BMJ Open

Hypocoagulable Coagulation Profile and Endogenous Heparinoids are Associated with Invasive Ventilation and Mortality in COVID-19

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-051971
Article Type:	Original research
Date Submitted by the Author:	03-Apr-2021
Complete List of Authors:	Premkumar, Madhumita; Post Graduate Institute of Medical Education and Research, Department of Hepatology Loganathan, Sekar; Post Graduate Institute of Medical Education and Research Kajal, Kamal; PGIMER, Department of Anaesthesia Hazarika, Amarjyoti; PGIMER, Department of Anaesthesia Soni, Shiv; PGIMER, Department of Anaesthesia Puri, Goverdhan ; Post Graduate Institute of Medical Education and Research Sehgal, Inderpaul Singh; PGIMER, Department of Pulmonary Medicine Suri, Vikas; Post Graduate Institute of Medical Education and Research Malhotra, Pankaj; PGIMER Singh, Virendra; PGIMER, Department of Hepatology Duseja, Ajay; PGIMER Bhalla, Ashish; Post Graduate Institute of Medical Education and Research, Internal Medicine Ahluwalia, Jasmina; PGIMER, Department of Hematology Kumar, Narender; PGIMER, Department of Hematology Kekan, Kushal; PGIMER, Department of Hepatology Ram, Sant; PGIMER, Department of Biochemistry Singla, Karan; PGIMER, Department of Anaesthesia Mahajan, Varun; PGIMER, Department of Anaesthesia Yaddanapudi, Narayana; Post Graduate Institute of Medical Education and Research
Keywords:	COVID-19, Anticoagulation < HAEMATOLOGY, INTENSIVE & CRITICAL CARE, Thromboembolism < CARDIOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Hypocoagulable Coagulation Profile and Endogenous Heparinoids are Associated with Invasive Ventilation and Mortality in COVID-19

Authors:

Madhumita Premkumar DM[1], Sekar L MD[2], Kamal Kajal MD[2*], Amarjyoti Hazarika MD[2], Shiv Soni DM[2], GD Puri PhD[2], Inderpaul Singh Sehgal DM[3], Vikas Suri MD[4],Pankaj Malhotra MD[4], Virendra Singh DM[1], Ajay Duseja DM[1], , Ashish Bhalla MD[4], Jasmina Ahluwalia MD[5], Narender Kumar MD[5], Kushal Kekan[1], Sant Ram MD[6], Karan Singla MD[2], Varun Mahajan MD [2], L Narayana Yaddanapudi MD[2].

Affiliations:

[1] Department of Hepatology,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[2] Department of Anaesthesia,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[3] Department of Pulmonary Medicine,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[4] Department of Internal Medicine,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[5] Department of Hematology,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[6] Department of Biochemistry,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

*Corresponding Author:

Dr.Kamal Kajal MD,

Department of Anaesthesia,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

Email: kamal.kajal@gmail.com

Phone No: +91 7087009545

Conflict of Interest and Source of Funding: All the authors have disclosed that they do not have any conflicts of interest.

Word count: 2883

ABSTRACT

Objective: To study the dynamic association of the coagulation abnormalities with respiratory failure and mortality in patients with COVID-19.

Design: Single center, prospective observational study.

Setting: Tertiary care hospital, North India.

Participants: Seventy four adult patients with COVID-19 pneumonia requiring intensive care (ICU) admissions from August 2020 to November 2020.

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

Primary outcome: To describe the coagulation states using SCTs and POC Sonoclot® test in COVID-19 patients.

Secondary outcome: Incidence of thromboembolic, bleeding events and presence of endogenous heparinoids generated due to cytokine storm. Predictability of clinical and laboratory parameters were also analysed for mortality at 28 days.

Results: Paired Sonoclot® assays in 33 patients demonstrated HLE in 17 (51.5%) and 20 (62.5%) at days 1 and 3 respectively. Presence of HLE, increased C-reactive protein and platelet function predicted mortality. Presence of HLE at day 1 predicted the need for invasive ventilation.

Conclusion: HLE contributes to hypocoagulable effect, need for invasive ventilation and mortality in patients with COVID-19.

Trial registration: Ethical clearance was obtained from the Institutional Review Board (PGI/IEC/2020/000997 dated 24 August 2020) and the study was registered in ClinicalTrials.gov.

Data availability statement:

Data not available due to legal restrictions.

Strengths and limitations of this study:

- This is the largest prospective study using paired point of care coagulation tests at for assessing the dynamic coagulation defect in COVID 19.
- This study will help in guiding anticoagulation therapy in patients with COVID-19.
- Paired Sonoclot® tests helps in identifying patients at risk of worse outcome.
- This study was restricted to patients admitted to intensive care, we did not have a control cohort of asymptomatic COVID-19 patients.

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection is a pandemic, which has affected approximately 54,771,888 persons, and claimed 1,324,249 lives till date.(1) Patients with COVID-19 have platelet abnormalities, endothelial dysfunction, clotting factors abnormalities and hyper fibrinolysis complicated by coexisting sepsis and renal failure. (2)This will lead to thromboembolic events both in the venous and arterial circulations .(3)

Nearly 20% of COVID-19 patients present with severe coagulation abnormalities.(4) Hypercoagulable state in COVID-19 is erroneously likened to an early DIC like state, due to an elevation of D-dimer and low platelet count. However, recent data from COVID-19 studies suggests that consumption of coagulation factors is absent, and patients had high fibrinogen and factor VIII levels.(5) Concomitant venous thromboembolism (VTE), a potential cause of unexplained deaths, has been reported in COVID-19 cases (5), but its management with antithrombotic therapy is challenging in the presence of bleeding risk.(6) In addition, there is conflicting evidence regarding the role of prophylactic vs therapeutic anticoagulation in COVID 19.

Tang et al. observed higher D-dimer and fibrin degradation product (FDP) level associated with the overall mortality.(7) A wide range of abnormalities in standard coagulation tests (SCTS) have been reported depending on the severity of the disease (8), suggesting the multifactorial dynamic pathology. Patients with severe COVID-19 pneumonia were associated with a hypercoagulable state rather than consumptive coagulopathy.(9) Viscoelastic testing of global coagulation in point-of care (POC) devices such as thromboelastometry and Sonoclot® has been proposed as a superior

tool to rapidly diagnose underlying pathophysiology of coagulation dysfunction and guide resuscitation with appropriate blood products or anticoagulation.(10)

A reduction in the activated clotting time (ACT) and maximum amplitude (MA) / peak amplitude (PA) is indicative of a procoagulant coagulopathy in global coagulation tests (GCT) like thromboelastography or Sonoclot®.(11) Spiezia et al. demonstrated a significant hypercoagulable thromboelastometry (ROTEM) profiles with a shorter clot formation time (CFT) in INTEM ($p = 0.0002$), EXTEM ($p = 0.010$) and increased maximum clot firmness (MCF) (p=0.001) that was associated with worse outcome. (12) Sonoclot® assessment of clot formation and clot lysis takes 30 to 60 minutes. Cytokine storm and sepsis trigger the release of endogenous heparinoids from the endothelium in various organs which is seen on global coagulation tests as a 'heparin-like effect' (HLE).(13) The production of endogenous heparinoids during cytokine storm 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 (PGIMER), Chandigarh, India between September 2020 and December 2020. We also aimed at creating an algorithm for management of hemostatic abnormalities in these patients based on the evidence generated from this prospective observational study.

METHODS

Study design and participants

Patients with SARS-Cov2 infection confirmed by reverse transcription polymerase chain reaction (RT-PCR), aged between 18 to 80 years, and either gender were recruited. A written informed consent was taken from all the study participants. Patients with a recent history of 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 demographic data, past medical history, clinical presentation, history of comorbid illness, drug therapy including use of antibiotics, antifibrinolytics and herbal medicines.

Blood, 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 in ClinicalTrials.gov. 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® test in COVID-19 patients. The secondary outcomes were the incidence of thromboembolic, bleeding events and the presence of endogenous heparinoids generated due to cytokine storm. The predictability of clinical and laboratory parameters were also analysed for mortality at 28 days.

Definitions:

Acute respiratory distress syndrome (ARDS) was defined as per the Berlin definition (14). DIC and major bleeding was defined as per recommendations of the International Society on Thrombosis and Hemostasis (ISTH) scoring system.(15) Sepsis was defined as per the Third International Consensus Definitions for Sepsis and Septic Shock. (16) *Major bleeding* was defined as patients with fatal bleeding, symptomatic bleeding and/or causing a fall in hemoglobin level of ≥2 g/dL or leading to transfusion of ≥2 units of packed red cells. (17)

Treatment protocols:

Oxygen therapy was titrated to target SpO2 > 93% with the use of oxygen delivery devices ranging from low flow devices (nasal prongs, venturi mask), high flow devices (high flow nasal cannula (HFNC), non-invasive ventilation (NIV)) and invasive ventilation. Awake prone positioning sessions were given to patients on non-invasive oxygen therapy. Standard medical care that includes steroids and anticoagulation were administered as per our institutional protocol. Few patients received drugs like tocilizumab (TCZ), remdesivir, mycobacterium indicus pranii (Immuvac), hydroxychloroquine (HCQ), plasma therapy as part of ongoing trials. Supportive care for critically ill patients in the form of advanced hemodynamic monitoring and support, enteral nutrition, glycaemic control, peptic ulcer prophylaxis were given in all eligible patients. Antibiotic and antifungal therapy were guided by culture and sepsis markers. Supplementary Figure 1 shows the treatment algorithm and Supplementary Figure 2 shows the anticoagulation algorithm at our centre.(18)

Assessment of Coagulation Parameters:

Complete automated blood cell count was performed on LH750/780 automated hematology analyzer (Beckman Coulter Inc., Fl, USA). SCTs measured include prothrombin time (PT), activated prothrombin time (APTT), international normalized ratio (INR), d-Dimer (Diagnostica Stago, France) and fibrinogen. (Sysmex CA 1500; Sysmex Corporation, Kobe, Japan). Sonoclot® (Sienco Inc., Arvada, CO, USA) was performed at the bedside. For performing glass-bead activated Sonoclot®340 µL of whole blood was added to the Sonoclot ®cuvette, pre-warmed to 37°C). Glass bead activated clotting time (gbACT), clot rate (CR), and platelet function (PF) were noted from the machine and time to peak (TP) and peak amplitude (PA) were calculated from the

Sonolcot signature.(19) Paired global coagulation traces (gbSonoclot®) and heparinase treated (hSonoclot®) were examined at days 1 and 3 and in case of clinical deterioration or bleeding. Sonoclot® ACT < 110 seconds and PA > 75 units were considered as the cut off for hypercoagulable state. The heparin like effect (HLE) due to endogenous heparinoids was calculated by the percentage correction of the gbACT with the heparinase treated hACT.(19)

$$\text{HLE (\%)} = \frac{\text{gb ACT} - \text{hACT (heparinase-treated)}}{\text{hACT}} \times 100$$

HLE was considered when the correction of the ACT 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 endogenous heparinoid effect.

Statistical methods:

Continuous data was represented as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Normality of quantitative data was checked using Kolmogorov Smirnov tests. The continuous data between groups were compared by using the ANOVA or its non-parametric equivalent, the Kruskal-Wallis test. 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 hazard ratios (HRs) and 95% confidence intervals (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 α=0.05.

Statistical analysis was performed with SPSS Statistics version 22 (IBM® SPSS® Statistics Armonk New York USA)

Sample size:

Sample size was estimated using G*Power, a statistical program. Assuming the incidence of hypercoagulable state in COVID-19 to be 10%, with an effect size of 0.5, alpha 0.05, and power 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. Of these 12 were excluded for already being anticoagulated before referral, 8 patients had recent blood and or blood component transfusion, 6 had refused consent, and 189 had incomplete coagulation assay data. After exclusion, 74 patients were included in this prospective observational study (Figure 1). The mean age of the participants was 54(42-67) years and 64% of them were male. The patients were divided into 3 groups based on the oxygen therapy into low flow (11, 14.9%), high flow (34, 45.9%) and invasive ventilation (29 39.2%) Mortality was observed only in 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.

Assessment of Coagulation function

The results of the conventional coagulation parameters are shown in Table 2. At days 1 and 3, 29.7% and 21.2% of the cohort of 74 patients had a hypercoagulable Sonoclot®, respectively. A procoagulant profile was seen in 45.5%, 32.4% and 20.7% in low-flow, high-flow, and invasive ventilation. At day 1, patients required invasive ventilation and high flow oxygen therapy had significant lower mean PF as compared with those maintained on low flow oxygen (p=0.000). In patients with secondary sepsis, we noted a prolongation of the ACT which was corrected in the hSonoclot®. The trace was partially corrected in the subjects with low oxygen requirement or noninvasive ventilation (Figure 3). The increased PA and the TP was shortened indicating a hypercoagulable trace in patients who required low flow oxygen (p=0.005). The paired Sonoclot results are shown in Table 3. HLE was noted in 17 (51.5%) and 20(62.5%) of 33 patients at days 0 and 3, respectively. Severe HLE was associated with patients who required invasive ventilation. (HR 1.2 CI 1.04-1.4 p=0.001).

Bleeding and Thrombotic Events in COVID-19

A total of 11 (14.8%) cases of thrombosis were seen in our cohort with 4 (5.4%) patients having echocardiographic and or radiological evidence of pulmonary thromboembolism. The rest of the patients had evidence of clotting of central venous catheters or dialysis catheters. Presence of endogenous heparinoids favored a bleeding phenotype. Episodes of major bleeding were seen in 12(16.2%) patients, with 75% having gastrointestinal bleeding and 25% having hematuria. Minor bleeding episodes were noted as ecchymosis, epistaxis, and endotracheal bleeding.

Association of Coagulation defects with mortality in COVID-19

In our cohort of 74 patients with COVID-19, the overall 28-day mortality was 21.6 %. Predictors of mortality at day 1 were presence of HLE (hazard ratio [HR] 1.02; CI 1.01- 1.04; p=0.002), CRP

(HR 1.01; CI 1.01-1.02 p=0.003), ACT (HR1.02; CI 0.1.04-1.4 p=0.001) and PF (HR 0.54; CI 0.29-0.90; p=0.010). In multivariate analysis, the presence of HLE (HR 1.02; CI 1.08-1.6; p=0.007), CRP (1.2; CI 1.1-1.4; p=0.014)], PF [HR 0.9; CI 0.7-1.1 p=0.050) remained significant. The presence of HLE at day 1 predicted the need for invasive ventilation (HR 1.4; CI 1.01-1.5; 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). Presence of HLE resulted in increased mortality. (p=0.001) (Figure 2). In Sonoclot assay, an ACT > 131 seconds (p=0.021), CR <27 units/min (p=0.027) and PA < 67.5 units predicted mortality. (Supplementary Table 1)

DISCUSSION

This study has demonstrated the association of endogenous heparinoids mediated HLE with the coagulation dysfunction in COVID-19 as diagnosed by global coagulation POC test. Ours is the largest prospective study using paired POC coagulation tests at two point time interval for assessing the dynamic coagulation defect in COVID 19 and its association with worsening of respiratory failure. This information provides us an etiopathological rationale for guiding anticoagulation therapy in COVID 19 patients.

Conventional coagulation profile were comparable among the three oxygen requiring groups despite significant difference in paired Sonoclot® on day 1 and day 3. Due to the dynamic nature of the disease, it is necessary to repeat the tests in the early disease course and whenever the clinical condition deteriorates.

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 only in low flow oxygen group compared to hypocogulable phenotype in high low and invasive group. Widespread endothelial injury from progressive COVID-19 resulting in release of endogenous

heparanoids, secondary sepsis induced coagulopathy may explain this change in coagulation phenotype to hypocoauglable state in the later groups. However, the influence of therapeutic anticoagulation in high flow and invasive groups cannot be rule out.

Patients with a procoagulant phenotype on Sonoclot® may benefit from early initiation of anticoagulation therapy and in patients those with a hypocoagulable Sonoclot® therapeutic anticoagulation can result in higher incidence of bleeding and thus increases morbidity and prolongation 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 day 1 and 3, we could guide the dose of anticoagulation, reduce bleeding risks and provide evidence-based transfusion thresholds for blood components like FFP and platelet concentrates in patients with major bleeding at the bedside thereby limiting blood and component transfusions This could reduce transfusion related complications including volume overload in patients with severe ARDS However, conventional tests did not help in diagnosing either the procoagulant or hypocoagulable phenotype in our cohort.

The existing pathophysiologic basis for coagulation dysfunction in patients with COVID 19 include disruption of cross-talk between immune and haemostatic relationship leading to widespread endothelial injury, pulmonary micro-thrombosis and early disseminated intravascular coagulation depending upon the severity of the disease.(15) However, significant proportion of severely ill Covid-19 patients had evidence of major bleeding that can be attributed to sepsis induced multi-organ dysfunction and anticoagulation regimen guided by SCTs. Considering the complex interplay between prothrombotic and anti-hemostatic pathways, their influence on outcome in terms of thrombosis or bleeding cannot be measured on SCTs. (20) Systemic inflammatory response syndrome disrupts endothelial matrix leading to release of endogenous heparinoids. (15)

SARS-CoV-2 infection induced induces cytokine storm, systemic inflammation and secondary sepsis leads to endothelial injury which releases a mixture of glycosaminoglycans by the endothelial cells that function like endogenous heparinoids.(21) The prolongation of the ACT and correction in the heparinase- modified test demonstrated the HLE in the Sonoclot® test. This HLE tilts the hemostatic balance to either the hypercoagulable or DIC state. Concomitant with 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 in patients with concurrent anticoagulation therapy.

HLE was noted in 66.7% of those who presented with clinical bleeding, and 33.3 % of those with pulmonary thrombosis. The GCTs showed HLE in 60% of patients and 13 (81%) deaths occurred in this subgroup.

Using ROC analysis, we found inflammatory markers like CRP, ferritin, LDH and Sonoclot® parameters as predictor for mortality. Increased ACT, d1gbACT and decreased peak amplitude, clot rate significantly predicted mortality with sensitivity and specificity better than the increased inflammatory markers.

A few limitations of our study merit mention. First, our centre is a tertiary care university hospital with many patients being referred here for intensive care. Many patients had received treatment elsewhere and the baseline native test may not be representative of the early coagulation abnormalities in patients who were shifted on mechanical ventilation. Secondly, the effect of blood component therapy between the two time points affecting the results of some coagulation tests cannot be ruled out. Thirdly, we did not estimate thrombin generation in this protocol, which should be evaluated in future research. Lastly, as our study was restricted to ICU patients, we did not have a control cohort of asymptomatic COVID-19 patients.

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. 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.(12,22)

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.

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 hypercoagulable profile in patients with COVID-19 predicts those who will benefit from early therapeutic anticoagulation. Our data argues for the need for careful evaluation of HLE and coagulation abnormalities in COVID-19 and to develop therapeutic strategies based on POC global coagulation tests rather than using the clinically less relevant SCTs like D-dimer or fibrinogen levels. Further work is also needed in the field of thrombin generation potential and anticoagulation protocol validation.

