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H3.1 Nucleosomes to Predict Renal Replacement Therapy and Mortality in Sepsis—A Secondary Analysis of the SISPCT Randomized Control Trial

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OBJECTIVES: The release of neutrophil extracellular traps is a key immune host defense mechanism that can contribute to organ damage during sepsis if excessive. We evaluated presentation H3.1 nucleosome levels in patients with sepsis, septic shock, and sepsis-associated acute kidney injury (AKI). We sought to determine if plasma H3.1 nucleosome levels could serve as predictive biomarkers for both renal replacement therapy (RRT) requirements and 28-day mortality.

DESIGN: A secondary analysis of prospectively collected samples from the large multicenter Effect of Sodium Selenite Administration and Procalcitonin-Guided Therapy on Mortality in Patients with Severe Sepsis or Septic Shock (SISPCT) trial.

SETTING: Patients from 33 ICUs in Germany recruited between November 6, 2009, and June 6, 2013, including a 90-day follow-up period.

PATIENTS: A total of 971 patients with complete data on plasma H3.1 admission levels with sepsis and septic shock, and 927 patients with complete data on RRT requirements.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: We evaluated associations between H3.1 levels and mortality and the time to commencement of RRT using multivariable Cox regression. A total of 443 patients (45.6%) presented with sepsis, and 520 patients (53.6%) had septic shock. Admission H3.1 levels were higher in patients with septic shock than sepsis (median, 921.84 vs. 432.71 ng/mL; $p < 0.001$). In a multivariable analysis for 28-day mortality, a one unit increase in log10 H3.1 levels was associated with a 48% increase in 28-day mortality (adjusted hazard ratio [HR], 1.48, 95% CI, 1.07–2.04; $p = 0.02$). Plasma H3.1 was also higher in patients with stage 3 AKI requiring RRT with septic shock vs. sepsis (1832 vs. 801.4 ng/mL; $p = 0.01$). In a multivariable Cox regression controlling for maximum lactate, procalcitonin, and C-reactive protein levels, plasma H3.1 levels were significantly higher in patients who developed AKI requiring RRT when compared with patients who did not develop AKI, with one log10 increase of H3.1 increasing the risk of 28-day RRT by 80% (adjusted HR, 1.8; 95% CI, 1.34–2.40; $p < 0.001$).

CONCLUSIONS: Elevated levels of H3.1 nucleosomes at admission are associated with mortality and AKI requiring RRT.

TRIAL REGISTRATION: The trial was registered in clinicaltrials.gov, identifier: NCT00832039.

KEYWORDS: biomarker; nucleosomes; renal replacement therapy; sepsis; sepsis-associated acute kidney injury; septic shock

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KEY POINTS

Question: Is H3.1 a marker for prediction of sepsis outcomes and severe sepsis-associated kidney dysfunction?

Findings: Elevated levels of H3.1 nucleosomes at admission are associated with mortality and AKI requiring renal replacement therapy (RRT).

Meaning: H3.1 nucleosomes are a promising novel biomarker for early mortality and severe kidney dysfunction in sepsis, with significantly higher levels found in septic shock than sepsis patients and in stage 3 acute kidney injury (AKI) with need for RRT vs. no AKI. The findings establish H3.1 nucleosomes as an independent predictor of 28-day mortality and need for RRT, suggesting its potential clinical utility for risk stratification, identification of treatable traits and subphenotypes, allowing early intervention in critically ill patients presenting with sepsis.

Sepsis remains a massive global health challenge, affecting approximately 48.9 million people annually and causing 11 million deaths worldwide (1). Sepsis is characterized by a dysregulated host response to infection (2), frequently leading to acute kidney injury (AKI). An estimated 19–33% of intensive care patients develop sepsis-associated AKI (SA-AKI) (3, 4). Although no universal definition of SA-AKI exists, we followed the international consensus definition of the presence of both sepsis-3 criteria and AKI using Kidney Disease Improving Global Outcomes (KDIGO) criteria (5). SA-AKI complication significantly increases mortality from 30% to 45% (6) and exceeds 60% when renal replacement therapy (RRT) is required (7). Long-term outcomes are equally concerning, with 20–30% of SA-AKI survivors progressing to chronic kidney disease (CKD) within 3–5 years (8). Early identification of SA-AKI and contributing to the understanding of AKI could allow for more targeted interventions and improved outcomes. These challenges underscore the urgent need for novel biomarkers to identify and characterize subtypes of sepsis, sepsis trajectories, and sepsis-associated organ dysfunction more accurately.

