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Glycocalyx biomarker syndecan-1 is a stronger predictor of respiratory failure in patients with sepsis due to pneumonia, compared to endocan

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ABSTRACT

Purpose: Endocan, a component of the endothelial glycocalyx (EG), has been linked with respiratory failure in sepsis. This study explored the temporal patterns of three EG biomarkers, including endocan, and their relationships with inflammation and respiratory failure.

Materials and methods: Plasma endocan, syndecan-1, and hyaluronan concentrations were measured in Emergency Department (ED) patients with sepsis due to pneumonia (n = 44) on ED arrival (T0), 1 h (T1), 3 h (T3) and 12–24 h (T24) later, with change over time tested using mixed regression models. Biomarker associations with inflammatory cytokine concentrations and with respiratory failure on days 1, 2 or 3, need for mechanical ventilation and 30-day mortality were also tested.

Results: Endocan concentration significantly decreased over time (T0–T24, $P = 0.003$) whereas both syndecan-1 (T0–T3, $P = 0.010$; T0–T24, $P < 0.001$) and hyaluronan (T0–T1, $P = 0.010$; T0–T3, $P < 0.001$; T0–T24, $P = 0.003$) significantly increased over time. Increased syndecan-1 was significantly correlated with neutrophil activation biomarkers and significantly increased the odds of respiratory failure (OR 1.18, 95% CI 1.05–1.33, $P = 0.004$), need for mechanical ventilation (OR 1.24, 95% CI 1.04–1.48, $P = 0.014$) and 30-day mortality (OR 1.29, 95% CI 1.07–1.55, $P = 0.008$).

Conclusion: Syndecan-1, but not endocan, was associated with neutrophil activation and was the best EG biomarker predictor of adverse clinical outcomes.

1. Introduction

Pneumonia and sepsis are leading causes of respiratory failure in critically ill patients [1]. Respiratory failure is characterized by pulmonary endothelial injury, including margination of leucocytes, hyperpermeability and protein-rich fluid leaking into the pulmonary interstitium [2]. Pro-inflammatory conditions such as sepsis promote shedding of the endothelial glycocalyx (EG), which is a mesh-like complex lining the luminal surface of the endothelium [3-5]. Shedding of the EG prompts endothelial activation and increases circulating concentrations of soluble EG components, such as glycosaminoglycans and proteoglycan fragments. Endothelial activation also upregulates synthesis and secretion of some of these EG components, therefore contributing further to circulating concentrations [6-10]. Additionally, EG components such as endocan and hyaluronan can have intrinsic pro-inflammatory properties and may contribute to organ injury when increased in circulation [7, 11].

Increased circulating EG biomarkers in patients with sepsis are associated with severity of illness [8, 12-15], organ dysfunction [12, 13, 15-18] and mortality [8, 13, 17, 19]. Endocan, in particular, appears heavily expressed in lung tissue [6, 20]. There is some evidence that plasma endocan concentration is positively associated with severity of pneumonia [21], as well as the need for mechanical ventilation for longer than 10 days in patients with Acute Respiratory Distress Syndrome (ARDS) [22]. However, other studies have reported negative associations between endocan concentration and clinical outcomes such as respiratory failure [23, 24]. It is difficult to know why these results are disparate, as timing of blood sampling in relation to time of illness

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onset and admission, and therapeutic intervention, is variable. The time course of endocan concentration in the early stages of sepsis treatment has not been described.

Studies conducted in the Intensive Care Unit (ICU) reporting the most frequently measured EG biomarkers, syndecan-1 and hyaluronan, typically describe decreasing concentrations over time in patients with sepsis [13, 16, 25]. In contrast, previous work by our group found that patients with sepsis had increasing syndecan-1 and hyaluronan concentrations from Emergency Department (ED) admission through the first 24 h [15]. We also identified an association between increasing hyaluronan concentration and fluid volumes administered in the first 3 h of treatment. However, increasing EG shedding early in the treatment of sepsis may also be related to the time course of systemic inflammation. Previous work by our group identified variation in the time course of inflammatory biomarker concentrations, where some biomarkers rapidly decreased while others increased during the first 24 h of treatment [26]. It is possible that the pattern of inflammatory biomarker release may explain some of the variations in EG biomarker shedding.

