




BMJ Open COVID-19-related dynamic coagulation disturbances and anticoagulation strategies using conventional D-dimer and point-of-care Sonoclot tests: a prospective cohort study

Madhumita Premkumar ¹, Sekar Loganathan ², Kamal Kajal ², Amarjyoti Hazarika,² Shiv Soni,² Goverdhan Dutt Puri,² Inderpaul Singh Sehgal,³ Vikas Suri,⁴ Pankaj Malhotra,⁵ Virendra Singh,¹ Ajay Duseja,¹ Ashish Bhalla,⁴ Jasmina Ahluwalia,⁶ Narender Kumar,⁶ Kushal Kekan,¹ Sant Ram,⁷ Karan Singla,² Varun Mahajan,² Narayana Yaddanapudi²

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For numbered affiliations see end of article.

Correspondence to

Dr Kamal Kajal;
kamal.kajal@gmail.com

ABSTRACT

Objectives Coagulation changes associated with COVID-19 suggest the presence of a hypercoagulable state with pulmonary microthrombosis and thromboembolic complications. We assessed the dynamic association of COVID-19-related coagulation abnormalities with respiratory failure and mortality.

Design Single-centre, prospective cohort study with descriptive analysis and logistic regression.

Setting Tertiary care hospital, North India.

Participants Patients with COVID-19 pneumonia requiring intensive care unit (ICU) admission between August 2020 and November 2020.

Primary and secondary outcome measures We compared the coagulation abnormalities using standard coagulation tests like prothrombin time, D-dimer, platelet count, etc and point-of-care global coagulation test, Sonoclot (glass beaded(gb) and heparinase-treated(h)). Incidence of thromboembolic or bleeding events and presence of endogenous heparinoids were assessed. Cox proportional Hazards test was used to assess the predictors of 28-day mortality.

Measurement All patients underwent Sonoclot (glass beaded) test at admission apart from the routine investigations. In patients at risk of thromboembolic or bleeding phenomena, paired tests were performed at day 1 and 3 with Sonoclot. Activated clotting time (ACT) <110 s and peak amplitude >75 units were used as the cut-off for hypercoagulable state. Presence of heparin-like effect (HLE) was defined by a correction of ACT ≥ 40 s in h-Sonoclot.

Results Of 215 patients admitted to ICU, we included 74 treatment naive subjects. A procoagulant profile was seen in 45.5% (n=5), 32.4% (n=11) and 20.7% (n=6) in low-flow, high-flow and invasive ventilation groups. Paired Sonoclot assays in a subgroup of 33 patients demonstrated the presence of HLE in 17 (51.5%) and 20 (62.5%) at day 1 and 3, respectively. HLE (day 1) was noted in 59% of those who bled during the disease course. Mortality was observed only in the invasive ventilation

Strengths and limitations of this study

- This is the largest prospective proof-of-concept study using paired point-of-care global coagulation tests for assessing the dynamic coagulation defect and predictors of outcomes in COVID-19.
- This study provides evidence-based data to guide anticoagulation therapy in patients with COVID-19.
- Point-of-care Sonoclot tests help in identifying patients at risk of worse prognosis and invasive ventilation.
- This study was restricted to treatment naive patients admitted to intensive care and we did not have a control cohort of asymptomatic COVID-19 and non-COVID-19 patients.
- The study was not designed to test the use of prophylactic versus therapeutic anticoagulation, but to assess the therapeutic window for using evidence-based anticoagulation safely.

group (16, 55.2%) with overall mortality of 21.6%. HLE predicted the need for mechanical ventilation (HR 1.2 CI 1.04 to 1.4 p=0.00). On multivariate analysis, the presence of HLE (HR 1.01; CI 1.006 to 1.030; p=0.025), increased C reactive protein (HR 1.040; CI 1.020 to 1.090; p=0.014), decreased platelet function (HR 0.901; CI 0.702 to 1.100 p=0.045) predicted mortality at 28days.

Conclusion HLE contributed to hypocoagulable effect and associated with the need for invasive ventilation and mortality in patients with severe COVID-19 pneumonia.

Trial registration NCT04668404; ClinicalTrials.gov. In. Available from <https://clinicaltrials.gov/ct2/show/NCT04668404>.

INTRODUCTION

The SARS-CoV-2 pandemic has affected approximately 225 024 781 persons and claimed 4 636 153 lives till 14 September



2021.¹ Patients with COVID-19 have platelet abnormalities, endothelial dysfunction, clotting factors abnormalities and hyperfibrinolysis complicated by coexisting sepsis.² This leads to thromboembolic events both in the venous and arterial circulation.³ About 20% of patients with COVID-19 present with venous thromboembolism (VTE), 13% had symptomatic VTE despite prophylactic anticoagulation.⁴

The typical coagulation defect is seen as elevated D-dimer concentration, mild thrombocytopenia and prolongation of prothrombin time. A series from China showed 46% of 560 patients had D-dimer >0.5 mg/L.⁵ In another observational study in 183 patients with COVID-19 from China, the mean D-dimer concentration was significantly higher (2.12 mg/L, range 0.77–5.27) in non-survivors compared with survivors.⁶