References:

1. WHO Coronavirus Disease (COVID-19) Dashboard | WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. [cited 2021 Feb 15]. Available from: https://covid19.who.int/?gclid=Cj0KCQiA1KiBBhCcARIsAPWqoSqA7NL1iSnMGGpdZETsEXWs2VRwMQdmjO0znx27cVKSImIrXvPEUc0aArSKEALw_wcB

2. Thachil J, Tang N, Gando S, Falanga A, Levi M, Clark C, et al. Laboratory haemostasis monitoring in COVID-19 [Internet]. Vol. 18, Journal of Thrombosis and Haemostasis. Blackwell Publishing Ltd; 2020 [cited 2021 Feb 15]. p. 2058–60. Available from: https://pubmed.ncbi.nlm.nih.gov/32324960/

3. Iba T, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of Coronavirus Disease 2019 [Internet]. Vol. 48, Critical Care Medicine. Lippincott Williams and Wilkins; 2020 [cited 2021 Feb 15]. p. 1358–64. Available from: https://pubmed.ncbi.nlm.nih.gov/32467443/

4. Hill JB, Garcia D, Crowther M, Savage B, Peress S, Chang K, et al. Frequency of venous thromboembolism in 6513 patients with COVID-19: A retrospective study. Blood Adv [Internet]. 2020 Nov 10 [cited 2021 Feb 15];4(21):5373–7. Available from: http://ashpublications.org/bloodadvances/article-

pdf/4/21/5373/1780288/advancesadv2020003083.pdf

5. Llitjos JF, Leclerc M, Chochois C, Monsallier JM, Ramakers M, Auvray M, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost [Internet]. 2020 Jul 1 [cited 2021 Feb 15];18(7):1743–6. Available from: https://pubmed.ncbi.nlm.nih.gov/32320517/

6. Moll M, Zon RL, Sylvester KW, Chen EC, Cheng V, Connell NT, et al. VTE in ICU Patients With COVID-19. Chest [Internet]. 2020 Nov 1 [cited 2021 Feb 15];158(5):2130–5. Available from: https://pubmed.ncbi.nlm.nih.gov/32710891/

7. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost [Internet]. 2020 Apr 1 [cited 2021 Feb 15];18(4):844–7. Available from: https://pubmed.ncbi.nlm.nih.gov/32073213/

8. Asakura H, Ogawa H. COVID-19-associated coagulopathy and disseminated intravascular coagulation. Int J Hematol [Internet]. 2021 Jan 1 [cited 2021 Feb 15];113(1):45–57. Available from: https://doi.org/10.1007/s12185-020-03029-y

9. Amgalan A, Othman M. Exploring possible mechanisms for COVID-19 induced thrombocytopenia: Unanswered questions [Internet]. Vol. 18, Journal of Thrombosis and Haemostasis. Blackwell Publishing Ltd; 2020 [cited 2021 Feb 15]. p. 1514–6. Available from: https://pubmed.ncbi.nlm.nih.gov/32278338/

10. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, et al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. J Thromb Haemost [Internet]. 2020 Jul 1 [cited 2021 Feb 15];18(7):1738–42. Available from:

https://pubmed.ncbi.nlm.nih.gov/32302438/

11. Song JC, Wang G, Zhang W, Zhang Y, Li WQ, Zhou Z. Chinese expert consensus on diagnosis and treatment of coagulation dysfunction in COVID-19. Mil Med Res [Internet]. 2020 Dec 20 [cited 2021 Feb 15];7(1):19. Available from: https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-020-00247-7

12. Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, et al. COVID-19-Related Severe Hypercoagulability in Patients Admitted to Intensive Care Unit for Acute Respiratory Failure. Thromb Haemost [Internet]. 2020 Jun 1 [cited 2021 Feb 15];120(6):998–1000. Available from: https://pubmed.ncbi.nlm.nih.gov/32316063/

13. Premkumar M, Bihari C, Saxena P, Devurgowda DR, Vyas T, Mirza R, et al. Heparin-like Effect Associated With Risk of Bleeding, Sepsis, and Death in Patients With Severe Alcohol-Associated Hepatitis. Clin Gastroenterol Hepatol [Internet]. 2020 Feb 1 [cited 2021 Feb 15];18(2):486-495.e3. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1542356519304896

14. Ferguson ND, Fan E, Camporota L, Antonelli M, Anzueto A, Beale R, et al. The Berlin definition of ARDS: An expanded rationale, justification, and supplementary material. Intensive Care Med [Internet]. 2012 [cited 2021 Feb 15];38(10):1573–82. Available from: https://pubmed.ncbi.nlm.nih.gov/22926653/

15. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation. Br J Haematol [Internet]. 2009 Apr [cited 2021 Feb 15];145(1):24–33. Available from: https://pubmed.ncbi.nlm.nih.gov/19222477/

16. Singer M, Deutschman CS, Seymour C, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3) [Internet].

Vol. 315, JAMA - Journal of the American Medical Association. American Medical Association; 2016 [cited 2021 Feb 15]. p. 801–10. Available from: https://jamanetwork.com/

17. Intagliata NM, Argo CK, Stine JG, Lisman T, Caldwell SH, Violi F. Concepts and Controversies in Haemostasis and Thrombosis Associated with Liver Disease: Proceedings of the 7th International Coagulation in Liver Disease Conference. In: Thrombosis and Haemostasis [Internet]. Georg Thieme Verlag; 2018 [cited 2021 Feb 15]. p. 1491–506. Available from: https://pubmed.ncbi.nlm.nih.gov/30060258/

18. Soni SL, Kajal K, Yaddanapudi LN, Malhotra P, Puri GD, Bhalla A, et al. Demographic & clinical profile of patients with COVID-19 at a tertiary care hospital in north India. Indian J Med Res [Internet]. 2020 Nov 19 [cited 2021 Feb 15]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/33208558

19. Premkumar M, Saxena P, Rangegowda D, Baweja S, Mirza R, Jain P, et al. Coagulation failure is associated with bleeding events and clinical outcome during systemic inflammatory response and sepsis in acute-on-chronic liver failure: An observational cohort study. Liver Int [Internet]. 2019 Apr 1 [cited 2021 Feb 16];39(4):694–704. Available from: https://pubmed.ncbi.nlm.nih.gov/30589495/

20. Premkumar M, Mehtani R, Divyaveer S, Kajal K, Kulkarni A V., Ahmed S, et al. Clinical Validation of Global Coagulation Tests to Guide Blood Component Transfusions in Cirrhosis and ACLF. http://www.xiahepublishing.com/ [Internet]. 2021 Feb 18 [cited 2021 Feb 22];0(000):0–0. Available from: http://www.xiahepublishing.com/2310-8819/JCTH-2020-00121

21. Magnani HN. Rationale for the Role of Heparin and Related GAG Antithrombotics in

COVID-19 Infection. Vol. 27, Clinical and Applied Thrombosis/Hemostasis. SAGE Publications Inc.; 2021.

22. Salem N, Atallah B, El Nekidy WS, Sadik ZG, Park WM, Mallat J. Thromboelastography findings in critically ill COVID-19 patients [Internet]. Journal of Thrombosis and Thrombolysis. Springer; 2020 [cited 2021 Feb 18]. Available from: https://pubmed.ncbi.nlm.nih.gov/33011896/

Foot note:

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

Funding: The authors do not declare a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest: None

List of Figures

1. Figure 1: Study design, patient selection and evaluation.

2. Figure 2: Kaplan Meir survival curves for patients (2A- Oxygen groups; 2B -Presence of heparin like effect)
3. Figure 3: Presence of HLE as defined as difference in Sonoclot® trace at days 0, 3 in patients. (Panel A-F)
4. Figure 4: ROC curves for predictors of mortality
5. Supplementary Figure 1: Patient risk assessment protocol and treatment allocation at our centre.
6. Supplementary Figure 2: Anticoagulation protocol at our centre based on conventional and POC global coagulation tests.

List of Tables

1. Table 1: Baseline characteristics, co-morbidities, and symptoms profile of the admitted patients with COVID-19.
2. Table 2: Conventional coagulation parameters and Sonoclot® results of the COVID-19 study participants (n=74)
3. Table 3: Dynamic Sonoclot® glass bead (gb) activated and heparinase (h) treated parameters on day 1 and day 3.
4. Supplementary Table 1: ROC analysis of predictors of mortality

Table 1: Baseline characteristics, co-morbidities, symptom profile 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	0.69
BMI (kgm-2)*	24.7(22.15-26.59)	23.8(22.99-26.14)	25.4(21.73-26.08)	24.4(22.7-27.7)	0.845	0.788
Sex# Male	47(64)	8(72.7)	23(67.6)	16(55.2)	0.752	0.213
SOFA score	4(3-5)	3(2.5-4)	3(2.8-5)	4(3-5)	0.397	0.116
Mortality	16(21.6)	0	0	16(55.2)	0.000	0.000
Bleeding and Thrombosis						
GI bleeding	9(12)	0(0)	5(14.7)	4(13.8)	0.177	0.260
Pulmonary micro-thrombi	6(8)	0	2(5.9)	4(13.8)	0.567	0.260
Thrombosis	5(7)	0(0)	1(2.9)	4(13.8)	0.756	0.260
Blood transfusion	20(27)	2(18.2)	5(14.7)	13(44.8)	0.782	0.120
Comorbidities						
Hypothyroidism	13(17.6)	0	7(20.6)	6(20.7)	0.101	0.102
COPD	1(1.4)	0	0	1(3.4)	-	0.725
Asthma	1(1.4)	0	0	1(3.4)	-	0.725
Obesity	27(36.48)	2(18.2)	5(14.7)	10(34.5)	0.555	0.315
Treatment						
Tocilizumab	32(43)	5(15.6)	17(53.1)	10(31.2)	0.534	0.522
Remdesivir	23(31)	3(27.3)	7(20.6)	13(44.8)	0.465	0.312
HCQ	16(22)	3(27.3)	11(33.3)	2(6.9)	0.709	0.117
Antibiotics	72(97)	10(90.9)	33(97.1)	29(100)	0.390	0.100
Antifungals	27(36)	0	12(35.3)	15(51.7)	0.021	0.003

Immuvac (mycobacterium indicus pranii)	31(42)	6(54.5)	19(55.9)	6(20.6)	0.938	0.866
Plasma therapy	2(3)	0	1(3)	1(3)	-	
Steroid duration(days)	6(0-7)	4(3-5)	1(0-5.75)	5(0-7)	0.059	0.873
Investigations*						
Hemoglobin (g/dl)	10.8(9.67-12.32	12(10.35-12.98)	10.85(10.02-11.97)	10.30(8.8-12.1)	0.130	0.060
NLR	16.38(8.17-30.84)	4.44(4.16-23.4)	17.09(10.75-34.23)	17.21(8.7-26.14)	0.099	0.084
Platelet count (x10^3 /L)	179(100.5-255.2)	198(111-239)	170(121-252)	174(61-256)	0.948	0.765
Urea (mg/dl)	38.5(26.75-58.25)	36(21.9-45)	35(25.7-54.72)	43(35-111)	0.667	0.044
Creatinine (mg/dl)	0.815(0.6-1.208)	0.740(0.5-1.05)	0.795(0.63-1.19)	0.880(0.6-1.79)	0.611	0.294
AST (U/L)	52.5(35-86.58)	79(40.5-115.5)	53.5(35.05-87.72)	46(31.6-75)	0.255	0.116
ALT (U/L)	40.95(32-65)	44(34.5-65)	38(32-61.62)	42(31-61.8)	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(247-589.5)	252(167-383)	474(295-556)	0.062	1.000
Total bilirubin (mg/d)	0.510(0.368-0.800)	0.670(0.41-0.83)	0.495(0.37-0.66)	0.600(0.37-0.8)	0.396	0.591
Total protein(g/dl)	6.135(5.515-6.715)	6.620(5.78-7.05)	6.175(5.57-6.78)	5.830(5.3-6.5)	0.302	0.038
Albumin (g/dl)	3.060(2.71-3.5)	3.3(2.9-3.85)	3.1(2.82-3.55)	3.01(2.65-3.16)	0.277	0.055
Lactate (mmol/l)	1.8(1.5-2.6)	1.7(1.3-1.9)	1.8(1.30-2.5)	2.0(1.8-2.6)	0.745	0.0381
CRP (mg/l)	86.50(33.25-165.5)	23(3.025-62.205)	49(23-96.9)	110(97.2-199)	0.247	0.001
Procalcitonin (ng/ml)	0.87(0.284-11.35)	0.869(0.284-2.85)	0.905(0.28-14.8)	1.108(0.45-4.55)	0.593	0.591
Ferritin (ng/ml)	559.5(127.5-944.75)	356(94.5-835)	235(100-649.25)	933(588-1424)	0.465	0.015

*Values are expressed as median (IQR) and compared using Mann-Whitney U test p value < 0.005 is considered as significant,
#Values are expressed as n (%) and compared using Chi-square test, p value < 0.005 is considered as significant.

Abbreviations: ARDS- acute respiratory distress syndrome; BMI-Body Mass Index; HTN- Hypertension; DM- Diabetes Mellitus; CAD- Coronary Artery Disease, CKD- Chronic Kidney Disease; CRP- C Reactive protein; COPD- Chronic Obstructive Pulmonary Disease, SOFA- Sequential Organ Failure Assessment; GI- Gastro Intestinal; HCQ-,Hydroxychloroquine; NLR- Neutrophil Lymphocyte Ratio; AST- aspartate transaminase; ALT- alanine transaminase; ALP- Alkaline Phosphatase; LDH- Lactate Dehydrogenas

Table 2: Conventional coagulation parameters and Sonoclot results of the COVID-19 study participants (n=74)

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)	1.16(1.1-1.2)	0.706	0.148
APTT (s)	32(29.2-35.1)	32.1(30.3-35.5)	31.7(28.8-34.3)	33.5(30.3-4)	0.593	0.788
D-Dimer (ng/ml)	1045(361.9-1982)	934(487.5-1134.5)	788.9(281.8-1608.6)	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

*Values are expressed as median (IQR) and compared using Mann-Whitney U test, p value < 0.005 is considered significant

ACT- Activated Clotting Time; aPTT- Activated Partial Thromboplastin Time; INR- International Normalised Ratio; CR- Clot Rate; PF- Platelet Function; PT, Prothrombin Time.

Table 3: Dynamic Sonoclot glass bead (gb) activated and heparinase (h) treated 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±63.277	102.50±18	135.67±28.71	197.71±66.10	0.004	0.060
gbCR	38.36±17.197	31.50±9.19	39.25±17.83	38.50±17.84	0.847	0.595
gbPF	2.09±1.673	6.40±4.52	2.08±1.31	1.68±0.836	0.000	0.000
gbPeak-Amp	77.73±21.032	107.5±24.74	98.0±17.8	65.24±8.72	0.000	0.000
gbTime to peak (s)	13.70±3.147	12.0±0.0	11.10±2.68	15.09±2.62	0.001	0.117
Day 1 hACT (s)	117±35.59	65±21	101±34	132±28	0.001	0.004
CR	36.5±12.389	34.0±11.31	38.8±10.64	35.4±13.68	0.729	0.888
PF	1.99±1.181	1.4±1.69	2.43±1.35	1.80±1.01	0.274	0.611
Peak-Amp	78.48±22.758	107.5±24.74	101.5±18.86	64.76±9.54	0.000	0.000
Day 3 gbACT (s)	162.58±56.877	92.50±17.67	125.00±32.61	187.14±53.39	0.001	0.023
gbCR	33.43±14.31	34.00±2.82	30.24±13.19	34.96±15.60	0.708	0.933
gbPF	1.577±1.023	2.40±1.69	1.91±1.36	1.30±0.634	0.161	0.060
gbPeak Amp	77.58±19.329	105±21.21	96.50±13.75	65.95±9.69	0.000	0.000
Time to peak (s)	14.7±4.7	9±1.41	10±2.86	17.47±3.02	0.000	0.001
Day 3 hACT (s)	109.58±39.35	65±0.00	82±38	129±28	0.000	0.005
hCR	36.66±14.9	34±9.89	35.67±15.99	37.44±15.43	0.931	0.764

Presence of HLE at Day 0 (n=33)	17(51.5%)	1(50%)	3(25%)	13(68.4%)	0.468	0.599
Presence of HLE at Day 3 (n=32)	20(62.5%)	1 (50%)	6(60%)	13(65%)	0.793	0.674
Procoagulant Sonoclot (Day 0) n=74 (ACT< 110)	22(29.7%)	5(45.5%)	11 (32.4%)	6 (20.7%)	0.430	0.117
Procoagulant Sonoclot (Day 3) (n=33)	7(21.2%)	2(100%)	4(40%)	1 (4.8%)	0.002	0.671

*Values are expressed as mean ± SD and compared using Student t-test, p value < 0.005 is considered is significant

ACT – Activated Clotting Time, CR – Clot Rate, PF – Platelet Function

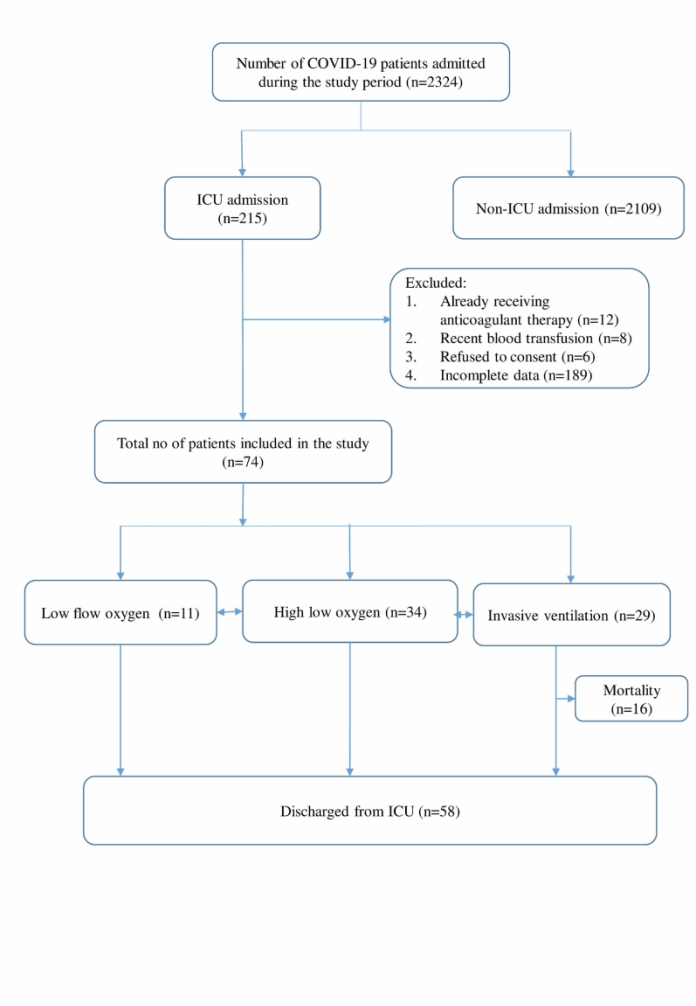


Figure 1: Study design, patient selection and evaluation.

