Poor microcirculatory flow dynamics are associated with endothelial cell damage and glycocalyx shedding following traumatic hemorrhagic shock

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ABSTRACT

Background

Endothelial cell damage and glycocalyx shedding following trauma can increase the risk of inflammation, coagulopathy, vascular permeability and death. Bedside sublingual video-microscopy may detect worse flow and perfusion associated with this endotheliopathy. We compared markers of endotheliopathy with physical flow dynamics following traumatic hemorrhagic shock (THS).

Methods

Sublingual incident dark field video-microscopy was performed at three time-points following injury (<10h; 10–30h; and 30–50h). Values for microcirculatory flow index (MFI), Point Of carE Microcirculation assessment (POEM) score, proportion of perfused vessels (PPV), microcirculatory heterogeneity index (MHI), perfused vessel density (PVD), and total vessel density (TVD) were obtained. ELISAs were performed to measure concentrations of thrombomodulin and syndecan-1 as biomarkers of endothelial cell damage and glycocalyx shedding respectively. Flow parameters were dichotomised to above and below average, and biomarkers compared between groups; below average MFI, POEM, PPV, PVD, and TVD, and above average MHI were considered poor microcirculatory flow dynamics.

Results

155 sublingual video-microscopy clips corresponding to 39 time-points from 17 trauma patients were analysed. Median age was 35 (IQR 25–52); 16/17 were male.

Within 10h of injury, syndecan-1 concentrations were significantly higher compared to 17 age and sex-matched healthy controls (30 (IQR 20–44) ng/ml) for worse TVD (78 (IQR 63–

417) ng/ml); PVD (156 (IQR 63–590) ng/ml); PPV (249 (IQR 64–578) ng/ml); MFI (249 (IQR 64–578) ng/ml); MHI (45 (IQR 38–68) ng/ml); and POEM scores (108 (IQR 44–462) ng/ml) (all $p<0.01$). Thrombomodulin was also raised within 10 hours of injury when compared to healthy controls (2.9 (IQR 2.2–3.4) ng/ml) for worse PPV (4.1 (IQR 3.4–6.2) ng/ml) and MFI (4.1 (IQR 3.4–6.2) ng/ml) (both $p<0.05$).

Conclusions

Endothelial cell damage and glycocalyx shedding are associated with worse flow, density, and heterogeneity within micro-vessels following THS. The clinical utility of these biomarkers and flow parameters at the bedside are yet to be elucidated.

Study type / level of evidence

Prognostic study, Level III

Key words

Endothelium, glycocalyx, trauma, hemorrhage, microcirculation

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Running head: Endotheliopathy and the microcirculation

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Conflicts of interest

All authors declare that they have no conflict of interest. Although some authors have current or previous affiliation to the UK Defence Medical Services, their opinions are their own, and do not necessarily represent those of the UK Defence Medical Services.

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BACKGROUND

There has been considerable interest in the endotheliopathy of trauma, since it is associated with increased vascular permeability[1], is an independent predictor of mortality[2], and may be a key determinant in the development of inflammation and coagulopathy[3]. In particular, endothelial glycocalyx shedding may have a role in microcirculatory dysfunction due to its essential role in the integrity and function of the oxygen exchange surface of all micro-vessels[4, 5]. Endotheliopathy of trauma is associated with sympathoadrenal activation[6, 7], and may be induced by the action of damage-associated molecular patterns (DAMPs) that are released into the circulation following tissue injury[8, 9], as well as raised levels of pro-inflammatory cytokines responsible for disruption of endothelial integrity[10]. Aside from the inflammatory and coagulopathic consequences of endotheliopathy, there may also be derangements in flow dynamics within the micro-vessels so that oxygen perfusion is inadequate, leading to hypoxia and acidosis. Prior to the current study, the relationship between endotheliopathy and microcirculatory flow dynamics following traumatic hemorrhagic shock (THS) has not been investigated.

Away from the patient, enzyme-linked immunosorbent assays (ELISA) can be performed for surrogate markers of endotheliopathy, such as thrombomodulin (CD141) and syndecan-1 (CD138). Thrombomodulin is a transmembrane glycoprotein in endothelial cells that acts as a cofactor during activation of Protein C by thrombin[6, 11]. It is not secreted by endothelial cells, but is present in the circulation due to endothelial cell damage[12]. This makes it a useful biomarker of endothelial injury, and has been used in this role for patients following sepsis[6, 13], myocardial infarction[14], cardiac arrest[15], and trauma[2, 7, 16]. Syndecan-1 is a transmembrane heparan sulfate glycoprotein whose extracellular domain forms part of the endothelial

glycocalyx. It has been used as a biomarker for glycocalyx shedding for patients following trauma[2, 10, 17].

At the bedside, non-invasive video-microscopy can be used to visualise the flow of red blood cells in the sublingual microcirculation. The latest incident dark field technology allows for the acquisition of high definition views of the flow and perfusion of these micro-vessels whose primary function is oxygen and substrate exchange. This technique has been used to examine the response of the microcirculation to critical illness, sepsis and shock[18]. More recently, investigators have demonstrated a prognostic value in this technology for hemorrhagic trauma[19], in particular in the detection of loss of hemodynamic coherence[20]. There have been no clinical studies that have tested whether glycocalyx shedding and endothelial cell damage are associated with poorer flow dynamics in the microcirculation.

The aim of the current study was to test whether endotheliopathy following traumatic hemorrhagic shock was associated with physical changes in the flow, density and perfusion of vessels in the microcirculation, from initial resuscitation to stabilization in the Intensive Care Unit (ICU). We hypothesized that there would be an association between endotheliopathy and poor flow dynamics of the microcirculation.

