Identifying tools to track hypercoagulability in COVID-19 patients: Exploring global haemostasis (ROTEM) and neutrophil extracellular traps (NETs) immunoassays

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INTRODUCTION

In around 20% of patients infected with SARS-CoV-02 leading to coronavirus disease 2019 (COVID-19) abnormal coagulation has been observed which is associated with poor outcomes [1]. Biologically, severe COVID-19 is characterised by the production of proinflammatory cytokines [2]. There is extensive cross talk between the inflammation and coagulation systems in response to invasion by pathogens [3,4,5]. Reflecting this, abnormalities in laboratory markers of coagulation and fibrinolysis in COVID-19 affected patients have been reported [6,7,8]. Retrospective data indicates that there is significantly more derangement in coagulation parameters (namely prothrombin time (PT) and D-Dimer) at the point of admission in patients who don't survive, versus those who do [1, 9]. These changes in individual coagulation parameters point to a disruption in haemostasis, but do not provide any guidance as to the biological effect of the changes. Traditional coagulation tests provide a snap-shot of a particular aspect of coagulation in cell depleted plasma, but do not provide an assessment of overall haemostasis. ROTEM® provides a composite assessment of the dynamic process of clot initiation, thrombin generation and whole blood clot formation which is arguably more representative of physiological processes [10].

AIM

(i) To investigate whether patients with COVID-19 demonstrate ROTEM parameters and or elevated NETs measurements, indicating prothrombotic phenotype, early in admission.

(ii) To investigate whether or not patients admitted to intensive therapy unit (ITU) have a significantly higher Maximum Clot Firmness (MCF) and NETs compared to those on the ward.

RESULTS

Rotational thromboelastometry demonstrated a hypercoagulable state compared to healthy controls. The demographics of the patient population can be seen in figure 2. On admission for FIBTEM CT ($p<0.001$) and MCF ($p<0.001$), EXTEM CT, CFT, MCF and ML ($p<0.002$) and INTEM CT ($p<0.028$), MCF ($p<0.004$) and ML ($p<0.001$). In our cohort MCF ($p=0.849$) was not significantly higher in those patients admitted to ITU compared to those on the ward however moderate significance was observed in the EXTEM CFT ($p=0.051$) and EXTEM ML ($p=0.056$). In the 3 individuals assessed with serial H3.1, the values closely track their clinical course (Figure 3).

COVID-19 / ROTEM (Demography and Admission)

Cohort = 33	
Gender	
Female	14 (42.4%)
Male	19 (57.8%)
Age	
Median (IQR)	52 years (41.0 - 62.5)
COVID-19 diagnosed	
On admission	25 (75.8%)
Later in ward	8 (24.2%)
Admission Unit	
Ward	25 (75.0%)
ITU	8 (25.0%)
Length of Stay (n=29)	
Median (IQR)	8 days (5 - 16)
Outcome / Event	
VTE	1 (3.0%)
Discharge home	29 (88.0%)
Deceased	4 (12.0%)

Figure 2: Demographics of COVID-19 patients admitted to hospital.

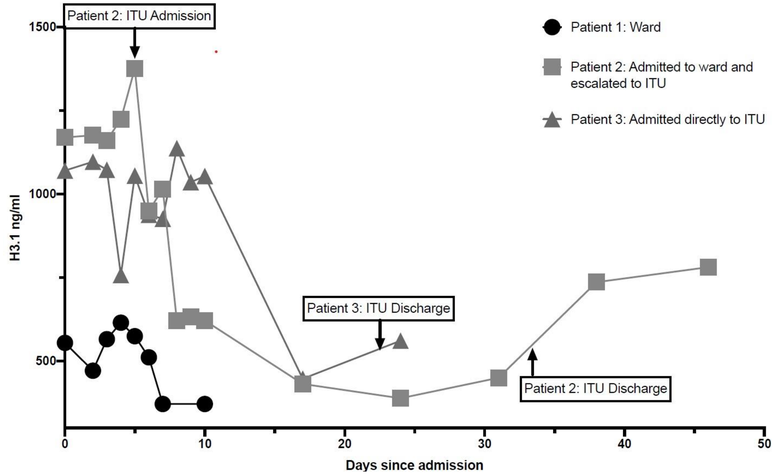

Figure 3: NETS testing was undertaken in 1 patient directly admitted to ITU, one transferred from the ward to ITU and one who remained on the ward. H3.1 values were noticeably higher in those patients admitted to ITU compared to the patient on the ward.

METHOD

Rotational thromboelastometry testing was undertaken in consenting patients on admission and daily until day 10 and weekly thereafter until discharge from hospital. For those who were not able to consent, assent was obtained from an independent medical practitioner not involved in the direct care of the patient. Plasma samples for NETs (neutrophil extracellular traps) testing were stored at -80ºC and batch tested. NETs were measured at all time points in 3 patients; 1 admitted directly to ITU, 1 admitted to ITU during the course of their stay and 1 who remained on the medical ward. NETs were measured using a commercially available immunoassay kit to measure H3.1 nucleosomes.

ROTEM

Rotational thromboelastometry measures evolving global clot firmness using a technology based on a fixed cylindrical cup and a permanently oscillating vertical axis. Rotation of the axis is driven by a motor which is connected to the axis by an elastic spring. The rotation is detected optically via a mirror plate at the upper end of the axis (Figure 1).

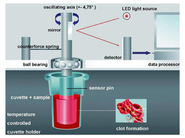

Figure 1: Pictorial representation of the principle used to measure overall clot quality using rotational thromboelastometry.

NEUTROPHIL EXTRACELLULAR TRAPS (NETS)

Levels of circulating H3.1 nucleosomes were measured using Nu.Q™ ELISA assays (Belgian Volition SRL, Isnes, Belgium). Plasma were incubated for 2 ½ hours at room temperature in a 96 well plate coated with a monoclonal antibody against Histone H3.1. Level of anti-nucleosome detection antibody (incubation 90 mins 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).

CONCLUSIONS

- COVID-19 patients demonstrated a hypercoagulable state compared to healthy controls as measured by ROTEM with increased MCF and hypofibrinolysis.
- Although this is a small, exploratory study, the H3.1 nucleosome findings suggest that it may be able to risk stratify on admission and track clinical course.

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CONTACT INFORMATION

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