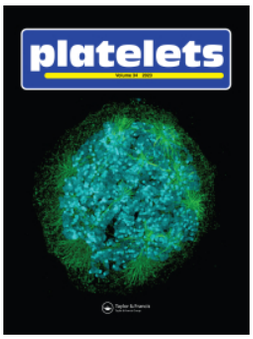

Platelets

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/iplt20

Assessment of whole blood platelet aggregation in patients with cirrhosis: challenges and opportunities

Alberto Zanetto, Elena Campello, Marco Senzolo & Paolo Simioni

To cite this article: Alberto Zanetto, Elena Campello, Marco Senzolo & Paolo Simioni (2023) Assessment of whole blood platelet aggregation in patients with cirrhosis: challenges and opportunities, Platelets, 34:1, 2178823, DOI: 10.1080/09537104.2023.2178823

To link to this article: https://doi.org/10.1080/09537104.2023.2178823

Published online: 20 Feb 2023.

http://www.tandfonline.com/iplt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

Platelets, 2023; 34(1): 2178823

DOI: https://doi.org/10.1080/09537104.2023.2178823

LETTER

Assessment of whole blood platelet aggregation in patients with cirrhosis: challenges and opportunities

Alberto Zanetto[1,2], Elena Campello[3], Marco Senzolo[1,2], & Paolo Simioni[3]

[1]Gastroenterology and Multivisceral Transplant Unit, Azienda Ospedale - Università Padova, Padova, Italy, [2]Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy, and, and [3]General Internal Medicine and Thrombotic and Hemorrhagic Diseases Unit, Department of Medicine, Padova University Hospital, Padova, Italy

Keywords

ACLF, coagulation, decompensation, hemostasis, platelet volume

History

Received 1 January 2023
Revised 27 January 2023
Accepted 6 February 2023

To the Editor:

We read with interest the letter by Scavone et al, published recently in *Platelets* [1], where the authors questioned the use of whole blood aggregometry (Multiplate™, WBA) in patients with thrombocytopenia. In their discussion, the authors included one prospective study by our group in which we assessed alterations of whole blood platelet aggregation in 203 patients with cirrhosis [2]. Since the majority of these patients were thrombocytopenic, and thus comparison with controls with normal platelet count was not possible, we expressed the results of WBA by a "PLT ratio" (i.e.: ADP-induced aggregation by Multiplate™ measured as AUC/platelet count). We found that the PLT ratio was higher in cirrhosis than chronic hepatitis and healthy subjects, and increased significantly with Child-Pugh stage. Furthermore, in the subgroup of decompensated patients, the baseline PLT ratio was an independent predictor of further decompensation and liver-related death during a 6-month follow-up. We thus concluded that [a] whole blood platelet aggregation, when expressed as a PLT ratio, is higher in cirrhosis than controls; [b] if our findings are confirmed by independent cohorts, the PLT ratio could become an independent prognostic biomarker in decompensated cirrhosis [2].

Scavone et al postulated that the assessment of whole blood platelet aggregation by Multiplate™ in patients with cirrhosis would depend not only on platelet aggregation and platelet count, but also on platelet volume [1]. They based this assumption on a weak/moderate correlation ($r = 0.4$) between mean platelet volume (MPV) and PLT ratio found in 29 patients with essential thrombocytopenia. Previous data on MPV in HBV-related cirrhosis indicate that some of these patients may have a higher MPV than healthy controls [3]. Since, per Scavone's hypothesis, the increased MPV would influence results of WBA when expressed as PLT ratio, they concluded that the use of such a ratio would overestimate platelet aggregation in cirrhosis.

