

## 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.<sup>1</sup>
- The composition of NETs varies depending on the stimulus.<sup>2</sup>
- Critical COVID-19 patients differ from septic shock at the admission in the ICU by presenting higher levels of IL-1 $\beta$  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.<sup>3</sup>

## 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<sup>®</sup> H3.1 and Nu.Q<sup>®</sup> 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

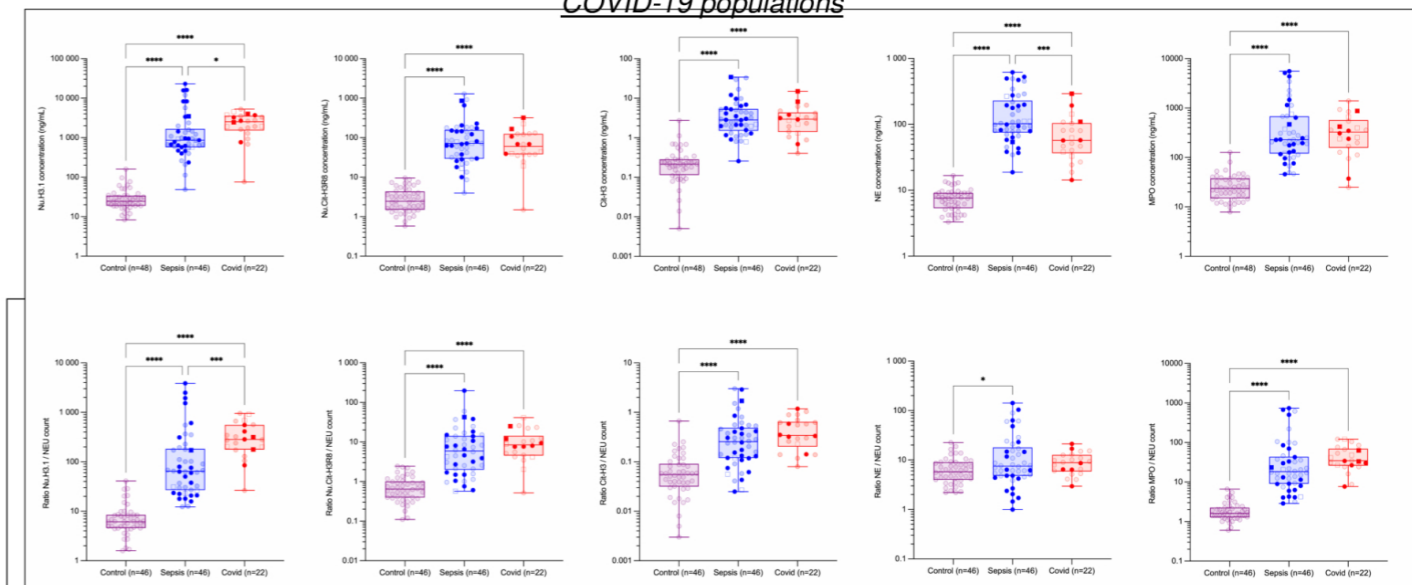
### Study population

	Control n=48	COVID-19 n=22	Sepsis n=48	p-value
<b>Demographics</b>				
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
<b>Medical History</b>				
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
<b>Outcome</b>				
30-day mortality	Not applicable	6 (27)	22 (46)	0.45
ICU length of stay (days)	Not applicable	29 $\pm$ 30	8 $\pm$ 9	< 0.01
Thromboembolic events (n, %)	Not applicable	6 (27)	4 (8)	0.06
TIMI major bleeding events (n, %) <sup>†</sup>	Not applicable	5 (23)	1 (2)	0.01
<b>ICU admission</b>				
Delays since symptoms	Not applicable	7.3 $\pm$ 3.2	2.6 $\pm$ 2.4	< 0.01
<b>Routine laboratory testing</b>				
Highest CRP (mg/dL)	Not reported	323 $\pm$ 119	313 $\pm$ 122	0.75
Creatinine (mg/dL)	Not reported	0.91 $\pm$ 0.59	2.19 $\pm$ 1.91	< 0.0
Hemoglobin (g/dL)	Not reported	11.62 $\pm$ 1.90	10.34 $\pm$ 2.05	0.02
Lowest Lymphocytes (103/ $\mu$ L)	Not reported	484 $\pm$ 335	469 $\pm$ 310	0.86
<b>Organ failure and severity scores</b>				
PaO <sub>2</sub> /FIO <sub>2</sub>	Not applicable	103 $\pm$ 37	225 $\pm$ 119	< 0.01
Ventilation duration (days)	Not applicable	27 $\pm$ 24	4 $\pm$ 7	< 0.01
Norepinephrine ( $\mu$ g/kg/min)	Not applicable	0.049 $\pm$ 0.105	0.330 $\pm$ 0.350	< 0.01
Norepinephrine duration (days)	Not applicable	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	Not applicable	15 $\pm$ 4	20 $\pm$ 7	< 0.01
SOFA score	Not applicable	4 $\pm$ 1	9 $\pm$ 3	< 0.01
SIC score	Not applicable	0 (0)	11 (24)	0.01
DIC score	Not applicable	0 (0)	7 (16)	0.09

<sup>†</sup>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; PaO<sub>2</sub>/FIO<sub>2</sub>, 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 25<sup>th</sup>-75<sup>th</sup> 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
<b>Nu.H3.1 (ng/mL)</b>							
Sepsis shock	766.4 (133.7-1277.9)	670.2 (215.9-1084.9)	1575.3 (641.4-1995.7)	577.9 (62.6-1172.9)	671.3 (396.9-1577.5)	1032.4 (612.7-1980.4)	828.6 (198.4-1608.7)
Critical COVID-19	2764.5 (877.9-4720.9)	1900.0 (516.9-4595.3)	2548.3 (809.2-4495.4)	2548.3 (809.2-4495.4)	1768.1 (481.3-357.3)		
adjusted p-value		0.0321			0.0025		
<b>Nu.Cit-H3R8 (ng/mL)</b>							
Sepsis shock	30.5 (13.3-108.9)	62.3 (18.8-362.7)	172.4 (19.0-2924.4)	31.8 (12.4-107.9)	61.4 (18.8-1033.1)	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 (1481.3-357.3)		
adjusted p-value		0.0005			< 0.0001		
<b>Cit-H3 (ng/mL)</b>							
Sepsis shock	31.6 (10.2-183.6)	70.2 (11.0-798.3)	86.2 (3.9-862.1)	28.6 (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 (76.6-218.4)	75.3 (19.2-131.4)			
adjusted p-value		< 0.9999		0.9538			
<b>NE (ng/mL)</b>							
Sepsis 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 (0.60-2.77)	2.96 (0.77-8.01)	2.96 (0.77-8.01)			
adjusted p-value		0.9666		< 0.9999			
<b>Nu.Cit-H3R8 / Nu.H3.1</b>							
Sepsis 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 (0.008-0.308)
Critical COVID-19	0.039 (0.011-0.094)	0.062 (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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