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

Laure Morimont [1,2], Mélanie Dechamps [3,4], Clara David [1], Céline Bouvy [1], Constant Gillot [2], Hélène Haguet [2], Julien Favresse [2], Lorian Ronvaux [2,5], Julie Candiracci [5], Marielle Herzog [5], Pierre-François Laterre [3,4], Julien de Poortere [4], Sandrine Horman [4], Christophe Beauloye [4,6], and Jonathan Douxfils[1,2]

[1]Qualiblood s.a., Research and Development Department, Namur, Belgium, [2]Department of Pharmacy, Namur Thrombosis and Hemostasis Center, Namur Research Institute for Life Sciences, University of Namur, Namur, Belgium, [3]Cardiovascular Intensive Care, Cliniques Universitaires St Luc, Brussels, Belgium, [4]Pôle de Recherche Cardiovasculaire, Institut de recherche expérimentale et clinique (IREC), Université Catholique de Louvain, Brussels, Belgium, [5] Belgian Volition SRL, Parc Scientifique Crealys, Isnes, Belgium, [6]Division of Cardiology, Cliniques Universitaires St Luc, Brussels, Belgium

## 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-1β 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 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.
- 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

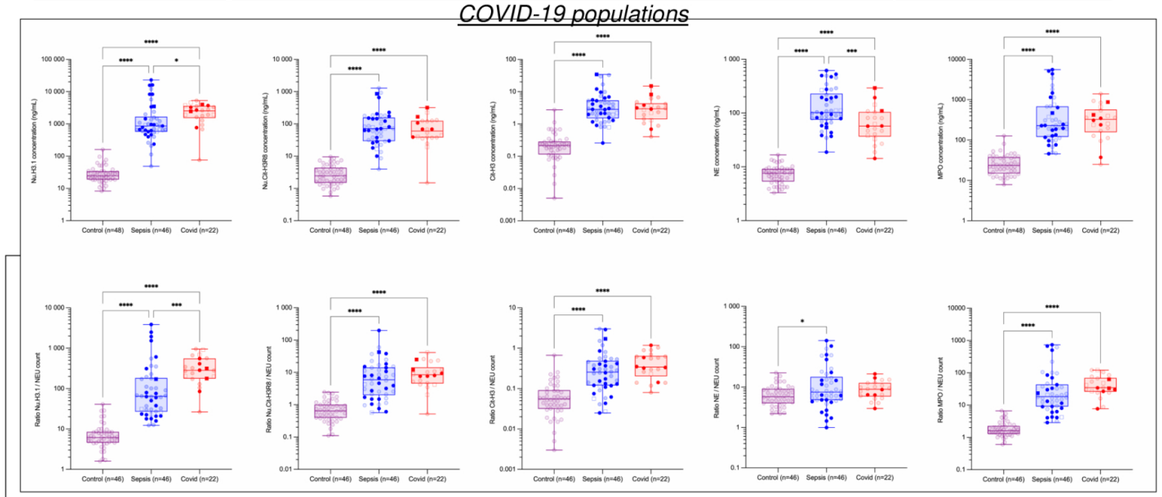
*Study population*

| | 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±14.5 | 59.9±10.3 | 65.0±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 | Not applicable | 6 (27) | 22 (46) | 0.45 |
| ICU length of stay (days) | | 29±30 | 8±9 | < 0.01 |
| Thromboembolic events (n, %) | | 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±3.2 | 2.6±2.4 | < 0.01 |
| **Routine laboratory testing** | | | | |
| Highest CRP (mg/dL) | Not reported | 323±119 | 313±122 | 0.75 |
| Creatinine (mg/dL) | | 0.91±0.59 | 2.19±1.91 | < 0.0 |
| Hemoglobin (g/dL) | | 11.62±1.90 | 10.34±2.05 | 0.02 |
| Lowest Lymphocytes (103/µL) | | 484±335 | 469±310 | 0.86 |
| **Organ failure and severity scores** | | | | |
| $PaO_2/FiO_2$ | Not applicable | 103±37 | 225±119 | < 0.01 |
| Ventilation duration (days) | | 27±24 | 4±7 | < 0.01 |
| Norepinephrine (µg/kg/min) | | 0.049±0.105 | 0.330±0.350 | < 0.01 |
| Norepinephrine duration (days) | | 1.2±3.4 | 4.8±6.1 | < 0.01 |
| Renal replacement therapy | | 5 (1) | 27 (13) | 0.04 |
