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Hemorrhagic shock drives glycocalyx, barrier and organ dysfunction early after polytrauma

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ABSTRACT

Polytrauma (PT) is frequently associated with hemorrhagic shock (HS), which increases morbidity and mortality. Although various aspects of HS have been addressed in PT patients, the impact of an additional HS is largely unknown regarding the development of multiple organ dysfunction associated with disturbed glycocalyx and barrier function early after trauma.

A prospective, longitudinal, mono-centered, observational study enrolling severely injured patients (Injury Severity Score, ISS = 38.1 ± 2.6) served for an in-depth analysis of blood (drawn on days 0, 1, 2, 3 and 5) and clinical data (up to 21 days) of 30 patients who were then stratified into PT with and without HS.

HS significantly enhanced signs of acute organ injury, assessed by increased serum concentrations of novel damage markers. Moreover, indicators of glycocalyx and tight-junction dysfunction were found in PT patients all of which were significantly enhanced in co-presence of HS. These markers revealed multiple significant correlations with specific barrier, fluid-balance, coagulation, inflammation, and clinical-outcome parameters. Strikingly, mucosa fragments, which affected clotting, could be detected in serum after PT/HS.

The results point to HS as a main driver for glycocalyx and barrier breakdown and suggest novel tools for the monitoring of organ dysfunction in the early course after PT.

1. Introduction

Hemorrhagic shock (HS) is a frequent early event in severely injured polytrauma (PT) patients and, despite modern damage control and shock-treatment strategies, HS remains associated with high morbidity and mortality rates [1,2]. In PT patients and experimental PT settings, HS has been proposed as an engine of systemic inflammation [3], gut-barrier disruption [3,4], danger-associated molecule pattern (DAMP) exposure [5], endotheliopathy [6-8], complementopathy and coagulopathy [9-12]. All these mechanisms may contribute to the development of multiple organ dysfunction syndrome (MODS). Although various proteomic and transcriptomic data sets from PT patients have been analyzed for inflammatory and infectious consequences [13,14], mechanisms of the molecular danger response due to HS directing towards early MODS development remain rather unknown. Concerning therapeutic strategies for HS in trauma, novel concepts address not only the inflammatory axes [15] and coagulation network [16,17], but also focus in particular on the endotheliopathy, *e.g.* by application of fresh-

Abbreviations: AIS, Abbreviated Injury Scale; AKI, acute kidney injury; aPTT, activated partial thromboplastin time; CC16, Clara Cell Protein 16; DAMP, danger-associated molecule pattern; GCS, Glasgow Coma Scale; HS, hemorrhagic shock; I-FABP, liver −/intestinal-type fatty acid binding protein; ISS, Injury Severity Score; L-FABP, liver-type fatty acid binding protein; MMP-13, matrix metalloproteinase 13; MODS, multiple organ dysfunction syndrome; NGAL, neutrophil gelatinase-associated lipocalin; PCT, procalcitonin; PT, polytrauma; RBC, red blood cell; ROTEM, rotational thromboelastometry; S1P, sphingosine-1-phosphate; SOFA, Sequential Organ Failure Assessment; TASH, Trauma Associated Severe Hemorrhage.

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frozen plasma, adiponectin or prothrombin-complex concentrates to restore the vascular barrier [18,19]. Other treatment approaches aimed to improve trauma −/HS-induced intestinal hypoperfusion, mucosal ischemia and gut-barrier breakdown and thereby MODS development, for example, by the glycosaminoglycan heparan sulfate, which in addition to exhibiting some anti-coagulatory effects could improve immunological functions [20]. Nevertheless, a precautionary approach is required for novel pathophysiological and therapeutic concepts in trauma −/HS-induced MODS [21]. Consequently, the organ damage and barrier reactions after PT require closer discriminative characterization in regard to the presence or absence of an additional HS and using reliable monitoring markers.

2. Materials and methods

2.1. Clinical study design

A subcohort of 30 patients was randomly selected from a prospective, mono-centered, observational cohort study including a total of 104 patients. Blood was drawn consecutively during the first 21 days after PT when admitted to the University Hospital Zurich (Trauma Level I Center). The study was carried out in accordance with local and international guidelines and regulations and the study protocol was approved by the Cantonal Ethic Commission Zurich (StV 26–2007). All patients were recruited into the study under informed consent as approved by the Cantonal Ethic Commission Zurich and international ethical guidelines (NCT02508272). Enrollment criteria were ISS > 17, age ≥ 18 y, and time after injury <6 h. A clinico-transcriptomic approach for septic complications after PT as primary endpoint was recently published [13,22]. In the present study, the subcohort was stratified for manifest HS [23-25] on day 0 as indicated by at least one of the following parameters: base excess < −6 mmol/l, lactate ≥2.5 mmol/l, red blood cell (RBC) transfusion >2 U, and/or a Trauma Associated Severe Hemorrhage (TASH) score ≥ 10. The patient stratification strategy identified $n = 10$ patients without and $n = 20$ with signs of manifest HS. For baseline levels, six healthy age- and sex-matched volunteers were included after informed and written consent (mean age ± standard error of the mean (s.e.m.): 43.3 ± 8.3 y; median, min.–max.: 44.5, 18–72; male/female 4/2). All included study patients received standard treatment based on the guidelines of the German Society of Trauma (DGU).