Concomitant VTE, a potential cause of unexplained deaths, had been reported in COVID-19 cases,⁷ but its management with anticoagulation is challenging in view of bleeding risk.⁸ In addition, there is conflicting evidence regarding the role of prophylactic versus therapeutic anticoagulation in COVID-19.⁹

Tang *et al* observed that higher D-dimer and fibrin degradation product levels were associated with the increased overall mortality in patients with COVID-19.¹⁰ A wide range of abnormalities in standard coagulation tests (SCTs) had been reported depending on the severity of the disease,¹¹ suggesting the multifactorial dynamic pathology. Patients with severe COVID-19 pneumonia were associated with a hypercoagulable state rather than consumptive coagulopathy.¹² Viscoelastic testing of coagulation function in point-of-care (POC) devices such as thromboelastometry and Sonoclot had been proposed as a superior tool to rapidly diagnose underlying pathophysiology of coagulation dysfunction and guide resuscitation with appropriate blood products or anticoagulation.¹³

A reduction in the activated clotting time (ACT) and maximum amplitude/peak amplitude (PA) is indicative of a procoagulant coagulopathy in global coagulation tests (GCT) like thromboelastography or Sonoclot.¹⁴ Spiezia *et al* demonstrated a significant hypercoagulable thromboelastometry profiles with a shorter clot formation time in the Intrinsic pathway thromboelastometry (INTEM) ($p = 0.0002$), extrinsic pathway thromboelastometry (EXTEM) ($p = 0.010$) and increased maximum clot firmness ($p = 0.001$) that was associated with worse outcome in COVID-19-affected patients.¹⁵ Sonoclot assessment of clot formation and clot lysis takes 30–60 min. Cytokine storm and sepsis trigger the release of endogenous heparinoids from the endothelium in various organs, which is seen on GCT as a 'heparin-like effect' (HLE).¹⁶ The production of endogenous heparinoids can be detected by using heparinase treated Sonoclot assays.

In this prospective trial, we aimed to study the coagulation abnormalities from SCTs and Sonoclot profiles in consecutive patients with severe COVID-19 admitted to the intensive care unit (ICU) of the Postgraduate Institute of Medical Education and Research, Chandigarh, India

between August 2020 and November 2020. We also aimed at creating an algorithm for management of haemostatic abnormalities in these patients based on the evidence generated from this prospective observational study.

METHODS

Study design and participants

Patients with SARS-CoV-2 infection, as confirmed by reverse transcription PCR, aged between 18 and 80 years, and of any gender with moderate to severe acute respiratory distress syndrome (ARDS), admitted to ICU were recruited. During the initial period of COVID-19 pandemic, patients with mild COVID-19 ARDS requiring low flow oxygen with Charlson's comorbidity index >6 and patients with COVID-19 who underwent major surgery were also admitted to ICU and considered eligible for recruitment. A written informed consent was taken from all the study participants/relatives. Patients with a recent history of anticoagulation therapy, blood and or blood component transfusion in the last 2 weeks, HIV infection, pregnancy or active malignancy in the last 5 years were excluded. We documented their demographic data, previous medical history, clinical presentation, comorbid illnesses and drug therapy including use of antibiotics, antifibrinolytics and herbal medicines. Blood and urine cultures, C reactive protein (CRP) and procalcitonin were measured within 12 hours of presentation to the ICU in the COVID-19 care facility.

The protocol was designed and followed in accordance with the Declaration of Helsinki. Ethical clearance was obtained from the Institutional Review Board (PGI/IEC/2020/000997 dated 24 August 2020) and the study was also registered at ClinicalTrials.gov. The study protocol is available from <https://clinicaltrials.gov/ct2/show/NCT04668404>. All the authors had access to the study data and approved the final manuscript.

Outcomes

The primary outcome was to describe the coagulation states using SCTs and POC Sonoclot tests in patients with severe COVID-19 pneumonia. The secondary outcomes were the incidence of thromboembolic episodes, bleeding events and the presence of HLE using Sonoclot. The predictability of clinical and laboratory parameters was also analysed for mortality at 28 days.

Definitions

ARDS was defined as per the Berlin definition.¹⁷ Disseminated intravascular coagulation (DIC) and major bleeding were defined as per recommendations of the International Society on Thrombosis and Hemostasis scoring system.¹⁸ Sepsis was defined as per the Third International Consensus Definitions for Sepsis and Septic Shock.¹⁹ Major bleeding was defined as patients with fatal bleeding, and/or symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular, pericardial or intramuscular with compartment

syndrome, and/or bleeding causing a fall in haemoglobin level of 2.0 g/L or more, or leading to transfusion of two or more units of whole blood or packed red cells.²⁰

Treatment protocols

Oxygen therapy was titrated to target SpO₂ >92% with the use of oxygen delivery devices ranging from low-flow devices (nasal prongs, simple face mask) to high-flow devices (venturi mask, high-flow nasal cannula, non-invasive ventilation) and invasive mechanical ventilation. Awake prone sessions were given to all patients on non-invasive oxygen therapy. Standard medical care including steroids and anticoagulation were administered as per our institutional protocol. Few patients received drugs like Tocilizumab, Remdesivir, Mycobacterium indicus pranii (Immuvac), Hydroxychloroquine, plasma therapy as part of ongoing trials.²¹ Supportive care for critically ill patients in the form of advanced haemodynamic monitoring and support, enteral nutrition, glycaemic control and stress ulcer prophylaxis were given in all eligible patients. Antibiotic and antifungal therapy were guided by cultures and sepsis markers. Online supplemental file 1 shows the patient triage and dynamic treatment algorithm at our centre. Online supplemental file 2 shows the anticoagulation algorithm followed at our centre.

