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BRITISH JOURNAL OF ANAESTHESIA, 121(5)

0007-0912

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2018-11-01

10.1016/j.bja.2018.05.072

Peer reviewed
Proteolysis in septic shock patients: plasma peptidomic patterns are associated with mortality

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# These authors contributed equally to this study.

Abstract

Background: Uncontrolled proteolysis contributes to cell injury and organ dysfunction in animal models of circulatory shock. We investigated in humans the relationship between septic shock, proteolysis, and outcome.

Methods: Intensive care patients with septic shock (n=29) or sepsis (n=6) and non-hospitalised subjects (n=9) were recruited as part of the prospective observational trial 'ShockOmics' (ClinicalTrials.gov Identifier NCT02141607). A mass spectrometry-based approach was used to analyse the plasma peptidomes and the origin of circulating peptides from proteolysis in the enrolled subjects.

Results: Evidence of systemic proteolysis was indicated by a larger number of circulating peptides in septic shock patients, compared with septic patients and non-hospitalised healthy subjects. The peptide count and abundance in the septic shock patients were greater in patients who died (n=6) than in survivors (n=23), suggesting an association between magnitude of proteolysis and outcome. In silico analysis of the peptide sequences and of the sites of cleavage on the proteins of origin indicated a predominant role for serine proteases, such as chymotrypsin, and matrix metalloproteases in causing the observed proteolytic degradation.

Conclusions: Systemic proteolysis is a novel fundamental pathological mechanism in septic shock. Plasma peptidomics is proposed as a new tool to monitor clinical trajectory in septic shock patients.

Clinical trial registration: NCT02141607.

Keywords: in-hospital mortality; mass spectrometry; peptidomics; proteolysis; septic shock
Sepsis is a life-threatening condition caused by a dysregulated host response to infection. Septic shock is the most severe form of sepsis, characterised by circulatory failure and tissue injury, and associated with high mortality.

Despite longstanding efforts to develop new treatments for septic shock, interventions are still based on haemodynamic support, antibiotics and anti-infective drugs, and source control (e.g. surgery). To date, no therapy addresses the root cause of septic shock, largely because of an incomplete understanding of the cascade leading to multiorgan dysfunction.

Uncontrolled proteolysis has recently been proposed as a fundamental pathological mechanism in shock, possibly mediated by digestive enzymes. Enteral blockade of pancreatic proteases in animals mitigates organ injury and improves outcome. I.V. serine protease blockade has also shown some efficacy in severe sepsis. Further, we recently reported the occurrence of systemwide proteolysis and the appearance of a large number of circulating peptides in a rat model of shock.

The objectives of this study are: (i) to investigate proteolysis in septic shock patients compared with septic patients without shock and healthy donors by assessment of plasma peptide concentrations from mass spectrometry-based peptidomics, and by in silico analysis of proteolytic activity; and (ii) to determine the association between proteolysis and inhospital mortality in septic shock.

**Methods**

**Study design and participants**

This study is part of the prospective observational trial ‘ShockOmics’ (ClinicalTrials.gov Identifier NCT02141607) and was approved by: the Geneva Regional Research Ethics Committee (study number 14-041); the Ethical committee of Hopital Erasme-Université Libre De Bruxelles (study number P2014/171); and the Mutua Terrassa Hospital Institutional Review Board (study number EO/1407).

Adult (>18 yr old) patients admitted between October 2014 and March 2016 to the ICU of Geneva University Hospitals, Geneva, Switzerland (38-bed, mixed) and Erasme University Hospital, Brussels, Belgium (36-bed, mixed) with septic shock diagnosis, Sequential Organ Failure Assessment (SOFA) score ≥6, arterial lactate ≥2 mmol L⁻¹ and a documented source of infection were screened for enrolment. Patients at high risk of death within the first 24 h after admission, systemic immunosuppression, haematological diseases, metastatic cancer, pre-existing dialysis, decompensated cirrhosis, or who received more than four units of red blood cells or any fresh frozen plasma were excluded. Given the stringency of the exclusion criteria, the thresholds on SOFA and lactate were chosen to include severe enough septic shock patients, while ensuring adequate patient enrolment and recruitment rate.

