Assessment of the Haemostatic Potential of Platelets Readied for Transfusion

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Dual antiplatelet therapy (DAPT) with aspirin and a P2Y12 antagonist such as clopidogrel has become a standard of care after percutaneous coronary intervention (PCI).[1] DAPT is associated with an increased bleeding risk,[2] which is a challenge if surgery is needed, either due to trauma or if bypass surgery is required. As both agents in DAPT are irreversible inhibitors, simply stopping DAPT will not restore haemostasis. A number of studies have investigated the use of platelet transfusions prior to surgery to prevent bleeding; however, these studies have failed to show any benefit.[3–5] It is not clear why transfusion of platelet concentrates does not restore haemostasis. To determine if the lack of benefit of platelet transfusions is due to the functionality of stored platelets, we investigated the response of stored platelets to different agonists.

We collected the residual platelet-rich plasma (PRP) from bags of platelets that were used for transfusion. All units were prepared by apheresis into PAS.[6] Platelet response was measured using the PL-12 Aggrestar platelet function analyser (Sinnowa, Nanjing, China).[7,8] The PL-12 performs sequential platelet counts, which it uses to calculate the maximum aggregation rate (MAR) and has been shown to correlate with other platelet function tests, although it is a more sensitive parameter.[8–10] While light transmission aggregometry measures platelet aggregation using light transmission as an indirect measure of changes in platelet concentration, the PL-12 measures these changes by directly counting the platelets using standard Coulter counting technology. ►**Fig. 1** shows that there was a significant difference between the response of stored platelets to different agonists (analysis of variance, $p < 0.0001$). Thrombin receptor-activating peptide (TRAP; 32 µM) produced a strong response in all bags of platelets ($76.3 \pm 6.8\%$, $n = 16$), whereas arachidonic acid (AA; 0.2 mg/mL) produced a more variable response ($36.2 \pm 23.7\%$, $n = 38$). In contrast, neither ADP (5 µM: $11.7 \pm 3.8\%$, $n = 38$; 50 µM: $26.5 \pm 18.7\%$, $n = 16$) nor adrenaline (ADR 100 µM; $12.4 \pm 6.8\%$, $n = 14$) produced a significant response. The response to TRAP was significantly different to that of all other agonists.

This lack of responsiveness may reflect the initial quality of the donated platelets or may be a loss of responsiveness due to storage (platelet storage lesion).[11] To address this, we collected blood from healthy volunteers ($n = 6$), prepared PRP by centrifugation and immediately measured the response to ADP and AA (<2-hour postdonation). As the PL-12 can also measure platelet aggregation in whole blood, it allowed the responsiveness of the original blood to be compared with the response in PRP. There was a strong response by platelets in whole blood to both 5 µM ($63.8 \pm 8.6\%$) and 10 µM ADP (61.1 ± 18.8); however, PRP failed to respond to 5 µM ADP ($15.9 \pm 5.6\%$, $p = 0.0001$ compared with whole blood), although there was a better response to 10 µM ADP (31.4 ± 10.4, $p = 0.02$). AA produced a similar response to ADP in whole blood (0.2 mg/mL: $60.9 \pm 14\%$ and 0.4 mg/mL: $65.1 \pm 5.4\%$) that was slightly decreased in PRP (0.2 mg/mL: 48.3 ± 23.6, $p = 0.4$ compared with whole blood; 0.4 mg/mL: $52.2 \pm 14.3\%$, $p = 0.1$; ►**Fig. 2**). To determine to what extent platelet function might recover

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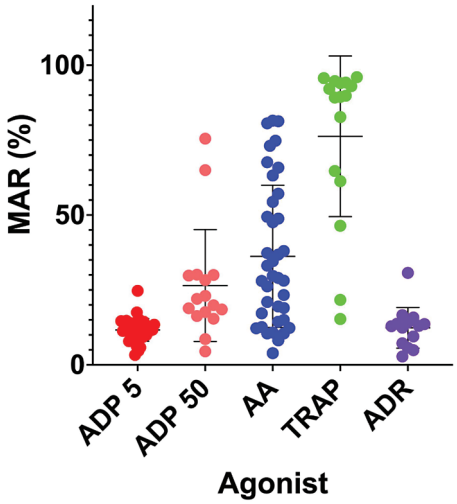

