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Circulating nucleosomes as a predictor of sepsis and organ dysfunction in critically ill patients

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SUMMARY

Objectives: Sepsis is a leading cause of death in critically ill patients, and apoptosis plays a major role in the pathophysiology of sepsis. Elevated levels of circulating nucleosomes released by apoptotic cells have been detected in patients with severe sepsis and septic shock. The aim of this study was to evaluate the diagnostic/prognostic value of circulating nucleosomes in sepsis.
Methods: Seventy-four newly admitted patients with an estimated length of stay in the intensive care unit of more than 48 h, were prospectively enrolled as cohort 1. The second independent cohort (cohort 2) consisted of 91 post-surgery patients. Patients receiving chemotherapy, those with AIDS, those on steroid treatment, and those undergoing transplants were excluded. Levels of circulating nucleosomes within 24 h of admission in both cohorts, and for cohort 1 also on days 3, 5, and 7 and a last time-point of ICU discharge or at imminent death, were measured and analyzed for their capacity to predict sepsis. The severity of the inflammatory response and organ dysfunction were assessed by cytokine levels and sepsis scores.
Results: Nucleosome levels on admission in septic patients were significantly higher than those in non-septic controls in both of the cohorts. The area under the receiver operating characteristic curve for admission nucleosome levels to differentiate septic patients from non-septic patients was 0.70 (95% confidence interval (CI) 0.51–0.88) in cohort 1, 0.66 (95% CI 0.55–0.79) in cohort 2, and 0.67 (95% CI 0.55–0.79) in all of the subjects. After multiple logistic regression analysis, circulating nucleosomes remained as an independent predictor of sepsis. Furthermore, the levels of circulating nucleosomes on admission were significantly correlated with the inflammatory response and organ dysfunction in sepsis. Meanwhile, a trend was observed for admission levels of circulating nucleosomes in non-survivors to be higher than those in survivors.
Conclusions: The level of circulating nucleosomes in the serum has a predictive value for sepsis and organ dysfunction and may serve as a candidate biomarker for the diagnosis/prognosis of sepsis. Further studies are warranted to confirm the present findings.

1. Introduction

Sepsis is a leading cause of morbidity and mortality in critical illness throughout the world.[1] The pathophysiology of sepsis is complex and remains to be delineated; the host immune response and apoptosis, as well as the coagulation/fibrinolysis system, are involved as key mechanisms.[1,2] Despite recent advances in supportive care and potential therapeutic strategies, the overall mortality rate of patients with septic shock remains high, ranging from 30% to 70%.[3–5] Early screening and diagnosis of sepsis are particularly important for those patients at high risk of sepsis.[6] Currently, useful biomarkers for predicting sepsis are limited to certain molecules, such as procalcitonin (PCT), C-reactive protein (CRP), and soluble triggering receptor expressed on myeloid cells (sTREM-1).[7] Investigators are still searching for new efficacious candidates for the prediction of sepsis.

Nucleosomes are complexes formed by DNA and histone proteins. The core particles of nucleosomes comprise an octamer of the double-represented histones H2A–H2B and H3–H4, surrounded by 146 base pairs of double-stranded DNA. Nucleosomes are mainly released by apoptotic cell death.[8,9] Under physiological conditions, the nucleosomes are packed into membrane-bound

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vesicles and engulfed by macrophages and neighboring cells. In the case of a high rate of apoptosis, these phagocytosing mechanisms are saturated or impaired, leading to higher concentrations of nucleosomes in the circulation.[9,10] Elevated levels of circulating nucleosomes have been detected in various non-malignant and malignant diseases, such as severe sepsis and septic shock, cerebral stroke, systemic lupus erythematosus, and cancer.[9,11–13] Of note, circulating nucleosomes have been demonstrated as a new diagnostic tool for the early estimation of the response to cytotoxic cancer therapy and as a new prognostic marker in early cerebral stroke.[8,12] However, the diagnostic/prognostic value of circulating nucleosomes in sepsis remains unknown.

In this study, we prospectively investigated circulating nucleosomes in critically ill patients, and assessed the potential role of circulating nucleosomes in predicting the incidence and outcome of sepsis.

2. Materials and methods

2.1. Study population

All patients who were newly hospitalized at two teaching hospitals in Zhejiang Province between June and November 2009, who had an estimated length of stay in the surgical intensive care unit (ICU) of more than 48 h, were prospectively enrolled in the study as cohort 1. The diagnosis of sepsis, severe sepsis, and septic shock met the criteria recommended by the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference.[14] Exclusion criteria were age younger than 18 years, advanced malignancy or other conditions resulting in a shortened life expectancy ($\leq$4 weeks), and pregnancy. Patients were also excluded if they were immunocompromised because of treatment with corticosteroids or chemotherapy, receipt of bone marrow or organ transplants, or AIDS.

