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Original Article

Plasma SCUBE2 as a novel biomarker associates with survival outcomes in patients with sepsis-associated acute kidney injury

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Abstract *Background:* The adverse effects of sepsis-associated acute kidney injury (SA-AKI) highlight the need for new biomarkers. Signal Peptide-Complement C1r/C1s, Uegf, Bmp1-Epidermal Growth Factor-like Domain-Containing Protein 2 (SCUBE2), important for angiogenesis and endothelial integrity, has been linked to increased mortality in models of lipopolysaccharide-induced lung injury. This research aimed to assess the utility of plasma SCUBE2 levels as a prognostic indicator for SA-AKI in intensive care unit (ICU) patients.
Methods: Between September 2020 and December 2022, our study enrolled ICU patients diagnosed with stage 3 SA-AKI. We collected demographic information, illness severity indices, and laboratory data, including plasma SCUBE2 and sepsis-triggered cytokine levels. We employed

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receiver operating characteristic curves and DeLong tests to assess the predictive accuracy for survival, Kaplan—Meier curves to evaluate the relative risk of death, and multivariate logistic regression to identify independent mortality predictors.
Results: Among the total of 200 participants, the survivors had significantly higher plasma SCUBE2 levels (115.9 ng/mL) compared to those who died (35.6 ng/mL). SCUBE2 levels showed a positive correlation with the anti-inflammatory cytokine IL-10 and a negative correlation with the APACHE II score, SOFA score, C-reactive protein, and monocyte chemoattractant protein-1. Multivariate analysis revealed that elevated SCUBE2 and IL-10 levels were independently protective against mortality, and associated with the most favorable 30-day survival outcomes.
Conclusions: In ICU patients with stage 3 SA-AKI, lower plasma levels of SCUBE2 were correlated with elevated pro-inflammatory factors, which impacted survival outcomes. This suggests that SCUBE2 could be a potential biomarker for predicting prognosis in patients with SA-AKI.

Introduction

In intensive care unit (ICU) settings, sepsis is a major cause of acute kidney injury (AKI), accounting for 45—70% of cases among critically ill patients and leading to severe negative outcomes.[1,2] Patients with sepsis-associated AKI (SA-AKI) face a higher risk of morbidity and mortality compared to those with AKI due to other etiologies.[3,4] Various studies have highlighted the potential of using biomarkers indicative of glomerular and tubular damage, such as cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), metalloproteinases 2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP7) to evaluate the risk for SA-AKI progression.[5—9] However, these biomarkers were not directly related to sepsis and were insufficient in predicting survival outcomes. This gap addressed the urgent need to identify novel biomarkers for patients with SA-AKI.

The Signal Peptide-Complement C1r/C1s, Uegf, Bmp1 (CUB)-Epidermal Growth Factor-like Domain-Containing Protein (SCUBE) gene family, expressed in endothelial cells, is found in highly vascularized tissues like lungs, kidneys, and liver.[10,11] This family includes the genes for SCUBE1—3, which encode glycoproteins structured into five domains: an N-terminal signal peptide, nine tandem epidermal growth factor-like repeats, a large spacer region, three cysteine-rich motifs, and a CUB domain at the C-terminus. Endothelial-derived SCUBE2 is a peripheral membrane protein that serves as a coreceptor for vascular endothelial growth factor (VEGF). It can also be cleaved and released into the bloodstream as a soluble form.[12]

SCUBE2 has been suggested to guide cancer therapy and predict prognosis in several cancers. Özcan discovered that SCUBE2 overexpression in breast cancer was associated with resistance to Taxane-based chemotherapy.[13] Furthermore, Cheng et al. reported that SCUBE2 suppresses breast tumor cell proliferation, and patients with SCUBE2 protein-expressing invasive breast cancer have better disease-free survival.[14] Lin et al. further demonstrated that SCUBE2 serves as a tumor suppressor by inhibiting breast cancer cell migration and invasion through the reversal of epithelial—mesenchymal transition.[15] Additionally, evidence has shown that reduced SCUBE2 expression is an independent predictor of poor prognosis in gastric cancer,[16] bladder cancer,[17] endometrial cancer,[18] and nasopharyngeal carcinoma.[19]

SCUBE2 also plays an important role in maintaining the endothelial barrier during inflammation. Downregulated SCUBE2 has been found in central nervous system tuberculosis and traumatic brain injury, leading to blood—brain barrier disruptions.[20,21] Recently, Lin et al. demonstrated that mice with endothelial-specific SCUBE2 deletion exhibited vascular leakage and leukocyte infiltration in response to lipopolysaccharide (LPS)-induced acute organ injury.[22] In the context of SA-AKI, endothelial dysfunction is a pivotal pathogenic factor.[23] Given that reduced endothelial SCUBE2 dysregulates microvascular integrity in sepsis animal models, we hypothesize that SCUBE2 may be associated with clinical outcomes in SA-AKI. Herein, we conducted a prospective study to evaluate the relationship between plasma SCUBE2 levels and various disease indices in stage 3 SA-AKI patients, particularly those with severe kidney damage and poor survival rates. Our objective is to determine the prognostic utility of plasma SCUBE2 in ICU settings.

Methods

Study design

We conducted this single-center prospective study between September 2020 and December 2022. Our inclusion criteria were as follows: (1) being admitted to the ICU on an emergency basis or for postsurgical care with an expected stay of >48 h; (2) fulfillment of sepsis criteria and (3) Kidney Disease: Improving Global Outcomes (KDIGO)-defined AKI stage 3. Exclusions were patients without sepsis, with AKI from non-septic causes, AKI not advancing to stage 3, baseline eGFR <15 mL/min/1.73 m^2 or on maintenance

dialysis, history of cancer, or under 20 years old. The patient enrollment process is depicted in Fig. 1. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (2020-09-002A).

