

# The efficacy of low molecular weight heparin is reduced in Covid-19

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## Abstract.

**BACKGROUND:** A significant degree of mortality and morbidity in Covid-19 is through thromboembolic complications, only partially mitigated by anticoagulant therapy. Reliable markers of infection severity are not fully established.

**OBJECTIVES:** This study investigated whether visco-elastic biomarkers predict disease severity on presentation to the Emergency Department (ED) and how they measure response to anticoagulation

**METHODS:** Patients testing positive for Covid-19 at a large University Teaching Hospital ED were recruited at presentation. Multiple blood samples were taken throughout hospital admission to monitor disease progression with end outcome recorded. Visco-elastic markers, fractal dimension ( $d_f$ ) and Time to Gel Point (TGP) which measure the properties of the incipient clot were compared in patients with and without anticoagulation by Low Molecular Weight Heparin (LMWH).

**RESULTS:** TGP and  $d_f$  did not predict severity of infection with Covid-19. Although LMWH prolonged TGP, there was no change in  $d_f$  indicating LMWH did not change clot microstructure.

**CONCLUSIONS:** Therapeutic efficacy of LMWH appears blunted in Covid-19 infection. This may be due to the inflammatory state creating a resistance to LMWH activity, which may explain why LMWH appears less effective in Covid-19 compared to other disease states. Covid-19 was not predicted by visco-elastic testing at the time of ED presentation.

Keywords: Covid-19, coagulopathy, visco-elastic testing, anticoagulation, Heparin resistance

## 1. Introduction

Covid-19 infection is frequently complicated by thromboembolic events. Beyond the acute lung inflammation and hypoxia, these events appear to be a major contributor to mortality and morbidity. There are a range of pathological processes implicated in this, Pulmonary Emboli (PE), and coronary thrombi have been observed without any evidence of preceding Deep Venous Thrombosis (DVT) suggesting de novo intravascular clot formation. Post-mortem findings in Covid-19 involve a wide range of thromboembolic complications including myocardial infarction (MI), PE and end organ microthrombi leading to multi-organ failure [1–3]. Techniques such as Thromboelastography (TEG) and Rotational Thromboelastometry (ROTEM) have been used to describe Covid-19 associated coagulopathy, and

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32 while a growing body of evidence suggests microthrombotic complications are caused as a result of  
33 fibrinolytic shutdown and an inability to break down clot mass [4–6], a range of other coagulopathic  
34 patterns were reported across studies [7, 8]. This heterogeneity in data indicates alternative measures  
35 of coagulation are necessary to fully examine the pathological effects of Covid-19.

36 Low Molecular Weight Heparin (LMWH) is routinely used as prophylactic treatment against Venous  
37 Thromboembolism (VTE), but in Covid-19 appears to be insufficient in preventing VTE [9]. Previous  
38 studies have demonstrated a significantly higher dosing of anticoagulant is required to prevent VTE in  
39 Covid-19 patients with a correspondingly higher target Anti-Xa concentration [10, 11]. And while this  
40 failure to achieve target Anti-Xa levels is more prevalent in patients on Intensive Care Units (ICUs),  
41 than on general medical wards, there remains a considerable cohort on general wards also under-  
42 anticoagulated as assessed by Anti-Xa levels [12, 13]. The certainty of these findings and the strength  
43 of recommendations possible from them has been limited as to date the majority of studies have either  
44 been small scale or non-peer reviewed articles. As we move further away from the earliest phases  
45 of the pandemic, there is a need to strengthen the evidence base with larger studies. Additionally,  
46 the majority of these studies tend to focus on a comparison of anticoagulant dose vs target Anti-Xa  
47 levels. Consequently, there is relatively little data investigating how established markers of coagulation  
48 respond to anticoagulant therapy [9].

