PROF. JAMES O'DONNELL (Orcid ID : 0000-0003-0309-3313)

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COVID-19 Coagulopathy in Caucasian patients

Helen Fogarty[1,2,3]*, Liam Townsend[4]*, Cliona Ni Cheallaigh[4], Colm Bergin[4], Ignacio Martin-Loeches[1,5], Paul Browne[6], Christopher L. Bacon[6],Richard Gaule[6], Alexander Gillett[6], Mary Byrne[2], Kevin Ryan[2], Niamh O'Connell[2], Jamie M. O'Sullivan[1], Niall Conlan[7], and James S. O' Donnell[1,2,3,6]

[1] Irish Centre for Vascular Biology, School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland, Dublin 2, Ireland.

[2] National Coagulation Centre, St James's Hospital, Dublin, Ireland.

[3] National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin, Ireland.

[4] Dept of Infectious Diseases, St James's Hospital, Trinity College Dublin, Ireland.

[5] Dept of Critical Care, St James's Hospital, Trinity College Dublin, Ireland.

[6] St James's Hospital, Trinity College Dublin, Ireland.

[7] Dept of Immunology, St James's Hospital, Trinity College Dublin, Ireland.

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Editorial correspondence should be addressed to:
Prof. James O'Donnell
National Coagulation Centre, St James's Hospital, Dublin 8, Ireland.
Tel +353 (1) 402 2209;
e-mail jamesodonnell@rcsi.ie

*H.F. and L.T. contributed equally to this study.

KEY POINTS

1. Race and ethnicity have major effects upon thrombotic risk, with significantly lower risk in Chinese compared to Caucasian individuals.

2. Severe COVID-19 infection is associated with a significant coagulopathy in Caucasian patients that correlates with disease severity.

3. Despite significantly increased D-dimers, progression to overt DIC in Caucasian COVID-19 patients maintained on prophylactic dose LMWH is rare.

ABSTRACT

Although the pathophysiology underlying severe COVID-19 remains poorly understood, accumulating data suggest that a lung-centric coagulopathy may play an important role. Elevated D-dimer levels which correlated inversely with overall survival were recently reported in Chinese cohort studies. Critically however, ethnicity has major effects on thrombotic risk, with a 3-4 fold lower risk in Chinese compared to Caucasians and a significantly higher risk in African-Americans. In this study, we investigated COVID-19 coagulopathy in Caucasian patients. Our findings confirm that severe COVID-19 infection is associated with a significant coagulopathy that correlates with disease severity. Importantly however, Caucasian COVID-19 patients on LMWH thrombo-prophylaxis rarely develop overt DIC. In rare COVID-19 cases where DIC does develop, it tends to be restricted to late stage disease. Collectively, these data suggest that the diffuse bilateral pulmonary inflammation observed in COVID-19 is associated with a novel pulmonary-specific vasculopathy which we have termed pulmonary intravascular coagulopathy (PIC) as distinct to DIC. Given that thrombotic risk is significantly impacted by race, coupled with the accumulating evidence that coagulopathy is important in COVID-19 pathogenesis, our findings

raise the intriguing possibility that pulmonary vasculopathy may contribute to the unexplained differences that are beginning to emerge highlighting racial susceptibility to COVID-19 mortality.

KEYWORDS

Coagulation parameter, D-dimer, novel coronavirus pneumonia, COVID-19

INTRODUCTION

COVID-19 infection was first described in December 2019 in Wuhan, China.[1,2] Since then, this disease has disseminated through most countries around the world and already caused more than 150,000 fatalities. The causative agent of COVID-19 ie Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2), a novel betacorona virus that shares similarities with SARS and Middle East Respiratory Syndrome (MERS) viruses which were previously responsible for endemics in 2003 and 2012.[3,4] Studies have estimated overall COVID-19 mortality rates ranging from 4.3% to 14.6%.[1,2,5] This mortality burden is predominantly attributable to a progressive bilateral pneumonia that can ultimately progress to acute respiratory distress syndrome (ARDS).