105x105mm (600 x 600 DPI)

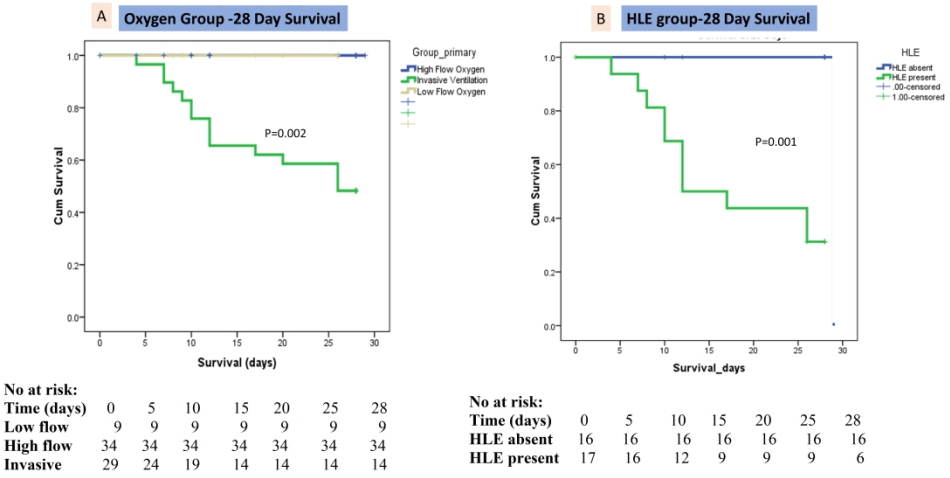


Figure 2: Kaplan Meir survival curves for patients (2A- Oxygen groups; 2B -Presence of heparin like effect)

499x295mm (300 x 300 DPI)

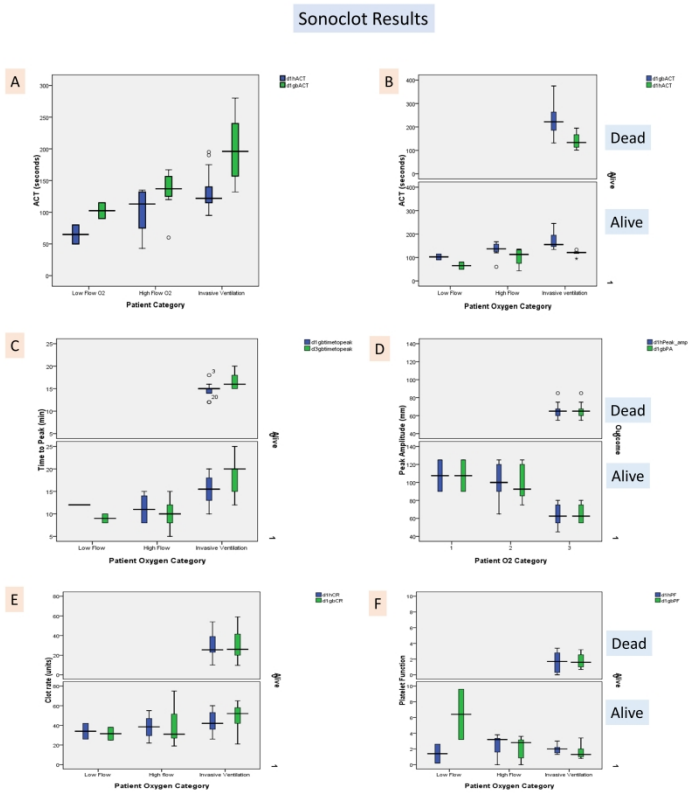


Figure 3: Presence of HLE as defined as difference in Sonoclot® trace at days 0, 3 in patients. (Panel A-F)

508x508mm (300 x 300 DPI)

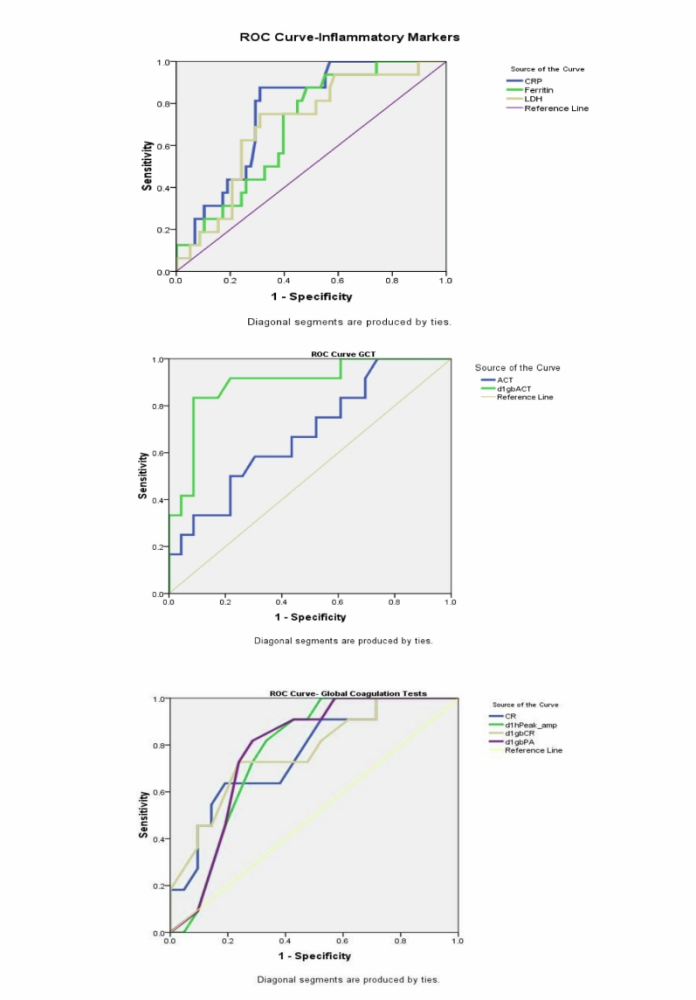


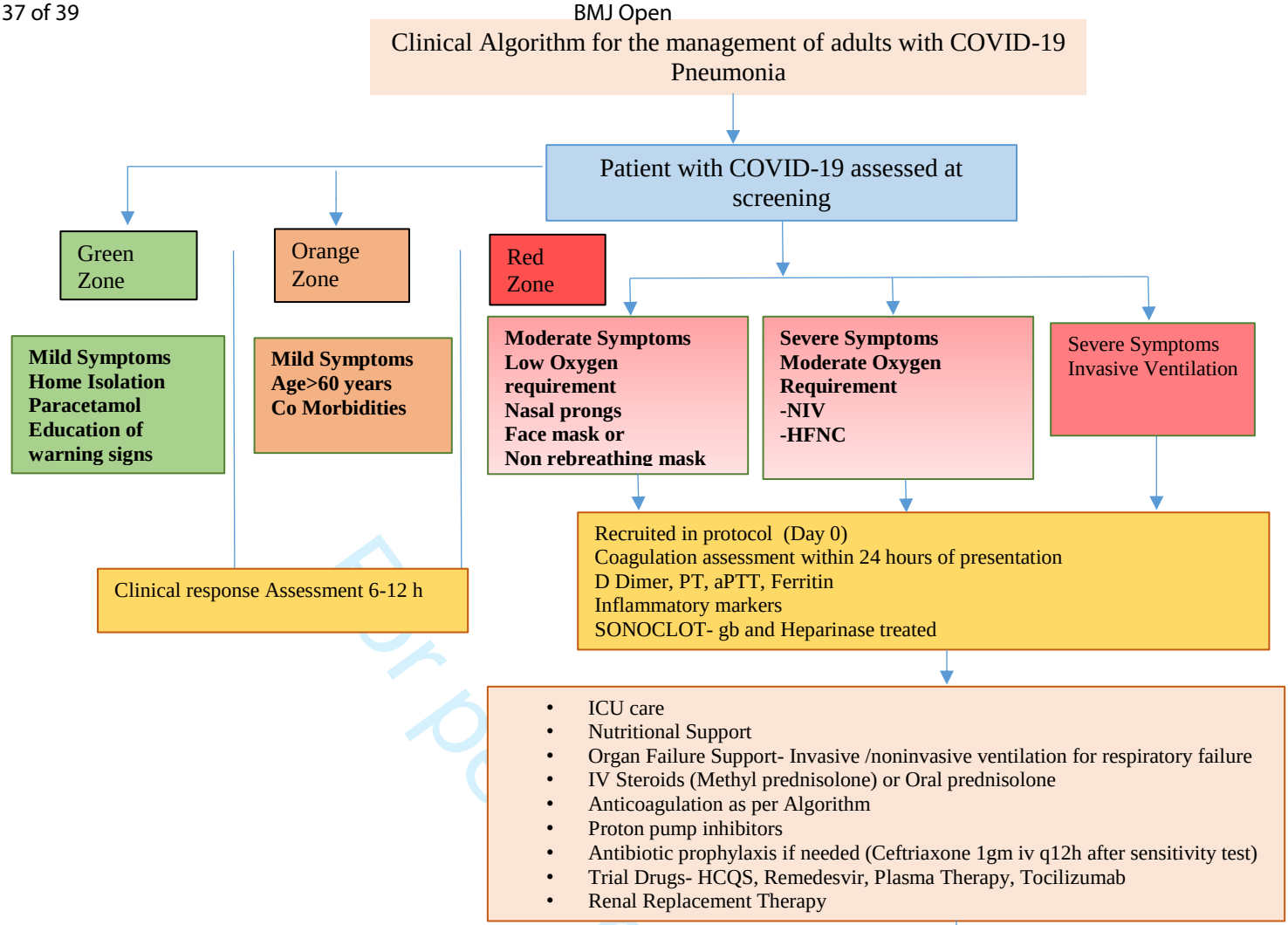
Figure 4: ROC curves for predictors of mortality

105x105mm (600 x 600 DPI)

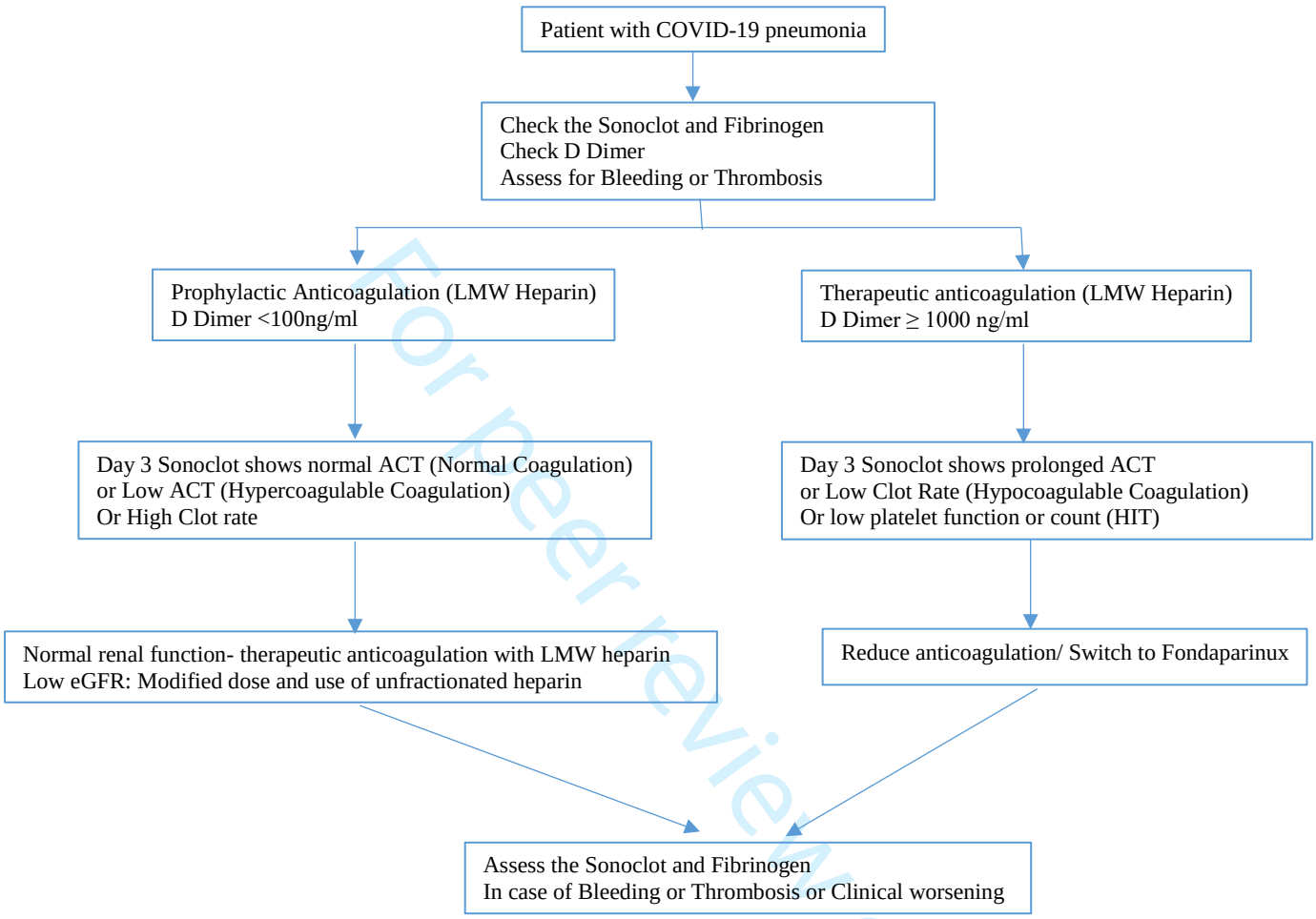
Supplementary Table 1: Predictors for mortality in COVID 19 patients based on ROC analysis

Parameter	Cut off value	AUC	Sensitivity	Specificity	P value
CRP	96·7 mg/l	0·767	87·5	69	0·001
Ferritin	587 ng/ml	0·690	75	61	0·021
LDH	405 U/L	0·700	75	70	0·015
CR	27·5 units/min	0·761	66·7	87	0·012
ACT	131 seconds	0·744	60	80	0021
D1hPeak-amp	72·5 units	0·736	81·8	65	0·029
D1gbACT	158 seconds	0·893	91·7	79	0·001
D1gbCR	27 units/min	0·730	63·7	79	0·027
gbPA	67·5 units	0·746	72·7	73	0·023

CRP, C Reactive Protein; ACT – Activated Clotting Time, CR – Clot Rate, PF – Platelet Function; PA, peak amplitude; LDH, lactate dehydrogenase·



Supplementary Figure 1: Patient risk assessment protocol and treatment allocation at our centre.



Supplementary Figure 2: Anticoagulation protocol at our centre based on conventional and POC global coagulation tests.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	03
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	03
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	06-07
Objectives	3	State specific objectives, including any prespecified hypotheses	07
Methods			
Study design	4	Present key elements of study design early in the paper	07-08
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	07-08
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	07
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	08-09
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	11-12
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	10
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	11
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11-12
		(b) Indicate number of participants with missing data for each variable of interest	11
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-12
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	21

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-051971.R1
Article Type:	Original research
Date Submitted by the Author:	25-Oct-2021
Complete List of Authors:	Premkumar, Madhumita; Post Graduate Institute of Medical Education and Research, Department of Hepatology Loganathan, Sekar; Post Graduate Institute of Medical Education and Research Kajal, Kamal; PGIMER, Department of Anaesthesia Hazarika, Amarjyoti; PGIMER, Department of Anaesthesia Soni, Shiv; PGIMER, Department of Anaesthesia Puri, Goverdhan ; Post Graduate Institute of Medical Education and Research Sehgal, Inderpaul Singh; PGIMER, Department of Pulmonary Medicine Suri, Vikas; Post Graduate Institute of Medical Education and Research Malhotra, Pankaj; PGIMER Singh, Virendra; PGIMER, Department of Hepatology Duseja, Ajay; PGIMER Bhalla, Ashish; Post Graduate Institute of Medical Education and Research, Internal Medicine Ahluwalia, Jasmina; PGIMER, Department of Hematology Kumar, Narender; PGIMER, Department of Hematology Kekan, Kushal; PGIMER, Department of Hepatology Ram, Sant; PGIMER, Department of Biochemistry Singla, Karan; PGIMER, Department of Anaesthesia Mahajan, Varun; PGIMER, Department of Anaesthesia Yaddanapudi, Narayana; Post Graduate Institute of Medical Education and Research
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Evidence based practice, Haematology (incl blood transfusion), Infectious diseases
Keywords:	COVID-19, Anticoagulation < HAEMATOLOGY, INTENSIVE & CRITICAL CARE, Thromboembolism < CARDIOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

COVID-19 Related Dynamic Coagulation Disturbances and Anticoagulation Strategies Using Conventional D-dimer and Point-of-Care Sonoclot® Tests: A Prospective Cohort Study

Authors:

Madhumita Premkumar DM[1], Sekar L MD[2], Kamal Kajal MD[2*], Amarjyoti Hazarika MD[2], Shiv Soni DM[2], GD Puri PhD[2], Inderpaul Singh Sehgal DM[3], Vikas Suri MD[4], Pankaj Malhotra MD[4], Virendra Singh DM[1], Ajay Duseja DM[1], Ashish Bhalla MD[4], Jasmina Ahluwalia MD[5], Narender Kumar MD[5], Kushal Kekan[1], Sant Ram MD[6], Karan Singla MD[2], Varun Mahajan MD [2], L Narayana Yaddanapudi MD[2].

Affiliations:

[1] Department of Hepatology,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[2] Department of Anaesthesia,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[3] Department of Pulmonary Medicine,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[4] Department of Internal Medicine,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[5] Department of Hematology,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[6] Department of Biochemistry,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

*Corresponding Author:

Dr.Kamal Kajal MD,

Department of Anaesthesia,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

Email: kamal.kajal@gmail.com

Phone No: +91 7087009545

Conflict of Interest and Source of Funding: All the authors have disclosed that they do not have any conflicts of interest.

Word count: 2883

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 (ICU) admission between August 2020 and November 2020.

Primary and secondary outcome measures: We compared the coagulation abnormalities using standard coagulation tests (SCTs) like prothrombin time, D-dimer, platelet count etc. and point-of-care (POC) 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 day1 and 3 with Sonoclot®. Activated clotting time (ACT) <110s 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 intensive care unit, 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 group (16, 55.2%) with overall mortality of 21.6%. HLE predicted the need for mechanical ventilation (HR 1.2 CI 1.04-1.4 p=0.00). On multivariate analysis, the presence of HLE (HR 1.01; CI 1.006-1.030; p=0.025), increased CRP (HR 1.040; CI 1.020-1.090; p=0.014)], decreased platelet function [HR 0.901; CI 0.702-1.100 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: Ethical clearance was obtained from the Institutional Review Board (PGI/IEC/2020/000997 dated 24 August 2020) and the study was registered at ClinicalTrials.gov.in (Trial registration no: NCT04668404. Available from https://clinicaltrials.gov/ct2/show/NCT04668404)

Data availability statement:

Data not available due to legal restrictions.

Strengths and limitations of this study:

1. 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.
2. This study provides evidence-based data to help guide anticoagulation therapy in patients with COVID-19.
3. Point-of-care Sonoclot® tests help in identifying patients at risk of worse prognosis and invasive ventilation.
4. 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 patients.
5. The study was not designed to test the use of prophylactic vs. therapeutic anticoagulation, but to assess the therapeutic window for using evidence-based anticoagulation safely.

INTRODUCTION

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has affected approximately 225,024,781 persons, and claimed 4,636,153 lives till September 14 2021.(1) Patients with COVID-19 have platelet abnormalities, endothelial dysfunction, clotting factors abnormalities and hyperfibrinolysis complicated by coexisting sepsis and renal failure.(2) This leads to thromboembolic events both in the venous and arterial circulation.(3) Nearly 20% of COVID-19 patients present with venous thromboembolism, 13% had symptomatic venous thromboembolism (VTE) despite prophylactic anticoagulation.(4)

The typical coagulation defect is seen as elevated D-dimer concentration, with 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 COVID-19 patients from China, the mean D-dimer concentration was significantly higher 2.12 mg/L (range 0·77–5·27) in non-survivors compared to survivors.(6)

Concomitant venous thromboembolism (VTE), a potential cause of unexplained deaths, has 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 vs therapeutic anticoagulation in COVID 19.(9)

Tang et al. observed higher D-dimer and fibrin degradation product (FDP) levels associated with the overall mortality.(10) A wide range of abnormalities in standard coagulation tests (SCTs) have 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 global coagulation in point-of care (POC) devices such as thromboelastometry and Sonoclot® has 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 (MA) / 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 (ROTEM) profiles with a shorter clot formation time (CFT) in the INTEM ($p = 0.0002$), EXTEM ($p = 0.010$) and increased maximum clot firmness (MCF) (p=0.001) that was associated with worse outcome.(15) Sonoclot® assessment of clot formation and clot lysis takes 30 to 60 minutes. Cytokine storm and sepsis trigger the release of endogenous heparinoids from the endothelium in various organs which is seen on global coagulation tests 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 (PGIMER), 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-Cov2 infection, as confirmed by reverse transcription polymerase chain reaction (RT-PCR), aged between 18 to 80 years, and of any gender with moderate to severe ARDS, admitted to intensive care unit (ICU) were recruited. During the initial period of

COVID-19 pandemic, patients with mild ARDS requiring low flow oxygen with Charlson's co-morbidity index > 6 and 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, human immunodeficiency virus (HIV) infection, pregnancy or active malignancy in the last 5 years were excluded. We documented their demographic data, past 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. NCT04668404 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® test in patients with severe COVID-19 pneumonia. The secondary outcomes were the incidence of thromboembolic episodes, bleeding events and the presence of "heparin-like-effect" (HLE) using Sonoclot®. The predictability of clinical and laboratory parameters were also analysed for mortality at 28 days.

