BRIEF REPORT

OPEN

Time-Dependent Changes in Pulmonary Turnover of Thrombocytes During Critical COVID-19

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OBJECTIVES (BACKGROUND): Under normal conditions, pulmonary megakaryocytes are an important source of circulating thrombocytes, causing thrombocyte counts to be higher in arterial than venous blood. In critical COVID-19, thrombocytes may be removed from the circulation by the lungs because of immunothrombosis, possibly causing venous thrombocyte counts to be higher than arterial thrombocyte counts. In the present study, we investigated time-dependent changes in pulmonary turnover of thrombocytes during critical COVID-19 by measuring arteriovenous thrombocyte differences. We hypothesized that the early stages of the disease would be characterized by a net pulmonary removal of circulating thrombocytes because of immunothrombosis and that later stages would be characterized by a net pulmonary release of thrombocytes as normal pulmonary function is restored.

DESIGN: Cohort study with repeated measurements of arterial and central venous thrombocyte counts.

SETTING: ICU in a large university hospital.

PATIENTS: Thirty-one patients with critical COVID-19 that were admitted to the ICU and received invasive or noninvasive mechanical ventilation.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: We found a significant positive association between the arteriovenous thrombocyte difference and time since symptom debut. This finding indicates a negative arteriovenous thrombocyte difference and hence pulmonary removal of thrombocytes in the early stages of the disease and a positive arteriovenous thrombocyte difference and hence pulmonary release of thrombocytes in later stages. Most individual arteriovenous thrombocyte differences were smaller than the variance coefficient of the analysis.

CONCLUSIONS: The results of this study support our hypothesis that early stages of critical COVID-19 are characterized by pulmonary removal of circulating thrombocytes because of immunothrombosis and that later stages are characterized by the return of normal pulmonary release of thrombocytes. However, in most cases, the arteriovenous thrombocyte difference was too small to say anything about pulmonary thrombocyte removal and release on an individual level.

KEYWORDS: blood platelets; COVID-19; megakaryocytes; respiratory insufficiency; thromboinflammation

It was first observed almost a century ago that thrombocyte counts are normally higher in arterial than in venous blood (1–3), which reflects the fact that pulmonary megakaryocytes produce up to 50% of circulating thrombocytes (4–6). A hallmark in the pathophysiology of critical COVID-19 is immunothrombosis, a process where activated thrombocytes interact with leukocytes and coagulation factors in the lungs and induce a dysregulated

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KEY POINTS

Question: Does the difference between arterial and central venous thrombocyte counts change with time in patients with critical COVID-19 as a sign of early pulmonary removal of circulating thrombocytes and later pulmonary release of thrombocytes?

Findings: In this cohort study on patients with critical COVID-19 receiving mechanical ventilation, we found a statistically significant positive association between the arteriovenous thrombocyte difference and time since symptom debut.

Meaning: Our findings suggest that early stages of critical COVID-19 are characterized by pulmonary removal of circulating thrombocytes and that later stages are characterized by the restoration of normal pulmonary release of thrombocytes.

inflammation that contributes to respiratory failure (7). In COVID-19, thrombocytopenia is associated with disease severity (8), and it has been suggested that immunothrombosis contributes to thrombocyte consumption in this disease (9). During COVID-19, the lungs may therefore cause a net removal of thrombocytes from the circulation, as opposed to the normal situation where the lungs release circulating thrombocytes.

Accurate measurements of pulmonary removal and release of circulating thrombocytes are difficult to obtain, but in critically ill patients with arterial and central venous catheters installed, blood entering and leaving the lungs is readily available. In the present study, we used the arteriovenous difference in thrombocyte counts (AV_{diff}) to investigate time-dependent pulmonary removal and release of thrombocytes during critical COVID-19. We hypothesized that early stages of the disease would be characterized by pronounced immunothrombosis and hence a net pulmonary removal of circulating thrombocytes and that later stages would be characterized by a net pulmonary release of thrombocytes as immunothrombosis resolves and normal pulmonary thrombocyte production is restored.