However, we would like to add some considerations to this hypothesis:

(1) The considerations by Scavone et al are mostly based on the "the fact that platelets from most cirrhotic patients with moderate/severe thrombocytopenia are large or even giant" [1]. In supporting this statement, the authors quoted a study from China including patients with HBV-related cirrhosis [3]. It is known, however, that HBV chronic infection has specific effects on both platelet activation and turn-over [4]. These effects are observed even before the development of cirrhosis with thrombocytopenia [4], and may potentially explain the association between HBV infection and a higher MPV. In our study, patients with HBV-related cirrhosis were 11% [2]. In the other study quoted by the authors [5], the MPV was comparable between cirrhosis and controls. In a prospective study from Italy including 75 patients with cirrhosis and baseline characteristics very close to those of our cohort, only 7% of the patients had MPV higher than normal [6]. Furthermore, the authors found no difference in MPV between compensated and decompensated patients, and no correlation between MPV and MELD score [6]. Additional studies found no evidence of a higher MPV in patients with clinically stable cirrhosis [7]. A higher MPV has been described in a study from a Chinese center including patients with HBV-related (again) acute-on-chronic liver failure (ACLF) [8]. However, ACLF is a distinct syndrome characterized by severe systemic inflammation and multiorgan failure [9,10]. Notably, patients with ACLF were not eligible for inclusion in our study [2].
(2) A second point made by Scavone et al is that "since the MPV is inversely proportional to the platelet count, it would emerge as a variable affecting the Multiplate™ results when

Correspondence: Paolo Simioni, Thrombotic and Hemorrhagic Diseases Unit, General Internal Medicine, Padova University Hospital, Padova, Italy. E-mail: paolo.simioni@unipd.it

they are expressed as AUC/platelet count ratio, which rules off the influence of platelet count" [1]. However, as also quoted by the authors, this was demonstrated - by a correlation between platelet count and mega-thrombocyte index - in healthy subjects [11]. Whether this is true in cirrhosis remains unknown. In the study by Giannini et al including a representative cohort of both compensated and decompensated patients, values of MPV were not associated with the presence of thrombocytopenia or severe thrombocytopenia, and were not correlated with platelet counts [6].

(3) *In vivo*, platelets exist in a multitude of different shapes and volumes according to their variable state of activation, which may also influence the assessment of MPV. In patients with cirrhosis, the increased platelet turnover [12] and the fact that the overall platelet activation state continuously and rapidly evolves according to multiple factors such as bacterial translocation, infections and renal dysfunction [13–15] may further influence results of MPV. Contrary to light-transmittance aggregometry (LTA), which *specifically* assesses platelet aggregation though requires an extensive pre-processing of samples (i.e. introducing potential artifacts due to platelets manipulation and assessment of their response to agonists outside their natural environment), WBA gives a relatively *less specific* yet more comprehensive assessment of the individual platelet state (i.e. takes into consideration red and white blood cells, coagulation factors, microvesicles etc., which may variably influence platelet function). Therefore, WBA by Multiplate™, and the PLT ratio, is not intended to provide a pure, specific assessment of platelet aggregation alone; instead, it is intended to measure the platelet response in an assay milieu that mimics *in vivo* conditions of platelet activation/aggregation [16]. In patients with cirrhosis, where a multitude of known and unknown alterations of hemostasis exist [17], we hypothesize that this type of information may ultimately be even more informative (and perhaps clinically relevant) than the assessment of platelet aggregation by LTA. For example, in an ancillary analysis of the same study, we showed that the PLT ratio was also associated with development of portal vein thrombosis independently of portal hypertension and chronic liver disease severity [18]. A baseline PLT ratio>0.75 had 83% sensitivity and 84% specificity for development of thrombosis within 1 year [18]. On the other hand, although the experiments conducted by LTA have been instrumental to improve our understanding of cirrhotic thrombocytopathy [17], it is now clear that the overall equilibrium of primary hemostasis in cirrhosis is influenced by additional elements that are not properly assessed by a static test such as aggregometry [19].

