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Sepsis and Major Abdominal Surgery Lead to Flaking of the Endothelial Glycocalix

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Background. **Recent evidence suggests that the endothelial glycocalix plays an important role in lethal outcomes following sepsis. We therefore tested if the endothelial glycocalix is shed in patients with sepsis compared with patients after major abdominal surgery and healthy volunteers.**

Material and Methods. **A total of 150 individuals were tested for levels of inflammatory markers (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], interleukin-6 [IL-6]) and glycocalix markers (syndecan-1, heparan sulfate). Three groups consisted of patients with severe sepsis or septic shock, patients after major abdominal surgery without systemic inflammatory response syndrome, and healthy volunteers. Blood was drawn, at the time of diagnosis or surgery, and 6, 24, and 48 h later. We correlated these markers to each other and to clinically used inflammation markers.**

Results. **Levels of inflammatory markers were markedly higher in patients with sepsis compared with patients after major abdominal surgery and healthy volunteers. After major abdominal surgery, glycocalix markers in human plasma were at levels comparable to patients with sepsis. In patients with sepsis, levels of IL-6 correlated with syndecan-1, ICAM-1, VCAM-1, and lactate, while ICAM-1 furthermore correlated with CRP and lactate levels.**

Conclusion. **High levels of glycocalix markers indicated that significant flaking of the endothelial glycocalix occurred in patients with sepsis, and to a lesser extent in patients after major abdominal surgery. This novel finding could explain the nonspecific capillary leaking syndrome of patients with sepsis and after major abdominal surgery, and may identify new targets for treating those patient populations.**

Key Words: **sepsis; glycocalix; syndecan-1; heparan sulfate; intercellular adhesion molecule-1; vascular cell adhesion molecule-1; major abdominal surgery.**

INTRODUCTION

Sepsis is still the single most common cause for death in intensive care units in the United States and Europe. Despite new therapeutic options, mortality from sepsis remains unacceptably high at approximately 40%. Many of the pathophysiologic changes during sepsis are related to inflammation and apoptosis [1]. Mortality is closely related to changes in vascular reactivity [2], decreased capillary density [3], decreased red blood cell velocity [3], activation of clotting factors [1], and tissue hypoxia [4]. A key pathophysiologic finding in patients with sepsis is microleakage of capillary blood vessels [5], leading to increased fluid requirements and edema. Furthermore, evidence is emerging for the influence of the glycocalix in the inflammatory microvascular compartment [2, 6].

As a result of its unique position directly between the serum and endothelial cells, the glycocalix is important in microvascular integrity and the interaction between the bloodstream and the endothelium [7]. Although the structure of the glycocalix was revealed by Luft and

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colleagues in 1966 [8], how the glycocalix interacts with the endothelium is not fully understood [8]. The glycocalix consists of glycolipids, glycopeptides, and proteoglycans that line the luminal side of endothelial cells [9]. It regulates vascular permeability [7], acts as a mechanotransducer regulating the release of nitric oxide [9], and serves as an important connection for plasma-derived molecules [9]. In animal experiments, flaking of the glycocalix leads to a broad spectrum of vascular changes, as seen in some lung diseases, diabetes, and lipoprotein changes [10-12]. In a rat model of lipopolysaccharide-induced sepsis, the glycocalix was significantly damaged and degraded. [2]. Syndecan-1 and heparan sulfate are considered valid markers for the integrity of the endothelial glycocalix. Although the endothelial glycocalix is clearly involved in many diseases, it remains to be elicited how to modify changes in the glycocalix that occur as part of those disease processes.

The aim of this study was to evaluate glycocalix changes in patients with sepsis, compared with patients after major abdominal surgery and healthy volunteers. We hypothesized that marked flaking of the endothelial glycocalix occurs, as evidenced by high glycocalix degradation products.

MATERIALS AND METHODS

Patient Collective

The study was approved by the ethics committee of the University of Heidelberg (S-123/2008). A total of 150 people were studied after giving their written consent, including 104 patients with severe sepsis or septic shock, according to the International Sepsis Definitions Conference [13] (referred to as the sepsis group, Table 1), 28 patients after major abdominal surgery (referred to as the surgery group, Table 1), and 18 healthy young volunteers without any signs of infection (referred to as the control group, Table 1). In the septic group, 55 (52.9%), patients survived for more than 28 d after diagnosis. The primary sites of infection were: lung (58.7%), genitourinary (11.6%), GI tract (3.8%), surgical site (6.7%), other (10.6%) and unknown (8.7%). Besides the patients with an infection at the surgical site, no other patients with sepsis had any recent surgeries done. The surgical patients mainly underwent abdominal surgeries (primary site of surgery: pancreas 46.4%, colon 17.9%, liver 7.1%, genitourinary 10.7%, other 17.9%). Blood for testing was drawn at the time of diagnosis of sepsis in the septic group, after surgery in the surgery group, and if results were negative for parameters of systemic inflammatory response syndrome, and 6, 24, and 48 h later. Blood testing for the healthy volunteers was performed once.

