

RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

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 cytosolic and granule proteins that are assembled on a scaffold of decondensed chromatin.¹
- The composition of NETs varies depending on the stimulus.²
- Critical COVID-19 patients differ from septic shock at the admission in the ICU by presenting higher levels of IL-1 β and T lymphocyte activation (including IL-7) whereas 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-1ra.³

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 yet.

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 syndrome (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 \geq 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.
- Nucleosome containing histone H3.1 or containing citrullinated nucleosome histone H3R8 were measured using the Nu.Q[®] H3.1 and Nu.Q[®] H3R8Cit ELISA assays from Volition (Belgian Volition). Free citrullinated histone H3 (Cit-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/ELA2 DuoSet 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

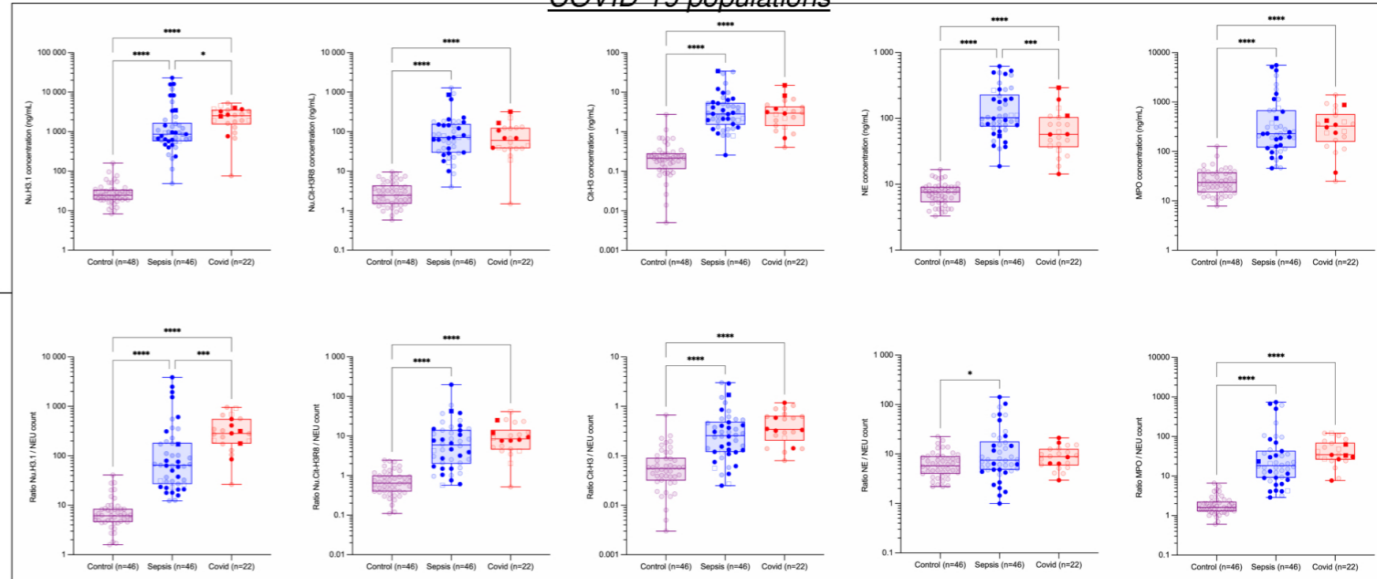
Study population

| | Control n=48 | COVID-19 n=22 | Sepsis n=48 | p-value |
|--|-----------------|-------------------|-------------------|----------|
| Demographics | | | | |
| Men (n, %) | 26 (54) | 15 (68) | 24 (50) | 0.36 |
| Women (n, %) | 22 (46) | 7 (32) | 24 (50) | |
| Age, years (n, sd) | 61.9 \pm 14.5 | 59.9 \pm 10.3 | 65.0 \pm 14.2 | 0.53 |
| Medical History | | | | |
| Hypertension (n, %) | 20 (42) | 12 (56) | 25 (52) | 0.48 |
| BMI $>$ 25 (n, %) | 26 (58) | 14 (74) | 26 (54) | 0.34 |
| Diabetes (n, %) | 11 (23) | 8 (36) | 5 (10) | 0.71 |
| History of smoking (n, %) | 10 (21) | 1 (5) | 15 (31) | 0.04 |
| COPD (n, %) | 4 (8) | 3 (14) | 5 (10) | 0.75 |
| CKD (n, %) | 9 (19) | 0 (0) | 10 (21) | 0.07 |
| Cancer (n, %) | 15 (31) | 0 (0) | 9 (19) | 0.01 |
| Outcome | | | | |
| 30-day mortality | | 6 (27) | 22 (46) | 0.45 |
| ICU length of stay (days) | | 29 \pm 30 | 8 \pm 9 | $<$ 0.01 |
| Thromboembolic events (n, %) | Not applicable | 6 (27) | 4 (8) | 0.06 |
| TIMI major bleeding events (n, %) [†] | | 5 (23) | 1 (2) | 0.01 |
| ICU admission | | | | |
| Delays since symptoms | Not applicable | 7.3 \pm 3.2 | 2.6 \pm 2.4 | $<$ 0.01 |
| Routine laboratory testing | | | | |
| Highest CRP (mg/dL) | | 323 \pm 119 | 313 \pm 122 | 0.75 |
