EDITORIAL II

Looking into the future of platelet transfusion in the presence of P2Y$_{12}$ inhibitors

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A method to evaluate platelet aggregation in vitro was first described by Professor Born at the Royal College of Surgeons in London. Over 50 years, his original method of light transmission (turbidometric) aggregometry (LTA) in anticoagulated platelet-rich plasma has been the gold standard for the assessment of platelet-related bleeding and thrombosis disorders. Pro-aggregatory mechanisms of platelets via thromboxane A$_2$ and adenosine 5′-diphosphate (ADP) have been confirmed by LTA, leading to clinical applications of antiplatelet drug such as aspirin (ASA) and, more recently, thienopyridine P2Y$_{12}$ inhibitors (clopidogrel, ticagrelor, prasugrel, etc.). Over the last decade, dual antiplatelet therapy (DAPT) using ASA and clopidogrel has become the standard of care in the management of acute coronary syndrome (ACS), and for the prevention of ischaemic events after percutaneous coronary intervention (PCI). A clinical use of a rapid whole blood platelet function test (Table 1) emerged as a potential way to prevent atherothrombotic events after PCI by monitoring therapeutic responses to ASA, clopidogrel, or both. A dose increase for clopidogrel and a switch to prasugrel or ticagrelor have been evaluated to achieve better platelet inhibition, but the standard approach has not been established for the management of high platelet reactivity (HPR) on DAPT. This is partly because an event rate of atherothrombosis after PCI is low, and HPR usually yields a low positive predictive value. Conversely, the lack of HPR yields a high negative predictive value for thrombosis, and is even associated with increased bleeding complications.

Screening for reduced platelet aggregation on DAPT is of particular interest to anaesthetists and surgeons because it can be helpful in gauging timing of an invasive procedure, or an indication for platelet transfusion. The assessment of platelet function after platelet transfusion is also an important consideration because transfused platelets can be affected by circulating platelet inhibitors. In the current issue of BJA, Hansson and colleagues report differences in the functional platelet recovery after in vitro addition of allogeneic platelets in whole blood samples from patients on ASA, ASA plus clopidogrel, or ASA plus ticagrelor. Platelet aggregation was evaluated in hirudin-anticoagulated whole blood using multi-electrode aggregometry (MEA) (Multiplate®, Munich, Germany). Antiplatelet effects of ASA and P2Y$_{12}$ inhibitors were presumably maximal when the specimens were collected within 2 h of the last drug ingestion (Table 2). This experiment mirrors the 'worst-case scenario' where an emergency coronary bypass grafting surgery (CABG) is being requested after failed PCI. Uninterrupted DAPT has been associated with haemorrhagic complications and increased transfusion requirements after CABG.

There were three principal findings in this study: (i) ADP-induced aggregation in the presence of clopidogrel or ticagrelor was minimally improved by platelet supplementation; (ii) antiplatelet effect of ASA was readily reversible with platelet supplementation, and (iii) P2Y$_{12}$ inhibitors interfere with the platelet recruitment process via thromboxane and thrombin receptors, but the reduced responsiveness to non-ADP agonists was improved by platelet supplementation. First, ADP-induced platelet aggregation (AU·min$^{-1}$) showed a statistically significant improvement after adding moderate and high platelet doses in both ASA plus clopidogrel or ASA plus ticagrelor samples. Mean peak values (21 and 16 AU·min$^{-1}$, respectively) remained far below the normal range of aggregability for ADP tests (43–100 AU·min$^{-1}$). A preoperative cut-off value of 31 AU·min$^{-1}$ has been associated with increased postoperative bleeding (>800 ml in 12 h) after cardiac surgery. It is thus speculated that more than 2–5 units of apheresis platelets would be required to restore ADP-induced platelet aggregation when active antiplatelet agents are high in circulation.

As discussed by the authors, VilaTjur and colleagues previously demonstrated in vitro that decreased platelet aggregation after 3 days of ASA plus clopidogrel was restored to >80% of normal after adding fresh platelet-rich plasma (PRP) equivalent to 2–3 units of apheresis platelets. It is notable that blood samples used for PRP supplementation were drawn at 72 h from the loading dose (ASA 325 mg, and clopidogrel 300 or 600 mg) and 24 h from the last dose. Near-complete reversal of platelet function in their study is thus partly attributable to the lack of active antiplatelet agents in the subjects’ blood. Time dependence of functional platelet recovery was also demonstrated for prasugrel. In
Zafar and colleagues’ study,24 platelet aggregation by LTA and VerifyNow-P2Y12 assay recovered only slightly when fresh PRP (equivalent to 3–4 apheresis units) was used to supplement the subjects’ blood obtained 2 h after a single loading dose (60 mg) of prasugrel plus ASA. Hansson and colleagues16 found that platelet supplementation was less effective in blood affected by ASA plus ticagrelor compared with ASA plus clopidogrel within 2 h from the last dose. However, adding a wait time of 6–24 h would allow natural recovery of platelets from ticagrelor,25 and improve the efficacy of transfused platelets based on its pharmacokinetics (Table 2).