TABLE 1

Baseline Data

	Sepsis group ($n = 104$)	Surgery group ($n = 28$)	Control group ($n = 18$)
Age, y	64.9 ± 12.4	62.9 ± 12.7	34.5 ± 8.6
Male gender	58 (55.8%)	15 (53.6%)	10 (55.6%)

Data are presented as mean ± standard deviation or number (%).

Measurement of Syndecan-1, Heparan Sulfate, Interleukin-6, Intercellular Adhesion Molecule-1, and Vascular Cell Adhesion Molecule-1 Concentrations in Blood Plasma

Concentrations of syndecan-1 (Diaclone; Besancon Cedex, France), interleukin-6 (IL-6) (R and D Systems, Minneapolis, MN), intercellular adhesion molecule-1 (ICAM-1) (Bender MedSystems, Vienna, Austria), and vascular cell adhesion molecule-1 (VCAM-1) (Bender MedSystems) were measured in human plasma using the corresponding enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions. Concentration of heparan sulfate was measured by ELISA (Seikagaku Corp., Tokyo, Japan) after pretreatment of plasma with actinase E (Sigma, St. Louis, MO).

Statistical Analysis

Values of continuous variables are reported with mean and standard error of the mean (SEM). Means of groups were compared by analysis of variance or paired t-test. Correlation analysis was performed according to Pearson. A result was denoted as statistically significant if the P value of its corresponding test statistic was less than 0.05.

Concerning symbolism and higher orders of significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All statistical computations were performed using Prism Graph (Version 5; GraphPad Software, La Jolla, CA).

RESULTS

Sepsis Causes Marked Increases in Inflammatory Markers Compared with Healthy Volunteers and Patients After Major Abdominal Surgery

Levels of the inflammatory markers, VCAM-1, ICAM-1, and IL-6, were significantly higher in the sepsis group compared with the surgery and control groups (Table 2, Fig. 1). The differences were less pronounced during early and late sepsis. Interestingly, IL-6 was elevated in the surgery group compared with the control group, while ICAM-1 and VCAM-1 did not differ between the surgery and control groups. Those results confirm the inflammatory process during sepsis and show that patients after major abdominal surgery also experience an inflammatory surge, but to a lesser extend.

Major Abdominal Surgery Causes Flaking of the Endothelial Glycocalix that is Comparable to Septic Patients

Levels of syndecan-1 and heparan sulfate, both markers for the integrity of the endothelial glycocalix, were markedly higher in the sepsis group and the surgery group compared with the control group (Table 2, Fig. 2). These results point towards glycocalix degradation during sepsis, as well as after major abdominal surgery. Surprisingly, the level of heparan sulfate was higher in the surgery group compared with the sepsis group. In contrast, syndecan-1 levels were higher in the sepsis group than in the surgery group. There was no difference in levels of syndecan-1 and heparan