The subjects in cohort 2 were post-surgery patients who were prospectively included in the study from the surgical ICUs of two hospitals of Zhejiang University from August to November 2011. The inclusion and exclusion criteria were the same as those for cohort 1.

The study was approved by the local institutional review boards and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Informed consent was obtained from all patients or their relatives.

2.2. Data collection

On admission to the surgical ICU, the following items were recorded for each patient: age, sex, reason for admission to the ICU, and principal diagnosis. The acute physiology and chronic health evaluation II (APACHE II) score was obtained within 24 h of admission. The sequential organ failure assessment (SOFA) score was calculated daily. The maximum SOFA score (SOFAmax) was defined as the highest score for the related system reached during the ICU stay and was used to express the worst organ dysfunction status attained during the ICU stay. Results of routine blood tests and microbiological culture were also recorded. Survival or death in the ICU was evaluated during a follow-up period of 28 days. All of the medical records of each patient were reviewed by two senior intensivists who were blinded to the circulating levels of nucleosomes. Agreement on the diagnosis was achieved in all cases.

2.3. Measurement of circulating nucleosomes, CRP, sTREM-1, PCT, and cytokines

Coagulated and ethylenediaminetetraacetic acid (EDTA) anticoagulated venous blood samples were drawn within 24 h of admission in both of the cohorts, and for cohort 1 also on days 3, 5, and 7 and a last time-point of ICU discharge or at imminent death in the non-survivors. The serum samples for nucleosome determination were centrifuged at 3000 *g* for 15 min and treated with 10 mmol/l EDTA (pH 8) immediately after centrifugation. Plasma was isolated by centrifugation at 3000 rpm for 3 min. Both of the serum and plasma samples were aliquoted and stored at −80 °C until further analysis.

The preanalytic handling of the serum samples for nucleosome measurement was performed as previously described.[15] Circulating nucleosome and sTREM-1 levels at each time-point in cohort 1 and the admission levels of these two parameters in cohort 2, and interleukin-6 (IL-6) and interleukin-10 (IL-10) concentrations at each time-point in cohort 1, were assayed using commercially available ELISA kits (nucleosomes: Cell Death Detection ELISA^{PLUS}, Roche, Mannheim, Germany; sTREM-1, IL-6, and IL-10: R&D Systems, Minneapolis, MN, USA). Plasma PCT concentrations on the day of admission in both of the cohorts were measured using an immunoassay technique and a chemiluminescent detection system, in accordance with the manufacturer's protocol (Brahms Diagnostica, Berlin, Germany). Serum levels of CRP on the day of admission in both of the cohorts were detected with the ultrasensitive latex immunoassay CRP Vario (Abbott Diagnostics). White blood cell (WBC) counts in whole blood were quantified routinely by standardized clinical biochemical methods.

Nucleosomes were quantified in arbitrary units (AU) in accordance with the manufacture's instructions. The maximum concentrations of IL-10 and IL-6 were defined as the highest values in the collected samples for each patient in cohort 1.

2.4. Statistical analysis

Data are presented as the median with the range unless stated otherwise. Differences in continuous variables were evaluated with the Mann–Whitney test or the Kruskal–Wallis test with Dunn's multiple comparison test. Differences in categorical data were compared using the Chi-square test and Fisher's exact test. Receiver operating characteristic (ROC) curves were constructed to explore the relationship between the sensitivity and specificity of circulating nucleosomes and sTREM-1, as well as PCT, CRP, and WBC counts, in discriminating patients with sepsis from those without sepsis. A multiple stepwise logistic-regression model was used to evaluate the diagnostic value of circulating nucleosomes for sepsis, which included admission levels of sTREM-1, PCT, and CRP, as well as WBC counts, as covariates. The relationships between circulating nucleosomes and clinical or biological features were assessed with Spearman's correlation test. The statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.00 for Windows (GraphPad Software Inc., La Jolla, CA, USA). A two-tailed *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the study population

During the study period, a total of 477 patients were admitted to the two ICUs. Three hundred and fifty-three patients stayed in the ICU for less than 48 h, 12 patients were younger than 18 years of age, 26 patients were admitted with advanced malignancy or other conditions resulting in a shortened life expectancy ($\leq$4 weeks), and three patients were pregnant; a further nine patients were immunocompromised because of treatment with corticosteroids or chemotherapy. Finally, 74 patients were included in the present study as cohort 1.