Definitions of SA-AKI and follow-up

Patients were identified as having SA-AKI if both consensus sepsis criteria and AKI criteria were met within 7 days of sepsis diagnosis. Sepsis was defined by Sepsis-3, requiring a confirmed infection with a Sequential Organ Failure Assessment (SOFA) score increase of $\geq$2 points.[24] In accordance with KDIGO guidelines, AKI was defined by an increase in serum creatinine (SCr) by $\geq$ 0.3 mg/dL within 48 h, an increase to $\geq$1.5 times baseline within 7 days, or urine output (UO) $<$ 0.5 mL/kg/h for 6–12 h.[25] The progression to stage 3 AKI was characterized by an increase in SCr to at least three times the baseline value, a rise to at least 4 mg/dL, UO of less than 0.3 mL/kg/h for 24 h, anuria for 12 h, or the need for acute dialysis.[26] In our study, baseline SCr was determined as the average of all SCr measurements from any hospital admissions within the year preceding the current admission or from outpatient blood tests conducted within the 6 months prior. In the absence of these data, the lowest SCr level recorded during the current hospital stay was used as the baseline. Hemodialysis was typically initiated in patients exhibiting azotemia with uremic symptoms, refractory oliguria or anuria, fluid overload, hyperkalemia, and metabolic acidosis, in accordance with standard clinical practice. The choice between intermittent hemodialysis and continuous renal replacement therapy (RRT) was based on the patient's hemodynamic stability. Follow-up of patients continued until discharge or death.

Demographic data and primary outcome

The patients' baseline demographic data were collected on the index day of ICU admission. These data included demographic factors such as age, sex, baseline SCr, and body mass index. We recorded the presence of comorbidities, such as hypertension, diabetes mellitus (DM), coronary artery disease (CAD), congestive heart failure (CHF), and CKD. Acute Physiology and Chronic Health Evaluation (APACHE) II score and SOFA score assessing illness severity were also examined. Additionally, we recorded the sources of infection, coexisting organ failures, and the types of antibiotics used. Besides the variations in SCr levels, our database also recorded UO and cumulative fluid overload when AKI progressed to stage 3. Notably, if a patient experienced a second episode of SA-AKI after discharge from the ICU, only the first episode was considered in our analysis. The primary outcome was in-hospital mortality, defined as any death during hospitalization.

Timeframe of blood sampling

We obtained blood samples within 2 h of diagnosing stage 3 AKI. Inflammatory markers such as white blood cell count (WBC), C-reactive protein (CRP), procalcitonin (PCT), and lactate levels were examined. Within 1 h after collection, the blood specimens used to measure plasma SCUBE2 and sepsis-triggered cytokines, including IL-1β, IL-6, IL-10, and monocyte chemoattractant protein (MCP)-1, were centrifuged, subjected to a single freeze–thaw cycle, and then stored at -80 °C.

Quantification of plasma SCUBE2

Anti-SCUBE2 monoclonal antibodies were developed in-house at the Institute of Biomedical Sciences, Academia Sinica, Taiwan.[27] Splenocytes from BALB/c mice immunized with purified recombinant SCUBE2-EGF-like domain (residues 177–321) GST-fusion protein were fused with myeloma cells to produce hybridomas. Four independent clones (SCUBE2-EGF-B2, C1, C2, and C3) were obtained, which specifically detected the recombinant full-length SCUBE2 protein expressed in HEK-293T cells by Western blotting and flow cytometry. Based on its higher sensitivity in sandwich ELISA, the SCUBE2-EGF-B2/C3 combination was used to evaluate SCUBE2 levels in plasma by multiplex immunoassay analysis. Briefly, anti-SCUBE2-EGF-B2 monoclonal antibody was conjugated to magnetic beads (Luminex, Austin TX, USA). Subsequently, 50 μL of each sample was incubated with the antibody-conjugated beads in 96-well microtiter plates for 2 h on an orbital shaker at room temperature. Following incubation, the plates were

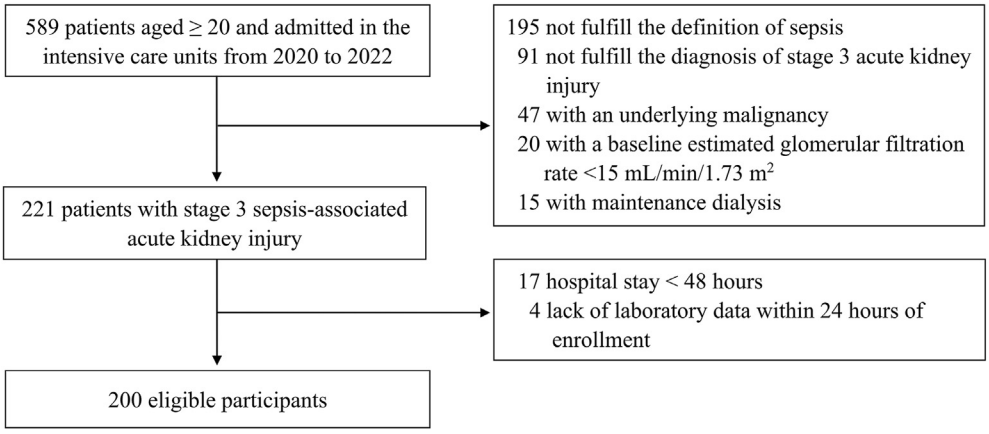


Figure 1. Flowchart for patient selection.

washed three times with assay wash buffer. Then, 40 μL of biotinylated anti-SCUBE2-C3 monoclonal antibody was added to each well and incubated for an additional hour. The wells were washed again, and 50 μL of Streptavidin-Phycoerythrin solution was added, followed by a 30-min incubation on the shaker, protected from light. Fluorescence levels of the beads were measured using a Luminex 200 instrument, and SCUBE2 concentrations were calculated using a standard curve.

Statistical analysis

The sample size needed for this study was calculated according to the following formula: n = $Z_{1-\alpha/2}$^2 * p (1 − p)/ d^2, where n is the sample size, $Z_{1-\alpha/2}$ is standard normal variate (*P* value less than 0.05 is considered significant; hence, 1.96 is used in this formula), p is expected prevalence (a recent epidemiological study demonstrated the prevalence of SA-AKI was 40.7% among critically-ill patients[2]), and d is precision (the precision in this study is 0.1).[28] Therefore, we had to enroll at least 93 subjects for this cohort study.

