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Influence of burn severity on endothelial glycocalyx shedding following thermal trauma: A prospective observational study

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ARTICLE INFO

Keywords:

Burns
Glycocalyx shedding
Metabolism
Fluid resuscitation

ABSTRACT

Objective: Severe burns cause hypermetabolic and inflammatory responses are treated with significant volume resuscitation. This study aimed to evaluate correlations between glycocalyx metabolites and the burn size as well as certain clinical parameters such as administered fluid volumes.

Study design: Severely burned patients with a total body surface area (TBSA) burned smaller and larger than 20% were included. Clinical parameters including length of stay, mortality, fluid administration and Sequential Organ Failure Assessment (SOFA) score as well as syndecan and heparansulfate, as laboratory parameters for endothelial damage, were obtained.

Results: A total of 39 patients (32 males, 7 females) with a mean age at burn of 45 ± 21 years were included. Syndecan levels decreased and heparansulfate levels increased over time. In both heparansulfate and syndecan, there was no significant difference between burns smaller and larger than 20% TBSA at any time point. Syndecan levels at 24 h after burn correlated significantly with IL-10 levels at admission ($R = 0.58$ and $p < 0.05$). There were significant linear correlations of %TBSA and cumulative administration of fluids after 24 h on syndecan levels after 48 h. Correlations between clinical parameters and syndecan or heparansulfate levels over time were not found.

Conclusions: This study shows that even though there are moderate correlations with burn size and administered fluid volume, levels of syndecan and heparansulfate are not predictive for clinical outcomes of burned patients in our cohort. Further studies with higher numbers evaluating the effect of large burns on glycocalyx shedding over a longer period of time are needed. Showing significant glycocalyx shedding in large burn including potentially correlations with clinical outcomes may yield new therapeutic targets.

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https://doi.org/10.1016/j.burns.2020.07.021
0305-4179/

1. Introduction

Severe burn injuries are frequently followed by an extensive hypermetabolic response [1,2]. Muscle loss has been shown to begin as early as 48 h after immobilization and peaks at 2 –3 weeks [3]. This results in decreased quality of life and delayed return to regular social activities [4].

In addition to local tissue destruction, severe burns with a total body surface area (TBSA) affected larger than 20% lead to an immune response with elevated levels of cytokines (including TNF-alpha, interleukin-6 and interleukin-10) and highly complex inflammatory cascades that lead to systemic effects on the whole organism [5]. Among other systemic pathophysiological responses, there is a generalized capillary leak due to damage to the vessel walls, which become increasingly permeable, causing large amounts of fluid to leak into the interstitial space [6]. If left untreated, this may lead to life-threatening intravascular volume deficiency shock or cardiac dysfunction [7,8].

Shedding of the endothelial glycocalyx appears to be a major pathomechanism when it comes to endothelial damage and capillary leak following severe burns [9–11]. This layer of various proteoglycans, glycosaminoglycans and glycoproteins, which is in contact with the luminal surface of endothelial cells, is essential for the permeability barrier of the vessel walls [12,13]. Damage to the glycocalyx with a resulting decrease in its integrity leads to an increased permeability of the vessel wall with the escape of liquid and macromolecules into the interstitial space [14].

The influence of glycocalyx shedding on pathomechanisms and outcomes have been reported in severe mechanical trauma and sepsis [15,16], but to date only few studies evaluated this effect following burns. Strong correlations between burn size and the level of glycocalyx shedding may have predictive value towards key outcomes and may yield a novel therapeutic target through preventing glycocalyx disruption. This may improve clinical care and outcomes of severely burned patients.

Therefore, this prospective observational study aimed to investigate whether the severity and extent of burns as well as certain clinical parameters such as administered fluid volume correlate with the level of endothelial glycocalyx shedding within the first 48 h after severe thermal trauma.

2. Methods

This study was approved by the local institutional review board prior to initiating the study (Protocol number: 837.387.14). Severely burned patients who were admitted to our institution between January and October 2015 were prospectively and consecutively included in this study. All participating patients consented or assented to participation in this study. All clinical and laboratory parameters were measured at admission to the burn unit and at 0, 8, 24 and 48 h after burn, respectively. These timepoints were selected because especially in the first 48 h following burn, appropriate fluid resuscitation is considered the single most effective therapeutic intervention [17].

