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NETs-associated H3.1-nucleosome measurements in plasma correlate with severity of Alzheimer's Disease progression

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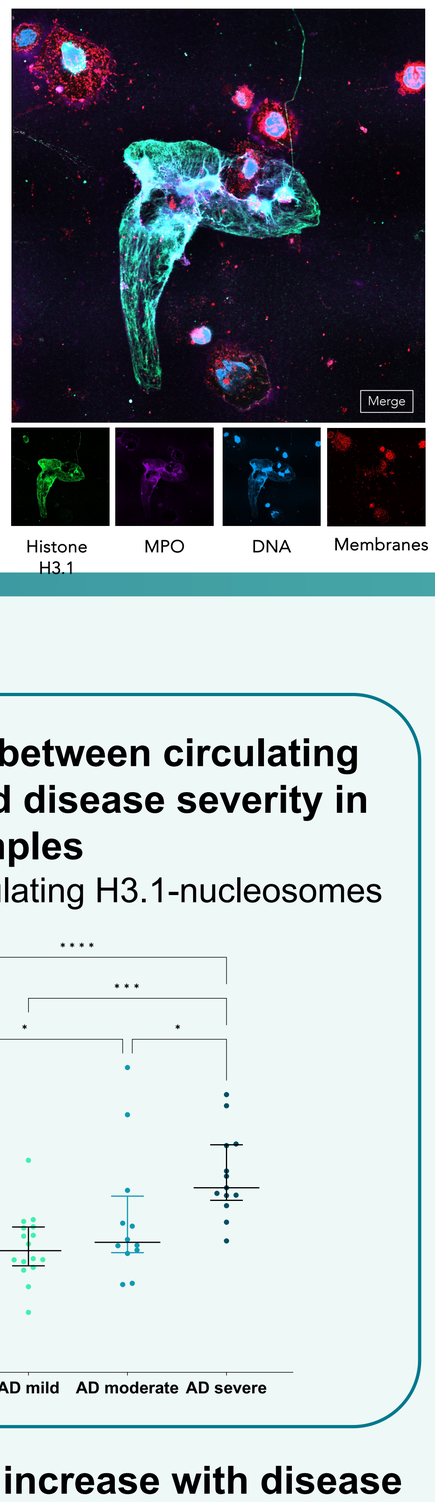
NETs in Alzheimer's disease

NETosis is a cell death mechanism leading to the formation of Neutrophil Extracellular Traps (NETs) and is involved in infectious and non-infectious diseases. NETs are composed of extruded chromatin comprising nucleosomes that can be citrullinated, and anti-microbial proteins such as myeloperoxidase (MPO) and neutrophil elastase (NE).

Inappropriate NETs production is associated with an uncontrolled inflammatory response resulting in diverse pathologies. An association between inflammation and NETs with Alzheimer's disease (AD) has been reported in human but circulating NETs levels in AD patients have not been investigated until now.

Immunofluorescence Imaging of NETs in HL-60 *in vitro* model

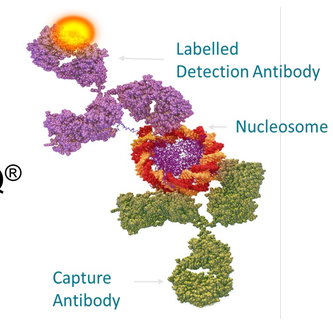


NETs Detection

We have developed the first analytically validated manual and automated immunoassay for the measurement of NETs. The assay has been CE-IVDD marked as a diagnostic tool to aid in the detection and evaluation of diseases associated with NETosis by quantifying circulating H3.1-nucleosomes in plasma.

Here, we investigated circulating NETs levels in Mild, Moderate and Severe AD by using the Nu.Q® NETs immunoassay.

Moreover, we compared the value of the Nu.Q® NETs assay with a variety of other potential NETs biomarkers: citrullinated nucleosomes, MPO, NE and cell-free DNA (cfDNA).



Methods

Nu.Q® NETs
Circulating NETs were quantified using the Nu.Q® NETs assay (CE-IVDD, Belgium Volition, SRL), an automated sandwich immunoassay involving a monoclonal anti-histone H3.1 capture antibody coated on magnetic beads and a labeled anti-nucleosome detection antibody.

H3R8cit- and H3R17cit-nucleosomes
Circulating H3R8cit- and H3R17cit-nucleosomes were quantified using the Nu.Q® H3R8cit and H3R17cit automated immunoassay prototypes (Belgian Volition, SRL).

MPO and NE
Human MPO and NE were quantified by Human MPO Quantikine™ ELISA Kit and Human NE/ELA2 DuoSet® ELISA (R&DSystems).

cfDNA
After extraction from plasma samples with the QIAamp® DSP Circulating NA kit (Qiagen), cfDNA was quantified using the Qubit™ 1X dsDNA HS assay Kit (ThermoFisher).

Cohort description
Retrospective study performed on K2EDTA plasma leftover samples from AD patients and age-matched control donors. Diagnosis were provided by biobank suppliers.