We compared the demographics of nonsurvivors and survivors. Between-group comparisons were analyzed using the χ^2 test for categorical variables and the independent t-test and Mann–Whitney U test for parametric and nonparametric continuous variables, respectively. We used Spearman's rank correlation test to assess the correlations of SCUBE2 with cytokines and other variables. The diagnostic accuracy of the variables in predicting in-hospital mortality was evaluated using receiver operating characteristic (ROC) curves, and the areas under the curves (AUC) were compared using the DeLong test. Youden Index was used to determine the optimal cutoff levels of the variables. Survival outcomes among the subgroups were compared using Kaplan–Meier curves and pairwise Wilcoxon tests. In the logistic regression models used to identify independent factors predicting mortality, all variables with *P* value < 0.1 from the univariate analysis and those deemed clinically significant were incorporated into the multivariate analysis. The parameters were dichotomized based on their best predictive cutoff values. The strength of each association was quantified as an odds ratio (OR) accompanied by a 95% confidence interval (CI). A two-tailed *P* value of <0.05 was statistically significant. All statistical analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC, USA) and Prism (version 9; GraphPad Software, San Diego, CA, USA).

Results

Demographic characteristics of the cohort

Among 200 patients with stage 3 SA-AKI, 104 (52%) died during hospitalization. The baseline characteristics of the study subjects are listed in Table 1. Respiratory failure (66.5%) was the most common coexisting organ failure, followed by heart failure (38.5%). The most common source of infection was the lungs (41%), followed by the bloodstream (19.5%). The top three antibiotics were cephalosporins (29.5%), glycopeptides (28.5%), and carbapenems (25.5%). The laboratory findings at AKI stage 3 included a median WBC count of 15,010/μL, albumin 2.8 g/dL, lactate 68.1 mg/dL, PCT 4.05 ng/mL, and CRP 19 mg/dL. The SCr levels rose to 3.86 mg/dL from a baseline of 1.25 mg/dL, with a median UO of 0.18 mL/kg/hr and a cumulative fluid overload of 1988 mL. The median time to RRT initiation following the diagnosis of AKI stage 3 was 60 h. Nonsurvivors exhibited significantly higher APACHE II score, more frequent coexisting respiratory failure, and elevated lactate, PCT, and CRP levels compared to survivors.

Plasma SCUBE2 and cytokine levels between survivors and nonsurvivors

Table 2 p*resents the log-transformed plasma biomarker profile of the patients. Plasma SCUBE2 and IL-10 levels were significantly higher in the survivors than in* nonsurvivors (P < 0.001). *By contrast, IL-1β, IL-6, and MCP-1 levels were significantly higher in the nonsurvivors than in the survivors. (IL-6,* P < 0.001; *IL-1β,* P $= 0.037$; *MCP-1,* P $= 0.021$).

Prediction of all-cause mortality

Fig. 2a presents the ROC curves assessing the performance of SCUBE2 and other disease indices in predicting survival outcomes. SCUBE2 demonstrated the highest AUC (0.877), indicating its potential as a prognostic marker in SA-AKI. While the AUC difference between SCUBE2 and APACHE II score was insignificant, the predictive performance of SCUBE2 for all-cause mortality was significantly superior to that of SOFA score, CRP, PCT, and lactate. Fig. 2b shows that the median plasma SCUBE2 levels were significantly higher in survivors than nonsurvivors ($P < 0.001$). The optimal cutoff values and diagnostic performance of the parameters are provided in Supplemental Table 1.

Correlation between SCUBE2 levels and disease markers

Fig. 3 displays the results of Spearman's correlation analysis. SCUBE2 demonstrated a positive correlation with anti-inflammatory IL-10 ($r = 0.35$) but negative correlations with APACHE II score ($r = -0.23$), SOFA score ($r = -0.2$), CRP ($r = -0.25$), and MCP-1 ($r = -0.23$). Similarly, IL-10 exhibited significantly negative correlations with APACHE II score ($r = -0.23$), SOFA score ($r = -0.19$), lactate ($r = -0.28$), procalcitonin ($r = -0.26$), IL-1β ($r = -0.28$), and MCP-1 ($r = -0.31$).

Independent risk factors for mortality

Table 3 presents the results of the *multivariate logistic regression identifying independent predictors of mortality. The higher APACHE II score (OR = 1.17; 95% CI = 1.06–1.29;* P $= 0.001$) *and CRP levels (OR = 11.92; 95% CI = 2.85–49.93;* P $= 0.003$) *significantly increased the risk of mortality. In contrast, SCUBE2 (OR = 0.04; 95% CI = 0.01–0.16;* P< 0.001) *and IL-10 (OR = 0.04; 95% CI = 0.01–0.21;* P < 0.001) *were identified as protective factors against mortality.*

Table 1 Patient characteristics at the time of developing stage 3 acute kidney injury.

