Plasma nucleosome measurement as a biomarker for NETs in intensive care patients

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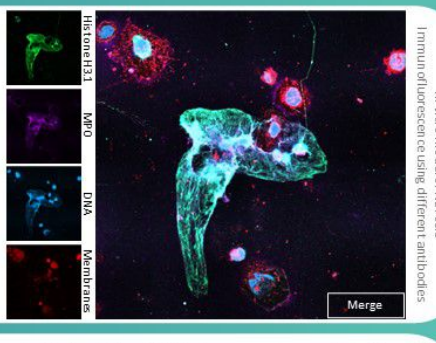
Introduction

NETosis is a regulated cell death mechanism leading to the formation of Neutrophil Extracellular Traps (NETs) and is a rapid and effective immunological response to infection. However, excessive production of NETs can lead to host tissue damage and, in severe cases, to sepsis and death. It is now well established that inappropriate NETs production may be associated with an uncontrolled inflammatory response and resulting pathologies.

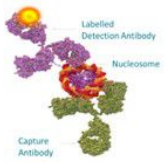
Circulating NETs have been measured using a variety of proxy biomarkers including MPO, MPO-DNA, NE, histones and cfDNA. These assays are not standardized and typically not analytically validated. We have developed the first analytically valid manual and automated immunoassay for nucleosomes which has been CE marked as a diagnostic tool to aid the detection and evaluation of diseases associated with NETosis.



Methods

Nu.Q® NETs Immunoassay.

NETs were measured using the quantitative Nu.Q® NETs assays (Belgium Volition, SRL). It consists of a sandwich immunoassay (manual and automated) involving a monoclonal anti-histone capture antibody and a labeled anti-nucleosome detection antibody.



Other blood biomarkers assays.

H3R8 citrullinated-nucleosomes were measured using the Nu.Q® H3R8Cit ELISA (Belgian Volition, SRL). Neutrophil elastase (NE) and myeloperoxidase (MPO) were measured using the Human Neutrophil Elastase/ELA2 DuoSet ELISA and the Human Myeloperoxidase Quantikine ELISA Kit (R&D systems).

Monitoring of removal of circulating NETs in a porcine model of sepsis using NucleoCapture™ Therapeutic Apheresis (Santersus AG).

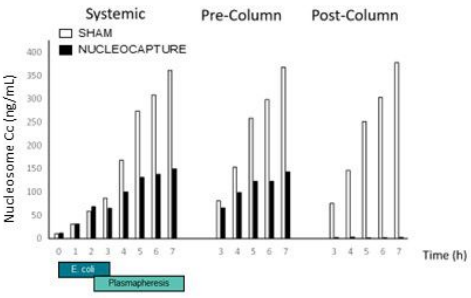
Sepsis was induced in 2 pigs by a 3h infusion of E. Coli. A test pig was treated to remove circulating NETs using a NucleoCapture H1-apheresis column for 5h. A control pig was treated using a sham column. NETs levels were measured using the Nu.Q® NETs assay. NucleoCapture is a novel first-in-class NETs depletion technology utilizing Linker Histone H1.3 binding.

Patients' and normal donors' population.

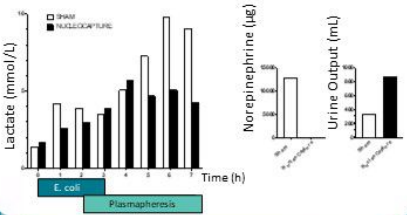
22 patients admitted to ICU for moderate or severe acute respiratory distress syndrome (ARDS-Berlin definition) due to SARS-CoV-2 infection (determined by RT-PCR on nasopharyngeal swabs) were included within 5 days of admission. 46 patients with septic shock (Sepsis-3 definition) admitted to ICU were included within 2 days of admission. 48 control patients matched for age, gender, and comorbidity were recruited at a central laboratory consultation.

Circulating nucleosomes are markers of NETs removal from blood

NucleoCapture was evaluated in a clinically relevant porcine critical care model of sepsis. Infusion of E. coli. over 3 hours resulted in a continuous increase in NETs levels in the sham treated pig during the experiment.
In contrast, passage of plasma through a NucleoCapture column removed 97.7-99.0% of NETs and prevented a continuous rise in the H1-apheresis treated pig with nucleosome levels plateauing.

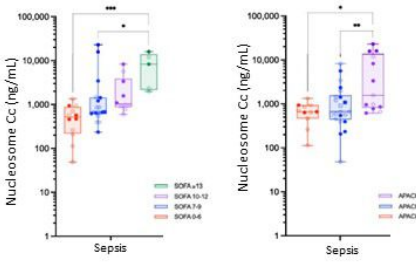


The reduced NETs levels in the treated pig were associated with an attenuation of septic shock as evidenced by reduced lactate levels, decrease total noradrenaline required and increased total urine output.



Circulating nucleosomes are markers of NETosis associated with disease severity in sepsis

Nucleosome levels measured in septic shock patients correlate with SOFA score (adjusted p-value 0.0025) and with APACHE II score (adjusted p-value 0.0321).

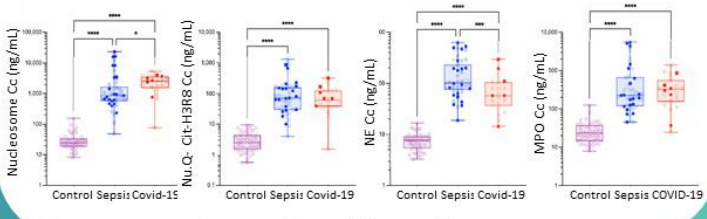


No correlation was observed for other biomarkers tested.

Nucleosome levels measured by Nu.Q® NETs assay were higher in COVID-19 and did not correlate with SOFA or APACHE-II scores.

	SOFA 0-6	SOFA 7-9	SOFA 10-12	SOFA ≥ 13	APACHE-II 0-15	APACHE-II 16-25	APACHE-II 25-35
Nucleosome Cc (ng/mL)							
Septic shock	518 (63-1214)	673 (397-15776)	1033 (613-7994)	8286 (1980-1669)	666 (134-1258)	670 (216-4899)	1575 (641-19956)
Critical COVID-19	2648 (689-4497)	1769 (1615-1938)	/	/	2765 (878-4721)	1904 (76-4555)	/
corrected p_value	*0,0025*	*0,025*	/	/	*0,0321*	*0,0321*	/

Nucleosomes (Nu.Q® NETs), Nu.Q® Cit-H3R8, NE and MPO were compared. All markers were statistically different in septic shock and critical COVID-19 compared to controls. Only nucleosomes and NE were different between septic shock and COVID-19 patients.



*: p-value <0.05; **: p <0.01; ***: p <0.001; ****: p <0.0001

Conclusions

- Nucleosome measurements using the Volition Nu.Q® NETs immunoassay may be useful in the management of sepsis patients.
- Nucleosome levels may be useful in evaluation of disease severity and could help in sepsis sub-classification.

** Further studies are ongoing to confirm clinical interests.*

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Reference

[1] Morimont, L.; et al., NETosis and Nucleosome Biomarkers in Septic Shock and Critical COVID-19 Patients: An Observational Study. Biomolecules 2022.