Neutrophil extracellular traps (NETs) were first reported in 2004 (9). Plasma H3.1 nucleosome levels are reported as a general marker of NETosis, and it has emerged as a potential biomarker candidate (10, 11). There has been growing interest in the role of extracellular histones and nucleosomes in the pathophysiology of sepsis (12, 13). During the innate immune response, histones are released extracellularly, either freely or within nucleosomes, functioning as damage-associated molecular patterns. These molecules exhibit cytotoxic and proinflammatory properties, contributing to endothelial dysfunction, coagulation abnormalities, immunothrombosis, and organ injury (14). Elevated circulating histone levels correlate with disease severity in meningococcal sepsis (15). Experimental studies demonstrate that histone injection induces AKI in animal models (16). The pathogenic mechanisms include direct renal tubular toxicity, pro-inflammatory cytokine induction, and promotion of intravascular coagulation (17).

Recent research indicates that nucleosomes may serve as more stable and specific sepsis biomarkers than free histones, with documented correlations between nucleosome levels and disease severity and mortality (18). The role of H3.1 nucleosomes in patients presenting with sepsis and SA-AKI has not yet been studied in a large population of critically unwell patients. This study used samples from the Effect of Sodium Selenite Administration and Procalcitonin-Guided Therapy on Mortality in Patients With Severe Sepsis or Septic Shock (SISPCT) randomized clinical trial to evaluate the potential of H3.1 nucleosome clinical utility as a biomarker for prediction of sepsis outcomes and severe SA-AKI with need for RRT.

MATERIALS AND METHODS

Aims

This analysis has four aims:

1) To quantify and evaluate the admission H3.1 nucleosome levels in patients with sepsis and septic shock.
2) To quantify and evaluate the admission H3.1 nucleosome levels in patients with stage 3 AKI requiring RRT and patients without AKI.
3) To investigate the admission plasma H3.1 nucleosome levels as a biomarker for prediction of RRT.
4) To determine the predictive values of plasma H3.1 nucleosome levels between admission and 28-day mortality.

Study Design and Study Population

We conducted a secondary analysis of circulating H3.1 nucleosomes in plasma samples collected from patients with sepsis and septic shock who participated

in the multicenter, randomized, bifactorial, prospective SISPCT trial (ClinicalTrials.gov identifier, NCT00832039) (19). The study design and population have been described previously in detail, and full eligibility criteria are provided in the **Tables S1 and S2; Figures S7, S8 and S10**; and **Supplemental Material** (https://links.lww.com/CCM/I26) (19). The trial was conducted from November 6, 2009, to June 6, 2013, and included a 90-day follow-up period. This secondary analysis from the SISPCT trial was accepted by the ethical committee of Friedrich-Schiller University Jena, Germany, on April 4, 2023 (registration number: 2023–2937–Material).

Blood Sample and Data Collection

Blood samples were collected on days 0–10 and on day 14 after randomization if the patient was still in the ICU. Citrate plasma samples were bio-banked in the Integrated Biobank Jena and stored at −80°C. All relevant data on demographics, comorbidities such as the Charlson Comorbidity Index, laboratory findings, diagnostic tests, and organ support in critically ill ICU patients (mechanical ventilation, administration of vasopressors, and RRT) and time of admission or transfer were extracted, reviewed, and recorded from the hospital database. Overall survival outcome was assessed for up to 90 days. In case of an earlier hospital discharge, patients received a follow-up call from study personnel as agreed on enrollment.

Nucleosome Measurements

According to the manufacturer's instructions, H3.1 nucleosome concentrations were measured in frozen citrate plasma samples using Nu.Q NETs immunoassay (CE-IVDD, Belgian Volition SRL, Isnes, Belgium). The instructions are predetermined before the study and are summarized in **Appendix A** (https://links.lww.com/CCM/I26).

Definition of Sepsis, Outcomes, and Acute Kidney Injury

Because the study was performed between 2009 and 2013, the primary sepsis definition was based on the Sepsis-2 criteria with severe sepsis, defined as a proven or suspected infection in combination with at least 2 Systemic Inflammatory Response Syndrome criteria and septic shock, defined as persistent hypotension (mean arterial pressure below 60 mm Hg, a systolic blood pressure below 90 mm Hg, or a decrease in systolic blood pressure of at least 40 mm Hg) despite adequate fluid resuscitation.

For this secondary analysis, sepsis was defined as an acute infection-related change in the Sequential Organ Failure Assessment (SOFA) score greater than or equal to 2 points and septic shock as persistent hypotension requiring vasopressors to maintain a mean arterial pressure greater than or equal to 65 mm Hg plus hyperlactatemia greater than 2 mmol despite adequate fluid administration in accordance with the Sepsis-3 criteria (2).