| Creatinine (mg/dL) | | 0.91 \pm 0.59 | 2.19 \pm 1.91 | $<$ 0.01 |
| Hemoglobin (g/dL) | Not reported | 11.62 \pm 1.90 | 10.34 \pm 2.05 | 0.02 |
| Lowest Lymphocytes (103/ μ L) | | 484 \pm 335 | 469 \pm 310 | 0.86 |
| Organ failure and severity scores | | | | |
| PaO ₂ /FIO ₂ | | 103 \pm 37 | 225 \pm 119 | $<$ 0.01 |
| Ventilation duration (days) | | 27 \pm 24 | 4 \pm 7 | $<$ 0.01 |
| Norepinephrine (μ g/kg/min) | | 0.049 \pm 0.105 | 0.330 \pm 0.350 | $<$ 0.01 |
| Norepinephrine duration (days) | | 1.2 \pm 3.4 | 4.8 \pm 6.1 | $<$ 0.01 |
| Renal replacement therapy | Not applicable | 5 (1) | 27 (13) | 0.04 |
| Apache II score | | 15 \pm 4 | 20 \pm 7 | $<$ 0.01 |
| SOFA score | | 4 \pm 1 | 9 \pm 3 | $<$ 0.01 |
| SIC score | | 0 (0) | 11 (24) | 0.01 |
| DIC score | | 0 (0) | 7 (16) | 0.09 |

[†]Major bleeding complications have been defined according to the TIMI definition. All bleeding complications in COVID-19 group occurred in ECMO-treated patients.

Abbreviations: APACHE, acute physiology and chronic health evaluation; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; CRP, C-reactive protein; DIC, disseminated intravascular coagulopathy; ICU, intensive care unit; PaO₂/FIO₂, arterial oxygen partial pressure/fractional inspired oxygen; SIC, sepsis-induced coagulopathy; SOFA, sepsis-related organ failure assessment; TIMI, Thrombolysis in Myocardial Infarction; VV ECMO, venovenous extracorporeal membrane oxygenation

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



Nu.H3.1, Nu.Cit-H3R8, Cit-H3, NE and MPO were compared. Results were expressed as absolute value or normalized by neutrophils level for each individual. All markers were statistically different in septic shock and critical COVID-19 compared to controls. Only Nu.H3.1 and NE were different between septic shock and critical COVID-19 patients. Boxes represent 25th-75th percentile with median. Whiskers represent min to max variation. Squares represent patients with a thromboembolic event and non-transparent symbols represent dead patients. *, **, ***, **** and **** represent p-value $<$ 0.05, $<$ 0.005, $<$ 0.0005 and $<$ 0.0001, respectively. Only differences which are statistically significant are reported. Some parameters were not available in all patients (n=2 in control group regarding neutrophil count and n=2 in sepsis patients regarding NET measurements).

