Platelet function point-of-care tests in post-bypass cardiac surgery: are they relevant?

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Background. Platelet dysfunction is an important cause of excessive bleeding after cardiac surgery. We assessed two platelet function point-of-care tests: the platelet function analyser (PFA-100) and the Hemostatus in patients with and without excessive bleeding after cardiac surgery with cardiopulmonary bypass.

Methods. Mediastinal chest tube drainage (MCTD) was measured for the first 6 h in the intensive care unit (ICU). Haematology and coagulation tests were done on arrival in the ICU, and when excessive bleeding occurred (MCTD >1 ml kg⁻¹ h⁻¹) or after 3 h.

Results. Eighteen patients bled excessively and 27 had normal MCTD. Hemostatus measurements were prolonged in those with excessive bleeding compared with the normal group. The times for PFA-100 adenosine diphosphate (ADP) and epinephrine were 91 vs 71 s (P = 0.004) and 155 vs 114 s (P = 0.02) in the bleeding and normal group s, respectively. None of the Hemostatus or PFA-100 values correlated with total MCTD. Depending on the agonist used, maximum aggregation was 33–81% and 52–86% in bleeding and normal groups, respectively. Only poor correlations were found between PFA-100 epinephrine and maximum aggregation in response to ADP (r = −0.52, P = 0.03) or to collagen (r = −0.48, P = 0.04).

Conclusion. Patients bleeding excessively in the ICU had abnormal measurements in point-of-care tests without a dramatic decrease in aggregation. Except for patients with increased risk of postbypass bleeding, point-of-care tests are not useful for routine use after cardiac surgery.

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Bleeding is a common complication of cardiac surgery with cardiopulmonary bypass (CPB), which can require transfusion of blood products and, in 3–6% of cases, mediastinal re-exploration.1 Of the causes of excessive bleeding, platelet dysfunction is considered to be the most important in the early postoperative period.

Two in vitro coagulation tests, the Hemostatus test and the platelet function analyser (PFA-100) are designed to provide simple and rapid assessment of platelet function, but their ability to predict excessive blood loss associated with CPB remains uncertain.2–5 However, the relevance of these tests when bleeding occurs in the intensive care unit (ICU) has been investigated less.7,8 We hypothesize that these point-of-care tests could help to detect poor platelet function and, with the help of guidelines,9,10 reduce the considerable variation in transfusion practice between institutions.11 We compared the PFA-100 and Hemostatus after cardiac surgery with CPB, in patients with and without excessive bleeding, in comparison with platelet aggregometry results.

Methods
The study was approved by our Institutional Ethics Committee. After obtaining informed consent, we recruited adult patients undergoing first-time cardiac surgery with CPB. We excluded patients who had taken antiplatelet drugs within 7 days of the operation, those on heparin or
anticoagulant therapy, or with bleeding diathesis or conditions that could affect platelet function. Because fibrinolytic activity can develop quickly, with maximal plasma levels of fibrin degradation products 30 min after infusion of protamine,\textsuperscript{12} patients who had diffuse bleeding from the surgical site without any identified source, after neutralization of heparin, were also excluded.

**Study groups**

Patients were recruited consecutively. We defined patients with excessive bleeding if they had mediastinal chest tube drainage (MCTD) >1 ml kg\(^{-1}\) h\(^{-1}\) for at least 1 h during the first 6 h after surgery. Patients were studied as those with and without excessive bleeding.

**Operative details**

Anaesthesia was induced and maintained with i.v. propofol (target controlled infusion), sufentanil citrate and atracurium. An ultra-low prophylactic dose of aprotinin (5\(\times\)10\(^5\) KIU) was administered before skin incision, and again during initiation of CPB. Patients were given heparin 300 UI kg\(^{-1}\) before CPB, and the activated clotting time (ACT), using kaolin as the activating agent (Medtronic, ACT II HemoTec, Rueil Malmaison, France) was maintained during CPB at a value greater than 450 s with additional doses of heparin as required. The extracorporeal circuit was primed with 1000–1200 ml lactated Ringer’s solution (Bioluz, St Jean de Luz, France) containing 4000 units of heparin. A membrane oxygenator was used and flows of 1.8–2.2 litre min\(^{-1}\) m\(^{-2}\) were obtained with a roller pump (Stöckert Instrumente, Munich, Germany) under mild hypothermic or normothermic conditions. After discontinuation of CPB, anticoagulation was reversed with protamine sulphate. Blood remaining in the CPB circuit was collected and infused into the patient before transfer to the ICU. Our practice was to maintain the haematocrit above 25\% after CPB as long as haemodynamic stability was maintained. Mediastinal blood loss was measured by the nurses every hour after arrival in the ICU. If bleeding was excessive, coagulopathy was treated as follows: (i) with additional protamine if the thrombin time was longer than 30 s, (ii) with 2–4 units of virally inactivated fresh frozen plasma if the activated partial thromboplastin time (aPTT) was greater than 50 s (1.5 times the normal value of our laboratory), (iii) with 8 units of platelet concentrate if the platelet count was less than 50 000\(\times\)10\(^9\) litre\(^{-1}\).