MATERIALS AND METHODS

Critically ill patients with COVID-19 admitted to ICU at Oslo University Hospital Ullevål, Oslo, Norway, were included. Inclusion criteria were age higher than 18 years, critical disease because of laboratory-confirmed COVID-19, and presence of arterial and central venous catheters. Critical disease was defined as severe COVID-19 (Pao_2/Fio_2 ratio < 300 mm Hg) with a need for invasive or noninvasive mechanical ventilation at the time of inclusion (10). No patients in the study received extracorporeal membrane oxygenation treatment.

Blood Sampling

Blood was sampled as soon as possible after inclusion and then preferably twice weekly while the patient was admitted to the ICU. Because of varying hospital capacity in the region during the COVID-19 pandemic, transfer of patients between hospitals was common. For this reason, there were sometimes considerable delays from ICU admission in another hospital to study inclusion in our hospital. Some patients were also transferred to other hospitals after inclusion and were not observed with blood samples until ICU discharge.

Blood was sampled in randomized order from arterial and central venous catheters into 4 mL 4.4 mmol/L K2 EDTA tubes. Thrombocytes were then automatically counted with impedance or fluorescence using a Sysmex XN 9000 instrument (Sysmex Europe, Norderstedt, Germany). Variation coefficients were less than 12% and less than 6% for thrombocyte counts under and over 100×10^9/L, respectively. Immediately after EDTA blood sampling, an arterial blood gas sample was obtained. Other biochemical analyses were registered from the routine blood samples obtained on the same day.

Statistics

The AV_{diff} was calculated as the percentage change in thrombocyte counts from central venous to arterial blood. Associations between the AV_{diff} and other variables were assessed using linear mixed models with random intercepts and fixed slopes, with the patient as the grouping variable. The coefficient β with 95% CI and *p* value for the independent variable is reported.

p values of less than 0.05 were considered statistically significant. Data were analyzed with Stata/SE 17.0 (StataCorp, College Station, TX).

Ethics

All patients were included in the Norwegian SARS-CoV-2 Study (NCT04381819), which was approved by the Regional Committee for Medical and Health Research Ethics on March 2, 2020 (ref. 106624). Before inclusion, an informed consent was obtained from the patient or the next of kin if the patient was unable to consent. The project was conducted in accordance with the approval of the institutional review board and the Helsinki Declaration of 1975.

RESULTS

During the study period, 36 patients were considered eligible. Four patients or their next of kin opposed participation, and one patient was excluded because central venous blood could not be sampled. The remaining 31 patients were included in the data analysis. Thrombocyte counts and other patient data are summarized in **Table 1**.

A positive association between the AV_{diff} and time since symptom debut was found (β = 0.11 [0.03–0.19]; p = 0.008; **Fig. 1*A***). To test if the association was biased because patients who survived were observed for longer and had less severe disease than patients who died, the analysis was repeated for patients who survived only. In this analysis, the association between the AV_{diff} and time from symptom debut remained in the same range (β = 0.15 [0.02–0.27]; p = 0.022).

To further investigate associations between the AV_{diff} and immunothrombosis, biochemical markers of inflammation (leukocytes, neutrophil granulocytes, lymphocytes, ferritin, and C-reactive protein [CRP]) or coagulation (international normalized ratio [INR], activated partial thromboplastin time [APTT], fibrinogen, and D-dimer) were included as the dependent variable. Negative associations between the AV_{diff} and CRP ($\beta = -9.5\times10^{-3}$ [-1.7×10^{-2} to -1.7×10^{-3}]; p = 0.017) and APTT (β = –0.14 [–0.23 to –0.05]; p = 0.003) were found (**Fig. 1*B***).

To investigate associations between the AV_{diff} and respiratory function, we included arterial oxygen saturation, Pao_2, or Pao_2/Fio_2 ratio as the dependent variable. No associations between the AV_{diff} and respiratory function were found.

