ISTH VIRTUAL CONGRESS 2021 JULY 17–21 ISTH2021.ORG
HOSTED FROM PHILADELPHIA
SCIENCE CONQUERING DISEASE

# CIRCULATING NUCLEOSOME IMMUNOASSAY: EVALUATING A CLINICALLY-APPLICABLE TEST TO RISK STRATIFY COVID-19 AND TARGET ANTICOAGULATION

C. Rea [1], S. Napoli [1], S. Mujib [1], F. Trovato [1], A. Zamalloa [1], J. Smith [1], D. Nagra [1], K. O'Gallagher [1], M. McPhail [1]
[1]Kings College NHS Foundation Trust, London, United Kingdom

NHS King's College Hospital NHS Foundation Trust

## INTRODUCTION

It has been suggested that therapeutic anticoagulation may decrease thrombotic and non-thrombotic complications in infection with SARS-CoV-2 (COVID-19), improving outcomes (1). Therapeutic anticoagulation of all COVID-19 patients would carry a significant bleeding risk. Accurately targeting only high-risk patients would improve safety of this approach and more widely aid with the targeting of therapies and appropriate resources to those at greatest risk. Dysregulated neutrophil extracellular traps (NETs) drive pulmonary inflammation, thrombosis and mortality and may therefore represent a useful biomarker. NETs determined via free DNA quantification are elevated in severe COVID-19 and correlate with outcome (2), but the technical requirements of these tests preclude their clinical use.

## AIM

We assessed the potential of a clinically-applicable immunoassay for the quantification of cell free H3.1-nucleosome as a NETs biomarker and predictor of thrombosis and mortality

## METHOD

Plasma samples on admission, day 3, 7 and 10 were evaluated from 20 patients with severe COVID-19 requiring organ support (severe cohort) and compared with 28 samples from COVID-19 patients requiring hospitalization, but not organ support (non-severe cohort).

Circulating H3.1-nucleosomes were measured using Nu.Q™ H3.1-nucleosome ELISA (Belgian Volition SRL, Isnes, Belgium) as per manufacturer's instruction Plasma samples (20µl in duplicate) were incubated for 2h30 at room temperature in a 96-well plate coated with a monoclonal antibody against Histone H3.1. After washing, the level of nucleosomes was quantified by adding 100µl of a HRP-labelled anti-nucleosome antibody (incubation 90min at room temperature). The wells were washed and a peroxidase substrate: 3,3',5,5'-Tetramethylbenzidine (TMB) was added. After 20 min, the colorimetric reaction was stopped by adding 100µl of Stop solution. The optical densities of the well were read at 450nm using a microplate reader (FLUOstar Omega, BMG Labtech). CV% was assessed. If unacceptable variation occurred samples were repeated. If values were above the detectable range, samples were diluted up to 1:10 with dilution plasma before being re-evaluated.

## RESULTS

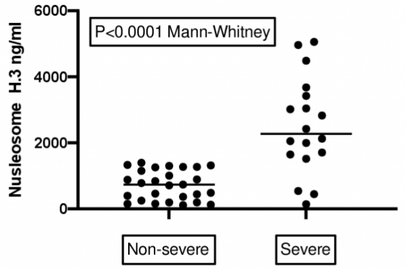


Figure 1a

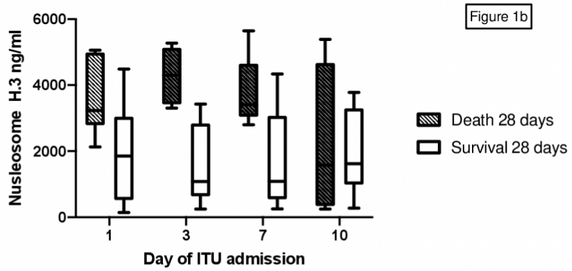


Figure 1b

Table 1: Patient Descriptive Data

| | Age- Years (Median, Range) | Female (Male) |
|---|---|---|
| Severe | 63 (43-84) | 5 (15) |
| Non-Severe | 51.5 (28-80) | 11 (19) |

**Table 1: Illustrates patient age and Sex distribution between Severe and Non-Severe groups.**

**Figure 1a: Illustrates H3.1-nucleosome levels (ng/ml) in non-severe versus severe COVID-19 patients and the group median.**

**Figure 1b: Charts the median and range of values for H3.1-nucleosome levels (ng/ml) over the first 10 days of ITU admission in severe patients who survived versus those who died up to 28 days.**

H3.1 nucleosome levels were significantly elevated in the ITU cohort versus ward cohort (figure 1a). H3.1 nucleosome levels could not be used to predict thrombotic outcome, but there was an association with 28-day mortality. Significantly higher H3.1 nucleosome levels were recorded upon ITU in patients who subsequently died (Mann-whitney P=0.014). In the six patients who dies following admission to ITU, comparatively higher H3.1 nucleosome values maintained during day 1-7 of ITU admission (Figure1b).

## CONCLUSIONS

The immunoassay NETs biomarker replicated findings of free DNA quantification studies; NETs correlate with disease severity.

No correlation was found with thrombotic events. This may reflect the limited sample size.

There is an indication that elevated NETs values predict poor outcomes in patient admitted to ITU and may be of value in risk stratifying to treatments such as therapeutic anticoagulation and tracking response to treatment

## ACKNOWLEDGEMENTS


- Belgian Volition SRL for provision of ELISA Kits
- Professor Shah, Dr O'Gallagher & Team: Cardiovascular Research. Dr McPhail & Team at the Institute of Liver Studies, Kings College London for collection, sample processing and access to facilities


## CONTACT INFORMATION

For further information or questions please contact:catherine.rea1@nhs.net

## REFERENCES

1: Nadkarni GN, Lala A, Bagiella E, Chang HL, Moreno PR, Pujadas E, Arvind V, Bose S, Charney AW, Chen MD, Cordon-Cardo C, Dunn AS, Farkouh ME, Glicksberg BS, Kia A, Kohli-Seth R, Levin MA, Timsina P, Zhao S, Fayad ZA, Fuster V. Anticoagulation, Bleeding, Mortality, and Pathology in Hospitalized Patients With COVID-19. J Am Coll Cardiol. 2020 Oct 20;76(16):1815-1826. doi: 10.1016/j.jacc.2020.08.041. Epub 2020 Aug 26. PMID: 32860872; PMCID: PMC7449655.

2: Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, Blair C, Weber A, Barnes BJ, Egeblad M, Woods RJ, Kanthi Y, Knight JS. Neutrophil extracellular traps (NETs) as markers of disease severity in COVID-19. medRxiv [Preprint]. 2020 Apr 14:2020.04.09.20059626. doi: 10.1101/2020.04.09.20059626. PMID: 32511633; PMCID: PMC7276989.