We reported 28-day mortality and the need for RRT within 28 days as a time-dependent outcome. AKI stages were defined according to the KDIGO guidelines (20) based on an increase in serum creatinine and a decrease in urine output. A priori, patients with end-stage renal disease who were already established on RRT before admission were excluded from the analysis regarding AKI. We did not discriminate between continuous and intermittent modes of RRT, and participating hospitals delivered RRT using either continuous venovenous hemodialysis or intermittent RRT. We considered RRT an objective endpoint for severe AKI. RRT was initiated based on absolute indications: severe electrolyte disturbances, persistent metabolic acidosis, fluid (diuretics resistant) overload, or severe uremia and subsequent complications based on individual clinician and unit practice.

Statistical Analysis

Distributions of each variable were reviewed as per the SISPCT trial statistical analysis plan (19). Admission H3.1 nucleosome levels were compared in sepsis and septic shock groups, in AKI (mild, moderate, and severe), no AKI groups, and across baseline characteristics. Continuous numerical variables were summarized using median values, and categorical variables as counts and percentages of total. The Mann-Whitney *U* test was used to determine whether there was a difference between the distribution of values for patients who had septic shock vs. sepsis.

Scatter plots and correlation analyses were undertaken to explore the relationship between admission

TABLE 1.
Baseline Demographics and Clinical Characteristics

Characteristics	Median (First Quartile–Third Quartile)	Sepsis ($n = 443$)	Septic Shock ($n = 520$)	p
Demographics				
Age	68 (57–75)	67 (56–74)	67 (58–76)	0.1264
Male, *n* (%)	631 (65)	295 (67)	330 (63)	0.3108
Body mass index, kg/m^2	26.47 (23.64–30.71)	27.02 (24.03–30.93)	26.12 (23.43–30.11)	0.0957
Charlson-Deyo comorbidity index	2.0 (1.0–4.0)	2.0 (1.0–4.0)	2.0 (1.0–4.0)	0.2187
Total SOFA score	10 (8–12)	9 (7–11)	10 (8–13)	< 0.001
SOFA subscores				
Circulatory	4 (3–4)	4 (3–4)	4 (4–4)	< 0.001
Pulmonary	3 (2–3)	3 (2–3)	3 (2–3)	0.002
Coagulation	0 (0–1)	0 (0–0)	0 (0–1)	< 0.001
Liver	0 (0–1)	0 (0–1)	0 (0–1)	< 0.001
Neurologic	0 (0–2)	0 (0–2)	0 (0–2)	0.6431
Renal	1 (0–3)	1 (0–2)	1 (0–3)	< 0.001
Simplified Acute Physiology Score II	61 (54–70)	59 (50–68)	63 (56–72)	< 0.001
Acute Physiology and Chronic Health Evaluation II	23 (19–28)	22 (17–26.5)	25 (20–30)	< 0.001
Pao_2/Fio_2, mm Hg	160 (110–235)	170 (120–246.8)	153 (104–218)	0.0008
Admission laboratory parameters				
Hemoglobin, mmol/L	5.84 (5.15–6.89)	5.84 (5.15–6.90)	5.82 (5.15–6.81)	0.7583
Platelets, 10^9/L	193 (124–288)	224 (155–324)	169 (107–245)	< 0.001
White cell count, 10^6/L	15.15 (10.5–21.2)	15.2 (10.6–20.85)	15.3 (10.2–21.8)	0.9784
Urea, mmol/L	9.296 (5.81–14.91)	8.134 (5.00–13.45)	10.126 (6.47–15.60)	< 0.001
Creatinine, µmol/L	114.92 (79.6–185.6)	97.24 (70.7–159.1)	140.72 (88.6–194.9)	< 0.001
Albumin, g/L	19 (15–23)	20 (16–24)	19 (15–23)	0.0109
Bilirubin, mg/dL	11.97 (7.9–22.2)	10.26 (6.8–18.8)	14.4 (8.6–27.4)	< 0.001
C-reactive protein, mg/L	227 (155–305)	223.5 (147–304)	229 (161–307)	0.2954
Procalcitonin, µg/L	6.67 (1.57–26.0)	2.60 (0.79–9.3)	13.17 (4.6–42.4)	< 0.001
Admission H3.1 levels, ng/mL	629.51 (257–1595)	432.71 (184–963)	921.84 (369–2394)	< 0.001
ICU admission sources (3), *n* (%)				
Emergency medicine	412 (42.4)	179 (40.4)	231 (44.4)	
Emergency surgery	449 (46.2)	201 (45.4)	242 (46.5)	
Elective surgery	110 (11.3)	63 (14.2)	47 (9.0)	
Cardiac arrest	33 (3.4)	6 (1.4)	26 (5.0)	

(*Continued*)

TABLE 1. (*Continued*)
Baseline Demographics and Clinical Characteristics

Characteristics	Median (First Quartile–Third Quartile)	Sepsis (*n* = 443)	Septic Shock (*n* = 520)	*p*
Sources of infection, *n* (%)				
Community acquired	427 (44.0)	172 (38.8)	251 (48.2%)	
Nosocomial ward	312 (32.1)	138 (31.2)	171 (32.9)	
Nosocomial intensive treatment unit	232 (23.9)	133 (30.0)	98 (18.8)	

SOFA = sequential organ failure assessment.
Continuous variables, including duration, are presented as median and interquartile ranges; categorical variables are presented as absolute value and percentage unless otherwise stated.

