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Critically ill COVID-19 infected patients exhibit increased clot waveform analysis parameters consistent with hypercoagulability

To the Editor:

Viral acute respiratory infections (ARI) are associated with thrombotic events,[1] and the pathophysiology of this association is multifactorial.[2] Although most ARIs are mild, subpopulation of patients can progress to severe disease with excessive proinflammatory response, and downstream uncontrolled cytokine storm being partially implicated for this severe manifestation.[3] As there is extensive crosstalk between inflammation and coagulation, it is likely the prothrombotic mechanisms in viral ARI could be further exacerbated in patients suffering from severe ARI. The novel coronavirus disease 2019 (COVID-19) is an evolving pandemic. Approximately one-fifth of the infected individuals develops severe to critical disease[4] requiring intensive care support. These critically ill patients often exhibit marked elevation of proinflammatory cytokines and C-reactive protein (CRP)[4] consistent with hyperinflammation. Nonetheless, the effects of COVID-19 infection on haemostatic functions remain unknown at the present moment.

Activated partial thromboplastin time (aPTT)-based clot waveform analysis (CWA) is a form of global haemostatic assay in which lower and higher CWA parameters are associated with bleeding and hypercoagulability respectively.[5] We postulated COVID-19 patients requiring intensive care unit (ICU) support would exhibit haemostatic disturbances and interrogated their aPTT-based CWA parameters as surrogates of their haemostatic functions.

In February 2020, three COVID-19 patients were admitted to ICU of Singapore General Hospital and Sengkang General Hospital, Singapore and we examined their clinical and hematological data. The aPTT tests were performed as part of their routine clinical management. The associated CWA data; maximum velocity (min1), maximum acceleration (min2) and maximum deceleration (max2) were retrieved from the CS2100i and CS2500 automated coagulation analysers (Sysmex Corporation, Kobe, Japan) from the respective hospitals. Dade Actin FSL (Siemens Healthcare, Marburg, Germany) reagent was used.

All three COVID-19 patients in the ICU at the time were included. All three patients did not have any pre-existing malignancy, bleeding or thrombotic conditions and were not on any antithrombotic drugs on admission. Two patients (ages 39 and 64) had no pre-existing conditions and one patient (age 54) had hypertension and hyperlipidemia. None had other superimposed infection or overt disseminated intravascular coagulation by the International Society of Thrombosis and Hemostasis criteria. Their clinical and laboratory features are summarized in Table 1. Only patient 2 had a single D-dimer value of 0.64 mg/L fibrinogen equivalent unit (FEU) (normal range: 0.19-0.55 mg/L FEU) performed upon the ICU admission. Whilst aPTT showed mild prolongation in most of the results and no biphasic waveform was noted, analyses of their CWA revealed interesting findings. All three patients had elevated min1 when their clinical conditions deteriorated to the point of requiring ICU support. Furthermore, all their CWA parameters became markedly raised as their ICU stay progressed. Serial CWA data for patient 1 shed some interesting light on how the overall dynamic haemostatic status changed with clinical deterioration; from fairly normal CWA on initial hospitalization to having markedly raised parameters in ICU. This suggested a positive association between the rise in CWA parameters and the worsening severity of COVID-19 infection.

On day 1 of hospitalization, although patient 1 had CWA parameters within normal intervals, COVID-19 infection resulted in some interesting differences in CWA, compared to CWA findings caused by other infections. Compared with other common viral ARI and bacterial infection,[7] min1 in COVID-19 infection was higher than other ARIs and tracked closer to bacterial infection. However, min2 and max2 were lower than the other infections. This suggests that COVID-19 infection causes dissimilar haemostatic derangement, even in non-critical cases, compared to other ARI. The higher min1 value could possibly suggest an overall elevated prothrombotic state in COVID-19 infection given our previous finding that min1, in comparison to min2 and max2, is more strongly associated with thrombotic events.[5]

Consistent with published reports,[4] all the patients expressed high CRP levels even on initial assessment during hospitalization, while procalcitonin remained unremarkable (data not shown). We did not observe a definitive pattern of association between CRP and ICU admission - patient 3 had a CRP upon ICU admission that was lower than that of patient 1 upon initial hospitalization. In contrast, as these patients' clinical conditions turned critical, min1 seemed to be the first CWA parameter to rise, with all three patients exhibiting raised min1 upon ICU admission. This raises the exciting possibility that high min1

TABLE 1 Laboratory results and clinical features of COVID-19 infected patients in reference to the normal intervals of clot waveform analysis (CWA) parameters and CWA data of patients with bacterial and other viral acute respiratory tract infections.