METHODS

Study design

A prospective longitudinal observational study was conducted in order to compare flow dynamic parameters of the microcirculation with serum markers of endothelial damage and glycocalyx shedding. All data are from a single site in the MICROSHOCK study[21]. This study had been granted prior ethical approval (Research Ethics Committee reference: 14/YH/0078; Yorkshire and the Humber Leeds West), and a protocol was published in advance[21].

Patient selection

Patients who were potentially eligible for inclusion at a single Major Trauma Center (University Hospitals Birmingham, UK) were identified through the screening of trauma activations. Trauma patients were eligible for inclusion if they required blood products, had been intubated, required admission to ICU, and had a point-of-care lactate of greater than 2mmol/l. They were enrolled into the study either in the Emergency Department (ED) or ICU depending on suitability for study observations. Selection bias was minimized by ensuring that there were no significant differences between patients enrolled in the study and those that were eligible but not included.

Data collection

Patient demographics (age, gender), injury details (including mechanism and timings), physiological parameters (heart rate and systolic blood pressure), and Glasgow Coma Scale (GCS) were recorded prospectively, and then corroborated using a combination of electronical and paper medical records. The most recent point of care lactate reading to each

time-point was also recorded, as well as the number and type of blood products used during the study period. Injury Severity Scores (ISS) were obtained from the Trauma Audit and Research Network, a central validated resource in the UK. Outcomes recorded for patients included ICU-free and hospital-free days (calculated by recording the number of days that the patient was present in ICU and hospital respectively during a period of 30 days), mortality within 30 days, and Sequential Organ Failure Assessment (SOFA) scores at days 3, 6, and 10.

Sublingual video-microscopy

A single operator (D.N.N.) conducted incident dark field (IDF) video-microscopy in order to acquire non-invasive video clips of the sublingual microcirculation (Cytocam, Braedius Medical B.V., Huizen, The Netherlands). Briefly, the camera was placed gently under the tongue until a clear view of the mucosal microcirculation was acquired, without blood or saliva artefact, with minimal pressure, and optimal focus and illumination according to consensus quality requirements for this technology[22]. Multiple clips of 100 frames each were recorded and stored on a computer for analysis. At least 5 good quality clips were recorded at each time-point in order to facilitate optimal analysis[23].

Microcirculatory analysis

Videos that had been acquired by IDF video-microscopy were exported for analysis and were individually graded for quality according to the most commonly reported domains (illumination, focus, content, duration, pressure, and stability)[24]. Of the highest quality clips, 3-5 from each patient time-point were kept for analysis. These clips were allocated random numbers to ensure that their analysis was blinded to patient, time-point, and clinical status. Semi-automated analysis was undertaken for each clip using dedicated computer software (Automated Vascular Analysis V.3.2, Microvision Medical, The Netherlands). All videos

were then un-blinded, and the time-points assigned average values for total vessel density (TVD, mm/mm^2), perfused vessel density (PVD, mm/mm^2), proportion of perfused vessels (PPV, %), microcirculatory flow index (MFI), and microcirculatory heterogeneity index (MHI) according to consensus guidelines for reporting sublingual microcirculation[23]. In addition, point of care microcirculation (POEM) scores (a composite score for flow and heterogeneity)[25] were allocated for each time-point. The methodology for the allocation of POEM scores is described in detail elsewhere[25]. In summary, flow is defined as "normal", "impaired", or "critical" for a video clip if <25%, 25–50%, or >50% of the vessels in the visual field respectively are sluggish or stopped. In addition, heterogeneity is either "present" or "absent" if >5 or <5 vessel segments have different flow to the remainder. This is repeated 4 times, and an algorithm calculates the overall score based on the majority of responses. A score is allocated from 1 (worst) to 5 (best).

Serum sampling

At the same time-point at which video-microscopy was performed, a 6 ml sample of peripheral blood was taken from an available venous access point. After a 30-minute incubation at room temperature, samples were spun at 1,620 x g for 10 minutes at 4^{o}C, after which aliquots of serum were stored in cryotubes at -80^{o}C. Serum samples were collected and stored in the same manner for 17 healthy volunteers, matched for age and sex to the patient cohort.

Enzyme-linked immunosorbent assays

Commercially available ELISAs were used according to their relevant protocols for the measurement of serum concentrations of syndecan-1 (CD 138; Abcam; product code ab46506, Cambridge, MA) as a surrogate marker of endothelial glycocalyx shedding, and

thrombomodulin (CD 141; Abcam; product code ab46508, Cambridge, MA) as a surrogate marker of endothelial cell damage.

Data analysis

Continuous data were tested for normality using the Shapiro-Wilk test. Normal data are presented as mean and standard deviation (SD), and non-normal data are presented as median and interquartile range (IQR). Spearman's rank correlation coefficient was used to determine the correlation between thrombomodulin and syndecan-1 concentrations for all patients, as well as the correlation between each of the biomarker concentrations and flow dynamics with lactate. Patient time-points were calculated from time of injury, and were divided into three groups: (i) <10 hours; (ii) 10 – 30 hours; and (iii) 30 – 50 hours since injury.