2.1. Fluid resuscitation

In all patients, fluid resuscitation was initiated preclinically and continued upon admission with crystalloid fluids according to the modified Brooke-formula (2 mL x kg body weight x % TBSA per 24 h). During the first 48 h, the infusion rate was re-evaluated every 2 h to ensure adequate resuscitation: measured through Pulse Contour Cardiac Output (PiCCO), target global enddiastolic index (GEDI) was $\geq$ 480 mL/m2, target cardiac index (CI) was $\geq$ 2.5 L/min/m2, target hourly urinary output (UO) was $\geq$ 0.5 mL/kg and target mean arterial pressure (MAP) was $\geq$

70 mmHg. In case one of the targets was not met, a 20% increase in hourly volume input was initiated. Over-accomplishment defined as GEDI $\geq$ 640 mL/m2, UO $\geq$ 1 mL/kg, triggered a step-wise reduction in infusion rate [18]. Colloid fluids were not administered before the first 8 h of resuscitation, substituting up to 1/3 of total crystalloid volume based on the algorithm if target criteria (GEDI, CI, MAP, and UO) could not be met with multiple 20% increases in hourly resuscitation volume re-evaluated every two hours. Additionally, use of catecholamines was considered if targets of CI (dobutamine) or MAP (norepinephrine) were not met.

2.2. Clinical parameters

Demographic and clinical data included age at burn, sex, % TBSA, body mass index (BMI), length of stay (LOS) at the intensive care burn unit, and the administered volumes of crystalloid and colloid fluids. Furthermore, the Sequential Organ Failure Assessment (SOFA) score was calculated for each timepoint.

2.3. Laboratory parameters

At each time point (admission, 8, 24 and 48 h, respectively), the following parameters were evaluated using Enzyme-linked Immunosorbent Assay (ELISA): heparansulfate (HA), syndecan, interleukin 6 (IL-6) and interleukin 10 (IL-10). Reference values for each of the evaluated parameters were reported as follows: HA = 1.96 $\pm$ 1.21 μg/mL [19], syndecan = 20.5 $\pm$ 5.05 ng/mL [19], IL-6 = 1.0 $\pm$ 0.1 pg/mL [20], IL-10 = 1.2 $\pm$ 0.2 pg/mL [21]. ELISA analyses were performed by trained laboratory technicians according to the manufacturers protocols (Echolon Biosciences Incorporated, California, USA; R&D Systems, Minnesota, USA; Diaclone SAS, Besancon Cedex, France).

2.4. Statistical analysis

Data are presented as mean values $\pm$ standard deviation (SD), or medians and ranges where applicable. Continuous outcome parameters were compared using Student's unpaired *t*-test, Mann Whitney *U* test, or one-way ANOVA, depending on normal distribution of datasets assessed by Shapiro-Wilk test. Paired repeated parametric measurements were analyzed with 2-way ANOVA and Tukey's post-hoc correction for multiple comparisons. Contingencies were analyzed using Fisher`s exact test. Pearson r correlations were used to assess statistical relationships between various outcome measures. Standard univariate and multivariate least-squares regression

models were then fit to continuous responses; for categorical outcomes, logistic regression models were fit. Statistical analyses were performed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla CA). Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Patient characteristics

A total of 39 patients (32 males, 7 females) with a mean age at burn of 45 ± 21 years were included in the study. General demographics are shown in Table 1. TBSA burned was 28 ± 19%. Patients stayed in the intensive care unit for 23.0 ± 25.3 days and had a mean SOFA-score of 3.6 ± 3.1. The overall mortality rate was 7.6% (n = 3), with all of the deceased patients having sustained burns larger than 20% TBSA. LOS ($p < 0.001$) and SOFA score ($p = 0.03$) were significantly larger in patients with burns larger than 20% TBSA. General demographic data of the total cohort and stratifiedby the burn size are shown in Table 1.

