031 (1.006–1.058)	0.017
FGF-2	0.806 (0.756–0.859)	<0.001	0.832 (0.772–0.896)	<0.001

aHR: adjusted hazard ratio; CI: confidence interval; FGF-2: fibroblast growth factor 2; HR: hazard ratio; IL-6: interleukin-6; TNF- α : tumor necrosis factor- α .

Table 7. Predictive performance of IL-6, TNF- α , FGF-2, and their combined models for disease progression and clinical outcomes

Biomarker	AUC	95% CI	Optimal cut-off value	Sensitivity	Specificity	<i>p</i>
Disease progression						
IL-6	0.897	0.822–0.948	>96.1 pg/mL	86.84% (71.29–95.62)	87.88% (78.12–94.31)	<0.001
TNF- α	0.863	0.782–0.923	>27.4 pg/mL	68.42% (51.73–82.54)	92.42% (84.75–97.25)	<0.001
FGF-2	0.802	0.712–0.874	$\leq$ 14.3 pg/mL	73.68% (56.91–86.67)	75.76% (65.45–84.44)	<0.001
Combined	0.919	0.850–0.964	NA	81.58% (66.61–92.07)	89.39% (80.44–95.35)	<0.001
Clinical outcomes						
IL-6	0.878	0.799–0.934	>104.2pg/mL	77.50% (61.45–89.27)	93.75% (86.50–97.97)	<0.001
TNF- α	0.902	0.828–0.951	>23.7 pg/mL	82.50% (67.72–92.71)	87.50% (78.27–94.07)	<0.001
FGF-2	0.891	0.814–0.943	$\leq$ 15.8 pg/mL	92.50% (79.46–98.47)	76.56% (65.56–85.56)	<0.001
Combined	0.962	0.906–0.990	NA	90.00% (76.33–97.23)	89.06% (80.52–95.04)	<0.001

^aAUC: area under the curve; CI: confidence interval; FGF-2: fibroblast growth factor 2; IL-6: interleukin-6; TNF- α : tumor necrosis factor- α .

**Figure 2. ROC curves of IL-6, TNF- α , FGF-2, and their combined probability for predicting disease progression and clinical outcomes**

DISCUSSION

This study systematically evaluated the predictive value of serum IL-6, TNF- α , and FGF-2 for disease progression and 28-day MAKE in patients with AKI. The findings demonstrated a clear association between the “pro-inflammatory-reparative” imbalance assessed on the day of AKI diagnosis and both the risk of short-term adverse outcomes and the timing of event occurrence. Specifically, IL-6 and TNF- α emerged as independent predictors of disease progression, remaining significant in the multivariate logistic regression model. TNF- α was identified as a factor independently associated with a shorter time to MAKE, whereas higher FGF-2 levels were associated with a delayed time to event occurrence. The combined measurement of all 3 biomarkers achieved a high predictive performance for 28-day MAKE (AUC = 0.962). These findings provide new clinical evidence for understanding the dynamic relationship between injury and repair during the course of AKI and offer a reference for developing multidimensional biomarker-based risk stratification models.

IL-6 and TNF- α were significantly associated with disease progression and MAKE in univariate analyses, and multivariate logistic regression confirmed both as independent risk factors for AKI stage progression. As a core mediator of the acute phase response, IL-6 has been shown in previous experimental studies to participate in renal tubular epithelial cell injury through activation of the JAK/STAT3 signaling pathway.^{24–26} TNF- α , meanwhile, is known to mediate inflammatory responses and apoptotic signal transduction via the TNFR1 receptor. In our observational data, these 2 cytokines showed differential associations with the timing of MAKE.^{27–29} Notably, these 2 cytokines exhibited differential effects on the timing of MAKE occurrence. In the multivariate Cox regression model, TNF- α remained an independent risk factor associated with earlier MAKE occurrence (aHR = 1.031), whereas IL-6 showed no significant impact on time-to-event (aHR = 1.009, $p=0.093$). This disparity may be attributable to distinct biological functions: TNF- α release is characterized by relatively rapid responsiveness, directly participating in processes such as endothelial dysfunction and microvascular permeability alterations.^{27,30} In contrast, IL-6 more broadly reflects the cumulative state of systemic

inflammatory burden, correlating with the risk of adverse events but demonstrating limited capacity to predict the precise timing of event occurrence.^{31,32} These findings suggest that even within the same pro-inflammatory cytokine family, differential roles may exist along the temporal dimension of the AKI course.