49 A range of techniques have been trialled to assess the severity of Covid-19 at early stages of the  
50 disease, and while they have each provided some utility, none have provided sufficient sensitivity and  
51 specificity to stand alone as a severity prognosticator. National Early Warning Score 2 (NEWS2) is a  
52 score generated from basic physiological measurements and was updated from an earlier National Early  
53 Warning Score (NEWS). Prior to the Covid-19 pandemic, NEWS was shown to be predictive of death,  
54 unanticipated ICU admission or cardiac arrest for patients already in hospital [14] and is also validated  
55 for use in the Emergency Department (ED) at the time of first presentation [15]. When specifically  
56 evaluated for its utility in Covid-19 patients, NEWS2 had good predictive value for assessing death or  
57 ICU admission within 24 hours of vital sign measurement without the need for additional covariates,  
58 or adjusting the weighting of parameters from the original scoring [16]. In much smaller scale studies  
59 designed to evaluate use of NEWS2 at the time of arrival to the ED for Covid-19 patients, NEWS2 on  
60 admission was closely associated with severe disease, defined as death whilst an inpatient, or admission  
61 to ICU at any stage of hospital stay [17]. While NEWS2 therefore appears to be a useful tool for the ED  
62 clinician in predicting risk of severe disease due to Covid-19, by definition it requires physiological  
63 deterioration in order to predict severity. Therefore a tool that is sensitive prior to deterioration is  
64 desirable.

65 Extensive work has gone into exploring the relationship between viral load and disease severity.  
66 While it appears that there is a link between older age and viral load, there has been no established  
67 relationship between severity and viral load [18]. Radiological techniques such as plain film or CT  
68 have also been assessed, and while there are a number of features which may indicate severe disease,  
69 imaging may also be unremarkable in early stages [19].

70 A range of laboratory markers have also been assessed, and an extensive list of markers have been  
71 associated with severity of disease, this includes D-dimer, white blood cell counts, CRP, renal function,  
72 liver function and cardiac markers [20, 21]. However, as these are markers, they are not direct targets  
73 for therapy and therefore there remains a need to establish modifiable testing parameters through  
74 which a goal directed therapy can be pursued. Other coagulation parameters might provide a direct  
75 target for therapy. As dysregulation of the coagulation system drives thrombogenicity, modifying these  
76 parameters might directly reduce the thrombogenic effect.

77 The aims of this study were therefore twofold. To evaluate whether visco-elastic measurements of  
78 clot quality and microstructure can predict severity of disease due to Covid-19 in the early stages of  
79 infection, and to describe changes to the therapeutic effect of heparin in Covid-19 infection.

## 2. Methodology

120 patients presenting to a single University Hospital ED with respiratory or febrile symptoms suspicious for Covid-19 infection were recruited, and tested for Covid-19 via PCR virology. Patients were excluded if they were on anticoagulant medication including warfarin and direct oral anticoagulants (DOACs) such as apixaban or rivaroxaban, or if they declined to participate. This study occurred before the roll out of Covid-19 vaccinations in the UK, and as such all patients were unvaccinated.

On arrival patients had Covid-19 PCR virology performed, once a PCR result was reported for a patient, further investigations were only performed if the result was positive for Covid-19. 96 patients tested positive for Covid-19, 1 patient was later found to have been on warfarin and was retrospectively removed from analysis.

On recruitment to the trial, patients had routine haematological and biochemical blood samples drawn. Alongside these, ROTEM and Rheometry were performed by the investigating team. Patients testing positive for Covid-19 on PCR virology and requiring admission received LMWH as per standard treatment protocols at the time. The time this was administered was recorded and patients stratified into those who had blood sampling before receiving LMWH and those who had already been given LMWH when recruited to the study. Clinical pathways also included corticosteroid therapy for any patient with a supplemental oxygen requirement, the standard therapy was dexamethasone orally or intra-venously unless the responsible clinician deemed another corticosteroid more appropriate. Patients were followed up through their admission with their length of stay and outcome recorded. The investigating team had no influence on the clinical decision making for patients in the study. Patients were retrospectively grouped based on their outcome. Those who died or required an ICU admission were classed as Critical, those who required a hospital stay of 5 days or more classed as Severe, and those discharged before 5 days were classed as Mild-Moderate.

Repeat blood sampling was performed at 24 hours, 3-5 days and 7 days if the patient remained in hospital at this time. Discharge decisions were made by the clinicians responsible for the patient with no input from the investigating team. Beyond 7 days no further blood samples were collected for the study even if the patient remained in hospital.

Blood samples were taken from the antecubital vein where possible using a 21 G butterfly needle. Two 2.7 mL samples were immediately transferred into PET 0.109M 3.2% citrated vacutainers (Becton Dickinson, Plymouth, UK Ref: 363095). Routine coagulation studies were performed on the first citrated vacutainer including PT, APTT, Clauss fibrinogen and were measured using a Sysmex CA1500 analyser within two hours of collection. D-dimer analysis was carried out using Latex immunoturbidimetric assay (Instrumentation Laboratory, Warrington, UK). 4 ml was collected into a plastic dipotassium EDTA vacuette (Becton Dickinson, Plymouth, UK Ref 367839) for FBC analysis using a Sysmex XE 2100 (Sysmex UK, Milton Keynes, UK). 5 mL was collected into a SST II Advance (Becton Dickinson, Plymouth, UK Ref 367954), testing of U&Es, LFTs, Bone Profile and CRP were performed on a Roche-Cobas 8000 Modular Analyser (Roche Diagnostics Ltd, UK).