3.2. Levels of interleukin, syndecan and heparansulfate over time

The levels of IL-6, IL-10, syndecan and HA are displayed in Figs. 1 (total cohort) and Fig. 2 (stratified by %TBSA).

HA levels continuously increased significantly in the total cohort over 48 h, whereas syndecan decreased after peaking at 24 h after burn. In both heparansulfate and syndecan, there was no significant difference between burns smaller and larger than 20% TBSA at any time point. In all patients IL-6 levels significantly increased whereas IL-10 levels significantly decreased over time compared to the baseline/timepoint 0. There were statistically significant higher levels of IL-6 at 48 h after burn ($p < 0.05$) and IL-10 at admission ($p < 0.05$) in patients with burns larger than 20% TBSA compared to those with burns smaller than 20% TBSA.

A larger burn size significantly correlated with higher levels of syndecan at 24 and 48 h after burn, respectively (both $R = 0.42$ and $p < 0.05$). Furthermore, syndecan levels at 8 h after burn significantly correlated with IL-10 levels at 8 ($R = 0.47$ and $p < 0.05$) and 24 h ($R = 0.44$ and $p < 0.05$) after burn, respectively. Syndecan levels at 24 h after burn correlated significantly with IL-10 levels at admission ($R = 0.58$ and $p < 0.05$). Syndecan levels at 48 h after burn significantly correlated with IL-6 levels at 48 h ($R = 0.43$ and $p < 0.05$) and IL-10 level at 24 h after burn ($R = 0.49$ and $p < 0.05$), respectively.

No significant correlations were found between levels of HA or syndecan and LOS.

3.3. Fluid administration

The cumulative amount of crystalloid and colloid fluids is shown in Fig. 3. In patients with burns encompassing less than 20% TBSA, the cumulative volume of crystalloids increased from 1119.4 ± 902.1 mL at admission to 2814.2 ± 1528.3 mL at 8 h, 5955.2 ± 2927.6 mL at 24 h and 8925.6 ± 4400.0 mL at 48 h, respectively. In patients with burns larger than 20% TBSA, the volume of crystalloids increased from 1761.9 ± 1189.7 mL at admission to 4956.2 ± 2235.2 mL at 8 h, 11361.8 ± 6033.3 mL at 24 h and 14829.7 ± 7503.9 mL at 48 h, respectively. Cumulative crystalloid fluid administration was significantly higher in patients with larger compared to smaller burns at 24 ($p < 0.001$) and 48 ($p < 0.001$) hours. The total fluid volume administered at 8 h after burn significantly correlated with syndecan levels at 24 h after burn ($R = 0.43$, $p < 0.05$).

3.4. Regression models

The relationship between burn size, administered fluid volume on one hand and syndecan and heparansulfate on the other was further examined using univariate linear regression. There was a significant linear effect of [%TBSA] ($R^2 = 0.173$, $p = 0.008$) and [cumulative administration of fluids in first 24 h] on syndecan levels at 48 h after burn ($R^2 = 0.163$, $p = 0.011$; Table 2).

Univariate logistic regression models to assess a relationship between HA or syndecan and length of stay or mortality yielded no significant results.

4. Discussion

In this study, we analyzed the influence of (severe) burn injuries on endothelial glycocalyx shedding. Our data show that glycocalyx shedding occurs shortly after burn and correlates with the estimate for high amounts of fluid resuscitation, similar to other etiologies such as severe trauma

Table 1 – Patients general demographics.

