Alterations of conjunctival glycocalyx and microcirculation in non-septic critically ill patients

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ABSTRACT

As of now the relationship between glycocalyx degradation and microcirculatory perfusion abnormalities in non-septic critical ill patients is unclear. In addition, conjunctival sidestream dark field-imaging for the purpose of glycocalyx thickness estimation has never been performed. We aimed to investigate whether changes in gly-cocalyx thickness in non-septic patients are associated with microcirculatory alterations in conjunctival and sublingual mucosa. In this single-centre prospective observational study, using techniques for direct in-vivo observation of the microcirculation, we performed a single measurement of microcirculatory perfusion para-meters and visualized glycocalyx thickness in both ocular conjunctiva and sublingual mucosa in mixed cardio surgical (n = 18) and neurocritical patients (n = 27) and compared these data with age-matched healthy controls (n = 20). In addition we measured systemic syndecan-1 levels. In the sublingual and conjunctival region we observed a significant increase of the perfused boundary region (PBR) in both neuro-critical and cardiac surgical ICU patients, compared to controls. There was a significant increase of syndecan-1 in ICU patients comparing with controls and in cardiac patients comparing with neurological (120.0[71.0–189.6] vs. 18.0[7.2–40.7], p < 0.05). We detected a weak correlation between syndecan-1 and sublingual PBR but no correlations between global glycocalyx damage and conjuctival glycocalyx thickness. We found significantly lower perfused vessel density (PVD) of small vessels in sublingual mucosa in patients after cardiac surgery in comparison with healthy subjects. In neuro-critical, but not cardiac surgery patients conjunctival TVD and PVD of small vessels were found to be significantly lower in comparison with controls.

1. Introduction

The glycocalyx is a glycolipids, glycoproteins, and proteoglycans-containing structure that coats all healthy vascular endothelium at the luminal side. This gel-like layer provides a micro-environment for many important vascular processes and is seen as a gatekeeper for endothelial function (Reitsma et al., 2007). It regulates vascular permeability, acts as a mechano-transducer to transmit shear stress forces to endothelial cells and carries out vascular protection via the inhibition of coagulation and leucocyte adhesion (Schott et al., 2016). From its functions a close interaction between the glycocalyx and microcirculatory perfu-sion can be assumed. Indeed, in experimental studies an association between glycocalyx degradation and decreased functional capillary density was demonstrated (Marechal et al., 2008; Cabrales et al., 2007), and in patients undergoing cardiac bypass surgery, glycocalyx de-gradation closely related to microvascular perfusion (Koning et al., 2016). Experimental and human data seem to confirm this relationship between microvascular perfusion and integrity of the glycocalyx during sepsis (De Backer et al., 2013). However, the relation between glyco-calyx degradation and microvascular perfusion in non-septic critical ill patients is less clear (Donati et al., 2013).

Fifty years ago Luft et al. was the first to visualize the glycocalyx directly in experimental models (Luft, 1966). In clinical research there are two known methodologies to assess glycocalyx thickness: indirect

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visualization of the glycocalyx or tracking its degradation products in serum. With the introduction of in-vivo videomicroscopy techniques, such as sidestream dark field (SDF)-imaging, and subsequent development of specialised software (Broekhuizen et al., 2009) a method for indirect visualization of the glycocalyx became available in the clinical setting. Most of the SDF-derived images for observation of the glycocalyx are obtained from the sublingual region. In general, sublingual glycocalyx thickness correlates well with systemic markers of glycocalyx degradation. However, to what extend sublingual microvascular changes reflect cerebral microvascular alterations, is largely unknown. In comparison to healthy volunteers, patients with white matter lesions had increased sublingual perfused boundary region (PBR) (Martens et al., 2013). Others have suggested a potential role for the estimation of glycocalyx thickness in the sublingual region as a marker for cerebral microcirculatory and brain-blood-barrier function (Haeren et al., 2016). However, such assumption remains controversial, having in mind that the systemic and cerebral microcirculation may not be affected equally during shock, due to protective mechanism of autoregulation in the brain (Wan et al., 2009).

