ORIGINAL ARTICLE

Comparison of Serum Nucleosomes and Tissue Inhibitor of Metalloproteinase1 (TIMP1) in Predicting Mortality in Adult Critically Ill Patients in Sepsis: Prospective Observational Study

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Abstract

Background: Sepsis is a life-threatening organ dysfunction due to dysregulated host response to infection. Timely identification is important for risk reduction and better outcomes in critically ill patients. Nucleosomes and tissue inhibitors of metalloproteinase1 (TIMP1) are the biomarkers whose validity and utility in predicting organ dysfunction and mortality in sepsis have been proven. However, which biomarker among these two has better predictive value in elucidating disease severity, organ dysfunction, and mortality in sepsis is yet to be answered, and further studies are needed.

Methods: Eighty patients with sepsis/septic shock, aged between 18 and 75 years admitted in intensive care unit (ICU) were recruited in this prospective observational trial. Quantification of serum nucleosomes and TIMP1 was done using enzyme linked immunosorbent assay (ELISA) within 24 hours of diagnosis of sepsis/septic shock. The primary outcome was to compare the predictability of nucleosomes and TIMP1 in estimating sepsis mortality.

Results: The area under the receiver operating characteristic curve (AUROC) for TIMP1 and nucleosomes to discriminate between survivors and non-survivors were 0.70 [95% Confidence interval (CI), 0.58–0.81] and 0.68 (0.56–0.80), respectively. Although independent, TIMP1 and nucleosomes have statistically significant capacity to discriminate between survivors and non-survivors ($p = 0.002$ and $p = 0.004$, respectively), superiority of one biomarker over the other in discriminating between survivors and non-survivors was not observed.

Conclusion: The median values of each biomarker showed statistically significant differences between survivors and non-survivors, superiority of one biomarker over other in predicting mortality was not observed. However, this was an observational study and larger studies are needed in the future to validate the findings of this study.

Keywords: Mortality, Nucleosomes, Sepsis, Septic shock, Tissue inhibitor of metalloproteinase1.

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Background

Sepsis is a life-threatening organ dysfunction due to a dysregulated host response to infection. A septic shock is characterized by hypotension despite an adequate volume resuscitation and requiring vasopressors for maintaining mean arterial pressure (MAP) more than or 65 mm Hg along with a serum lactate level more than 2 mmol/L (18 mg/dL). The hospital mortality for septic shock is approximately 40%.[1] It is one of the leading etiologies of morbidity and mortality throughout the world.[2]

The recent statistics show that the actual prevalence of sepsis is as high as 6% in hospitalized adults, while the reliable predictions suggest that its future incidence may nearly double in the next 30 years.[3,4] Early identification of sepsis is important as mortality rates as high as 7% per hour delay in diagnosis for the first 6 hour.[5]

Traditionally, blood cultures have represented the only reliable means for establishing bloodstream infection. However, the yield is positive only in 30–40% of the cases and the blood cultures remain sterile in approximately one-third of sepsis cases.[6,7] Long turnaround time is another hindrance in timely detection of sepsis. Due to these limitations, a large armamentarium of biomarkers has been investigated over the past decade, out of the plethora of biomarkers, procalcitonin (PCT) and C-reactive protein (CRP) have been the most commonly used ones, but even these have limited sensitivity and

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specificity to distinguish sepsis from other inflammatory conditions and in predicting prognosis. Biomarkers are the molecules that are objectively quantified and are used as the markers of various processes (biological and pathogenic), or to assess responses to a therapy.[8] Currently, despite the presence of multiple novel and promising biomarkers, very few have been validated for the use in clinical practice with acceptable sensitivity and specificity.