	All patients (n = 39)		< 20% TBSA (n = 18)		> 20% TBSA (n = 21)		p-value
	Mean	SD	Mean	SD	Mean	SD	
Age	44.5	21.2	42.1	20.7	46.7	21.9	0.510
% male	82.1		77.80		85.70		0.683
TBSA %	27.7	19	12.7	5.7	38.6	16.6	**<0.001**
BMI	26	4.8	26.7	5.6	25.3	4.1	0.359
LOS	23	25.3	11	11.3	33.3	29.36	**<0.001**
%Mortality	7.6		0		14.3		0.235
SOFA admission	3.6	3.1	2.5	2.6	4.6	3.1	**0.033**
Inhalation injury n (%)	4	(10.3)	0	(0)	4	(22)	**0.037**

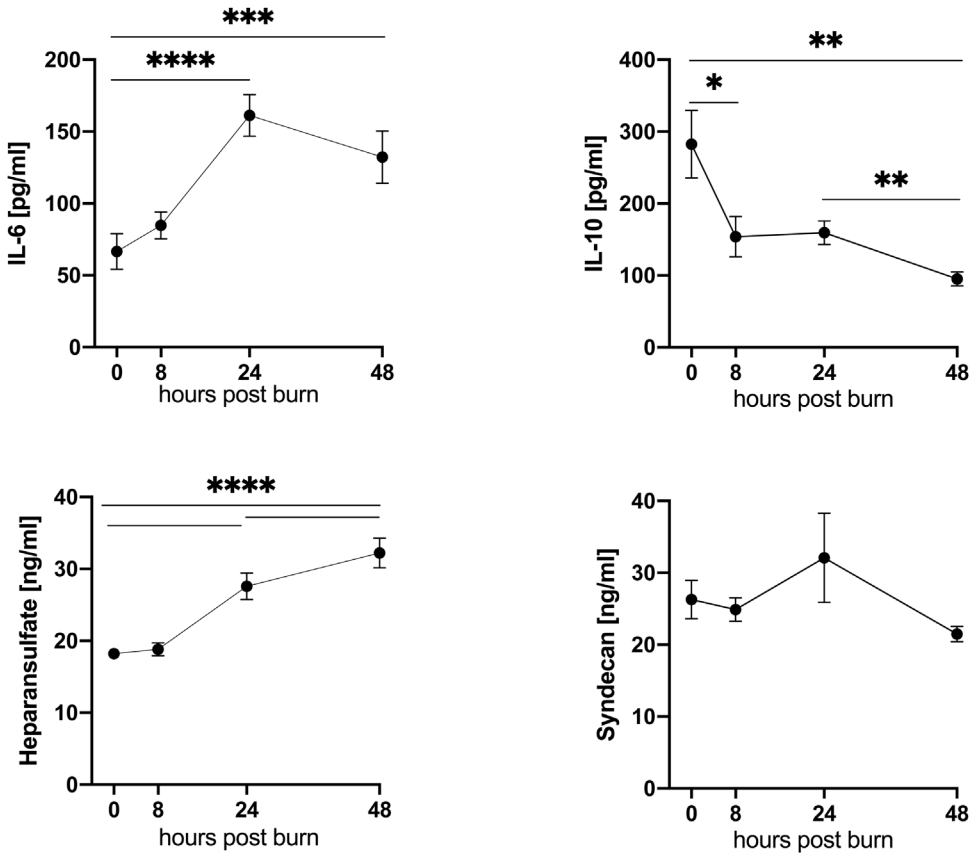

Fig. 1 – Total syndecan, heparansulfate, IL-6 and IL-10 levels over time (* = $p < 0.05$ between indicated timepoints).

