Evolution of serum hyaluronan and syndecan levels in prognosis of sepsis patients

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ARTICLE INFO

ABSTRACT

Objectives: Endothelial glycocalyx shedding has been recognized as a contributor in sepsis pathophysiology. Hence, we attempted to analyze hyaluronan and syndecan (glycocalyx components) as markers of morbidity and prognosis of sepsis by performing serial measurements in these patients.

Design and methods: Subjects were community acquired sepsis, severe sepsis and septic shock patients (150) admitted to ICU of our tertiary care hospital and controls were 50 healthy volunteers. Serum concentrations of markers were measured on days 1, 3, 5, 7 of ICU admission. Survival was assessed after 90 days. Statistical analysis was performed by SPSS version 17.

Results: Hyaluronan and syndecan levels were significantly elevated in all categories of sepsis patients as compared to healthy controls (p < 0.001). Levels of both markers were increased in severe sepsis and septic shock patients as compared to sepsis patient group at all time-points. Hyaluronan and syndecan differentiated survivors from non-survivors (p < 0.001). Unlike non-survivors, in the survivor group, median hyaluronan and syndecan levels decreased significantly (p < 0.001) in subsequent measurements. ROC analysis for the prediction of mortality identified cut-offs of 441 ng/ml and 898 ng/ml for hyaluronan and syndecan respectively. The specificity and negative predictive values were 90% and 90% for hyaluronan and 86% and 91% for syndecan respectively. Kaplan Meier curves revealed similar results. Both markers correlated significantly with APACHE II and SOFA scores.

Conclusions: These observations indicate that serial measurements of hyaluronan and syndecan are significant prognostic markers for morbidity and survival in sepsis. Future therapeutic interventional possibilities need to be explored in experimental interventional prospective multi-centric trials.

1. Introduction

Sepsis is one of the most serious conditions among hospitalized critically ill patients. It contributes significantly to morbidity and mortality, despite improved and more intense critical care management settings and newer modalities of diagnosis and therapy [1,2,3,4]. Refractory hypotension and organ failure remains an area of concern in sepsis patients, reported widely as the major cause of high mortality rates [5,6]. It has been described in experimental studies that along with other pathophysiological events, disruption of vascular endothelium in the vital organs often trigger organ failure [7,8]. The vascular endothelium is lined by a protective latticework on its luminal surface, the glycocalyx. It is known to maintain endothelial permeability, regulating leukocyte migration and inhibiting intravascular coagulation [9,10]. This is comprised of glycoproteins, proteoglycans, glycosaminoglycans and associated plasma proteins. Hyaluronan (a glyocosaminoglycan) and syndecan (a proteoglycan) are important in maintaining endothelial glycocalyx integrity [11,12]. In sepsis, pathogens behave as proteinases, disrupting the linkages in the mesh of glycocalyx proteins that protects the endothelial surface from direct insult. The denuded endothelium probably leads to an increased "leakiness" contributing to organ failure. Few studies have demonstrated a positive correlation of plasma concentrations of these glycocalyx components in sepsis patients early in the course of disease [13,14,15]. However, it is important to determine whether this derangement is just an early transient phenomenon or persists during the course of the disease, especially in severe sepsis and shock, so as to provide relevant information for future diagnostic or therapeutic avenues. Even though one time estimations at the time of admission have been performed in earlier studies, it is important to assess their prognostic value in subsequent estimation so as to enhance management of these critically ill sepsis patients. This information shall be helpful to complement the existing biomarkers such as procalcitonin (PCT) and C-reactive protein (CRP). In the current study, we estimated

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hyaluronan and syndecan in the serum of sepsis patients, taking serial blood samples and correlated them with progression of disease, survival status and organ failure.

2. Materials and methods

2.1. Site of the study and patients

We performed a prospective, cohort study of community acquired sepsis patients, consecutively admitted to the intensive care unit (ICU) of a tertiary care center for a period of two years. Subjects included 50 healthy controls and 150 patients. The latter were screened and identified using the definition of American College of Chest Physicians and Society of Critical Care Medicine and the 2001 International Sepsis Definition [16,17]. Inclusion Criteria were age ≥18 years, diagnosis of sepsis, severe sepsis and septic shock at the time of ICU admission. Exclusion criteria were patients transferred from wards and other ICU's, post-operative cases, immunocompromised patients and those with malignancy. Patients with bilateral pneumonia (suspected viral infection) and diagnosed tropical diseases such as malaria, dengue, leptospira and rickettsia were also excluded. The study protocol was approved by the institutional ethics committee. Written informed consent was obtained from all the patients or their relatives if the patient was unconscious. Enrolled patients were followed up for 90 days and categorized into survivor and non-survivor groups.