TABLE 1.
Patient Characteristics, Thrombocyte Counts, and Other Patient Data

Patients (n = 31)	Value
Male sex (n)	23 (74 ± 8)
Age (yr)	61 (31-77)
Died before hospital discharge (n)	10 (32 ± 8)
Time to first blood sampling	
From symptom debut (d)	17 (6-51)
From hospital admission (d)	9 (1-36)
From ICU admission (d)	7 (1-34)
Blood sample pairs per patient (n)	2 (1-6)
Blood sample pairs (n = 73)	**Value**
Site of arterial blood sampling	
Radial artery (n)	64 (88 ± 4)
Brachial artery (n)	9 (12 ± 4)
Site of central venous blood sampling	
Superior vena cava (n)	70 (96 ± 2)
Common iliac artery (n)	3 (4 ± 2)
Thrombocyte counts	
Arterial (× 10^9/l)	271 (27-780)
Central venous (× 10^9/l)	277 (26-826)
AV_{diff}	
Measured in × 10^9/l	1 (-46 to +31)
Measured in %	0.5 (-15.6 to +10.1)
Larger than analytical variation coefficient (n)	6 (8 ± 3)

Characteristics of patients included in the study (n = 31) and sites of blood sampling, thrombocyte counts, and arteriovenous thrombocyte differences (AV_{diff}) for the total number of blood sample pairs collected (n = 73). Continuous variables are expressed as median (range). Categorical variables are expressed as absolute number (percent of total ± SE).

DISCUSSION

In this study, we found a positive association between the AV_{diff} and time since symptom debut, which suggests that time-dependent changes in pulmonary turnover of thrombocytes occur during critical COVID-19. This supports our hypothesis that early stages of the disease are characterized by pulmonary