Assessment of coagulation parameters

Complete automated blood cell count was performed on LH750/780 automated haematology analyzer (Beckman Coulter, Florida). SCTs measured include prothrombin time (PT), activated PT (aPTT), international normalised ratio (INR), D-dimer (DiagnosticaStago, France) and fibrinogen (Sysmex CA 1500; Sysmex Corporation, Kobe, Japan). Sonoclotanalysis of whole blood sample (non-heparinised) (Sienco, Arvada, Colorado) was performed at the bedside. For performing glass-bead activated Sonoclot, 340 µL of whole blood was added to the Sonoclot cuvette, prewarmed to 37°C. Glass bead ACT (gbACT), clot rate (CR) and platelet function (PF) were noted from the results and time to peak and PA were calculated from the Sonoclot signatures.²² Paired global coagulation traces, that is, (gbSonoclot) and heparinase-treated (hSonoclot) were examined at days 1 and 3 in patients with rapid clinical deterioration and or clinical major bleeding. Sonoclot ACT <110 s and PA >75 units were considered as the cut-off for hypercoagulable state. The HLE due to endogenous heparinoids was calculated by the percentage correction of the gbACT with the hACT.¹⁶

$$\text{HLE (\%)} = \frac{\text{gbACT} - \text{hACT}}{\text{hACT}} \times 100$$

HLE was considered when the HLE% was greater than 20%, and severe HLE was defined as a value greater than 50%. In addition, corrected ACT (gbACT-hACT) >40 s was used to define the presence of endogenous heparinoids.

Statistical methods

Continuous data were represented as mean with SD or median with IQR, as appropriate. Normality of quantitative

data was checked using Kolmogorov Smirnov tests. The continuous data between groups were compared by using the Analysis of Variance (ANOVA) with a post-hoc Bonferroni test applied for differences between the three groups. Survival curves were constructed by Kaplan Meier analysis & compared by log-rank method. Proportions were compared using χ^2 or Fisher's exact test. Independent predictors for mortality were identified on univariate analysis, and the variables with p value <0.10 were subjected to multivariate logistic regression analysis. We computed the Cox proportional Hazards test with adjusted HRs and 95% CI to estimate the association of each predictor to the clinical event or death. Predictive values of tests for outcomes were done by creating receiver operating characteristics (ROC) curves. All statistical tests were two sided and performed at a significance level of $\alpha=0.05$. Statistical analysis was performed with SPSS Statistics V.22 (IBM SPSS Statistics Armonk, New York, USA).

Sample size

Sample size was estimated using G*Power, a statistical programme. Assuming the incidence of hypercoagulable state in COVID-19 to be 5%–15%,⁵ with an effect size of 0.5, alpha of 0.05 and power of 0.85, it was estimated that a total sample size of 60 patients were required. Seventy patients were recruited to account for 10% with incomplete data and or attrition.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

RESULTS

A total of 2324 patients with COVID-19 were screened from August 2020 to November 2020. Of them, 215 patients with COVID-19 required admission to the ICU based on admission criteria. Of these, 12 were excluded for being on anticoagulation therapy before referral, 8 patients had recent blood and or blood component transfusion, 6 had refused consent and 189 had incomplete POC coagulation assay data or were referred late after initial management to our centre for management in critically ill state. After exclusion, 74 treatment-naive patients were included in this prospective observational study (figure 1). The median age of the participants was 54 (42–67) years and 64% of them were men. The patients were divided into three groups based on the oxygen therapy into low-flow (11, 14.9%), high-flow (34, 45.9%) and invasive ventilation (29, 39.2%) groups. Mortality was observed only in the invasive ventilation group (16, 55.1%) with overall mortality of 21.6% in the cohort of 74 patients.

The baseline clinical and biochemical characteristics in the three oxygen therapy groups are shown in table 1. Online supplemental file 3 shows the comorbid illness and treatment details of the cohort.

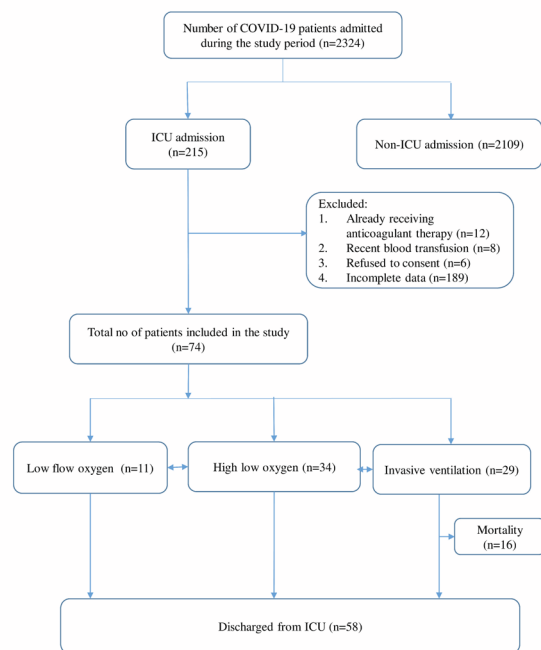


Figure 1 Study design, patient selection and evaluation. ICU, intensive care unit.