As of now, conjunctival SDF-imaging for the purpose of glycocalyx thickness estimation has not been performed. It is conceivable that evaluation of conjunctival glycocalyx thickness and microcirculatory blood flow could be more sensitive to the changes in the cerebral microcirculation, due to its close anatomical proximity and common circulation route (Tamosuitis et al., 2016).

In this study we aimed to investigate two main objectives: 1. To what extend is glycocalyx thickness affected in critically ill patients under non-septic conditions? 2. Is the conjunctival and sublingual glycocalyx equally affected in a clinical model of systemic and cerebral perfusion abnormalities?

2. Material and methods

2.1. Setting and patient population

This prospective observational study was performed in an 18-bed neurosurgical ICU and in an 18-bed cardiac surgery ICU in a tertiary teaching hospital. The study was carried out in compliance with the Helsinki Declaration and approved by the Ethics Committee of Lithuanian University of Health Sciences and by the Kaunas Regional Biomedical Research Ethics Committee (number BEC-MF-30; BE-2-17). Informed consent was given by the patients or their next-of-kin, according to applicable laws. Exclusion criteria were the presence of sepsis, oral bleeding, age < 18 years or advanced malignancy. Demographic and general data were collected. Forty-five ICU patients were subject to a single evaluation of the sublingual and conjunctival microcirculation and microvascular glycocalyx during their ICU stay. The control group consisted of twenty healthy volunteers with no reported ocular pathology. After a 30 minute resting time hemodynamic parameters and ocular and sublingual microvascular evaluation were performed. None of the control group individuals was locally anaesthetized; none of them reported any discomfort related to the ocular conjunctival evaluation.

2.2. Evaluation of the microcirculation

Sublingual and conjunctival microcirculation images were obtained using a Cytocam®-IDF device (Braedius Medical, Huizen, The Netherlands). This device is technically and optically optimized for visualization the microcirculation on the surfaces of organs. IDF-imaging is based on the principle that emitted green light (wavelength 530 nm) is absorbed by the hemoglobin content in red blood cells. Therefore, red blood cells are seen as black or gray bodies during imaging. The vessel walls are not visualized, so vessels can only be detected by the presence of red blood cells (Goedhart et al., 2007). A recently published validation study demonstrated that Cytocam-IDF

imaging yielded better image quality than SDF-imaging (Aykut et al., 2015).

All conjunctival microcirculatory images were obtained in accordance with a local research protocol, selecting the nasal side of the conjunctival area. This allows steady imaging by carefully using the patient's nose as a resting point for the hand of the camera operator. Selecting the same conjunctival area is also done to avoid large variations in microcirculatory parameters (van Zijderveld et al., 2014).

We followed published expert recommendations for quality and analysis of obtained images (De Backer et al., 2007). Steady images from at least three areas were acquired and stored on a computer. Image clips were exported for analysis using validated AVA® v3.0 software (Microvision Medical, Amsterdam, The Netherlands) (Dobbe et al., 2008). Four investigators blindly analyzed the video clips offline in random order to prevent coupling. Each image was divided into four equal quadrants. Flow in small vessels was classified semi-quantitatively (no flow: 0; intermittent flow: 1; sluggish flow: 2; continuous flow: 3). We calculated the microvascular flow index (MFI) as the sum of each quadrant score divided by the number of quadrants in which the vessel type was visible. We calculated the total vessel density (TVD) of small vessels using the AVA software package and a cut-off diameter for small vessels (mostly capillaries) of < 20 µm. Perfused small vessel density (PVD) was calculated as the number of crossings of perfused small vessels per total length of three equidistant horizontal and vertical lines.

