Evaluation and comparison of NETosis biomarkers in sepsis and COVID-19 patients

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INTRODUCTION

- Neutrophil extracellular traps (NETs) are large, extracellular, web-like structures composed of cytotoxic and granule proteins that are assembled on a scaffold of decondensed chromatin.
- The composition of NETs varies depending on the stimulus.
- Critical COVID-19 patients differ from septic shock at the admission in the ICU by presenting higher levels of IL-1β and TNF-α, and lower levels of IL-10 and IL-6, which septic shock display higher levels of IL-6, IL-8, and a more significant myeloid response (including triggering receptors expressed on myeloid cells-1 (TREM-1) and IL-1R.

AIM

While both conditions have been linked to excessive NETosis, the direct comparison of NETosis biomarkers including nucleosomes in these two infectious conditions has not been described before.

METHOD

- 48 controls, 22 COVID-19 patients and 48 sepsis patients were included.
- Patients with critical COVID-19 who were admitted to the ICU for moderate or severe acute respiratory distress syn-drome (ARDS) due to SARS-COV-2 infection were included within five days of admission. ARDS was diagnosed according to the Berlin definition, and SARS-COV-2 infection was demonstrated by real-time reverse transcription PCR on nasopharyngeal swabs.
- Septic shock was defined according to the Sepsis-3 definition as sepsis with vasopressor therapy needed to elevate the mean arterial pressure ≥ 65 mmHg and lactate levels > 2 mmol/L despite adequate fluid resuscitation of 30 ml/kg of intravenous crystalloids within 6 hours. Patients with septic shock admitted to the ICU were included within two days of admission.
- Control patients with matched age, gender, and comorbidities were recruited at a central laboratory consultation.
- Nucleosomes containing histone H3.1 or containing citrullinated nucleosome histone H3R8 were measured using the NuQ® H3.1 and NuQ® H3R8 Cit ELISA assays from Human (Belgian Volition). Free citrullinated histone H3 (K3-H3) (citrullinated at R2, R8 and R17) were measured using the Cayman citrullinated histone H3 ELISA kit (Cayman Chemical). Neutrophil elastase and MPO were measured using the Human Neutrophil Elastase/Elastase 2 Direct ELISA and the Human Myeloperoxidase Quantikine ELISA Kit (R&D systems). Cytokines and chemokines were measured using the Bio-Plex Pro Human Cytokine 27-plex Assay and ICAM-1 and VCAM-1 were measured by mixing Bio-Plex Pro Human cytokines ICAM-1 and VCAM-1 sets (ICAM-VCAM) on a Bio-Plex 200 (Bio-Rad Laboratories N.V.).

RESULTS

Study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=16</th>
<th>COVID n=22</th>
<th>Sepsis n=48</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td><strong>Neutrophil elastase (EU/L)</strong></td>
<td>160±27</td>
<td>204±56</td>
<td>208±53</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Neutrophil myeloperoxidase (mmol/L)</strong></td>
<td>8.0±1.6</td>
<td>8.0±2.5</td>
<td>8.0±2.5</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Nucleosomes histones H3.1</strong></td>
<td>Not reported</td>
<td>19.9±4.4</td>
<td>22.1±6.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Nucleosomes histones H3R8</strong></td>
<td>Not reported</td>
<td>23.1±5.4</td>
<td>23.1±5.4</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Levels of circulating nucleosomes and neutrophil activation biomarkers in control, septic shock and critical COVID-19 populations

**Figure 1:** Nucleosomes, NE, HE and MPO were compared. Results were expressed as absolute value or normalised to neutrophil level for each individual. All markers were significantly different in septic shock compared to COVID-19. Significant differences were observed in critical COVID-19 and septic shock patients. **Present** and **Bold** indicate significant differences (p<0.05) from COVID-19. Circulating nucleosomes and cytokines were measured using different assays. Squares represent patients with a thrombocytopenic event and a significant neutrophil response demonstrated during the time. **** represents p-value of 0.0001 < p < 0.0005 and < 0.00001. **p-value**. Only differences which are statistically significant are reported. Some parameters were not available in all patients (nC-reactive protein, neutrophil count and MPO in septic patients regarding NE measurements).

CONCLUSIONS

- Circulating H3.1-nucleosomes and Cit-H3R8-nucleosomes appear to be interesting markers of global cell death and neutrophil activation when combined.
- H3.1-nucleosomes levels permit the evaluation of disease severity and differ between critical COVID-19 and septic shock patients reflecting two potential distinct pathological processes in these ARDS conditions.
- Normalization of H3.1-nucleosomes on the neutrophil count permit to better discriminate these different populations, reflecting the higher contribution of neutrophils to generate nucleosomes in septic shock patients.
- Further studies are required to confirm if measurement of nucleosomes and citrullinated nucleosomes may predict disease severity and help in categorizing patients at early stage of the disease.

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REFERENCES


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