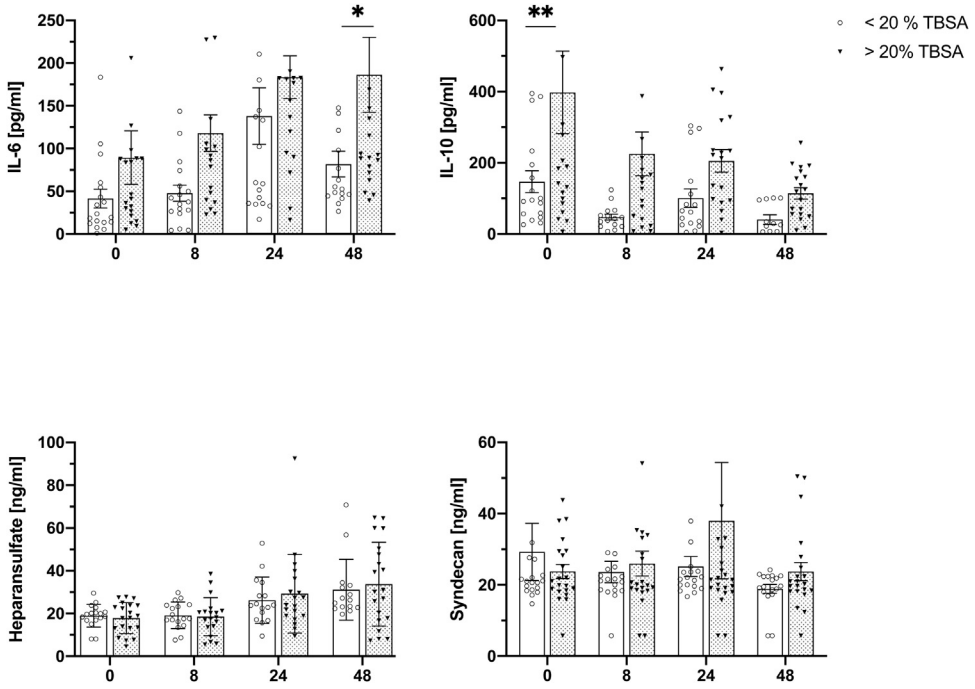

Fig. 2 – Syndecan, heparansulfate, IL-6 and IL-10 levels divided by burn size over time (* = $p < 0.05$ between indicated timepoints).

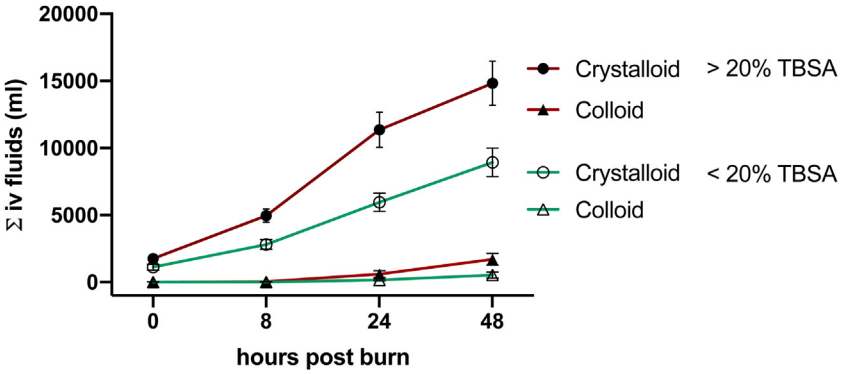

Fig. 3 – Cumulative fluid administration divided by burn size.

Table 2 – Regression models.

	Estimate	Std. Error	p-value	R^2
%TBSA -> Syndecan 48 h				
(Intercept)	15.65	2.513	< **0.0001**	
%TBSA	0.219	0.078	**0.008**	**0.173**
Fluid volume 24 h -> **Syndecan** 48 h				
(Intercept)	15.35	2.262	< **0.0001**	
∑ Volume 24 h	0.0007	0.0003	**0.011**	**0.163**

or sepsis [15,22,23]. However, these increased indicators of glycocalyx disruption do not seem to predict clinical outcomes of the patients in the present study.

After identifying a potential target for vascular permeability alterations at the endothelial surface level in the animal model [24], this prospective observational study described glycocalyx shedding within the first 48 h after burns of different severity. Osuka et al. recently reported an age-dependency and correlations with higher fluid estimates for syndecan levels in severely burned patients [25]. Studies from different fields demonstrated associations between microcirculatory alterations and tissue malperfusion and mortality, respectively [15,22].

One aim of the study was to evaluate if levels of released components of the glycocalyx such as HA and syndecan can predict clinical outcomes such as length of hospital stay or mortality after severe burns. Even though there was a correlation between burn size and syndecan levels, clinical outcomes could not be predicted. The association between the level of glycocalyx shedding and clinical outcomes was evaluated in numerous previous studies dealing with other traumas than burns [15,23,26]. In a study with 424 severely injured patients, Johansson et al. reported that endotheliopathy was an independent predictor of mortality [15].