The second citrated sample was used for Rotational Thromboelastometry, performed using a ROTEM Delta Whole Blood Haemostasis System (TEM Innovations GmbH, 2011) with testing carried out according to manufacturer recommendations and analysed on ROTEM Delta Software v1.6.3. The markers selected for analysis were Extem and Intem Clotting Time (CT), Clot Formation Time (CFT), Maximum Clot Firmness (MCF), Alpha Angle (Alpha) and Maximum Lysis (ML). This was designed to evaluate clot formation kinetics, mechanical structure and clot breakdown, mirroring the values selected by other studies. This data has been more fully reported in a separate article [4].

A 6.6 mL aliquot of whole blood was immediately loaded into a TA instruments AR-G2 controlled-stress rheometer (TA Instruments, New Castle, DE, USA) at 37°C ( $\pm 0.1^\circ\text{C}$ ) for visco-elastic testing.

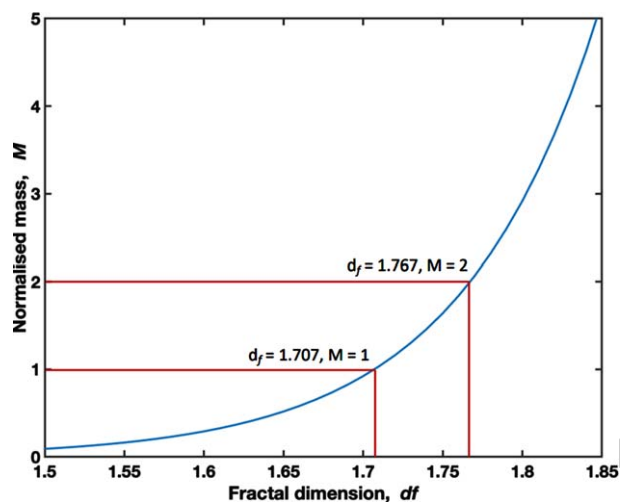


Fig. 1. A graph displaying the relationship between  $d_f$  and the Normalised Mass, where a Normalised Mass of 1.00 is set at the mean  $d_f$  for the study cohort (1.707).

127 This technique has been fully described previously in the literature, has been validated as a reliable  
 128 technique for describing the visco-elastic properties of blood and acts as a compound marker of clot  
 129 initiation, the templating effect and mechanical properties of the mature clot [22–24]. Briefly, visco-  
 130 elastic measurement is performed using small amplitude oscillatory shear measurements at varying  
 131 frequencies. The gel point is defined as the transition point from visco-elastic liquid to visco-elastic  
 132 solid and is indicative of formation of the incipient clot. Time to Gel Point (TGP) measures the time  
 133 taken to reach this transition. A mathematical relationship exists between the visco-elastic properties  
 134 at the gel point and a structural parameter, the Fractal Dimension ( $d_f$ ) which quantitatively describes  
 135 the framework of the incipient clot – a higher value represents a denser and more branched fibrin  
 136 network, whereas a lower  $d_f$  represents a looser and more open fibrin network. The incipient clot mass  
 137 scales as a power-law relationship with  $d_f$  as illustrated in Fig. 1 [22]. Herein the incipient clot mass  
 138 is normalised to a value of 1 for  $d_f = 1.707$ . Crucially  $d_f$  has been shown to be sensitive to the effects  
 139 of in-vitro administration of heparin to whole blood in healthy volunteers, with increased levels of  
 140 heparin, as measured by anti-Factor Xa, leading to lower values of  $d_f$  representative of incipient clots  
 141 with significantly less mass [22].