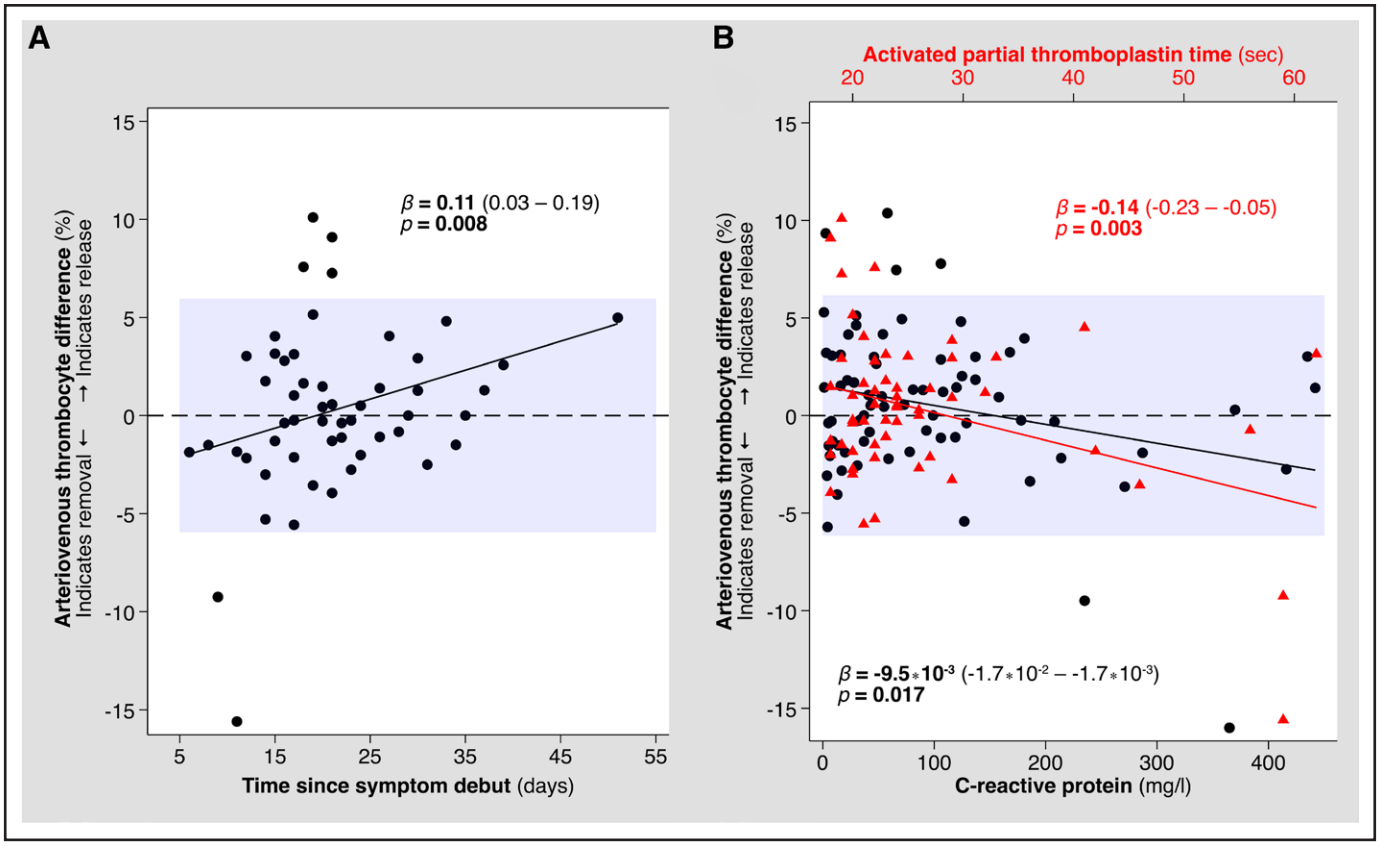

Figure 1. Associations between arteriovenous thrombocyte difference and other variables, with mixed models regression lines, coefficients, 95% CIs, and corresponding *p* values. The *light blue backgrounds* indicate the variance coefficient of 6% for thrombocyte counts greater than 100×10^9/L. In all six cases where the arteriovenous thrombocyte difference was greater than ± 6%, arterial thrombocyte counts were greater than 100×10^9/L. **A**, Arteriovenous thrombocyte difference and time since symptom debut. **B**, Arteriovenous thrombocyte difference, C-reactive protein, and activated partial thromboplastin time that were measured on the same day. *Black dots* indicate measurements of C-reactive protein and *red triangles* indicate measurements of activated partial thromboplastin time.

removal of circulating thrombocytes because of pronounced immunothrombosis and that later stages are characterized by the gradual return of normal pulmonary release of thrombocytes. We also found negative associations between the AV_{diff}, CRP, and APTT, which further suggests a link between more pulmonary removal of thrombocytes and increasing immunothrombosis. However, most measurements of the AV_{diff} were smaller than the analytical variance coefficient. While this does not preclude significant associations on the group level, the AV_{diff} can, in most cases, not be used to say anything about the degree of pulmonary removal or release of thrombocytes on an individual level.

Our results are very similar to the results from a previous study conducted on patients requiring mechanical ventilation because of acute respiratory failure of different etiologies (11). In that study, a shift from a negative to a positive AV_{diff} was found 4 days after intubation, suggesting that time-dependent changes in pulmonary turnover of thrombocytes are similar in COVID-19 and acute respiratory failure of other causes.

The association between thrombocytopenia and disease severity in COVID-19 is well established, but the causal relation between the two remains elusive (8, 12). Because we found a net pulmonary removal of thrombocytes in the stage of COVID-19 where immunothrombosis is presumably most pronounced, we speculate that immunothrombosis with pulmonary thrombocyte removal explains the association between COVID-19 severity and thrombocytopenia (9).

While our findings can be explained by an early immunothrombosis that is gradually resolved, other mechanisms may have contributed: During COVID-19, the number of pulmonary megakaryocytes is increased (13), but their ability to produce thrombocytes may nevertheless be impaired (14), which could

contribute to a more negative AV_{diff}. Additionally, it has been shown in mice with sepsis that thrombocytes trapped in the lungs can return to the circulation (15). If this also happens in humans with COVID-19, a more positive AV_{diff} in later stages of the disease could be expected. It is not possible to derive from our data which mechanisms contributed more to our findings.