It is well established in previous studies of microcirculatory flow dynamics that healthy volunteers score near-maximum values of the traditional parameters of choice (such as PPV close to 100%, MFI equal to or close to 3.0, and heterogeneity near to 0)[26-28]. Comparison of flow dynamics between healthy controls and critically unwell patients is therefore unlikely to yield surprising or meaningful results. Of greater interest would be the difference between patients within the critically unwell cohort. In order to test the association of flow dynamics and endothelial cell damage and glycocalyx shedding amongst the injured cohort in the current study, flow dynamic parameters were dichotomised above and below the average value at each time-point (the median for non-normal data and the mean for normal data) in a manner similar to that previously reported for microcirculatory parameters[29]. The "worse" group were considered to have poor microcirculatory flow dynamics. Concentrations of syndecan-1 and thrombomodulin were compared between these "above" and "below" average groups. Multiple pairwise comparisons of biomarkers between patients and healthy controls were made using Dunn's multiple comparison's tests. Comparison of flow dynamics

between time points was made using the Skillings-Mack test for repeated measures of non-normal data that allows for some missing values. A *p*-value of <0.05 was considered statistically significant. Box and whisker graphs are presented according to the Tukey method, with horizontal bars as the median value, boxes as the interquartile range, and the upper and lower whiskers being 1.5 times the interquartile range above and below the 75th and 25th percentile respectively.

RESULTS

Patient characteristics

Analysis was performed for 155 sublingual video-microscopy clips that corresponded to 39 time-points from 17 trauma patients. Six patients had data from all three time points, 10 had data from two time points, and one patient died after the first time point. Patient characteristics are shown in Table 1.

Endothelial cell damage and glycocalyx shedding

When pairs of thrombomodulin and syndecan-1 concentrations for all healthy controls and patients were compared, there was a significant correlation between them (*rho* = 0.634; $p < 0.001$) (Supplementary Digital Content Figure 1), indicating an association between glycocalyx shedding and endothelial cell damage.

Endotheliopathy, flow disruption and perfusion

Thrombomodulin and syndecan-1 were both significantly correlated with lactate concentrations (Figure 1) (*rho* = 0.331; $p < 0.05$; and *rho* = 0.440; $p < 0.01$ respectively),

indicating that endotheliopathy is associated with perfusion mismatch. When flow dynamics were compared to lactate concentrations, there were significant correlations with PVD (*rho* = -0.416; $p < 0.01$); PPV (*rho* = -0.440; $p < 0.01$); MFI (*rho* = -0.464; $p < 0.01$); MHI (*rho* = 0.363; $p < 0.05$); and POEM score (*rho* = -0.440; $p < 0.01$). There was no significant correlation with TVD (*rho* = -0.312; $p = 0.053$).

Glycocalyx shedding and endothelial cell damage over time

Figure 2 illustrates the concentrations of syndecan-1 and thrombomodulin for all patients over time. When syndecan-1 concentrations were compared with healthy controls (30 (IQR 20 – 44) ng/ml), they were significantly higher at the 10h (63 (IQR 41 – 296) ng/ml), 10-30h (61 (IQR 38 – 109) ng/ml) and 30-50h (51 (IQR 34 – 90) ng/ml) time-points. In contrast, there were no significant differences between thrombomodulin concentrations for healthy controls (2.9 (IQR 2.2 – 3.4) ng/ml) and patients at the <10h (3.7 (IQR 2.8 – 4.7) ng/mL), 10-30h (3.9 (IQR 3.4 – 4.5) ng/ml), or 30-50h (2.9 (IQR 2.4 – 6.0) ng/ml) time-points.

Flow parameters improve over time

Flow dynamics for all three time-points are illustrated in Figure 3. There was a significant improvement over time for measures of flow (MFI; $p < 0.001$), perfusion (PPV; $p < 0.001$), heterogeneity (MHI; $p < 0.001$), and combined flow and heterogeneity (POEM score; $p < 0.01$). There were no significant differences over time for measures of total or perfused vessel density (TVD ($p = 0.276$) and PVD ($p = 0.389$) respectively).

Glycocalyx shedding and flow dynamics

Figure 4 illustrates the concentrations of syndecan-1 at each time-point relative to flow dynamic parameters. Higher heterogeneity of flow (MHI), as well as worse perfusion (PPV)

and flow (MFI) were associated with glycocalyx shedding at all three time-points. The most significant differences were observed within 10h of injury; at this time-point, syndecan-1 concentrations were significantly higher than healthy controls (30 (IQR 20 – 44) ng/ml) when there was worse TVD (78 (IQR 63 – 417) ng/ml); PVD (156 (IQR 63 – 590) ng/ml); PPV (249 (IQR 64 – 578) ng/ml); MFI (249 (IQR 64 – 578) ng/ml); MHI (45 (IQR 38 – 68) ng/ml); and POEM scores (108 (IQR 44 – 462) ng/ml) (all $p<0.01$). Below average TVD and PVD were associated with glycocalyx shedding at <10h and 10-30h (all $p < 0.05$), but not at the 30-50h time-points.

Endothelial cell damage and flow dynamics

Figure 5 illustrates the concentration of thrombomodulin at each time-point relative to flow dynamic parameters. In contrast with syndecan-1, thrombomodulin was only raised within 10 hours of injury when compared to healthy controls (2.9 (IQR 2.2 – 3.4) ng/ml) for worse PPV (4.1 (IQR 3.4 – 6.2) ng/ml) and MFI (4.1 (IQR 3.4 – 6.2) ng/ml) (both $p<0.05$). Below average density (TVD) and functional density (PVD) were associated with endothelial damage only at the 10-30h time-point (3.7 (IQR 3.3 – 3.9) ng/ml and 3.7 (IQR 3.3 – 3.9) ng/ml respectively), but not at the <10h or 30-50h time-points. No associations were found between endothelial cell damage and heterogeneity of flow (MHI) or POEM scores (*p* values all non-significant).