Definitions:

Acute Respiratory Distress Syndrome (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 (ISTH) 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 hemoglobin level of 2.0 g/L or more, or leading to transfusion of two or more units of whole blood or 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), high flow devices (venturi mask, high flow nasal cannula (HFNC), non-invasive ventilation (NIV)) 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 (TCZ), Remdesivir, Mycobacterium indicus pranii (Immuvac), Hydroxychloroquine (HCQ), plasma therapy as part of ongoing trials.(21) Supportive care for critically ill patients in the form of advanced hemodynamic monitoring and support, enteral nutrition, glycemic control, and stress ulcer prophylaxis were given in all eligible patients. Antibiotic and antifungal therapy were guided by cultures and sepsis markers. Supplementary figure 1 shows the patient triage and dynamic treatment algorithm at our centre. Supplementary figure 2 shows the anticoagulation algorithm followed at our centre.

Assessment of Coagulation Parameters:

Complete automated blood cell count was performed on LH750/780 automated hematology analyzer (Beckman Coulter Inc., Fl, USA). SCTs measured include prothrombin time (PT), activated prothrombin time (aPTT), international normalized ratio (INR), D-dimer (Diagnostica Stago, France) and fibrinogen. (Sysmex CA 1500; Sysmex Corporation, Kobe, Japan). Sonoclot® analysis of whole blood sample (non-heparinised) (Sienco Inc., Arvada, CO, USA) was performed at the bedside. For performing glass-bead activated Sonoclot®340 µL of whole blood was added to the Sonoclot® cuvette, pre-warmed to 37°C. Glass bead activated clotting time (gbACT), clot rate (CR), and platelet function (PF) were noted from the results and time to peak (TP) and peak amplitude (PA) were calculated from the Sonolcot signatures. (22) Paired global coagulation traces i.e. (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 seconds and PA > 75 units were considered as the cut off for hypercoagulable state. The heparin like effect (HLE) due to endogenous heparinoids was calculated by the percentage correction of the gbACT with the heparinase treated hACT. (16)

$$\text{HLE (\%)} = \frac{\text{gb ACT} - \text{hACT (heparinase-treated)}}{\text{hACT}} \times 100$$

HLE was considered when the correction of the ACT 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 was represented as mean with standard deviation (SD) or median with interquartile range (IQR), as appropriate. Normality of quantitative data was checked using Kolmogorov Smirnov tests. The continuous data between groups were compared by using the

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 hazard ratios (HRs) and 95% confidence intervals (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 α=0.05. Statistical analysis was performed with SPSS Statistics version 22 (IBM® SPSS® Statistics Armonk New York USA).

Sample size:

Sample size was estimated using G*Power, a statistical program. Assuming the incidence of hypercoagulable state in COVID-19 to be 5-15%, (5) with an effect size of 0.5, alpha 0.05, and power 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 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 of 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 male. The patients were divided into 3 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. Supplementary table 1 shows the comorbid illness and treatment details of the cohort.

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 between 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 ventilation group. This can be seen as reduction in the prolonged ACT in the hSonoclot assay. (Figure 2, panel A). 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 patients requiring invasive ventilation. (HR 1.200 CI 1.040-1.400 p=0.001) and an uncorrected ACT was associated with increased risk of death (Figure 2, panel B).

Bleeding and Thrombotic Events in COVID-19

Eleven (14.8%) of 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, normalized D-dimer, NLR, 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 outcomes in any regression model. Predictors of mortality at day 1 were presence of HLE (HR 1.02; CI 1.01- 1.04; p=0.002), increased CRP (HR 1.04; CI 1.02-1.09 p=0.003), elevated ACT (HR1.02; CI 0.1.04-1.4 p=0.001) and decreased PF (HR 0.54; CI 0.29-0.90; p=0.010). In multivariate analysis, the presence of HLE (HR 1.02; CI 1.08-1.6; p=0.007), raised CRP (1.2; CI 1.1-1.4; p=0.014) and reduced PF [HR 0.9; CI 0.7-1.1 p=0.045) remained significant. (Supplementary Table 2) 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 1.01-1.5; 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 seconds (p=0.021), CR <27 units/min (p=0.027) and PA < 67.5 units predicted mortality. (Supplementary Table 3)

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 shows that information generated by POC tests provides an etiopathological rationale for appropriate use of anticoagulation in COVID 19 patients. Firstly, patients with hypercoagulable tests benefit from anticoagulation, whereas those with HLE or hypocoagulable profile on Sonoclot have higher likelihood of bleeding and require invasive ventilation. Secondly, the use of D-dimer as a marker of a procoagulant state is fallacious, as it is elevated in patients with thrombosis, hyperfibrinolysis and disseminated intravascular coagulation (DIC). Thirdly, our data shows 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 anticoagulation did not improve survival at 30 days, and the rate of bleeding events were 8% and 2%, respectively in the 2 comparators. The limitations of this study were relatively rate recruitment of patients (within a 2-week interval post COVID diagnosis) and lack of testing for fibrinolysis, and DIC at inclusion which introduced population heterogeneity. The differential use of intravenous unfractionated heparin vs. 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 and normalized 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 multicentric 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%. (25)

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 to 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 disseminated intravascular coagulation. Damaged endothelial cells with microangiopathy releases a mixture of glycosaminoglycans that function like endogenous heparinoids. (26) 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. (27) 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. 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 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 day 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 minimized 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 can be attributed to sepsis induced coagulopathy and anticoagulation regimen guided by SCTs. Considering the complex interplay between prothrombotic and anti-hemostatic pathways, their influence on outcome in terms of thrombosis or bleeding cannot be measured on SCTs. (27,28)

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 is 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. (29,30) Increased d1gbACT, decreased PA, decreased clot rate 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 global coagulation tests 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.

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. 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. (15,31)

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.

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.

Ethics statements:

Patient consent for publication:

Consent had been obtained before the enrolment to the study from the study participants / patient's relatives.

Ethics approval:

Institutional Ethics Committee, Postgraduate Institute of Medical Education and Research, Chandigarh, India ID: (PGI/IEC/2020/000997)

References:

1. WHO Coronavirus Disease (COVID-19) Dashboard | WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. [cited 2021 Feb 15]. Available from:

https://covid19.who.int/?gclid=Cj0KCQiA1KiBBhCcARIsAPWqoSqA7NL1iSnMGGpdZETsEXWs2VRwMQdmjO0znx27cVKSImIrXvPEUc0aArSKEALw_wcB

2. Thachil J, Tang N, Gando S, Falanga A, Levi M, Clark C, et al. Laboratory haemostasis monitoring in COVID-19 [Internet]. Vol. 18, Journal of Thrombosis and Haemostasis. Blackwell Publishing Ltd; 2020 [cited 2021 Feb 15]. p. 2058–60. Available from: https://pubmed.ncbi.nlm.nih.gov/32324960/

3. Iba T, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of Coronavirus Disease 2019 [Internet]. Vol. 48, Critical Care Medicine. Lippincott Williams and Wilkins; 2020 [cited 2021 Feb 15]. p. 1358–64. Available from: https://pubmed.ncbi.nlm.nih.gov/32467443/

4. S M, M C, TF van H, M F, AP V, MCA M, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost [Internet]. 2020 Aug 1 [cited 2021 Sep 11];18(8):1995–2002. Available from: https://pubmed.ncbi.nlm.nih.gov/32369666/

5. M L, J T, T I, JH L. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol [Internet]. 2020 Jun 1 [cited 2021 Sep 17];7(6):e438–40. Available from: https://pubmed.ncbi.nlm.nih.gov/32407672/

6. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med [Internet]. 2020 Apr 30 [cited 2020 Aug 26];382(18):1708–20. Available from: https://pubmed.ncbi.nlm.nih.gov/32109013/

7. Llitjos JF, Leclerc M, Chochois C, Monsallier JM, Ramakers M, Auvray M, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19

patients. J Thromb Haemost [Internet]. 2020 Jul 1 [cited 2021 Feb 15];18(7):1743–6. Available from: https://pubmed.ncbi.nlm.nih.gov/32320517/

8. Moll M, Zon RL, Sylvester KW, Chen EC, Cheng V, Connell NT, et al. VTE in ICU Patients With COVID-19. Chest [Internet]. 2020 Nov 1 [cited 2021 Feb 15];158(5):2130–5. Available from: https://pubmed.ncbi.nlm.nih.gov/32710891/

9. RD L, PGM de BES, RHM F, AVS M, B B, LP D, et al. Therapeutic versus prophylactic anticoagulation for patients admitted to hospital with COVID-19 and elevated D-dimer concentration (ACTION): an open-label, multicentre, randomised, controlled trial. Lancet (London, England) [Internet]. 2021 Jun 12 [cited 2021 Sep 17];397(10291):2253–63. Available from: https://pubmed.ncbi.nlm.nih.gov/34097856/

10. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost [Internet]. 2020 Apr 1 [cited 2021 Feb 15];18(4):844–7. Available from: https://pubmed.ncbi.nlm.nih.gov/32073213/

11. Asakura H, Ogawa H. COVID-19-associated coagulopathy and disseminated intravascular coagulation. Int J Hematol [Internet]. 2021 Jan 1 [cited 2021 Feb 15];113(1):45–57. Available from: https://doi.org/10.1007/s12185-020-03029-y

12. Amgalan A, Othman M. Exploring possible mechanisms for COVID-19 induced thrombocytopenia: Unanswered questions [Internet]. Vol. 18, Journal of Thrombosis and Haemostasis. Blackwell Publishing Ltd; 2020 [cited 2021 Feb 15]. p. 1514–6. Available from: https://pubmed.ncbi.nlm.nih.gov/32278338/

13. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, et al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of

thromboelastography findings and other parameters of hemostasis. J Thromb Haemost [Internet]. 2020 Jul 1 [cited 2021 Feb 15];18(7):1738–42. Available from: https://pubmed.ncbi.nlm.nih.gov/32302438/

14. Song JC, Wang G, Zhang W, Zhang Y, Li WQ, Zhou Z. Chinese expert consensus on diagnosis and treatment of coagulation dysfunction in COVID-19. Mil Med Res [Internet]. 2020 Dec 20 [cited 2021 Feb 15];7(1):19. Available from: https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-020-00247-7

15. Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, et al. COVID-19-Related Severe Hypercoagulability in Patients Admitted to Intensive Care Unit for Acute Respiratory Failure. Thromb Haemost [Internet]. 2020 Jun 1 [cited 2021 Feb 15];120(6):998–1000. Available from: https://pubmed.ncbi.nlm.nih.gov/32316063/

16. Premkumar M, Bihari C, Saxena P, Devurgowda DR, Vyas T, Mirza R, et al. Heparin-like Effect Associated With Risk of Bleeding, Sepsis, and Death in Patients With Severe Alcohol-Associated Hepatitis. Clin Gastroenterol Hepatol [Internet]. 2020 Feb 1 [cited 2021 Feb 15];18(2):486-495.e3. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1542356519304896

17. Ferguson ND, Fan E, Camporota L, Antonelli M, Anzueto A, Beale R, et al. The Berlin definition of ARDS: An expanded rationale, justification, and supplementary material. Intensive Care Med [Internet]. 2012 [cited 2021 Feb 15];38(10):1573–82. Available from: https://pubmed.ncbi.nlm.nih.gov/22926653/

18. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation. Br J Haematol [Internet]. 2009 Apr [cited 2021 Feb 15];145(1):24–33. Available from:

https://pubmed.ncbi.nlm.nih.gov/19222477/

19. Singer M, Deutschman CS, Seymour C, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3) [Internet]. Vol. 315, JAMA - Journal of the American Medical Association. American Medical Association; 2016 [cited 2021 Feb 15]. p. 801–10. Available from: https://jamanetwork.com/

20. S S, C K. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost [Internet]. 2005 Apr [cited 2021 Sep 17];3(4):692–4. Available from: https://pubmed.ncbi.nlm.nih.gov/15842354/

21. Soni SL, Kajal K, Yaddanapudi LN, Malhotra P, Puri GD, Bhalla A, et al. Demographic & clinical profile of patients with COVID-19 at a tertiary care hospital in north India. Indian J Med Res [Internet]. 2020 Nov 19 [cited 2021 Feb 15]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/33208558

22. Premkumar M, Saxena P, Rangegowda D, Baweja S, Mirza R, Jain P, et al. Coagulation failure is associated with bleeding events and clinical outcome during systemic inflammatory response and sepsis in acute-on-chronic liver failure: An observational cohort study. Liver Int [Internet]. 2019 Apr 1 [cited 2021 Feb 16];39(4):694–704. Available from: https://pubmed.ncbi.nlm.nih.gov/30589495/

23. S C, S C, X L, S L, F W. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J Thromb Haemost [Internet]. 2020 Jun 1 [cited 2021 Sep 20];18(6):1421–4. Available from: https://pubmed.ncbi.nlm.nih.gov/32271988/

24. Schiavone M, Gasperetti A, Mancone M, Curnis A, Mascioli G, Mitacchione G, et al. Oral anticoagulation and clinical outcomes in COVID-19: An Italian multicenter experience. Int J Cardiol [Internet]. 2021 Jan 15 [cited 2021 Sep 20];323:276–80. Available from: http://www.internationaljournalofcardiology.com/article/S0167527320337359/fulltext

25. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol [Internet]. 2020 Jun 1 [cited 2021 Sep 20];7(6):e438–40. Available from: http://www.thelancet.com/article/S2352302620301459/fulltext

26. Magnani HN. Rationale for the Role of Heparin and Related GAG Antithrombotics in COVID-19 Infection. Vol. 27, Clinical and Applied Thrombosis/Hemostasis. SAGE Publications Inc.; 2021.

27. M A, SE V, M K, A H, T W, F L, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med [Internet]. 2020 Jul 9 [cited 2021 Sep 20];383(2):120–8. Available from: https://pubmed.ncbi.nlm.nih.gov/32437596/

28. Premkumar M, Mehtani R, Divyaveer S, Kajal K, Kulkarni A V., Ahmed S, et al. Clinical Validation of Global Coagulation Tests to Guide Blood Component Transfusions in Cirrhosis and ACLF. http://www.xiahepublishing.com/ [Internet]. 2021 Feb 18 [cited 2021 Feb 22];0(000):0–0. Available from: http://www.xiahepublishing.com/2310-8819/JCTH-2020-00121

29. M S, A G, M M, AV K, C G, G M, et al. Redefining the Prognostic Value of High-Sensitivity Troponin in COVID-19 Patients: The Importance of Concomitant Coronary

Artery Disease. J Clin Med [Internet]. 2020 Oct 12 [cited 2021 Sep 20];9(10):3263. Available from: https://pubmed.ncbi.nlm.nih.gov/33053826/

30. E P, KB L, R D, G S, J P, R B, et al. The relationship between coronary artery disease and clinical outcomes in COVID-19: a single-center retrospective analysis. Coron Artery Dis [Internet]. 2021 [cited 2021 Sep 20];32(5):367–71. Available from: https://pubmed.ncbi.nlm.nih.gov/32732512/

31. Salem N, Atallah B, El Nekidy WS, Sadik ZG, Park WM, Mallat J. Thromboelastography findings in critically ill COVID-19 patients [Internet]. Journal of Thrombosis and Thrombolysis. Springer; 2020 [cited 2021 Feb 18]. Available from: https://pubmed.ncbi.nlm.nih.gov/33011896/

Foot note:

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

Funding: The authors do not declare a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest: None

List of Figures

List of Tables

.

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*						
Hemoglobin (g/dl)	10.8(9.7-12)	12(10. 4-13)	10.8(10-12)	10.3(8.8-12.1)	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 (x10^3 /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.

Abbreviations: ARDS- acute respiratory distress syndrome; BMI-Body Mass Index; HTN- Hypertension; DM- Diabetes Mellitus; CAD- Coronary Artery Disease, CKD- Chronic Kidney Disease; CRP- C Reactive protein; COPD- Chronic Obstructive Pulmonary Disease, SOFA- Sequential Organ Failure Assessment; GI- Gastro Intestinal; HCQ-, Hydroxychloroquine; NLR- Neutrophil Lymphocyte Ratio; AST- aspartate transaminase; ALT- alanine transaminase; ALP- Alkaline Phosphatase; LDH- Lactate Dehydrogenase

Table 2: Conventional coagulation parameters and Sonoclot results of the COVID-19 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 v.s 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	10 (59%)	0	0	10 (77%)	0.024	0.024

1, % within HLE)						

*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; INR- International Normalised Ratio; CR- Clot Rate; PF- Platelet Function; PT, Prothrombin Time.

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

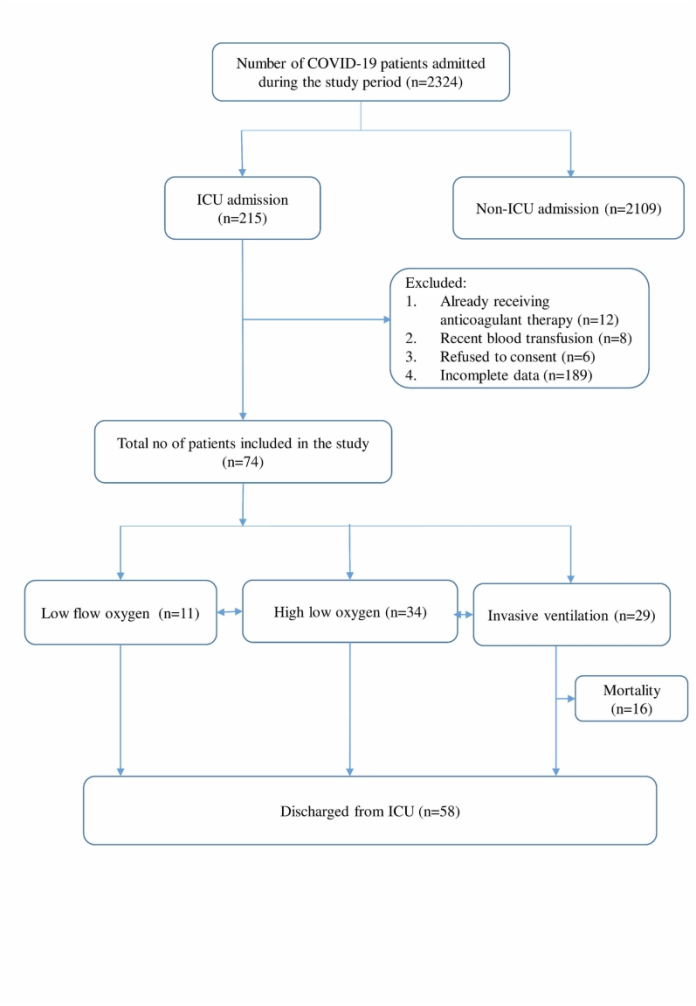


Figure 1: Study design, patient selection and evaluation.