Assessment of coagulation function

The results of the conventional and POC coagulation parameters are shown in [table 2](#). At day 1, 29.7% of the cohort of 74 patients had a hypercoagulable Sonoclot profile. There were no significant differences in the conventional coagulation tests among the oxygen groups. The paired Sonoclot results in subgroup of 33 patients are shown in [table 3](#). In patients belong to the invasive ventilation group, we observed a prolongation of the ACT, which was corrected in the hSonoclot. However, the trace was partially corrected in the subjects with low-flow oxygen and high-flow oxygen group. This can be seen as reduction in the prolonged ACT in the hSonoclot assay ([figure 2A](#)). HLE was noted in 17 (51.5%) and 20 (62.5%) of 33 patients at day 1 and 3, respectively ([table 3](#)). Severe HLE was associated with requirement for invasive ventilation (HR 1.200 CI 1.040 to 1.400 p=0.001) and an uncorrected ACT was associated with increased risk of death ([figure 2B](#)).

Bleeding and thrombotic events in COVID-19

Eleven (14.8%) patients developed thrombotic complications during the hospital course, with 4 (5.4%) patients having echocardiographic evidence of pulmonary thromboembolism. The rest of the patients had evidence of clotting of central venous lines or dialysis catheters. Presence of HLE favoured a bleeding phenotype (p=0.024) ([table 2](#)). Episodes of major bleeding were seen in 12 (16.2%) patients, with 9 (75%) having gastrointestinal

bleeding and 3 (25%) having haematuria. Minor bleeding episodes (14%) were noted as ecchymosis, epistaxis and tracheal bleeds during suction ([table 1](#)).

Association of coagulation defects with mortality in COVID-19

In our cohort of 74 patients with COVID-19, the overall 28-day mortality was 16 (21.6 %). Age, gender, respiratory rate, blood pressure, IL-6, ferritin, D-dimer, normalised D-dimer, neutrophil lymphocyte ratio, fibrinogen, creatinine, bilirubin, aminotransferases and lactate did not predict mortality on Cox proportional Hazard analysis. SCTs like platelet count, PT, INR, aPTT and D-dimer did not predict mortality in any regression model. Predictors of mortality at day 1 were presence of HLE (HR 1.02; CI 1.01 to 1.04; p=0.002), increased CRP (HR 1.04; CI 1.02 to 1.09 p=0.003), elevated ACT (HR 1.02; CI 0.1.04 to 1.4 p=0.001) and decreased PF (HR 0.54; CI 0.29 to 0.90; p=0.010). In multivariate analysis, the presence of HLE (HR 1.02; CI 1.08 to 1.6; p=0.007), raised CRP (1.2; CI 1.1 to 1.4; p=0.014) and reduced PF (HR 0.9; CI 0.7 to 1.1 p=0.045) remained significant (online supplemental file 4). Presence of HLE resulted in increased mortality (p=0.001) ([figure 3](#)). The presence of HLE at day 1 predicted the need for invasive ventilation (HR 1.4; CI 1.01 to 1.5; p=0.002). On ROC analysis, CRP >96.7 mg/dL (p=0.001), ferritin >587 mg/dL (p=0.021) and LDH>405 U/L (p=0.015) predicted mortality ([figure 4](#)). In Sonoclot assay, an ACT >131 s (p=0.021), CR <27 U/min (p=0.027) and PA <67.5 units predicted mortality (online supplemental file 5).

DISCUSSION

This prospective cohort study has demonstrated the association of endogenous heparinoids with the coagulation dysfunction in COVID-19, using paired POC coagulation tests at two time points. Our data show that information generated by POC tests provides an etiopathological rationale for appropriate use of anticoagulation in COVID-19 patients. First, patients with hypercoagulable tests benefit from anticoagulation, whereas those with HLE or hypocoagulable profile on Sonoclot have higher likelihood for bleeding and require invasive ventilation. Second, the use of D-dimer as a marker of a procoagulant state is fallacious, as it is elevated in patients with thrombosis, hyperfibrinolysis and DIC. Thirdly, our data show that the procoagulant tendencies are seen early in the course of illness, and there exists a therapeutic window for use of anticoagulation in patients with COVID-19-related coagulation dysfunction. In late stages of COVID-19 the coagulation profile switches to a hypocoagulable phenotype with the onset of the cytokine storm, secondary infections and organ failures.

