Platelet aggregates, a marker of severe COVID-19 disease

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ABSTRACT
Thrombocytopenia is common in an intensive care unit (ICU) setting due to endogenous and iatrogenic factors. Despite that, thrombocytopenia in patients with severe COVID-19 infections is surprisingly uncommon. By examining the blood film of 20 ICU patients with COVID-19, we observed the presence of platelet aggregates and macrothrombocytes indicating increased platelet activity. We compared these findings with 20 blood films of non-severe COVID-19 cases where these findings were absent. These morphology features could be consistent with severe COVID-19 infection and is further evidence of the important role that platelets play when COVID-19 manifests with thrombotic complications or respiratory failure.

Significant thrombocytopenia in patients with severe COVID-19 infection is surprisingly uncommon, with only around 5% of hospitalised patients and 8% of those on the intensive care unit (ICU) developing a platelet count below 100×10^9/L.[1] This is inconsistent with our expectation of patients with serious infective/inflammatory conditions where endogenous and iatrogenic factors affect the platelet count; for example, liver impairment, sepsis, heparin, antibiotics, antivirals and other commonly used agents.[2]

The coagulopathy associated with COVID-19, with very elevated D-dimers, is different from classic disseminated intravascular coagulation (DIC), where platelet and fibrinogen levels fall as a result of consumption in the coagulation process. Autopsies from patients who have died with COVID-19 pneumonia show microvascular thrombosis throughout the small vessels of the lungs and alveolar capillaries, suggesting thrombi are likely to be the end point of localised inflammation.[3] This inflammation points toward an immune response which can lead to dysregulation of P-selectin, fibrin, von Willebrand factor and d-dimers resulting in a hypercoagulable state.[4 5] Overt DIC can also develop after prolonged hospitalisation, while a fall in platelet count has been associated with severe COVID-19 disease, high mortality and indicates a high intravascular clotting risk.[4 6–9]

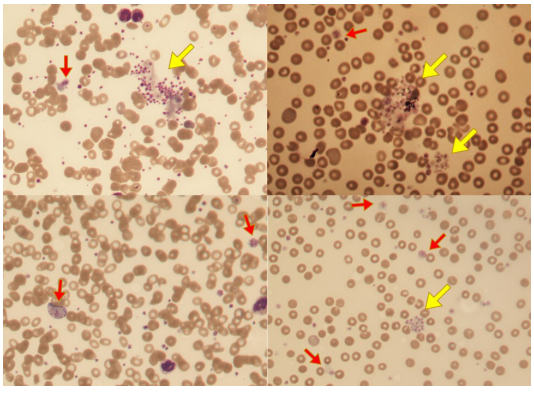

Figure 1 Peripheral films showing platelet aggregates (yellow arrows) and macrothrombocytes (red arrows).

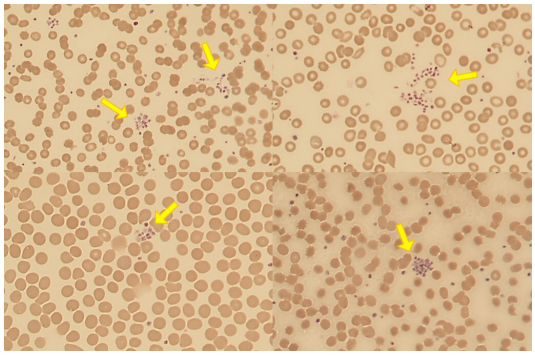

Figure 2 Peripheral films showing platelet aggregates (yellow arrows).

To help understand the relative preservation of the platelet count, we examined 20 random blood films from patients undergoing invasive ventilation comparing those to 20 random films from stable patients with COVID-19 who had ward-based care or were discharged after the initial assessment, and identified the nadir platelet count reached by all patients positive for COVID-19 hospitalised in our centre. The diagnosis of COVID-19 was made either via a throat swab or PCR of bronchioalveolar lavage following high clinical suspicion.