| Apache II score | | 15 ± 4 | 20 ± 7 | < 0.01 |
| SOFA Score | | 4 ± 1 | 9 ± 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 25-35 | SOFA 0-6 | SOFA 7-9 | SOFA 10-12 | SOFA ≥13 |
|---|---|---|---|---|---|---|---|
| **Nu.H3.1 (ng/mL)** | | | | | | | |
| Septic shock | 666.4 (133.7-1257.8) | 670.0 (215.9-4898.9) | 1575.3 (641.4-19955.7) | 517.9 (62.6-1213.9) | 673.0 (396.9-15775.5) | 1032.8 (612.7-7993.8) | 8285.6 (1980.4-16068.7) |
| Critical COVID-19 | 2764.5 (877.8-4720.9) | 1904.0 (76.0-4555.3) | / | 2648.3 (689.2-4496.4) | 1769.1 (1615.1-1937.8) | / | / |
| adjusted p-value | 0.0321 | | | 0.0025 | | | |
| **Nu.H3.3 / neutrophils** | | | | | | | |
| Septic shock | 50.5 (13.2-108.9) | 62.3 (18.8-362.7) | 172.4 (19.0-2934.4) | 31.8 (12.4-107.9) | 61.4 (18.0-1533.1) | 132.7 (28.7-2025.4) | 553.7 (353.0-1933.7) |
| Critical COVID-19 | 3222.6 (1353.5-826.3) | 177.4 (26.4-914.7) | / | 299.5 (86.9-888.9) | 236.0 (204.4-272.4) | / | / |
| adjusted p-value | 0.0005 | | | <0.0001 | | | |
| **Nu.Cit-H3R8 (ng/mL)** | | | | | | | |
| Septic shock | 31.6 (10.2-183.6) | 70.2 (11.0-798.3) | 86.2 (39.3-862.1) | 28.6 (5.4-161.1) | 68.5 (16.5-226.3) | 79.1 (38.0-893.3) | 143.7 (72.3-1286.0) |
| Critical COVID-19 | 68.4 (21.9-271.3) | 41.1 (1.5-165.9) | / | 60.6 (25.6-218.6) | 75.3 (19.2-131.4) | / | / |
| adjusted p-value | >0.9999 | | | 0.9038 | | | |
| **Cit-H3 (ng/mL)** | | | | | | | |
| Septic shock | 2.12 (0.79-9.28) | 2.58 (1.1-25.8) | 3.18 (0.52-24.5) | 2.04 (0.94-8.92) | 2.84 (0.79-12.1) | 2.99 (1.24-27.6) | 4.16 (0.91-32.7) |
| Critical COVID-19 | 3.04 (0.94-11.7) | 2.77 (0.40-2.77) | / | 2.96 (0.71-8.01) | 3.74 (1.32-6.15) | / | / |
| adjusted p-value | 0.9966 | | | >0.9999 | | | |
| **Nu.Cit-H3R8 / Nu.H3.1** | | | | | | | |
| Septic shock | 0.076 (0.027-0.209) | 0.084 (0.034-0.0165) | 0.055 (0.009-0.121) | 0.079 (0.040-0.172) | 0.083 (0.010-0.214) | 0.072 (0.043-0.166) | 0.031 (0.009-0.108) |
| Critical COVID-19 | 0.030 (0.011-0.064) | 0.040 (0.010-0.090) | / | 0.031 (0.013-0.079) | 0.028 (0.010-0.081) | / | / |
| 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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## CONTACT

jonathan.douxfils@qualiblood.eu
+32 81/44.49.92
Rue du Séminaire 20a, 5000 Namur (BE)