Abbreviations: Cit-H3, citrullinated histone H3 (citrullinated in R2, R8 and R17); MPO, myeloperoxidase; NE, neutrophil elastase; Nu.Cit-H3R8, citrullinated H3R8-nucleosome; Nu.H3.1, H3.1-nucleosome

| | APACHE II 0-15 | APACHE II 16-25 | APACHE II 26-35 | SOFA 0-4 | SOFA 5-9 | SOFA 10-12 | SOFA \geq 13 |
|------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|-----------------------|----------------------|
| Nu.H3.1 (ng/mL) | | | | | | | |
| Septic shock | 766.4 (133.7-1227.9) | 670.2 (215.6-1084.9) | 1575.3 (641.4-1995.7) | 577.9 (62.6-1273.9) | 671.3 (396.9-1577.5) | 1032.4 (612.7-1980.4) | 825.6 (198.4-1608.7) |
| Critical COVID-19 | 2764.5 (877.9-4720.9) | 1500.0 (516.9-4556.3) | 2548.3 (889.2-4495.4) | 2548.3 (889.2-4495.4) | 1768.1 (611.3-357.3) | | |
| adjusted p-value | | 0.0321 | | 0.0025 | | | |
| Nu.Cit-H3R8 (ng/mL) | | | | | | | |
| Septic shock | 50.5 (13.3-108.9) | 62.3 (18.8-362.7) | 172.4 (19.0-2924.4) | 31.8 (12.4-107.9) | 61.4 (18.8-103.1) | 132.7 (28.7-2025.4) | 333.2 (133.0-1933.7) |
| Critical COVID-19 | 328.9 (158.5-826.3) | 1774 (26.4-914.7) | 299.3 (86.9-888.9) | 299.3 (86.9-888.9) | 29.0 (204.4-222.4) | | |
| adjusted p-value | | 0.0005 | | $<$ 0.0001 | | | |
| Cit-H3 (ng/mL) | | | | | | | |
| Septic shock | 31.6 (10.2-183.6) | 70.2 (11.0-798.3) | 86.2 (3.9-862.1) | 26.6 (5.4-161.1) | 68.5 (16.5-226.3) | 79.1 (38.0-893.3) | 152.7 (72.5-1266.0) |
| Critical COVID-19 | 68.4 (21.9-277.3) | 41.3 (1.5-165.9) | 40.6 (25.6-218.4) | 75.3 (19.2-131.4) | | | |
| adjusted p-value | | $<$ 0.9999 | | 0.9538 | | | |
| NE (ng/mL) | | | | | | | |
| Septic shock | 0.12 (0.79-2.9) | 2.38 (1.1-25.8) | 1.8 (0.32-24.5) | 2.01 (0.94-8.92) | 2.81 (0.79-12.1) | 2.99 (1.24-27.6) | 4.81 (0.91-33.7) |
| Critical COVID-19 | 3.04 (0.94-11.7) | 2.77 (0.40-2.77) | 2.96 (0.77-8.01) | 2.96 (0.77-8.01) | | | |
| adjusted p-value | | 0.9666 | | $<$ 0.9999 | | | |
| Ratio MPO/NEI (count) | | | | | | | |
| Septic shock | 0.076 (0.027-0.209) | 0.084 (0.034-0.173) | 0.055 (0.009-0.121) | 0.079 (0.040-0.177) | 0.083 (0.010-0.214) | 0.072 (0.045-0.166) | 0.051 (0.009-0.308) |
| Critical COVID-19 | 0.039 (0.011-0.094) | 0.060 (0.010-0.090) | 0.031 (0.013-0.079) | 0.031 (0.013-0.079) | | | |
| adjusted p-value | | 0.0002 | | 0.0038 | | | |

Circulating nucleosomes and histones parameters in septic shock and critical COVID-19 patients according to APACHE-II and SOFA scores.

Abbreviations: Cit-H3, citrullinated histone H3; MPO, myeloperoxidase; NE, neutrophil elastase; Nu.Cit-H3R8, citrullinated nucleosome H3R8; Nu.H3.1, nucleosome H3

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 differs 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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