	Total (n = 200)	Survivors (n = 96)	Nonsurvivors (n = 104)	*P*
Age (year)	64 (49, 83)	62 (46, 78)	66 (55, 85)	0.351
Male (%)	132 (66.0)	72 (76.6)	60 (56.6)	0.196
Body mass index (kg/m^2)	60 (52, 70)	25.7 (23.1, 28.5)	23.0 (21.3, 25.4)	0.077
Body temperature (°C)	37.7 (37.2, 38.3)	37.7 (37.2, 38.3)	37.7 (37.2, 38.3)	0.911
Heart rates (beats/min)	111 (96, 128)	103 (96, 127)	115 (96, 128)	0.880
Respiratory rates (breaths/min)	25 (22, 30)	26 (22, 29)	25 (22, 30)	0.719
MAP (mmHg)	52 (46, 68)	56 (46, 76)	48 (43, 61)	0.833
GCS	7 (5, 8)	7 (5, 8)	6.5 (5, 8)	0.726
APACHE II score	23 (16, 29)	18 (15, 23)	28 (22.5, 31.5)	<0.001
SOFA score	10.5 (8.5, 13)	10 (8, 11)	11.5 (9, 15)	0.082
Coexisting organ failures				
Respiratory failure (%)	133 (66.5)	52 (39.1)	81 (77.9)	<0.001
Heart failure (%)	77 (38.5)	42 (43.8)	35 (33.7)	0.143
Liver failure (%)	39 (19.5)	24 (25)	15 (14.4)	0.088
Source of infection				
Lungs (%)	82 (41)	36 (37.5)	46 (44.2)	0.334
Bloodstream (%)	39 (19.5)	20 (20.8)	19 (18.3)	0.647
Urinary tract (%)	20 (10)	13 (13.5)	7 (6.7)	0.109
Soft tissue (%)	13 (6.5)	7 (7.3)	6 (5.8)	0.663
Abdomen (%)	12 (6)	7 (7.3)	5 (4.8)	0.460
CNS (%)	2 (1)	1 (1)	1 (1)	0.955
Unidentified (%)	32 (16)	12 (12.5)	20 (19.2)	0.195
Types of antibiotic use				
Cephalosporins (%)	59 (29.5)	33 (31.7)	26 (27.1)	0.472
Glycopeptides (%)	57 (28.5)	28 (29.2)	29 (27.9)	0.841
Carbapenems (%)	51 (25.5)	27 (28.1)	24 (23.1)	0.413
Tetracyclines (%)	37 (18.5)	16 (16.7)	21 (20.2)	0.413
Penicillins (%)	35 (17.5)	14 (14.6)	21 (20.2)	0.297
Fluoroquinolones (%)	29 (14.5)	18 (18.8)	11 (10.6)	0.101
Sulfonamides (%)	26 (13)	14 (14.6)	12 (11.5)	0.522
Macrolides (%)	16 (8)	9 (9.4)	7 (6.7)	0.491
Laboratory parameters				
WBC (10^3/μL)	15.01 (6.43, 16.52)	14.21 (6.22, 14.35)	15.57 (6.52.18.63)	0.338
Albumin (g/dL)	2.8 (2.1, 3.2)	2.7 (2.2, 3.1)	2.8 (2.4, 3.0)	0.855
Blood pH value	7.25 (7.15, 7.26)	7.23 (7.16, 7.48)	7.26 (7.15, 7.44)	0.977
Bicarbonate (mg/dL)	15.7 (10.2, 19.2)	15.2 (11.5, 19.7)	16.7 (10.3, 18.4)	0.460
Lactate (mmol/L)	68.1 (57.6, 83.9)	62.5 (51.0, 78.9)	73.9 (64.2, 86.1)	<0.001
Potassium (mmol/L)	4.6 (3.5, 5.9)	4.1 (3.3, 5.1)	4.8 (3.9, 6.1)	0.193
Phosphate (mg/dL)	6.4 (2.5, 8.1)	6.2 (2.4, 7.9)	6.6 (2.6, 8.6)	0.283
AST (U/L)	53 (25, 172)	52 (22, 166)	56 (23, 175)	0.662
PCT (ng/mL)	4.05 (3.30, 5.22)	3.66 (3.18, 4.22)	4.87 (3.61, 5.70)	<0.001
CRP (mg/dL)	19 (15.5, 25.4)	16.8 (14.2, 19.7)	23.6 (17.4, 28.0)	<0.001
SCr (mg/dL)	3.86 (2.97, 4.27)	3.87 (2.65, 4.16)	3.84 (2.62, 6.81)	0.989
Urine output (mL/kg/h)	0.18 (0.01, 0.24)	0.16 (0.01, 0.22)	0.19 (0.02, 0.27)	0.174
Cumulative fluid overload (mL)	1988 (378, 3040)	2030 (460, 3215)	1950 (466, 3142)	0.196
Time to RRT initiation (hour)	60 (12, 110)	55 (7, 101)	62 (12, 113)	0.236

The data are presented in frequency (percentage) and median (interquartile range) values.
Abbreviations: SCr, serum creatinine; MBP, mean blood pressure; GCS, Glasgow coma scale; APACEII, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; CNS, central nervous system; WBC, white blood cell; AST, aspartate aminotransferase; PCT, procalcitonin; CRP, C-reactive protein; RRT, renal replacement therapy.

Fig. 4 *illustrates the Kaplan–Meier curves, stratifying the cohort into four subgroups based on SCUBE2 and IL-10 levels, using median values as cutoffs. The pairwise comparison of* P *values revealed that the subgroup with elevated levels of both SCUBE2 and IL-10 exhibited the most favorable 30-day survival outcomes following the diagnosis of SA-AKI.*

Discussion

To evaluate the potential of SCUBE2 as a biomarker associated with survival outcomes in SA-AKI, this study examined the relationship between plasma SCUBE2 levels, cytokines, illness severity, and patient survival. We found that increased plasma SCUBE2 and anti-inflammatory IL-10

Table 2 Log-transformed plasma levels of SCUBE2 and various cytokines in stage 3 sepsis-associated acute kidney injury survivors and nonsurvivors.

Biomarkers	Survivors (n = 96)	Nonsurvivors (n = 104)	*P*
Log (SCUBE2, in pg/mL)	4.98 ± 0.29	4.5 ± 0.34	<0.001
Log (IL-1β, in pg/mL)	1.35 ± 0.37	1.84 ± 0.42	0.037
Log (IL-6, in pg/mL)	2.34 ± 0.53	2.85 ± 0.51	<0.001
Log (MCP-1, in pg/mL)	2.29 ± 0.51	2.57 ± 0.45	0.021
Log (IL-10, in pg/mL)	2.22 ± 0.34	1.5 ± 0.32	<0.001

The data are presented in terms of the mean ± standard deviation values.
Abbreviations: SCUBE2, signal peptide-complement C1r/C1s, Uegf, Bmp1-epithelial growth factor-like domain-containing protein 2; IL, interleukin; MCP-1, monocyte chemoattractant protein-1.

levels were associated with higher survival rates. In contrast, elevated levels of pro-inflammatory cytokines, including IL-1β, IL-6, and MCP-1, were associated with an increased risk of death. SCUBE2 demonstrated the highest performance for survival prediction, exceeding the capabilities of the APACHE II score, CRP, PCT, lactate, and SOFA score.[29–32] Multivariate logistic regression and subgroup analysis also indicated that patients with both elevated SCUBE2 and IL-10 had the lowest risk of death. These findings suggest that SCUBE2 could be a valuable biomarker associated with survival outcomes in patients with SA-AKI.