Nucleosomes consists of deoxyribonucleic acid (DNA) and histone proteins. The core is an octamer comprising of histones H2A–H2B and H3–H4, surrounded by double-stranded DNA.[9,10] The whole complex is integrated into vesicles bounded by membrane. This complex is ingested by the macrophages under physiological conditions. In sepsis, the high rate of apoptosis leads to saturation and impairment of phagocytosis mechanism producing higher concentrations of nucleosomes in the circulation.[10,11] Multiple prospective, observational studies have shown that the patients with sepsis and septic shock had raised levels of plasma nucleosomes.[12–14]

Tissue inhibitors of metalloproteinases (TIMPs) are selective inhibitors of matrix metalloproteinases (MMPs).[15] The various studies have shown positive correlation between mortality and elevated levels of TIMP1, TIMP2, and MMPs in sepsis patients.[16–19] Nucleosomes and TIMP1 are the two such biomarkers, whose validity and utility in predicting severity, organ dysfunction, and mortality in sepsis has been proven in multiple previous studies. However, given the paucity of literature answering better biomarker, we planned this prospective, observational study to compare nucleosomes vs TIMP1 in predicting mortality in sepsis.

Methods

After obtaining institute ethics committee approval and written informed consent from participants or their legally acceptable representatives, 80 patients were recruited in this prospective observational study from May 2018 to July 2019. The study was registered in the National Clinical Trial Registry of India (www.ctri.nic.in CTRI/REF/CTRI/2018/05/013770). Patients in sepsis/septic shock, aged between 18 and 75 years of both genders were included in this study. Patients with advanced malignancy, immunocompromised or transplant recipient, radiation therapy, coronary artery disease, and heart failure were excluded.

The patient in sepsis or septic shock on admission to ICU or who went into sepsis or septic shock subsequently in ICU was included in this study and quantification of biomarker was done within 24 hours of diagnosis of sepsis/septic shock. In this study, sepsis was defined using systemic inflammatory response syndrome (SIRS) criteria. The patients were recruited in the study if they met at least two of the following four criteria: Temperature, >100.4°F or <96.8°F; heart rate, >90/min; white blood cell (WBC) count, >12,000/μL or <4,000/μL or with 10% immature/band forms and respiratory rate >20 breaths/min or arterial carbon dioxide tension ($PaCO_2$) <32 mm Hg. In this study, SIRS was chosen over quick sequential organ failure assessment (qSOFA) as studies have shown that qSOFA is more specific but less sensitive than having two of four SIRS criteria for early identification of infection induced organ dysfunction.[20]

Data Collection

Once the patient was recruited in the study, the following baseline characteristics was recorded demographic characteristics (age, gender, and weight), type and source of admission, primary diagnosis, comorbidities, source of infection (clinically), blood culture report (if available), day of ICU admission, day of inclusion in the study, acute physiology and chronic health evaluation II (APACHE II) score, and sequential organ failure assessment (SOFA) score within 24 hours of inclusion in the study. Subsequently, the worst reading of APACHE II and SOFA score, TLC and maximum temperature each day was recorded. Also, the duration of vasopressors, culture sensitivity, duration of antibiotics, length of stay (LOS), and total length in hospital were recorded.

Sampling

A 10 mL of venous blood, either from percutaneous route or central line was obtained within 24 hours of the diagnosis of sepsis or septic shock. Whole blood was collected in a covered test tube. After collecting whole blood, it was allowed to clot by keeping it undisturbed at room temperature for 15–30 minutes. The sample was centrifuged at 1,000–2,000*g* for 10 minutes in a refrigerator refrigerated and the clot was separated. The resulting supernatant serum was immediately transferred into a clean polypropylene tube by using a Pasteur pipette. The sample was maintained at 2–8°C while handling. If the serum was not analyzed immediately, it was kept in 0.5 mL aliquots and stored at –20°C. The quantification of the nucleosomes and TIMP1 was done using ELISA (Orgentec ELISA Immunoblot).