The aim of this study was to characterize the change over time in plasma endocan concentration in patients with sepsis due to pneumonia, and compare this to patterns of shedding of two other EG biomarkers (syndecan-1 and hyaluronan). Given that we found different patterns of shedding over time between the EG biomarkers in this study, we explored associations with inflammatory and endothelial activation biomarker concentrations to further understand these differences. The secondary aim was to test associations between each EG biomarker and respiratory outcomes in patients with pneumonia, including respiratory failure, need for mechanical ventilation and mortality. Given that endocan is heavily expressed in the lung, we hypothesized that endocan concentration would be the best predictor of respiratory failure, compared to other EG biomarkers.

2. Materials and methods

2.1. Participant selection

Patients meeting criteria for sepsis in the ED were identified from the Critical Illness and Shock Study (CISS) (HREC permit number 2009-080). Written informed consent was gained from the participant or next-of-kin for inclusion in the study. The CISS methodology has been described in detail elsewhere [27]. In brief, CISS is an ED-based observational study of patients presenting to two urban EDs and meeting predefined physiologic criteria consistent with critical illness. Among the CISS cohort, sepsis was defined as having at least 2 of 4 Systemic Inflammatory Response Syndrome (SIRS) criteria [28]; temperature > 38 °C or <36 °C, heart rate > 90 bpm, respiratory rate > 20 bpm or white cell count $>12 \times 10^9$/L or $<4 \times 10^9$/L, as well as clinical suspicion of infection as the primary admitting diagnosis and administration of intravenous antibiotics.

Participants enrolled in CISS from March 2011 to July 2013 with sepsis due to pneumonia were identified from a larger cohort in a previously reported study that measured EG biomarkers over time [15]. Criteria for pneumonia included regional or lobar pulmonary infiltrates present on chest radiographs consistent with acute infection plus one or more of; cough, sputum production, chest pain or shortness of breath. Cases of suspected infective exacerbation of chronic airway disease without localizing radiographic signs were excluded.

Healthy control samples were also utilized from the study previously described [15]. As a part of the original study, these samples were selected based on age and sex-stratification to match the cohort with sepsis.

2.2. Participant data collection

The Sequential Organ Failure Assessment (SOFA) score [29] and CURB-65 score [30] were calculated from parameters collected on the first day of hospitalization. Presence of respiratory failure, as defined by a $Pa0_2/Fi0_2$ (PF) ratio < 300, an $Sp0_2/Fi0_2$ (SF) ratio < 315 or $Sp0_2$ < 90% on >6 L/min supplemental oxygen, was assessed on days 1, 2 and 3 of hospitalization. Mechanical ventilation was defined as the use of invasive positive pressure ventilation at any point during the first 3 days of hospitalization. Length of ICU stay did not include hospitalization in high-dependency wards that provided non-invasive respiratory support. White cell count, C-reactive protein and lactate concentration were retrieved from medical records on the first day of hospitalization. Charlson Comorbidity Score [31] was retrieved from the CISS database.

2.3. Biomarker analysis

Samples collected at ED enrolment included serum and EDTA plasma, which were stored at −80 °C until batched analysis. Plasma endocan was measured using a commercial ELISA kit (Lunginnov, Lille, France). Serum syndecan-1 and hyaluronan concentrations were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) as part of a previous study [15]. Serum inflammatory and endothelial activation biomarker concentrations (interleukin-6, interleukin-10, neutrophil gelatinase-associated lipocalin (NGAL), intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1)) were measured for a previous biomarker study [26] and were available from the CISS database. As an additional inflammatory biomarker, plasma neutrophil myeloperoxidase was measured using a commercial ELISA kit (R&D Systems, Minneapolis, MN). Healthy control samples (n = 28) were available from the previous studies [15, 26] and were included to provide a frame of reference, with data either retrieved from the CISS database or biomarkers (endocan, myeloperoxidase) measured specifically for this study. Assays were performed according to manufacturer's instructions and samples were only used if they had undergone no more than two freeze-thaw cycles.