**Haematologic assays**

Native whole-blood samples were taken from a radial arterial catheter on arrival in the ICU for all patients (T\(_1\)), and when excessive bleeding was diagnosed or after 3 h in the ICU (T\(_2\)). Haemoglobin concentration, haematocrit and platelet count (Coulter Diagnostics, Villepinte, France) were measured in the two groups at T\(_1\) and T\(_2\). Anti-Xa activity was measured at T\(_1\) in all patients. A routine coagulation profile including prothrombin time, aPTT and fibrinogen concentration was done in all patients at T\(_1\) and T\(_2\).

The Hemostatus test was performed at T\(_2\) using the Hepron/Hemostatus\textsuperscript{TM} apparatus (Medtronic, Rueil-Malmaison, France). Six channels with cartridges containing 0.05 M calcium chloride, kaolin 4\% (w/v), and heparin 3 IU ml\(^{-1}\) were used. A syringe containing 3 ml native whole blood was mounted on the machine, and 0.35 ml automatically dispensed into each of six channels of the cartridge to measure the platelet-activated clotting time (PACT) in the presence and absence of platelet activating factor (PAF). Channels 1 and 2 did not contain PAF (control ACT) whereas channels 3–6 contained increasing concentrations of PAF (1.25, 6.25, 12.5 and 150 nM). Heparin was used to increase the sensitivity of the method by prolonging the Hemostatus values. Mechanical shear stress activation was obtained by a plunger mechanism contained in each channel. Hemostatus values were expressed directly as the clotting time (s).

The closure time was measured at T\(_2\) with the PFA-100 (Dade-Behring Inc., Miami, FL, USA). This device comprises a microprocessor-controlled instrument and disposable test cartridges with a bioactive membrane coated with type-I equine collagen and, depending on the cartridge, epinephrine or adenosine diphosphate (ADP). The instrument uses a constant vacuum to aspirate a citrated whole-blood sample introduced into the cartridge, through a capillary and a microscopic aperture cut into the membrane. The time from the start of the test until the occlusion of the aperture by a platelet plug was reported as the PFA-100 closure time (s).

Part of the citrated whole-blood samples (4.5 ml) taken from each patient at T\(_2\) was used for platelet aggregometry. Samples were centrifuged at 180 g for 10 min at room temperature to separate platelet-rich plasma. Platelet aggregation of platelet-rich plasma was assessed using the PAP-4 aggregometer (PAP-4 model; BIO/DATA Corporation, Paris, France), in the presence of different commonly used chemical activators: ADP (5 \(\mu\)m litre\(^{-1}\), Sigma A88.5-5), arachidonic acid (500 \(\mu\)m ml\(^{-1}\), Taussart et Matignon, France), collagen (16 \(\mu\)g ml\(^{-1}\), Sigma A88.6), epinephrine (8 \(\mu\)m litre\(^{-1}\), Sigma A88.5-5), and ristocetin (1.5 mg ml\(^{-1}\), Stago501). Maximum aggregation (MA) was registered for each agonist after 5 min.

Physicians were unaware of the results of Hemostatus, PFA-100 and aggregometry when treating the excessive MCTD.

**Data collection**

Patient characteristics and perioperative data were collected on a standardized questionnaire, and included age, sex, height, weight, duration of CPB, haematology and haemos-
tasis results, doses of heparin and protamine, core body temperature during the stay in the ICU, hourly MCTD for the 6 h after surgery, intravascular volume replacement with colloids (medium-molecular weight hydroxyethyl starch solution, 200 000 Da, degree of substitution 0.6), blood products given, and treatment with aprotinin (10^8 KIU) as part of our treatment plan to control postbypass bleeding.

**Statistical analysis**

Qualitative variables were compared between bleeding and non-bleeding groups using Fisher’s exact test, and continuous variables using the student’s t test or the Wilcoxon rank-sum test. Spearman’s rank correlation test was used to assess the relationship between Hemostatus and PFA-100 measurements and either platelet aggregation results or total MCTD. P<0.05 was considered statistically significant. The statistical analysis was done using STATA software.

**Results**

Excessive bleeding occurred in 18 patients and 27 patients did not have excessive bleeding. Age, sex and weight did not differ between groups (Table 1). Surgical procedures did not differ between the groups. Mean duration of CPB was not significantly different: 77 min in the bleeding group vs 83 min in the normal group. Mean heparin and protamine doses did not differ between the two groups.