(4) We investigated whole blood platelet aggregation in the portal blood of patients with decompensated cirrhosis undergoing trans-jugular intrahepatic portosystemic shunt [20]. In this study, despite the platelet count was significantly lower in the portal vs. peripheral vein (57×10^9/L vs. 92×10^9/L), whole blood platelet aggregation, expressed as AUC by Multiplate™, was perfectly comparable between the two districts. Therefore, the PLT ratio was higher in the portal vs. peripheral vein, indicating increased aggregation and again highlighting the usefulness of using a ratio to compare results of WBA in patients with thrombocytopenia. The higher platelet response in the portal blood likely reflects a greater stimulation by the lipopolysaccharide derived from the leaky gut [13,21], whereas there is no reason to suspect a different MPV in portal vs. peripheral vein *in the same patient*.

In conclusion, LTA and WBA are complementary techniques to assess different aspects of the complex alterations of platelet function occurring in patients with cirrhosis. Regarding WBA, we suggest that more data on MPV are required prior to draw any conclusion regarding its potential effect on the PLT ratio. Awaiting such studies, our results indicate that patients with cirrhosis have a significantly higher PLT ratio than controls, and that the PLT ratio predicts the risk of further decompensation and death independently of liver disease severity [2]. We now look forward to further studies to test our findings.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

1. Scavone M, Podda GM, Tripodi A, Cattaneo M. Whole blood platelet aggregation measurement by multiplate: potential diagnostic inaccuracy of correcting the results for the sample platelet count. Platelets. 2023;34(1):2156493. doi:10.1080/09537104.2022.2156493.
2. Zanetto A, Campello E, Bulato C, Gavasso S, Farinati F, Russo FP, Tormene D, Burra P, Senzolo M, Simioni P. Increased platelet aggregation in patients with decompensated cirrhosis indicates higher risk of further decompensation and death. J Hepatol. 2022;77(3):660–669. doi:10.1016/j.jhep.2022.03.009.
3. Qi XT, Wan F, Lou Y, Ye B, Wu D. The mean platelet volume is a potential biomarker for cirrhosis in chronic hepatitis B virus infected patients. Hepatogastroenterology. 2014;61(130):456–459.
4. Wang X, Jiang W, Li F, Hua F, Zhan Y, Li Y, Ji L, Zou S, Min Z, Song D, et al. Abnormal platelet kinetics are detected before the occurrence of thrombocytopaenia in HBV-related liver disease. Liver Int. 2014;34(4):535–543. doi:10.1111/liv.12309.
5. Luzzatto G, de Franchis G, Fabris F, Gerunda GE, Girolami A. Increased proportion of giant platelets and platelet distribution width are better indicators of altered platelet homeostasis than mean platelet volume in liver cirrhosis. Folia Haematol Int Mag Klin Morphol Blutforsch. 1988;115(5):719–726.
6. Giannini EG, Moscatelli A, Brunacci M, Zentilin P, Savarino V. Prognostic role of mean platelet volume in patients with cirrhosis. Dig Liver Dis. 2016;48(4):409–413. doi:10.1016/j.dld.2015.10.018.
7. Jorgensen B, Fischer E, Ingeberg S, Hollaender N, Ring-Larsen H, Henriksen JH. Decreased blood platelet volume and count in patients with liver disease. Scand J Gastroenterol. 1984;19(4):492–496. doi:10.1080/00365521.1984.12005758.
8. Han L, Han T, Nie C, Zhang Q, Cai J. Elevated mean platelet volume is associated with poor short-term outcomes in hepatitis B virus-related acute-on-chronic liver failure patients. Clin Res Hepatol Gastroenterol. 2015;39(3):331–339. doi:10.1016/j.clinre.2014.10.006.
9. Zanetto A, Pelizzaro F, Campello E, Bulato C, Balcar L, Gu W, Gavasso S, Saggiorato G, Zeuzem S, Russo FP, et al. Severity of systemic inflammation is the main predictor of ACLF and bleeding in individuals with acutely decompensated cirrhosis. J Hepatol. 2022;78(2):301–311. doi:10.1016/j.jhep.2022.09.005.
10. Campello E, Zanetto A, Bulato C, Maggiolo S, Spiezia L, Russo FP, Gavasso S, Mazzeo P, Tormene D, Burra P, et al. Coagulopathy is not predictive of bleeding in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. Liver Int. 2021;41(10):2455–2466. doi:10.1111/liv.15001.
11. Karpatkin S. Heterogeneity of human platelets. VI. Correlation of platelet function with platelet volume. Blood. 1978;51(2):307–316. doi:10.1182/blood.V51.2.307.307.
12. Pradella P, Bonetto S, Turchetto S, Uxa L, Comar C, Zorat F, De Angelis V, Pozzato G. Platelet production and destruction in liver cirrhosis. J Hepatol. 2011;54(5):894–900. doi:10.1016/j.jhep.2010.08.018.
13. Raparelli V, Basili S, Carnevale R, Napoleone L, Del Ben M, Nocella C, Bartimoccia S, Lucidi C, Talerico G, Riggio O, et al. Low-grade endotoxemia and platelet activation in cirrhosis. Hepatology. 2017;65(2):571–581. doi:10.1002/hep.28853.
14. Zanetto A, Campello E, Bulato C, Gavasso S, Saggiorato G, Shalaby S, Burra P, Angeli P, Senzolo M, Simioni P. Global