The basic characteristics of the study subjects are shown in Table 1. Twenty-nine of the cohort 1 patients (39.2%) did not suffer

Table 1
Patient characteristics

Characteristics	Cohort 1			Cohort 2		
	Sepsis (n=45)	Control (n=29)	p-Value	Sepsis (n=70)	Control (n=21)	p-Value
Age (years)	54 (24–87)	44.5 (20–75)	0.009	59 (20–89)	57 (24–88)	0.75
Sex, n (%)			0.81			1.00
Male	28 (62.2)	17 (58.6)		56 (80)	17 (81.0)	
Female	17 (37.8)	12 (41.4)		14 (20)	4 (19.0)	
APACHE II on admission	19 (7–37)	19 (1–31)	0.79	19 (6–34)	15 (2–30)	0.053
SOFAmax score	7.5 (2–18)	5 (1–18)	0.15	7 (1–23)	3.5 (0–19)	0.0017
Length of ICU stay (days)	8 (2–35)	3 (2–6)	0.0002	12.5 (2–43)	5 (2–11)	0.0019
28-day mortality rate, n (%)	13 (28.9)	2 (6.9)	0.036	21 (30)	4 (19.0)	0.41

Data are expressed as the median (range), or number (%) where indicated. APACHE II, acute physiology and chronic health evaluation II score; SOFAmax, maximum sequential organ failure assessment score; ICU, intensive care unit.

from sepsis during their ICU stay, which mainly resulted from trauma and major surgery. In the sepsis group, multiple trauma (51.1%) was the most frequent initial diagnosis for the septic patients, followed by peritonitis (20%) and severe acute pancreatitis (11.1%). Microbiological evidence showed that 37 of 45 septic patients experienced a documented infection, of whom 22 had a Gram-negative infection, eight had a Gram-positive infection, and seven had a fungal infection. The remaining eight patients had clinically suspected infections as stated by the senior intensivist. The major sources of infection were the respiratory tract (40%) and the bloodstream (33.3%). In addition, out of the 45 patients, 18 (40%) developed sepsis, 16 (35.6%) severe sepsis, and 11 (24.4%) developed septic shock.

Cohort 2 consisted of 91 post-surgery patients; 70 were diagnosed with sepsis and 21 patients served as controls (Table 1). In cohort 2 patients, multiple trauma (25.3%) was the most frequent reason for the surgery, followed by severe acute pancreatitis (20.9%), intestinal obstruction (20.9%), and other reasons (32.9%). In the sepsis group, 37 (52.8%) had a Gram-negative infection, 11 (15.7%) had a Gram-positive infection, and 13 (18.6%) had a fungal infection. The other nine patients had clinically suspected infections as stated by the senior intensivist. The major sources of infection were the invasive vessel (22.8%), the bloodstream (21.4%), the respiratory tract (17.1%), and the abdomen (14.3%). In addition, 34 patients (48.6%) developed severe sepsis and 14 (20%) developed septic shock.

3.2. Circulating nucleosome levels and the incidence of sepsis

Changes in the serum levels of circulating nucleosomes and plasma levels of sTREM-1 in the septic patients and controls in cohort 1 on days 1, 3, 5, 7, and the last time point (discharge from the ICU or imminent death) are shown in Figure 1. In septic patients, the serum levels of circulating nucleosomes showed a trend to decline during the ICU stay, while plasma levels of sTREM-1 remained high. Since only six control patients were still in the ICU on day 3 and only one control patient was still in the ICU on day 6, the results for the change in serum levels of circulating nucleosomes and plasma levels of sTREM-1 may be compromised in this control group.

For evaluating the diagnostic value of circulating nucleosomes, the admission levels of the potential biomarkers were analyzed. For cohort 1, the levels of circulating nucleosomes, sTREM-1, and CRP on admission in the septic patients were significantly higher than those in the controls (Table 2). The capacity of these parameters to differentiate the septic patients from non-septic patients was assessed with a ROC curve analysis. Areas under the ROC curves were 0.70 (95% confidence interval (CI) 0.51–0.88) for nucleosomes, 0.68 (95% CI 0.46–0.89) for sTREM-1, and 0.72 (95% CI 0.56–0.87) for CRP, as well as 0.64 (95% CI 0.45–0.84) for PCT and 0.40 (95% CI 0.20–0.60) for WBC count (Figure 2A). The best cutoff value of circulating nucleosomes for predicting sepsis was 2.09 AU, which had a sensitivity of 64% and a specificity of 76%.