2.2. Clinical data

Concurrent to the blood sampling, clinical data were collected prospectively. Corresponding scores were retrospectively determined from the patients' records. As recently described [13], various scores were applied: ISS, Glasgow Coma Scale (GCS) [26], Sequential Organ Failure Assessment (SOFA) score [27], systemic inflammation score [13], and the TASH score [23]. Systemic inflammatory response syndrome and sepsis were defined in accordance with previously established guidelines [28-30].

2.3. Enzyme-linked immunosorbent assay analyses of patient samples

Blood from patients and healthy volunteers was centrifuged at 2000×*g* for 10 min at 4 °C and serum was stored at −80 °C. Samples were assayed using the following kits for human proteins according to manufacturers' instructions: FABP2/I-FABP, Lipocalin-2/NGAL, Angiopoietin-2, Syndecan-1, C-reactive protein, MMP-13, serum albumin (all DuoSet ELISA, R&D, Wiesbaden-Nordenstadt, Germany), IL-6 (BD Biosciences, Heidelberg, Germany) CC16 (Clara Cell Protein 16, Elabscience, Bethesda, MD, USA), Heparan Sulfate (Amsbio, Abingdon, UK), L-FABP (Hycultec, Beutelsbach, Germany), Claudin-5 (Cusabio, College Park, MD, USA), ADM/Adrenomedullin (LifeSpan BioSciences, Seattle, WA, USA), MUC2 (Aviva Systems Biology, San Diego, CA, USA) and Sphingosine 1 Phosphate (MyBiosource, San Diego, CA, USA).

2.4. Rotational thromboelastometry (ROTEM) analyses

To assess the impact of dilution by high-volume resuscitation, whole blood anticoagulated with sodium citrate (Sarstedt, Nuembrecht, Germany) was drawn from healthy volunteers. The respective hematocrit was adjusted to 33% or 25% with Jonosterile (Fresenius Kabi, Bad Homburg, Germany) and incubated with heparan sulfate (Amsbio, Abingdon, UK), human recombinant syndecan-1 (R&D) and mucin-2 (Sigma-Aldrich, Darmstadt, Germany) for 30 min. The activity of the intrinsic pathway (INTEM test) was then analyzed using a ROTEM® delta device (Tem, Munich, Germany).

2.5. Statistics

Patient characteristics in the two groups were compared using the Chi-square test in case of categorical variables and using Student's *t*-test in case of continuous parameters. Correlation analyses were performed using Pearson Product Moment Correlation. ROTEM parameters of blood samples with adjusted hematocrit were compared using one-way ANOVA followed by Student-Newman-Keuls post-hoc test and paired t-test. These statistical analyses were performed using SigmaPlot (Version 11.0, Systat Software, Erkrath, Germany). To evaluate whether HS significantly changed overall plasma values of barrier and organ molecules during the time course after trauma, we used repeated-measures ANOVA employing SAS (Version 9.3, SAS, Cary, NC, USA). We applied no formal statistical test on normality due to their very limited validity regarding our available sample size [31]. Instead, we assessed graphically by means of QQ-plotting the model residuals whether there is suspicion of any severe violation of the model assumptions. Overall, we found no apprehensive deviation from the model assumptions for all the variables tested. The alpha level was 0.05 for all analyses. Results are presented as mean ± s.e.m.

3. Results

3.1. Patient cohort

When compared to the total cohort of 104 patients described by Rittirsch et al. [13], the 30 patients in our randomly selected subcohort were not significantly different regarding any demographic parameter (Table 1). Six age- and sex-matched volunteers were enrolled as healthy controls. We stratified the 30 patients into those with and those without clear signs of HS employing established clinical parameters [23-25]: base excess (< −6 mmol/l), lactate (≥2.5 mmol/l), RBC transfusion (>2 U on day 0), and/or TASH score (≥10) of which at least one had to be fulfilled to define HS. All key patient parameters are presented in Table 1. When comparing the two groups with ($n = 20$) and without ($n = 10$) HS, we found that although the ISS was similar in both groups (38.7 ± 2.8 *vs.* 36.2 ± 6.3, $p = 0.54$), the Abbreviated Injury Scale (AIS) of the abdomen/lumbar spine and the maximal SOFA until day 21 were significantly increased in the cohort with HS (2.8 ± 0.3 *vs.* 1.1 ± 0.4, $p = 0.01$, and 10.5 ± 1 *vs.* 6 ± 1.1, $p = 0.009$, respectively). Furthermore, the length of stay in the intensive care unit and in the hospital were significantly longer for patients with HS (19.2 ± 2.8 *vs.* 5.9 ± 1.3, p = 0.01, and 34 ± 4.1 *vs.* 17.2 ± 3.2, $p = 0.001$, respectively). As expected from our stratification, the data confirmed that HS patients had a higher initial TASH score ($p = 0.004$), lactate levels ($p = 0.007$) and hematocrit values ($p = 0.013$), required more total RBC transfusions ($p < 0.001$) and had impaired coagulation as indicated by reduced Quick values (calculated from the thromboplastin time; lower Quick relates to longer thromboplastin time) ($p = 0.008$) compared to patients without manifest HS (Table 1).