2.4. Statistical analysis

Normality of data was assessed by visual inspection of histograms and Q-Q plots. Participant characteristics were summarized using number (percentage) for categorical variables, and median [Q1–Q3] or mean (95% confidence interval) for continuous variables depending on normality. Between-group differences for participant characteristics were tested using Student's *t*-test for age, chi-square test for sex and negative binomial regression for Charlson Comorbidity Score.

Endothelial glycocalyx biomarker concentrations were log-transformed to generate normal or approximately normal distributions. For each EG biomarker, change in concentration over time was tested using linear mixed regression models incorporating maximum likelihood estimation. Raw data was summarized using predicted mean (95% confidence interval) generated by the regression model. A sensitivity analysis was performed for each model that included only participants with complete blood sampling to T24, to ensure that dropout of participants prior to T24 did not significantly skew the pattern in change over time.

Associations between EG and inflammatory biomarker concentrations at T0 and T24 were tested using Spearman's rank correlation, with only correlations at rho > 0.5 being considered significant. Associations between EG biomarker concentrations at T0 and respiratory failure on days 1, 2 and 3 were tested using ordinal logistic mixed effects regression models. Where there was no significant interaction between respiratory failure and time (days 1, 2 and 3), a single odds ratio was reported for association across time. Associations between EG biomarker concentrations and mechanical ventilation, as well as 30-day mortality, were tested using logistic regression. Associations with the above clinical outcomes were also tested for both CURB-65 score and lactate concentration as a frame of reference, as these clinical severity surrogates are predictive of mortality in ED patients with pneumonia [32-34].

All analyses were performed using Stata 14 (College Station, TX, USA), with significance set at $P < 0.05$.

3. Results

3.1. Participant characteristics

This study included 44 patients with sepsis due to pneumonia and 28 healthy controls. Participant characteristics are provided in Table 1. There was no significant difference in age ($P = 0.91$) or sex ($P = 0.53$) between patients and controls. Patients had a significantly increased Charlson Comorbidity Score ($P < 0.001$) compared to controls. Research blood samples were available at T0 and T1 for 44 participants, at T3 for 34 participants and at T24 for 24 participants.

3.2. Biomarker concentrations over time

Endocan concentration did not significantly change from T0 in the first 3 h of the study (T0 to T1, $P = 0.09$; T0–T3, $P = 0.32$), however, endocan significantly decreased at T24 (T0–T24, $P = 0.003$)(Fig. 1). In contrast, both syndecan-1 and hyaluronan significantly increased over time (Fig. 1). Specifically, syndecan-1 increased at T3 (T0–T3, $P = 0.010$) and at T24 (T0–T24, $P < 0.001$). Hyaluronan increased from T0 at all subsequent time points (T0–T1, $P = 0.010$; T0–T3, $P < 0.001$; T0–T24, $P = 0.003$). Summarized healthy control biomarker concentrations are provided for reference in Supplementary data.

Participants that had respiratory failure on day 1 did not have any significant differences in the pattern of EG biomarker change over time, compared to participants that did not have respiratory failure on day 1 (data not shown).

3.3. Associations with inflammation and endothelial activation

Syndecan-1 concentration was the only EG biomarker to show moderate correlation with inflammatory biomarkers (Table 2). Syndecan-1 was moderately correlated with NGAL at both T0 (rho 0.52, $P < 0.001$) and T24 (rho 0.52, $P = 0.01$), with resistin at T0 (rho 0.54, $P < 0.001$) and T24 (0.53, $P = 0.007$), and with myeloperoxidase at T0 (rho 0.54, $P < 0.001$). Syndecan-1 also showed moderate correlation with endothelial activation biomarker VCAM-1 at T24 (rho = 0.51, $P = 0.011$), as did endocan at T24 (rho = 0.52, $P = 0.009$). Summarized inflammatory biomarker concentrations are provided in Supplementary data.