To validate the above findings, we replicated the same analysis in a second independent post-surgery cohort (cohort 2). Compared to the controls, the septic patients showed significantly elevated levels of circulating nucleosomes, sTREM-1, and CRP on the day of admission (Table 2). Areas under the ROC curves for nucleosomes, sTREM-1, CRP, PCT, and WBC count were 0.66 (95% CI 0.55–0.79), 0.67 (95% CI 0.52–0.81), 0.69 (95% CI 0.57–0.81), 0.48 (95% CI 0.34–0.62), and 0.57 (95% CI 0.43–0.71), respectively (Figure 2B). The best cutoff value of circulating nucleosomes for predicting sepsis was 0.78 AU, which yielded a sensitivity of 86% and a specificity of 52%.

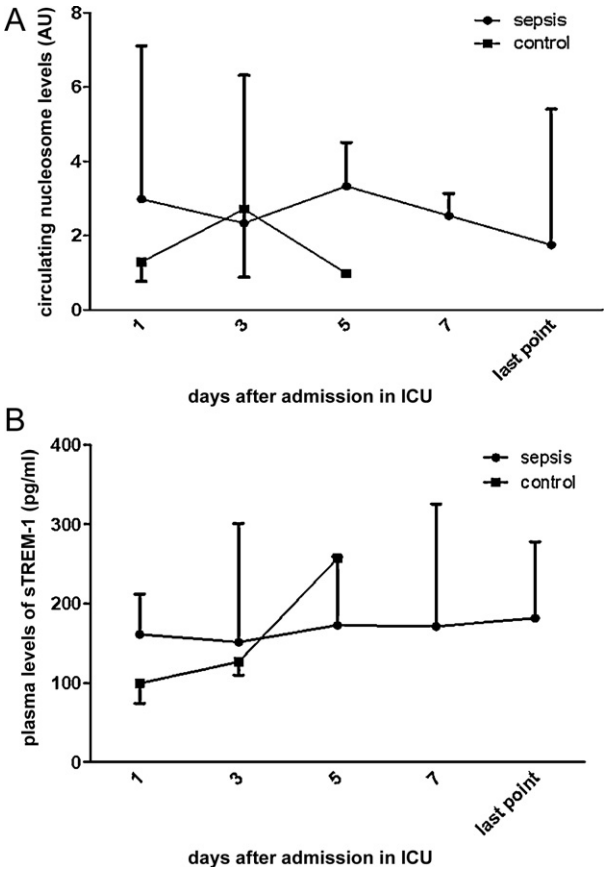

Figure 1. Time course of (A) circulating nucleosome levels and (B) plasma levels of sTREM-1, in septic patients and controls. Data are expressed as the median (interquartile range).

Table 2
Comparison of potential biomarkers on the day of admission for predicting sepsis in the studied cohort

Parameters	Cohort 1			Cohort 2		
	Sepsis ($n=45$)	Control ($n=29$)	p-Value	Sepsis ($n=70$)	Control ($n=21$)	p-Value
Nucleosome (AU)	2.98 (0.30–12.60)	1.29 (0.11–9.86)	0.0028	1.86 (0.40–10.27)	0.78 (0.35–9.69)	0.028
sTREM-1 (pg/ml)	161.30 (60.03–1123.00)	99.42 (37.08–276.30)	0.0003	92.09 (26.31–476.30)	72.60 (16.82–432.80)	0.023
CRP (mg/l)	124.50 (1.89–420.00)	54.46 (12.46–110.80)	0.038	110.20 (7.61–267.00)	54.04 (3.26–161.00)	0.008
PCT (μg/l)	2.24 (0.02–93.60)	1.23 (0.03–4.64)	0.21	0.98 (0.02–100.00)	1.17 (0.04–41.87)	0.80
WBC ($\times 10^9$/l)	13.10 (2.40–35.60)	13.10 (6.20–29.00)	0.75	13.00 (1.20–43.40)	9.70 (3.40–37.40)	0.33

Data are expressed as the median (range). AU, arbitrary units; sTREM-1, soluble triggering receptor expressed on myeloid cells 1; CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cell count.