Figure 1 shows the changes of MCTD and core body temperature over time in the ICU. In bleeding patients, MCTD reached the maximal value (204 ml) 2 h after surgery, then sharply decreased during the third hour and, according to our criterion, returned to normal bleeding rate (60 ml) after 4 h. Nine patients started to bleed excessively during the first hour, and nine others during the second hour in the ICU. The median duration of bleeding was 3 h in this group. Median blood loss in the first 6 h was 580 ml in the bleeding group and 125 ml in the non-bleeding group (P<0.001). The mean time of T$_2$ was 156 (SD 55) min in the bleeding group and 192 (33) min in the normal group. Core body temperature of patients with bleeding was lower than in those without bleeding, especially during the third hour after surgery, but no statistically significant difference was shown during the stay in the ICU. All patients reached a core body temperature of 37°C by 4 h.

Treatment of excessive bleeding is shown in Table 2. Median volumes of red blood cells and virally inactivated fresh frozen plasma given were 460 and 200 ml, respectively. No patients required surgical re-exploration.

Table 3 summarizes haemoglobin concentration, platelet count and routine coagulation values at T$_1$ and T$_2$. At T$_1$, differences were found between bleeding and normal groups in haemoglobin concentration (10.7 vs 11.1 g litre$^{-1}$, P<0.05), and mean fibrinogen concentration (2.5 vs 3.3 g litre$^{-1}$, P<0.01). Mean platelet count did not differ between the two groups on arrival in the ICU (127 vs 154×10$^9$ litre$^{-1}$, respectively). At T$_2$, all haematology values reported in Table 3 were significantly less in bleeding patients than in normal patients, except for aPTT, which did not differ between the two groups (46.7 vs 43.4 s, respectively).

The results of Hemostatus and PFA-100 at T$_2$ are shown in Table 4. In bleeding patients, PACT values were significantly prolonged in all channels compared with normal patients, and PFA-100 ADP and epinephrine closure time values reached 91 vs 71 s (P<0.01) and 155 vs 114 s, respectively (P<0.05). However, after adjusting PACT and PFA-100 closure time values at T$_2$ for haemoglobin concentration and platelet count by logistic regression, there was no difference between groups. The Hemostatus or PFA-100 values did not correlate with total MCTD.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bleeding patients (n=18)</th>
<th>Normal patients (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range) (years)</td>
<td>67 (50–82)</td>
<td>67 (46–77)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>83</td>
<td>74</td>
</tr>
<tr>
<td>Mean (SD) weight (kg)</td>
<td>73 (3)</td>
<td>72 (2)</td>
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<tr>
<td>Type of surgery (%)</td>
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<td></td>
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<tr>
<td>Coronary artery bypass grafting</td>
<td>44</td>
<td>48</td>
</tr>
<tr>
<td>Valve</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>Combined procedure</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Mean (SD) duration of CPB (min)</td>
<td>77 (6)</td>
<td>84 (5)</td>
</tr>
<tr>
<td>Median heparin dose (×10$^3$ IU)</td>
<td>25 (21–30)</td>
<td>24 (22–27)</td>
</tr>
<tr>
<td>Mean (SD) protamine dose (mg)</td>
<td>27 (16)</td>
<td>23 (11)</td>
</tr>
</tbody>
</table>

**Fig 1** Median mediastinal chest tube drainage (MCTD) and mean (SD) core body temperature over time in the intensive care unit in bleeding and normal patients.
Table 5 shows the results of platelet aggregometry performed at T2 with collagen, ristocetin and arachidonic acid as chemical activators in bleeding and normal patients. Platelet MA was significantly lower in bleeding patients compared with normal patients, in the presence of ADP (50.1% vs 61.2%, \( P<0.05 \)) and epinephrine (32.9% vs 51.9%, \( P<0.05 \)).

Table 6 shows the relationship between Hemostatus, PFA-100 closure time results and MA in bleeding patients. None of the Hemostatus values correlated with MA. No correlation was found between PFA-100 closure time and MA results except for PFA-100 epinephrine which correlated with MA in the presence of ADP (\( r=-0.52, P<0.05 \)) and collagen (\( r=-0.48, P=0.05 \)).

**Discussion**

We found that point-of-care tests of platelet function are significantly prolonged when excessive bleeding occurs. These tests did not correlate with total MCTD, and only a weak correlation was found between PFA-100 epinephrine and laboratory-based platelet aggregation. Depending on the agonist used, MA was 33–81% in the bleeding group and 52–86% in the non-bleeding group. No significant difference existed between the groups, except for ADP and epinephrine tests.