A convenience sampled cohort of patients with sepsis (infection with inflammatory response and one organ dysfunction), but not in shock, was included as control. Healthy donors were enrolled at Mutua Terrassa Hospital, Terrassa, Spain for an additional non-sepsis control. Informed consent was obtained from patients or proxies. Patients in the ICU were managed according to international guidelines. Patient characteristics, organ function, and haemodynamic data were prospectively collected into a custom-made electronic case report form. In-hospital mortality was assessed by consultation of the local death registry, or by telephone call to the patient or proxies.

**Sample collection**

Blood (total volume 18 ml for the multiomics analyses of the ‘ShockOmics’ project) was collected from either the arterial or venous line in septic shock and sepsis patients; venous blood was drawn from healthy donors. For peptidomics, plasma (0.5 ml) was isolated within 30 min of sample collection in K₂-EDTA tubes (BD Biosciences, San Jose, CA, USA) by centrifugation (twice at 1200g for 10 min) to pellet cellular elements. COmplete Protease Inhibitor Cocktail (Roche Applied Sciences, Mannheim, Germany) was added and samples were stored at −80°C until in-batch analyses. Plasma samples collected from the septic shock patients at three time points were analysed: within 16 h of ICU admission (T₁); at 48 h (T₂) after ICU admission; and before discharge from the ICU or on Day 7 at latest (T₃). Only one sample was collected from the sepsis patients (at T₁) and healthy donors.

**Liquid chromatography–mass spectrometry analysis**

Residual protease activity in the plasma samples was tested by a fluorometric assay (Supplementary material S.1). Peptides were extracted by filtration (Supplementary material S.2), separated by reverse phase liquid chromatography, and analysed by mass spectrometry (Supplementary material S.3) in the Orbitrap (Thermo Fisher Scientific, Bremen, Germany).

**Peptide and protein identification: qualitative approach**

Proteins and peptides were identified (Supplementary material S.4) using Mascot search engine (v2.3.0.1, Matrix Science, Boston, MA, USA) against the SwissProt Human (SPH) database (v160127). Venn diagrams of peptide counts were generated using R packages.

**Peptide and protein quantification: label-free approach**

Raw data were processed with Progenesis QI for Proteomics (Non-Linear Dynamics, Waters, Milford, MA, USA). Pool samples were used as alignment reference. A total of 219 825 mass spectra (z>1 and Rank<5) were considered for database search. Mascot search engine was used against SPH (Supplementary material S.4).
Both the total number (i.e. the sum of peptides found in the subjects belonging to a group) and the number of exclusive peptides identified (i.e. the sum of peptides found exclusively in such group) in the septic shock, sepsis, and healthy donor groups were compared.

The level of proteolysis is related to the intensity of the peptide signal detected by the mass spectrometry, which is proportional to the peptide abundance in a sample, as calculated by Progenesis. The abundance of a cleaved protein is the cumulative abundance of all peptides derived uniquely from cleavage of this protein. Then, the median abundance of each protein in a patient group is computed, and the total protein abundance is obtained from the sum of the medians of all the cleaved proteins in the patient group. This serves as an estimate of proteolysis: an incremental change in the abundance of cleaved proteins can be interpreted as an incremental change in proteolysis.

### Table 1 Clinical parameters. *Mean (SD) and \(^n\) (%). Blood samples were drawn at three different time points. T1: within 16 h of ICU admission; T2: at 48 h after ICU admission; T3: before discharge from the ICU or on Day 7 at latest. SS, septic shock