If the two cohorts were combined, the area under the ROC curve for these variables to differentiate the septic patients from non-septic patients was 0.67 (95% CI 0.55–0.79) for nucleosomes, 0.67 (95% CI 0.55–0.78) for sTREM-1, 0.70 (95% CI 0.60–0.79) for CRP, 0.54 (95% CI 0.43–0.66) for PCT, and 0.52 (95% CI 0.41–0.64) for WBC count (Figure 2C). In a multiple logistic regression analysis, circulating nucleosomes remained an independent predictor of sepsis (odds ratio 4.60, 95% CI 1.62–12.78; $p = 0.004$).

In addition, there was no significant difference in the admission levels of circulating nucleosomes between Gram-negative and Gram-positive sepsis in both of the cohorts (data not shown).

3.3. Circulating nucleosome levels and the inflammatory response in sepsis

The relationship between serum levels of circulating nucleosomes and the immune response was investigated in cohort 1. The changes in levels of cytokines IL-6 and IL-10 over time in septic patients and controls are shown in Figure 3A and Figure 3B; these did not correlate with the evolution of circulating nucleosomes or sTREM-1 ($p > 0.05$). When the admission levels of circulating nucleosomes and the severity of the inflammatory response were analyzed, we found that at a cutoff value of 2.09 AU for circulating nucleosomes, the septic patients with higher levels of nucleosomes

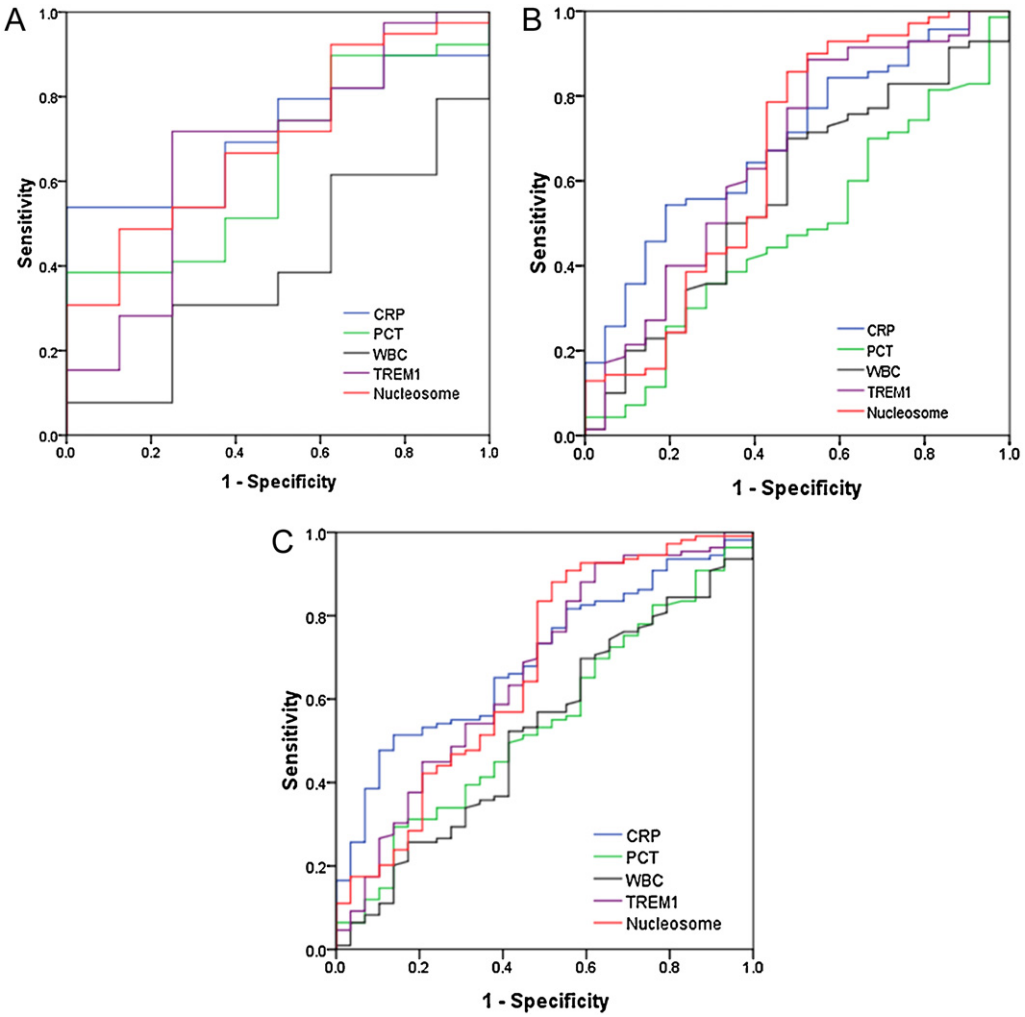

Figure 2. The receiver operating characteristic (ROC) curve for admission levels of circulating nucleosomes, sTREM-1, and CRP, as well as PCT and WBC, in differentiating septic and non-septic patients in (A) cohort 1, (B) cohort 2, and (C) the entire study population.