Recently, large multicentric trials have assessed the role of anticoagulation in COVID-19 with varying success. Their use of SCTs like D-dimer to guide prophylactic and therapeutic anticoagulation protocols has inherent flaws. In the ACTION trial by Lopes *et al*, therapeutic

Table 1 Baseline characteristics, co-morbidities, symptom profile, laboratory parameters and treatment details of the admitted patients with COVID-19

Parameter	Total Patients (n=74)	Low flow oxygen group 1 (n=11)	High flow oxygen group 2 (n=34)	Invasive ventilation group 3 (n=29)	P value 1 vs 2	P value 1 vs 3
Age (years)*	54 (42–67)	54 (39–67.5)	55 (45–68.5)	53 (36–65)	0.725	0.690
BMI (kgm ²)*	24.7 (22.2–26.6)	23.8 (23.0–26.1)	25.4 (21.7–26.1)	24.4 (22.7–27.7)	0.845	0.788
Sex						
Male	47 (64)	8 (73)	23(68)	16(55)	0.752	0.213
SOFA score	4 (3–5)	3 (2–4)	3 (2–5)	4 (3–5)	0.397	0.116
Mortality	16 (22)	0	0	16 (55)	0.000	0.000
Bleeding and thrombosis						
Major bleeding	12 (16%)	0	5 (15)	7 (24)	0.313	0.159
Minor bleeding	10 (14%)	0	2 (6%)	8 (28%)	1.000	0.080
Pulmonary micro-thrombosis	6 (8)	0	2 (6)	4 (14)	0.567	0.260
Thrombosis	5 (7)	0 (0)	1 (3)	4 (14)	0.756	0.260
Blood transfusion	20 (27)	2 (18)	5 (15)	13 (45)	0.782	0.120
Investigations*						
Haemoglobin (g/L)	108 (97–120)	120 (104–130)	108 (100–120)	103(88–121)	0.130	0.060
NLR	16.4 (8.2–30.8)	4.4 (4.2–23.4)	17.1 (10.7–34.2)	17.2 (8.7–26.1)	0.099	0.084
Platelet count (x109/L)	179 (100.5–255.2)	198 (111–239)	170 (121–252)	174 (61–256)	0.948	0.765
Urea (mg/dL)	38.5 (26.8–58.3)	36 (21.9–45)	35 (25.7–54.7)	43 (35–111)	0.667	0.044
Creatinine (mg/dL)	0.8 (0.6–1.2)	0.7 (0.5–1.0)	0.8 (0.6–1.1)	0.9 (0.6–1.8)	0.611	0.294
AST (U/L)	52 (35–86)	79 (40–115)	53 (35–87)	46 (31–75)	0.255	0.116
ALT (U/L)	40 (32–65)	44 (34–65)	38 (32–61)	42 (31–61)	0.825	0.743
ALP (U/L)	107 (70–137)	111 (86–136)	110.5 (65–136)	107 (86–126)	0.745	1.000
LDH (U/L)	352 (221–507)	523 (247–589)	252 (167–383)	474 (295–556)	0.062	1.000
Total bilirubin (mg/d)	0.5 (0.4–0.8)	0.7 (0.4–0.8)	0.5 (0.4–0.7)	0.6 (0.4–0.8)	0.396	0.591
Total protein(g/dL)	6.1 (5.5–6.7)	6.6 (5.8–7.0)	6.2 (5.8–6.8)	5.8 (5.3–6.5)	0.302	0.038
Albumin (g/dL)	3.1 (2.7–3.5)	3.3 (2.9–3.9)	3.1 (2.8–3.6)	3.0 (2.7–3.2)	0.277	0.055
Lactate (mmol/L)	1.8 (1.5–2.6)	1.7 (1.3–1.9)	1.8 (1.3–2.5)	2.0 (1.8–2.6)	0.745	0.0381
CRP (mg/L)	86 (33–165)	23 (3–62)	49 (23–96)	110 (97–199)	0.247	0.001
Procalcitonin (ng/mL)	0.9 (0.3–11.3)	0.9 (0.3–2.9)	0.9 (0.3–14.8)	1.1 (0.5–4.6)	0.593	0.591
Ferritin (ng/mL)	559 (127–944)	356 (94–835)	235 (100–649)	933 (588–1424)	0.465	0.015

*Values are expressed as median (IQR) and compared using Mann-Whitney U test p value<0.05 is considered as significant.

†Values are expressed as n (%) and compared using Chi-square test, p value<0.05 is considered as significant.

ALP, alkaline phosphatase; ALT, alanine transaminase; ARDS, acute respiratory distress syndrome; AST, aspartate transaminase; BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; DM, diabetes mellitus; GI, gastro intestinal; HCQ, hydroxychloroquine; HTN, hypertension; LDH, lactate dehydrogenase; NLR, neutrophil lymphocyte ratio; SOFA, sequential organ failure assessment.

anticoagulation did not improve survival at 30 days, and the rate of bleeding events were 8% and 2%, respectively in the two comparators. The limitations of this study were variable recruitment of patients, within a 2 week interval post COVID-19 diagnosis, which introduced some population heterogeneity in terms of duration of COVID-19 diagnosis before enrollment. Also, we were unable to test for fibrinolysis, platelet aggregation and thrombin generation at inclusion due to limitations of laboratory support in the COVID-19 facility.