H3.1 nucleosome levels and other continuous numeric variables. A multivariable Cox proportional hazards regression model was employed against multiple time-to-mortality and time-to-first RRT outcomes within 28 days, and hazard ratio (HR) and 95% CIs were reported adjusting for biologically determined confounders such as maximum lactate levels, C-reactive protein (CRP), and procalcitonin levels. For time-to-event analyses, patients were right censored at hospital discharge or last known follow-up if they were lost to follow-up before the analyzed time point. Subsequently, receiver operating characteristic (ROC) curve analysis was undertaken with area under the curve (AUROC), and its 95% CIs were calculated. Optimal cutoff points were determined by leveraging maximally selected rank statistics using *surv_cutpoint* function from the *survminer* R package and *maxstat* R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) [https://cran.r-project.org/web/packages/survminer/index.html; https://cran.r-project.org/web/packages/maxstat/index.html].

Please see **Appendix E** (https://links.lww.com/CCM/I26) for detailed methodology. All statistical analyses were performed using R 4.1.0 (R Core Team). The Consolidated Standards of Reporting Trials (CONSORT) best practice for trials guidelines for reporting was followed (21).

RESULTS

Study Population

Plasma samples were available for analysis from 971 patients from the SISPCT trial. The baseline characteristics and clinical features are summarized in **Table 1**. In our cohort of 971 critically ill patients, 443 patients (45.6%) presented with sepsis, 520 patients (53.6%) with septic shock, and eight patients' sepsis and septic shock status were unable to be classified due to missing data on lactate levels. The median age of patients was 68 years, and the majority (65%) were male. The sex distribution, median body mass index, Charlson-Deyo comorbidity index, and SOFA scores were comparable between the groups (Table 1).

Admission H3.1 nucleosome levels were higher in patients with septic shock compared with patients with sepsis (median values, 921.84 vs. 432.71 ng/mL; $p < 0.001$) (Table 1). **Table 2** summarizes the comparison of admission H3.1 nucleosome levels between the sepsis and septic shock populations according to a patient's comorbidities. **Table 3** summarizes H3.1 nucleosome levels across complications of sepsis, interventions, and mortality. The highest levels of nucleosomes were detected in the septic shock population across various diagnoses, sources of infection, and hospital admission statuses. Pneumonia, at 29.4%, was the most common respiratory diagnosis, with significantly higher H3.1 nucleosome levels among those affected in the septic shock group vs. sepsis group (1056 vs. 407.7 ng/mL; $p < 0.001$). With regard to AKI, the highest admission H3.1 levels were determined in AKI 3 RRT groups compared with patients without AKI.

Correlation of H3.1 Levels With White Cell Count (WCC) and Organ Severity Scores

Admission H3.1 nucleosome levels showed a weak, statistically significant positive correlation with white cell

TABLE 2.
Admission H3.1 Levels Across Comorbidities

Characteristics	Total, *n* (%)	Median (First Quartile–Third Quartile) Admission H3.1 Values, ng/mL		*p*	Sepsis-2 Definition, Number of Patients, *n* (%)		Missing
		Sepsis	Septic Shock		Sepsis	Septic Shock	
	971	432.7	921.8	< 0.0001	443	520	8
Comorbidities							
Respiratory	392 (40.4)	405.3 (177.3–956.1)	1041 (353.3–2725)	< 0.001	182 (41.1)	209 (40.2)	1
Neoplasms	290 (29.9)	475.5 (196.7–841.6)	888.3 (323.9–2062)	0.0004	127 (28.7)	162 (31.2)	1
Circulatory	282 (29.0)	462.6 (177.4–1063)	1066 (397.4–2038)	< 0.001	136 (30.7)	143 (27.5)	3
Neurologic	123 (12.7)	334.7 (176.7–740.6)	971.4 (367.3–2667)	0.0009	58 (13.1)	65 (12.5)	0
Rheumatological	9 (0.9)	201.1 (177.9–224.3)	1120 (365.3–1784)	NA	3 (0.7)	6 (1.2)	0
Gastrointestinal	188 (19.4)	637 (171.1–1335)	1081 (401–2613)	0.0068	67 (15.1)	121 (23.3)	0
Genitourinary	90 (9.3)	460.2 (312.8–852)	1143 (485.4–2457)	0.0097	32 (7.2)	57 (11.0)	1
Gynecological or obstetric	13 (1.3)	330.7 (184.8–389.3)	2489 (448.3–3241)	0.0734	6 (1.4)	7 (1.3)	0
Endocrine and metabolic	247 (25.4)	429.3 (184.2–1041)	1102 (415.2–3532)	< 0.001	108 (24.4)	139 (26.7)	0
Immunocompromised	27 (2.8)	391 (191.4–744.1)	1115 (630.4–3840)	0.008	12 (2.7)	15 (2.9)	0