Sample Size Calculation and Statistical Analysis

The sample size was calculated based on a study by Nino et al. in which they used a multivariate model to elucidate the role of TIMP1 in predicting mortality in septic patients.[13] Calculating the mean ± Standard deviation (SD) of TIMP1 in the survivor group of 294.8 pg/μL and in the non-survivor group of 497.5 pg/μL and taking power of study to be 80% and alpha error of 5%, 37 cases were required. However, the higher incidence of sepsis associated mortality of 55–65% has been reported in two studies from the Indian subcontinent.[21,22] We assumed a higher overall mortality of 50 ± 5% in our study (as opposed to 12% of the reference study), a sample size of 80 was calculated.

All collected data were tabulated in the Microsoft Excel™ (Microsoft Corp., Redmond, WA). The data was analyzed by using statistical software Stata 14.0. Normality of data was assessed by drawing normal distribution curve. The data were presented as median and inter-quartile range (IQR) for continuous variables and as absolute numbers or percentages for categorical variables (sex, comorbidities, etc.) Wilcoxon rank–sum test was used to compare the values of biomarker between survivor and non-survivor. Categorical data were expressed as frequency and percentage. The receiver operating characteristics (ROC) curves were constructed for serum nucleosomes and TIMP1 as a predictor of mortality and best cut-off values were obtained from Youden's index (Sensitivity + Specificity − 1) for obtaining specificity and sensitivity and to discriminate between survivors and non-survivors. Using appropriate cut-off values, logistic regression analysis was carried out to estimate the odds of mortality. DeLong test was used for comparison between the ROC curve for two biomarkers; $p \leq 0.05$ was considered statistically significant.

Results

In this prospective observational trial, 80 patients were recruited during the study period. The baseline demographic parameters of the patients admitted with sepsis or septic shock are displayed

in Table 1. The mean age of the patients included in the study was 46.1 ± 19.6 years. A total of 51.2% of the enrolled patients were male. Pneumonia, including ventilator associated pneumonia (VAP), community acquired pneumonia (CAP), and hospital acquired pneumonia (HAP), along with abdominal and urosepsis was the predominant diagnosis at the admission contributing 31.2 and 30%, respectively. Postoperative complications, trauma, tropical illness, encephalopathies, and liver disease contributed to the rest of the diagnosis at admission. Septic shock was present in 76.1% of the cases during the inclusion in the study. The mean values of APACHE II and SOFA scores were 17.8 ± 6.9 and 7.9 ± 2.7, respectively.

Comparison of Serum Nucleosomes and Tissue Inhibitor of Metalloproteinase1 in Predicting Mortality

The optimal cut-off point for TIMP1, as determined by ROC curve analysis, was 149.4 pg/μL. Using the above cut-off, sensitivity was 71.4% and specificity was 53.3%. Similarly, the optimal cut-off point for nucleosomes was 215 pg/μL, providing sensitivity and specificity of 71.4 and 57.7% respectively. The AUROC for TIMP1 and nucleosomes to discriminate between survivors and non-survivors were 0.70 (95% CI, 0.58–0.81) and 0.68 (0.56–0.80), respectively Figure 1.

For comparison between the ROC curve for two biomarkers, DeLong test was used. Although independent, TIMP1 and nucleosomes have statistically significant capacity to discriminate between survivors and non-survivors (*p*-values 0.002 and 0.004, respectively), superiority of one biomarker over other in discriminating between survivors and non-survivors was not observed (Table 3). Tissue inhibitor of metalloproteinase1 and nucleosomes sensitivity and specificity calculation with odds predicting death are displayed in Tables 4 and 5. With the cut-off for the biomarkers (as determined by ROC curve analysis), the odds ratio (OR) for predicting death was 2.86 (95% CI, 1.13–7.21) and 3.42 (1.35–8.68) for TIMP1 and nucleosomes, respectively.

Table 1: Baseline demographics of patients (*n* = 80)

Characteristics	*n (%)*
*Age (Mean ± SD)	(46.1 ± 19.6)
Gender	
Male	41 (51.2)
Female	39 (48.8)
Diagnosis at ICU admission	
Pneumonia (HAP/CAP/VAP)	25 (31.2)
Abdominal/urosepsis	24 (30)
Postoperative complications	8 (10)
Encephalopathy	9 (11.2)
Trauma	6 (7.5)
Tropical illness	8 (10)
Liver disease	5 (6.2)
Others*	11 (13.9)
Septic shock	61 (76.2)
Severity score at admission [Mean (± SD)]	
SOFA	7.9 (2.7)
APACHE II	17.8 (6.1)

Data presented as number (percentage) and *mean ± standard deviation.

