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Abstract:
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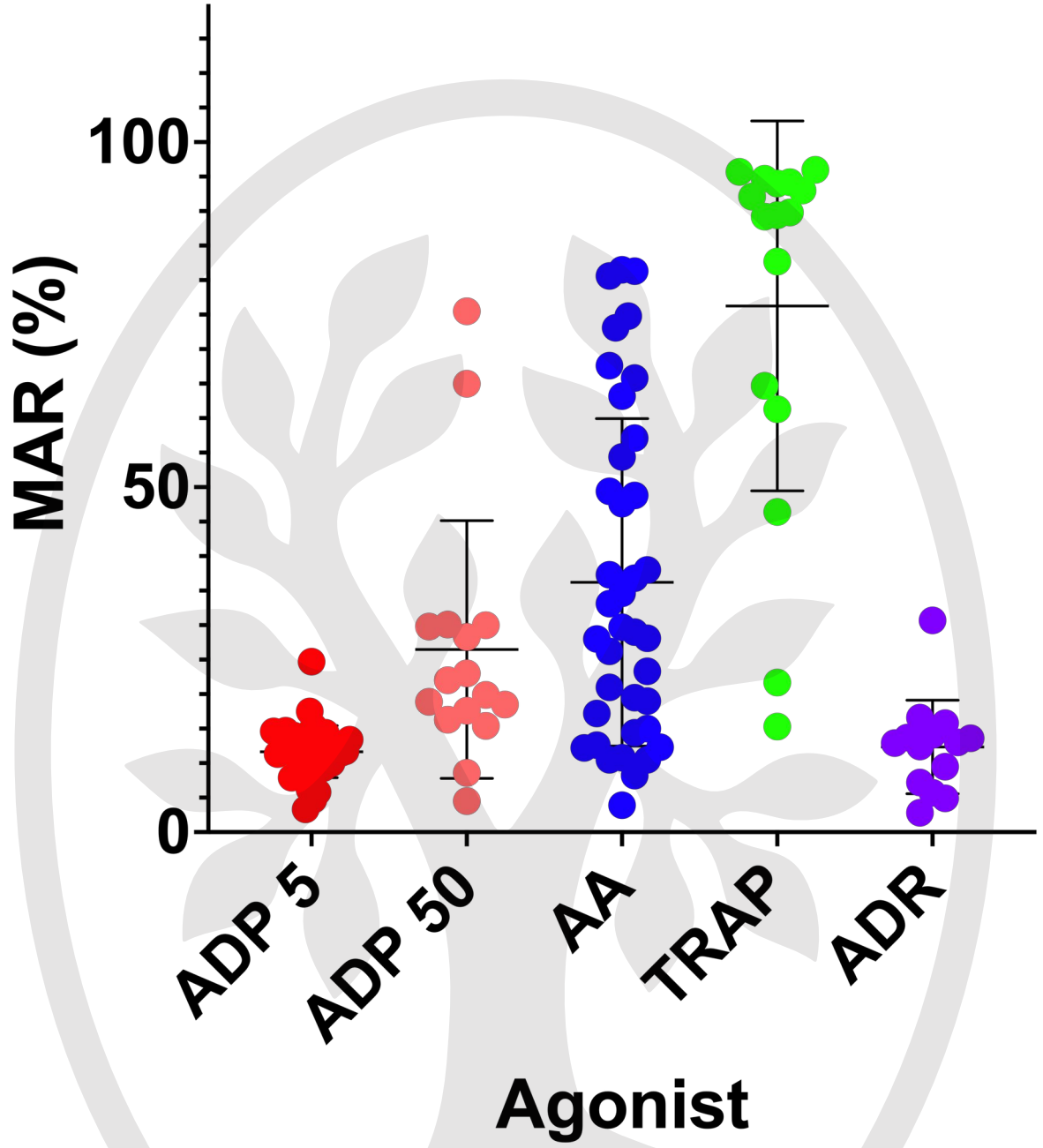