NA = not available.

counts (WCCs; Pearson correlation coefficient $r = 0.13$ [0.06–0.19]; $p < 0.05$), indicating likely independence between NET levels and WCCs. Further correlation analysis demonstrated a weak positive association between admission H3.1 nucleosomes and organ severity scores such as Acute Physiology and Chronic Health Evaluation II, SOFA, and Simplified Acute Physiology Score II, and similarly with procalcitonin, CRP, and admission lactate (**Fig. S1** and Table S1, https://links.lww.com/CCM/I26).

28-Day Mortality

Overall, 73 patients (16.5%) out of 443 died in the sepsis group and 107 patients (20.6%) out of 520 died in the septic shock group within 28 days. For 90-day mortality, 115 deaths (26%) out of 443 were observed in the sepsis group and 175 patients (33.7%) out of 520 in the septic shock group.

In a multivariable Cox model, log-10 transformed admission H3.1 levels were significantly associated with 28-day mortality outcome (HR, 1.86; 95% CI, 1.41–2.47; $p = 0.019$; **Fig. 1**). The 28-day mortality analysis adjusting for log10 thrombocytes, leukocytes, maximum lactate levels, procalcitonin, and CRP levels revealed H3.1 levels as the strongest predictor (adjusted HR, 1.80; 95% CI, 1.35–2.41; $p = 0.019$) with high platelet counts protective (adjusted HR, 0.33; 95% CI; 0.22–0.48; $p < 0.001$; **Table 4**).

Analysis of the ROC curves for five biomarkers: admission H3.1 levels, WCC, maximum lactate, CRP, and procalcitonin, revealed varying predictive capabilities for survival outcomes across different time points (**Figs. S2–S6**, https://links.lww.com/CCM/I26).

TABLE 3.
Admission H3.1 Levels Across Organ Support and Mortality

Characteristics	Total, *n* (%)	Sepsis	Septic Shock	*p*
Disseminated intravascular coagulation	128 (13.2)	450.5 (276.2–1260)	1115 (411.5–2631)	0.0232
Cardiogenic shock	26 (2.7)	829.9 (448.7–3318)	1619 (474.3–3113)	0.776
AKI stage: AKI 0	567 (58.4)	385.6 (172.8–833.1)	679.1 (295.8–1562)	< 0.001
AKI 1	67 (6.9)	484.6 (239.4–956.1)	589.3 (310.9–1998)	0.2702
AKI 2	28 (2.9)	469.7 (322.9–880.7)	592.5 (392.5–2235)	0.3943
AKI 3	219 (22.6)	742.4 (388–1821)	1712 (805.7–4384)	0.0006
AKI status unknown	90 (9.3)	NA	NA	NA
Renal replacement therapy	254 (26.2)	801.4 (406.4–2518)	1832 (674–4297)	0.0102
P/F ratio: > 40 kPa	138 (14.2)	285.7 (158.6–611.6)	647.8 (293.5–1371)	0.0017
P/F ratio: 26.7 kPa < to ≤ 40 kPa	201 (20.7)	396.3 (153.2–966.6)	646.7 (295.8–1522)	0.0044
P/F ratio: 13.3 kPa < to ≤ 26.7 kPa	436 (44.9)	465.5 (185.1–961.3)	921.6 (377.5–2442)	< 0.001
P/F ratio: < 13.3 kPa	196 (20.2)	540.1 (256.9–1356)	1306 (557.6–3435)	< 0.001
Noninvasive ventilation	63 (6.5)	475.5 (266.3–1798)	868.7 (356–2843)	0.5129
Invasive mechanical ventilation	693 (71.4)	453.9 (176.6–985.9)	1023 (402.8–2613)	< 0.001
Survival outcome				
Critical care mortality	170 (17.5)	665 (312.1–1458)	1201 (506.9–3200)	0.0144
Hospital mortality	234 (24.1)	699.8 (327.6–1956)	1118 (439.5–3011)	0.0519
28-day mortality	180 (18.5)	699.8 (336.5–2071)	1133 (477.1–3200)	0.0672
90-day mortality	290 (29.9)	610.3 (271.6–1478)	1084 (403.6–2547)	0.0042

AKI = acute kidney injury, NA = not available.