DISCUSSION

We present for the first time an association between endotheliopathy and impaired microcirculatory perfusion following traumatic hemorrhagic shock, the effects of which were most striking within 10 hours of injury. The correlation between serum lactate readings and both endothelial biomarkers and microcirculatory flow dynamics suggests a concurrent oxygen perfusion deficit associated with endotheliopathy and microcirculatory flow disruption. Deterioration in flow may represent one of the mechanisms of microcirculatory dysfunction attributable to endotheliopathy, and poor flow dynamics following shock may be responsible for worsening endotheliopathy. Although the current study does not attribute causality, it is likely that poor flow, glycocalyx shedding and endothelial cell disruption are mutually detrimental, and together contribute to derangement in oxygen exchange, inflammatory dysregulation and coagulopathy following trauma and hemorrhagic shock.

Other investigators have reported a strong association between injury severity, catecholamine-driven sympathoadrenal response, hypocoagulability, and raised levels of syndecan-1 and thrombomodulin as markers of glycocalyx shedding and endothelial cell injury[2, 30]. Trauma-induced coagulopathy has been observed in patients with glycocalyx shedding, and it has been postulated that endogenous autoheparinization may be responsible for this[31], as well as the generation of thrombin and activation of protein C[32]. The term "shock-induced endotheliopathy" (SHINE) has been used to describe the same phenotype observed in several different critical illnesses characterised by shock[33]. These observed effects of endotheliopathy may be considered in the context of the microcirculatory flow disruption observed in the current study, which are likely to exacerbate the pathologic process by reducing the efficient flow of red cells and oxygen delivery to tissues.

It is notable that most of the flow parameters showed dramatic improvement over the three time-points (Figure 3), and yet the glycocalyx does not appear to have been restored to normality according to the levels of syndecan-1 (Figure 2). Although the median levels of syndecan-1 improved over time, they were still raised above that of healthy controls, a finding in keeping with a previous study[10]. Restoration of flow parameters may be a reflection of a pressure-based resuscitation strategy and goal-directed therapy aimed at restoring oxygen perfusion and reducing acidosis, rather than any therapy deliberately targeted at repairing the endothelium. The exact consequences of incomplete endothelial restoration are unknown, but it seems likely that any inflammatory and coagulopathic processes caused by the injured endothelium would continue to have an effect on subsequent clinical progression until the endothelium was returned to its normal state.

The flow parameters observed in this study included those used for research but also a point of care tool that may be used at the bedside[25]. Other investigators have also proposed bedside utilisation of more traditional research parameters[34, 35]. Sublingual video-microscopy is safe to be performed for patients following injury, even in the ED for haemodynamically unstable patients[36]. If conducted in real time, these parameters may be able to aid in the identification of patients likely to suffer from ongoing consequences of endotheliopathy by directly visualising the dynamic changes from initial resuscitation through to ICU. Since endotheliopathy may predict poor patient outcome[2, 13], this information is of potential value in the clinical context.

A recent experimental swine model of trauma and hemorrhagic shock showed that even with identical injury and hemorrhage, there was a wide variation in microcirculatory dysfunction between animals from the outset[29], confirming the findings of an earlier small animal

model[37]. The level of microcirculatory dysfunction was not predictable based on injury-specific details, but instead may be subject to genetic susceptibility. The same may also be true for injured humans; for example, age is associated with a different biomarker profile related to circulating inflammatory and sympathoadrenal mediators[38]. Diagnostic techniques may be desirable if they can distinguish between patients at risk of endotheliopathy and those who are not. In the current study there was a wide variation in biomarker levels and flow dynamics parameters, with some overlap in values between healthy volunteers and injured patients. Furthermore, although statistically significant, the correlations between the lactate and the study parameters of interest are not perfect. These are finding that might be anticipated in a trauma population due to the heterogeneity of injury patterns and severity with differences in genetic susceptibility between patients. Biomarkers and flow dynamics alone may not be sensitive or specific enough to determine clinical decisions during resuscitation, but may have a role in the overall assessment of patients in the context of their other physiological and biochemical parameters. This may be especially relevant if endothelial and microcirculatory behaviour is subject to genetic variation between individuals in the presence of the same injury patterns.

The current management strategy for traumatic hemorrhagic shock involves an early damage control resuscitation (DCR) phase that begins during the pre-hospital evacuation of the casualty, followed by fluid resuscitation with empirical ratios of packed red cells, plasma, and platelets. Following empirical resuscitation, a more bespoke, individualized approach to ongoing resuscitation is favorable, using additional diagnostic modalities such as thromboelastography[39]. Currently there is no point-of-care test for endotheliopathy of trauma, but it appears that sublingual video-microscopy has the potential to provide information relating to this important pathology during individualized resuscitation. Previous

investigators have described a restoration of the endothelial glycocalyx with plasma-based fluid resuscitation[40-43], and the ideal resuscitation fluid for restoring microcirculatory flow dynamics should act to restore the endothelial glycocalyx[44]. Whether additional microcirculatory data might direct fluid resuscitation towards a plasma-based strategy in the clinical situation is yet to be determined. Further clinical investigation of this is warranted.

Limitations

This study included a relatively small number of patients, with all of the associated statistical limits. Analysis was performed on an individual time-point (rather than individual patient) level in order to increase the number of data points to address our research question. The patients included in the current study were profoundly unwell, with high injury severity scores and poor physiological parameters on admission. The data from the current study may not necessarily be translatable for patients with less severe injuries and physiological burden. Sublingual video-microscopy is not currently in widespread clinical use, and the utility of these data in the clinical context are yet to be examined in prospective clinical trials.