2.3. Glycocalyx evaluation: measurement of the perfused boundary region (PBR)

The PBR includes the dimension of the permeable part of the endothelial glycocalyx, which allows the penetration of flowing red blood cells (RBC). Erythrocytes usually have limited access into an intact glycocalyx. When the integrity of the glycocalyx is compromised and starts losing its protective capacity, its permeability increases, allowing circulating cells to approximate the luminal endothelial membrane. Glycocalyx degradation leads to a deeper penetration of circulating RBCs towards the endothelial surface, resulting in an increased PBR (Vink and Duling, 1996). An SDF videomicroscope (Microscan®, Microvision Medical, Amsterdam, The Netherlands) connected to a glycocalyx measurement system (GlycoCheck ICU®, Maastricht University Medical Center, Maastricht, The Netherlands) was used to visualize the sublingual and conjunctival microcirculation. A series of 10 video sequences (40 frames each) were recorded and automatically analyzed to calculate the PBR, as described elsewhere (Martens et al., 2013). In each subject, GlycoCheck ICU® analyzing software automatically measured the RBC column in at least 3000 vessel segments. For each measurement segment, 840 radial intensity profiles were obtained for measurement of RBC column width, and PBR was calculated as the distance of median (P_{50}) RBC column width to the (estimated) outer edge of the RBC-perfused lumen. Vessel segments were classified in 1-µm-wide diameter classes and median PBR values were determined for each diameter class before calculating the average PBR over the 5- to 25-µm diameter range.

2.4. Serum measurements of glycocalyx damage marker

Concentrations of syndecan-1 (Human Syndecan-1(CD138) ELISA kit, BioVendor, Brno, Czech Republic) were measured in serum by using the corresponding ELISA kits in accordance with the instructions of the manufacturer.

3. Statistics

Data were analyzed with Statistical Package for Social Sciences (SPSS 22 for Windows, Chicago, USA). With respect to small numbers, data are presented as the median [25th–75th percentiles] and analyzed

Table 1
General data.

	All patients (n = 45)	Neuro critical care (n = 27)	Cardiac surgery (n = 18)
Age (years)	63[49–74]	65[48–76]	60[48–71]
Gender (n male; n female)	28; 17	17; 10	11; 7
Admission diagnosis			
Surgery	27	9	18
Stroke	11	11	
SAH	7	7	
Time after admission (hours)	24[6–72]	30[3–84]	24[3–49]
APACHE II score	17[13 – 23]	17[11 – 23]	17[13–27]
SOFA score	5[3–7]	5[3–7]	6[3–9]
Temperature (°C)	36.7[36.1–36.8]	36.8[36.5–37.0]*	36.2[35.9–36.5]
Heart rate (bpm)	77[70–94]	79[70–93]	74[63–96]
Mean arterial pressure (mm Hg)	88[74–99]	92[77–103]*	78[71–84]
Cardiac index (L/min/m^2)	2.5[2.0–3.1]	2.7[2.2–3.2]*	1.8[1.2–2.2]
PaO2/FiO2	320[235–391]	323[234–395]	310[268–400]
Creatinine (mmol/L)	84[74–144]	80[70–137]	94[80–172]
Bilirubin (mmol/L)	12.0[7.3–24.8]	12.0[7.3–24.8]	11.0[6.0–15.0]
Glasgow Coma Scale	15[7–15]	10[7–15]*	15[15–15]
Platelet count ($*10^9$/L)	212[157–244]	217[156–248]	202[165–240]
Leukocyte count ($*10^9$/L)	10.0[7.2–13.4]	10.8[9.1–13.4]	8.5[6.0–13.8]
Lactate (mmol/L)	0.9[0.7–1.5]	0.9[0.7–1.25]	1.6[0.9–2.2]
Norepinephrine (n; μg/kg/min)	3; 0.06[0.02–0.08]	0; 0	3; 0.06[0.02–0.08]
Adrenaline (n; μg/kg/min)	3; 0.05[0.03–0.06]	0; 0	3; 0.05[0.03–0.06]
Dopamine (n; μg/kg/min)	3; 3.0[2.0–4.0]	0; 0	3; 3.0[2.0–4.0]
Crystalloids (mL)[a]		1000[700–1300]	1000[1000 – 2000]
Glucocorticoids (n, %)		1; 4	0; 0

SAH subarachnoid hemorrhage; APACHE acute physiology and chronic health evaluation; SOFA sequential organ failure assessment.

  * p < 0.05 in comparison to cardiac surgery patients.