142 Statistical analysis was performed on IBM SPSS Statistics v28.0.0.0(190), Shapiro-Wilk testing was  
 143 performed to assess for normality, and t-tests or ANOVA were used to assess for differences in groups  
 144 when appropriate. These data are presented as a mean  $\pm$  standard deviation. Where non-parametric  
 145 testing was appropriate, data is presented as a median  $\pm$  interquartile range and Mann-Witney or  
 146 Kruskal-Wallis testing performed in place of a t-test or ANOVA respectively. Statistical signifi-  
 147 cance throughout the paper is defined as  $p < 0.05$ . In graphs \*, \*\* and \*\*\* signify  $p < 0.05$ ,  $p \leq 0.01$   
 148 and  $p \leq 0.001$  respectively. Where comparisons between 3 groups indicate 1 group is significantly  
 149 different to both other groups, but those two groups do not have a significant difference, the signifi-  
 150 cant differences are denoted by <sup>a</sup> and <sup>b</sup> next to the values which differ with the p value indicated  
 151 below.

152 Graphs and tables were produced in GraphPad Prism 9 v9.3.1(471).

153 Ethical approval was given by the South West Wales Research Ethics Committee (Wales REC 6),  
 154 IRAS 216266, REC reference 17/WA/0123 [25].

Table 1a  
Patient cohort characteristics for each group

	Mild-Moderate	Severe	Critical
n	29	46	20
Age (yrs)	50.0 ± 14.6 <sup>ab</sup>	64.3 ± 12.5 <sup>a</sup>	69.2 ± 13.6 <sup>b</sup>
Male %	38	39	55
Diabetes %	17	22	25
COPD %	3	4	30
Hypertension %	14	30	50
IHD / Angina %	7	4	20
Heart failure %	3	0	0
Hypercholesterolaemia	7	7	5
CVA %	0	2	5
PE / DVT %	3	2	5
Cancer %	7	11	5
Received LMWH before recruitment %	31	39	30

<sup>a</sup> and <sup>b</sup>,  $p < 0.001$ .

Table 1b  
Standard blood testing results taken on arrival to the ED

	Mild-Moderate	Severe	Critical
<b>Full Blood Count</b>			
Haemoglobin (g/dL)	139 ± 16	134 ± 18	137 ± 18
Platelets (x10 <sup>9</sup> /L)	238 ± 74	282 ± 97	228 ± 80
White Cell Count	6.7 (5.15 - 8.55)	7.25 (5.325 - 9.850)	7.9 (6.525 - 11.075)
Neutrophils	4.9 (3.55 - 7.05)	6.15 (4.15 - 8.65)	6.4 (4.85 - 10.4)
<b>Coagulation Screen</b>			
PT (s)	10.6 (10.2 - 11.15)	10.6 (10.4 - 11.0)	10.9 (10.225 - 11.2)
APTT (s)	23.8 (21.7 - 25.45)	24.6 (20.35 - 25.6)	24.05 (22.3 - 26.9)
Fibrinogen (g/L)	5.3 (4.1 - 6.15)	5.65 (4.8 - 6.625)	5.9 (4.025 - 6.35)
D-dimer (µg/L)	731 (544 - 1456.5)	1149 (597.5 - 2182.75)	981.5 (632 - 1520.75)
Anti-Xa (units/mL)	0.03 (0.01 - 0.0775)	0.04 (0.01 - 0.275)	0.04 (0.01 - 0.1675)
<b>Inflammatory Markers</b>			
CRP (mg/L)	44 (5 - 101) <sup>ab</sup>	88 (38.25 - 229) <sup>a</sup>	149.5 (63.5 - 200.25) <sup>b</sup>

<sup>a</sup> and <sup>b</sup>,  $p < 0.01$ .

### 3. Results

Table 1a shows descriptive characteristics for each group. Age was significantly different in the mild-moderate group compared with both other groups, and is a well-known risk factor for disease severity. There was no significant difference in the age of the severe and critical groups. The relatively low proportion of patients with a previous CVA or PE is likely reflective of the fact that taking anticoagulant medication was an exclusion criteria for the study. Table 1b details the results of the first blood samples taken from each patient. The only significant difference in routine blood testing was CRP, which was significant different in the mild-moderate group compared to both other groups ( $p = 0.009$  vs severe,  $p = 0.002$  vs critical), the severe and critical groups did not have a statistically significant

