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-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

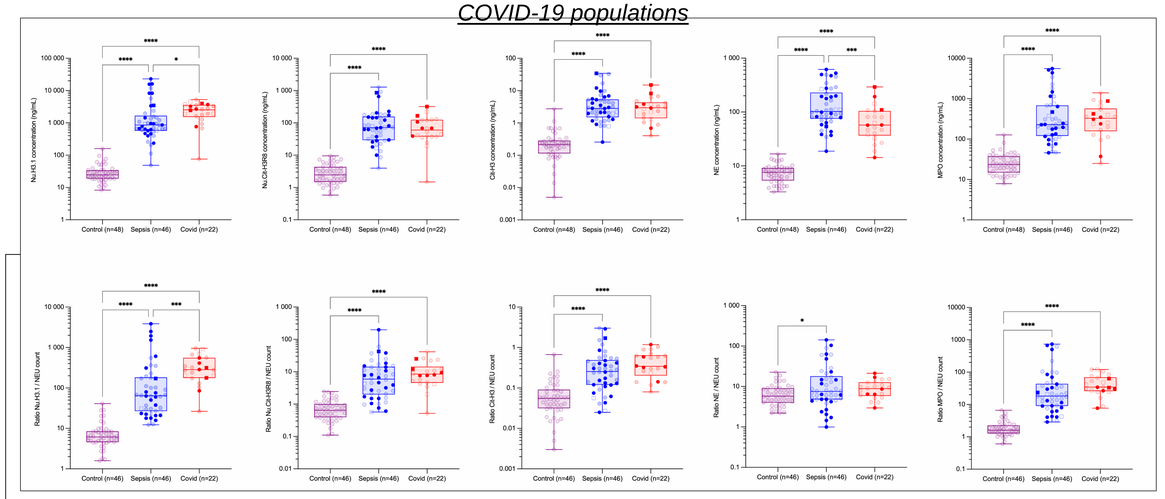
Study population

	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 ≤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 (396.9-13775.5)	1032.8 (612.7-7993.8)	8285.6 (1980.4-16068.7)
Critical COVID-19	2764.5 (877.8-4720.9)	1904.0 (76.0-4553.3)		2048.3 (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 (28.7-2023.4)	553.7 (353.6-1933.7)
Critical COVID-19	322.6 (155.5-826.3)	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)	80.2 (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 (21.9-271.5)	41.1 (1.5-165.9)		60.6 (25.6-218.6)	75.3 (19.2-131.4)		
adjusted p-value	*>0.9999*			*0.9538*			
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)	4.14 (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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