Renal endothelial cells undergo molecular and phenotypic changes in the development of SA-AKI. During sepsis, the activated endothelial cells upregulate adhesion molecules and chemokines, initiating systemic inflammation. This promotes immune cell adhesion, exacerbating inflammation and increasing microvascular permeability.[33–36] Phenotypically, the endothelial cells exhibit barrier loss, shape changes, and a pro-coagulant state, leading to microvascular thrombosis. Lin et al. reported that endothelial SCUBE2 acts as a co-receptor for VEGF, facilitating its binding to VEGF receptor-2. This interaction promotes angiogenesis, essential for repairing microvascular damage in inflammatory settings.[27,37] Additionally, human umbilical vein endothelial cells treated with LPS and pro-inflammatory cytokines show downregulated SCUBE2 expression, compromising the cell-cell junctions.[10] Recently, Lin et al. unveiled the function of endothelial SCUBE2 in preserving vascular barrier integrity by recruiting vascular endothelial (VE) protein tyrosine phosphatase to dephosphorylate VE-cadherin, thereby stabilizing adherens junctions. To determine the role of SCUBE2 during sepsis *in vivo*, they showed that endothelial-specific knockout mice had impaired baseline endothelial barrier function and exacerbated vascular leakiness in the lungs, heart, and kidney tissues in LPS-induced sepsis model.[22] The essential role of SCUBE2 in maintaining the vascular barrier integrity of these vital organs during sepsis aligns with our findings that higher plasma SCUBE2 levels may protect against mortality in patients with SA-AKI.

Our findings reveal that SCUBE2 and the anti-inflammatory IL-10 are negatively correlated with pro-inflammatory cytokines. These results align with prior research indicating that hypoxia-induced increases in SCUBE2 within endothelial cells can alleviate oxidative stress-induced apoptosis and reduce the production of pro-inflammatory cytokines by inhibiting the mitogen-activated protein kinase (MAPK) pathways.[27] Additionally, IL-10 plays a crucial anti-inflammatory role by suppressing the production of adhesion molecules and cytokines through NF-κB and MAPK pathways, reducing the adhesion of activated immune cells to the endothelium.[38–41] IL-10 also regulates the endothelial cell cycles via the PI3K/Akt and JAK/STAT3 signaling pathways.[42,43] Furthermore, studies on IL-10-knockout mice have shown a lesser increase in superoxide levels, thereby alleviating LPS-induced endothelial

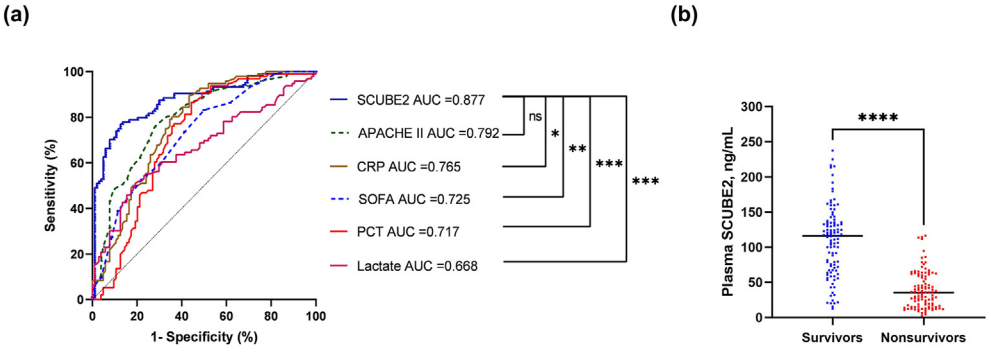


Figure 2. Predictive power of plasma SCUBE2 level for in-hospital mortality in patients with sepsis-associated acute kidney injury. (a) Receiver operating characteristic curve analyses compare the accuracy of the SCUBE2 level with that of other parameters in mortality prediction. The difference in the AUC for each variable was assessed using the DeLong test. ns, non-significant, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. (b) Survivors had significantly higher SCUBE2 levels (115.9 ng/mL) than non-survivors (35.6 ng/mL), ****$p < 0.0001$.

