Detection and evaluation of diseases associated with NETosis in Human Plasma

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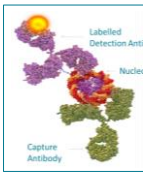
Introduction

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.

The aim of the first study was to evaluate circulating nucleosome levels as a biomarker of NETosis in patient populations with different NET-related pathologies (sepsis, COVID-19 infection, autoimmune diseases, organ injury, inflammatory disorders and other diseases associated with NETs) using a rapid nucleosome quantitative test in plasma.

Septic shock and COVID-19 are two well-studied diseases involving an excessive inflammatory reaction and release of NETs. The inflammatory reaction in critical COVID-19 and septic shock patients differs on admission to ICU [1] but no direct comparison of NETosis biomarkers has been described.

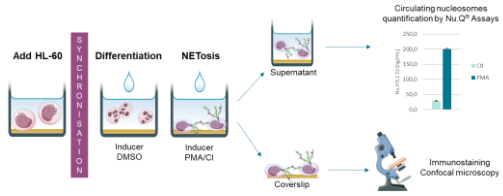
The aim of the second study was to evaluate NETosis biomarkers in these conditions and compare the results with SOFA (Sequential Organ Failure Assessment) and APACHE-II (Acute Physiology And Chronic Health Evaluation) scores.



Nu.Q® sandwich ImmunoAssay.
Circulating nucleosomes containing histone H3.1 will be measured using quantitative Nu.Q® ImmunoAssay. These consist of sandwich ELISA involving monoclonal anti-histone H3.1 capture antibody and labeled anti-nucleosome detection antibody.
The ability of these two antibody to recognize nucleosomes-containing NETs was evaluated NETs standard produced *in vitro* in HL-60 model and data are presented on the right panel.

Visualization of nucleosomes as NETs component using antibodies contained in Nu.Q® Assays

Synchronized HL-60 (48 h serum starvation) are differentiated during 6 days with 1.5 % DMSO (renew every 2 days). NETosis is finally induced by 100 nM of PMA (phorbol myristate acetate) or 4 µM of CI (calcium ionophore) [2].



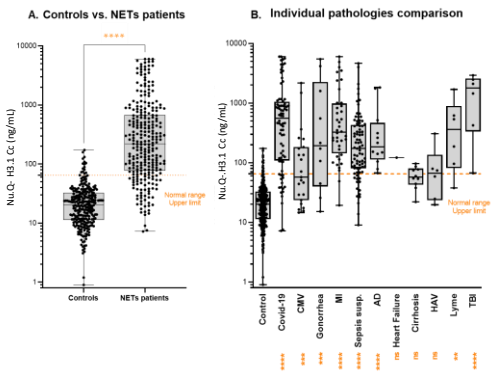
Nucleosomes containing H3.1 histone are part of NETs.
A. Presence of H3.1 histones on NETs produced *in vitro* and co-localization with two other NETs components: DNA and MPO (myeloperoxidase). **B.** Co-localization of H3.1 histones and nucleosomes on web-like DNA structure using capture and detection antibodies used the Nu.Q® H3.1 assays.

Circulating nucleosomes in NETs-related diseases

Study population. Frozen EDTA plasma samples from 269 controls (self-certified as healthy donors from EFS (Etablissement Français du Sang)) and 275 patients with diseases associated with NETosis such as COVID-19 (n=80), Sepsis suspicion (n=81), Cirrhosis or NASH (n=10), Cytomegalovirus Infection (n=23), Gonorrhoea Infection (n=10), Myocardial Infarction (n=40), Alzheimer's disease (n=12), HAV Infection, (n=6), Lyme Infection (n=6), Traumatic Brain Injury (n=6), heart failure (n=1) were included.

Blood biomarkers assays. Nucleosomes containing histone H3.1 (Nu.Q® H3.1) were measured using the Nu.Q® H3.1 ChLIA assay from Volition (Belgian Volition, SRL).

Results. Assessment of circulating nucleosome levels in the control population, compared to the population of patients with NETs-related pathologies. **A.** Significantly elevated levels of circulating nucleosomes were found in patients with NETs-related diseases compared to control population (mean 732,2 ng/mL vs 26,1 ng/mL, p <0.0001). **B.** All pathologies were individually compared to controls.