Table 1
Baseline characteristics of participants, either presenting to an Emergency Department with sepsis due to pneumonia or healthy control.

Characteristic	Pneumonia (N = 44)	Control (N = 28)
Age (y)	66 (61–71)	65 (60–71)
Male	25 (57)	18 (64)
CCS	2 [1–4]	0 [0–1]
Infection severity*		
Simple infection	5 (11)	–
Sepsis	19 (43)	–
Septic shock	20 (46)	–
White cell count ($10^9/\mu L$)	10.8 (8.7–13.3)	–
C-reactive protein (mg/mL)	200 [65–350]	–
SOFA score	4 [2–8]	–
Respiratory failure		
Day 1	30 (68)	–
Day 2	22 (55)	–
Day 3	12 (32)	–
CURB-65 score	3 [2–3]	–
Lactate (mmol/L)	2.1 [1.3–4.5]	–
Glasgow Coma Scale	15 [14–15]	–
Mechanical ventilation	9 (21)	–
Admission to ICU	15 (34)	–
Length of ICU stay (days)	4.9 [0.5–11]	–
30-day mortality	11 (25)	–
1-year mortality	15 (34)	–

Data are presented as either mean (95% confidence interval) or median [Q1–Q3] for continuous variables, or No. (%) for binary variables.
Abbreviations: CCS, Charlson Comorbidity Score; SOFA, sequential organ failure assessment; ICU, intensive care unit.
* Simple infection was defined as a SOFA score < 2, sepsis as a SOFA score ≥ 2 and septic shock as systolic blood pressure < 100 m Hg despite >20 mL/kg of intravenous fluid.