We found no association between the AV_{diff} and respiratory function. It is possible that the degree of immunothrombosis and thrombocyte removal by the lungs is not directly related to the degree of respiratory failure. However, an association between Pao_2/Fio_2 ratio and the formation of neutrophil extracellular traps, an essential component in immunothrombosis, has previously been found in COVID-19 (16). Most likely, we observed no similar association because the AV_{diff} is not a sufficiently accurate measure of pulmonary thrombocyte turnover.

This study has several limitations. The number of patients is low, and many patients were transferred between our hospital and other hospitals during their critical disease, which rendered our longitudinal dataset somewhat incomplete. Additionally, venous blood was primarily sampled from the superior vena cava and not from the pulmonary arteries. Deposits of thrombocytes have been found in the kidneys, liver, and spleen in patients with lethal COVID-19 (17), and thrombocyte removal or release by organs draining blood to the inferior vena cava could cause changes in the AV_{diff} that we would not be able to distinguish from AV_{diff} changes caused by the lungs. We do, however, note that our results are very similar to the results obtained by Huval et al (6), where venous blood was sampled from the pulmonary arteries.

CONCLUSIONS

In this study on patients with critical COVID-19, we found a positive association between AV_{diff} and time since symptom debut. This finding supports our hypothesis that early stages of the disease are characterized by immunothrombosis with pulmonary removal of thrombocytes from the circulation and that later stages are characterized by the restoration of normal pulmonary release of thrombocytes. However, in most cases, the AV_{diff} was too small to say anything about pulmonary thrombocyte removal or release on an individual level.

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REFERENCES

1. Tocantins LM: Arterial, venous and cutaneous blood platelet counts in men and dogs under normal and abnormal conditions. *Am J M Sc* 1936; 192:150–151
2. Howell WH, Donahue DD: The production of blood platelets in the lungs. *J Exp Med* 1937; 65:177–203
3. Kallinikos-Maniatis A: Megakaryocytes and platelets in central venous and arterial blood. *Acta Haematol* 1969; 42:330–335
4. Lefrançais E, Ortiz-Muñoz G, Caudrillier A, et al: The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature* 2017; 544:105–109
5. Lefrançais E, Looney MR: Platelet biogenesis in the lung circulation. *Physiology (Bethesda)* 2019; 34:392–401
6. Zucker-Franklin D, Philipp CS: Platelet production in the pulmonary capillary bed: New ultrastructural evidence for an old concept. *Am J Pathol* 2000; 157:69–74

7. Bonaventura A, Vecchié A, Dagna L, et al: Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nat Rev Immunol* 2021; 21:319–329
8. Lippi G, Plebani M, Henry BM: Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. *Clin Chim Acta* 2020; 506:145–148
9. Xu P, Zhou Q, Xu J: Mechanism of thrombocytopenia in COVID-19 patients. *Ann Hematol* 2020; 99:1205–1208
10. Wu Z, McGoogan JM: Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; 323:1239
11. Huval WV, Lelcuk S, Shepro D, et al: Role of serotonin in patients with acute respiratory failure. *Ann Surg* 1984; 200:166–172
12. Pranata R, Lim MA, Yonas E, et al: Thrombocytopenia as a prognostic marker in COVID-19 patients: Diagnostic test accuracy meta-analysis. *Epidemiol Infect* 2021; 149:e40
13. Gelon L, Fromont L, Lefrançais E: Occurrence and role of lung megakaryocytes in infection and inflammation. *Front Immunol* 2022; 13:1029223
14. Shan C, Yu F, Deng X, et al: Biogenesis aberration: One of the mechanisms of thrombocytopenia in COVID-19. *Front Physiol* 2023; 14:1100997
15. Cloutier N, Allaeys I, Marcoux G, et al: Platelets release pathogenic serotonin and return to circulation after immune complex-mediated sequestration. *Proc Natl Acad Sci U S A* 2018; 115:E1550–E1559
16. Middleton EA, He XY, Denorme F, et al: Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood* 2020; 136:1169–1179
17. Schurink B, Roos E, Radonic T, et al: Viral presence and immunopathology in patients with lethal COVID-19: A prospective autopsy cohort study. *Lancet Microbe* 2020; 1:e290–e299