The differential use of intravenous unfractionated heparin versus low molecular heparin in the critically ill subgroup makes universal applicability of results difficult.⁹ Cui *et al* reported that a D-dimer cut-off value of 1.5 µg/mL had a sensitivity of 85% and specificity of 88.5% to predict COVID-19 related VTE with a negative predictive value of 94.7%.²³

In our study, SCTs were comparable among the three oxygen requiring groups despite the significant difference in paired Sonoclot tests on days 1 and 3. The D-dimer

**Table 2** Conventional coagulation parameters and Sonoclot results of the study participants

Parameter*	Total Patients (n=74)	Low flow oxygen (n=11)	High flow oxygen (n=34)	Invasive ventilation (n=29)	P value 1 vs 2	P value 1 vs 3
PT(s)	15.5 (14.5–16.4)	15.2 (14.1–16.2)	15.2 (14.6–16.7)	15.6 (15.2–16.2)	0.575	0.385
INR	1.1 (1.0–1.2)	1.02 (0.9–1.2)	1.08 (1–1.9)	1.2 (1.1–1.2)	0.706	0.148
APTT (s)	32 (29.2–35.1)	32.1 (30–35.5)	31.7 (28.8–34.3)	33.5 (30.3–4)	0.593	0.788
D-Dimer (ng/mL)	1045 (362–1982)	934 (488–1135)	789 (282–1609)	1200 (652–3443)	0.907	0.124
Fibrinogen (g/L)	5.0 (4.5–6.5)	5.0 (3.5–6.1)	5.2 (4.6–6.4)	4.9 (4.6–6.1)	0.866	0.612
ACT (s)	120 (101–145)	119 (94–133)	120 (92.75–135)	121 (112–145)	0.907	0.419
CR	39 (28–51.3)	42 (33–48)	39 (29.5–48.5)	37 (26–52)	0.765	0.676
PF	2.7 (1.5–3.4)	2.9 (1.5–3.3)	3.0 (2.7–3.6)	1.6 (1.3–2.4)	0.367	0.229
Procoagulant Sonoclot (day 1) (ACT <110 and PA >75 units)	22 (30%)	5 (46%)	11 (32%)	6 (21%)	0.430	0.117
Presence of HLE at day 1 (n=33)	17 (52%)	1 (50%)	3 (25%)	13 (68%)	0.468	0.599
Bleeding episodes (patients with HLE at day 1, % within HLE)	10 (59%)	0	0	10 (77%)	0.024	0.024

*Values are expressed as median (IQR) and compared using Mann-Whitney U test, p value<0.05 is considered significant. ACT, activated clotting time; aPTT, activated partial thromboplastin time; CR, clot rate; INR, international normalised ratio; PF, platelet function; PT, prothrombin time.

and normalised D-dimer were not predictive of VTE or mortality in our cohort. Due to the dynamic nature of the disease, it is necessary to repeat the tests whenever the clinical condition deteriorates. A large Italian multi-centric study showed that use of heparin (n=394, 46.6%) was associated with a better chance of survival (OR 0.60 [0.38–0.94], p<0.001), suggesting COVID-19 related microthrombosis could be managed with timely anticoagulation.²⁴ Coagulopathy associated with COVID-19 is a combination of low-grade DIC and localised pulmonary thrombotic microangiopathy, which could have a substantial impact on organ dysfunction. In critically ill patients, the incidence of thromboembolic complications ranges from 5% to 15%.⁵

We found that there was a significant difference in the presence of HLE observed among the groups. In our cohort of patients, hypercoagulable coagulation phenotype was observed predominantly in the low-flow oxygen group compared with hypocoagulable phenotype in the high-flow and invasive group. SARS-CoV-2 infection induces cytokine storm, with systemic inflammation and secondary sepsis, leading to endothelial injury and early DIC. Damaged endothelial cells with microangiopathy release a mixture of glycosaminoglycans that function like endogenous heparinoids.²⁵ Ackermann *et al* demonstrated a distinctive vascular pathology in COVID-19 lungs, including severe endothelial injury, presence of intracellular virus and disrupted cell membranes. The pulmonary vessels showed widespread thrombosis, with alveolar capillary microthrombi.²⁶ The COVID-19

microangiopathy can be assessed by testing for HLE, seen as prolongation of the ACT with correction in the heparinase-modified Sonoclot test. The presence of HLE tilts the haemostatic balance to either the hypercoagulable or DIC like state. Similar to HLE, inflammatory markers like CRP, LDH, ferritin and oxygen requirement were also found to increase with disease severity in our cohort. In our study, we found >50% of patients have evidence of HLE on Sonoclot. The effects of endogenous heparinoids are not routinely well appreciated by SCTs like D-dimer in patients on anticoagulation therapy. Patients with a procoagulant phenotype on Sonoclot should benefit from early initiation of anticoagulation therapy and in patients those with a hypocoagulable Sonoclot, therapeutic anticoagulation should result in higher incidence of bleeding and thus increases morbidity and prolongation of hospital stay. The use of POC tests can rapidly identify the type of coagulation defect reliably and reproducibly. By performing serial POC coagulation tests on days 1 and 3, we could guide the dose of anticoagulation, reduce bleeding risks and provide evidence-based transfusion thresholds for blood components like fresh frozen plasma and platelet concentrates, thereby limiting blood component transfusions. The POC test-based algorithm minimised transfusion related complications like volume overload in patients with severe ARDS, whereas SCTs like D-dimer did not help in diagnosing either the procoagulant or hypocoagulable phenotype in our cohort.

