Received: 7 July 2021 | Revised: 24 August 2021 | Accepted: 4 September 2021

DOI: 10.1111/ijlh.13705

ISLH International Journal of Laboratory Hematology WILEY

LETTER TO THE EDITOR

Change in platelet indices in patients with Coronavirus disease-2019 (COVID-19): A reflection of platelet activation and contribution to immunothrombosis?

Dear Editors,

In December 2019, several cases of pneumonia of unknown aetiology were reported in Wuhan, China. The causative coronavirus was subsequently isolated and named the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the disease, coronavirus disease-2019 (COVID-19).[1] COVID-19-related coagulopathy is a poor prognostic feature and reflects an underlying pathological process termed immunothrombosis.[2] This process differs from infection-related disseminated intravascular coagulation (DIC) as it manifests primarily with thrombosis while thrombocytopenia and bleeding remain modest.[3] The coagulation activation in COVID-19 relates to endothelial dysfunction, complement activation and ensuing cytokine storm with contributions from secondary infections and organ dysfunction.[1,2] D-dimer values above 0.5 mg/L signify poor patient outcomes.[3,4]

Platelets are essential for normal haemostasis and become activated when exposed to the sub-endothelial matrix at sites of vessel injury with the formation of platelet plugs, which support coagulation factor rich thrombi.[5] Platelets are however also inflammatory cells with key innate and adaptive functions displaying several pattern recognition receptors (PRR) and serve as first-line responders in the defence against pathogens, including viruses.[6]

Viral infections cause platelet activation through different pathophysiological processes including direct interactions with the viral pathogen, effects of inflammatory mediators such as interleukin-3 and interleukin-6 and exposure to viral antigen-antibody complexes.[6] Platelets in turn release chemokines which promote endothelial signalling and leukocyte tissue migration.[6] There are several ways to monitor platelet activation in the laboratory including flow cytometric analysis of platelet activation markers. Platelet activation however also alters routine laboratory platelet indices such as mean platelet volume (MPV), platelet-large cell ratio (P-LCR), platelet distribution width (PDW) and platelet crit (PCT).[7] A proportion of platelets also consists of "young," hyper-reactive reticulated platelets, which can be quantified in the laboratory as the immature platelet fraction (%IPF).[8] Changes in these indices reflect an increased average size and density of platelets as the proportion of immature platelets with greater prothrombotic activity increases in response to infection.[7,9] Deranged laboratory platelet parameters reflecting platelet activation are potential prognostic biomarkers in multiple disease processes including malignancies, critical illness and venous and arterial thromboses.[10]

The role of platelets in COVID-19-related immunothrombosis is an area of active research and thrombocytopenia is a biomarker of poor outcome and increased disease severity.[2,8]

The current study aimed to determine whether changes in platelet indices on routine full blood count (FBC) analyses were potential surrogate markers of activation of the coagulation system in COVID-19 patients. We utilized fibrin degradation products, D-dimers, as a reflection of coagulation activation.

This study was a retrospective record review. All adult patients who underwent a COVID-19 RT-PCR (Cobas, Roche® or GeneXpert, Cepheid®) test on nasal swabs with an associated D-dimer and FBC at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a quaternary referral centre in South Africa, between June and December 2020 were included. Approval for the study was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (Protocol M201010).

The patients were divided into 3 groups for comparison namely COVID-19-positive patients with elevated D-dimers of greater than 0.5 mg/L (n=281), COVID-19-positive patients with normal D-dimers of less than 0.5 mg/L (n=72) and COVID-19-negative patients with elevated D-dimers of greater than 0.5 mg/L (n=51).

Blood samples were collected in Becton Dickinson®, Plymouth, UK (BD®) vacutainers with ethylenediaminetetraacetic acid (EDTA) for FBC and trisodium citrate for D-dimer analyses, respectively, and hand-delivered to the National Health Laboratory Service (NHLS) laboratory at CMJAH. Samples were not stored but analysed within 4 hours of collection on automated haematology (Sysmex XN9000®, Sysmex® Kobe, Japan) and coagulation (STAGO STA-R Max®, STAGO Diagnostica®, Paris, France) analysers. The laboratory is a fully South African National Accreditation System (SANAS) accredited laboratory, and all quality control measures were adhered to during the analysis of samples.