<table>
<thead>
<tr>
<th></th>
<th>SS non-Survivors (n=6)</th>
<th>SS survivors (n=23)</th>
<th>Sepsis (n=6)</th>
<th>Healthy controls (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)(^*)</td>
<td>62–93</td>
<td>19–85</td>
<td>41–90</td>
<td>31–51</td>
</tr>
<tr>
<td>Sex (female)(^1)</td>
<td>2 (33.3%)</td>
<td>7 (30.4%)</td>
<td>2 (33.3)</td>
<td>4 (44.4%)</td>
</tr>
<tr>
<td>BMI (Kg m(^{-2}))(^*)</td>
<td>25.5 (0.93)</td>
<td>27.3 (6.20)</td>
<td>21.4 (6.43)</td>
<td>—</td>
</tr>
<tr>
<td>SOFA(^*)</td>
<td>T1 13.2 (1.94)</td>
<td>T2 11.6 (2.79)</td>
<td>5.33 (3.01)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T2 10.5 (3.51)</td>
<td>T3 7.65 (2.62)</td>
<td>6.67 (3.98)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 6.50 (3.62)</td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>APACHE II (T1)(^*)</td>
<td>27.2 (5.85)</td>
<td>23.4 (7.08)</td>
<td>13.0 (3.16)</td>
<td>—</td>
</tr>
<tr>
<td>Temperature (ºC)(^*)</td>
<td>T1 37.9 (1.3)</td>
<td>T2 37.5 (1.3)</td>
<td>37.0 (0.7)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 37.5 (1.1)</td>
<td></td>
<td>36.6 (0.4)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T1 37.3 (2.0)</td>
<td></td>
<td>37.6 (0.9)</td>
<td>—</td>
</tr>
<tr>
<td>Lactate (mmol L(^{-1}))(^*)</td>
<td>T1 6.25 (2.94)</td>
<td>T2 4.22 (2.20)</td>
<td>3.23 (2.79)</td>
<td>—</td>
</tr>
<tr>
<td>Heart rate (beats min(^{-1}))(^*)</td>
<td>T1 2.77 (0.90)</td>
<td>T2 1.52 (0.64)</td>
<td>0.75 (0.07)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 1.56 (0.51)</td>
<td></td>
<td>1.16 (0.56)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Heart rate (beats min(^{-1}))(^*)</td>
<td>T1 109 (19)</td>
<td>T2 107 (26)</td>
<td>95 (21)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 92 (25)</td>
<td></td>
<td>74 (32)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T1 82 (24)</td>
<td></td>
<td>104 (19)</td>
<td>—</td>
</tr>
<tr>
<td>MAP (mm Hg)(^*)</td>
<td>T1 59 (7)</td>
<td>T2 59 (6)</td>
<td>80 (17)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 67 (6)</td>
<td></td>
<td>68 (18)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T1 73 (15)</td>
<td></td>
<td>76 (13)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Patients on vasopressor(^1)</td>
<td>T1 6 (100)</td>
<td>T2 4 (66.7)</td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 2 (33.3)</td>
<td></td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td>Patients on mechanical ventilation(^1)</td>
<td>T1 6 (100)</td>
<td>T2 18 (78.3)</td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 6 (100)</td>
<td></td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Renal replacement therapy(^1)</td>
<td>T1 3 (50.0)</td>
<td>T2 13 (56.5)</td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T3 3 (50.0)</td>
<td></td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td>Source of infection(^1)</td>
<td>Abdominal 1 (16.7)</td>
<td>Respiratory 5 (83.3)</td>
<td>Urinary tract 0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 (34.8)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 (30.4)</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 (34.8)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Mortality(^1)</td>
<td>ICU 5 (83.3)</td>
<td></td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Hospital 6 (100)</td>
<td></td>
<td>0 (0.0)</td>
<td>—</td>
</tr>
</tbody>
</table>

### In silico protease effector estimation

In order to estimate the proteases responsible for protein cleavage, we split the peptide dataset into two: protein fragments with median abundance ratio higher or exclusive to the healthy group (357 peptides) and protein fragments with median higher or exclusive to the septic shock group (500 peptides). We extracted the C-terminus amino acid of every identified peptide, obtaining P1\(_{C\text{-term}}\) amino acids and P1\(_{N\text{-term}}\) amino acids (immediately before each peptide N-terminus position) by matching the peptides to the SPH database using a variant of a Perl Script.\(^{15}\) The analysis of proteases responsible for both C- and N-terminus cleavage focused specifically on matrix metalloproteases (MMPs), for their role in shock pathophysiology,\(^{16}\) and on serine proteases, which could have a dual role of cleaving proteins and activating pro-MMPs.
Statistical analysis

For group homogeneity, calculated P-values correspond to Student’s t-test and χ² test for numerical and categorical data, respectively.

In order to assess the spread of the data of cleaved proteins and derived peptides, data variance was tested by one-way analysis of variance (ANOVA) after log₂-transformation of the dataset. Proteins showing significant differences (P<0.05) between groups by the ANOVA test were evaluated for normality through the Shapiro-Wilk test. For non-normally distributed protein abundances, comparisons of the mean abundances between groups were evaluated by Wilcoxon (Mann—Whitney) test; for normally-distributed protein abundances, comparisons were evaluated by Student’s t-test (P<0.05). Statistical analyses were performed using R packages.14

Results

Study participants

Out of 529 screened patients, 29 patients with septic shock (of whom six died during the hospital stay) and six septic controls were enrolled in the study. Healthy blood donors (n=9) served as additional controls (Table 1). At T1, patients with septic shock not surviving to hospital discharge were older than survivors (P=0.034), with higher acute physiology and chronic health evaluation II (APACHEII) (P=0.00091) and SOFA (P=0.0005) scores than septic patients not in shock. No significant difference in the SOFA score was found between septic shock survivors and non-survivors at either T1 or T2. At T2, lactate was higher (P=0.017) in the non-survivors. No patient in the sepsis group not in shock died during the hospital stay.