TABLE 2

Markers of Inflammation and Glycocalix Shedding

	Group	Baseline	6 h	24 h	48 h
ICAM-1 (ng/mL)	Control	250 ± 65.7	250 ± 65.7	250 ± 65.7	250 ± 65.7
	Sepsis	583 ± 354	652 ± 427	746 ± 460	641 ± 343
	P value	< 0.001	< 0.001	< 0.001	< 0.001
	Control	250 ± 65.7	250 ± 65.7	250 ± 65.7	250 ± 65.7
	Surgery	244 ± 91.1	250 ± 81.0	378 ± 293	324 ± 181
	P value	0.81	0.99	0.08	0.13
	Sepsis	583 ± 354	652 ± 427	746 ± 460	641 ± 343
	Surgery	244 ± 91.1	250 ± 81.0	378 ± 293	324 ± 181
	P value	< 0.001	0.004	0.02	0.008
VCAM-1 (mg/mL)	Control	1.81 ± 1.11	1.81 ± 1.11	1.81 ± 1.11	1.81 ± 1.11
	Sepsis	1.71 ± 2.00	6.38 ± 3.41	6.54 ± 3.80	2.18 ± 2.10
	P value	0.82	< 0.001	< 0.001	0.50
	Control	1.81 ± 1.11	1.81 ± 1.11	1.81 ± 1.11	1.81 ± 1.11
	Surgery	1.46 ± 0.544	1.70 ± 0.866	1.64 0.867	1.30 ± 0.454
	P value	0.20	0.77	0.64	0.18
	Sepsis	1.71 ± 2.00	6.38 ± 3.41	6.54 ± 3.80	2.18 ± 2.10
	Surgery	1.46 ± 0.544	1.70 ± 0.866	1.64 ± 0.867	1.30 ± 0.454
	P value	0.57	< 0.001	< 0.001	0.20
IL-6 (pg/mL)	Control	0.709 ± 1.37	0.709 ± 1.37	0.709 ± 1.37	0.709 ± 1.37
	Sepsis	318 ± 274	425 ± 208	243 ± 204	79.2 ± 137
	P value	< 0.001	< 0.001	< 0.001	0.02
	Control	0.709 ± 1.37	0.709 ± 1.37	0.709 ± 1.37	0.709 ± 1.37
	Surgery	213 ± 169	118 ± 86.5	74.3 ± 50.5	84.4 ± 56.1
	P value	< 0.001	< 0.001	< 0.001	< 0.001
	Sepsis	318 ± 274	425 ± 208	243 ± 204	79.2 ± 137
	Surgery	213 ± 169	118 ± 86.5	74.3 ± 50.5	84.4 ± 56.1
	P value	.09	< 0.001	0.006	0.91
Syndecan-1 (ng/mL)	Control	20.5 ± 5.05	20.5 ± 5.05	20.5 ± 5.05	20.5 ± 5.05
	Sepsis	160 ± 109	141 ± 87.3	161 ± 99.4	165 ± 86.5
	P value	< 0.001	< 0.001	< 0.001	< 0.001
	Control	20.5 ± 5.05	20.5 ± 5.05	20.5 ± 5.05	20.5 ± 5.05
	Surgery	50.5 ± 46.9	59.6 ± 59.8	85.6 ± 131	26.6 ± 16.9
	P value	0.01	0.009	0.04	0.16
	Sepsis	160 ± 109	141 ± 87.3	161 ± 99.4	165 ± 86.5
	Surgery	50.5 ± 46.9	59.6 ± 59.8	85.6 ± 131	26.6 ± 16.9
	P value	< 0.001	0.01	0.16	< 0.001
Heparan sulfate (μg/mL)	Control	1.96 ± 1.21	1.96 ± 1.21	1.96 ± 1.21	1.96 ± 1.21
	Sepsis	3.23 ± 2.43	4.99 ± 2.75	5.68 ± 2.43	3.45 ± 1.86
	P value	0.03	< 0.001	< 0.001	0.006
	Control	1.96 ± 1.21	1.96 ± 1.21	1.96 ± 1.21	1.96 ± 1.21
	Surgery	7.96 ± 3.26	8.48 ± 3.33	8.49 ± 3.46	6.77 ± 2.63
	P value	< 0.001	< 0.001	< 0.001	< 0.001
	Sepsis	3.23 ± 2.43	4.99 ± 2.75	5.68 ± 2.43	3.45 ± 1.86
	Surgery	7.96 ± 3.26	8.48 ± 3.33	8.49 ± 3.46	6.77 ± 2.63
	P value	< 0.001	0.007	0.05	< 0.001

Data are presented as mean ± standard deviation.
ICAM-1 = intercellular adhesion molecule-1; IL-6 = interleukin-6; VCAM-1 = vascular cell adhesion molecule-1.

sulfate between patients who survived or died in the sepsis group (data not shown).

Syndecan-1 Correlates with Interleukin-6 Levels in Septic Patients

In patients with sepsis, we found a significant correlation between levels of IL-6 and syndecan-1 ($P = 0.004$), IL-6 and ICAM-1 ($P < 0.001$), IL-6 and VCAM-1 ($P < 0.001$), and IL-6 and lactate ($P = 0.02$). In addition, in patients with sepsis, the level of ICAM-1 correlated with the level of lactate ($P = 0.02$) and C-reactive protein ($P < 0.001$, data not shown).