Parameters	Reference Intervals	Bacterial Infection#	Other Viral ARI>	Results of Patients with COVID-19 Infection								
				Patient 1					Patient 2			Patient 3
CRP, mg/L	0.2 – 9.1			178.8	221.0	315.5	311.0	272.0	218.8	94.4	99.6	140
Procalcitonin, µg/L	< 0.50			0.35	0.30	0.48	0.39	0.50	0.51	0.37	0.37	0.63
Platelet, x 10^9/L	140 – 440			280	332	352	293	364	232	333	381	169
PT, s	9.9 – 11.4			10.4	10.9	10.4	10.7	11.2	10.8	10.0	10.4	9.9
APTT, s	25.7 – 32.9	33.0 ± 7.3	30.0 ± 3.9	38.4	36.7	37.4	34.3	33.3	36.5	26.5	24.6	34.5
Min1, %/s	3.12 – 6.87*	6.92 ± 1.02	6.19 ± 1.32	6.74	7.52	7.93	8.28	9.18	6.97	9.35	10.49	7.04
Min2, %/s²	0.51 – 1.05*	1.04 ± 0.28	0.95 ± 0.21	0.90	1.08	1.14	1.22	1.42	0.94	1.49	1.76	1.00
Max2, %/s²	0.40 – 0.91*	0.82 ± 0.24	0.73 ± 0.18	0.67	0.81	0.92	1.04	1.20	0.72	1.27	1.53	0.73
Day of hospitalisation				1st	3rd	4th	5th	6th	3rd	5th	6th	3rd
Clinical features				Admission to hospital	Admission to ICU	In ICU^	In ICU^	In ICU^	Admission to ICU	In ICU^	In ICU^	Admission to ICU

* Reference intervals were established locally based on 191 healthy individuals in accordance with the Clinical and Laboratory Standards Institute Guidelines.

\# Based on data collected from 52 patients with bacterial infection (manuscript under review).[14] Data presented in mean ± standard deviation.

> Based on data collected from 13 patients with common viral ARI (manuscript under review).[14] Data presented in mean ± standard deviation.

^ On daily enoxaparin 40mg.

Abbreviations: CRP, C-reactive protein; ARI, acute respiratory tract infection; ICU, intensive care unit.

may be a useful biomarker to predict severity in COVID-19 infected patients, but further study is needed.

From the second day of ICU admission onward, all the CWA parameters became markedly elevated for at least the ensuing 4 days of ICU stay. Many of the levels were at least as high as what we noted in acute venous thromboembolism, which had an odds ratio of four or greater for thrombotic events.[5] Of note, although none of our cases developed thrombosis, our patients' CWA parameters remained remarkably high despite the use of thromboprophylaxis during their ICU stay. It is possible that CWA and other thrombin generation assays might not be sensitive enough to detect the haemostatic changes caused by the standard prophylactic dose of low molecular weight heparin. All three patients recovered from COVID-19 infection.

Thatour findings of markedly raised CWA parameters in critically ill infected cases are possibly consistent with hypercoagulability is not unexpected. Such patients exhibit hyperinflammation and cytokine overdrive, and extensive crosstalk is known to exist in the cytokines, the inflammatory system, and coagulation.[6] Critically ill COVID-19 patients have been shown to have increased proinflammatory cytokines including IL-2 and TNF-α,[4] and these factors could upregulate the coagulation system.[6] We speculate that this could partially account for the CWA changes observed.

Although our findings are limited by the relatively few patients and data points and by the lack of other correlation studies with other coagulation assays, we believe there are still valuable points to take away. Many of the specialized and research haemostatic assays cannot be safely and easily performed on samples collected from COVID-19 patients in view of laboratory biosafety concerns. As COVID-19 infection is spreading relentlessly worldwide, there is an urgent need for rapid and readily accessible biomarkers that can aid clinical stratification and management. So, CWA represents a simple, automated and rapid test, which fulfills these biosafety criteria. Whenever an aPTT is performed, an aPTT waveform is generated automatically by commonly used optical analysers worldwide.