105x105mm (600 x 600 DPI)

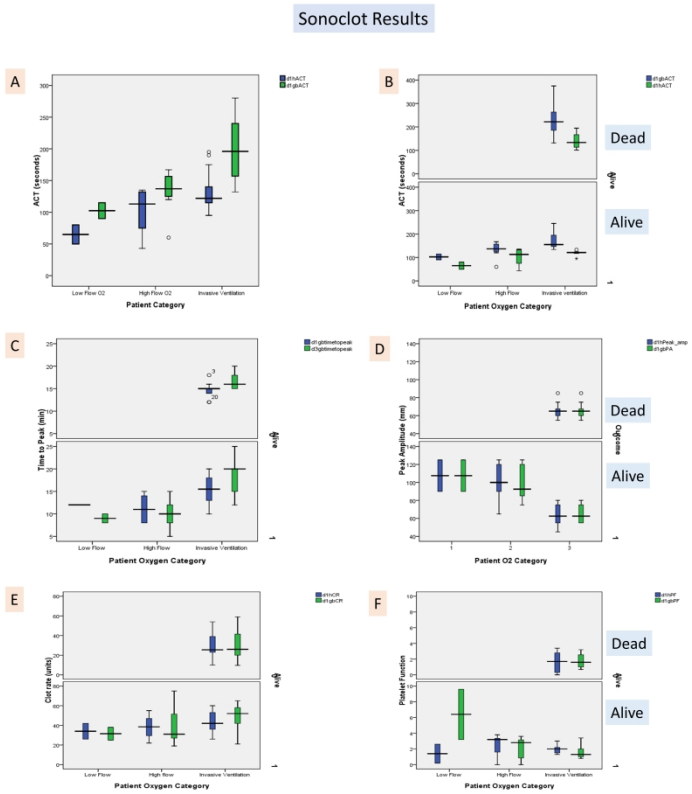


Figure 2: Presence of HLE as defined as difference in Sonoclot® trace at days 0, 3 in patients. (Panel A-F)

508x508mm (300 x 300 DPI)

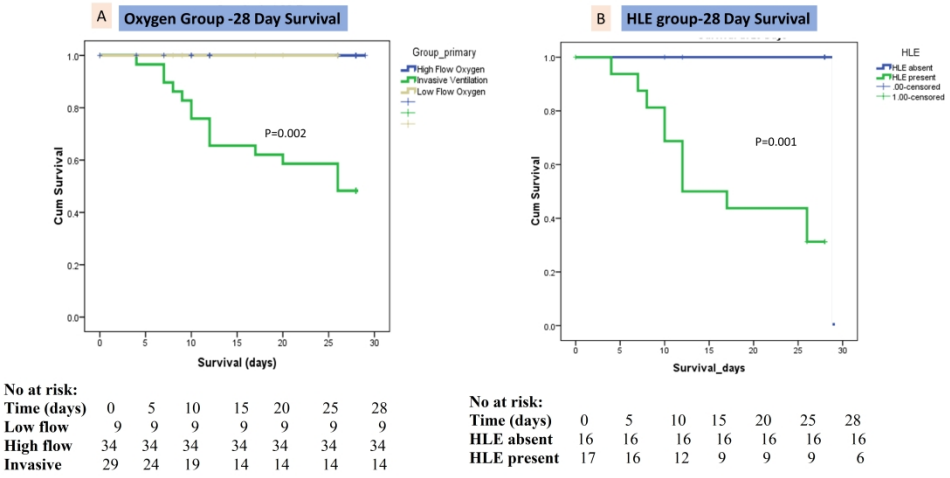


Figure 3: Kaplan Meir survival curves for patients (2A- Oxygen groups; 2B -Presence of heparin like effect)

499x295mm (300 x 300 DPI)

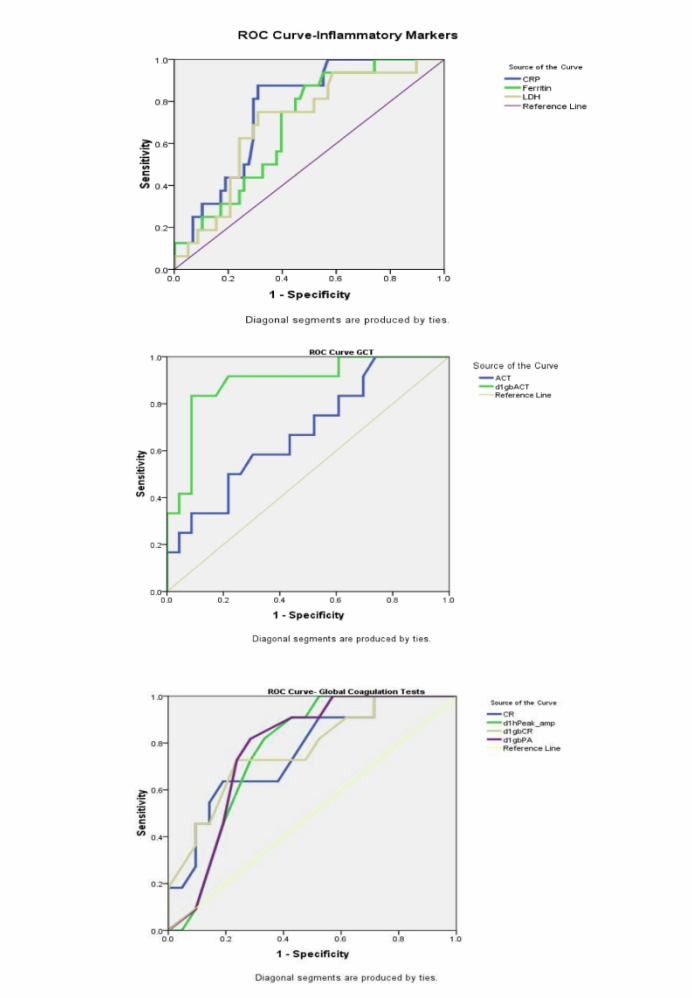


Figure 4: ROC curves for predictors of mortality

105x105mm (600 x 600 DPI)

Supplementary Information

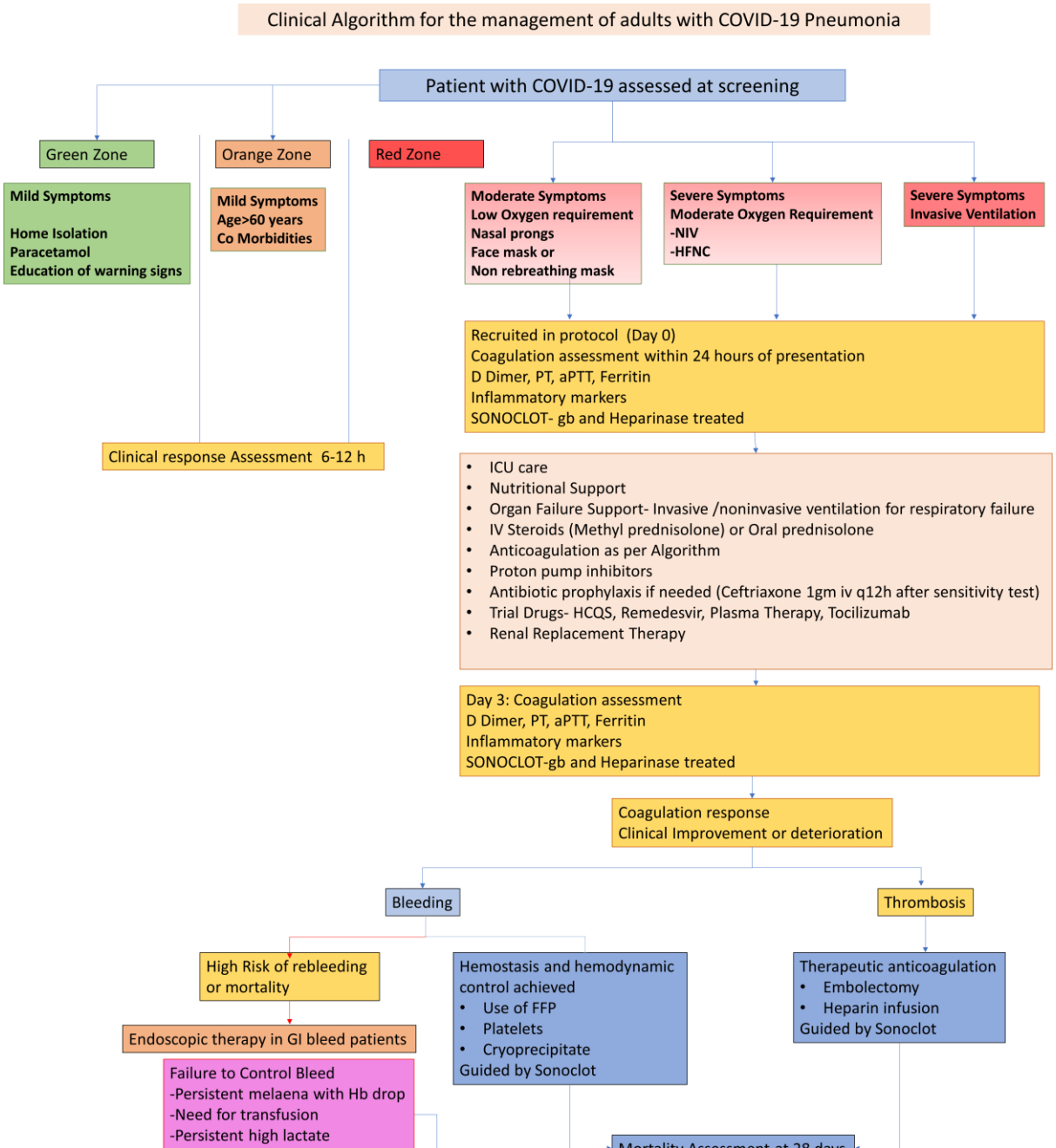
COVID-19 Related Dynamic Coagulation Disturbances and Anticoagulation Strategies Using Conventional D-dimer and Point-of-Care Sonoclot® Tests: A Prospective Cohort Study

Dynamic patient risk assessment protocol and treatment allocation at our centre.

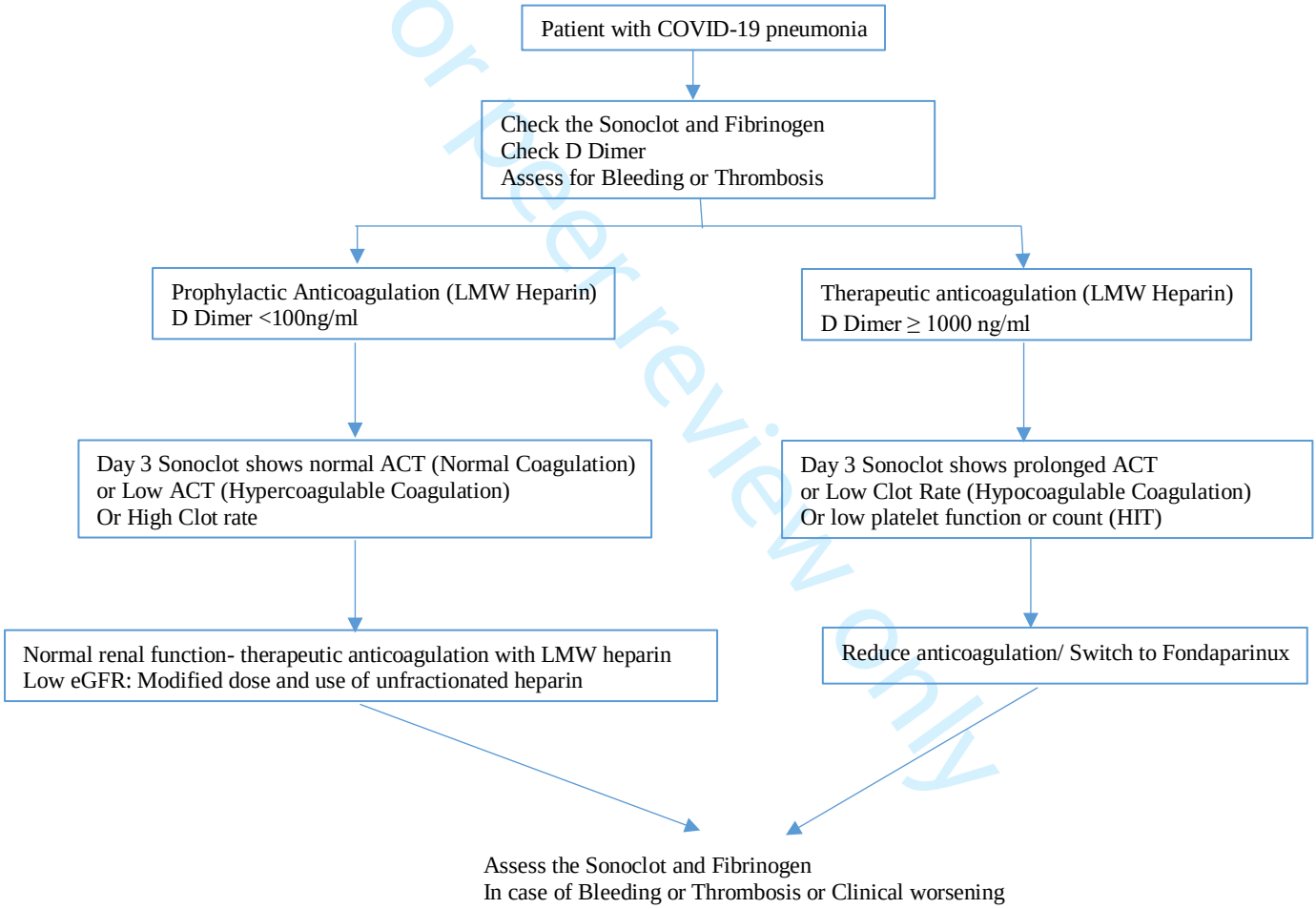
Patients were brought in directly to the PGIMER emergency or were taken in as referred cases for management of advanced COVID-19 pneumonia. As such the hospital was divided into three triage zones. The Green Zone had patients with mild symptoms who were managed symptomatically at home. Patients in the Orange Zone had mild symptoms or were classed as those having comorbidities or were older individuals (>60 years). Such patients were admitted in a holding ward called the Severe Acute Respiratory Illness ward for an observation period. After assessment for 24-48 hours, they were either moved to the Red Zone or were discharged if clinically improved. Patients who were critically unwell, or in need or invasive or non-invasive ventilation or had high oxygen requirements were admitted to the Red zone directly or moved in from the Green or Orange zones in case of clinical worsening. The Red zone directly received critically ill patients from other centres as well for advanced management.

In the initial phase of the pandemic all patients with COVID-19 RT PCR positivity were admitted as inpatients regardless of the symptom status. This was in accordance with the Government of India directive to ensure patient isolation to minimize spread. Thus, the protocol reflects a dynamic policy which was modified in a dynamic way as per availability of new data, clinical treatments, and public health policy.

Supplementary Figure 1: Hospital Triage Areas for COVID-19 and clinical algorithm for management of COVID-19 pneumonia.



Supplementary Figure 2: Anticoagulation protocol at our centre based on conventional and POC global coagulation tests.



Supplementary Table 1: Baseline characteristics, co-morbidities, 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**
Comorbidities						
Hypothyroidism	13(17.6)	0	7(20.6)	6(20.7)	0.101	0.102
COPD	1(1.4)	0	0	1(3.4)	-	0.725
Asthma	1(1.4)	0	0	1(3.4)	-	0.725
Obesity	27(36.48)	2(18.2)	5(14.7)	10(34.5)	0.555	0.315
Treatment given in each group						
Tocilizumab	32(43)	5(15.6)	17(53.1)	10(31.2)	0.534	0.522
Remdesivir	23(31)	3(27.3)	7(20.6)	13(44.8)	0.465	0.312
HCQS	16(22)	3(27.3)	11(33.3)	2(6.9)	0.709	0.117
Antibiotics	72(97)	10(90.9)	33(97.1)	29(100)	0.390	0.100
Antifungals	27(36)	0	12(35.3)	15(51.7)	0.021	0.003

Immuvac (mycobacterium indicus pranii)	31(42)	6(54.5)	19(55.9)	6(20.6)	0.938	0.866
Plasma therapy	2(3)	0	1(3)	1(3)	-	
Steroid duration(days)	6(0-7)	4(3-5)	1(0-5.75)	5(0-7)	0.059	0.873

Abbreviations: COPD, chronic obstructive pulmonary disease; HCQS, hydroxychloroquine

Supplementary Table 2: Predictors of Mortality using Cox Proportional Hazards Analysis

Univariate Analysis			**Multivariate Analysis**	
Covariate	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
CRP	1.007 (1.005- 1.016)	0.003	1.040 (1.020-1.090)	0.014
PF	0.540 (0.291-0.901)	0.429	0.901 (0.702-1.100)	0.045
HLE	1.020 (1.007-1.034)	0.002	1.010 (1.006-1.030)	0.025
PF Ratio	0.989 (0.979- 0.999)	0.038		
Ferritin	1.001 (1.000- 1.010)	0.037		
NLR	1.027 (1.007- 1.047)	0.009		
D-Dimer	1.000 (1.000- 1.000)	0.027		
LDH	1.003 (1.000- 1.000)	0.021		
Bilirubin	1.147 (1.010- 1.303)	0.035		
ACT	1.012(1.004- 1.020)	0.005		
d1hACT	1.033 (1.013- 1.054)	0.001		
d1hCR	0.956 (0.908- 1.006)	0.081		
d1h Peak amplitude	0.960 (0.920- 1.002)	0.062		
D1gbACT	1.022 (1.011- 1.033)	0.000		
D1gb Peak-Amplitude	0.948 (0.898-1.000)	0.051		

Abbreviations: ACT, activated clotting clotting time; CRP, C Reactive Protein; CR, clot rate; LDH, lactate dehydrogenase; PF, platelet function; HLE, heparin like effect; NLR, neutrophil lymphocyte ratio; hACT,

heparinase treated; hCR, heparinase treated CR; hPF, heparinase treated platelet function; PF Ratio, PaO_2/ FiO_2 ratio;

Supplementary Table 3: Predictors for mortality in COVID 19 patients based on Receiver Operating Characteristics (ROC) analysis

Parameter	Cut off value	AUC	Sensitivity	Specificity	P value
CRP	96·7 mg/l	0·767	87·5	69	0·001
Ferritin	587 ng/ml	0·690	75	61	0·021
LDH	405 U/L	0·700	75	70	0·015
CR	27·5 units/min	0·761	66·7	87	0·012
ACT	131 seconds	0·744	60	80	0021
D1hPeak-amp	72·5 units	0·736	81·8	65	0·029
D1gbACT	158 seconds	0·893	91·7	79	0·001
D1gbCR	27 units/min	0·730	63·7	79	0·027
gbPA	67·5 units	0·746	72·7	73	0·023

Abbreviations: CRP, C Reactive Protein; ACT – Activated Clotting Time, CR – Clot Rate, PF – Platelet Function; PA, peak amplitude; LDH, lactate dehydrogenase·

Response to Editor and Reviewer's comments:

Editor(s)' Comments to Author:

Please note that declarative titles are not part of the journal format. As such, please revise the title of your manuscript to include the research question, study design and setting. This is the preferred format of the journal. See published articles for examples.

Reply: We have changed the title of the manuscript as suggested.
The new title is 'COVID-19 Related Dynamic Coagulation Disturbances and Anticoagulation Strategies Using Conventional D-dimer and Point-of-Care Sonoclot® Tests: A Prospective Cohort Study'. This is in accordance with the journal style.

Reply to the review

We thank the Editors and the Reviewers for the critical appraisal of the manuscript. We have complied with all the instructions and made the appropriate changes.

Reviewers' comments:

Reviewer: 1
Dr. Giovanni Battista Forleo, Luigi Sacco University Hospital
Comments to the Author:
BMJ REVIEW COVID HLE

In the article entitled "Hypocoagulable Coagulation Profile and Endogenous Heparinoids are Associated with Invasive Ventilation and Mortality in COVID-19", Premkumar et al. provided an interesting analysis on the dynamic association of the coagulation abnormalities with respiratory failure and mortality in patients with COVID-19. This was a single center, prospective observational study enrolling 74 adult patients with COVID-19 pneumonia requiring intensive care (ICU) admissions from August 2020 to November 2020. The primary outcome was to describe the coagulation states using SCTs and POC Sonoclot® test in COVID-19 patients. Secondary outcomes were deemed the incidence of thromboembolic or bleeding events and presence of endogenous heparinoids generated due to cytokine storm and the predictability of clinical and laboratory parameters. Through different measurements, the authors concluded that HLE contributes to hypocoagulable effect, need for invasive ventilation and mortality in patients with COVID-19.
This is an interesting topic and the authors should be congratulated on their work. Nevertheless, the article must improve under certain aspects. I have some suggestions to further improve this manuscript:

Reply: We thank the reviewer for the insightful appraisal. We have modified the manuscript extensively to explain and modify the flow of presentation of our data. Since there have been many updates in the vastly changing field of coagulation in COVID-19, we have added several new citations and compared our methodology for initiating prophylactic or therapeutic anticoagulation using evidence-based point-of-care testing.

1) The small sample size is a very significant limitation of this analysis and it should be surely included in the limitation section; I understand the clinical classification in low flow vs high flow vs invasive ventilation (11 vs 34 vs 29), but maybe a comparison between two groups (non-invasive vs invasive) would have been more appropriate. It is very difficult to draw definite conclusions when the groups are two small. Are the results the same if you stratify patients into invasive vs non-invasive ventilation? If instead you are willing to keep a three group comparison, stat analysis is not appropriate. An ANOVA or Chi squared per groups, with a post hoc analysis on Tukey or Bonferroni per groups should be definitely performed to confirm your results.