  [a] Total amount administered within 24 h before the glycocalyx assessment.

with non-parametric tests. Within-group differences were tested with a Mann–Whitney *U* test. Correlations between changes in glycocalyx dimensions and microcirculation parameters were tested with Pearson correlations test. A p value of < 0.05 was considered significant.

4. Results

4.1. General data

Baseline characteristics of 45 ICU patients are shown in Table 1. Twenty-seven patients had a non-infectious neurological disorder as primary reason for ICU admission; eighteen patients were admitted after cardiac surgery. All cardiac surgery patients were subject to non-pulsatile cardiopulmonary bypass (CPB)-assisted surgical procedures. No significant difference was found in general characteristics between these subgroups, with the exception of the cardiac index, mean arterial blood pressure, Glasgow Coma Scale and temperature. Six patients after cardiac surgery were on vasopressors.

4.2. Microcirculation

We found a significantly lower PVD of small vessels in the sublingual mucosa of patients after cardiac surgery in comparison with

healthy subjects (Table 2). In neuro-critical, but not in cardiac surgery patients, conjunctival TVD and PVD of small vessels were found to be significantly lower in comparison with controls (Table 2). We did not find any changes in other microcirculation parameters in sublingual mucosa of both subgroups. The association between the Glasgow Coma Scale and conjunctival TVD was non-significant (r = 0.254; p = 0.266).

4.3. Glycocalyx

The overall ICU population and both subgroups separately showed a significant increase of syndecan-1, sublingual and conjunctival PBR in comparison with age-matched healthy subjects (Table 3, Fig. 2). Patients after cardiac surgery showed a higher sindecan-1 value compared to neuro-critical care patients (Table 3).

4.3.1. Correlations between parameters of microvascular perfusion and glycocalyx thickness

In the total ICU population a significant, but weak correlation was found between syndecan-1 and sublingual PBR (r = 0.40, p = 0.002). No correlations between sindecan-1 and conjunctival PBR, as well as between sublingual and conjunctival PBR were observed. However, a significant correlation was found between syndecan-1 and conjunctival TVD of small vessels (r = 0.63, p < 0.0001, Fig. 1) and conjunctival

Table 2
Microvascular variables in ICU patients and healthy subjects.

	Neuro-critical care (n = 27)		Cardiac surgery (n = 18)		Healthy volunteers (n = 20)	
	Sublingual	Conjunctival	Sublingual	Conjunctival	Sublingual (n = 20)	Conjunctival (n = 12)
TVD s (mm/mm^2)	20.9[17.1–23.9]	12.4[10.3–17.9]*	17.4[12.6–19.7]	15.7[14.3–18.6]	17.7[16.3–18.2]	16.5[15.6–18.3]
PVD s, (1/mm)	14.0[12.4–15.7]	6.8[6.0–10.8]*	11.2[9.3–12.5]*,[a]	8.7[7.2–12.0]	15.7[13.8–17.5]	10.9[9.2–11.8]
PPV s, (%)	97.6[95.0–99.3]	100[96.2–100]	98.5[92.9–100]	94.4[90.0–97.7]	94.8[92.4–98.5]	98.0[97.0–99]
MFI s	3.00[2.87–3.00]	3.00[3.00–3.00]	3.00[2.93–3.00]	3.00[2.60–3.00]	3.00[2.80–3.00]	3.00[3.00–3.00]

TVD total vessel density; PVD perfused vessel density; PPV percentage of perfused vessls; MFI microvascular flow index; s small vessels (< 20 μm).

  * p < 0.05 in comparison to healthy controls.

  [a] p < 0.05 in comparison to neuro-critical care patients.

Table 3
Perfused boundary region in ICU patients and healthy subjects.

	All ICU patients	Neuro-critical care	Cardiac surgery	Healthy volunteers
Syndecan-1 (ng/ml)	23.6[11.6–101.6]*	18.0[7.2–40.7]*	120.0[71.0–189.6]*,a	8.2[2.4–15.1]
PBR s (μm)	2.20[2.04–2.42]*	2.15[2.00–2.39]*	2.35[2.18–2.53]*	1.76[1.63–2.08]
PBR c (μm)	2.19[2.01–2.36]*	2.20[2.03–2.34]*	2.1[1.80–2.50]*	1.70[1.61–2.00]

PBR perfused boundary region; s sublingual; c conjunctival.