Preliminary studies reported a great sensitivity of Hemostatus and PFA-100 tests to major platelet adherence or aggregation abnormalities. With the Hemostatus test, Coiffric and colleagues found impairment of GPIIb–IIIa receptors by c7E3 (chimeric Fab fragments of a murine monoclonal antibody), lengthening the PACT by up to 1500 s, when mean PACT values of the 18 controls ranged from 517 (SD 121) s to 350 (124) s (channels 1 and 2, and 3–6, respectively). Others reported lengthening of PFA-100...
closure time, by impairment of von Willebrand factor or inhibition of GPIb and GPIIb–IIIa receptors. 14–15 In the present study, in spite of a significant difference between the groups, Hemostatus and PFA-100 values lay in ranges described for healthy volunteers. 16–17 These data should be compared with laboratory-based platelet aggregation tests that are very sensitive to the impairment of platelet function after CPB. 18 Although these tests are highly dependent on sample preparation and operator, 19 they do not show a severe impairment of GPIb and IIb–IIIa receptors of patients bleeding excessively in this study. MA in response to ADP and epinephrine differed at T 2 but this is probably not clinically significant. This result may be explained by the fact that aggregometry is performed on platelet-rich plasma, while the PFA-100 closure time is explained by the response to ristocetin fits well with normal functional responses of GPIb and GPIIb–IIIa. For these reasons, major abnormalities of platelet membrane receptors are unlikely to be responsible for the slight increase of Hemostatus and PFA-100 measurements observed in patients with bleeding in the present study. On the whole, our results failed to show a correlation between point-of-care tests and aggregometry in patients with bleeding, especially between PFA-100 epinephrine and platelet aggregometry induced by epinephrine. This may be explained by the fact that aggregometry is performed on platelet-rich plasma, while the PFA-100 closure time is done on native whole-blood samples and under high shear conditions very different from those of platelet aggregometry.

Other factors, apart from platelet defects, can affect these point-of-care tests on whole blood, such as altered haematocrit and platelet count. 13–22 Red blood cells can also affect platelet function, related to stimulation of platelet production of thromboxane A 2 , 23 or to shear-induced release of ADP when haematocrit is 25–35%. 24 Hemostatus should reliably detect platelet dysfunction only if the platelet count is greater than 50–70×10 9 litre −1 and the haematocrit exceeds 30%. 21 To our knowledge, neither the manufacturers nor the literature provide information about correction of Hemostatus results for altered haematocrit. In our study, platelet count and haematocrit were significantly lower in bleeding patients, without exceeding the limits of interpretation of the point-of-care tests. However, because haemodilution is common after cardiac surgery, we investigated the possibility that differences between groups could be reduced by adjusting Hemostatus and PFA-100 values for haemoglobin concentration and platelet count by logistic regression. Our results suggest these two variables are an important source of variation in the results of Hemostatus and PFA-100, and could explain the differences between bleeding and normal patients.

The use of aprotinin in the present study may be questioned too, since the protective mechanism of aprotinin on platelet function is controversial. 25–26 However, the initial elimination half-life of aprotinin is about 1 h, so platelet function is unlikely to be affected by the ultra-low prophylactic dose administered before skin incision and during initiation of CPB. Furthermore, blood samples for aggregometry and point-of-care tests were collected before any possible therapeutic use of aprotinin, as part of our treatment plan to control postbypass bleeding in the ICU.

Preliminary reports on the ability of Hemostatus and PFA-100 tests to predict excessive blood loss associated with CPB gave mixed results. 2–5 Our primary hypothesis
was that these tests could detect a platelet function defect, and be used to rationalize transfusion practices. The fact that no relationship exists between Hemostatus and PFA-100 results and total MCTD does not necessarily mean that these tests are not specific and reliable, because defects after bypass are generally thought to be secondary to the activation, consumption or dilution of several constituents of haemostasis. We found discrepancies in the results of point-of-care tests of bleeding and normal groups, and an overall lack of correlation with MCTD. In patients with a low risk for postoperative bleeding, who account for about 60% of patients, the routine use of Hemostatus and PFA-100 is unlikely to improve transfusion practice. However, these tests would be more efficient when patients are at high risk of perioperative bleeding, for example patients with von Willebrand disease.27,28

In our study, which excluded patients receiving antiplatelet drugs, anticoagulant therapy or a preoperative bleeding diathesis, we conclude that these point-of-care tests should not be used for routine evaluation when excessive bleeding occurs after cardiac surgery. Clinical assessment and transfusion algorithms, with continuous assessment of mediastinal drainage, are essential in modern management. However, because they can detect severe platelet dysfunction, further studies are necessary to assess these tests for the management of patients with von Willebrand disease or receiving anti-GPIIb–IIIa drugs.

Acknowledgements

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