Reply: Thank you for the suggestion. We agree that the sample size is small, hence this fact is now mentioned in the Limitations as suggested by the reviewer. (Page 17 Lines 9-11)
Naturally, while planning a trial with a research question on dosing protocol of heparin, i.e., prophylactic vs. therapeutic doses, a much larger sample size is needed which will require pooled data from multiple centres. We needed to keep three groups for analysis of **low flow vs high flow vs invasive ventilation (11 vs 34 vs 29)** as patients showed a transition from one group to the other in case of clinical improvement or worsening, and this would require a change in the anticoagulation dose. We did use an ANOVA with a post hoc Bonferroni test applied for differences between the three groups. The Bonferroni test was chosen as we have smaller numbers for comparison. This is mentioned in the methods section on page 10 lines1-3.

2) The authors state that all patients in this study were restricted to patients admitted to intensive care. How is it possible to find patients in ICU with a low-flow oxygen support?

Reply: Thank you for the observation. Patients with SARS-Cov2 infection, as confirmed by reverse transcription polymerase chain reaction (RT-PCR), aged between 18 to 80 years, and of any gender with moderate to severe ARDS, admitted to intensive care unit (ICU) were recruited. During the initial period of COVID-19 pandemic, patients with mild ARDS requiring low flow oxygen with Charlson's co-morbidity index > 6 and who underwent major surgery were also admitted to ICU and considered eligible for recruitment. This is mentioned on Page no 7 Lines 21-23 and page no 8 and lines 1-3.

3) I cannot find the results of the univariate and multivariable analysis in a specific table. Severe HLE was associated with patients who required invasive ventilation, reporting a HR of 1.2 (CI 1.04-1.4 p=0.001), but I was not able to find the whole output of the regression. The authors should report in table what has been analyzed in this regression, which is the main point of the manuscript, in order to understand the real "weight" of HLE on clinical outcomes.

Reply: Thank you for the suggestion. We agree that the table should provide complete information regarding the predictors of mortality. We have now added the Cox Proportional Hazards table to the document (Supplementary Table 2). We are providing the complete results for the Cox Regression for survival below for the scrutiny of the reviewer.

Predictors of Mortality using Cox Proportional Hazards Analysis

Univariate Analysis			Multivariate Analysis	
Covariate	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
CRP	1.007 (1.005- 1.016)	0.003	1.040(1.020-1.090)	0.014
PFR	0.989 (0.979- 0.999)	0.038	0.892 (0.576- 1.381)	0.609
Age	1.007 (0.975- 1.039)	0.681		
Sex	1.312 (0.487- 3.530)	0.591		
Ferritin	1.000 (1.000- 1.000)	0.037	0.984 (0.960-1.010)	0.220
RR	1.035 (0.970- 1.104)	0.298		
MAP	0.975 (0.939- 1.012)	0.189		
SBP	0.981 (0.958- 1.004)	0.108		
DBP	0.985 (0.945- 1.025)	0.453		
IL6	0.965 (0.842- 1.106)	0.965		
NLR	1.027 (1.007- 1.047)	0.009	1.052 (0.534- 2.075)	0.883

D-Dimer	1.000 (1.000- 1.000)	0.027		
Fibrinogen	1.066 (0.851- 1.336)	0.576		
Creatinine	0.999 (0.798- 1.252)	0.996		
LDH	1.003 (1.000- 1.000)	0.021	1.009 (0.940- 1.082)	0.809
Bilirubin	1.147 (1.010- 1.303)	0.035	0.824 (0.018- 38.316)	0.921
AST	0.997 (0.990- 1.005)	0.493		
ALT	0.996 (0.987- 1.005)	0.407		
Normalised D-dimer	0.687 (0.237- 1.992)	0.490		
Serum lactate	0.948 (0.560- 1.605)	0.844		
ACT	1.012(1.004- 1.4010)	0.001		
CR	0.975 (0.942- 1.009)	0.146		
PF	0.54 (0.291-0.901)	0.429	0.901 (0.702-1.100)	0.045
Platelet count	0.999 (0.995- 1.003)	0.596		
PT	1.014 (0.804- 1.280)	0.904		
aPTT	1.002 (0.973- 1.032)	0.880		
INR	2.267 (0.106- 48.301)	0.600		
Procalcito	0.996 (0.987- 1.005)	0.371		

nin				
d1hACT	1.033 (1.013- 1.054)	0.001	1.167 (0.302- 4.507)	0.822
d1hCR	0.956 (0.908- 1.006)	0.081	1.432 (0.253- 8.116)	0.685
d1hPF	0.885 (0.541- 1.447)	0.626		
d1h Peak-amp	0.960 (0.920- 1.002)	0.062	2.644 (0.090- 77.270)	0.572
D1gbACT	1.022 (1.011- 1.033)	0.000	1.207 (0.859- 1.698)	0.278
D1gbPF	0.858 (0.542- 1.358)	0.513		
D1gb Peak-Amp	0.948 (0.898-1.000)	0.051	0.149 (0.002- 9.973)	0.149
D1gb time to peak	1.075 (0.884- 1.308)	0.468		
HLE	1.020 (1.007-1.034)	0.002	1.01 (1.006-1.030)	0.025
HLE percent	1.005 (0.995-1.015)	0.338		

Abbreviations: NLR- Neutrophil Lymphocyte Ratio; AST- aspartate transaminase; ALT- alanine transaminase; ALP- Alkaline Phosphatase; LDH- Lactate Dehydrogenase, CRP- C Reactive protein, ACT- Activated Clotting Time; aPTT- Activated Partial Thromboplastin Time; INR- International Normalized Ratio; CR- Clot Rate; PF- Platelet Function; PT, Prothrombin Time, HLE- Heparin Like Effect. P value less than 0.05 is considered significant.

The following Supplementary Table 2 has been added to the final manuscript:

Univariate Analysis			Multivariate Analysis	
Covariate	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
CRP	1.007 (1.005- 1.016)	0.003	1.040 (1.020-1.090)	0.014

PF	0.54 (0.291-0.901)	0.429	0.901 (0.702-1.100)	0.045
HLE	1.020 (1.007-1.034)	0.002	1.01 (1.006-1.030)	0.025
PFR	0.989 (0.979- 0.999)	0.038		
Ferritin	1.001 (1.000- 1.010)	0.037		
NLR	1.027 (1.007- 1.047)	0.009		
D-Dimer	1.000 (1.000- 1.000)	0.027		
LDH	1.003 (1.000- 1.000)	0.021		
Bilirubin	1.147 (1.010- 1.303)	0.035		
ACT	1.012(1.004- 1.020)	0.005		
d1hACT	1.033 (1.013- 1.054)	0.001		
d1hCR	0.956 (0.908- 1.006)	0.081		
d1h Peak-amp	0.960 (0.920- 1.002)	0.062		
D1gbACT	1.022 (1.011- 1.033)	0.000		
D1gb Peak-Amp	0.948 (0.898-1.000)	0.051		

Abbreviations: NLR- Neutrophil Lymphocyte Ratio; AST- aspartate transaminase; ALT- alanine transaminase; ALP- Alkaline Phosphatase; LDH- Lactate Dehydrogenase, CRP- C Reactive protein, ACT- Activated Clotting Time; aPTT- Activated Partial Thromboplastin Time; INR- International Normalized Ratio; CR- Clot Rate; PF- Platelet Function; PT, Prothrombin Time, HLE- Heparin Like Effect. P value less than 0.05 is considered significant.

4) The evidence that heparin (more than other AC drugs) could be associated with a better prognosis in patients with severe COVID-19 was reported here: Oral anticoagulation and clinical

outcomes in COVID-19: An Italian multicenter experience. Int J Cardiol. 2020 Sep 8:S0167-5273(20)33735-9. doi: 10.1016/j.ijcard.2020.09.001. Please cite and discuss this study accordingly.

Reply: Thank you for the suggestion. The document is cited on page 15 lines 4-10.

5) Coagulation abnormalities and thromboembolism are one the major determinants of CV involvement in COVID-19. For example, COVID-19 may lead to ACS for macrothrombosis, microthrombosis and supply/demand imbalance as emerged in relevant studies that have been published in the last months. Please discuss this aspect referring also to these works, analyzing potential advantages/disadvantages of AC therapy, also in the light of the pleiotropic effect that for example heparin may show (as reported also above): Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol. 2020 doi: 10.1016/s2352-3026(20)30145-9. --- Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J. Thromb. Haemost. 2020;18:1421–1424. doi: 10.1111/jth.14830. --- povid-19. N. Engl. J. Med. 2020 doi: 10.1056/nejmoa2015432).

Reply: Thank you for the suggestion. We agree with the reviewer that the manuscript needs to be updated as the paper was submitted to the journal over 4 months ago. We have extensively revised the manuscript, added the above three studies in the Discussion section on page 14 lines 18-20, page 16 lines 15-19.

6) Findings about D-dimer elevation (that was mentioned) could be better explored, since in other articles it has been reported that it may sometimes predict a vascular involvement, coagulopathy and or silent microthrombosis. D-dimer elevation is somehow capable of predicting worse outcomes, but its role should be further analyzed as well as subsequent anticoagulant therapy in most severe COVID-19 cases. Please discuss in detail your findings on this topic.

Reply: Thank you for the suggestion. D-dimer can be elevated in two situations, one when there is formation of a clot with usual lysis or in disseminated consumptive coagulation failure where there is primary and secondary hyperfibrinolysis. Therefore although the use of D-dimer is predictive of disease severity, the quantification of the elevated levels cannot reliably be used to guide use of anticoagulation. In patients with hyperfibrinolysis, with elevated D-dimer, the use of therapeutic anticoagulation will be associated with bleeding manifestations.

The current study emphasizes the use of global coagulation tests like Sonoclot with heparinase modifications to determine the true status of coagulation function. Therefore the use of our POC algorithms will be able to refine the use of heparin or novel oral anticoagulants over a long term basis. In accordance with the reviewer's instruction, we have described the use of D-dimer as a conventional starting point to determine which patients had significant coagulation disturbances.

7) This conclusion: "Our data argues for the need for careful evaluation of HLE and coagulation abnormalities in COVID-19 and to develop therapeutic strategies based on POC global coagulation tests rather than using the clinically less relevant SCTs like D-dimer or fibrinogen levels", should be definitely toned down. It is indeed very unlikely that in routine clinical practice HLE could replace D-dimer or fibrinogen analysis.

Reply: Thank you for the comment. We agree with the reviewer that point-of care coagulation testing is not available in all centres and will not enter direct practice guidelines in primary and secondary care levels. SCTs like D dimer or fibrinogen levels are routinely available in most centres. However, the use of POC tests like blood gas analysis, and use of ultrasound using lung and abdominal POC imaging, and echocardiography has vastly improved our understanding and management of critical illness in intensive care practice.

We have modified the statement to indicate 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. Page 18, lines 5-12.

8) Could the authors present baseline coronary artery disease data of the enrolled population and troponin values data across the cohorts? CAD is one of the most common comorbidities resulting in a worse prognosis and cardiac troponin elevation is one major points of COVID-19 involvement, representing a mirror of myocardial injury which could definitely lead to worse outcomes, especially in patients with CAD, as reported here: Redefining the Prognostic Value of High-Sensitivity Troponin in COVID-19 Patients: The Importance of Concomitant Coronary Artery Disease - J. Clin. Med. 2020, 9, 3263; doi:10.3390/jcm9103263" – "Prognostic significance of cardiac injury in COVID-19 patients with and without coronary artery disease. Coron Artery Dis. 2020. doi:10.1097/MCA.0000000000000914." – "The relationship between coronary artery disease and clinical outcomes in COVID-19: a single-center retrospective analysis. Coron Artery Dis. 2020 Jul 23. doi: 10.1097/MCA.0000000000000934." Please analyze your findings accordingly.
Reply: We completely agree with the reviewers comments that coronary macro thrombosis is also a possible cause for cardiac related mortality in COVID-19. The use of biomarkers like hs-troponin can certainly refine the diagnosis of known or unknown coronary artery disease in patients with COVID-19 and the resultant myocardial injury can contribute to shock and lead to mortality. We did not test for troponin routinely in our cohort. But we have mentioned this pertinent observation in the Discussion section on page 14 Lines 14-24 and added the suggested citations.

Minor comments:
1) Unless it is a specific journal policy, I would format the abstract in a more standard scheme (e.g. background, methods, results, conclusion) and I would expand results and conclusion sections.
Reply: Thank you for the suggestion. We have changed the abstract section with the standard scheme as per the journal style.

2) Please fix syntax and grammar errors.
Reply: Thank you for the suggestion. We have extensively revised the manuscript and reassessed for inadvertent errors.

3) Please fix spaces and punctuation all along the manuscript (e.g. spaces between ref number and full stops/commas); also references should be written according to the journal policies.
Reply: Thank you for the suggestion. We have extensively revised the manuscript and reassessed for inadvertent errors.

Reviewer: 2
Dr. Antonio Brandão, Beneficencia Portuguesa de Sao Paulo, Universidade de Sao Paulo Instituto do Cancer do Estado de Sao Paulo
Comments to the Author:
The Premkumar et al. manuscript describes a very well-designed study which analyzes the heparin-like effect (HLE) in COVID-19 patients treated in the ICU setting. The authors used point of care (POC) coagulation tests which are capable of detecting some hemostatic abnormalities that are not evaluated by standard coagulation tests (SCTs). There is a current gap of evidence in this setting and this study may help to elucidate some questions.
The authors observed that patients with severe COVID-19 may present HLE and hypocoagulability more frequently than patients who require lower oxygen flows, and this observation is supported by the results presented. Of note, hypocoagulability was not present at SCTs routinely performed in the study patients, thus highlighting the importance of evaluating POC tests whenever available. These findings are particularly important since critically ill patients with COVID-19 may be exposed to anticoagulants, even at therapeutic dose, and the optimal anticoagulant strategy to these patients is still a matter of debate.
As stated above, this manuscript contains an interesting study. Unfortunately, a major review will be needed, mainly regarding some references to be improved, a few results to be better clarified and an additional study limitation which was not pointed out by the authors that could be included in the article. These points are detailed as follows:
Reply: We thank the reviewer for the insightful appraisal. We have modified the manuscript extensively to explain and modify the flow of presentation of our data. We have added new references and brought the Discussion section up to date with comparison of the new data generated from the large multicentric trials on diagnostic and therapeutic anticoagulation.

Major issues:
1. Page 7, line 24: the authors use the reference no. 4 to describe that nearly 20% of COVID-19 patients present with severe coagulation abnormalities. That statement however is not mentioned in the above reference. I would suggest citing Middeldorp et al. (J Thromb Haemost. 2020;18(8):1995-2002) who described that almost 20% of its cohort of hospitalized COVID-19 patients developed venous thromboembolism.

Reply: Thank you for the suggestion. We have added the suggested reference on page 6, line 5-8, and reference no 4.

2. Page 8, line 53: I would suggest using the ISTH definition of major bleeding which is as follows: 1 Fatal bleeding, and/or 2 Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome, and/or 3 Bleeding causing a fall in hemoglobin level of 2.0 g/L or more, or leading to transfusion of two or more units of whole blood or red cells. This definition was published by Schulman et al. (J Thromb Haemost . 2005 Apr;3(4):692-4.).

Reply: Thank you for the suggestion. We have added the suggested reference on page 9, lines 2-4, and reference no 18.

3. Page 11, line 11: the reference no. 19 mentioned by the authors does not contain the information described, which is present at the reference no. 13. Thus, I suggest using the reference no. 13 to better inform the reader about that topic.

Reply: Thank you for the suggestion. We have used the suggested reference.

4. Page 12, line 41: the authors mention that 74 patients were included in the study although they also mention that 189 of 215 patients admitted to ICU were excluded due to incomplete data. Hence, I would suggest checking this data because it may bring a major impact in this study results. This data is also described at figure 1, at page 32.

Reply: Thank you for the suggestion. The reasons for incomplete data was, large number of patients referred to our ICU with prior hospital stay of more than one week. Hence, we included only those treatment naive patients who were admitted to our hospital directly without any prior hospital admissions whose baseline laboratory values were available. This information is explained in the supplementary information. 9Supplementary figure 1)

5. Page 13, line 43: the authors mention that the presence of endogenous heparinoids favored a bleeding phenotype. I found difficulties to find the data that support this statement at the result section, although the authors mention at the discussion section (page 16, line 23) that HLE was noted in 66.7% of those who presented with clinical bleeding. That said, I would suggest also describing this data in the results section.
Reply: The suggested changes had been added in the results section.

6. Page 13, line 56 and page 14 line 2: the authors describe that CRP, ACT and PF at day 1 are predictors of mortality although they do not discriminate whether they are increased or decreased in the patients who have died.

Reply: Appropriate correction was made in the manuscript.

7. Page 29 (table 3): the authors describe a total of 33 patients analysed, although at page 30 line 10, they mention a total of 74 patients with an available Sonoclot. This information presented in that way may not be clear enough to the reader. Also, the data in this table does not show that the hypercoagulable coagulation phenotype was observed only in low flow oxygen group (page 14, line 53), since it shows that 32.4% of the patients in high-flow oxygen group and 20.7% of the patients in the invasive ventilation group had an ACT < 110 (defined by the authors as a procoagulant Sonoclot).
Reply: Out of 74 patients included in the study, paired Sonoclot tests were performed in subgroup of 33 patients and this data was shown in table 3.

8. I would suggest that the lack of a comparison of those coagulation parameters with non-COVID-19 patients may be interpreted as a limitation of the study.
Reply: The limitation as suggested is incorporated in the manuscript at page 17 lines 15-17.

Minor issues:

1. Page 12, line 12: the authors describe that the incidence of hypercoagulable state in COVID-19 to be 10%, although at the introduction section (page 7 line 24) the incidence cited is nearly 20%. I would suggest clarifying the evidence which base the assumption used to the sample size calculation.

Reply: The incidence of coagulation abnormality in COVID-19 is around 20% (hypercoagulable and hypocoagulable). However, sample size was calculated, assuming an incidence of hypercoagulable state in COVID-19 is nearly 10%.
Reference had been added: Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol. 2020 doi: 10.1016/s2352-3026(20)30145-9.

2. Page 12, line 32: the authors inform that the patients were screened from August 2020 to November 2020 however in the introduction (page 8, line 38) they describe that the study period was between September 2020 and December 2020.

Reply : The study period was from **August 2020 to November 2020** And it has been corrected in the manuscript.

3. Page 13, line 28: the authors describe that the HLE was evaluated at day 0, which differs from the methods sections (page 11, line 4 – day 1). I would suggest standardizing the moment of evaluation as day 1 throughout the manuscript.