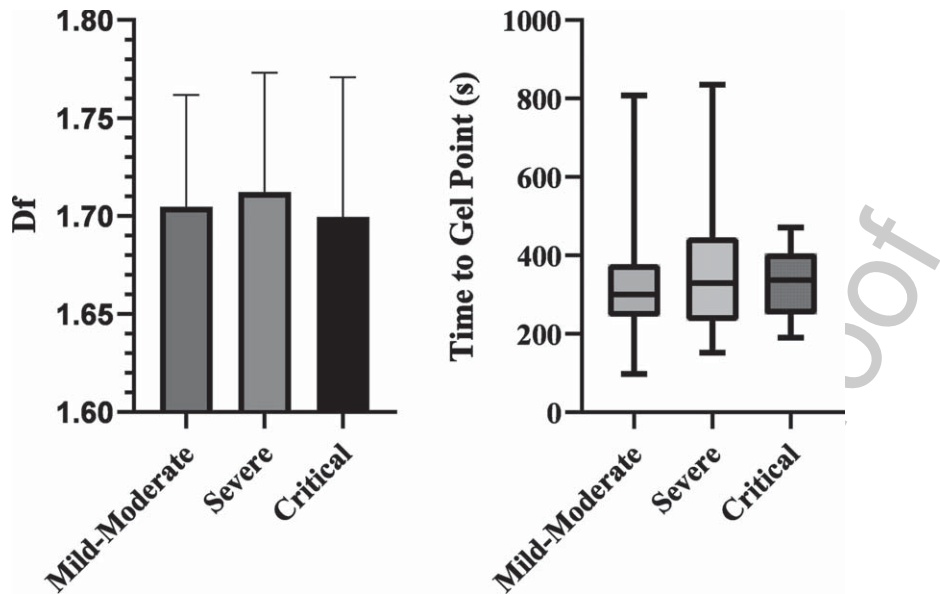


Fig. 2. Bar chart of  $d_f$  and box plot of TGP based on outcome severity. No significant differences were detected.

164 difference. Previously published data from this study cohort demonstrated the critical outcome group  
 165 had a significantly reduced capacity for fibrinolysis compared to the other outcome groups [4], whilst  
 166 other ROTEM parameters were not different across the outcome groups. Comparisons across the  
 167 sampling time points indicated ROTEM parameters did not change throughout hospital admission in  
 168 any outcome group.

169 Figure 2 compares the  $d_f$  and TGP values on arrival between the groups. There were no differences  
 170 in either  $d_f$  or TGP between the difference outcome groups.

171 The patients who had received LMWH before blood sampling were compared to those who had  
 172 been given no anti-coagulation before blood sampling and is shown in Fig. 3. In the LMWH group,  
 173 mean time between administration of LMWH and blood sampling was 8.7 hours. Anti-Xa levels were  
 174 significantly different between the groups (0.299 vs 0.031,  $p < 0.001$ ). Figure 3 shows the  $d_f$  and TGP  
 175 between these groups. There was no significant difference in the  $d_f$  between these two groups, but  
 176 TGP was significantly prolonged in those who had received LMWH, 409 s vs 301 s ( $p < 0.001$ ). Full  
 177 details are shown in Table 2.

178 Anti-Xa levels were compared to TGP,  $d_f$  and Normalised Mass. These values include sampling at all  
 179 time points within the study. Once patients were commenced on LMWH it was continued throughout  
 180 the study with no doses missed. These results are shown in Fig. 4. A positive relationship was observed  
 181 between TGP and Anti-Xa ( $R^2 = 0.020$ ,  $P = 0.03$ ), whilst no relationship was seen between Anti-Xa  
 182 and either  $d_f$  or Normalised Mass.

183 Normalised Mass was compared against ROTEM and this data is seen in Fig. 5. In the context of  
 184 clot lysis, it is appropriate to compare ROTEM findings to Normalised Mass in addition to  $d_f$ . There  
 185 was a significant negative correlation between Normalised Mass and ROTEM clot lysis markers. As  
 186 a greater Normalised Mass is indicative of a denser clot, this indicates rate of clot lysis is related to clot  
 187 density.

188 As previously published, Table 3 describes the results of CTPAs performed on patients in the study,  
 189 and their detection rates for PE [4]. These CTPAs were performed at the discretion of the clinical team  
 190 caring for the patient independently of the research team. This data was not a primary outcome of the