On this clinical cohort, based on a normal range up to 65 ng/mL, the clinical performances are about 79,3% sensitivity at a specificity of 94,1 %.

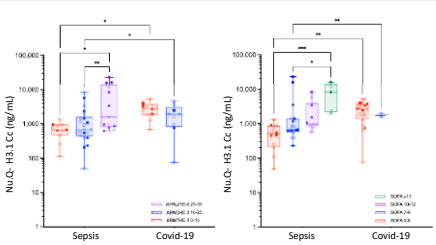
Boxes represent 25th-75th percentile with median. Whiskers represent min to max variation. *, **, *** and **** represent p-value < 0.05, < 0.005, < 0.0005 and < 0.0001, respectively.

Conclusions.
The Nu.Q® H3.1 assay targets nucleosome-containing NETs and nucleosome metabolites of NETs and may be used as a biomarker for sepsis, COVID-19, organ injury, inflammatory disorders and other diseases associated with NETs.
*The assay is also validated on fresh EDTA plasma in an independent cohort (Normal range ≤ 24 ng/mL, sensitivity of 79,4 % at 93,8 % specificity).

Circulating nucleosomes are markers of NETosis associated with disease severity in sepsis [3]

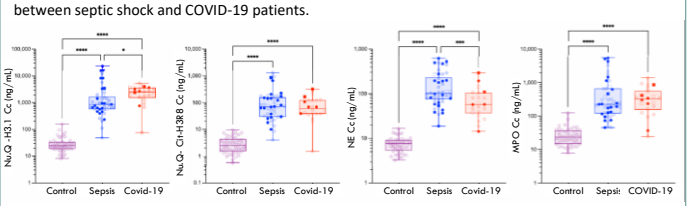
Study population. 22 patients with critical COVID-19 admitted to the ICU for moderate or severe acute respiratory distress syndrome (ARDS) due to SARS-CoV-2 infection were included within five days of admission. ARDS was diagnosed according to the Berlin definition, and SARS-CoV-2 infection was demonstrated by real-time reverse transcription PCR on nasopharyngeal swabs. 46 patients with septic shock (defined according to the Sepsis-3 definition) admitted to the ICU were included within two days of admission. 48 control patients with matched age, gender, and comorbidities were recruited at a central laboratory consultation.

Blood biomarkers assays. Nucleosome containing histone H3.1 (Nu.Q® H3.1) or citrullinated histone H3R8 (Nu. Q® Cit-H3R8) were measured using the Nu.Q® H3.1 and Nu.Q® H3R8Cit ELISA assays from Volition (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).



Results.
Nu.Q®H3.1 nucleosome levels measured in septic shock patients correlated 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.
Nu.Q®H3.1 levels were higher in COVID-19 and did not correlate with SOFA or APACHE-II scores.

Nu.Q®H3.1, 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 Nu.Q®H3.1 and NE were different between septic shock and COVID-19 patients.



Boxes represent 25th-75th percentile with median. Whiskers represent min to max variation. *, **, *** and **** represent p-value < 0.05, < 0.005, < 0.0005 and < 0.0001, respectively.

Conclusions.
Circulating Nu.Q®H3.1 and Nu.Q®Cit-H3R8-nucleosomes appear to be interesting markers of global cell death and neutrophil activation.
Nu.Q®H3.1-nucleosomes levels permit the evaluation of disease severity and differ between critical COVID-19 and septic shock patients reflecting two potential distinct pathological processes in these ARDS conditions.
Further studies are required to confirm nucleosome measurements as predictors of disease severity at an early stage of the disease.

References

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[3] Morimont, L.; Dechamps, M.; David, C.; Bouvy, C.; Gillot, C.; Haguet, H.; Favresse, J.; Ronvaux, L.; Candiracci, J.; Herzog, M.; et al., NETosis and Nucleosome Biomarkers in Septic Shock and Critical COVID-19 Patients: An Observational Study. Biomolecules 2022.

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