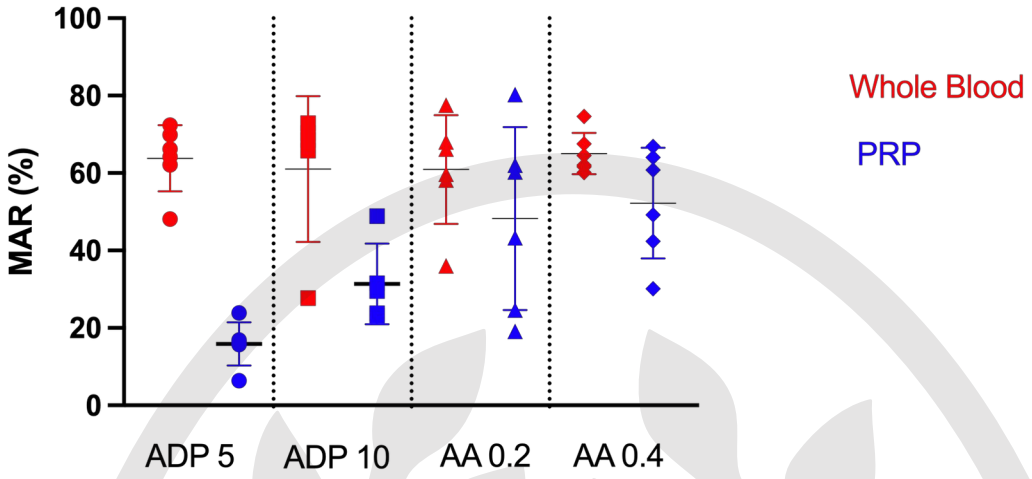

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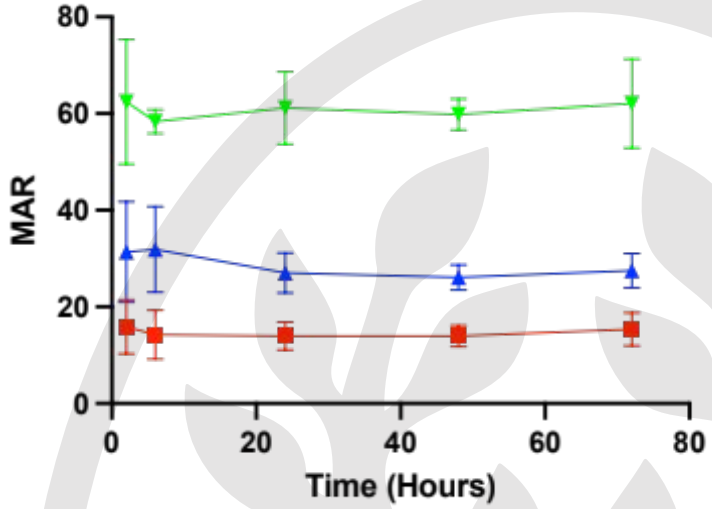

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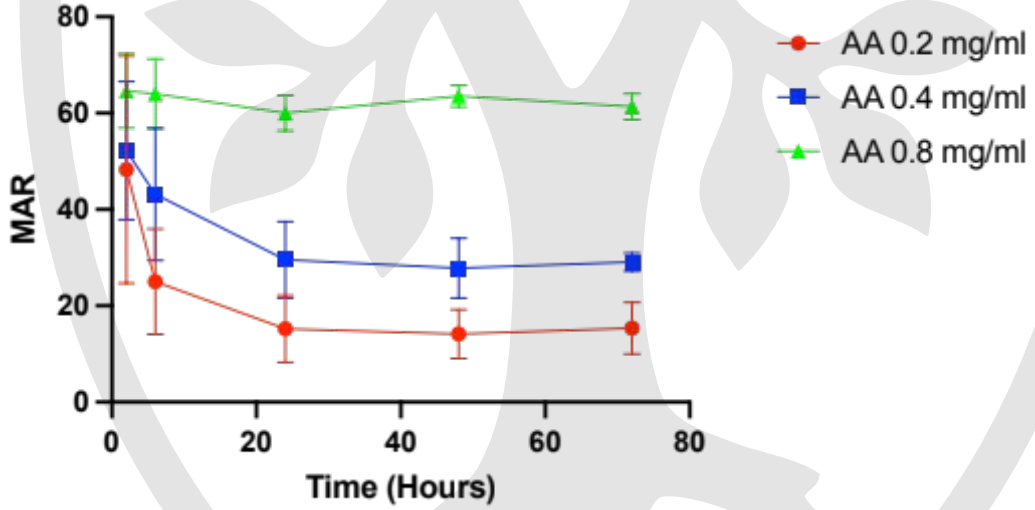

Assessment of the haemostatic potential of platelets readied for transfusion

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Dual anti-platelet therapy (DAPT) with aspirin and a P2Y12 antagonists such as clopidogrel, has become standard of care post-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]. The PL-12 performs sequential platelet counts which it uses to calculate the maximum aggregation rate (MAR). Figure 1 shows that there was significant difference between the response of stored platelets to different agonists (ANOVA, $p<0.0001$). Thrombin receptor activating peptide (TRAP; 32 μM) produced a strong response in all bags of platelets (76.3±6.8%, n=16), while arachidonic acid (AA; 0.2 mg/ml) produced a more variable response (36.2±23.7%, n=38). In contrast neither ADP (5 μM: 11.7±3.8%, n=38; 50 μM 26.5±18.7%, n=16) nor adrenaline (ADR 100 μM; 12.4±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)[8]. To address this, we collected blood from healthy volunteers (n=6), prepared PRP by centrifugation and immediately measured the response to ADP and AA (<2hrs post-donation). As the PL-12 can also measure platelet aggregation in whole blood it allowed the responsiveness of the original blood to be compared to the response in PRP. There was a strong response by platelets in whole blood to both 5 μM (63.8±8.6%) and 10 μM ADP (61.1±18.8), however, PRP failed to respond to 5 μM ADP (15.9±5.6%, p=0.0001 compared to 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±14% and 0.4 mg/ml: 65.1±5.4%) which 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±14.3%, p=0.1). (see Fig 2).

To determine to what extent platelet function might recover post-infusion, 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[9] especially with respect to the response to AA.

The PL-12 platelet function analyser has been used to monitor patients on anti-platelet agents. Zheng and co-workers monitored the response to ADP in patients undergoing PCI on DAPT. In these patients MAR with ADP was 34.7±15.8% (n=421)[10], 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)[11] while 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[8] 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 post-transfusion however, there is a paucity of data on their haemostatic potential post-transfusion[12]. 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 post-transfusion.

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