We present for the first time an association between endotheliopathy (including endothelial cell damage and glycocalyx shedding) and alterations in flow, density, functional density, proportion of perfused vessels, and micro-vessel heterogeneity. This microcirculatory dysfunction may explain in part some of the mechanisms of the oxygen perfusion deficit that is attributable to endotheliopathy. The clinical utility of these flow parameters at the bedside is yet to be elucidated.

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Author contributions

DNN, MJM, SDH and PH designed and implemented the current study. Ethical and financial applications and amendments were made by SDH and DNN. DNN conducted all of the video-microscopy and subsequent computer analysis of acquired video clips. DNN and JH conducted the ELISAs and their analyses. DNN wrote the manuscript, and JH, MJM, SDH, and PH critically appraised and edited the manuscript. All authors agreed the final version.

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Figure legends

Figure 1. The relationship between (a) thrombomodulin and (b) syndecan-1 concentrations and lactate for all patients.

Figure 2. Concentrations of (a) syndecan-1 and (b) thrombomodulin over the study time-points for all patients compared to healthy controls (HCs).

*$p < 0.05$; **$p < 0.01$ *vs* HCs using Dunn's multiple comparison's test

Figure 3. Flow dynamics for all patients over time, including (a) Microcirculatory Flow Index; (b) Proportion of Perfused Vessels; (c) Microcirculatory Heterogeneity Index; and (d) Point of Care Microcirculation score.

**$p < 0.01$; ***$p < 0.001$ using the Skillings-Mack test

Figure 4. Concentrations of syndecan-1 at each time-point, dichotomised to "above" and "below" the average values of (a) Microcirculatory Heterogeneity Index; (b) Proportion of Perfused Vessels; and (c) Point of Care Microcirculation score; and (d) Microcirculatory Flow Index compared to healthy controls (HCs).

*$p < 0.05$, **$p < 0.01$; ***$p < 0.001$ *vs* HCs using Dunn's multiple comparison's test

Figure 5. Concentrations of thrombomodulin at each time-point, dichotomised to "above" and "below" the average values of (a) Total Vessel Density; (b) Perfused Vessel Density; (c) Proportion of Perfused Vessels; and (d) Microcirculatory Flow Index compared to healthy controls (HCs).

*$p < 0.05$ *vs* HCs using Dunn's multiple comparison's test

Supplementary Digital Content Figure 1. The relationship between thrombomodulin and syndecan-1 concentrations for all patients and healthy controls.

Table 1. Study patient characteristics

Patient characteristic	All
Age, years	35 (25–52)
Sex, male:female	16:1
Mechanism of injury	
Road traffic accident	10
Stabbing	4
Fall	2
Crush	1
Injury Severity Score	27 (23–34)
Physiological parameters on arrival	
Heart rate, min^{-1}	108 (98–118)
Systolic blood pressure, mmHg	91 (61–108)
Glasgow Coma Scale	9 (3–14)
Serum lactate, mmol/l	6.0 (3.6–10.2)
Blood products required in ED	
Packed red cells, units	3 (2–4)
Fresh frozen plasma, units	2 (0–4)
Outcomes	
ICU-free days	11 (0–19)
Hospital-free days	0 (0–5)
SOFA score, day 3	6 (8 – 9)
SOFA score, day 6	4 (3 – 6)
SOFA score, day 10	3 (3 – 4)
30 day mortality	3

Summary data are presented as median and interquartile range in parentheses; categorical data are reported as N. ICU- and Hospital-free days are calculated for a 30-day period.
ED: Emergency Department; ICU: Intensive Care Unit; SOFA: Sequential Organ Failure Assessment

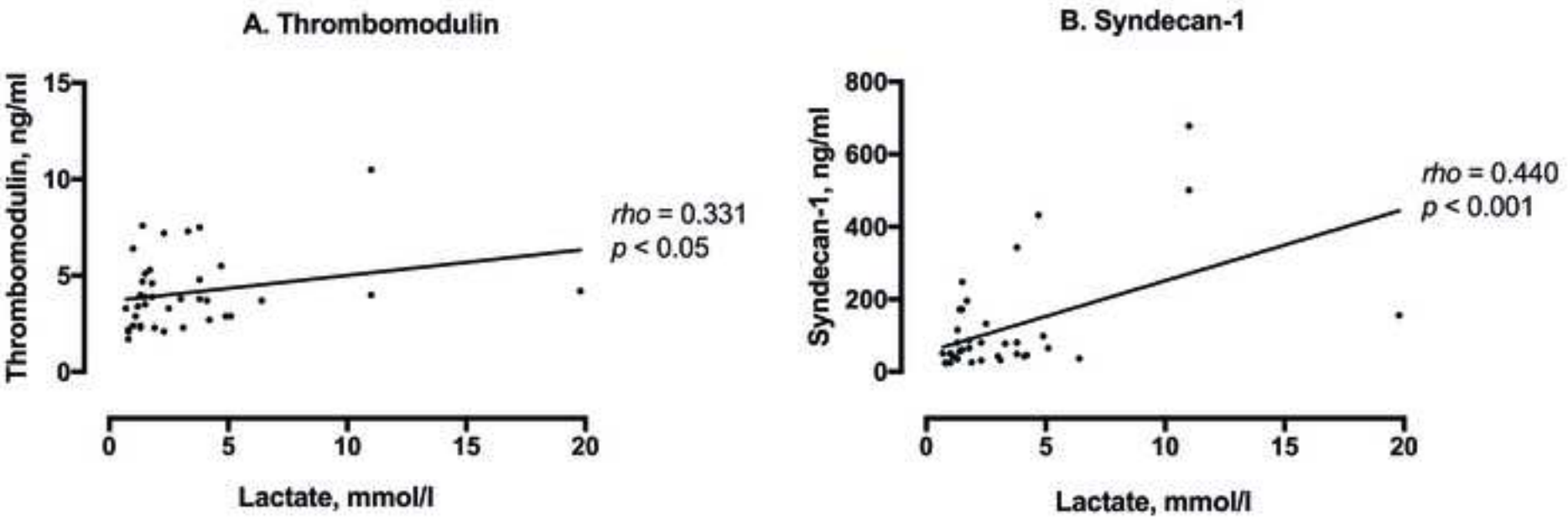


Figure 1. The relationship between (a) thrombomodulin and (b) syndecan-1 concentrations and lactate for all patients.