Although the underlying pulmonary pathophysiology remains incompletely understood, severe COVID-19 infection is associated with a marked alveolar inflammatory cell infiltrate, together with a systemic cytokine storm response.[6] Several studies have also reported evidence of a COVID-19 associated coagulopathy.[5,7,8] Furthermore, multivariate regression analysis in Chinese COVID-19 cohorts reported that elevated plasma levels of fibrin degradation D-dimers constituted an independent biomarker for poor prognosis in COVID-19.[8] Consistent with the hypothesis that coagulation activation may play a role in COVID-19 pathogenesis, post-mortem studies have highlighted marked pathological changes specifically involving the lung microvasculature, including disseminated micro-thrombi and significant hemorrhagic necrosis.[9,10] Moreover, emerging data suggest that severe COVID-19 is also associated with a significant increased risk for developing deep vein thrombosis and pulmonary embolism.[11,12]

Critically, the COVID-19 coagulopathy data published to date have been predominantly derived from studies of Chinese patients.[2,5,7,8] This is important because race and ethnicity have major effects upon thrombotic risk.[13,14] In particular, epidemiological studies have shown that the incidence of venous thromboembolism (VTE) is approximately 3-4 fold lower in Chinese compared to Caucasian individuals.[13,15] Conversely, VTE risk is significantly higher in African-Americans compared to Caucasians.[14] This effect has been consistently observed, even in individuals of different ethnicities living within the same geographical location.[13] Importantly, reduced VTE prevalence also contributes to the fact that thromboprophylaxis is utilised less frquently in Chinese hospitals. Given these data, it is clearly important to determine whether there are differences in coagulopathic features in COVID-19 infected Caucasian compared to Chinese patients. In addition, the utility of D-dimer levels as a prognostic marker in hospitalised Caucasian patients maintained on low molecular weight heparin (LMWH) thromboprophylaxis also needs to be validated.

METHODS

Consecutive adult patients with COVID-19 were recruited from St James's Hospital between 13th March and 10th April. Inclusion criteria were indiviuals with a positive COVID-19 polymerase chain reaction test in patients aged 18 years or older. The study was approved by the St James's Hospital Research Ethics Committee and informed consent was obtained from all participants. Criteria for hospital admission were defined as those requiring inpatient care as a result of the severity of illness based on laboratory and radiological parameters as well as clinical gestalt. Following admission, all patients received supportive care in line with best international practice, which included the use of supplemental oxygen where indicated. In addition, hospitalised patients with COVID-19 received weight- and renally- appropriate doses of LMWH thromboprophylaxis unless contra-indicated as part of standard of care (enoxaparin 20mg OD if <50kg; enoxaparin 40mg OD if 50-100kg; 40mg BD if 101-150kg; 60mg BD if >150kg). Eight patients had renal impairment on admission and were therefore treated with enoxaparin 20mg OD. Four patients were on therapeutic anticoagulation on admission (2 on apixaban, 1 on edoxaban and 1 on warfarin). Epidemiological, demographic, treatment and outcome data were derived from the hospital electronic patient record using a standard data collection form. For each subject, samples were collected at time of admission and at timepoints during their subsequent admission.

All hemostasis testing was performed in the National Coagulation Laboratory in St James' Hospital, Dublin. Assays included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, and D-dimer levels. The PT, APTT and Fibrinogen assays were measured on the ACL Top 550TM analyser using HemosIL(r) RecombiPlasTin 2G, HemosIL(r) SynthASil and HemosIL(r) Fibrinogen-C reagents respectively. D-dimer levels were measured in ng/mL Fibrinogen Equivalent Units (FEU) using the D-Dimer HS 500 assay on the ACL Top 550 analyser. Between patient sub-groups, normally and non-normally distributed quantitative data were compared using the Student's t test and Mann-Whitney U test, respectively. Results were tablated as the mean ± standard deviation, median (interquartile range) or number (percentage) as appropriate. Data were analysed using GraphPad Prism 8, and a *P* value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