FGF-2 was negatively correlated with both disease progression and MAKE in univariate analysis; however, it did not demonstrate independent predictive value for disease progression in multivariate logistic regression. This finding does not negate the potential value of FGF-2 as a reparative biomarker. The repair effects mediated by FGF-2 primarily operate during the stages of renal tubular regeneration and vascular remodeling, whereas AKI stage progression reflects acute declines in glomerular filtration function over a short period—a process predominantly driven by acute hemodynamic disturbances and tubular injury, during which repair mechanisms have not yet been fully activated.³³ This suggests that the reparative capacity represented by FGF-2 primarily exerts its effects during the recovery phase following injury, rather than during the acute deterioration phase; its delaying effect on the timing of MAKE occurrence further supports this interpretation. In the multivariate Cox regression model, FGF-2 was independently associated with delayed event occurrence. This finding carries several potential clinical implications. First, even among patients who ultimately experience adverse outcomes, those with higher FGF-2 levels may have a relatively delayed time to event occurrence, potentially providing a therapeutic window for clinical intervention. Second, the association with FGF-2 persisted after adjustment for IL-6 and TNF- α , suggesting that reparative capacity may represent a prognostic dimension relatively independent of inflammatory burden. This observation aligns with the biological functions of FGF-2, which exerts tissue repair-related effects during the post-AKI recovery phase through mechanisms including endothelial progenitor cell mobilization, promotion of peritubular capillary repair, and regulation of fibroblast activation.³⁴

In this study, the combined model yielded a further improvement in AUC compared to the best single indicator. This observation suggests that IL-6, TNF- α , and FGF-2 may reflect distinct dimensions of AKI prognosis: IL-6 is associated with systemic inflammatory burden, TNF- α correlates with the degree of acute injury, and FGF-2 relates to tissue repair

reserve. The combined measurement of these 3 biomarkers may facilitate a more comprehensive assessment of patients' overall prognostic status. Nevertheless, the stability and generalizability of this combined predictive model require further validation.

From a translational perspective, the proposed 3-biomarker panel (IL-6, TNF- α , and FGF-2) is potentially feasible for clinical integration. All 3 cytokines can be measured using commercial ELISA kits, with a turnaround time of approximately 4 to 6 hours, allowing same-day results. The estimated cost per patient is around \$30 to \$50 USD, which is reasonable for high-risk AKI stratification. From a decision-making standpoint, the combined model (AUC = 0.962 for 28-day MAKE) could guide earlier nephrology referral, intensified monitoring, avoidance of nephrotoxic insults, and timely preparation for renal replacement therapy. These practical considerations support the potential utility of this panel pending prospective validation.

This study has several limitations. The single-center, retrospective design may introduce selection bias and unmeasured confounding. Biomarkers were measured only at a single time point on the day of AKI diagnosis, without longitudinal sampling to capture dynamic changes in the inflammatory-reparative balance. The primary outcome was limited to 28-day MAKE without longer-term endpoints (e.g., 60- or 90-day MAKE), which restricts extrapolation to late renal recovery or CKD transition. Although the sample size met the minimum requirements for multivariate regression (40 MAKE events), the limited number of events raises concerns about model overfitting, particularly for the combined biomarker model (AUC = 0.962). Internal validation (e.g., bootstrapping) was not performed, and the absence of an external validation cohort warrants cautious interpretation. Detailed inflammatory laboratory markers, such as C-reactive protein (CRP) and procalcitonin (PCT), and microbiological data were not available, limiting our ability to fully adjust for underlying infection or systemic inflammation. Etiology-specific subgroup analyses (e.g., septic vs. ischemic AKI) were not conducted due to insufficient power, despite potential differences in biomarker profiles. Formal interaction analyses (e.g., IL-6 $\times$ FGF-2) were not performed for the same reason. Incremental prognostic value (e.g., NRI, IDI) and model calibration (e.g., Hosmer-Lemeshow test) were not evaluated beyond AUC. Finally, treatment heterogeneity, such as

renal replacement therapy (RRT) timing and vasopressor use, could not be fully controlled due to the retrospective design. Future prospective, multicenter studies with serial biomarker sampling, longer follow-up, robust internal validation, and calibration assessments are needed to confirm and extend these findings.

In conclusion, this study identified significant associations between serum levels of IL-6, TNF- α , and FGF-2 measured on the day of AKI diagnosis and both disease progression and 28-day MAKE. IL-6 and TNF- α jointly contributed to the prediction of AKI stage progression; however, their effects on the timing of MAKE occurrence differed: TNF- α was independently associated with a shorter time to MAKE, whereas IL-6 showed no significant impact on time-to-event. Although FGF-2 did not demonstrate independent predictive value for short-term renal function deterioration, it exhibited a significant association with delayed MAKE occurrence, an effect that remained independent of classical inflammatory cytokines. The combined measurement of IL-6, TNF- α , and FGF-2 improved the predictive performance for 28-day MAKE compared to individual biomarkers alone. Future studies should employ multicenter, prospective cohort designs with dynamic monitoring of biomarker trajectories and further explore the clinical value of individualized risk stratification strategies based on "injury-repair" balance assessment.

This study not only reveals the differential associations of IL-6, TNF- α , and FGF-2 with distinct levels of clinical endpoints but also systematically differentiates, through multivariate analysis, the distinct roles of inflammatory versus reparative factors in predicting disease progression versus the timing of outcomes. The findings confirm that TNF- α and FGF-2 independently influence the acceleration or delay of MAKE occurrence, respectively, while IL-6 primarily reflects the overall risk of the event. The combined measurement of these 3 biomarkers demonstrates a high predictive performance for MAKE, offering a critical clinical rationale and translational foundation for integrating multi-dimensional biomarkers into AKI management pathways. This approach could facilitate a shift in risk assessment from predicting whether an event will occur to estimating when it might occur, enabling more refined risk stratification.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of The Second Medical Center of Chinese PLA General Hospital (Approval No.: S2023-725-01).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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