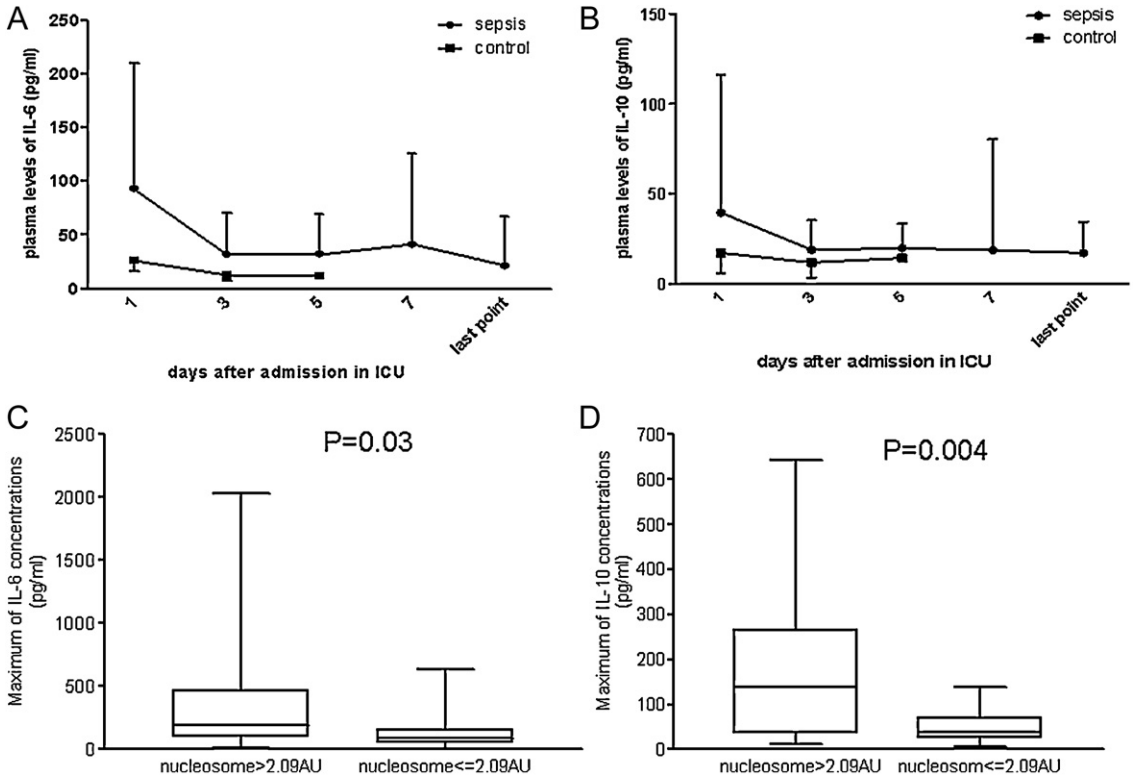

Figure 3. Time course of plasma levels of (A) IL-6 and (B) IL-10 in septic patients and controls from cohort 1, and (C) the maximum IL-6 concentrations and (D) the maximum IL-10 concentrations in septic patients with nucleosome levels higher than 2.09 and those with nucleosome levels lower than 2.09.

experienced a more severe inflammatory response (reflected by the maximum of IL-10 and IL-6 levels at the sampled time-points) than those with lower levels of nucleosomes (median of IL-6 maximum: 194.90 pg/ml vs. 87.64 pg/ml, $p = 0.03$; median of IL-10 maximum: 137.30 pg/ml vs. 37.91 pg/ml, $p = 0.004$; Figure 3C and Figure 3D). However, the admission levels of sTREM-1 were not associated with the cytokine levels.

3.4. Circulating nucleosome levels and the severity/outcome of sepsis

To evaluate the relationship of circulating nucleosome levels and the severity/outcome of sepsis, the two cohorts were combined in the further analysis. The admission level of circulating nucleosomes was associated with the APACHE II score (nucleosome: $r = 0.24$, $p = 0.01$; Figure 4A). The organ function in septic patients was evaluated using the SOFA score. The level of circulating nucleosomes on admission was significantly correlated with SOFAmax during the ICU stay (nucleosome: $r = 0.21$, $p = 0.03$; Figure 4B).