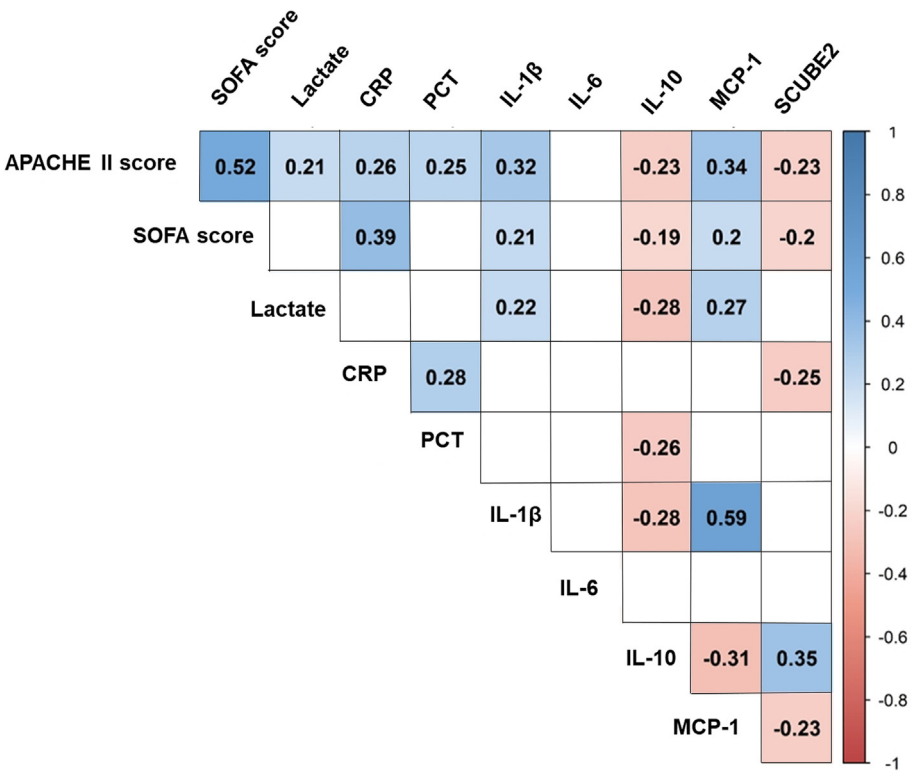


Figure 3. Correlation matrix of sepsis severity, SCUBE2 level, and pro- and anti-inflammatory cytokine levels in patients with sepsis-associated acute kidney injury. The associations between the variables were investigated through the Spearman correlation analysis. Positive correlations are in blue, whereas negative ones are in red. Blank spaces represent non-significant associations.

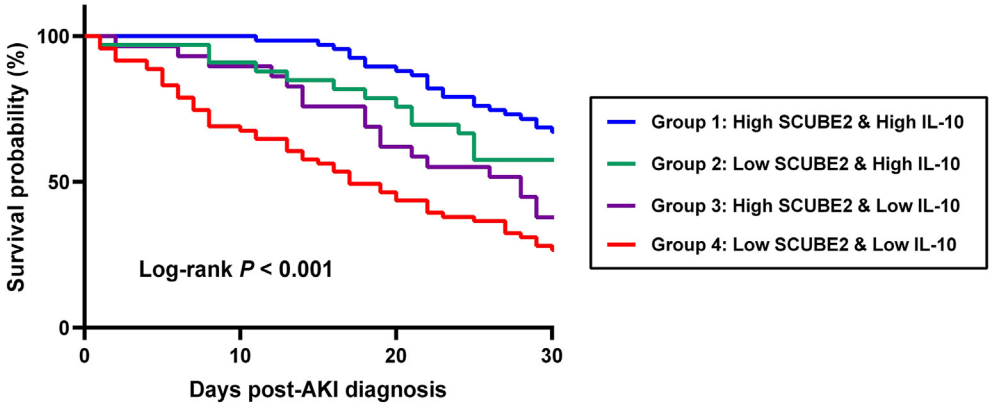
Table 3 Multivariate logistic regression of independent variables associated with in-hospital mortality in patients with stage 3 sepsis-associated acute kidney injury.

Variables	Failure to survive discharge (OR, 95% CI)	*P*
Age	1.02 (0.98, 1.06)	0.331
Male	1.93 (0.53, 7.02)	0.319
Respiratory failure	3.51 (0.86, 14.43)	0.082
APACHE II	1.17 (1.06, 1.29)	0.001[a]
PCT (High vs. Low)	2.84 (0.79, 10.18)	0.108
Lactate (High vs. Low)	1.75 (0.42, 7.32)	0.442
CRP (High vs. Low)	11.92 (2.85, 49.93)	0.001[a]
SCUBE2 (High vs. Low)	0.04 (0.01, 0.16)	<0.001[a]
IL-1β (High vs. Low)	1.94 (0.49, 7.66)	0.345
IL-6 (High vs. Low)	3.18 (0.8, 12.61)	0.099
MCP-1 (High vs. Low)	3.26 (0.83, 12.8)	0.090
IL-10 (High vs. Low)	0.04 (0.01, 0.21)	<0.001[a]

[a] $P < 0.05$.
Biomarkers dichotomized according to their best predictive cutoff values determined by Youden's index.
Abbreviations: APACHE, Acute physiologic and Chronic Health Evaluation; PCT, procalcitonin; CRP, C-reactive protein; SCUBE2, signal peptide-complement C1r/C1s, Uegf, Bmp1-epithelial growth factor-like domain-containing protein 2; IL, interleukin; MCP-1, monocyte chemoattractant protein-1.

dysfunction.[44,45] Our study uniquely demonstrates that the synergistic protective effects of SCUBE2 and IL-10 were associated with improved survival outcomes, highlighting their potential roles in mitigating systemic inflammation during sepsis and AKI. The potential interplay between IL-10 and SCUBE2 within the endothelial context, and its relevance to other diseases needs further investigation.

This study has some limitations. First, the optimal timing for collecting specimens is during the acute phase of sepsis, when cytokine levels are likely at their highest. However, it's challenging to determine the exact time of sepsis onset. Therefore, we set the sample collection timing at AKI stage 3, which typically corresponds with the patient's condition's peak severity. Second, the secretory source of plasma SCUBE2 remains to be elucidated. Given that sepsis activates systemic immune responses and induces subsequent endothelial dysfunction, the upregulation of SCUBE2 may represent a compensatory mechanism to protect microvasculature against end-organ damage. However, due to the critical condition of the ICU patients, performing kidney tissue biopsies to investigate the intra-renal expression of SCUBE2 was not feasible. Thus, plasma SCUBE2 alone may not suffice as a specific biomarker for distinguishing SA-AKI from other organ dysfunctions. Additional research is warranted to elucidate the cellular sources and detailed molecular mechanisms of SCUBE2 in SA-AKI. Third, we did not compare SCUBE2 with other AKI biomarkers regarding survival outcomes, such as NGAL,



Pairwise P-value for comparison between the subgroups

	Group 1	Group 2	Group 3	Group 4
Group 1	-	-	-	-
Group 2	<0.05	-	-	-
Group 3	<0.01	0.58	-	-
Group 4	<0.001	<0.05	<0.05	-

Figure 4. Kaplan—Meier curves illustrate the cumulative survival of patients stratified by SCUBE2 and IL-10 levels (median cutoff values: 62 ng/mL and 56 pg/mL, respectively). The y-axis presents the probability of survival, whereas the x-axis presents the days after AKI diagnosis. The Wilcoxon test was performed to calculate the pairwise *p* values for multiple comparisons.