Moreover, a significant proportion of severely ill COVID-19 patients had evidence of major bleeding that

Table 3 Dynamic Sonoclot glass bead (gb)-activated, heparinase (h) treated (paired Sonoclot) parameters on day 1 and day 3

Sonoclot variable*	Total patients (n=33)	Low flow oxygen (n=2)	High flow oxygen (n=12)	Invasive ventilation (n=19)	P value 1 vs 2	P value 1 vs 3
Day 1						
gbACT (s)	171 (63)	102 (18)	135 (28)	197 (66)	0.004	0.060
gbCR	38.4 (17.2)	31.5 (9.2)	39.3 (17.8)	38.5 (17.8)	0.847	0.595
gbPF	2.1 (1.7)	6.4 (4.5)	2.1 (1.3)	1.7 (0.8)	0.000	0.000
gbPeak-Amplitude	77 (21)	107 (24)	98 (17)	65 (8)	0.000	0.000
gbTime to peak (s)	13 (3)	12 (2)	11 (3)	15 (3)	0.001	0.117
Day 1						
hACT (s)	117 (35)	65 (21)	101 (34)	132 (28)	0.001	0.004
CR	36.5 (12.4)	34 (11.3)	38.8 (10.6)	35.4 (13.7)	0.729	0.888
PF	2 (1.2)	1.4 (1.7)	2.4 (1.6)	1.8 (1.0)	0.274	0.611
Peak amplitude	78 (23)	108 (25)	102 (19)	65 (10)	0.000	0.000
Day 3						
gbACT (s)	163 (57)	93 (18)	125 (33)	187 (53)	0.001	0.023
gbCR	33.4 (14.3)	34 (2.8)	30.2 (13.2)	35 (15.6)	0.708	0.933
gbPF	1.6 (1.0)	2.4 (1.7)	1.9 (1.4)	1.3 (0.6)	0.161	0.060
gbPeak amplitude	78 (19)	105 (21)	97 (14)	66 (10)	0.000	0.000
Time to peak (s)	15 (5)	9 (1)	10 (3)	18 (3)	0.000	0.001
Day 3						
hACT (s)	110 (39)	65 (15)	82 (38)	129 (28)	0.000	0.005
hCR	36.7 (14.9)	34 (10)	35.7 (16)	37 (15.4)	0.931	0.764
Presence of HLE at day 3 (n, %)	20(63)	1 (50)	6 (60)	13(65)	0.793	0.674
Procoagulant Sonoclot (day 3) (n, %)	7 (21)	2 (100)	4 (40)	1 (5)	0.002	0.671

*Values are expressed as mean±SD and compared using Student t-test, p value<0.05 is considered is significant. ACT, activated clotting time; CR, clot rate; PF, platelet function.

can be attributed to sepsis induced coagulopathy and anticoagulation regimen guided by SCTs. Considering the complex interplay between prothrombotic and anti-haemostatic pathways, their influence on outcome in terms of thrombosis or bleeding cannot be measured on SCTs.^{26 27}

HLE was noted in 59% of those who presented with clinical bleeding. The GCTs showed HLE in 60% of patients and 13 (81%) deaths occurred in this subgroup. (table 3) Using ROC analysis, we found inflammatory markers like CRP, ferritin, LDH and Sonoclot parameters predicted outcomes.

Previous data on the prognostic role of troponin elevation due to macrothrombosis in the coronary circulation are based on the same coagulation dysregulation mechanism representing a mirror of myocardial injury which could definitely lead to worse outcomes, especially in patients with CAD.^{28 29} Increased dIgbACT, decreased PA, decreased CR significantly predicted mortality with sensitivity and specificity better than the inflammatory markers.

A few limitations of our study merit mention. Our centre is a tertiary care university hospital with many critically ill patients being referred for intensive care. Therefore, patients who had received treatment elsewhere (>7 days after diagnosis) and those who were already on mechanical ventilation were not included in our study as the baseline native test may not be representative of the early coagulation abnormalities in COVID-19. Our study sample is small, but it was calculated as a proof-of-concept study regarding pro-and anticoagulant mechanisms in COVID-19 and is not powered to assess the efficacy of anticoagulation. At the time of study initiation, we had no data regarding the use of POC GCT in COVID-19. In patients who received blood component therapy between the two time points, the results of some coagulation tests may have been affected. We did not estimate thrombin generation in this protocol, which should be evaluated in future research. Lastly, as our study was restricted to COVID-19-ICU patients, we did not have a control cohort of asymptomatic COVID-19 and non-COVID patients.