Results were retrospectively captured in a Microsoft Excel spreadsheet (Microsoft®, Redmond, Washington, USA). Quantitative data were tabulated and summarized with standard statistics. Further statistical analyses were performed with Statistical Package for the Social Sciences® software (SPSS®, Chicago, IL, USA). The normality of distribution of the data was tested in each group of samples using the Kolmogorov-Smirnov test. Chi-square cross tabulation was used to categorize the data based on age, sex and D-dimer concentration. Platelet count and platelet indices namely MPV, P-LCR, PDW and PCT were compared between the COVID-19-positive groups with and

without raised D-dimers and with the COVID-19-negative group with raised D-dimers using an independent t-test. The %IPF is only analysed by the automated analysers if the platelet count is < $50x10^9$/L, but the results of this parameter were also captured if it was available. Comparisons with p-values of <0.05 were considered significant.

Over the 6-month study period in 2020, 281 patients with COVID-19 and elevated D-dimers, 72 patients with COVID-19 and normal D-dimers and 51 patients without documented COVID-19 but with elevated D-dimers were included in the study analysis. The overall median age and gender distributions were similar in the 3 groups.

The COVID-19-positives patients (n=353) had statistically significant increases in MPV, P-LCR and PDW compared with the COVID-19-negative patients (n=51). Platelet counts and PCTs were decreased in COVID-19-positive patients compared with the COVID-19-negative patients, but these differences were not statistically significant (Table 1). The %IPF was analysed in 4 (1.5%) patients with COVID-19 with elevated D-dimers and the mean result was 10.63% (%IPF normal: 1.1-6.1%).

The mean platelet counts and PCT values were lower, and MPV, P-LCR and PDW higher in COVID-19-positive patients with elevated D-dimers compared with COVID-19 patients with normal D-dimers, but the differences were not statistically significant (Table 2).

Where available, the MPV was also collected at the time of discharge or proximal to a repeat negative SARS-CoV-2 test (median of 11 days of in-hospital treatment, n=85) and a significant decrease in the MPV from a mean of 10.69 to a mean of 10.04 (p<0.001) was observed.

COVID-19 is characterized by significant activation of the coagulation system with micro- and macrovscular thromboses being more prevalent than bleeding diatheses.[11] These thrombotic events are more common in patients with severe COVID-19 infection and are predictive of poorer clinical outcomes.[3] The pathogenesis of COVID-19 coagulation activation is multifactorial, but endothelial damage and cytokines are of pivotal importance in this pathological process termed immunothrombosis.[2,12]

The current study documented changes in routine platelet parameters in patients with COVID-19, which were more pronounced in those with elevated D-dimers, although this change did not reach statistical significance. The changes in platelet indices were however statistically different from patients without COVID-19 but with elevated D-dimers and probably is reflective of the contribution of platelets to the immunothrombotic process in COVID-19. Further multi-centre studies on larger cohorts, including %IPF analysis, are however indicated to determine the prognostic value of the changes in platelet parameters in patients with COVID-19. This retrospective study had some limitations. The cross-sectional nature means that certain patients who tested negative for SARS-CoV-2 may have had either pre-existing disease with a lower viral load or may subsequently have tested positive. In addition, the underlying clinical disease processes in patients with negative SARS-CoV-2 tests but with increased D-dimers were not available. This study, does however, suggests a potential pathogenic role of platelets in SARS-CoV-2-associated disease and a potential role as a novel biomarker.

TABLE 1 Independent t-test of platelet parameters in COVID-19-positive (with and without elevated D-dimers) and COVID-19-negative patients with elevated D-dimers

	COVID-19 Positive (n=353)		COVID-19 Negative (n=51)		
Platelet parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**
MPV (fL)	10.8	±1	10.08	±0.73	<.001
P-LCR (%)	31.06	±7.82	25.26	±6.09	<.001
PDW (fL)	12.74	±2.52	11.31	±1.63	<.001
PCT (%)	0.28	±0.12	0.29	±0.09	.53
Platelet count (10^9/L)	264.16	±124.15	291.45	±91.23	.14

n, number of patients; SD, standard deviation; MPV, mean platelet volume; P-LCR, platelet large cell ratio; PDW, platelet distribution width; PCT, platelet-crit.