* p < 0.05 in comparison to healthy subjects.
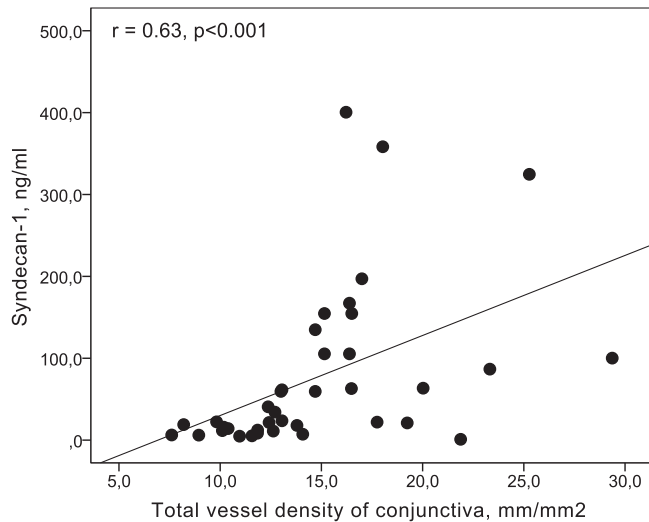a p < 0.05 in comparison to neuro-critical care patients.

Fig. 1. Correlation between conjunctival total vessels density of small vessels and syndecan-1 in all ICU patients.
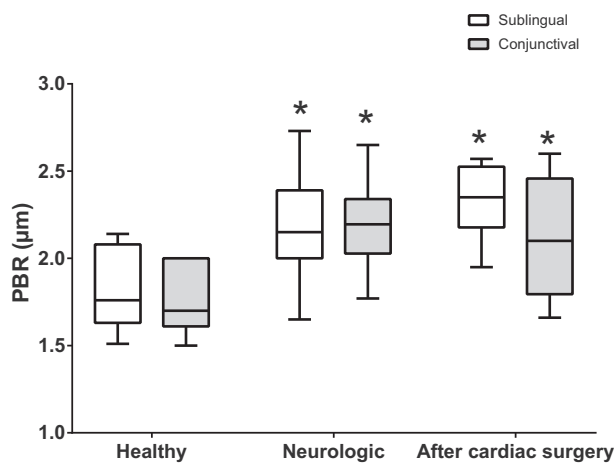

Fig. 2. Perfused boundary region in all ICU patients and healthy volunteers.
* p < 0.05 in comparison to healthy subjects.

PVD of small vessels (r = 0.59, p < 0.0001).

5. Discussion

To the best of our knowledge we are the first to evaluate conjunctival glycocalyx thickness and to compare this with systemic markers of glycocalyx shedding as well as with variables of sublingual microvascular perfusion in (subsets of) non-septic ICU patients. Compared to healthy controls we observed a significant increase in conjunctival PBR, sublingual PBR and systemic syndecan-1 in all ICU patients, suggesting loss of glycocalyx integrity, irrespective of the reason for ICU admission. In addition, markers of altered conjunctival microvascular perfusion were only present in neuro-critical care patients, whereas markers of altered sublingual microvascular perfusion were restricted to post-cardiac surgery patients. These observations are in line with the idea that the microvascular effects of glycocalyx shedding (as detected with elevated systemic syndecan-1 levels and increased PBR) is organ or site specific. Substantial rises in syndecan-1 levels after cardiac surgery are not accompanied by changes in conjunctival microvascular perfusion, despite significant loss of conjunctival glycocalyx integrity. On the other hand, shedding of glycocalyx during cerebral insults is not accompanied by alterations in the sublingual microcirculation. Such findings suggest a multifactorial origin of microvascular alterations under conditions of glycocalyx shedding.