There were 575 hospitalised patients with confirmed COVID-19 from 10 March 2020 to 20 June 2020 in Oxford University Hospitals. Overall, the nadir platelet counts are shown in table 1. Only 8% had platelet counts $<100\times10^9$/L and 2.2% had counts $<50\times10^9$/L. Even in non-survivors, significant thrombocytopenia was only seen in 6% and in 9% of ICU patients with COVID-19.

Examination of the peripheral blood film (figure 1) showed the presence of macrothrombocytes in all films, with median Mean Platelet Volume (MPV) of 10.65 fL (IQR: 10.35–11.47), dense granules and occasional large proplatelet fragments. Large platelet aggregates of around 7–30 platelets throughout the film were seen in all the blood films from the ICU patients (figures 1 and 2). This finding was not present in any of the 20 comparative blood films from stable

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Table 1 Nadir platelet count of patients diagnosed with COVID-19 treated in ICU and non-ICU (ward)

Platelet count	<50×10^9/L	50–100×10^9/L	100–150×10^9/L	>150×10^9/L	Total
Ward survivors	2	10	54	275	341
Ward non-survivors	4	17	40	98	159
Ward total	**6**	**27**	**94**	**373**	**500**
ICU survivors	0	2	7	41	50
ICU non-survivors	7	3	3	12	25
ICU total	**7**	**5**	**10**	**53**	**75**
Grand total	**13**	**32**	**104**	**426**	**575**

ICU, intensive care unit.

patients with COVID-19, while their MPV was 10.3 fL (IQR: 9.91–11.25).

All ICU patients had a degree of anaemia with a median haemoglobin of 86.5 g/L (IQR: 72.25–98.25), with polychromasia and nucleated red blood cells present in all films. None of the films showed schistocytes. Their duration of illness and relevant medication are shown in table 2.

This study suggests a high turnover of platelets, with macrothrombocytes consistent with increased production, and the relative preservation of the platelet count due to a balance of increased consumption, in microangiopathic thrombosis, and production, likely driven by the effect of the cytokine storm enhancing hepatic production of thrombopoietin. The dense granules and platelet aggregates present in all ICU patients suggest heightened platelet activity, and the absence of red cell fragments is in keeping with the microvascular thrombi being a result of inflammation rather than a typical thrombotic microangiopathy. These changes can be seen in ICU patients, but they are almost always associated with DIC and concurrent thrombocytopenia.[10]

The large proplatelet fragments may indicate abnormal fragmentation of megakaryocytes. The lungs have been identified as a primary site of terminal platelet production, accounting for approximately 50% of total platelet production.[11] One could postulate that the damage to the lung results in disordered megakaryocyte fragmentation or disruption of the normal filtration of megakaryocytes in the pulmonary circulation, leaving increased megakaryocytes in the blood. Indeed, a large number of megakaryocytes have been found in the pulmonary capillaries at autopsy.[12] Additionally, megakaryocytes are a rich source of cytokines and growth factors that have the potential to influence inflammatory or fibrotic lung diseases. Interestingly RNA-sequence analysis has revealed that lung megakaryocytes are skewed toward an innate immunity function.[13] Mechanical ventilation can aggravate this process and lead to increased inflammation as the inflation and deflation of the lung can induce proplatelet cleavage and release of proinflammatory particles.[14]

Despite the relatively normal platelet counts seen in patients with severe COVID-19 disease, blood film appearances support significant involvement of platelets in the disease process. The presence of platelet aggregates may be a useful marker of worsening disease.

Table 2 Intensive Treatment Unit (ITU) patient characteristics that had blood film examined

Characteristics	
Age (years, median)	62 (IQR: 55–74)
Antiplatelets (no of patients)	4/20
Anticoagulation (no of patients)	0/20
Duration of illness (days, median)	9 (IQR: 8–16)
Previous history of arterial or venous thrombosis (no of patients)	0/20

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