TABLE 2 Independent t-test of platelet parameters and D-dimers of COVID-19-positive patients with and without elevated D-dimer levels

	COVID-19 patients with elevated D-dimers (n=281)		**COVID-19 patients with normal D-dimers (n=72)**		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**
MPV (fL)	10.84	±1.02	10.69	±0.90	.26
P-LCR (%)	31.37	±7.99	30.14	±7.28	.52
PDW (fL)	12.87	±2.66	12.37	±2.04	.49
PCT (%)	0.28	±0.12	0.29	±0.11	.45
Platelet count (x 10^9/L)	260.98	±127.78	273.40	±113.28	.47

n, number of patients; SD, standard deviation; MPV, mean platelet volume; P-LCR, platelet large cell ratio; PDW, platelet distribution width; PCT, platelet-crit.

KEYWORDS
COVID-19, immunothrombosis, laboratory parameters, platelets

ACKNOWLEDGMENTS
None.

CONFLICT OF INTEREST
The authors have no competing interests.

FUNDING INFORMATION
No funding was received for this retrospective record review study.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author.

Michael Mezgebe[1]
Barry Frank Jacobson[1]
Elizabeth Sarah Mayne[2]
Susan Louw[1]

[1]*Department of Molecular Medicine and Haematology, School of Health Sciences, University of the Witwatersrand (WITS), National Health Laboratory Service (NHLS), Johannesburg, South Africa*
[2]*Department of Immunology, School of Health Sciences, University of the Witwatersrand (WITS), National Health Laboratory Service (NHLS), Johannesburg, South Africa*

Correspondence
Susan Louw, Department of Molecular Medicine and Haematology, School of Health Sciences, University of the Witwatersrand (WITS), National Health Laboratory Service (NHLS), PO Box 94, Cresta, 2118, RSA, Johannesburg, South Africa.
Email: Susan.louw@nhls.ac.za

ORCID
Elizabeth Sarah Mayne https://orcid.org/0000-0002-6360-3488
Susan Louw https://orcid.org/0000-0002-4315-1496

REFERENCES

1. Wu D, Wu T, Liu Q, Yang Z. The SARS-CoV-2 outbreak: What we know. *Int J Infect Dis*. 2020;94:44–48.
2. Gu SX, Tyagi T, Jain K, et al. Thrombocytopathy and endotheliopathy: crucial contributors to COVID-19 thromboinflammation. *Nat Rev Cardiol*. 2021;18(3):194–209.
3. Al-Ani F, Chehade S, Lazo-Langner A. Thrombosis risk associated with COVID-19 infection, A scoping review. *Thromb Res*. 2020;192:152–160.
4. 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*. 2020;18(4):844–847.
5. Holinstat M. Normal platelet function. *Cancer Metastasis Rev*. 2017;36(2):195–198.
6. Hottz ED, Bozza FA, Bozza PT. Platelets in Immune Response to Virus and Immunopathology of Viral Infections. *Front Med (Lausanne)*. 2018;5:121.
7. Thorup CV, Christensen S, Hvas AM. Immature Platelets As a Predictor of Disease Severity and Mortality in Sepsis and Septic Shock: A Systematic Review. *Semin Thromb Hemost*. 2020;46(3):320–327.
8. Cohen A, Harari E, Cipok M, et al. Immature platelets in patients hospitalized with Covid-19. *J Thromb Thrombolysis*. 2021;51(3):608–616.
9. Iba T, Levy JH. Inflammation and thrombosis: roles of neutrophils, platelets and endothelial cells and their interactions in thrombus formation during sepsis. *J Thromb Haemost*. 2018;16(2):231–241.
10. Pogorzelska K, Kretowska A, Krawczuk-Rybak M, Sawicka-Zukowska M. Characteristics of platelet indices and their prognostic significance in selected medical condition - a systematic review. *Adv Med Sci*. 2020;65(2):310–315.
11. Iba T, Levy JH, Connors JM, Warkentin TE, Thachil J, Levi M. The unique characteristics of COVID-19 coagulopathy. *Crit Care*. 2020;24(1):360.
12. Skendros P, Mitsios A, Chrysanthopoulou A, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J Clin Invest*. 2020;130(11):6151–6157.