KIM-1, urinary TIMP-2 and IGFBP7. Fourth, heterogeneity is inherent in critical care and our single-center design. Despite adjusting for confounders in our analysis, residual bias from unmeasured factors may still exist. In addition, all our patients were Taiwanese individuals, which may restrict the generalizability of our findings to other ethnic populations. Finally, the interaction of inflammatory cytokines with endothelial dysfunction during sepsis is intricate, and the observational nature of our study prevented us from establishing any causal relationship. Despite these limitations, our study might pioneer in unveiling SCUBE2's potential to forecast survival outcomes in SA-AKI. Targeting SCUBE2 and IL-10 may provide a potential strategy to improve survival outcomes for ICU patients.

Conclusions

Among patients with stage 3 SA-AKI, plasma SCUBE2 levels were inversely related to illness severity and pro-inflammatory markers. Higher plasma concentrations of SCUBE2 and the anti-inflammatory cytokine IL-10 correlated with decreased mortality risk. These observations highlight the urgent need for further studies on the role of SCUBE2 in alleviating sepsis-induced immune dysregulation.

CRediT authorship contribution statement

Kuo-Hua Lee: Data curation, Formal analysis, Writing — original draft. **Yuh-Charn Lin:** Investigation, Methodology, Validation. **Ming-Tsun Tsai:** Formal analysis, Methodology, Writing — review & editing. **Cheng-Fen Tu:** Investigation, Validation. **Shuo-Ming Ou:** Data curation, Formal analysis, Methodology, Writing — review & editing. **Huan-Yuan Chen:** Data curation, Investigation, Resources, Software. **Fu-An Li:** Investigation, Validation. **Wei-Cheng Tseng:** Conceptualization, Validation, Writing — review & editing. **Yao-Ping Lin:** Data curation, Methodology, Visualization. **Ruey-Bing Yang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing — review & editing. **Der-Cherng Tarng:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision.

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References

1. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA* 2005;**294**(7):813—8.
2. Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med* 2015;**41**(8):1411—23.
3. Bellomo R, Kellum JA, Ronco C, Wald R, Martensson J, Maiden M, et al. Acute kidney injury in sepsis. *Intensive Care Med* 2017;**43**(6):816—28.
4. Zarbock A, Nadim MK, Pickkers P, Gomez H, Bell S, Joannidis M, et al. Sepsis-associated acute kidney injury: consensus report of the 28th Acute Disease Quality Initiative workgroup. *Nat Rev Nephrol* 2023;**19**(6):401—17.
5. Bagshaw SM, Bennett M, Haase M, Haase-Fielitz A, Egi M, Morimatsu H, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med* 2010;**36**(3):452—61.
6. Ostermann M, Zarbock A, Goldstein S, Kashani K, Macedo E, Murugan R, et al. Recommendations on acute kidney injury biomarkers from the acute disease quality initiative consensus conference: a consensus statement. *JAMA Netw Open* 2020;**3**(10):e2019209.
7. Molinari L, Del Rio-Pertuz G, Smith A, Landsittel DP, Singbartl K, Palevsky PM, et al. Utility of biomarkers for sepsis-associated acute kidney injury staging. *JAMA Netw Open* 2022;**5**(5):e2212709.
8. Molinari L, Landsittel DP, Kellum JA. ProCess, Pro G-AKII. Use of kidney injury molecule-1 for sepsis-associated acute kidney injury staging. *Nephrol Dial Transplant* 2023;**38**(6):1560—3.
9. Honore PM, Nguyen HB, Gong M, Chawla LS, Bagshaw SM, Artigas A, et al. Urinary tissue inhibitor of metalloproteinase-2 and insulin-like growth factor-binding protein 7 for risk stratification of acute kidney injury in patients with sepsis. *Crit Care Med* 2016;**44**(10):1851—60.
10. Yang RB, Ng CK, Wasserman SM, Colman SD, Shenoy S, Mehraban F, et al. Identification of a novel family of cell-surface proteins expressed in human vascular endothelium. *J Biol Chem* 2002;**277**(48):46364—73.
11. Grimmond S, Larder R, Van Hateren N, Siggers P, Hulsebos TJ, Arkell R, et al. Cloning, mapping, and expression analysis of a gene encoding a novel mammalian EGF-related protein (SCUBE1). *Genomics* 2000;**70**(1):74—81.
12. Lin YC, Sahoo BK, Gau SS, Yang RB. The biology of SCUBE. *J Biomed Sci* 2023;**30**(1):33.
13. Ozcan G. SCUBE2 as a marker of resistance to taxane-based neoadjuvant chemotherapy and a potential therapeutic target in breast cancer. *Eur J Breast Health* 2023;**19**(1):45—54.
14. Cheng CJ, Lin YC, Tsai MT, Chen CS, Hsieh MC, Chen CL, et al. SCUBE2 suppresses breast tumor cell proliferation and confers a favorable prognosis in invasive breast cancer. *Cancer Res* 2009;**69**(8):3634—41.
15. Lin YC, Lee YC, Li LH, Cheng CJ, Yang RB. Tumor suppressor SCUBE2 inhibits breast-cancer cell migration and invasion through the reversal of epithelial-mesenchymal transition. *J Cell Sci* 2014;**127**(Pt 1):85—100.
16. Wang X, Zhong RY, Xiang XJ. Reduced expression of SCUBE2 predicts poor prognosis in gastric cancer patients. *Int J Clin Exp Pathol* 2018;**11**(2):972—80.
17. Ottley EC, Pell R, Brazier B, Hollidge J, Kartsonaki C, Browning L, et al. Greater utility of molecular subtype rather than epithelial-to-mesenchymal transition (EMT) markers for prognosis in high-risk non-muscle-invasive (HGT1) bladder cancer. *J Pathol Clin Res* 2020;**6**(4):238—51.
18. Skrzypczak M, Lattrich C, Haring J, Schuler S, Ortmann O, Treeck O. Expression of SCUBE2 gene declines in high grade endometrial cancer and associates with expression of steroid hormone receptors and tumor suppressor PTEN. *Gynecol Endocrinol* 2013;**29**(12):1031—5.
19. Chen Y, Zhou C, Li H, Li H, Li Y. Identifying key genes for nasopharyngeal carcinoma by prioritized consensus differentially expressed genes caused by aberrant methylation. *J Cancer* 2021;**12**(3):874—84.
20. Brilha S, Ong CWM, Weksler B, Romero N, Couraud PO, Friedland JS. Matrix metalloproteinase-9 activity and a down-regulated Hedgehog pathway impair blood-brain barrier function in an in vitro model of CNS tuberculosis. *Sci Rep* 2017;**7**(1):16031.
21. Wu MY, Gao F, Yang XM, Qin X, Chen GZ, Li D, et al. Matrix metalloproteinase-9 regulates the blood brain barrier via the hedgehog pathway in a rat model of traumatic brain injury. *Brain Res* 2020;**1727**:146553.
22. Lin YC, Chang YJ, Gau SS, Lo CM, Yang RB. SCUBE2 regulates adherens junction dynamics and vascular barrier function during inflammation. *Cardiovasc Res* 2024 Jun 13:cvae132.
23. Molema G, Zijlstra JG, van Meurs M, Kamps J. Renal microvascular endothelial cell responses in sepsis-induced acute kidney injury. *Nat Rev Nephrol* 2022;**18**(2):95—112.
24. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016;**315**(8):801—10.
25. Kellum JA, Lameire N, Group KAGW. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care* 2013;**17**(1):204.
26. Zarbock A, Nadim MK, Pickkers P, Gomez H, Bell S, Joannidis M, et al. Sepsis-associated acute kidney injury: consensus report of the 28th Acute Disease Quality Initiative workgroup. *Nat Rev Nephrol* 2023;**19**(6):401—17.
27. Lin YC, Liu CY, Kannagi R, Yang RB. Inhibition of endothelial SCUBE2 (signal peptide-CUB-EGF domain-containing protein 2), a novel VEGFR2 (vascular endothelial growth factor receptor 2) coreceptor, suppresses tumor angiogenesis. *Arterioscler Thromb Vasc Biol* 2018;**38**(5):1202—15.
28. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? *Indian J Psychol Med* 2013;**35**(2):121—6.
29. Del Bufalo C, Morelli A, Bassein L, Fasano L, Quarta CC, Pacilli AM, et al. Severity scores in respiratory intensive care: Apache II predicted mortality better than SAPS II. *Respir Care* 1995;**40**(10):1042—7.
30. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med* 1998;**26**(11):1793—800.
31. Livingston BM, MacKirdy FN, Howie JC, Jones R, Norrie JD. Assessment of the performance of five intensive care scoring models within a large Scottish database. *Crit Care Med* 2000;**28**(6):1820—7.
32. Beck DH, Smith GB, Pappachan JV, Millar B. External validation of the SAPS II, Apache II and Apache III prognostic models in South England: a multicentre study. *Intensive Care Med* 2003;**29**(2):249—56.
33. Kang S, Kishimoto T. Interplay between interleukin-6 signaling and the vascular endothelium in cytokine storms. *Exp Mol Med* 2021;**53**(7):1116—23.
34. Jang HR, Rabb H. Immune cells in experimental acute kidney injury. *Nat Rev Nephrol* 2015;**11**(2):88—101.
35. Groeneveld AB, Raijmakers PG, Hack CE, Thijs LG. Interleukin 8-related neutrophil elastase and the severity of the adult respiratory distress syndrome. *Cytokine* 1995;**7**(7):746—52.