Reply: The suggested change is made throughout the manuscript**.**

4. A minacious language review to correct some typos, grammar and spelling issues is welcome.

Reviewer: 1
Competing interests of Reviewer: None

Reviewer: 2
Competing interests of Reviewer: Nothing to disclose.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	03
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	03
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	06-07
Objectives	3	State specific objectives, including any prespecified hypotheses	07
Methods			
Study design	4	Present key elements of study design early in the paper	07-08
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	07-08
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	07
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	08-09
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	11-12
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	10
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	11
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11-12
		(b) Indicate number of participants with missing data for each variable of interest	11
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-12
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	21

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-051971.R2
Article Type:	Original research
Date Submitted by the Author:	16-Feb-2022
Complete List of Authors:	Premkumar, Madhumita; Post Graduate Institute of Medical Education and Research, Department of Hepatology Loganathan, Sekar; Post Graduate Institute of Medical Education and Research Kajal, Kamal; PGIMER, Department of Anaesthesia Hazarika, Amarjyoti; PGIMER, Department of Anaesthesia Soni, Shiv; PGIMER, Department of Anaesthesia Puri, Goverdhan ; Post Graduate Institute of Medical Education and Research Sehgal, Inderpaul Singh; PGIMER, Department of Pulmonary Medicine Suri, Vikas; Post Graduate Institute of Medical Education and Research Malhotra, Pankaj; PGIMER Singh, Virendra; PGIMER, Department of Hepatology Duseja, Ajay; PGIMER Bhalla, Ashish; Post Graduate Institute of Medical Education and Research, Internal Medicine Ahluwalia, Jasmina; PGIMER, Department of Hematology Kumar, Narender; PGIMER, Department of Hematology Kekan, Kushal; PGIMER, Department of Hepatology Ram, Sant; PGIMER, Department of Biochemistry Singla, Karan; PGIMER, Department of Anaesthesia Mahajan, Varun; PGIMER, Department of Anaesthesia Yaddanapudi, Narayana; Post Graduate Institute of Medical Education and Research
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Evidence based practice, Haematology (incl blood transfusion), Infectious diseases
Keywords:	COVID-19, Anticoagulation < HAEMATOLOGY, INTENSIVE & CRITICAL CARE, Thromboembolism < CARDIOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

COVID-19 Related Dynamic Coagulation Disturbances and Anticoagulation Strategies Using Conventional D-dimer and Point-of-Care Sonoclot® Tests: A Prospective Cohort Study

Authors:

MadhumitaPremkumar DM[1], Sekar L MD[2], Kamal Kajal MD[2*], Amarjyoti Hazarika MD[2], Shiv Soni DM[2], GD Puri PhD[2], Inderpaul Singh Sehgal DM[3], Vikas Suri MD[4], Pankaj Malhotra MD[4], Virendra Singh DM[1], Ajay Duseja DM[1], Ashish Bhalla MD[4], Jasmina Ahluwalia MD[5], Narender Kumar MD[5], Kushal Kekan[1], Sant Ram MD[6], Karan Singla MD[2], Varun Mahajan MD [2], L Narayana Yaddanapudi MD[2].

Affiliations:

[1] Department of Hepatology,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[2] Department of Anaesthesia,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[3] Department of Pulmonary Medicine,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[4] Department of Internal Medicine,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[5] Department of Hematology,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

[6] Department of Biochemistry,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

*Corresponding Author:

Dr.Kamal Kajal MD,

Department of Anaesthesia,

Postgraduate Institute of Medical Education and Research,

Chandigarh, India, 160012

Email: kamal.kajal@gmail.com

Phone No: +91 7087009545

Conflict of Interest and Source of Funding: All the authors have disclosed that they do not have any conflict of interest.

Word count: 2883

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 (ICU) admission between August 2020 and November 2020.

Primary and secondary outcome measures: We compared the coagulation abnormalities using standard coagulation tests (SCTs) like prothrombin time, D-dimer, platelet count etc. and point-of-care (POC) 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 day1 and 3 with Sonoclot®. Activated clotting time (ACT) <110s 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 intensive care unit, 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 group (16, 55.2%) with overall mortality of 21.6%. HLE predicted the need for mechanical ventilation (HR 1.2 CI 1.04-1.4 p=0.00). On multivariate analysis, the presence of HLE (HR 1.01; CI 1.006-1.030; p=0.025), increased CRP (HR 1.040; CI 1.020-1.090; p=0.014)], decreased platelet function [HR 0.901; CI 0.702-1.100 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: Ethical clearance was obtained from the Institutional Review Board (PGI/IEC/2020/000997 dated 24 August 2020) and the study was registered at ClinicalTrials.gov.in (Trial registration no: NCT04668404. Available from https://clinicaltrials.gov/ct2/show/NCT04668404)

Data availability statement:

Data are not available due to legal restrictions.

Strengths and limitations of this study:

1. 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.
2. This study provides evidence-based data to guide anticoagulation therapy in patients with COVID-19.
3. Point-of-care Sonoclot® tests help in identifying patients at risk of worse prognosis and invasive ventilation.
4. 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.
5. The study was not designed to test the use of prophylactic vs. therapeutic anticoagulation, but to assess the therapeutic window for using evidence-based anticoagulation safely.

INTRODUCTION

The Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) pandemic has affected approximately 225,024,781persons, and claimed 4,636,153 lives till September 14 2021.(1)Patients with COVID-19 have platelet abnormalities, endothelial dysfunction, clotting factors abnormalities and hyperfibrinolysis complicated by coexisting sepsis.(2) This lead to thromboembolic events both in the venous and arterial circulation.(3) About 20% of COVID-19 patients present with venous thromboembolism, 13% had symptomatic venous thromboembolism (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 an another observational study in 183 COVID-19 patients from China, the mean D-dimer concentration was significantly higher (2.12 mg/L, range 0·77–5·27) in non-survivors compared to survivors.(6)

Concomitant venous thromboembolism (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 vs therapeutic anticoagulation in COVID-19.(9)

Tang et al. observed that higher D-dimer and fibrin degradation product (FDP) 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 (MA) / 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 (ROTEM) profiles with a shorter clot formation time (CFT) in the INTEM ($p = 0.0002$), EXTEM ($p = 0.010$) and increased maximum clot firmness (MCF) (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 to 60 minutes. Cytokine storm and sepsis trigger the release of endogenous heparinoids from the endothelium in various organs which is seen on global coagulation tests 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 intensivecare unit (ICU) of the Postgraduate Institute of Medical Education and Research (PGIMER), 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-Cov2 infection, as confirmed by reverse transcription polymerase chain reaction (RT-PCR), aged between 18 to 80 years, and of any gender with moderate to severe

ARDS, admitted to intensive care unit (ICU) were recruited. During the initial period of COVID-19 pandemic, patients with mild COVID-19 ARDS requiring low flow oxygen with Charlson's co-morbidity index > 6 and COVID-19 patients 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, human immunodeficiency virus (HIV) infection, pregnancy or active malignancy in the last 5 years were excluded. We documented their demographic data, past 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. (Trial registration no: NCT04668404) 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 "heparin-like-effect" (HLE) using Sonoclot®. The predictability of clinical and laboratory parameters were also analysed for mortality at 28 days.

Definitions:

Acute Respiratory Distress Syndrome (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 (ISTH) 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 hemoglobin 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), high flow devices (venturi mask, high flow nasal cannula (HFNC), non-invasive ventilation (NIV)) 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 (TCZ), Remdesivir, Mycobacterium indicus pranii (Immuvac), Hydroxychloroquine (HCQ), plasma therapy as part of ongoing trials.(21) Supportive care for critically ill patients in the form of advanced hemodynamic monitoring and support, enteral nutrition, glycemic control, and stress ulcer prophylaxis were given in all eligible patients. Antibiotic and antifungal therapy were guided by cultures and sepsis markers. Supplementary file 1 shows the patient triage and dynamic treatment algorithm at our centre. Supplementary 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 hematology analyzer (Beckman Coulter Inc., Fl, USA). SCTs measured include prothrombin time (PT), activated prothrombin time (aPTT), international normalized ratio (INR), D-dimer (DiagnosticaStago, France) and fibrinogen (Sysmex CA 1500; Sysmex Corporation, Kobe, Japan). Sonoclot®analysis of whole blood sample (non-heparinised) (Sienco Inc., Arvada, CO, USA) was performed at the bedside. For performing glass-bead activated Sonoclot® 340 µL of whole blood was added to the Sonoclot® cuvette, pre-warmed to 37°C. Glass bead activated clotting time (gbACT), clot rate (CR), and platelet function (PF) were noted from the results and time to peak (TP) and peak amplitude (PA) were calculated from the Sonolcot signatures. (22) Paired global coagulation traces i.e. (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 seconds and PA > 75 units were considered as the cut off for hypercoagulable state. The heparin like effect (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 was represented as meanwith standard deviation(SD) or median with interquartile range (IQR), as appropriate. Normality of quantitative data was checked using Kolmogorov Smirnov tests. The continuous data between groups were compared by using the 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 hazard ratios (HRs) and 95% confidence intervals (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 α=0.05. Statistical analysis was performed with SPSS Statistics version 22 (IBM® SPSS® Statistics Armonk New York USA).

Sample size:

Sample sizewas estimated using G*Power, a statistical program. Assuming the incidence of hypercoagulable state in COVID-19 to be 5-15%,(5) with an effect size of 0.5, alpha 0.05, and power 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 male. The patients were divided into 3 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. Supplementary file 3 shows the comorbid illness and treatment details of the cohort.

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 2, panel A). 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 1.040-1.400 p=0.001) and an uncorrected ACT was associated with increased risk of death (Figure 2, panel B).

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, normalized D-dimer, NLR, fibrinogen, creatinine, bilirubin, aminotransferases, and lactate did not predict mortality on Cox Proportional Hazard analysis. SCTs like platelet count, PT, INR, aPTTand 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- 1.04; p=0.002), increased CRP (HR 1.04; CI 1.02-1.09 p=0.003), elevated ACT (HR1.02; CI 0.1.04-1.4 p=0.001) and decreased PF (HR 0.54; CI 0.29-0.90; p=0.010). In multivariate analysis, the presence of HLE (HR 1.02; CI 1.08-1.6; p=0.007), raised CRP (1.2; CI 1.1-1.4; p=0.014) and reduced PF [HR 0.9; CI 0.7-1.1 p=0.045) remained significant. (Supplementary 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 1.01-1.5; 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 seconds (p=0.021), CR <27 units/min (p=0.027) and PA < 67.5 units predicted mortality. (Supplementary 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 shows that information generated by POC tests provides an etiopathological rationale for appropriate use of anticoagulation in COVID 19 patients. Firstly, 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. Secondly, the use of D-dimer as a marker of a procoagulant state is fallacious, as it is elevated in patients with thrombosis, hyperfibrinolysis and disseminated intravascular coagulation (DIC). Thirdly, our data shows 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 anticoagulation did not improve survival at 30 days, and the rate of bleeding events were 8% and 2%, respectively in the 2 comparators. The limitations of this study were variable recruitment of patients, within a 2-week interval post COVID 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 vs. 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 and normalized 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 multicentric 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%. (25)

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 to 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 disseminated intravascular coagulation. Damaged endothelial cells with microangiopathy release a mixture of glycosaminoglycans that function like endogenous heparinoids. (26) 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.(27) 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. 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 day 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 minimized 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 can be attributed to sepsis induced coagulopathy and anticoagulation regimen guided by SCTs. Considering the complex interplay between prothrombotic and anti-hemostatic pathways, their influence on outcome in terms of thrombosis or bleeding cannot be measured on SCTs. (27,28)

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 is 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. (29,30)Increased d1gbACT, decreased PA, decreased clot rate 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 global coagulation tests 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.

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. 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. (15,31)

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.

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.

Ethics statements:

Patient consent for publication:

Consent had been obtained before the enrolment to the study from the study participants / patient's relatives.

Ethics approval:

Institutional Ethics Committee, Postgraduate Institute of Medical Education and Research, Chandigarh, India ID: (PGI/IEC/2020/000997)

Foot note:

MP, SL and KK equally contributed to this study.

Author statement: (Contributorship 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

Funding: The authors do not declare a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest: None

References:

1. WHO Coronavirus Disease (COVID-19) Dashboard | WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. [cited 2021 Feb 15]. Available from: https://covid19.who.int/?gclid=Cj0KCQiA1KiBBhCcARIsAPWqoSqA7NL1iSnMGGpdZETsEXWs2VRwMQdmjO0znx27cVKSImIrXvPEUc0aArSKEALw_wcB

2. Thachil J, Tang N, Gando S, Falanga A, Levi M, Clark C, et al. Laboratory haemostasis monitoring in COVID-19 [Internet]. Vol. 18, Journal of Thrombosis and Haemostasis. Blackwell Publishing Ltd; 2020 [cited 2021 Feb 15]. p. 2058–60. Available from: https://pubmed.ncbi.nlm.nih.gov/32324960/

3. Iba T, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of Coronavirus Disease 2019 [Internet]. Vol. 48, Critical Care Medicine. Lippincott Williams and Wilkins; 2020 [cited 2021 Feb 15]. p. 1358–64. Available from: https://pubmed.ncbi.nlm.nih.gov/32467443/

4. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Muller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020;18(8):1995-2002.

5. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol. 2020;7(6):e438-e40.

6. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med [Internet]. 2020 Apr 30 [cited 2020 Aug 26];382(18):1708–20. Available from: https://pubmed.ncbi.nlm.nih.gov/32109013/

7. Llitjos JF, Leclerc M, Chochois C, Monsallier JM, Ramakers M, Auvray M, et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost [Internet]. 2020 Jul 1 [cited 2021 Feb 15];18(7):1743–6. Available from: https://pubmed.ncbi.nlm.nih.gov/32320517/

8. Moll M, Zon RL, Sylvester KW, Chen EC, Cheng V, Connell NT, et al. VTE in ICU Patients With COVID-19. Chest [Internet]. 2020 Nov 1 [cited 2021 Feb 15];158(5):2130–5. Available from: https://pubmed.ncbi.nlm.nih.gov/32710891/

9. Lopes RD, de Barros ESPGM, Furtado RHM, Macedo AVS, Bronhara B, Damiani LP, et al. Therapeutic versus prophylactic anticoagulation for patients admitted to hospital with COVID-19 and elevated D-dimer concentration (ACTION): an open-label, multicentre, randomised, controlled trial. Lancet. 2021;397(10291):2253-63.

10. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost [Internet]. 2020 Apr 1 [cited 2021 Feb 15];18(4):844–7. Available from: https://pubmed.ncbi.nlm.nih.gov/32073213/

11. Asakura H, Ogawa H. COVID-19-associated coagulopathy and disseminated intravascular coagulation. Int J Hematol [Internet]. 2021 Jan 1 [cited 2021 Feb 15];113(1):45–57. Available from: https://doi.org/10.1007/s12185-020-03029-y

12. Amgalan A, Othman M. Exploring possible mechanisms for COVID-19 induced thrombocytopenia: Unanswered questions [Internet]. Vol. 18, Journal of Thrombosis and Haemostasis. Blackwell Publishing Ltd; 2020 [cited 2021 Feb 15]. p. 1514–6. Available from: https://pubmed.ncbi.nlm.nih.gov/32278338/

13. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, et

al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. J Thromb Haemost [Internet]. 2020 Jul 1 [cited 2021 Feb 15];18(7):1738–42. Available from: https://pubmed.ncbi.nlm.nih.gov/32302438/

14. Song JC, Wang G, Zhang W, Zhang Y, Li WQ, Zhou Z. Chinese expert consensus on diagnosis and treatment of coagulation dysfunction in COVID-19. Mil Med Res [Internet]. 2020 Dec 20 [cited 2021 Feb 15];7(1):19. Available from: https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-020-00247-7

15. Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, et al. COVID-19-Related Severe Hypercoagulability in Patients Admitted to Intensive Care Unit for Acute Respiratory Failure. Thromb Haemost [Internet]. 2020 Jun 1 [cited 2021 Feb 15];120(6):998–1000. Available from: https://pubmed.ncbi.nlm.nih.gov/32316063/

16. Premkumar M, Bihari C, Saxena P, Devurgowda DR, Vyas T, Mirza R, et al. Heparin-like Effect Associated With Risk of Bleeding, Sepsis, and Death in Patients With Severe Alcohol-Associated Hepatitis. Clin Gastroenterol Hepatol [Internet]. 2020 Feb 1 [cited 2021 Feb 15];18(2):486-495.e3. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1542356519304896

17. Ferguson ND, Fan E, Camporota L, Antonelli M, Anzueto A, Beale R, et al. The Berlin definition of ARDS: An expanded rationale, justification, and supplementary material. Intensive Care Med [Internet]. 2012 [cited 2021 Feb 15];38(10):1573–82. Available from: https://pubmed.ncbi.nlm.nih.gov/22926653/

18. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation. Br J Haematol [Internet]. 2009

Apr [cited 2021 Feb 15];145(1):24–33. Available from: https://pubmed.ncbi.nlm.nih.gov/19222477/

19. Singer M, Deutschman CS, Seymour C, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3) [Internet]. Vol. 315, JAMA - Journal of the American Medical Association. American Medical Association; 2016 [cited 2021 Feb 15]. p. 801–10. Available from: https://jamanetwork.com/

20. Schulman S, Kearon C, Subcommittee on Control of Anticoagulation of the S, Standardization Committee of the International Society on T, Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost. 2005;3(4):692-4.

21. Soni SL, Kajal K, Yaddanapudi LN, Malhotra P, Puri GD, Bhalla A, et al. Demographic & clinical profile of patients with COVID-19 at a tertiary care hospital in north India. Indian J Med Res [Internet]. 2020 Nov 19 [cited 2021 Feb 15]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/33208558

22. Premkumar M, Saxena P, Rangegowda D, Baweja S, Mirza R, Jain P, et al. Coagulation failure is associated with bleeding events and clinical outcome during systemic inflammatory response and sepsis in acute-on-chronic liver failure: An observational cohort study. Liver Int [Internet]. 2019 Apr 1 [cited 2021 Feb 16];39(4):694–704. Available from: https://pubmed.ncbi.nlm.nih.gov/30589495/

23. Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J Thromb Haemost. 2020;18(6):1421-4.

24. Schiavone M, Gasperetti A, Mancone M, Curnis A, Mascioli G, Mitacchione G, et al. Oral anticoagulation and clinical outcomes in COVID-19: An Italian multicenter experience. Int J Cardiol [Internet]. 2021 Jan 15 [cited 2021 Sep 20];323:276–80. Available from: http://www.internationaljournalofcardiology.com/article/S0167527320337359/fulltext

25. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol [Internet]. 2020 Jun 1 [cited 2021 Sep 20];7(6):e438–40. Available from: http://www.thelancet.com/article/S2352302620301459/fulltext

26. Magnani HN. Rationale for the Role of Heparin and Related GAG Antithrombotics in COVID-19 Infection. Vol. 27, Clinical and Applied Thrombosis/Hemostasis. SAGE Publications Inc.; 2021.

27. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 2020;383(2):120-8.

28. Premkumar M, Mehtani R, Divyaveer S, Kajal K, Kulkarni A V., Ahmed S, et al. Clinical Validation of Global Coagulation Tests to Guide Blood Component Transfusions in Cirrhosis and ACLF. http://www.xiahepublishing.com/ [Internet]. 2021 Feb 18 [cited 2021 Feb 22];0(000):0–0. Available from: http://www.xiahepublishing.com/2310-8819/JCTH-2020-00121

29. Schiavone M, Gasperetti A, Mancone M, Kaplan AV, Gobbi C, Mascioli G, et al. Redefining the Prognostic Value of High-Sensitivity Troponin in COVID-19 Patients: The Importance of Concomitant Coronary Artery Disease. J Clin Med. 2020;9(10).

30. Peterson E, Lo KB, DeJoy R, Salacup G, Pelayo J, Bhargav R, et al. The relationship between coronary artery disease and clinical outcomes in COVID-19: a single-center retrospective analysis. Coron Artery Dis. 2021;32(5):367-71.

31. Salem N, Atallah B, El Nekidy WS, Sadik ZG, Park WM, Mallat J. Thromboelastography findings in critically ill COVID-19 patients [Internet]. Journal of Thrombosis and Thrombolysis. Springer; 2020 [cited 2021 Feb 18]. Available from: https://pubmed.ncbi.nlm.nih.gov/33011896/

List of Figures

List of Tables

Supplementary materials:

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*						
Hemoglobin (g/dl)	10.8(9.7-12)	12(10. 4-13)	10.8(10-12)	10.3(8.8-12.1)	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 (x10^3 /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.

Abbreviations: ARDS- acute respiratory distress syndrome; BMI-Body Mass Index; HTN- Hypertension; DM- Diabetes Mellitus; CAD- Coronary Artery Disease, CKD- Chronic Kidney Disease; CRP- C Reactive protein; COPD- Chronic Obstructive Pulmonary Disease, SOFA- Sequential Organ Failure Assessment; GI- Gastro Intestinal; HCQ-, Hydroxychloroquine; NLR- Neutrophil Lymphocyte Ratio; AST- aspartate transaminase; ALT- alanine transaminase; ALP- Alkaline Phosphatase; LDH- Lactate Dehydrogenase

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 v.s 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	10 (59%)	0	0	10 (77%)	0.024	0.024

HLE)						

*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; INR- International Normalised Ratio; CR- Clot Rate; PF- Platelet Function; PT, Prothrombin Time.