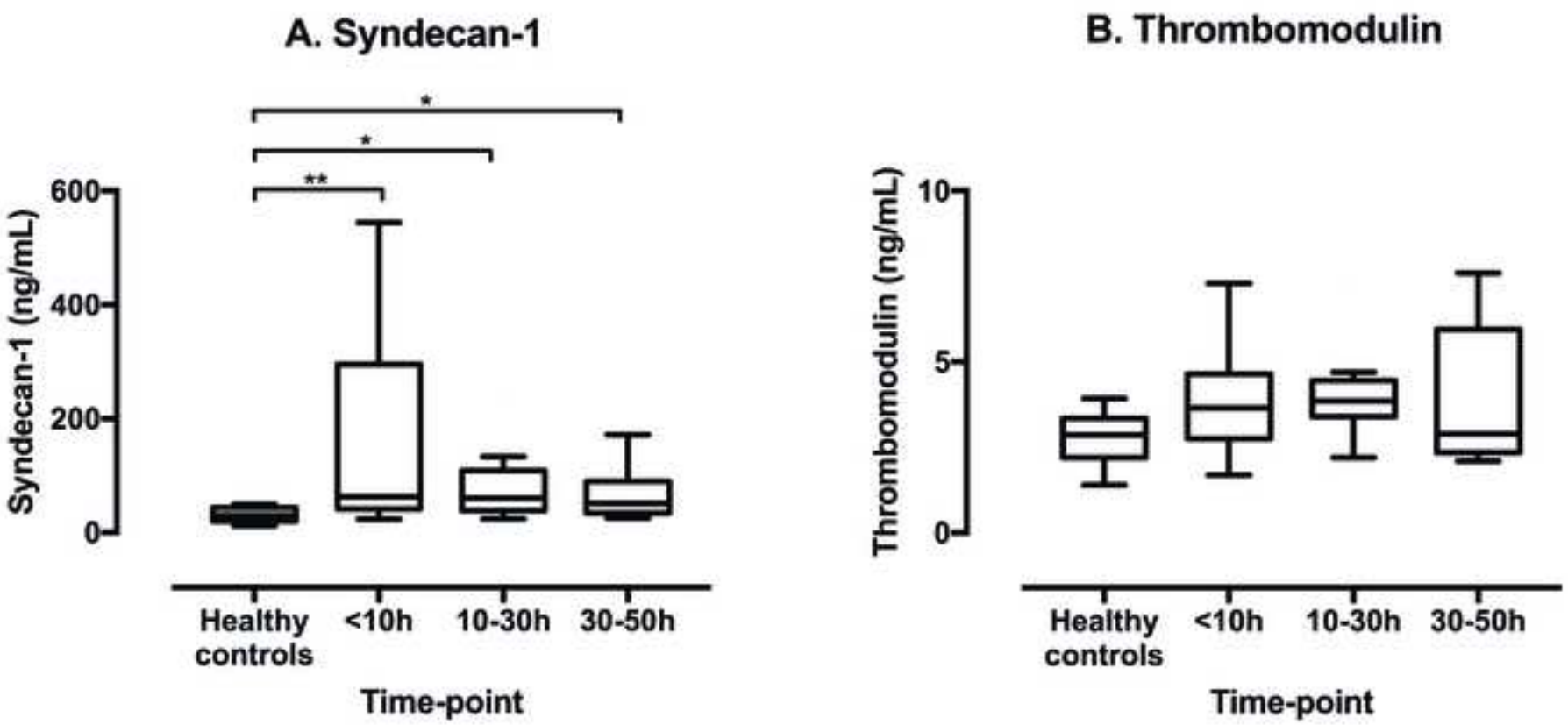
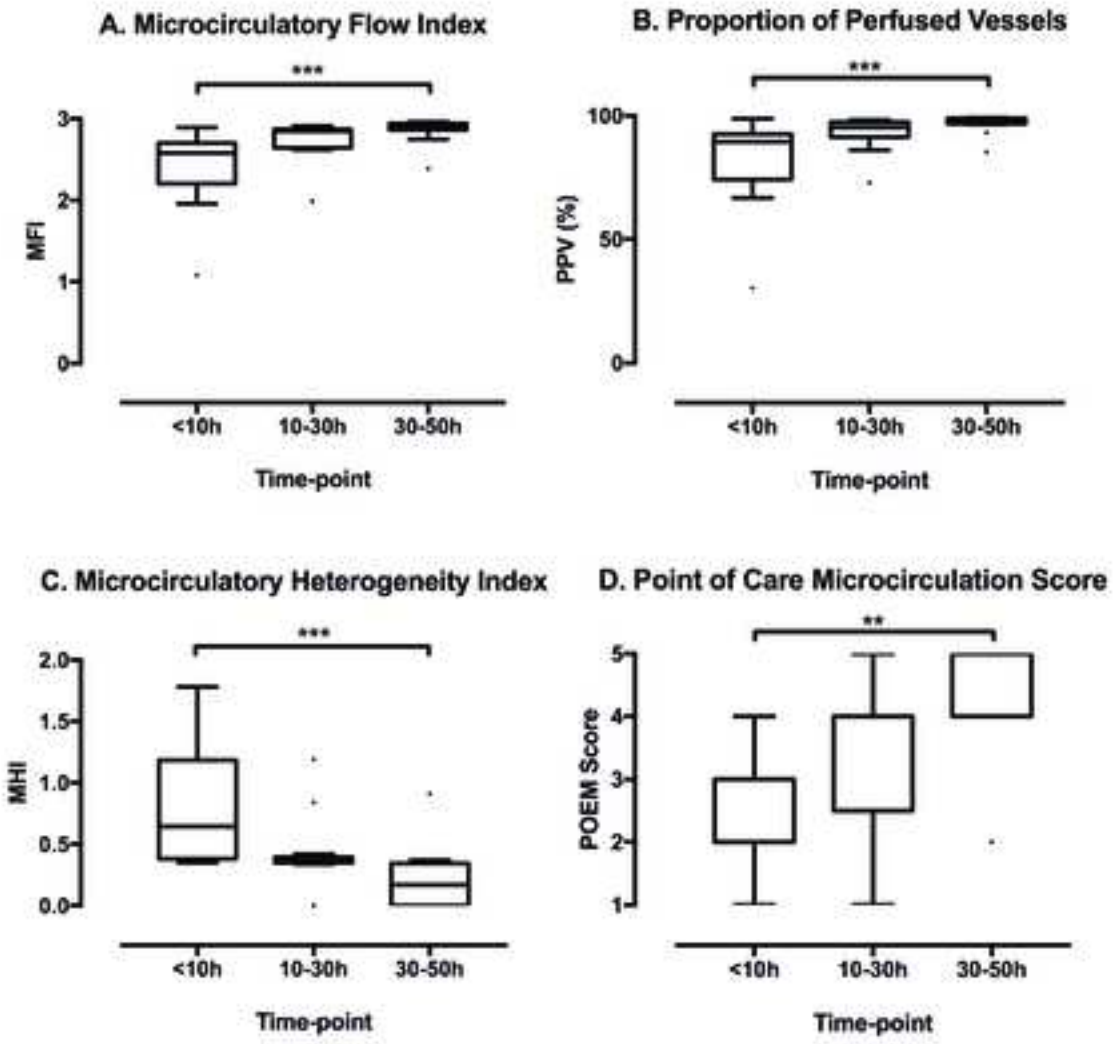