The biomarkers demonstrate modest discriminatory power, with areas under the curve (AUC) ranging from 45% to 62%. Maximum lactate showed comparable performance, with its highest AUC of 61.02% (95% CI, 51.18–70.86%) observed at day 7, followed by sustained performance through subsequent time points.

SA-AKI Outcomes

Of the 971 patients with plasma samples available, 33 patients were excluded a priori due to preexisting CKD in receipt of regular intermittent hemodialysis, and 11 patients had missing RRT time series data, leaving 927 patients eligible for the 28-day RRT analysis. In this cohort of 927 patients, 314 patients developed AKI (n = 67 stage 1, n = 28 stage 2, and n = 219 stage 3, of whom 222 patients received RRT-216 required continuous RRT, and six patients received intermittent dialysis). A total of 567 patients did not develop AKI. After removing all cases with missing values for H3.1 nucleosomes, platelets, and urine output, a complete case analysis is available for 831 patients, of whom 198 received continuous RRT, six patients received intermittent dialysis, and 627 received no RRT (**Fig. S9** CONSORT diagram, https://links.lww.com/CCM/I26). H3.1 levels above 2500 ng/mL were associated with a consistently elevated risk of RRT, with hazard ratios ~3.5 to 4.0 at 7, 14, 28, and 90 days (**Fig. 2**).

In a multivariable Cox model adjusting for H3.1 levels along with thrombocytes, creatinine, leukocyte levels, maximum lactate levels, procalcitonin, and CRP levels, log-10 transformed admission H3.1 levels were significantly associated with time to first RRT (HR, 1.801; 95% CI, 1.34–2.41; $p < 0.05$).

On ROC analysis for RRT, admission H3.1 levels alone demonstrated moderate discriminatory performance with an AUC of 70.38% (95% CI, 66.13–74.64%). When compared with other individual biomarkers, H3.1's performance was comparable but not superior to traditional markers such as serum creatinine and urine output, although these variables were part of

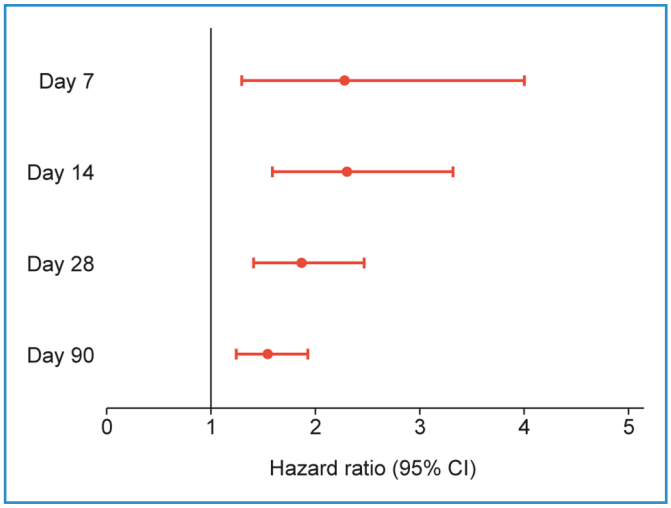

Figure 1. Association between log-transformed H3.1 nucleosome levels at admission and mortality risk over time in sepsis patients. Forest plot demonstrating the hazard ratios (HRs) with 95% CIs for mortality at days 7, 14, 28, and 90 in relation to admission H3.1 nucleosome levels (log10-transformed) in sepsis and septic shock patients. The analysis shows the strongest association between H3.1 levels and early mortality (day 7: HR, 2.28; 95% CI, 1.30–4.01; day 14: HR, 2.30; 95% CI, 1.59–3.32), with a gradual attenuation over time (day 28: HR, 1.86; 95% CI, 1.41–2.47; day 90: HR, 1.54; 95% CI, 1.24–1.93). All associations remained statistically significant ($p < 0.05$) throughout the 90-day follow-up period. The raw data are shown in **Table S3** (https://links.lww.com/CCM/I26).

the AKI definition. Initial creatinine level showed the strongest individual predictive capability with an AUC of 78.31% (95% CI, 75.01–81.6%), while initial lactate level (AUC, 67.65%; 95% CI, 63.56–71.74) and CRP (AUC, 54.39%; 95% CI, 49.79–58.98) demonstrated lower predictive value (**Fig. S11** https://links.lww.com/CCM/I26).

DISCUSSION

In this secondary analysis of the SISPCT trial with 971 patients, we demonstrated that admission H3.1 nucleosome levels have prognostic value in patients with sepsis and septic shock, particularly for 28-day mortality and need for RRT. This is the first observation from a large multicenter clinical trial evaluating the role of H3.1 in sepsis and SA-AKI.