If the septic patients were divided into three subgroups – sepsis, severe sepsis, and septic shock – the admission levels of circulating nucleosomes were comparable among these groups. Furthermore, no statistically significant differences were seen in the levels of circulating nucleosomes when the patients developed sepsis or severe sepsis or septic shock during their ICU stay (data not shown).

The association between the admission levels of circulating nucleosomes and mortality in sepsis was also assessed. There was a trend towards admission levels of circulating nucleosomes in non-survivors being higher than those in survivors (median 2.58 AU vs. 1.97 AU; $p = 0.06$).

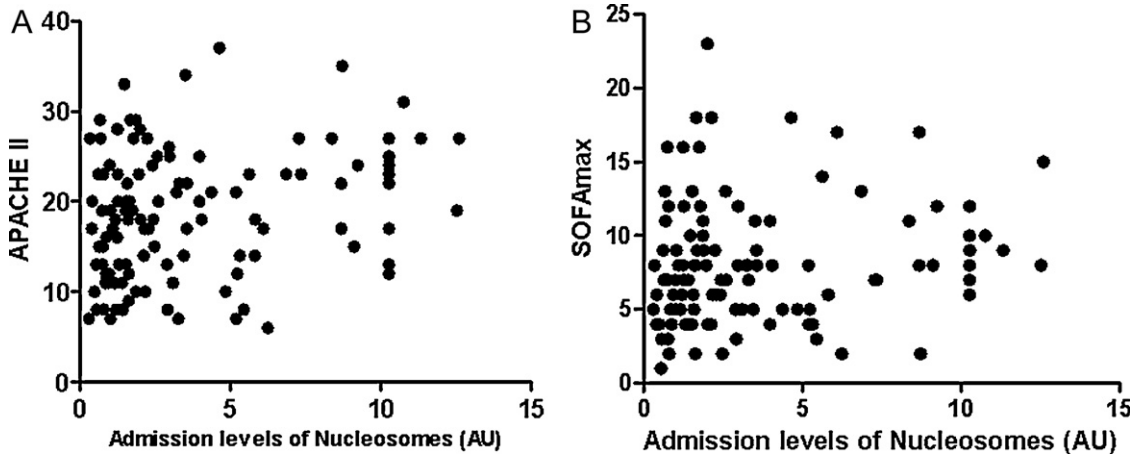

Figure 4. The correlation of circulating nucleosome levels on admission with (A) APACHE II score ($r = 0.24$, $p = 0.01$) and (B) SOFAmax score ($r = 0.21$, $p = 0.03$).

4. Discussion

The current prospective study demonstrated that serum levels of circulating nucleosomes on admission were significantly elevated in septic patients when compared to those in non-septic patients. The area under the ROC curve for circulating nucleosomes to discriminate patients with sepsis from those without sepsis was 0.67 (95% CI 0.55–0.79). Multiple logistic regression analysis confirmed circulating nucleosomes as an independent predictor of sepsis. Furthermore, the higher levels of circulating nucleosomes on admission were significantly correlated with a more immunosuppressive response and worse organ function in septic patients. These findings suggest that circulating nucleosomes may serve as a valuable candidate biomarker for predicting sepsis and organ dysfunction.

Sepsis is thought to be the most noteworthy clinical disorder in which apoptosis occurs.[16] A marked increase in apoptosis has been observed in patients with sepsis compared with critically ill patients without sepsis and healthy controls.[17] Apoptosis presents a major mechanism of nucleosome liberation. This extraordinary apoptosis in sepsis releases numerous nucleosomes, which may overload the nucleosome clearance mechanism, eventually resulting in elevated levels of nucleosomes in the circulation. Hence it was not surprising that septic patients displayed high levels of nucleosomes, which were correlated with the APACHE II score. Furthermore, the apoptosis during sepsis, especially the deletion of T and B cells, not only impairs the adaptive immune response, but also induces anergy and immunosuppression by releasing anti-inflammatory cytokines such as IL-10.[17] Therefore, it was not surprising that septic patients with higher levels of circulating nucleosomes experienced more severe immunoparalysis (as indicted by the maximum of the IL-10 concentrations). In addition, studies have found that non-immune cells such as gut mucosal epithelial cells, lung epithelial cells, hepatocytes, and endothelial cells, exhibit apoptotic changes during clinical and experimental sepsis.[17] This may contribute to the organ dysfunction following the onset of sepsis and explain the correlation between circulating levels of nucleosomes and the severity of organ dysfunction, which was assessed by SOFA score in the current study.