Proteolysis in septic shock

Both the total (2743) and exclusive (1855) peptide population counts in the septic shock group at T1 were higher than in the sepsis and healthy groups (Fig. 1A). The mean value of exclusive peptides per patient was computed to account for the different group sizes. Septic shock patients had approximately a three-fold larger peptide population than the sepsis control and healthy donor groups.

Septic shock peptidome: correlation with outcome and source of infection

The mean values of the peptides exclusive for each group (Fig. 1B and C) were lower for survivors than non-survivors at
all times. In addition, survivors showed a decrease from T1 to T2, while the non-survivor group showed a large increase at T2, the time when the highest peptide count was reached in non-survivors.

To investigate the relationship between proteolysis and outcome, we compared the peptidomes of survivors vs non-survivors at T1 and T2. This quantitative analysis identified 218 proteins as the source for 913 peptides detected in the plasma samples. At both time points, the proteolysis levels, estimated from the abundance of the cleaved proteins, were increased in the non-survivor group (Fig. 2a) vs survivors.

Further, when comparing the sources of infection, the magnitude of proteolysis was higher in respiratory-initiated and abdominal-initiated septic shock compared with urosepsis (Fig. 2b). In the urinary and abdominal septic shock subgroups, the numbers of total, exclusive peptides and mean of exclusive peptides decreased from T1 to T3 (Table 2). The respiratory subgroup displayed a different behaviour. Both survivors and non-survivors in this subgroup presented a higher number of peptides at T2 than at T1, although the increase was larger in the non-survivors, which displayed the highest number of peptides at T2. Except for the respiratory non-survivors, the number of peptides at T3 was smaller than at T1 in all groups.

No protein from the urosepsis group was characterised by significantly increased proteolysis either at T1 or T2, while we found five proteins subject to significantly increased proteolysis in the abdominal septic shock group at T1, and 10 proteins with significantly increased proteolysis in the group that had a respiratory-initiated septic shock at T2 (Supplementary Table S2).

Group differences in proteolysis levels were assessed by computing the difference between protein abundance in the two groups at T2 (Fig. 3), the time point at which the two groups of survivors and non-survivors clearly separated. At T1 there was no appreciable difference between the two groups. At T2, six proteins showed significantly increased proteolysis in non-survivors (Fig. 3a–f), and one protein in survivors (Fig. 3g).

**Effector proteases estimation**

In total, 644 and 929 P1$_{N/C}$-term amino acids for the healthy and septic shock groups, respectively, were classified according to the protease-specific cleavage annotated in databases$^{17}$ and the literature.$^{7,18}$ Despite comparable protease cleavage distributions, the contribution of chymotrypsin-like enzymes to proteolysis was larger in shock (42% of the circulating peptides) than in the healthy controls (37%) (Fig. 4).

**Discussion**

This study demonstrates that plasma from septic shock patients displayed approximately a three-fold increment in total peptide count compared with healthy individuals, as well as 40% higher peptide abundance, indicating elevated proteolysis above physiological levels in septic shock. The protein fragments resulted mostly from the proteolytic action of serine proteases, such as chymotrypsin-like enzymes, and MMPs, in

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Table 2 | Source of infection and number of circulating peptides. Blood samples were drawn at three different time points. T1: within 16 h of ICU admission; T2: at 48 h after ICU admission; T3: before discharge from the ICU or on Day 7 at latest (T3)