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

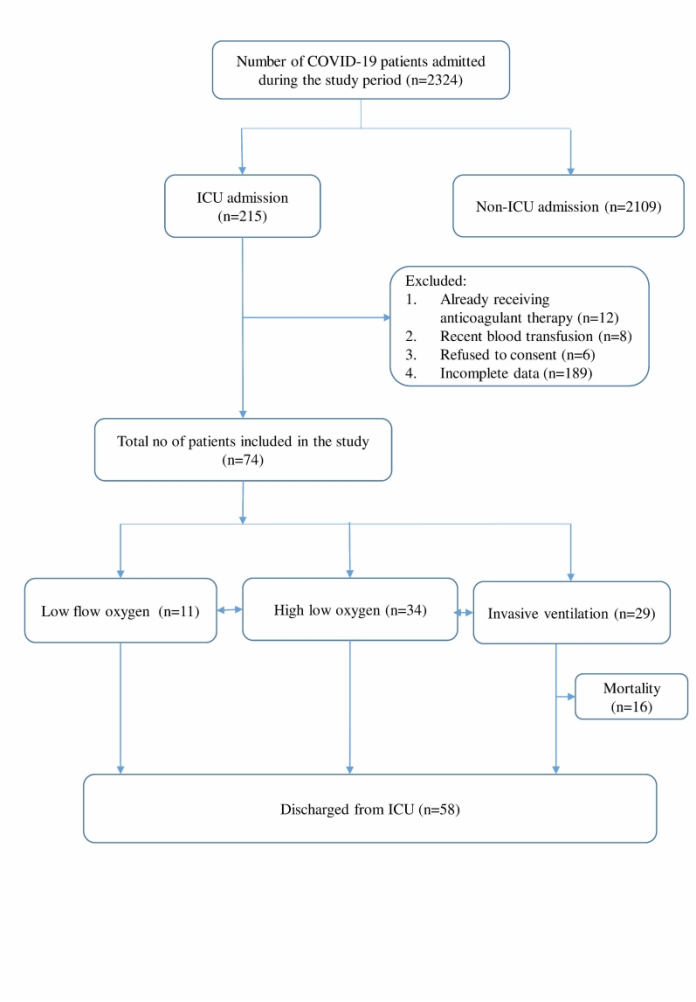


Figure 1: Study design, patient selection and evaluation.

105x105mm (1200 x 1200 DPI)

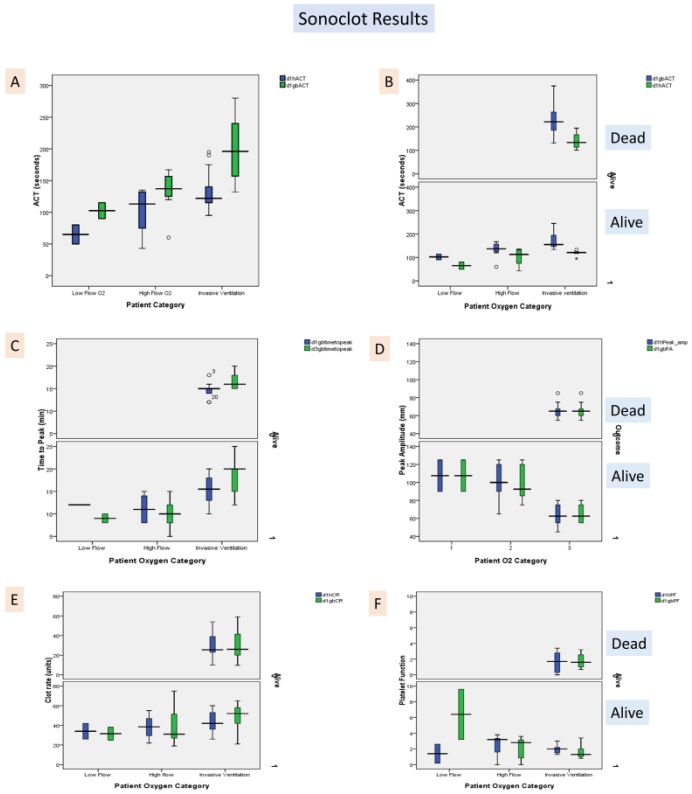


Figure 2: Presence of HLE as defined as difference in Sonoclot® trace at days 0, 3 in patients. (Panel A-F)

508x508mm (300 x 300 DPI)

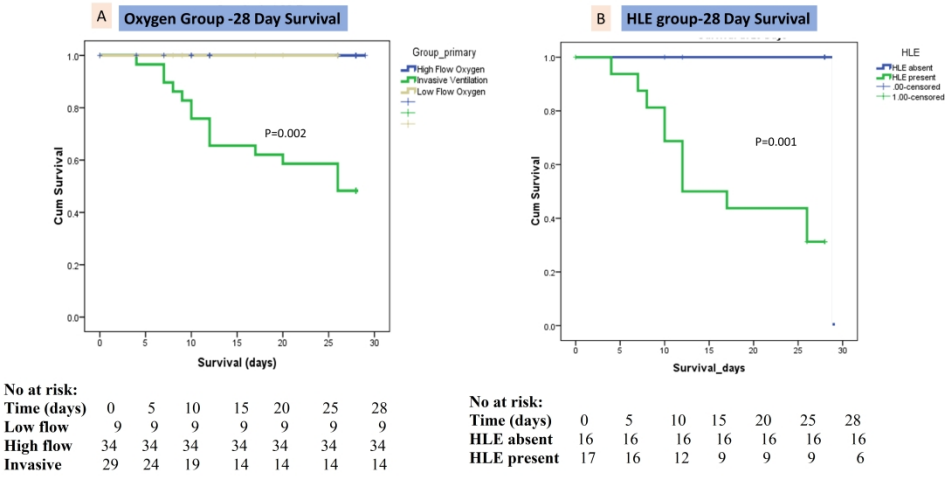


Figure 3: Kaplan Meir survival curves for patients (2A- Oxygen groups; 2B -Presence of heparin like effect)

499x295mm (300 x 300 DPI)

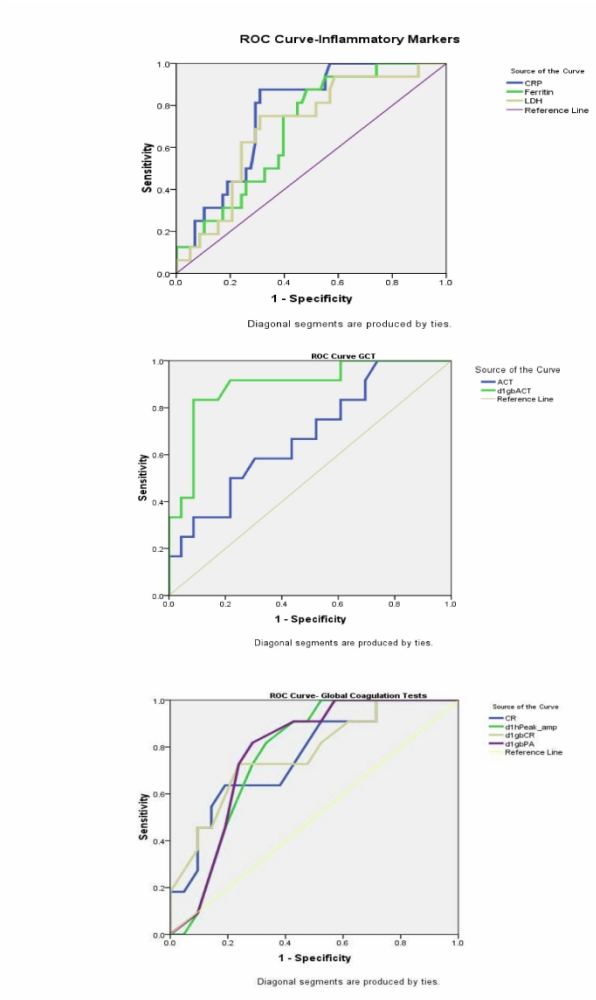
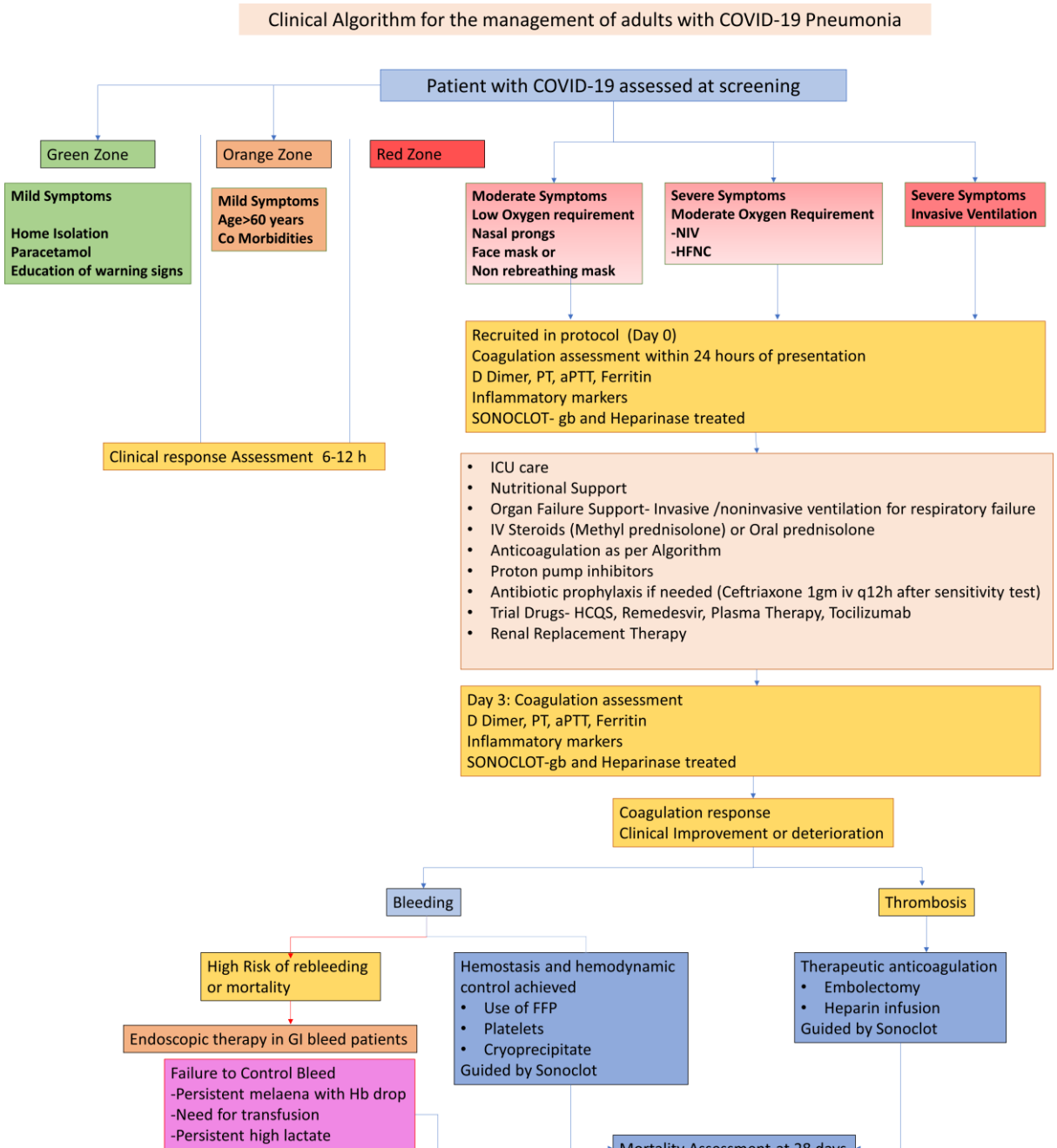


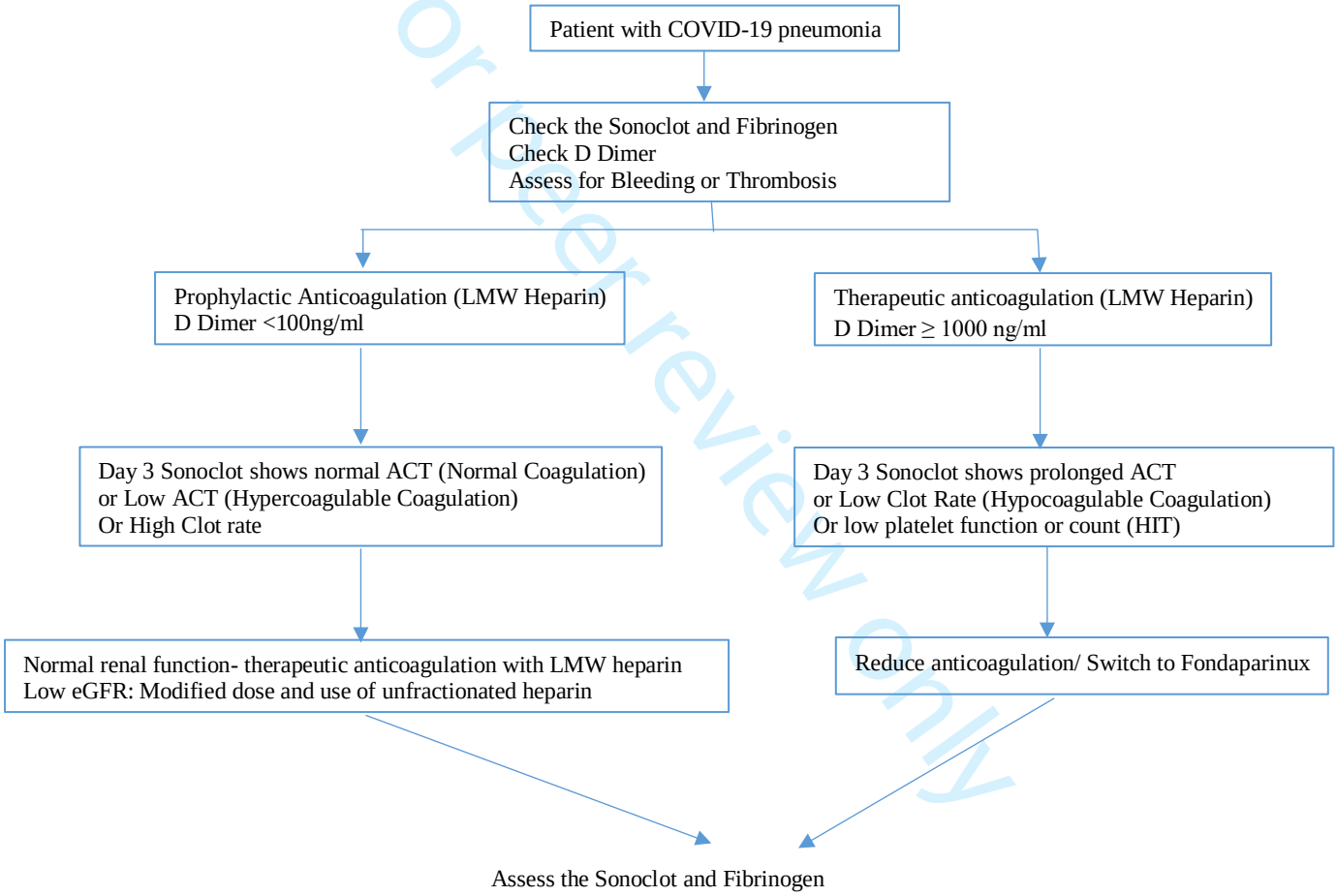
Figure 4: ROC curves for predictors of mortality

105x105mm (1200 x 1200 DPI)

Supplementary File 1: Hospital Triage Areas for COVID-19 and clinical algorithm for management of COVID-19 pneumonia



Supplementary File 2: Anticoagulation protocol at our centre based on conventional and POC global coagulation tests.



Supplementary File 3: Baseline characteristics, co-morbidities, 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
Comorbidities						
Hypothyroidism	13(17.6)	0	7(20.6)	6(20.7)	0.101	0.102
COPD	1(1.4)	0	0	1(3.4)	-	0.725
Asthma	1(1.4)	0	0	1(3.4)	-	0.725
Obesity	27(36.48)	2(18.2)	5(14.7)	10(34.5)	0.555	0.315
Treatment given in each group						
Tocilizumab	32(43)	5(15.6)	17(53.1)	10(31.2)	0.534	0.522
Remdesivir	23(31)	3(27.3)	7(20.6)	13(44.8)	0.465	0.312
HCQS	16(22)	3(27.3)	11(33.3)	2(6.9)	0.709	0.117
Antibiotics	72(97)	10(90.9)	33(97.1)	29(100)	0.390	0.100
Antifungals	27(36)	0	12(35.3)	15(51.7)	0.021	0.003

Immuvac (mycobacterium indicus pranii)	31(42)	6(54.5)	19(55.9)	6(20.6)	0.938	0.866
Plasma therapy	2(3)	0	1(3)	1(3)	-	
Steroid duration(days)	6(0-7)	4(3-5)	1(0-5.75)	5(0-7)	0.059	0.873

Abbreviations: COPD, chronic obstructive pulmonary disease; HCQS, hydroxychloroquine

Supplementary File 4: Predictors of Mortality using Cox Proportional Hazards Analysis

Univariate Analysis			**Multivariate Analysis**	
Covariate	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
CRP	1.007 (1.005- 1.016)	0.003	1.040 (1.020-1.090)	0.014
PF	0.540 (0.291-0.901)	0.429	0.901 (0.702-1.100)	0.045
HLE	1.020 (1.007-1.034)	0.002	1.010 (1.006-1.030)	0.025
PF Ratio	0.989 (0.979- 0.999)	0.038		
Ferritin	1.001 (1.000- 1.010)	0.037		
NLR	1.027 (1.007- 1.047)	0.009		
D-Dimer	1.000 (1.000- 1.000)	0.027		
LDH	1.003 (1.000- 1.000)	0.021		
Bilirubin	1.147 (1.010- 1.303)	0.035		
ACT	1.012(1.004- 1.020)	0.005		
d1hACT	1.033 (1.013- 1.054)	0.001		
d1hCR	0.956 (0.908- 1.006)	0.081		
d1h Peak amplitude	0.960 (0.920- 1.002)	0.062		
D1gbACT	1.022 (1.011- 1.033)	0.000		
D1gb Peak-Amplitude	0.948 (0.898-1.000)	0.051		

Abbreviations: ACT, activated clotting clotting time; CRP, C Reactive Protein; CR, clot rate; LDH, lactate dehydrogenase; PF, platelet function; HLE, heparin like effect; NLR, neutrophil lymphocyte ratio; hACT,

heparinase treated; hCR, heparinase treated CR; hPF, heparinase treated platelet function; PF Ratio, PaO_2/ FiO_2 ratio;

Supplementary File 5: Predictors for mortality in COVID 19 patients based on Receiver Operating Characteristics (ROC) analysis

Parameter	Cut off value	AUC	Sensitivity	Specificity	P value
CRP	96·7 mg/l	0·767	87·5	69	0·001
Ferritin	587 ng/ml	0·690	75	61	0·021
LDH	405 U/L	0·700	75	70	0·015
CR	27·5 units/min	0·761	66·7	87	0·012
ACT	131 seconds	0·744	60	80	0021
D1hPeak-amp	72·5 units	0·736	81·8	65	0·029
D1gbACT	158 seconds	0·893	91·7	79	0·001
D1gbCR	27 units/min	0·730	63·7	79	0·027
gbPA	67·5 units	0·746	72·7	73	0·023

Abbreviations: CRP, C Reactive Protein; ACT – Activated Clotting Time, CR – Clot Rate, PF – Platelet Function; PA, peak amplitude; LDH, lactate dehydrogenase·

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	03
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	03
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	06-07
Objectives	3	State specific objectives, including any prespecified hypotheses	07
Methods			
Study design	4	Present key elements of study design early in the paper	07-08
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	07-08
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	07
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	08-09
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	11-12
		(*c*) Explain how missing data were addressed	-
		(*d*) If applicable, explain how loss to follow-up was addressed	-
		(*e*) Describe any sensitivity analyses	10
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	11
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11-12
		(b) Indicate number of participants with missing data for each variable of interest	11
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-12
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	21

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.