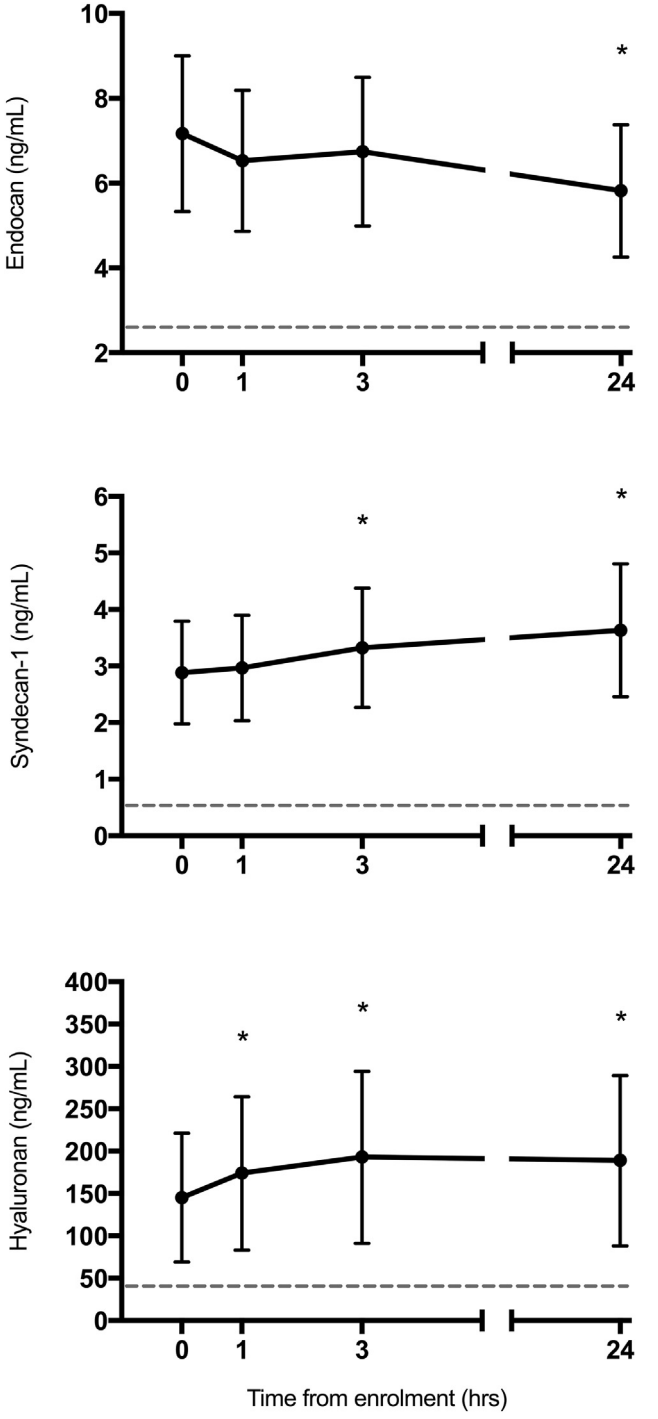

Fig. 1. Endothelial glycocalyx biomarker concentrations (predicted mean, 95% confidence interval) in participants with sepsis due to pneumonia (n = 44) at admission to an Emergency Department (0 h, N = 44), 1 h (N = 44), 3 h (N = 34) and 12–24 h (N = 24) later. Asterisks denote significant change ($P < 0.05$) from 0 h. Dashed lines represent median concentration of healthy controls.

Table 2
Correlations between endothelial glycocalyx biomarkers (endocan, syndecan-1 and hyaluronan) and inflammatory biomarkers in patients with sepsis due to pneumonia, measured at admission to the Emergency Department (T0) and 12–24 h later (T24).

Biomarker	Endocan				Syndecan-1				Hyaluronan			
	T0		T24		T0		T24		T0		T24	
	rho	*P*	rho	*P*	rho	*P*	rho	*P*	rho	*P*	rho	*P*
Interleukin-6	0.26	0.09	0.36	0.09	0.25	0.11	0.41	0.048	0.35	0.019	0.21	0.33
Interleukin-10	0.24	0.11	0.29	0.17	0.10	0.54	0.40	0.052	0.15	0.34	0.25	0.24
NGAL	0.18	0.25	0.40	0.052	**0.52**	**<0.001**	**0.52**	**0.010**	0.39	0.008	0.25	0.25
Resistin	0.19	0.21	0.37	0.079	**0.54**	**<0.001**	**0.53**	**0.007**	0.45	0.002	0.32	0.13
MPO	0.07	0.64	0.14	0.50	**0.54**	**<0.001**	0.38	0.067	0.42	0.005	0.48	0.019
ICAM-1	0.18	0.24	0.20	0.35	0.21	0.17	0.32	0.13	0.27	0.079	0.09	0.66
VCAM-1	0.34	0.025	**0.52**	**0.009**	0.24	0.13	**0.51**	**0.011**	0.32	0.034	0.37	0.076

Values in bold indicate significant correlations at rho > 0.5.
Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; MPO, myeloperoxidase; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

3.4. Associations with clinical outcomes

Associations between biomarker concentrations at T0 and respiratory failure on day 1 were not significantly different to days 2 and 3, therefore a single odds ratio over time is reported (Table 3). No significant associations were identified between endocan concentration and clinical outcomes. In contrast, increased syndecan-1 concentration was associated with higher odds of respiratory failure over the first 3 days ($P = 0.004$), mechanical ventilation ($P = 0.014$) and 30-day mortality ($P = 0.008$). Also, increased hyaluronan concentration was associated with higher odds of 30-day mortality ($P = 0.018$). Clinical severity surrogates, CURB-65 score and lactate concentration, were associated with 30-day mortality ($P = 0.004$, $P = 0.035$, respectively) but not respiratory failure over the first 3 days.