A total of 83 patients (55 males and 28 females) were enrolled in the study with a median age of 64 (range 26-92) years **(Table 1)**. Sixty-seven patients (81%) were Caucasian, 10 (12%) were Asian, 5 (6%) were African and 1 (1%) patient was of Latino/Hispanic ethnicity. Underlying co-morbidities were identified in sixty-seven (80.7%) of the cohort. At time of writing, 50 patients (60.2%) had fully recovered and were discharged from hospital, while 20 (24.1%) remained in hospital and 13 (15.7%) had died. Fifty patients (60.3%) were discharged without requiring ICU admission, 23 patients (27.7%) were admitted to ICU during their disease course and 10 patients (12%) required ICU support but were deemed clinically unsuitable for ICU admission.

Coagulation testing performed on admission in the total cohort demonstrated normal PT and APTT **(Table 1).** In contrast, plasma D-dimer levels were significantly increased (median 732; range 200 to 10,000 ng/ml). D-dimer levels were above the normal range in 67% of the total cohort on admission **(Table 1)**. Importantly however, despite the increased D-dimers, disseminated intravascular coagulopathy (DIC) was not evident. In particular, platelet counts were within the normal range in 83.1% with a platelet count <100 x 10^9/L observed in only 5 patients on admission. Fibrinogen levels were significantly increased (median 4.7; range 3.0 to 9.9 g/L) on admission, with levels remaining persistently elevated throughout hospitalisation. No patient had a fibrinogen level < 2.0 g/L at any time point. This increase in fibrinogen levels is probably due to an acute phase response, as significantly elevated C-reactive protein levels (median 56 mg/L; normal range 0-5 mg/L) were also observed **(Table 1)**. Thus, despite the fact that thrombotic risk is much higher in Caucasian patients and the significant elevated levels of D-dimers observed, overt DIC as defined according to the ISTH SSC DIC score[16] was present in none of our COVID-19 patients at time of admission. Nevertheless, our data confirm that severe COVID-19 infection is associated with a significant coagulopathy in Caucasian patients that appears to be similar in magnitude to that previously reported in the original Chinese cohorts.[5,7,8]

To assess whether COVID-19 coagulopathy at time of admission was indicative of future clinical course, we divided our cohort into 2 groups based on requirement of ICU admission for ventilatory support or death due to COVID-19 infection versus those who were discharged without requiring ICU support. Median age of non-survivors was 75.2 years (range 63.5 to 92) compared to 60.2 years (range 26.9 to 89) in survivors. Those whose admission resulted in an ICU stay or death were also more likely to have underlying co-morbidities compared with those who survived and did not require ICU admission **(Table 1).** In keeping with the previous Chinese data,[7] we observed that abnormal coagulation parameters on admission were also associated with a poor prognosis in Caucasian patients with COVID-19 infection. In particular, D-dimer levels were significantly higher in the subgroup who eventually needed ICU admission (median 1003 versus 804 ng/mL; p = 0.018) **(Table 1)**. Similarly, fibrinogen and CRP levels were also both significantly elevated in the poor prognosis group. While no significant difference was observed in PT between the two groups on admission, the PT was higher in the adverse prognostic group by Day +4 (median 13.1 versus 12.5sec; p=0.007) **(Figure 1).** In addition, D-dimer levels remained significantly higher in the poor prognosis group at Day +4 **(Figure 1).** Cumulatively, these data support the hypothesis that COVID-19 associated coagulopathy probably contributes to the underlying pulmonary pathogenesis.