DISCUSSION

In this study of 150 individuals, the endothelial glycocalix was shed significantly in patients with sepsis. As expected, all measured markers of inflammation were significantly elevated during sepsis, while only IL-6 was elevated after major abdominal surgery (Fig. 1). This corresponds to the generalized inflammation that occurs in patients during sepsis, while patients after major abdominal surgery experience only a mild activation of the inflammatory system [14]. As hypothesized, we found significantly increased glycocalix degradation

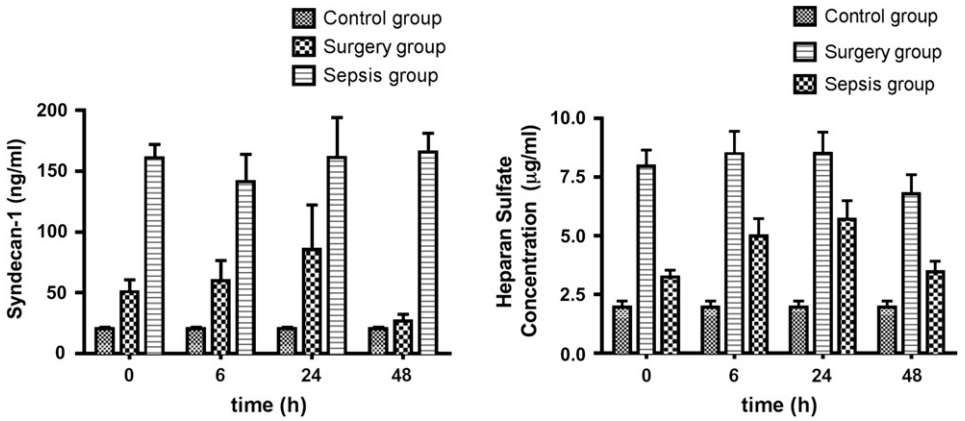

FIG. 1. Markers of inflammation. The concentrations of ICAM-1, VCAM-1, and IL-6 are shown in the control, surgery, and sepsis groups. Levels of these three inflammatory markers were markedly higher in patients with sepsis compared with the other groups. Blood samples were drawn 0, 6, 24, and 48 h after surgery or diagnoses of sepsis. ICAM-1 = intercellular adhesion molecule-1; IL-6 = interleukin-6; VCAM-1 = vascular cell adhesion molecule-1.

products, namely, heparan sulfate, and syndecan-1 in patients with sepsis and patients after surgery [15]. Marechal and colleagues [2] showed similar results in the rat, but these findings have not previously been reported in humans or in individuals after major abdominal surgery.

Interestingly, patients after major abdominal surgery experienced similar flaking of the glycocalix as patients with sepsis, but to a lesser extent. In patients after surgery, the increase in syndecan-1 mirrored the one in patients with sepsis but did not reach the same levels. Furthermore, we detected markedly increased levels of heparan sulfate in patients after major abdominal surgery, while the increase in patients with sepsis was significant, but not to the same extent (Fig. 2). That heparan sulfate was higher in patients after major abdominal surgery compared with patients with sepsis, while syndecan-1 levels were lower, could be explained