Koning et al. demonstrated an increase in PBR immediately after closure of the sternal wound during non-pulsatile CPB, as opposed to pulsatile CPB-assisted cardiac surgery (Koning et al., 2016). Our results confirm the presence of sublingual microvascular flow alterations and markers of systemic glycocalyx damage in patients after non-pulsatile CPB-assisted surgery. Martens et al. found an increase in PBR of the sublingual microvascular glycocalyx in lacunar stroke patients with white matter lesions but not in lacunar stroke patients per se (Martens et al., 2013). However, in our study signs of glycocalyx injury were present in all neuro-critical care patients, irrespective of the specific underlying disease state. Our data suggest that glycocalyx damage may be present in multiple sites, in case systemic biomarkers of glycocalyx are elevated in critically ill patients. We showed that the conjunctival area is another easy approachable site for microscopic assessment of glycocalyx thickness. Although experimental studies show associations between glycocalyx damage and microcirculatory alterations (Cabrales et al., 2007; Marechal et al., 2008), our observations highlight the importance of the underlying mechanisms. Decreased sublingual functional capillary density can be explained by the more severe systemic glycocalyx damage in the group of patients after cardiac surgery in comparison to neurologic critical ill patients. Loss of endothelial glycocalyx volume may lead to increased permeable plasma volume and loss of vascular autoregulation (Cabrales et al., 2007). This is accompanied by redistribution of RBC flow in the capillary network, leading to a significant increase in the presence of RBC-empty plasma flowing through capillaries, as well as increased distance between capillaries. The significant difference in Syndecan-1 between subgroups could be explained by a complex of mechanisms of systemic injury to endothelium in cardiac patients. Contributing factors to this systemic endothelial injury include ischemia/reperfusion following major cardiac surgery (Rehm et al., 2007) and cardiogenic shock or low cardiac output with elevated adrenalin and noradrenalin levels (Ostrowski et al., 2013). In contrast, all neuro-critical care patients were hemodynamically stable with a significantly better cardiac output. According to the more mild elevation of syndecan-1 levels, their endothelial damaged was more regional and related to cerebral hypoperfusion. This is not in contrast with the existing concept of the conjunctival microcirculation as a mirror of cerebral blood flow, as suggested in both animal models (Ohtani, 1996) and clinical research in various conditions (Schaser et al., 2003). However, it must be stressed the common origin of brain circulation and conjunctival circulation from the

internal carotid is not enough to assume that the two vascular beds will automatically respond similarly during various disease states. The preservation of conjunctival blood flow in angiography-confirmed brain dead patients exemplifies the complexity of the interplay between the circulation of the brain and conjunctiva (Tamosuitis et al., 2016).

The main limitation of this study is related to the fact, that assessment of glycocalyx was performed with SDF-imaging, whereas microcirculatory perfusion was observed with IDF-imaging. Since SDF-imaging is reported to yield lower resolution in comparison to SDF, it is conceivable that glycocalyx shedding has been more pronounced than observed with the current technology. However, as of now, SDF-derived PBR measurement is the only available clinical tool for assessment of glycocalyx thickness. In addition, it must be underlined that this technique provides indirect markers of glycocalyx degradation. Direct in-vivo measurement is simply not yet feasible. Furthermore, it must be stressed that the observed associations between markers of glycocalix degradation between two organs (e.g. conjunctiva and brain) do not allow to conclude that the two compartments are equal. Further research is needed to elaborate on the underlying mechanisms. Lastly, we acknowledge that we cannot provide quantitative markers of cerebral (vascular) lesions.

6. Conclusions

In non-septic ICU patients we observed signs of conjunctival microvascular glycocalyx degradation. Conjunctival microcirculatory perfusion abnormalities were present in patients with cerebral pathology, whereas sublingual microvascular alterations occured during systemic endothelial damage. Alternatively, syndecan-1 levels during cerebral pathology were simply not high enough to provoke sublingual microvascular alterations. These results merit further investigation.

Author's contribution

AP was responsible for data acquisition, SDF imaging, image blinding and analysis and design of the study; he wrote the first draft; TT, NB, DD performed SDF imaging and analysis, TT wrote some aspects of the first draft; ESN, AV, ES critically revised the manuscript and participated in data interpretation; VP participated in the design of the study; CB contributed to the design of the study and wrote the final manuscript. All authors read and approved the final manuscript.

Conflicts of interest

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

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