Figure 2. Concentrations of (a) syndecan-1 and (b) thrombomodulin over the study time-points for all patients compared to healthy controls (HCs).

*$p < 0.05$; **$p < 0.01$ *vs* HCs using Dunn's multiple comparison's test

Figure 3



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$^{**}p < 0.01$; $^{***}p < 0.001$ using the Skillings-Mack test

Figure 4

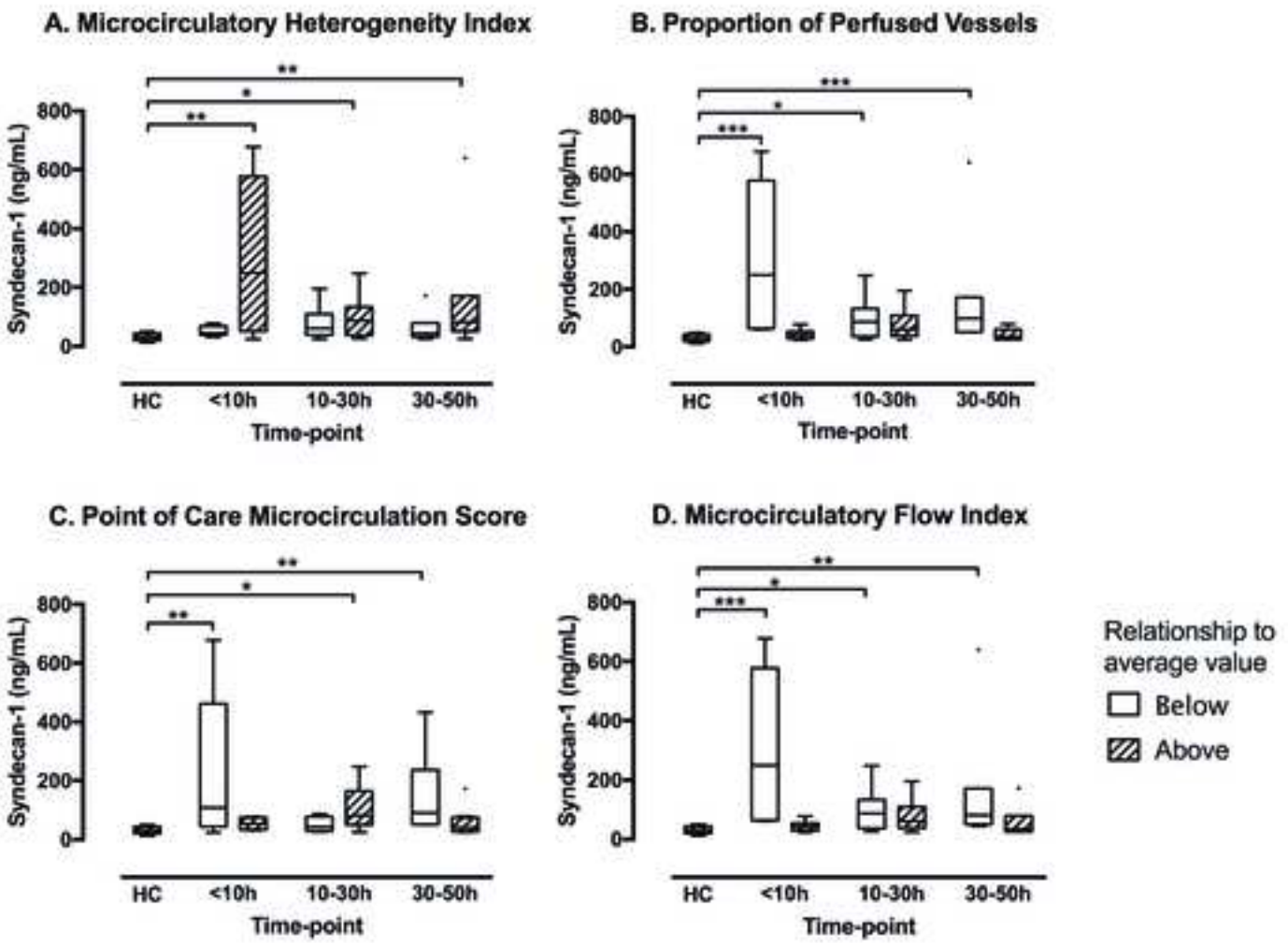

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Figure 5