2.2. Data collection

Principal diagnosis, data including age, gender, presenting complaints, suspected source of infection, physiologic scores were noted.

Blood samples were obtained on days 1, 3, 5 and 7 from patients and at one time-point from healthy volunteers. Serum levels of hyaluronan and syndecan were measured in all enrolled subjects. Acute Physiologic Assessment and Chronic Health Evaluation (APACHE II) score was calculated from worst values of the parameters within first 24 h of ICU admission [18]. Sequential Organ Failure Assessment (SOFA) scores were calculated on days 1, 3, 5 and 7.

2.3. Sample processing and biochemical assays

Blood samples were obtained in evacuated gel tubes. After collection, samples were allowed to clot and then centrifuged at 1300 g for 10 min at room temperature for the separation of serum. Serum samples were transferred to Eppendorf tubes and stored at −80 °C until analysis.

Concentrations of hyaluronan and syndecan were measured using double antibody sandwich ELISA (TECO medical; Switzerland for hyaluronan, Diaclone; Besancom Cedex, France for syndecan). Samples above the highest detection range of the kit were diluted and rerun as required.

2.4. Data analysis and statistics

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA) version 17. Non-parametric data is presented as median with 25%–75% interquartile range (IQRs). Chi-square followed by proportion test was used to analyze qualitative data (gender distribution, mortality difference). Statistical significance of differences for non-parametric quantitative data was determined using Mann–Whitney U test (two groups) or Kruskal–Wallis (three groups). Wilcoxon signed rank test was applied to compare related sample data. Receiver operating characteristic analysis (ROC) curves were plotted to assess the efficacy of these markers for the prediction of mortality. Youden index was used to calculate best cut off point. At the best cut off point, sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios were calculated. The Kaplan Meier survival curves were generated at all the significant cut offs as per the ROC curves so as to identify the time-point at which these analytes most accurately predicts survival. Log rank test was applied to compare mortality between subgroups. Spearman rank correlation analysis was performed to evaluate the association between scores and markers. p value < 0.05 considered significant and < 0.005 highly significant.

3. Results

3.1. Clinical and demographic characteristics of population

The study included 150 adult patients with community acquired sepsis (n = 15), severe sepsis (n = 45) and septic shock (n = 90). 50 healthy controls were also enrolled in the study. Baseline characteristics, outcome and scores of the patient groups are shown in Table 1. In the overall patient population, subjects were of a median age of 56 years with interquartile range (IQR) 44.5–65 years. The median APACHE II score of the patients was 22 (IQR 15–28). There was no significant difference in the age and gender distribution in the three sepsis groups. Median APACHE II score was significantly different in the three groups with a highest score of 25 in septic shock group. Mortality rates were significantly different between the three groups (p = 0.004) with the highest mortality observed in septic shock (38%). 41 patients died within the 90 days follow-up.

3.2. Comparison of hyaluronan and syndecan levels in study groups at different time-points

Serum levels of hyaluronan and syndecan were significantly elevated in all categories of sepsis patients as compared to healthy volunteers (p < 0.001). This increase was more prominent in severe sepsis and septic shock patients as compared to sepsis patients.

Sepsis versus severe sepsis and septic shock: Levels of hyaluronan and syndecan were found significantly higher till day 5 of sampling when sepsis group was compared with severe sepsis group. On day 7, the difference between sepsis and severe sepsis did not achieve significance. Similarly in septic shock group as compared to sepsis group both

Table 1
Baseline subject characteristics, outcome and scores.

Characteristics	Sepsis	Severe sepsis	Septic shock	Healthy controls	p value
Number	15	45	90	50	–
Gender M/F	8/7	27/18	56/34	30/20	ns[a]
Age (years), Median (IQR)	56 (36–67)	56 (46–63)	56 (44.5–65)	36.1 (23.7–45)	<0.001[a]
Mortality, n (%)	1 (6.66%)	6 (13.3%)	34 (37.7%)	–	0.002[a]
APACHE II, Median (IQR)	10 (8–12)	20 (14.5–24)	25 (20.75–30)	–	<0.001[b]
SOFA (day 1) Median (IQR)	6 (4–8)	8.5 (5–11)	12 (10–14)	–	<0.001[b]

M: male, F: female APACHE II — acute physiology and chronic health evaluation score, SOFA — sequential organ failure assessment score; data is presented as median (interquartile range) or n (percentage) as appropriate. p values are given for comparison of three sepsis groups, p < 0.05 (significant), and p < 0.005 (highly significant).

[a] — χ^2 test.
[b] — Kruskal Wallis test.