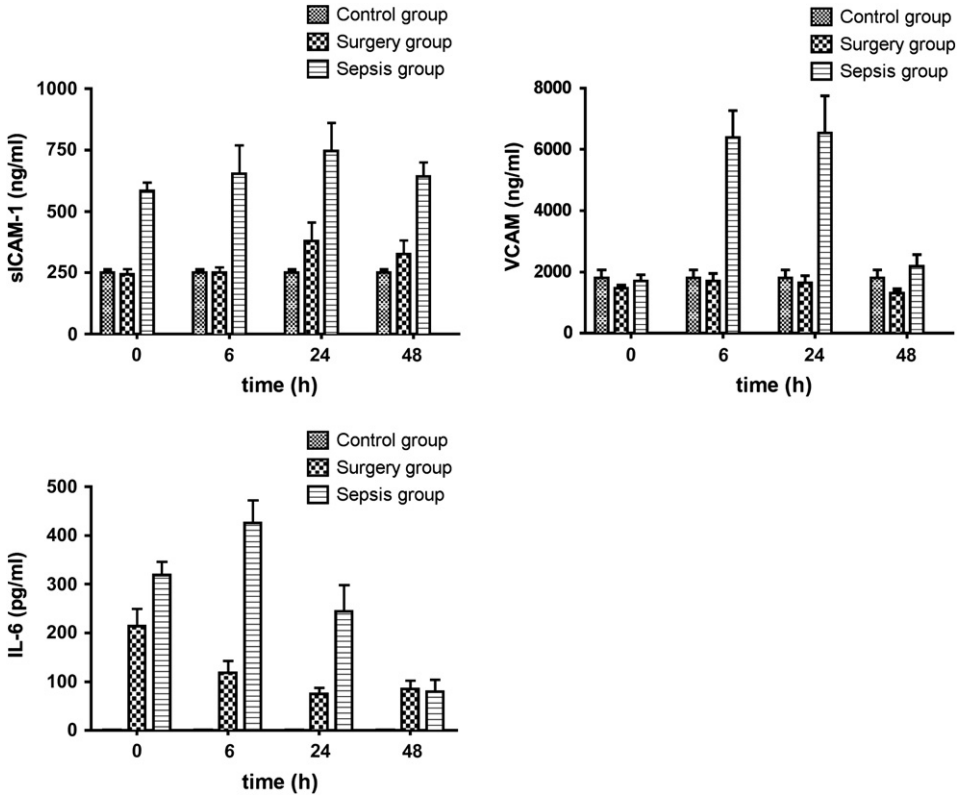

FIG. 2. Markers of glycocalix shedding. The concentrations of syndecan-1 and heparan sulfate are shown in the control, surgery, and sepsis groups. Blood samples were drawn 0, 6, 24, and 48 after surgery or diagnoses of sepsis. Levels of syndecan-1 were higher in the sepsis group compared with the surgery group. However, levels of heparan sulfate were higher in the surgery group compared with the sepsis group.

by the fact that heparan sulfate lines the inside of the glycocalix, while syndecan-1 is a transmembranous protein [7]. One could argue that heparan sulfate is shed first and more vigorously, while syndecan-1 is shed later, and indicates more substantial damage of the glycocalix. Keeping in mind that patients after major abdominal surgery also suffer from capillary leakage syndrome, flaking of the glycocalix appears to play a major role in this pathophysiology. Another explanation for the different pattern of heparan sulfate and syndecan-1 could be that heparan sulfate simply is shed so early that the peak has already passed before we were able to obtain the specimen from the patients. Furthermore, there could be different patterns of transcribing of syndecan-1 and heparan sulfate that may be triggered by nuclear factor κB production. To further investigate this issue, it will be necessary to conduct a study focusing on molecular changes of those markers, especially gene expression of syndecan-1 and heparan sulfate. Finally heparin, which is given widely to patients with sepsis, but not during surgery, could be responsible for the earlier breakdown of heparan sulfate in patients with sepsis. Therefore, syndecan-1 could be more specific in this patient population.

Therapeutic options for patients with sepsis are very limited. Besides antibiotics, there are few therapies proven to improve survival [16]. An integral part of sepsis therapy is the liberal administration of fluids with crystalloid or colloid solutions to maintain the patient's intravascular volume (measured by mean arterial blood pressure or central venous pressure), to counteract the extravasation of fluid due to capillary leakage syndrome. Patients with sepsis experience a breakdown of tight junctions between cells, leading to leaking of fluid into the interstitial tissue. We have now demonstrated that in patients with sepsis and in those after major abdominal surgery, there also occurs a significant breakdown of the endothelial glycocalix. This explains third spacing of fluids after major abdominal surgery, due to loss of the barrier function of the glycocalix from nonspecific breakdown. Given this finding, stabilization of the glycocalix could be a new target for patients with sepsis and those after major surgery. Of side note is that there were no differences between survivors and nonsurvivors in our data regarding shedding of the glycocalix.

The strong positive correlation between IL-6 and syndecan-1 indicates the connection between glycocalix flaking leading to capillary leakage and inflammation in general. This might further explain the formation of refractory edema in patients with sepsis. As expected, we also found a strong correlation between IL-6 and ICAM-1, VCAM-1, and lactate, which emphasizes the close association between these inflammatory markers.

A limitation of our study is that we did not directly visualize the glycocalix in tissue obtained from patients with sepsis or after surgery. However, given the specificity of heparan sulfate and syndecan-1 as markers for glycocalix breakdown, we feel it is justified to consider them as valid surrogates for glycocalix shedding [9, 15].

In summary, we have demonstrated that the endothelial glycocalix is significantly shed in patients with sepsis and those after major abdominal surgery. This novel finding could explain the nonspecific capillary leaking syndrome of patients after surgery and with sepsis. Therefore, this is a potential new target to treat those patient populations. Furthermore, the strong correlation between syndecan-1 and IL-6 indicates the connection between inflammation and nonspecific flaking of the glycocalix. Understanding these complex changes in sepsis and after major abdominal surgery is a key point to adequately treating patients and to developing new therapeutic strategies to improve the outcome of patients with sepsis and those after major surgery.

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