*Others: Acute liver failure, sickle cell, rheumatic heart disease, interstitial lung disease, systemic lupus erythematosus.

APACHE II, acute physiology and chronic health evaluation II; SD, standard deviation; SOFA, sequential organ failure assessment

The median values of biomarkers were compared between survivors and non-survivors (Table 2). The median value of TIMP1 was significantly lower in survivors than non-survivors [136.9 (84.6–1585.5) pg/μL vs 217.8 (92.6–1352.2), $p = 0.002$]. Similarly, nucleosomes values also showed significant difference between survivors and non-survivors [185.0 (68.0–1721.0) pg/μL vs 345.0 (65.0–1584.2), $p = 0.004$]

Bivariate Analysis

Bivariate analysis was done between biomarkers and the clinical parameters such as temperature, TLC, SOFA, APACHE II, duration

Table 2: Comparison of biomarkers between survivors and non-survivors

	Survivors	*Non-survivors*	
Biomarker	*Median (min-max)*	*Median (min-max)*	*p*
TIMP1 (pg/μL)	136.9 (84.6–1585.5)	217.8 (92.6–1352.2)	0.002
Nucleosomes (pg/μL)	185.0 (68.0–1721.0)	345.0 (65.0–1584.2)	0.004

*Statistically significant when $p < 0.05$; *p* value calculated by two-sample Wilcoxon rank–sum (Mann–Whitney) test. TIMP1, tissue inhibitor of metalloproteinase1

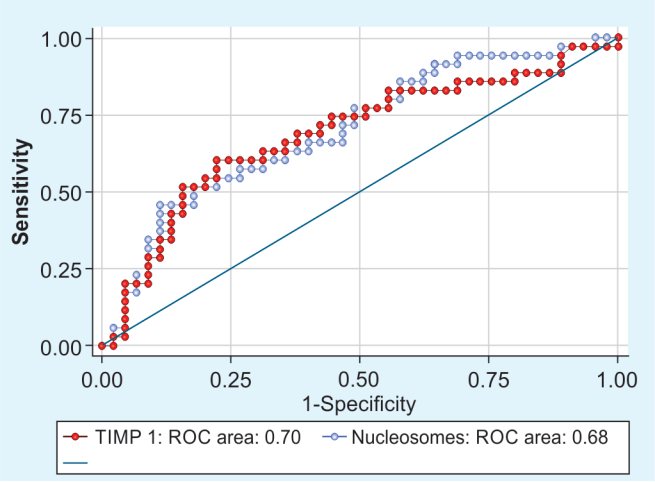

Fig. 1: Comparison of serum nucleosomes and TIMP1 in predicting mortality by an AUC–ROC curve

Table 3: Primary outcome: Comparison of serum nucleosomes and TIMP1 in predicting mortality

Biomarker	*Cut-off*	*Sensitivity*	*Specificity*	*AUC–ROC*	*p*
TIMP1 (pg/μL)	149.4	71.4	53.3	0.70 (0.58– 0.81)	0.69
Nucleosomes (pg/μL)	215.0	71.4	57.7	0.68 (0.56–0.80)	

Sensitivity and specificity expressed as percentage. TIMP1, tissue inhibitor of metalloproteinase1; AUC–ROC, area under curve–receiver operator characteristic curve

Table 4: Tissue inhibitor of metalloproteinase1 sensitivity and specificity calculation with odds predicting death

TIMP1 (pg/μL)	*Survivors*	*Non-survivors*	*OR (95% CI)*	*Sensitivity (95% CI)*	*Specificity (95% CI)*	*p*
≤149.47	24	10	1	71.4 (53.7–85.4)	53.3 (37.9–68.3)	0.026
>149.47	21	25	2.86 (1.12–7.30)			