4. Discussion

This study found that patients with sepsis due to pneumonia admitted to the ED had decreasing endocan concentration over the first 24 h of hospitalization, whereas both syndecan-1 and hyaluronan concentration increased over time. Notably, syndecan-1 showed the most consistent association with inflammatory biomarkers, including neutrophil-derived cytokines NGAL, resistin and myeloperoxidase. Additionally, syndecan-1 concentration appeared superior to other EG biomarkers and clinical severity surrogates for predicting three meaningful clinical outcomes: respiratory failure, mechanical ventilation and 30-day mortality.

It has been postulated that circulating endocan concentration is a useful indicator of severity of lung disease due to evidence of its strong expression in human lung tissue and upregulation in response to inflammation [7, 20, 35]. In this study, endocan concentrations decreased over time, in contrast to other EG biomarkers, and the pattern in change over time did not differ between patients with respiratory failure, and those without. Additionally, endocan concentration was not a predictor of respiratory failure, in contrast to syndecan-1. It is possible that endocan secretion is not as 'lung-specific' as previously thought, and may simply represent systemic glycocalyx shedding or endothelial activation. Studies have identified endothelial endocan expression in tissues other than lung, such as kidney, intestine and fat [6, 36]. Positive associations reported by others between circulating endocan concentration and respiratory failure may simply reflect severity of systemic illness [7, 8, 37, 38].

Circulating endocan may be present in multiple forms. It is likely that the type of endocan measured by ELISA in most previous studies [5, 6, 8, 21, 22], including this one, is that which is induced and actively secreted by endothelial cells. The molecular weight of endothelial cell surface endocan is in the range of 14-20 kDa, whereas actively secreted endocan is ~50 kDa [6]. The ELISA used in the present study quantifies the latter form and increased concentrations may more accurately reflect ongoing endothelial activation, rather than being a reliable indicator of real-time EG shedding. Measurement of smaller molecular weight endocan, such as 14 kDa, would also not serve as an ideal EG biomarker, as it may simply have been cleaved in circulation [39]. Regardless, given previous evidence that circulating endocan measured by assays currently available is associated with lung injury, our purpose was to describe the time course of this type of endocan, compared to other biomarkers associated with disruption of the endothelium.

Syndecan-1 concentration showed moderate correlation with markers of neutrophil activation; NGAL, resistin and myeloperoxidase. The increasing concentration of syndecan-1 concentration over time also parallels increasing concentrations of both NGAL and resistin (see Supplementary data, Table 1). It is well established that NGAL plays an important role in the neutrophil's early response to bacterial infection [40] and increased plasma NGAL concentration has been associated with severity of pneumonia [33]. Neutrophils have also been recently identified as a major source of resistin in bacterial infection [41]; a biomarker that has also been associated with severity of sepsis [26]. We measured myeloperoxidase in this study as another marker of neutrophil activation, which is a neutrophil-derived cytotoxic enzyme that may contribute to lung injury in pneumonia [42]. An association between these important neutrophil activation markers and syndecan-1

Table 3
Associations (odds ratio (95% confidence interval)) between variables at Emergency Department admission (glycocalyx biomarker, CURB-65 score or lactate) and clinical outcomes in patients with sepsis due to pneumonia.

	Respiratory failure		Mechanical ventilation		30-day mortality	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Endocan (ng/mL)	1.04 (0.99–1.13)	0.053	1.04 (0.97–1.10)	0.27	1.05 (0.98–1.11)	0.16
Syndecan-1 (ng/mL)	1.18 (1.05–1.33)	**0.004**	1.24 (1.04–1.48)	**0.014**	1.29 (1.07–1.55)	**0.008**
Hyaluronan (μg/mL)	1.27 (0.96–1.69)	0.092	1.37 (0.91–2.07)	0.13	1.69 (1.09–2.60)[a]	**0.018**
CURB-65 score	1.33 (0.97–1.83)	0.077	1.32 (0.72–2.43)	0.36	3.74 (1.51–9.22)	**0.004**
Lactate (mmol/L)	1.04 (0.92–1.16)	0.55	1.35 (1.07–1.70)	**0.010**	1.24 (1.02–1.51)	**0.035**