36. Ince C, Mayeux PR, Nguyen T, Gomez H, Kellum JA, Ospina-Tascon GA, et al. The endothelium in sepsis. *Shock* 2016;**45**(3):259—70.
37. Lin YC, Chao TY, Yeh CT, Roffler SR, Kannagi R, Yang RB. Endothelial SCUBE2 interacts with VEGFR2 and regulates VEGF-induced angiogenesis. *Arterioscler Thromb Vasc Biol* 2017;**37**(1):144—55.
38. Raychaudhuri B, Fisher CJ, Farver CF, Malur A, Drazba J, Kavuru MS, et al. Interleukin 10 (IL-10)-mediated inhibition of inflammatory cytokine production by human alveolar macrophages. *Cytokine* 2000;**12**(9):1348—55.
39. Shames BD, Selzman CH, Meldrum DR, Pulido EJ, Barton HA, Meng X, et al. Interleukin-10 stabilizes inhibitory kappaB-alpha in human monocytes. *Shock* 1998;**10**(6):389—94.
40. Wang P, Wu P, Siegel MI, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem* 1995;**270**(16):9558—63.
41. Krakauer T. IL-10 inhibits the adhesion of leukocytic cells to IL-1-activated human endothelial cells. *Immunol Lett* 1995;**45**(1—2):61—5.
42. Chavakis E, Dimmeler S. Regulation of endothelial cell survival and apoptosis during angiogenesis. *Arterioscler Thromb Vasc Biol* 2002;**22**(6):887—93.
43. Short WD, Steen E, Kaul A, Wang X, Olutoye 2nd OO, Vangapandu HV, et al. IL-10 promotes endothelial progenitor cell infiltration and wound healing via STAT3. *FASEB J* 2022;**36**(7):e22298.
44. Gunnett CA, Heistad DD, Berg DJ, Faraci FM. IL-10 deficiency increases superoxide and endothelial dysfunction during inflammation. *Am J Physiol Heart Circ Physiol* 2000;**279**(4):H1555—62.
45. Gunnett CA, Lund DD, Faraci FM, Heistad DD. Vascular interleukin-10 protects against LPS-induced vasomotor dysfunction. *Am J Physiol Heart Circ Physiol* 2005;**289**(2):H624—30.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.07.006.