TREM-1 is a member of the immunoglobulin superfamily and is expressed on myeloid cells. sTREM-1 is a soluble form of this protein and is shed from the membrane of activated myeloid cells.[18,19] Studies have shown that sTREM-1 is a new emergent biomarker in infectious and inflammatory diseases such as sepsis.[20,7] Due to the persistently activated myeloid cells and the upregulated expression of TREM-1 on myeloid cells during sepsis, the concentration of sTREM-1 remains at high levels in septic patients. Although the role of elevated levels of sTREM-1 in sepsis remains unclear, a previous study in a mouse model of septic shock showed that the administration of the soluble form of TREM-1 was protective against hyper-responsiveness and death.[21] sTREM-1 may be linked to the blocking of cell surface TREM-1 binding to its endogenous ligand, which eventually lessens the inflammatory reaction. Therefore, plasma levels of sTREM-1 are not associated with the severity of the inflammatory response in sepsis.

There are several advantages to the potential clinical application of detecting circulating nucleosomes. First, circulating nucleosomes are assayed in serum. Apoptosis is characterized by the deformation of the cell membrane, cell shrinkage, chromatin condensation, nuclear fragmentation, and disruption of the mitochondrial transmembrane potential.[16] Assessment of these apoptotic changes is mainly performed in cells. However, apoptosis in sepsis occurs not only in peripheral blood cells, but also in various organs, the latter making measurement of apoptosis unpractical in septic patients. Serum samples are easily obtainable and it has been shown that shaking, rolling, and storing of serum does not impact the levels of circulating nucleosomes.[8] Second, the assay of circulating nucleosomes can be done using commercially available ELISA kits, with high reproducibility. Although quantification of cell-free DNA by real-time PCR is accepted as the standard for the evaluation of apoptosis, measuring circulating nucleosomes with an ELISA technique showed a good correlation with the standard method and has its merits in terms of cost, time, and labor.[22] Third, circulating nucleosomes have the diagnostic capacity to discriminate sepsis from non-sepsis comparable to that of sTREM-1, a well-characterized biomarker of sepsis.[7,23] Efforts have been made in recent decades to identify suitable early markers of sepsis, however only a few biomarkers have been applied in clinical practice. The early elevated circulating levels of nucleosomes in patients newly admitted to the ICU will be very helpful to screen those at high risk of sepsis and to guide physicians in clinical decision-making.

Zeerleder and colleagues reported that compared to patients with fever and systemic inflammatory response syndrome, patients with severe sepsis and septic shock had stepwise elevated plasma levels of nucleosomes.[11] However in Zeerleder's study, the lack of a group of patients only with sepsis made it unclear whether the increased levels of nucleosomes resulted from sepsis or organ dysfunction. Furthermore, a correction for multiple comparisons among the four defined groups was absent in the statistical analysis, which may explain the discrepant findings between the two studies. In addition, Zeerleder et al. used an in-house developed ELISA method to detect the plasma levels of nucleosomes, while a commercial ELISA kit was used to assay the serum levels of nucleosomes in the current study. The different techniques and samples applied may also have contributed to the diverse findings between the two studies. Of note, a trend towards higher nucleosome levels in non-surviving septic patients than in survivors was observed in both studies.

There are limitations in the current study that should be noted. First, previous studies have shown plasma cell-free DNA to be a predictor of mortality in severe sepsis and septic shock.[24,25] Recently, cell-free DNA/neutrophil extracellular traps, as well as extracellular histones, were elaborated to play important roles in the immune defense against bacteria and inflammation.[26,27] Extracellular histones were found to be major mediators of death in sepsis.[27] Combined measurement of plasma DNA, histones, and nucleosomes may provide new insight into the diagnosis and prognosis of sepsis. Limited by the analytical methodology, the current study did not detect histone molecules in the circulation. Second, the levels of circulating nucleosomes were not absolutely quantified, which may hamper the application of this predictor in clinical diagnosis. The third limitation is the small study population, which may compromise the results of the relationship of the nucleosome levels and the severity/outcome of sepsis. A much larger study cohort is required to confirm the present findings and would also allow the prognostic relevance of changes in circulating nucleosomes to be assessed. Further studies addressing the questions raised above will be undertaken.

In summary, the present study demonstrated that plasma levels of circulating nucleosomes on admission could distinguish septic patients from non-septic critically ill patients, and that these levels are correlated with the severity of the immunosuppressive response and organ dysfunction in sepsis. These findings suggest that circulating nucleosomes may serve as a candidate biomarker for predicting sepsis and organ dysfunction.

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