Odds ratios are for an increase of 1 ng/mL for endocan and syndecan-1, 1 μg/mL for hyaluronan and 1 mmol/mL for lactate. Bold *P* values indicate a significant ($P < 0.05$) association.
Respiratory failure was determined on days 1, 2 and 3 of hospitalization by a $Pa0_2/Fi0_2$ ratio < 300, $Sp0_2/Fi0_2$ < 315 or $Sp0_2$ < 90% on >6 L/min oxygen.
[a] Biomarker concentration log-transformed due to non-linearity.

raises the possibility that activated neutrophils are a source of shed syndecan-1 ectodomains. Syndecan-1 belongs to a family of heparan sulfate proteoglycans, which have been identified as important binding sites on the surface of neutrophils for various inflammatory mediators [43, 44]. Neutrophil expression of syndecan-1 has also been shown to increase in patients with diabetes [45], and therefore may increase in other disease states as well. It is possible that syndecan-1 plays a dual role as an inflammatory biomarker and as an indicator of EG shedding. The relative contribution of neutrophil-derived syndecan-1 to total circulating concentrations in critically ill patients requires further exploration.

Further to the associations shown with neutrophil activation, syndecan-1 concentration in this study showed stronger associations with clinical outcomes compared to other EG biomarkers, CURB-65 score and lactate concentration. Other studies investigating sepsis have also shown strong positive associations between circulating syndecan-1 and organ failure [12, 16]. In another ED-based study, syndecan-1 concentration in patients with sepsis was also significantly associated with higher odds of intubation [19]. There is a paucity of studies directly comparing circulating EG biomarkers, but the results of this study suggest that syndecan-1 is superior for predicting clinical outcome in patients with sepsis secondary to pneumonia.

Examining relationships between EG biomarkers and inflammation is difficult in the clinical setting due to variability in the time of onset of disease. This study has identified some parallel patterns in biomarker change over time (*e.g.* syndecan-1 and NGAL) but, if a causal relationship exists, cannot differentiate between the instigator and the responder. For example, it has been demonstrated *in vitro* that shedding of the EG increases endothelial activation, leucocyte adhesion, upregulation of NFκb and inflammatory cytokine production [46-48]. However, it is also well established that application of lipopolysaccharide (LPS) or inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), to endothelium can also cause shedding of the EG [5-9, 20, 49-52]. It is likely that the process is dynamic and further work is needed to determine if therapy aimed at protecting the EG will reduce the pro-inflammatory response and, potentially, reduce the risk of multiple organ failure. Also, future studies should consider multiple modes of detecting glycocalyx shedding in clinical patients, beyond measurement of circulating biomarkers, as these soluble proteoglycan and glycosaminoglycans can be shed from various sources and may simply reflect cell activation.

Strengths of this study are the early enrolment in the ED, making it a point of difference to most other studies that have delayed enrolment in the ICU, as well as application of predefined inclusion criteria, and serial blood sampling. Limitations include convenience sampling, observational design and a relatively small sample size that may increase the risk of selection bias. Sensitivity analysis did not indicate a significant influence of participant drop-out before T24 blood sampling on the pattern of biomarker concentration change over time, however, we cannot excluded bias generated by participant drop-out. Although this study is exploratory in nature, multiple comparisons made between many biomarkers increases the risk of Type 1 error and results should be interpreted with caution. The findings of this study should be considered as hypothesis-generating only and prospective validation is required. Also, the respiratory failure criterion was expanded to include decreased $Sp0_2$, as arterial blood sampling was not always possible. Use of SF ratio has been validated in other studies [53-56] but this expansion of criteria may have led to some variability of classification of patients.

In summary, endocan and syndecan-1 showed different patterns of shedding in patients with sepsis due to pneumonia. Syndecan-1 showed the strongest associations with neutrophil activation biomarkers as well as relevant clinical outcomes. Further work is needed to better understand the various sources of EG biomarkers, as well as the mechanisms causing their rise during sepsis and acute lung injury.

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Conflicts of interest and source of funding

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