Fig. 1 Residual platelets in transfusion bags (<8 days old) were tested for response to agonists (maximum aggregation rate; MAR) using the Sinnowa PL-12 platelet function analyser. The agonists used were thrombin receptor-activating peptide (TRAP 32 μM, $n = 16$), arachidonic acid (AA; 0.2 mg/mL, $n = 38$), ADP (5 μM, $n = 38$; 50 μM, $n = 16$), and adrenaline (ADR; 100 μM, $n = 14$).

postinfusion, PRP was separated from whole blood and replaced with stored platelets (50:50 ratio), and platelet function was determined. The MAR of stored platelets with ADP was 7.9 ± 3.0% ($n = 21$) and was 6.2 ± 7.5% when added to red blood cells (RBC), which was not significantly different. In the case of AA-induced aggregation, the MAR for stored platelets was 26.3 ± 25.1% ($n = 21$) and when added to RBCs was 43.0 ± 29.3 ($p = 0.02$, paired t-test). This supports the potential role for RBCs in platelet aggregation,[12] especially with respect to the response to AA.

To determine the time course of the change in platelet function we prepared PRP from healthy volunteers and tested it with different concentrations of ADP and AA over a 72-hour period (►**Fig. 3**). During the 72-hour period, the response to ADP did not change; however, there was a gradual decline in the response to AA.

The PL-12 platelet function analyser has been used to monitor patients on antiplatelet agents. Zheng et al monitored the response to ADP in patients undergoing PCI on

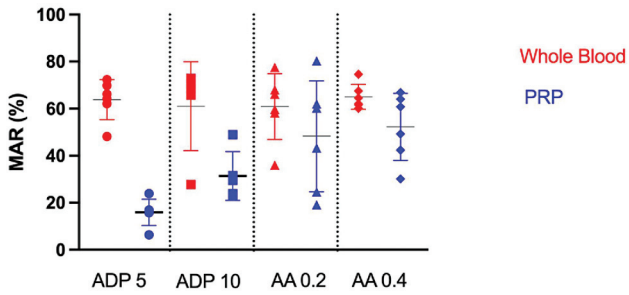

Fig. 2 Blood was collected from healthy volunteers and PRP was prepared from it ($n = 6$). Both the blood and corresponding PRP were stimulated with ADP (5 and 10 μM) and arachidonic acid (0.2 and 0.4 mg/mL). The response to agonist is presented as maximum aggregation rate (MAR).

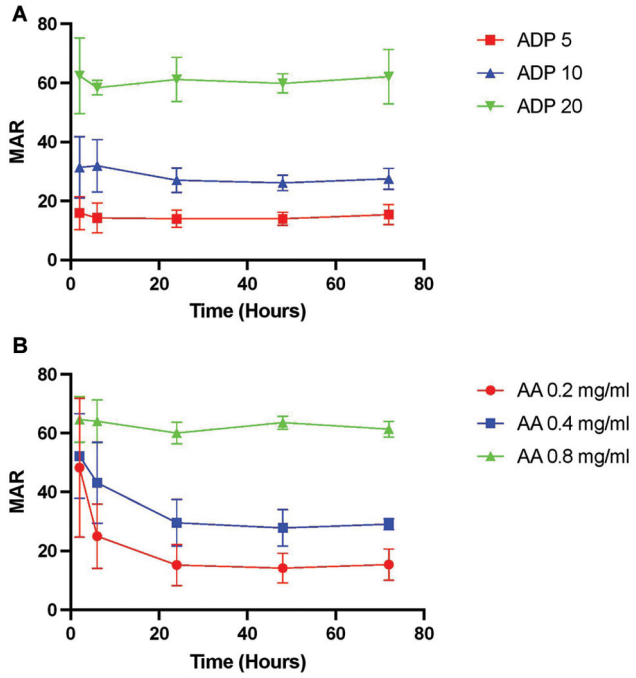

Fig. 3 Blood was collected from healthy volunteers and PRP was prepared ($n = 6$) and stored at room temperature for 72 hours. PRP was stimulated with agonist immediately after preparing PRP ($t = 0$) and over 72 hours. Aggregation response is presented as maximum aggregation rate (MAR). (**A**) PRP stimulated with 5, 10, and 20 μM ADP. (**B**) PRP stimulated with 0.2. 0.4, and 0.8 mg/mL arachidonic acid.

DAPT. In these patients, MAR with ADP was 34.7 ± 15.8% ($n = 421$),[13] which is significantly higher than the transfused platelets (11.7 ± 3.8%) in our study. In a study of patients with stroke who were being treated with aspirin, their MAR in response to AA was 49.23 ± 7.2% ($n = 197$),[10] whereas the MAR for the AA in stored platelets in our study was 36.2 ± 23.7%.