hemostatic profiling in patients with decompensated cirrhosis and bacterial infections. JHEP Rep. 2022;4(7):100493. doi:10.1016/j.jhepr.2022.100493.

15. Zanetto A, Rinder HM, Campello E, Saggiorato G, Deng Y, Ciarleglio M, Wilson FP, Senzolo M, Gavasso S, Bulato C, et al. Acute kidney injury in decompensated cirrhosis is associated with both hypo-coagulable and hyper-coagulable features. Hepatology. 2020;72(4):1327–1340. doi:10.1002/hep.31443.
16. Zanetto A, Senzolo M, Campello E, Bulato C, Gavasso S, Shalaby S, Gambato M, Vitale A, Cillo U, Farinati F, et al. Influence of hepatocellular carcinoma on platelet aggregation in cirrhosis. Cancers (Basel). 2021;13(5):1150. doi:10.3390/cancers13051150.
17. Zermatten MG, Fraga M, Moradpour D, Bertaggia Calderara D, Aliotta A, Stirnimann G, De Gottardi A, Alberio L. Hemostatic alterations in patients with cirrhosis: from primary hemostasis to fibrinolysis. Hepatology. 2020;71(6):2135–2148. doi:10.1002/hep.31201
18. Zanetto A, Campello E, Burra P, Senzolo M, Simioni P. Increased platelet ratio in patients with decompensated cirrhosis indicates a higher risk of portal vein thrombosis. Liver Int. 2022;77:S358–359. doi:10.1016/S0168-8278(22)01072-8.
19. Lisman T, Bongers TN, Adelmeijer J, Janssen HL, de Maat MP, de Groot PG, Leebeek FW. Elevated levels of von willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity. Hepatology. 2006;44(1):53–61. doi:10.1002/hep.21231.
20. Shalaby S, Zanetto A, Campello E, Gavasso S, Barbiero G, Battistel M, Feltracco P, Bizzaro D, Burra P, Simioni P, et al. Reply to "peripheral versus central venous blood sampling does not influence the assessment of platelet activation in cirrhosis". Platelets. 2022;33(7):1104–1106. doi:10.1080/09537104.2022.2060499.
21. Queck A, Carnevale R, Uschner FE, Schierwagen R, Klein S, Jansen C, Meyer C, Praktiknjo M, Thomas D, Strassburg C, et al. Role of portal venous platelet activation in patients with decompensated cirrhosis and TIPS. Gut. 2020;69(8):1535–1536. doi:10.1136/gutjnl-2019-319044.