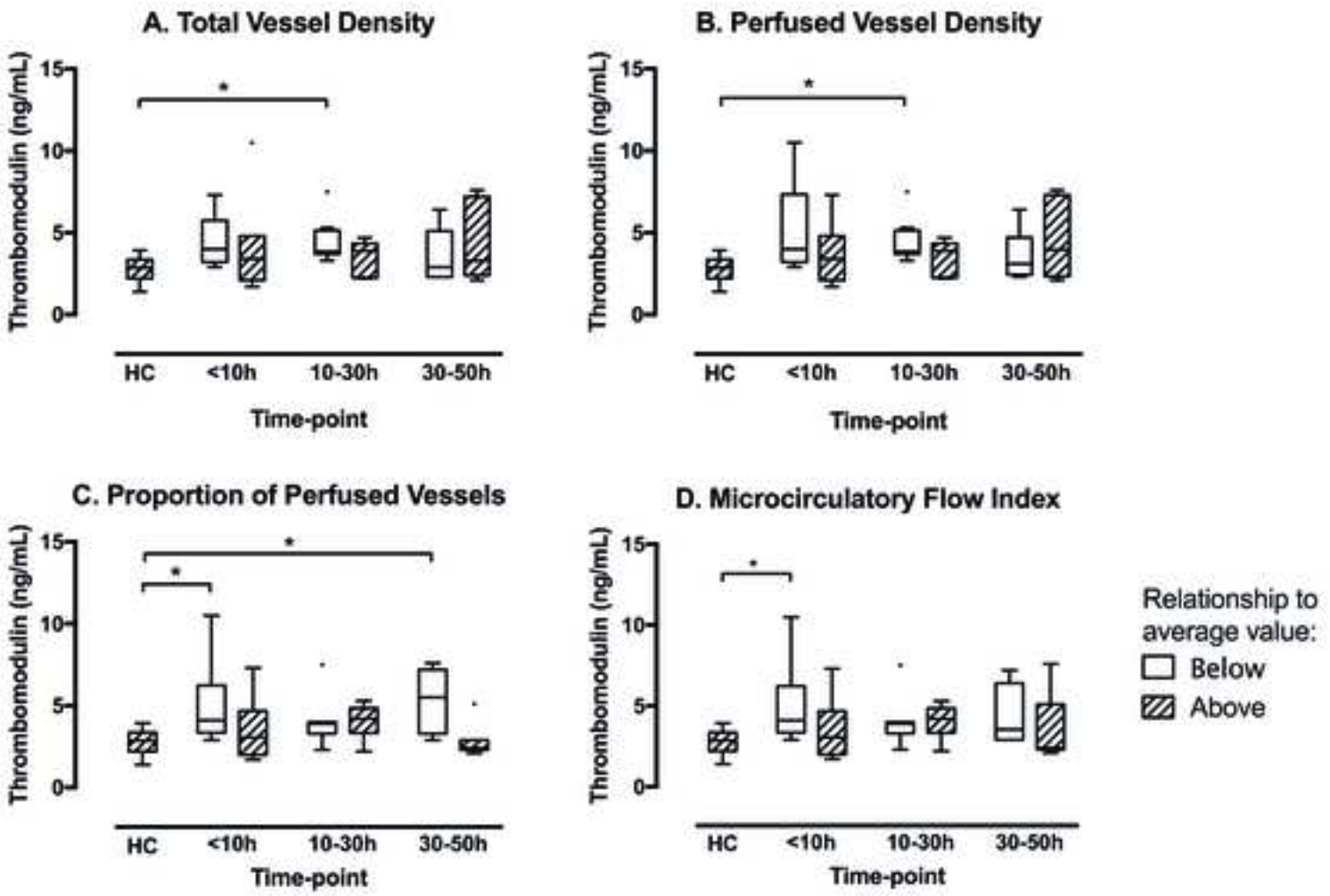

Figure 5. Concentrations of thrombomodulin at each time-point, dichotomised to "above" and "below" the average values of (a) Total Vessel Density; (b) Perfused Vessel Density; (c) Proportion of Perfused Vessels; and (d) Microcirculatory Flow Index compared to healthy controls (HCs).

*$p < 0.05$ *vs* HCs using Dunn's multiple comparison's test

Supplemental Data File (.doc, .tif, pdf, etc.)