In a previous study using the VerifyNow platelet analyser, platelet transfusions were found to restore responsiveness in patients treated with aspirin but not in those treated with clopidogrel.[3] Using bleeding time as an in vivo measure of platelet function, Cohn et al found that platelet transfusion had no effect on bleeding time in clopidogrel-treated volunteers.[4] Our results would predict these responses, as we found that stored platelets did not respond to ADP but did respond to AA.

Thus, stored platelets fail to respond to ADP and have a reduced response to AA, which are lower than the response of platelets from patients on DAPT. So, it is not surprising that platelet transfusions fail to restore platelet function in patients on DAPT. The reason for this loss of response to ADP in stored platelets is not clear but is not due to platelet storage lesion,[11] as it happens immediately upon separation of PRP from whole blood. Platelets are collected and stored under conditions that are optimised for maximum platelet survival posttransfusion; however, there is a paucity of data on their haemostatic potential posttransfusion.[14] Further studies are necessary to understand the effectiveness of platelet transfusions and to determine collection and storage

conditions that optimise both survival and haemostatic potential posttransfusion.

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Conflict of Interest
None declared.

References

1 Banerjee S, Angiolillo DJ, Boden WE, et al. Use of antiplatelet therapy/DAPT for post-PCI patients undergoing noncardiac surgery. J Am Coll Cardiol 2017;69(14):1861–1870
2 Shpigelman J, Proshkina A, Daly MJ, Cox D. Personalized dual antiplatelet therapy in acute coronary syndromes: striking a balance between bleeding and thrombosis. Curr Cardiol Rep 2023;25(07):693–710
3 Taylor G, Osinski D, Thevenin A, Devys J-M. Is platelet transfusion efficient to restore platelet reactivity in patients who are responders to aspirin and/or clopidogrel before emergency surgery? J Trauma Acute Care Surg 2013;74(05):1367–1369
4 Cohn SM, Jimenez JC, Khoury L, Perez JM, Panzo M. Inability to reverse aspirin and clopidogrel-induced platelet dysfunction with platelet infusion. Cureus 2019;11(01):e3889
5 Nagalla S, Sarode R. Role of platelet transfusion in the reversal of anti-platelet therapy. Transfus Med Rev 2019;33(02):92–97
6 van der Meer PF, de Korte D. Platelet additive solutions: a review of the latest developments and their clinical implications. Transfus Med Hemother 2018;45(02):98–102
7 Offiah C, et al. Performance metrics of the AGGRESTAR PL-12 platelet function analyser in patients with TIA or ischaemic stroke on commonly-prescribed antiplatelet regimens. Thromb Haemost 2021;5:1057
8 Guan J, Cong Y, Ren J, et al. Comparison between a new platelet count drop method PL-11, light transmission aggregometry, VerifyNow aspirin system and thromboelastography for monitoring short-term aspirin effects in healthy individuals. Platelets 2015;26(01):25–30
9 Ma L, Chen W, Pan Y, et al. Comparison of VerifyNow, thromboelastography, and PL-12 in patients with minor ischemic stroke or transient ischemic attack. Aging (Albany NY) 2021;13(06): 8396–8407
10 Yue C, Lin Z, Lu C, Chen H. Efficacy of monitoring platelet function by an automated PL-12 analyzer during the treatment of acute cerebral infarction with antiplatelet medicine. Clin Appl Thromb Hemost 2021;27:10760296211001119
11 Caram-Deelder C, Kreuger AL, Jacobse J, van der Bom JG, Middelburg RA. Effect of platelet storage time on platelet measurements: a systematic review and meta-analyses. Vox Sang 2016;111(04): 374–382
12 Weisel JW, Litvinov RI. Red blood cells: the forgotten player in hemostasis and thrombosis. J Thromb Haemost 2019;17(02): 271–282
13 Zheng Y-Y, Wu TT, Yang Y, et al. Personalized antiplatelet therapy guided by a novel detection of platelet aggregation function in stable coronary artery disease patients undergoing percutaneous coronary intervention: a randomized controlled clinical trial. Eur Heart J Cardiovasc Pharmacother 2020;6(04): 211–221
14 Cornelissen LL, Caram-Deelder C, Meier RT, Zwaginga JJ, Evers DDutch Blood Transfusion Related Research Consortium. Platelet transfusion and tranexamic acid to prevent bleeding in outpatients with a hematological disease: a Dutch nationwide survey. Eur J Haematol 2021;106(03):362–370