<table>
<thead>
<tr>
<th>Source of infection</th>
<th>n</th>
<th>Total no. peptides</th>
<th>Exclusive peptides</th>
<th>Exclusive peptides/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary tract: survivors</td>
<td>T1</td>
<td>8</td>
<td>1016</td>
<td>352</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>8</td>
<td>885</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>3</td>
<td>512</td>
<td>85</td>
</tr>
<tr>
<td>Abdominal: survivors</td>
<td>T1</td>
<td>8</td>
<td>1429</td>
<td>601</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>8</td>
<td>1119</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>6</td>
<td>785</td>
<td>182</td>
</tr>
<tr>
<td>Respiratory: survivors</td>
<td>T1</td>
<td>7</td>
<td>1442</td>
<td>522</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>7</td>
<td>1556</td>
<td>663</td>
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<td></td>
<td>T3</td>
<td>5</td>
<td>704</td>
<td>140</td>
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<tr>
<td>Respiratory: non-survivors</td>
<td>T1</td>
<td>5</td>
<td>1265</td>
<td>344</td>
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<tr>
<td></td>
<td>T2</td>
<td>5</td>
<td>1689</td>
<td>634</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>4</td>
<td>1252</td>
<td>343</td>
</tr>
</tbody>
</table>
accordance with previous evidence. The distribution of protease effectors responsible for proteolytic cleavage in shock was similar to the one in healthy controls: the largest number of circulating peptides were derived by proteolytic cleavage because of chymotrypsin-like enzymes in both groups, while elastase-like/MMPs, and MMPs and trypsin-like enzymes contributed to a similar extent to proteolysis in both groups, with a slight reduction of the contribution of MMPs and trypsin-like enzymes and a slight increase of elastase-like/MMPs. These results: (i) hint at a pathological shift in the concentration or activity of circulating enzymes or in the balance between endogenous protease inhibitors (the reduced presence of which in shock could also explain the increased proteolysis) and active proteases; (ii) confirm in humans the concept, previously proposed in experimental models of shock, that increased enzymatic activity and diffuse proteolysis are associated with mortality, and (iii) are consistent with previous reports on the effectiveness of anti-protease treatments in acutely ill humans. Therefore, this body of evidence points to a novel pathological mechanism with evident therapeutic implications in the complex framework of septic shock.

The comparison between non-survivors and survivors yielded a significantly larger total number of circulating proteases in non-survivors than in survivors. This was observed in multiple protease effectors, including cathepsin L1, glyceraldehyde-3-phosphate dehydrogenase, oncoprotein-induced transcript 3 protein, immunoglobulin kappa variable 2D-28, tissue inhibitor of metalloproteases 1, hornerin, and basic salivary proline-rich protein 3.
peptides in the non-survivor group at admission to the ICU, which increased further at 48 h after ICU admission (Figs 1 and 2). This result is even more striking in light of the low number of subjects in the non-survivor group and reinforces the notion that protein cleavage is increased in lethal septic shock.

Several important proteins were differentially cleaved between non-survivors and survivors. Specifically, at T2, six proteins were more proteolysed in non-survivors, suggesting the pathologic disruption of important biological processes (Table 3). Most of these proteins are physiologically expressed in multiple tissues, and they are involved in the regulation of several functions known to be altered in shock, such as immune response, complement activation, coagulation, toll-like receptor signalling, etc. Of interest in the context of our findings, several proteins are involved in the regulation of proteolytic activity, especially by acting as endopeptidase inhibitors. For instance, the increased cleavage of glyceraldehyde-3-phosphate dehydrogenase (Fig. 3b) may explain the reduction of aspartic-type endopeptidase inhibitor activity, and the increased cleavage of tissue inhibitor of metalloproteases 1 (Fig. 3e) may reflect a higher release of MMP1 into plasma of non-surviving individuals. The cleavage of inhibitors (inter-alpha-trypsin inhibitors, alpha-1-antichymotrypsin, alpha-1-antitrypsin, alpha-2-antiplasmin), even in the absence of significant differences in the proteolytic levels between subgroups of patients, supports the evidence of an imbalance between the pathologically increased proteolytic activity and the physiological endogenous inhibitory potential, which should trigger the testing of proteases as new therapeutic targets in septic shock.

A fragment of the activation peptide of cathepsin L1 (residues 75–89) also had increased abundance in non-survivors (Fig. 3a), along with immunoglobulin kappa variable 2D-28, hornerin, and the liver-specific oncprotein-induced transcript 3 protein.

Only one protein was more proteolysed in the survivors group (Fig. 3g) (i.e. basic salivary proline-rich protein 3, a salivary gland secreted protein involved in a ‘first barrier’ immune response to infection, given its role in the saliva as receptor for the Gram-negative Fusobacterium nucleatum). The identification of this protein is based on the qSLNEDVS-QEESPVISGKPEG peptide sequence (residues 17–39 of the protein), which corresponds to the protein N-terminus after signal peptide release, detected with modified N-terminal glutamine (q) to pyroglutamate.