	Control	AD Mild	AD Moderate	AD Severe
Number of samples (n)	33	16	12	13
Median age (years)	65	76	79	80
Gender (female)	13 (39%)	8 (50%)	6 (50%)	8 (62%)

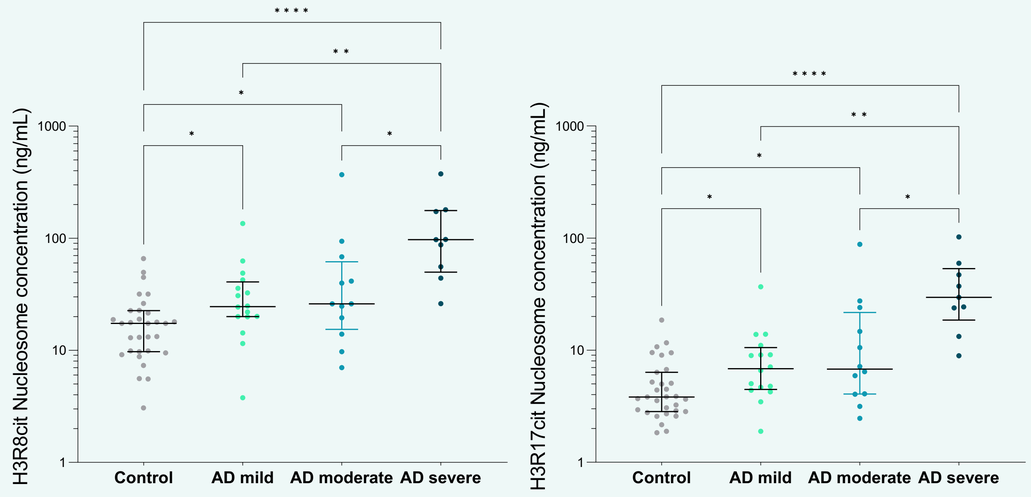
Results

A. Association between circulating NETs levels and disease severity in AD plasma samples

Quantified by circulating H3.1-nucleosomes

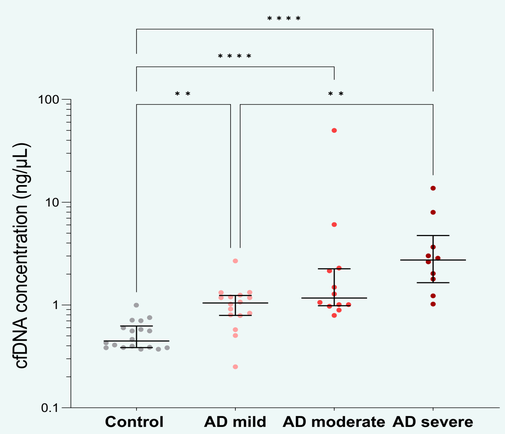
B. Similar results were observed for circulating H3R8cit- and H3R17cit-nucleosomes

These markers were also elevated in early stage (Mild AD)

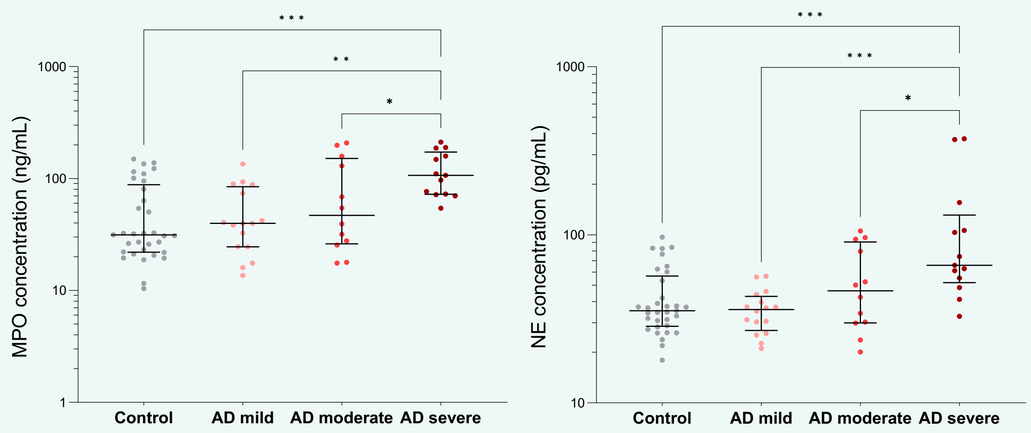


C. cfDNA levels increase with disease severity in AD plasma samples

cfDNA measurement requires DNA extraction method and larger plasma volume compared to Nu.Q® assays



D. MPO and NE levels were elevated only in the more advanced stage (AD Severe)



E. In AD cohort, all the results are strongly correlated with the Nu.Q® NETs assay

Spearman r = 0,88 (H3R8cit); 0,87 (H3R17cit); 0,75 (cfDNA); 0,73 (MPO) and 0,70 (NE); p<0,0001.

Conclusions

- **Circulating NETs levels are determined by H3.1-nucleosome measurement in plasma samples.**
- **Elevated levels of circulating NETs are observed in AD plasma samples.**
- **Circulating NETs levels correlate with disease severity in contrast to MPO and NE.**
- **Nu.Q® NETs measurements may have value for the assessment and management of AD patients*.**

** Further studies are required to confirm clinical interests*