Another potential confounding element in platelet transfusion is the quality of platelets. In this regard, Prüller and colleagues26 demonstrated that transfusion of 3-day-old apheresis platelets (2 units) in subjects who received a 3-day course of ASA plus clopidogrel did not immediately improve platelet aggregation (LTA) induced by arachidonic acid or ADP, but they moderately improved aggregation (31–38%) in 24 h. The platelet apheresis devices used by Prüller’s group26 and Hansson’s group16 were the same, and therefore it is speculated that clinical efficacy of stored platelet transfusion could be overestimated by the experimental use of fresh platelet concentrates (<6 h of collection).

In addition to ADP-induced aggregation, Hansson and colleagues16 reported arachidonic acid responses to incremental doses of platelets. ASA-induced platelet inhibition was readily reversible with the lowest platelet dose, but inhibitory effects of ASA plus clopidogrel or ASA plus ticagrelor required medium–high platelet doses to approach normal aggregation. As mentioned above, excellent platelet responsiveness to arachidonic acid underlies the quality of fresh platelet concentrate used by Hansson and colleagues,16 but stored platelets could be less efficacious than fresh ones.26 The safety of preoperative ASA was assessed in the prospective study of non-cardiac surgical patients with cardiovascular risks. ASA (75 mg qd; n=145) or placebo (n=146) was randomly assigned to patients 10 days before the scheduled surgery. Approximately 70 and 30% of all patients were chronically on ASA and clopidogrel. There

<table>
<thead>
<tr>
<th>Method (manufacturer)</th>
<th>End point</th>
<th>Sample type/anti-coagulant</th>
<th>Detectable platelet inhibition/defect</th>
<th>Agonist(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregometry Various manufacturers</td>
<td>Turbidity change or impedance change</td>
<td>PRP/citrate or WB/citrated</td>
<td>Aspirin, P2Y12 inh, GPIIb/IIIa inh, GPIb, or vWF defect</td>
<td>ADP, Collagen, or Risto</td>
</tr>
<tr>
<td>Multiplate Roche; Indianapolis, IN, USA</td>
<td>Impedance change</td>
<td>WB/hirudin</td>
<td>Aspirin, P2Y12 inh, GPIIb/IIIa inh</td>
<td>ADP, Collagen, or TRAP</td>
</tr>
<tr>
<td>VerifyNow Accumetrics, San Diego, CA, USA</td>
<td>Fibrinogen bead aggregation</td>
<td>WB/citrate</td>
<td>Aspirin, P2Y12 inh, GPIIb/IIIa inh</td>
<td>ADP, arachidonic acid, or TRAP</td>
</tr>
<tr>
<td>Plateletworks Helena; Beaumont, TX, USA</td>
<td>Platelet count change</td>
<td>WB/citrate</td>
<td>Aspirin, P2Y12 inh, GPIIb/IIIa inh</td>
<td>ADP, arachidonic acid, or Collagen</td>
</tr>
<tr>
<td>PlateletMapping Haemonetics; Niles, IL, USA</td>
<td>Viscoelastic change</td>
<td>WB/heparin</td>
<td>Aspirin, P2Y12 inh, GPIIb/IIIa inh</td>
<td>ADP, or arachidonic acid (+-Reptilase/Factor XIII)</td>
</tr>
<tr>
<td>PFA-100 Siemens; Tarrytown, NY, USA</td>
<td>Closure of micropore under high shear</td>
<td>WB/citrate</td>
<td>Aspirin, GPIb, or vWF defect</td>
<td>Epi/ADP, or Epi/ Collagen under shear</td>
</tr>
<tr>
<td>T-TAS PL Zacros, Tokyo, Japan</td>
<td>Flow pressure elevation attributable to capillary occlusion under shear</td>
<td>WB/hirudin</td>
<td>Aspirin, P2Y12 inh, prostacyclin, GPIb, or vWF defect</td>
<td>Collagen under shear</td>
</tr>
</tbody>
</table>

Table 1 Platelet function tests. WB, whole blood; PRP, platelet-rich plasma; P2Y12 inh, P2Y12 inhibitors including clopidogrel, prasugrel, and ticagrelor; GPIIb/IIIa inh, glycoprotein IIb/IIIa inhibitors including epifibatide and tirofiban; GPIb, glycoprotein Ib receptor; vWF, von Willebrand factor; ADP, adenosine 5′-diphosphate; Epi, epinephrine; Risto, ristocetin; TRAP, thrombin receptor agonist peptide.  |
were no differences between continuation and interruption of ASA in terms of major thrombotic or bleeding events within 30 days of surgery. It is recommended to continue ASA in preoperative patients with cardiovascular risks because the antithrombotic benefit of ASA is considerably greater than the risk of bleeding.\cite{27} Monotherapy with ASA does not increase the risk of haematoma in patients who undergo regional anaesthesia (e.g. spinal block).\cite{28,29}