We also found that 10 proteins were only proteolysed in survivors at T1 or T2, while in contrast, no protein was exclusively proteolysed in non-survivors. The proteolysis of these 10 proteins (Supplementary Table S1) may have beneficial roles in the progression of shock and be part of enhanced recovery in survivors. Among these proteins, a 29-amino acid peptide fragment of lipopolysaccharide-binding protein (P18428) was found in 57% of survivors at T1. The identification of this protein is based on the ANPQLVAR-ITDKGLQYAAQEGLLALQSEL peptide. As a protein that
facilitates binding of lipopolysaccharide (LPS) to recognition receptors (e.g. CD14, TLR4),\textsuperscript{27} P18428 degradation may signal a reduced response to Gram-negative endotoxin-mediated signalling and its absence in non-survivors could indicate complete degradation of the protein into fragments below the peptide range in the current analysis. The significance of this protein in survival remains to be investigated with independent measurements of LPS concentrations in patients.

Peptidomic profiles were different according to the source of infection. Uroseptic shock typically has better prognosis than shock derived from pulmonary or abdominal origins.\textsuperscript{28} Consistently, proteolysis was more limited in the uroseptic patients, further confirming the association between severity of septic shock, outcome, and extent of proteolysis. Reduced proteolysis in these patients could be related to a favourable response to the early therapeutic interventions which, when successful, limit hypoperfusion and protect the tissues, including the gut.\textsuperscript{29} Furthermore, in hyperdynamic states such as urosepsis, blood flow to heart, gut, and kidney is better preserved.\textsuperscript{30}

In contrast, septic shock of pulmonary origin accounted for five of the six deaths and it was the most common source of infection, similar to previous observations.\textsuperscript{28} Comparison of proteolysis in abdominal and respiratory infection showed that five proteins were significantly more cleaved in the abdominal group at T1, while 12 proteins were significantly more cleaved in the respiratory-initiated shock patients (Supplementary Table S2). Despite the same overall level of proteolysis at T1, these differences could indicate a broader impact of proteolysis on physiologic and repair processes in the respiratory group and therefore explain the association between outcome and proteolysis. These observations indicate that, much like in urosepsis, the patterns of proteolysis in the pulmonary and abdominal groups were consistent with their clinical severity.

The present study acknowledges some limitations. First, non-survivors were on average older than survivors and of healthy donors. Still, the data on circulating peptides detected in the whole septic shock group (survivors and non-survivors together), as well as in both the abdominal and respiratory septic shock subgroups at T1, do not point to a positive correlation between age and levels of proteolysis. This is consistent with previously reported evidence of the negative correlation between ageing and proteasome activity,\textsuperscript{31} and of the decreasing trypsin and MMP-9 activity with age\textsuperscript{32} (with possible pathological implications), which is also accompanied by increased alpha-1-antitrypsin concentration. Second, the number of enrolled patients was not very large. However, this work can serve as a guide for future studies designed to validate the peptidomic approach and its significance in larger and more diverse cohorts.

**Conclusions**

We propose a novel peptidomics-based analysis to test proteolysis in septic shock patients as a fundamental pathological mechanism contributing to outcome. Our data suggest an enhanced proteolytic activity with an association between proteolysis and mortality.

Even though the current analysis was limited to plasma only, the main findings of our study confirm previous animal reports. We observed that the increased proteolysis is the result of the activity of serine proteases and MMPs, and could also be explained by the systematic cleavage of endogenous protease inhibitors. Thus, a patient’s peptidomic profile could serve as a useful tool for interpreting the trajectory and outcome of patients in septic shock and to design new therapeutic treatments aimed to target proteases and support protease inhibition.

**Authors’ contributions**

Draft of the first manuscript: J.B.M., F.A.
Proteolysis in septic shock and mortality

Acknowledgements
The authors acknowledge the European Union-funded ‘ShockOmics’ consortium (Principal Investigator: Professor Giuseppe Baselli, Politecnico di Milano), and the Spanish Proteomics Network.

Declaration of interest

Funding
The 7th Framework Program of the European Union under the ‘ShockOmics’ grant #602706; the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development and Career Development Award (CDA2) 1IK2BX001277-01A1; the Instituto de Salud Carlos III Proteomics Network.

Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.bja.2018.05.072.

References


*Handling editor: J.P. Thompson*