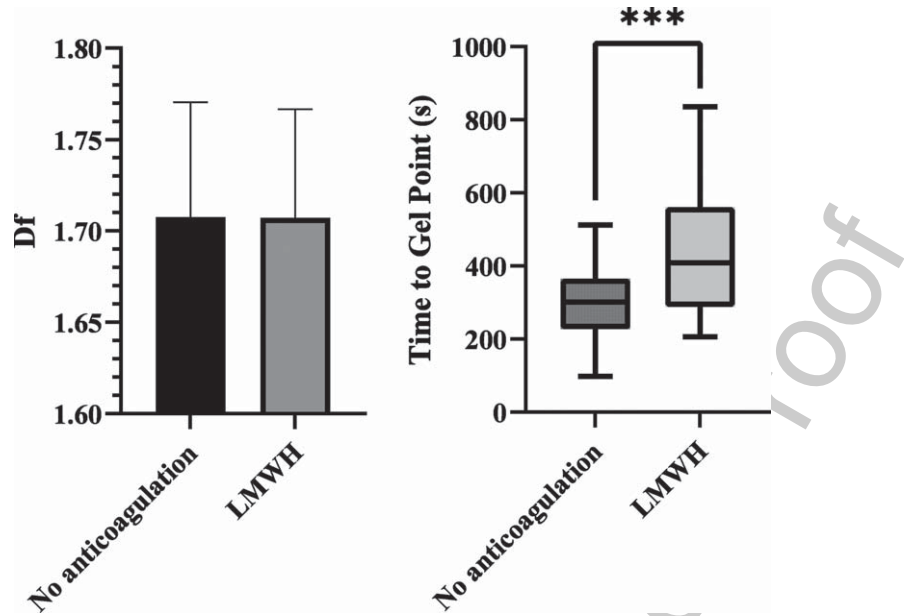


Fig. 3. Bar chart of  $d_f$  and box plot of TGP comparing Covid positive patients who had received LMWH before blood sampling against those with no anticoagulation.  $d_f$  was not significant. TGP was significantly prolonged in the LMWH group ( $p < 0.001$ ).

Table 2

Comparisons of  $d_f$  and TGP in patients who had received LMWH before blood sampling vs patients who had received no anticoagulation at the time of sampling

	No Anticoagulation	LMWH
n	59	32
$d_f$	$1.71 \pm 0.06$	$1.70 \pm 0.06$
TGP (s)	301 (227 - 365)***	409 (288 - 561)***
Anti-Xa (units/mL)	0.031 (0.010 - 0.060)***	0.299 (0.130 - 0.578)***

study, however is included for completeness and is broadly comparable to previous systematic reviews on similar patient cohorts [26].

#### 4. Discussion

In this study, there has been no evidence that assessment of clot microstructure, quality and strength in using novel dynamic visco-elastic markers such as  $d_f$  at the time of presentation predicts the severity of the subsequent disease progression in Covid-19. This is further evidence that despite a significant burden of thromboembolic disease in Covid-19, it is atypical in how this disease is reflected by coagulation assays. In sepsis, it appears the initial trend in visco-elastic testing is towards a hypercoagulable state with dense clots and shortened clot formation times, but that with deterioration to septic shock, there is a reversal of this trend to weak clots generated by a very loose fibrin network [27]. However, in Covid-19 there appears to be no differences in clot microstructure as measured by  $d_f$ , even in severe disease with significant thromboembolic complications. Mortality and morbidity in Covid-19 do not