CI, confidence interval; OR, odds ratio

Table 5: Nucleosomes sensitivity and specificity calculation with odds predicting death

Nucleosomes (pg/μL)	*Survivors*	*Non-survivors*	*OR (95% CI)*	*Sensitivity (95% CI)*	*Specificity (95% CI)*	*p*
≤215.05	26	10	1	71.4 (53.7–85.4)	57.7 (42.2–72.3)	0.009
>215.05	19	25	3.42 (1.33–8.77)			

CI, confidence interval; OR, odds ratio

Table 6: Correlation of biomarkers with clinical parameters

	TIMP1		*Nucleosomes*	
Variables	*R*	*p**	*R*	*p**
Temperature (°F)	0.07	0.52	0.05	0.57
TLC (cells/mm^3)	0.16	0.13	0.14	0.20
SOFA	0.16	0.13	0.08	0.46
APACHE II	0.17	0.13	0.10	0.34
Antibiotics (days)	0.37	0.20	0.05	0.61
Vasopressors (Noradrenaline) (days)	0.27	0.03	0.17	0.18
LOS (days)	0.22	0.04	0.07	0.51

*Statistically significant when $p < 0.05$; APACHE II, acute physiology and chronic health evaluation II; LOS, length of stay; *R*, Spearman's correlation coefficient; SOFA, sequential organ failure assessment

of vasopressors, duration of antibiotics, and LOS. The correlation coefficient and *p* values for each variable is shown in Table 6. For TIMP1, the correlation was found between TIMP1 value and the duration of vasopressor and LOS ($r = 0.27$, $p = 0.03$; and $r = 0.22$, $p = 0.04$, respectively). For nucleosomes, there was no significant correlation between nucleosomes value and the variables.

Comparison of Biomarkers between Variates

A comparison of biomarkers between the variates (such as gender, presence or absence of septic shock, hospital outcome, type, and source of admission culture results) is shown in Tables 7 and 8. None of the mentioned variates showed a significant difference between the level of biomarkers.

Logistic Regression Model for Association between Covariates and ICU Outcome

A logistic regression model was built with a purposeful selection of covariates which showed that the presence of septic shock and positive cultures were statistically associated with ICU mortality (OR = 10.0, 95% CI = 2.1–47.1, $p = 0.001$ and OR = 3.3, CI = 1.25–9.04, $p = 0.014$, respectively).

Independent Predictors for ICU Mortality: Multivariate Logistic Regression Model

Multivariate logistic regression model was constructed by the method of intentional selection of covariates obtaining the end model with four statistically significant variables (culture positive, nucleosomes levels, septic shock, and tropical fever) as the independent predictors for ICU mortality.

Table 7: Comparison of TIMP1 between other variates

Variables	*TIMP1(pg/μL) Median (min-max)*	*p*
Male	146.5 (85.7–1271.5)	0.15
Female	189.0 (84.6–1585.5)	
Septic shock absent	172.0 (84.6–1585.5)	0.50
Septic shock present	171.0 (84.7–1352.2)	
Hospital discharge	217.8 (92.6–1352.2)	0.65
Hospital death	243.0 (125.0–320.5)	
Type of admission		
Medical	172.0 (84.7–1271.5)	0.32*
Surgical elective	160.0 (84.6–1585.5)	
Surgical emergency	207.0 (96.0–789.7)	
Trauma	104.4 (94.6–177.6)	
Source of admission		
Emergency	153.8 (84.6–1585.5)	0.15*
Ward	199.1 (96.0–1271.5)	
OT	789.8 (789.7–789.9)	
Culture positive	199.2 (98.2–1352.2)	0.28
Culture negative	153.8 (84.6–1585.5)	

OT, operation theatre; *p* value derived by Wilcoxon rank–sum test; **p* value derived by Kruskal–Wallis test