In a study with 458 burn and non-burn patients, Welling et al. showed that endotheliopathy is correlated to the shock phase rather than the mechanism of trauma itself [23]. In our study, syndecan higher levels were significantly and positively correlated with burn size. In contrast to this, Welling et al. reported that the severity of glycocalyx shedding is correlated to the presence of inhalation injury rather than the burn size [23]. The effect of a larger burn size may be clinically more relevant when comparing patients with smaller and larger burns over a longer period of time. In the present study, there was no effect of the presence of inhalation injury on levels of glycocalyx metabolites. This may be due to the small number and the overall small burn size.

There was a significant correlation between the cumulatively administered fluid volume over time and syndecan levels which is with a proxy for the degree of glycocalyx shedding. However, the correlation that was shown in the present study was rather weak and needs to be confirmed in a larger cohort. It has been shown that microcirculatory derangements can already be expected even if the macrohemodynamics are still within normal limits [27]. The impact of fluid resuscitation on glycocalyx shedding has been reported in different patient cohorts [26,28–30]. Chappell et al. reported that cardiac surgery patients with good cardiopulmonary function had significantly increased levels of syndecan, HA and hyaluronan after volume loading [31]. Also, Johansson et al. reported a strong correlation between syndecan levels and outcomes following severe trauma [26]. However, it is not clear if glycocalyx shedding and therefore microcirculatory alterations in burn patients lead to a higher fluid demand or if the extensive administration of fluids following burn favors glycocalyx shedding. This needs to be investigated further in suitable animal models.

This study also yielded significant, but overall weak correlations between syndecan levels and interleukin (IL-6 and IL-10) levels at several time points. Many studies reported the hypermetabolic and hyperinflammatory response with increased interleukin levels in severely burned patients [32,33]. There may be a correlation between burn severity, hyperinflammatory response and therefore the level of glycocalyx shedding with release of syndecan and HA. Even though a regulation of IL-6 on syndecan expression has been shown in B lymphoid cells [34], the exact mechanisms and the clinical value is not known in severely burned patients.

4.1. *Limitations*

Although this study brings new insights into the dynamics of endothelial glycocalyx shedding after severe burn injury, the findings should be interpreted in the context of the study's limitations like the small number of included patients and its single-center nature. Therefore, inferences regarding causality of the findings cannot be made. A further limitation is that the endothelial biomarkers were only measured at admission to the burn unit and up to 48 h after burn. Consequently, conclusions of the course of levels of glycocalyx products over a longer time cannot be drawn. However, this study aimed to evaluate the ability of syndecan and HA levels to predict clinical outcomes within the first two days of treatment. Additionally, the correlations that were found in the present study were overall weak and need to be confirmed in future studies with larger numbers of severely burned patients.

5. Conclusion

Following severe burns, the body experiences a hypermetabolic and hyperinflammatory response with intense microcirculatory alterations. Serum indicators of glycolcalyx disruption follow a characteristic pattern over the first 48 h after burn. However, the results of the present study show that even though there are moderate correlations with burn size and administered fluid volume, levels of syndecan and HA are not predictive for clinical outcomes of burned patients in our cohort. This may me due to the small number of patients with large burns. However, showing the correlation between burn size and syndecan levels at 48 h after burn, further studies with larger numbers, especially with larger burns, may be able to show a clinical effect when protecting the glycocalyx pharmaceutically and decreasing fluid demands.

Authors contributions

Study conception and design: JFH, JH, UK, VH, MS, TK; acquisition of the data: CT, JH, JV, TK, GH; statistical analysis: CT, JH, GH; analysis and interpretation of the data: CT, JFH, VH, TH, GH; drafting of the manuscript: CT, JFH, JH, UK, TK, GH; critical revision: JH, UK, VH, JV, MS, TK, GH. All authors approved the final version of the manuscript.

Funding sources

None.

Ethics approval

This study was approved by the local institutional review board prior to initiating the study (Protocol number: 837.387.14). All participating patients consented or assented to participation in this study.

Declarations of interest

None.

Acknowledgements

None.

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