Limited data are available regarding serial follow-up of coagulation parameters in COVID-19 positive patients. Tang *et al* observed a progressive increase in PT and D-dimers over the first 7 days following admission in Chinese patients who did not survive.[7] In contrast, in COVID-19 survivors, both PT and D-dimer levels remained consistent over this period. Similarly, we observed a progressive increase in D-dimer levels in our poor prognostic COVID-19 group, but not in the subgroup who did not require ICU admission **(Figure 1)**. In contrast to the Chinese data however, we observed no progressive increase in PT in the adverse prognostic group **(Figure 1)**. We postulate that these different results probably reflect the fact that our cohort are predominantly Caucasian in origin, and also that our patients were commenced on LMWH at time of admission. Critically, despite the evidence of a progressive COVID-19 coagulopathy over time, none of our cohort maintained on prophylactic LMWH developed systemic DIC. This is evident from the fact that platelet count, APTT and fibrinogen levels do not differ over this time period **(Figure 1).**

In conclusion, our findings demonstrate that severe COVID-19 infection is associated with a significant coagulopathy that correlates with disease severity. The marked increase in D-dimer levels is consistent with progressive coagulation activation, along with concurrent activation of fibrinolysis within the lungs. Critically however, COVID-19 patients on prophylactic dose LMWH do not typically develop overt DIC. In rare COVID-19 cases where DIC does develop, it tends to be restricted to late stage disease. Collectively, these data suggest that the diffuse bilateral pulmonary inflammation observed in COVID-19 is associated with a novel pulmonary-specific vasculopathy which we recently termed pulmonary intravascular coagulopathy (PIC) as distinct to DIC.[17] Although the biological mechanisms underlying COVID-19 pulmonary-vasculopathy remain poorly understood, the ACE-2 receptor utilized by COVID-19 is expressed on both type II pneumocytes and vascular endothelial cells (EC) within the lungs,[3,18] raising the interesting possibility that the pathobiology may include direct pulmonary EC infection, activation and/or damage. In addition, the cytokine storm associated with COVID-19 infection will have major impacts upon thrombin gereneration and fibrin deposition within the lung. In the context of this lung-centric vasculopathy, we hypothesise that the refractory ARDS phenotype observed in severe COVID-19 is due to concurrent *'double-hit'* pathologies targeting both ventilation (V) and perfusion (Q) within the lungs where alveoli and pulmonary microvasculature exist in close anatomical juxtaposition. Our data suggest that, at least at standard prophylactic doses, LMWH does not significantly impact the progressive increase in D-dimer levels observed in patients with severe COVID-19. Further adequately-powered randomised controlled studies will be required to determine whether more intensive anticoagulation and/or targeted anti-inflammatory therapies may be useful in attenuating PIC in selected patients with severe COVID-19.[19] Given that thrombotic risk is significantly impacted by race, coupled with the accumulating evidence that coagulopathy is important in COVID-19 pathogenesis, our findings raise the intriguing possibility that pulmonary vasculopathy may contribute to the unexplained differences that are beginning to emerge highlighting racial susceptibility to COVID-19 mortality.

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AUTHORSHIP

Contribution: HF, LT, CNC, CB, PB, CLB, RG, AG, MB, KR, NOC, NC, JOS & JOD - conception, patient enrollment, data collection and interpretation. All authors contributed to literature review, final draft writing and critical revision. All the authors have participated sufficiently in this work, take public responsibility for the content and have made substantial contributions to this research

Conflict-of-interest disclosure:

J.S.O'D has served on the speaker's bureau for Baxter, Bayer, Novo Nordisk, Boehringer Ingelheim, Leo Pharma, Takeda and Octapharma. He has also served on the advisory boards of Baxter, Bayer, Octapharma CSL Behring, Daiichi Sankyo, Boehringer Ingelheim, Takeda and Pfizer. J.S.O.D has also received research grant funding awards from Baxter, Bayer, Pfizer, Shire, Takeda and Novo Nordisk.