Discussion

This study was designed to compare between the two biomarkers, TIMP1 and nucleosomes, in predicting mortality in sepsis. The validity of both biomarkers has been established separately in independent studies on disease severity, organ dysfunction, and mortality in sepsis. The area under the ROC curve for TIMP1 and nucleosomes to discriminate between survivors and non-survivors were 0.70 (95% CI, 0.58–0.81) and 0.68 (0.56–0.80), respectively. Although independent, TIMP1 and nucleosomes have statistically significant capacity to discriminate between survivors and non-survivors ($p = 0.002$ and $p = 0.004$, respectively), superiority of one biomarker over the other in discriminating between survivors and non-survivors was not observed.

In this study, mean and median values of TIMP1 was 309.9 pg/μL and 171.5 ng/mL respectively. Nino et al., in a multicentric prospective cohort analysis, observed the mean value of 320.1 ng/mL.[16] A higher mean value of TIMP1 was observed in another study

Table 8: Comparison of nucleosomes between other variates

Variables	*Nucleosomes (pg/μL) Median (min-max)*	*p*
Male	209.8 (68.0–1263.0)	0.29
Female	248.7 (65.0–1721.0)	
Septic shock present	248.0 (65.0–1584.2)	0.50
Septic shock absent	215.2 (74.0–1721.0)	
Hospital discharge	345.0 (65.0–1584.2)	0.79
Hospital death	302.2 (185.0–396.2)	
Type of admission		
Medical	218.2 (65.0–1473.0)	0.69
Surgical elective	234.2 (85.2–1721.0)	
Surgical emergency	245.0 (65.0–900.1)	
Trauma	162.4 (75.0–321.5)	
Source of admission		
Emergency	218.2 (68.0–1721.0)	0.16*
Ward	218.2 (65.0–1198.7)	
OT	834.0 (767.9–900.1)	
Culture positive	248.5 (65.0–1584.2)	0.83*
Culture negative	215.2 (68.0–1271.0)	

OT, operation theatre; *p* value derived by Wilcoxon rank–sum test; **p* value derived by Kruskal–Wallis test

analyzing TIMPs in plasma obtained from patients with severe sepsis, where the mean value of 429 ng/mL.[17] Similarly, Bojic, et al., in a prospective, observational study, observed a median TIMP1 value of 558.7 ng/mL in their sepsis cohort.[18] Higher mean/median values in their study could be explained by relatively sick patient population in the latter studies, in which the mean values of APACHE II scores were 25.8 and 21.5 which was higher than this study, in which the mean value of APACHE II scores was 17.8. The mean value of nucleosomes in this study was 354.1 ± 367.9 pg/μL. Most of the studies mention the concentration of nucleosomes in terms of units/mL, as the ELISA kits used in them have quantified the values in units/mL.[13,14] So, the direct comparison is not feasible in view of the differences in units between the studies.

The mean value of TIMP1 was lower in survivors than non-survivors (mean ± SD; 233.2 ± 298.6 pg/μL vs 408.5 ± 386.7, $p = 0.002$) in this study. Similar results have been observed in other studies. The mean values for TIMP1 were lower in survivors than non-survivors [294.8 (95% CI, 273.1 ± 316.6 ng/mL) vs 497.5 (95% CI, 411.8 ± 583.2) $p = <0.0001$] in a multicentric prospective study.[16] In a study done recently, the authors noted a significant difference in nucleosome concentrations between survivors and non-survivors ($p = 0.007$).[12]