In conclusion, the rise of CWA parameters precedes and coincides with ICU admission and warrant further study to confirm its utility in the routine management of COVID-19 patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Chuen Wen Tan[1,2] (iD), Jenny Guek Hong Low[3,4], Wan Hui Wong[1], Ying Ying Chua[3,5], Sher Li Goh[2], Heng Joo Ng[1]

[1]Department of Haematology, Singapore General Hospital, Singapore
[2]Department of Pathology, Sengkang General Hospital, Singapore
[3]Department of Infectious Diseases, Singapore General Hospital, Singapore
[4]Programme in Emerging Infectious Diseases, Duke NUS Medical School, Singapore
[5]Department of Infectious Diseases, Sengkang General Hospital, Singapore

Correspondence

Chuen W. Tan, Department of Hematology, Singapore General Hospital, Outram Road, Singapore 169608.
Email: tan.chuen.wen@singhealth.com.sg
DOI 10.1002/ajh.25822

ORCID

Chuen Wen Tan https://orcid.org/0000-0003-0643-8460

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Blood and blood product use during COVID-19 infection

To the Editor:

The COVID-19 pandemic is worsening in severity globally. Singapore confirmed its first imported case of COVID-19 on 23 Jan 2020. As of 29 March 2020, Singapore had 844 confirmed cases of COVID-19 infection with the majority being treated in the National Centre for Infectious Diseases (NCID). Blood and blood products are precious resources and the World Health Organization (WHO) has released guidelines on the management of blood supply in response to the COVID-19 pandemic.[1] We evaluated the indications and requirements of blood and blood products in patients with COVID-19 infection at the NCID, as well as the monthly blood component usage at HealthCity Novena; a central campus comprising of the NCID, Renci Community Hospital and Tan Tock Seng Hospital, a 1700-bed acute care hospital.

Out of 572 patients with COVID-19 infection, 19 required ICU treatment. Nine out of the 572 cases required transfusions; five were male and four were female, and the median age was 70 years. None had clinical evidence of hemolysis. Here we present the clinical profiles of the nine patients who required transfusion (Figure 1).

Patient 1 was a 70-year-old male with hypertension and diabetes. He was admitted to the Intensive Care Unit (ICU) for COVID-19 pneumonia complicated by acute respiratory distress syndrome (ARDS). He suffered a non-ST-elevation myocardial infarction, atrial fibrillation, a cardioembolic stroke and acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT). There was no overt bleeding. Packed red blood cell (PRBC) transfusions were initiated at the ICU physician's discretion due to hemoglobin decreasing to as low as 8.1 g/dL.

Patient 2 was a 39-year-old male with no significant medical history. He was admitted for severe COVID-19 pneumonia complicated by ARDS, septic shock, AKI requiring CRRT and multi-organ failure. He suffered recurrent coffee ground nasogastric tube aspirates and was treated conservatively with intravenous esomeprazole infusions, with plans for endoscopy in the event of massive bleeding. Bone marrow examination performed for evaluation of pancytopenia showed adequate trilineage hematopoiesis. The cause of his anemia was multifactorial and attributed to anemia of inflammation and gastrointestinal bleeding. He required a total of 24 units of PRBC and 1 unit of pooled platelets.

Patient 3 was a 64-year-old male with asthma, hypertension, hyperlipidemia and ischemic heart disease. He was admitted for severe COVID-19 pneumonia with ARDS and AKI requiring CRRT. Veno-venous extracorporeal membrane oxygenation (ECMO) was initiated with no requirements for transfusions but was then abandoned when the patient developed major bleeding. Fresh frozen plasma (FFP) and pooled platelets were transfused but the patient succumbed to multi-organ failure.

Patient 4 was a 74-year-old with hypertension, hyperlipidemia, previous transient ischemic attack and hypertensive nephrosclerosis. He was admitted for severe COVID-19 pneumonia with ARDS and developed ST-elevation myocardial infarction. Coronary angiography revealed triple vessel disease, and he was placed on medical therapy with plans for coronary artery bypass grafting when stable. Dual antiplatelet agents and low molecular weight heparin were started; thereafter he developed a drop in hemoglobin requiring 2 units of PRBC.

Patient 5 was a 77-year-old female with hypertension, hyperlipidemia, ischemic heart disease and pan-atrophic gastritis confirmed on endoscopy in 2019. She was admitted for severe COVID-19 pneumonia with ARDS, AKI requiring CRRT, melena secondary to severe gastritis on endoscopy and right sided colitis. She also developed limb gangrene and a distal limb deep vein thrombosis. She required 9 units of PRBC and unfortunately succumbed to COVID-19 pneumonia with multiorgan failure.

Patient 6 was an 86-year-old female with hypertension. She was admitted for severe COVID-19 pneumonia complicated by ARDS, acute liver and kidney injury. She had worsening anemia attributed to the severe infection and developed bleeding from a central line site, requiring 2 units of PRBC.

Patient 7 was a 71-year-old male with type 2 diabetes mellitus. He was admitted for severe COVID-19 pneumonia with ARDS complicated by right upper limb deep vein thrombosis secondary to a right