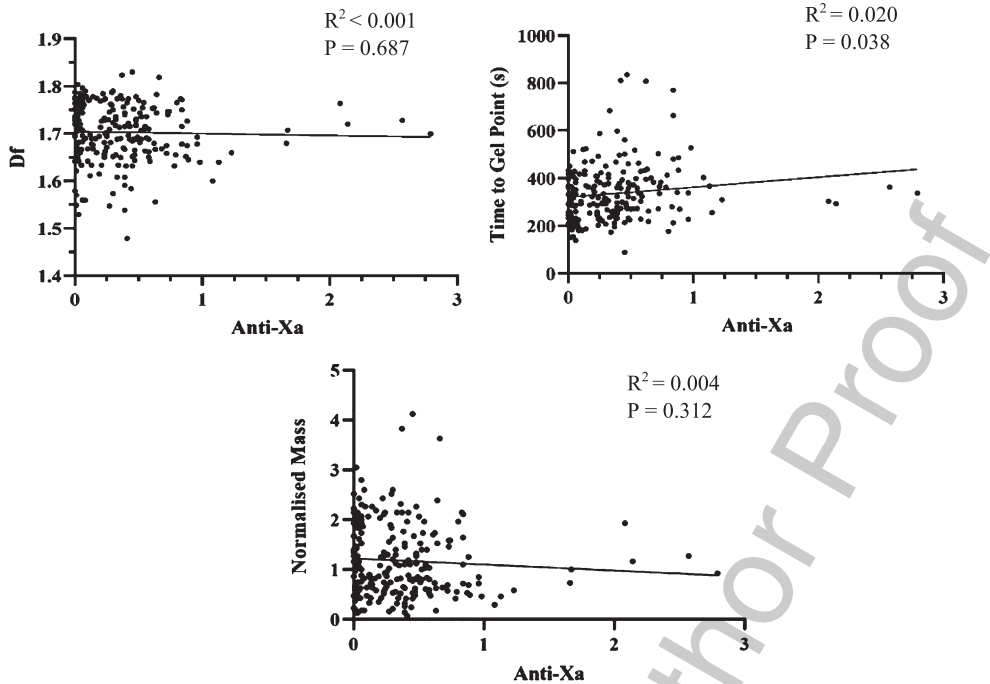


Fig. 4. Scatterplots of  $d_f$ , TGP and Normalised Mass against Anti-Xa levels. There was a very weak relationship between TGP and Anti-Xa, but no significant correlation between Anti-Xa and  $d_f$  and TGP.

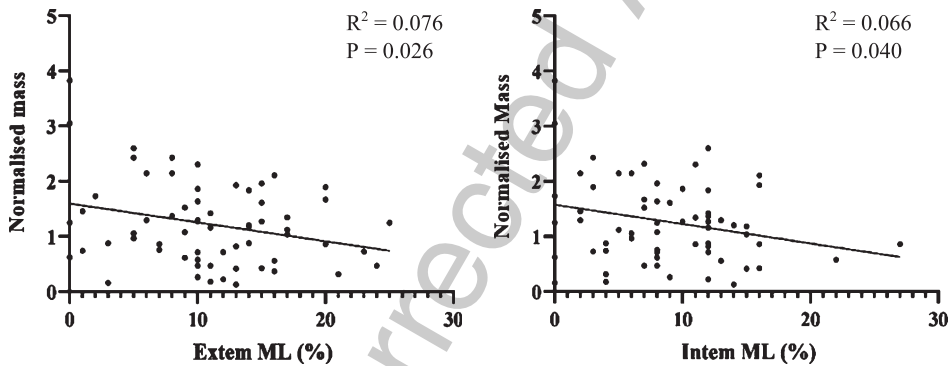


Fig. 5. Scatterplots of Normalised Mass against Extrem and Intem Maximum Lysis (%). A statistically significant negative correlation was seen in both cases ( $p < 0.05$ ).

203 appear to follow the standard model of mortality in septic shock due to bacterial pathogens, and as  
 204 such it may not be appropriate to mirror strategies for treating sepsis in the management of severe  
 205 Covid-19.

206 Fractal analysis describes microstructure of the incipient clot at the gel point where the clot transitions  
 207 from fluid like behaviour to solid like behaviour. The microstructure at this stage goes on to establish  
 208 the template for the mature clot which follows. The negative correlation of Normalised Mass with  
 209 maximum clot lysis on ROTEM (Fig. 5) is of significance because it reinforces this link between the  
 210 incipient clot and its mechanical and elastic properties once formed as a mature clot.



Table 3

A table showing the percentage of patients in each severity group who had a CTPA during admission, and the percentage which were positive

	Mild-moderate	Severe	Critical
CTPAs performed	34%	30%	40%
Positive rate	20%	21%	25%

211 However, there is ample evidence to suggest that in Covid-19 there is a reduced efficacy of LMWH  
 212 in these patients. This is counter to previous findings in healthy volunteers that *in vitro* heparin alters  
 213 microstructure [20]. These studies also demonstrated a consistent relationship between Anti-Xa and  
 214 both  $d_f$  and TGP, this relationship does not appear to be maintained in Covid-19 infection. While a  
 215 relationship between Anti-Xa and TGP is maintained, there is no relationship between Anti-Xa and  
 216  $d_f$ . Although the previous study administered heparin *in vitro*, whilst this study measured  $d_f$  following  
 217 the *in vivo* administration of LMWH, we believe the lack of alteration to clot microstructure following  
 218 administration of LMWH is more likely a pathological effect of Covid-19 infection.