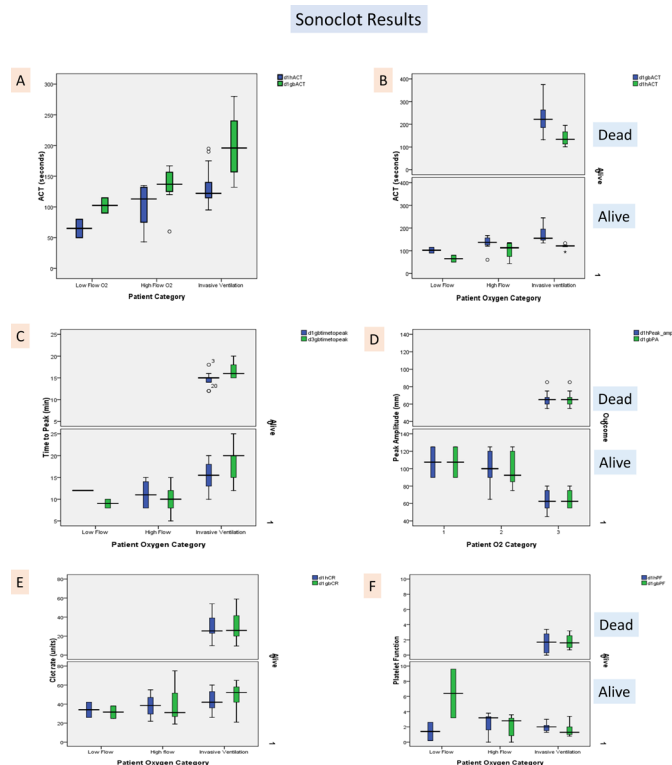


Figure 2 Results of global coagulation tests (gb and hSonoclot) in the low flow (N=11), high flow (n=34) and invasive ventilation (n=29) oxygen requiring subgroups. Presence of heparin-like effect (HLE) as defined as difference in Sonoclot trace at days 1, 3 in patients. The prevalence of HLE and changes in Sonoclot test was assessed as per oxygen requirement and survival (A–F).

In our study, patients with COVID-19 requiring high flow oxygen support and invasive ventilation had worse global coagulation parameters as compared with those with uncomplicated disease. The presence of endogenous heparinoids was associated with secondary sepsis, bleeding and increased mortality. As described previously, coagulation failure and cytokine storm portend a poor prognosis in COVID-19.^{15 30}

The potential therapeutic implications of testing global coagulation in COVID-19 include the appropriate use of blood products prior to invasive procedures and appropriate dosing of anticoagulation.

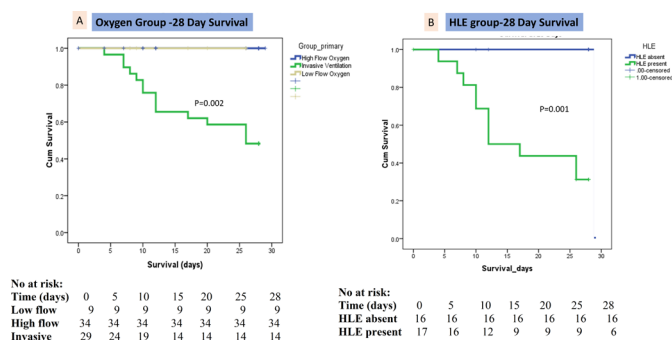


Figure 3 Kaplan-Meier survival curves for patients (A—oxygen groups; B—presence of heparin-like effect).

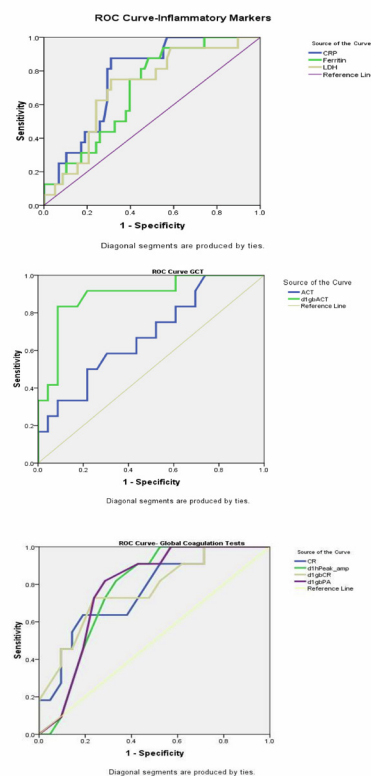


Figure 4 Receiver operating characteristic (ROC) curves for predictors of mortality in the COVID-19 cohort (n=74). CRP, C reactive protein; LDH, lactate dehydrogenase; gbCR, glass-bead test Clot Rate ; gbPA, glass-bead test Peak Amplitude.

CONCLUSION

Our results demonstrate a spectrum of coagulation derangement in COVID-19 with a procoagulant phenotype with a good prognosis at one end and a hypocoagulable phenotype in patients who required invasive ventilation, with high mortality, at the other end. The presence of a hypercoagulable profile in patients with COVID-19 indicates those who will benefit from early therapeutic anticoagulation. POC algorithms are better suited to determine the need and dose strategy (prophylactic vs therapeutic), but these tests remain complementary to SCT-based treatment decisions in primary practice. Further work is also needed in the field of thrombin generation potential and anticoagulation protocol validation.

Author affiliations

- Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
- Department of Anaesthesia and Intensive Care, Postgraduate Institute of Medical Education and Research, Chandigarh, India
- Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India
- Department of Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India
- Department of Clinical Haematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
- Department of Haematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
- Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Contributors MP, SL and KK equally contributed to this study. Author statement: MP, KK and SL: designed the trial, data curation, verified the underlying data, wrote the manuscript and approved submission. AH, SS, ISS, PM, AB, GDP and VS: provided logistical support, treated patients, provided experimental data and approved the final draft of the manuscript and submission. JA, VS, AD, NK, SR, KS, VM and YLN: resources and coordinated activities, KK: data curation. KK, the guarantor of this study, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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ORCID iDs

Madhumita Premkumar <http://orcid.org/0000-0003-2961-4148>

Sekar Loganathan <http://orcid.org/0000-0003-1937-788X>

Kamal Kajal <http://orcid.org/0000-0003-3271-0122>

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