NETs are essential to pathogen clearance. In excessive formation, NETs are able to encourage further destructive signaling through interaction with other tissue components and the immune system (22). Antimicrobial histones, constituents within the NETs structure, impose a direct cytotoxic effect on tissues (23). To date, there have been numerous accounts of NETosis as "inflammatory cell death mechanisms" being present in diseases of major organs (22). First, we observed significantly elevated H3.1 nucleosome levels in patients with septic shock compared with those with sepsis alone across all primary diagnosis categories (Tables 1 and 2). Both preclinical and observational studies have demonstrated the association between admission thrombocytopenia and mortality during sepsis (24). Immunothrombosis related to NETs has been observed in sepsis-related acute lung injury as well as in AKI (17, 25). Our multivariable Cox model for 28-day mortality demonstrated thrombocytopenia and NETs as statistically and clinically significant predictors with mortality, confirming the earlier hypotheses at a larger scale in real-world sepsis patients.

Similarly, our findings of higher circulating H3.1 nucleosome levels in sepsis and septic shock align with studies in smaller cohorts of critically unwell patients but in a large multicentre cohort of sepsis patients across different diagnosis categories (26).

The involvement of NETosis in different organ injuries and potential therapeutic measures targeting NETosis remain at the forefront of current investigation. The prognostic value of admission H3.1 nucleosome levels was most pronounced in the first days following ICU admission, with a gradual attenuation of effect over time. This temporal pattern suggests that nucleosome levels may be helpful in identifying patients at the highest risk of early deterioration. Notably, patients with very high admission levels (> 20,000 ng/mL) exhibited 100% mortality within 14 days, while those with levels below 1,000 ng/mL had significantly better survival (93% survived). To our knowledge, these are some of the highest measurements of NETs observed in in vivo samples, and this suggests a potential role in using NETs to identify patients requiring more aggressive early intervention. Therefore, NETs not only offer a mechanistic biomarker of organ injury in sepsis and septic shock but also allow a potential treatable trait to phenotype sepsis patients and provide targeted therapy such as extracorporeal NET removal or medications directed at reducing NETosis.

H3.1 nucleosomes demonstrated comparable or superior prognostic performance for 28-day mortality when compared with conventional biomarkers. Although maximum lactate showed a stronger

TABLE 4.
Multivariable Cox Proportional Hazards Model for 28-Day Mortality and Time to First Renal Replacement Therapy Outcomes

Variables	Hazard Ratio	Lower 95% CI	Upper 95% CI	*p*
Time to renal replacement therapy				
H3.1_first_log10	1.8010	1.3487	2.4050	6.68e-05
Thrombocytes_first_log10	0.3263	0.2202	0.4835	2.40e-08
Creatinine_first_log10	23.9897	10.8969	52.8140	2.97e-15
Leukocytes_first_log10	1.7699	1.0132	3.0918	0.0449
Lakmax_log10	1.4999	0.9061	2.4828	0.1149
Procalcitonin_first_log10	1.1055	0.8854	1.3804	0.3759
Crp_first_log10	1.0930	0.6342	1.8837	0.7489
Urea_first_log10	0.7138	0.3613	1.4101	0.3317
28-day mortality				
H3.1_first_log10	1.4763	1.0662	2.0442	0.01897
Thrombocytes_first_log10	0.4617	0.2857	0.7459	0.00159
Leukocytes_first_log10	0.9864	0.522	1.8639	0.96631
Lakmax_log10	1.2989	0.6957	2.4252	0.41167
Procalcitonin_first_log10	1.0427	0.8099	1.3426	0.74556
Crp_first_log10	0.5661	0.3202	1.0008	0.05032

association with 28-day mortality (HR, 2.64 vs. 1.86), H3.1 nucleosomes outperformed CRP and provided independent predictive value. Our analysis using log-10 transformed H3.1 levels offers higher measurement granularity than lactate levels. This increased precision, combined with nucleosomes' unique role in linking cell death and inflammation (independent from lactate synthesis), positions nucleosomes as a novel and complementary tool for risk stratification.

Notably, in contrast to previous studies (26), H3.1 nucleosome levels showed only weak correlation with WCC (Pearson $r = 0.13$), suggesting processes beyond "simple leukocytosis." Similarly, this strengthens the use of NETs as a complementary biomarker. While serum creatinine levels at admission could represent AKI or CKD, the weak correlation with initial creatinine (Pearson $r = 0.09$) but strong association with subsequent RRT suggests nucleosomes may indicate evolving organ dysfunction rather than just current injury. A simple comparison of H3.1 and serum creatinine in AKI prediction leads to a circular conclusion given serum creatinine as a predictor and as an AKI definition, with published literature addressing the shortcomings of using creatinine or urine output (27). For this reason, we decided to evaluate the prediction of RRT as a clear maximal extent of the highest AKI severity using H3.1 levels.