219 In blood, a lower  $d_f$  represents a looser fibrin network with fewer branch points, and as such,  
 220 a developing clot will take longer to transition from displaying fluid-like to solid-like properties.  
 221 The pro-inflammatory state seen in Covid-19 appears to disrupt the widely held relationship seen in  
 222 polymers – both biological and non-biological - between  $d_f$  and TGP in which a change in one variable  
 223 predictably corresponds with a change in the other. A prolonged TGP would ordinarily be accompanied  
 224 by a lower  $d_f$ , and yet in Covid-19, LMWH is able to prolong TGP without a change in  $d_f$ . Across  
 225 a range of disease states, this relationship between  $d_f$  and TGP has been maintained even when the  
 226 pathophysiology of disease leads to a coagulopathy. Covid-19 appears to be an outlier in disrupting  
 227 this relationship. Studies in healthy volunteers with ex-vitro addition of heparin also demonstrated  
 228 reciprocal changes to  $d_f$  and TGP, so it does not appear likely that heparin is the causative agent  
 229 for this disruption. The only previous instance of a similar finding was in patients receiving warfarin  
 230 therapy following first episode of VTE [28]. Comparing these patients to patients receiving warfarin for  
 231 non-VTE indications (e.g atrial fibrillation or metallic heart valve), there was a significant difference in  
 232  $d_f$  but not in TGP. In both VTE and non-VTE patients, TGP was appropriately prolonged by warfarin.  
 233 But only in the non-VTE group was  $d_f$  reduced below the normal healthy range. Further analysis of the  
 234 VTE group indicated a sub-group of patients in which  $d_f$  remained high despite warfarin therapy, and  
 235 patients who subsequently suffered recurrent VTE despite anticoagulation were all from this sub-group  
 236 in which  $d_f$  remained high following warfarin therapy. The factor which uncoupled  $d_f$  from TGP in  
 237 recurrent VTE was not identified, recurrence was not due to sub-therapeutic INR readings. Both the  
 238 VTE study and this work in Covid-19 would indicate there are pathways by which coagulation can be  
 239 modified which remain poorly understood.

240 It is not known what factor has led to the disruption of the relationship between  $d_f$  and TGP.  
 241 But understanding this change may offer new insights into thromboembolic diseases and provide a  
 242 better understanding of the reasons why anticoagulant therapies are unsuccessful in the treatment  
 243 or prevention of thromboembolic disease in some patients. This additional understanding may lead  
 244 on to the identification of new targets for pharmacological agents, or the development of additional  
 245 biomarkers in coagulation.

246 Although this study has not linked this reduction in efficacy with an increased incidence of PE or  
 247 DVT, it was not powered or designed to do so. While there has been a link between increased throm-  
 248 bogenicity and patient outcome in Covid-19, an important step would be to link these via pathological  
 249 processes such as PE and DVT. This follow up work may provide evidence to warrant studies into

250 LMWH dosing in Covid-19 patients and whether more aggressive anticoagulation strategies are of  
251 benefit.

252 As seen across a wide range of the literature, demographic factors such as age and co-morbidity,  
253 and inflammatory markers such as CRP continue to be predictive of severe disease and death.

## 254 **5. Limitations**

255 Some patients were not admitted to ICU at the discretion of the clinical team if pre-existing co-  
256 morbidity and functional status indicated an ICU admission would not be in their best interests. This  
257 may mean some patients were misclassified based on the classification criteria used. In some cases this  
258 did not affect the analysis, e.g a patient who was not admitted to ICU, and subsequent died on the ward  
259 and therefore would be classed as critical in either case. However, there were other patients whose  
260 physiological condition would normally indicate an ICU admission, but due to baseline functional  
261 status were not escalated to ICU, and then survived a ward based stay in hospital. In these instances  
262 critically unwell patients were downclassed as a severe case meaning a type II statistical error was  
263 more likely.

264 While the study did not collect blood beyond day 7 of admission, there were some patients who  
265 either died later in their stay, or deteriorated later in their admission and only then required an ICU  
266 admission. In these patients it is therefore unknown whether visco-elastic testing was deranged as their  
267 clinical condition worsened. While the majority of deaths occurred within or close to the first 7 days,  
268 the longest period between admission and death was 25 days.

## 269 **6. Conclusions**

270 At the time of presentation to the ED, visco-elastic testing does not predict the severity of Covid-19  
271 infection. However, there is significant evidence to suggest that LMWH therapy does not induce the  
272 same effect in Covid-19 patients as it would in other cohorts. It is likely that this explains some of  
273 the additional burden of thromboembolic disease seen in these patients. Further studies are required  
274 to translate this laboratory finding into clinical practice.

275 Additionally, the coagulopathy of Covid-19 appears to induce unique pathophysiological changes  
276 to clot microstructure. Understanding how these changes are generated may offer crucial insights into  
277 thromboembolic diseases and the possibility to develop new biomarkers in coagulation or provide  
278 additional pharmaceutical targets to treat disease.

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