The optimal cut-off point for TIMP1, as determined by ROC curve analysis, was 149.4 pg/μL which gives the rea under curve–receiver operating characteristic curve (AUC–ROC) of 0.70 (95% CI. 0.58-0.81) to discriminate between survivors and non survivors. Similar to our study, other studies have found significantly elevated levels of TIMP1 in non-survivors.[16,18,19] The ability of TIMP1 to discriminate patient's death within the first 30 days was analyzed and result similar to this study was observed in the study conducted by Nino et al., where AUC–ROC for TIMP1 was 0.68 (95% CI, 61.9–75.6).[16] These findings conclude that there is moderate-to-poor performance of TIMP1 to discriminate between survivors and non-survivors. A marginally better result was obtained in a study by Hoffman, in which ROC curves for TIMP1 measurements confirmed that TIMP1 was a good predictor for outcome (AUC = 0.78; $p < 0.01$).[19] However, the optimal cut-off for TIMP1, as determined by ROC curve analysis, was 3,200 ng/mL, which was significantly higher than the cut-off of 149.4 ng/mL used in this study. The higher cut-off in this study could be explained by the fact that the mean values of APACHE II score was 23 ± 4 which is significantly higher than the mean values of APACHE II score of 17.8 ± 6.9 in our study. Hence, the higher severity of illness could have contributed to the difference in values.

Similarly, the optimal cut-off point for nucleosomes by ROC curve analysis was 215 pg/μL. The AUC–ROC in this study was 0.68 (95% CI, 0.56–0.80) which showed the result that was similar to the TIMP1, in which nucleosomes had a moderate-to-poor ability to discriminate between survivors and non-survivors. Mortality prediction models was utilized by the study by Duplessis to predict 28-days mortality using nucleosomes obtained at baseline in sepsis patients, in which an AUC value of 0.75 (0.62–0.87) was obtained.[12]

In a multicentric prospective cohort study by Nino et al.,[16] they performed a bivariate analysis using logistic regression with a TIMP1 value against survival at 30 days. The OR and *p* value for predicting survival at 30 days were 1.002 and less than 0.001, respectively. The OR for predicting death was 3.42 (95% CI, 1.35–8.68; $p = 0.009$) for nucleosomes. There has been no direct analysis computing OR for nucleosomes in predicting survival and death. However, Chen et al.,[14] in a multiple logistic regression analysis observed circulating nucleosomes to be an independent predictor of sepsis (OR, 4.60; 95% CI, 1.62–12.78; $p = 0.004$).

Bivariate analysis showed significant correlation between TIMP1 and duration of vasopressor as well as that between TIMP1 and LOS ($r = 0.27$, $p = 0.03$ and $r = 0.22$, $p = 0.04$, respectively). A similar finding was observed in study by Bojic,[18] where a significant correlation was found between TIMP1 and duration of vasopressors. Similar to this study, analyses performed by Hoffman[19] and Ashoori[17] also did not find a significant association between TIMP1 and other covariates.

There was no significant correlation between nucleosomes value and the clinical parameters. Chen et al.[14] observed a significant correlation between the admission level of circulating nucleosomes and APACHE II score ($r = 0.24$, $p = 0.01$), but not with SOFA score. This could be explained by a relative higher APACHE II score (mean of 19) in their study compared to mean of 17.8 in this study.

Comparison between the levels of biomarkers with other categorical variables (such as gender, presence or absence of septic shock, hospital outcome, type, and source of admission culture results) was performed using Wilcoxon rank–sum test and Kruskal–Wallis test (depending on the variables in categorical parameter). None of the mentioned variates showed a significant difference between the level of biomarkers.

Multivariate logistic regression with adjusted OR was used to find the independent factors associated with ICU mortality. An end model with four statistically significant variables (culture positive, nucleosomes levels, septic shock, and tropical fever) emerged as the independent predictors for ICU mortality (Tables 9 and 10).

Reviewing across the different studies of biomarkers (TIMP1 and nucleosomes) for discriminating between survivors and non-survivors using AUC–ROC, both TIMP1 and nucleosomes showed moderate-to-poor ability, with both having approximately similar values (0.70 and 0.68, respectively). An ideal biomarker should be characterized by high sensitivity and specificity. Unfortunately, neither of the two markers can predict the outcomes with perfect diagnostic accuracy. The sensitivity and specificity of each of