FIGURE LEGENDS

Table 1. Demographics and coagulation parameters of COVID-19 patients on admission and Day +4

P values are for Survivors and non-ICU compared to Non-survivors and/or ICU admission. Data are presented as mean ± standard deviation or median (interquartile range) as appropriate (ns; not significant *; p<0.05, ** p<0.01; *** p<0.001). PT = prothrombin time; APTT = activated partial thromboplastin time.

†Underlying co-morbidities - cardiovascular conditions including hypertension, ischemic heart disease, stroke, type 2 diabetes mellitus and hyperlipidemia were most common. Other conditions included asthma, chronic obstructive pulmonary disease, solid organ or haematological cancer, hyperlipidemia and obesity.

Figure 1. Serial coagulation parameters in COVID-19 patients following admission. Patient data is graphed as the median and interquartile range. Dotted lines represent the upper limit of the local normal range for PT, APTT, D-dimers and fibrinogen levels. Dotted line represents the lower limit of the normal range for platelet count. Survivors and patients not requiring ICU admission are in blue compared to Non-survivors and/or those needing ICU admission in red. As many non-ICU patients had been discharged, no Day +14 data are presented for that group.

TABLE 1: Demographics and Coagulation parameters of COVID-19 patients on admission and Day +4.

Parameters	Normal range	Total (n=83)	Survivors and non-ICU n=50	Non-survivors and/or ICU n=33	P value
Age (years)		62 ± 16.3	60.5 ± 17.7	67.9 ± 11.9	0.02*
Sex (male/female)		55/28	33/17	22/11	
Underlying co-morbidities†		67 (80.7%)	38 (76%)	29 (87.9%)	
On admission					
PT (sec)	9.9-13.1	12.9 (12-14.5)	12.6 (11.7-14.5)	12.9 (12.2-14.5)	0.11
APTT (sec)	24-36	31 (28.2-31.9)	31.3 (29.3-33.1)	30.4 (28.2-32.2)	0.52
Fibrinogen (g/L)	1.9-3.5	4.7 (4.4-6.6)	4.5 (3.7-6.2)	5.6 (4.4-6.6)	0.045*
D-dimer (nanogram/mL)	0-500	732 (553-1580)	804 (513-1290)	1003 (536.5-1782)	0.018*
Platelets (x 10^9/L)	140-450	196 (162.3-227)	201 (161-251)	196 (153-289)	0.47
C-reactive protein (mg/L)	0-5	56.1 (12.5-122.5)	37.9 (7.9-92.1)	94.8 (35-158.5)	0.0005***
Day 4 of admission					
PT (sec)	9.9-13.1	12.8 (12.4-13.8)	12.5 (12.2-14.1)	13.1 (12.5-14.5)	0.007**
APTT (sec)	24-36	31.1 (29.8-33.1)	31.3 (29.5-35.3)	30.8 (28.3-33.4)	0.35

Fibrinogen (g/L)	1.9-3.5	4.9 (4.6-7.6)	5 (3.6-6.5)	5 (4.2-7.1)	0.29
D-dimer (nanogram/mL)	0-500	881 (738.5-3459)	803 (529-1549)	1210 (603.5-3623)	0.003**
Platelets (x 10^9/L)	140-450	212 (153-309)	203 (153-323)	231 (154-328.8)	0.52
C-reactive protein (mg/L)	0-5	71 (33.3-225.7)	46.1 (21-105.9)	107 (58.1-222.5)	0.001**