Moderate AUROC of 70.38% of H3.1 for RRT is comparable to urine and blood neutrophil gelatinase-associated lipocalin (NGAL; AUC 0.720 [95% CI, 0.638–0.803] and 0.755 [95% CI, 0.706–0.803], respectively) for prediction of RRT use in critically unwell patients (28). However, the optimal circumstances for whether and when to start RRT remain unclear, and recent studies demonstrated no survival benefit at day 90 in an accelerated renal-replacement strategy vs. standard strategy (29). However, we believe the strengths and utility of H3.1 measurement are not in its role as an AKI biomarker but more in its role as a dysfunction of innate immune response causing organ injury. A study by He et al on 136 SA-AKI patients demonstrated that plasma NET markers may independently predict 28-day mortality in this cohort (30); thus, 28-day mortality may be mediated by NET levels as a marker of organ dysfunction through RRT.

The study has several strengths: First, its large sample size, multicenter design, and comprehensive clinical characterization and extensive exploration

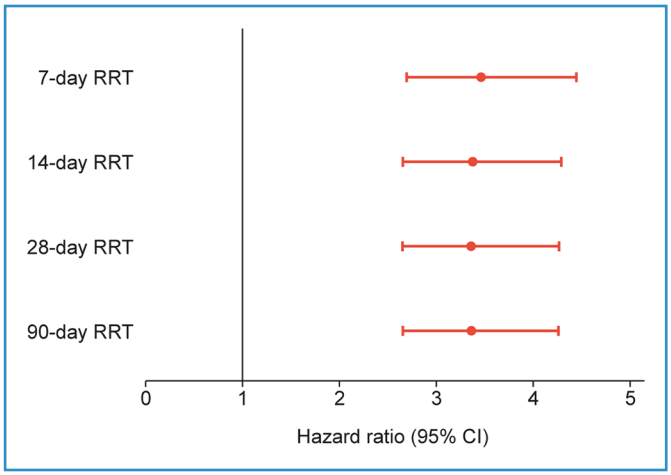

Figure 2. Forest plots of hazard ratios for renal replacement therapy outcomes. Forest plot showing adjusted hazard ratios with 95% CIs for renal replacement therapy (RRT) outcomes at different time points after admission in sepsis and septic shock patients ($n = 831$). The analysis demonstrates consistent hazard ratios of approximately 3.5–4.0 across all time periods (7, 14, 28, and 90 d), indicating that elevated admission H3.1 nucleosome levels (> 2500 ng/mL) are associated with increased risk of requiring RRT. The sustained elevation in hazard ratios suggests that admission H3.1 levels may have prognostic value for predicting RRT requirements throughout the critical care stay. The raw data are shown in **Table S4** (https://links.lww.com/CCM/I26).

with mortality. The use of standardized H3.1 nucleosome measurement and predefined clinical endpoints enhances reliability. However, as a secondary analysis, the findings are hypothesis-generating and require prospective validation. Second, we only analyzed admission nucleosome levels; longitudinal measurements might provide additional prognostic information. Third, while associations with outcomes are clear, the precise mechanisms linking nucleosome levels to organ dysfunction remain to be fully elucidated. Finally, in this study, no other AKI biomarkers were evaluated such as damage biomarkers, for example, kidney injury molecule-1 (31), NGAL or L-type fatty acid-binding protein (32), C–C motif chemokine ligand 14 (7, 33) stress biomarkers, for example, tissue inhibitor of metalloprotease-2 and insulin-like growth factor-binding protein-7 (34), and functional markers, for example, proenkephalin A 119-159 (35), and we thus recommend future studies to evaluate such biomarkers of (sub-)clinical renal damage. Similarly, a large molecular size of H3.1 nucleosomes (molecular size of NETs) means that they are unlikely to be filtered by RRT, potentially allowing utility for patients commenced on RRT.

Our findings have clinical implications; first, the early prognostic value of H3.1 nucleosomes could help identify high-risk patients requiring more intensive monitoring or aggressive intervention. Second, the association with RRT might guide the timing of renal replacement initiation, though this requires prospective evaluation. Additionally, nucleosome levels could serve as a stratification tool for future clinical trials in sepsis and SA-AKI.

Future studies should also evaluate serial nucleosome measurements to characterize their dynamic changes during sepsis progression. Investigating potential therapeutic implications, such as nucleosome-targeted interventions or the timing of RRT initiation based on nucleosome levels, may yield clinical benefits. Additionally, exploring the relationship between nucleosome levels and long-term outcomes, including CKD risk, would be valuable.

CONCLUSIONS

Our study establishes H3.1 nucleosomes as a biomarker delineating organ dysfunction in sepsis, particularly for early mortality and RRT. The clear stratification of risk and independence from conventional markers suggests clinical utility, though prospective validation is needed. These findings advance our understanding of nucleosome biology in critical illness and help inform future therapeutic strategies.

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