Finally, Hansson and colleagues\cite{16} observed that platelets from patients on DAPT yielded $\approx 25\%$ lower aggregation in response to thrombin receptor agonist peptide (TRAP). Jakubowski and colleagues\cite{30} had previously shown that mean platelet aggregation (LTA) induced by TRAP was reduced by 27\% with prasugrel plus ASA, and by 15\% with clopidogrel plus ASA. Cross-interactions among different platelet agonists and receptors are important in the formation of haemostatic plugs or pathological thrombus (Fig. 1).\cite{31} In this regard, Hosokawa and colleagues\cite{32,33} recently developed a microchip-based flow-chamber system that enables evaluation of platelet thrombus formation influenced by ASA, P2Y\textsubscript{12} inhibitors, or their combination under physiological shear rates (1000–2000 s\textsuperscript{-1}). Perfused platelets in hirudin-anticoagulated whole blood are activated by shear over the collagen-coated capillary, and thrombus (i.e. platelet aggregates) is subsequently formed after the endogenous release of thromboxane A\textsubscript{2} and ADP from adherent platelets (Fig. 1). This new monitoring approach appears to yield reasonable correlations with the MEA-collagen test for ASA and the MEA-ADP test for DAPT.\cite{33}

High negative predictive value (>90\%), and low positive predictive value (<20\%) for atherothrombotic events have been demonstrated with many platelet assays.\cite{2,4,5} Similarly, a MEA-ADP test cut-off of 31 AU x min was found to have 92\% negative predictive value, but 29\% positive predictive value for bleeding after cardiac surgery.\cite{22} These findings suggest that most patients do not develop thrombotic complications despite HPR on DAPT, and most patients with low platelet aggregability do not suffer from bleeding complications. Currently available platelet function tests are limited to the assessment of platelet aggregation and adhesion, and platelet-mediated haemostatic activities, including microparticle release and procoagulant reactions, cannot be ascertained.\cite{34} Other bleeding risk factors besides platelet function should also be considered in the perioperative management of haemostasis in cardiovascular patients. Corrections of hypofibrinogenaemia and reduced vitamin K-dependent clotting factors using fibrinogen concentrate and prothrombin complex concentrate are particularly important in the presence of thrombocytopenia, platelet dysfunction, or both.\cite{35}

**Fig 1.** Thrombus formation at vascular injury site. At the site of vascular injury, platelets adhere to subendothelial collagen via interactions between von Willebrand factor (vWF) and the platelet-surface glycoprotein receptor (GP, GPIb/IX). The platelet integrin receptor ($\alpha\text{IIb}\beta\text{3}$) reinforces binding to collagen. Trace amounts of thrombin are generated during the initiation phase of coagulation by FXa via interactions between circulating FVIIa and tissue factor (TF) expressed on subendothelial pericytes and fibroblasts. Platelets adherent to the vessel wall are activated by collagen and thrombin, releasing adenosine 5’-diphosphate (ADP), and thromboxane A\textsubscript{2} (TXA\textsubscript{2}). These mediators activate platelet GPIIb/IIIa on the surface of platelets in the vicinity (note: initial interaction via vWF between wall-adherent platelets and circulating platelets is not shown). Transiently formed platelet thrombus via vWF is subsequently stabilized by fibrinogen binding to GPIIb/IIIa receptors. ASA, aspirin; TP, prostanoid TXA\textsubscript{2} receptor.
The combination of global coagulation tests (thromboelastometry or thromboelastography) and MEA is one exemplary strategy to manage the use of factor concentrate plasma and platelets rationally. Recombinant activated factor VII and antifibrinolytic therapy may be also useful for bleeding associated with platelet inhibitors.

Clinical management for the patients with cardiovascular risks has been rapidly evolving with the introduction of novel antithrombotic and antiplatelet agents. A wide variety of platelet function tests with different techniques and end points is commercially available, but they are not routinely utilized to guide perioperative antiplatelet therapy at most medical institutions. This is largely attributable to the lack of standardization of platelet testing, and to the weak positive predictive value for atherothrombosis and bleeding. Platelets are empirically transfused, and their efficacy is guessed from an increase in platelet count. Individualized platelet transfusion is more desirable in terms of reducing the overall cost and the risk of allergic reaction, pathogen transmission, and immunomodulation associated with allogeneic platelets. The observations by Hansson and colleagues clearly demonstrate that the efficacy of platelet transfusion is strongly affected by the type of platelets (fresh vs stored), volume of transfusion, and circulating antiplatelet agents (active metabolites). Although still preliminary, these data collectively point to our future goal of individualized platelet transfusion therapy, in which optimal indications and doses of platelet transfusion can be selected in a timely fashion.

Declaration of interest
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Fibrinogen concentrate: clinical reality and cautious Cochrane recommendation

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A recent systematic review, ‘Fibrinogen concentrate in bleeding patients’, published by The Cochrane Collaboration, aimed to assess the benefits and harms of fibrinogen concentrate compared with placebo or other treatments in bleeding patients.1 We agree with the authors finding that fibrinogen concentrate reduces transfusion requirements for allogeneic blood products, as this is in line with previously published work.2 3 In addition, the review did not identify any adverse events associated with fibrinogen concentrate; again, this is in line with previous findings.4 5 However, we feel that the review also made a number of incorrect or misleading assertions which merit further discussion and clarification.