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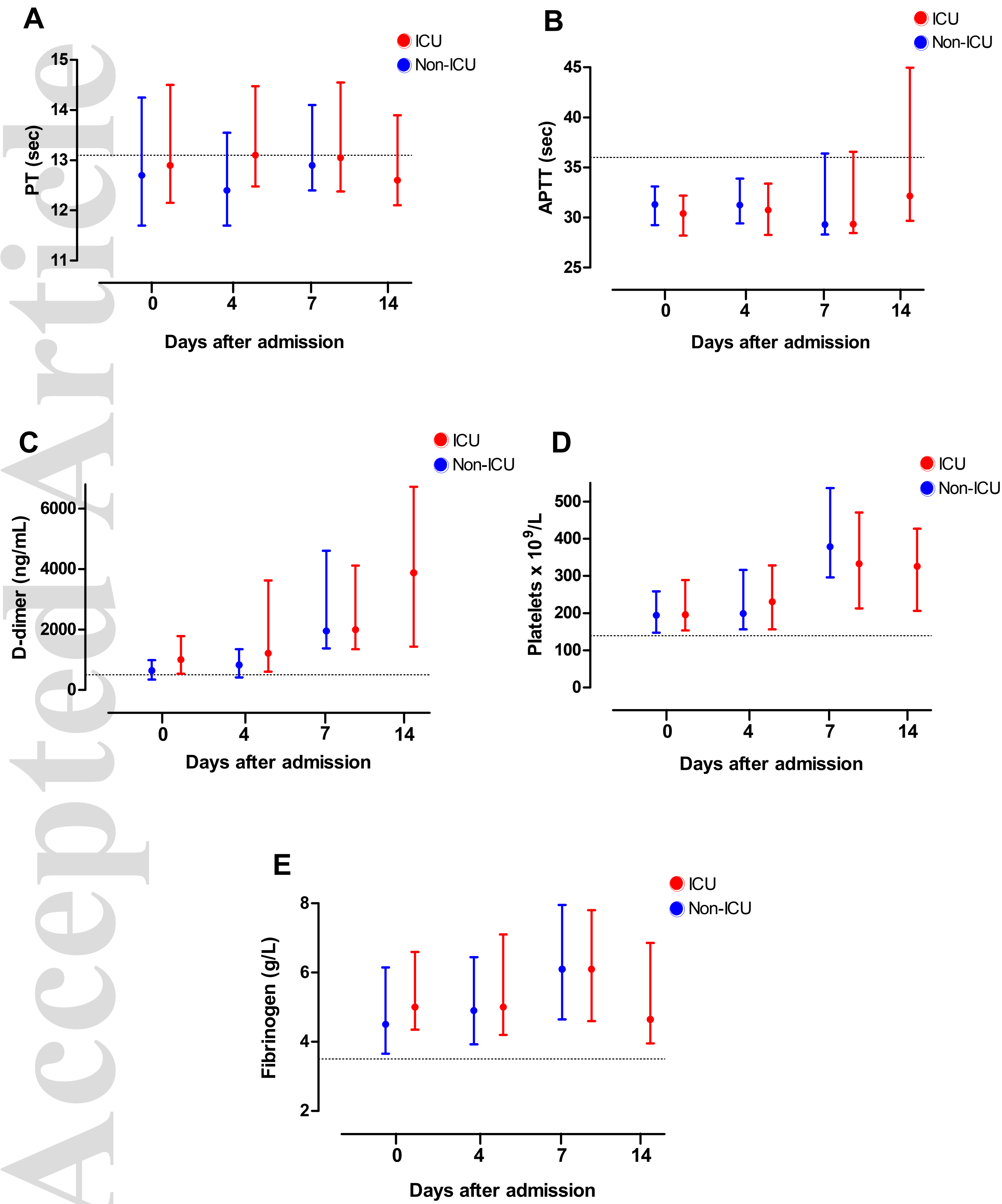
Figure 1


A
ICU
Non-ICU
PT (sec)
15
14
13
12
11
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7
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Days after admission
B
ICU
Non-ICU
APTT (sec)
45
40
35
30
25
0
4
7
14
Days after admission
C
ICU
Non-ICU
D-dimer (ng/mL)
6000
4000
2000
0
0
4
7
14
Days after admission
D
ICU
Non-ICU
Platelets x 10^9/L
500
400
300
200
100
0
0
4
7
14
Days after admission
E
ICU
Non-ICU
Fibrinogen (g/L)
8
6
4
2
0
4
7
14
Days after admission