Table 9: Independent associated factors for outcome using logistic regression

	Outcome (ICU)			
Variables	*Discharge*	*Death*	*Unadjusted OR (95% CI)*	*p*
Male	22 (53.6)	19 (46.3)	0.80 (0.33–1.95)	0.63
Female	23 (58.9)	16 (41.0)		
Septic shock absent	17 (89.4)	2 (10.5)	10.0 (2.1–47.1)	0.001
Septic shock present	28 (45.9)	33 (54.1)		
Type of admission				
Medical	25 (55.5)	20 (44.4)	0.94 (0.59–1.48)	0.18
Surgical elective	8 (66.6)	4 (33.3)		
Surgical emergency	8 (42.1)	11 (57.9)		
Trauma	4	0		
Source of admission				
Emergency	35 (49.3)	24 (40.7)	1.80 (0.81–4.03)	0.29
Ward	10 (52.6)	9 (47.4)		
OT	0 (0.0)	2 (100)		
Culture negative	36 (66.5)	19 (34.5)	3.3 (1.25–9.04)	0.014
Culture positive	9 (36.0)	16 (64.0)		

CI, confidence interval; ICU, intensive care unit; OR, odds ratio; OT, operation theatre

Table 10: Multivariate logistic regression

Variable	*OR (95% CI)*	*p*
Culture positive	3.68 (1.14–11.99)	0.030
Nucleosomes	4.03 (1.31–12.43)	0.015
Septic shock	14.78 (2.30–95.03)	0.005
Tropical fever	11.55 (1.56–85.30)	0.016

CI, confidence interval; OR, odds ratio

these analyzed biomarkers are quite low. However, it is possible that the predictive value might increase significantly when both these biomarkers are analyzed in a combination. The primary objective of this study was to compare nucleosomes and TIMP1 in predicting mortality in sepsis. To the best of our knowledge, this is the first study comparing the outcomes between these two biomarkers. The optimal cut-off was obtained by ROC curve analysis and DeLong test was used for comparison between the ROC curve for two biomarkers. The analysis confirmed that although median values of TIMP1 and nucleosomes have statistically significant capacity between survivors and non-survivors ($p = 0.002$ and $p = 0.004$, respectively), superiority of one biomarker over the other in discriminating between survivors and non-survivors was not observed ($p = 0.69$).

Limitations

This study has some limitations. First, this study involved single-point analysis of biomarkers in a single-center ICU. The changes in the dynamics of the biomarkers with changes in disease severity was not assessed. Also, observation for the values of biomarkers was done only in a single group of patients and there was no control group; hence, confounding variables or bias could not have been effectively eliminated. Second, a larger study population is required to validate the findings of this study and to allow the prognostic relevance of changes in biomarkers to be assessed. Third, we did not exclude various factors that may have influenced the estimation of biomarkers, including the ongoing medical therapies (e.g., heparin-based anticoagulants) and/or postsampling auto-degradation.

Conclusion

To conclude, the two biomarkers, nucleosomes and TIMP1 showed statistically significant ability to discriminate between survivors and non-survivors; however, superiority of one biomarker over other in predicting mortality was not observed.

Author Contributions

Concept and design: KL and KP; Conduct of study: RN, KL, and KP; Statistical analysis: KP; Manuscript writing: RN, KL, and KP; Manuscript editing: KL, KP, SD, SK, ARK, and YSK; Sample processing and analysis: SMK. All authors read and approved the final manuscript.

Availability of Data and Materials

Available on request.

Ethics Approval and Consent to Participate

Institute Ethics approval was obtained from Institute Ethics Committee for Post-Graduate Research, All India Institute of Medical Sciences, New Delhi (Ref No. IECPG-170/23.08.2018, RT-26/07.09,2017, letter dated 18 September 2017; Chairman Prof. SC Tiwari). Informed consent was obtained from the participants or their legally acceptable representatives for participation in the study.

Consent for Publication

Written, informed consent was obtained from the participants or their legally acceptable representatives for publication of the manuscript. Individual consent form is kept by the author and

clinical notes with the institute and these are available on request for review by Editor-in-Chief.

Competing Interests

The authors declare that they have no competing interests.

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