Assessment of thrombocytopenic disorders using the Platelet Function Analyzer (PFA-100®)*

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Summary. The Platelet Function Analyzer (PFA-100®) was used to measure platelet function in paediatric patients with destructive versus underproduction thrombocytopenia. Closure time (CT) and total volume (TV) measurements with standard 150 μm apertures discriminated between patients with similar platelet counts from 30 to 150 × 10^9/l. However, at platelet counts < 30 × 10^9/l, a 100-μm aperture (experimental) gave the best assessment of platelet function. TV results could be analysed even when CTs were indeterminate. Further investigations are warranted to more fully understand the relationships among platelet function as measured by the PFA-100® in standard/experimental modes, bleeding and transfusion outcome in thrombocytopenia.

Keywords: platelet function, PFA-100®, thrombocytopenia, ITP.

Platelets are essential in haemostatic plug formation at sites of vessel injury and quantitative platelet abnormalities may result in clinically significant bleeding (Rintels et al, 1994). The contribution of platelet function to bleeding risk in thrombocytopenia is difficult to assess with standard in vitro tests (Rand & Dean, 1998) and the skin bleeding time is poorly reproducible and a poor predictor of bleeding risk (Lind, 1991; Peterson et al, 1998).

The Platelet Function Analyzer (PFA-100®; Dade Behring, Deerfield, IL, USA) was developed as a quantitative, rapid, in vitro test of platelet function at high shear rates (Kundu et al, 1994, 1995, 1996; Mammen et al, 1995). Citrated whole blood is aspirated through a 150 μm diameter aperture in a membrane coated with collagen and epinephrine (Col/Epi) or adenosine 5′-diphosphate (Col/ADP); a platelet plug forms that occludes the aperture (Kundu et al, 1996) and the closure time (CT) is recorded.

By lowering platelet counts in vitro, Kundu et al (1996) and Harrison et al (1999) showed a progressive increase in CTs with decreasing platelet count. Here, the standard aperture was compared with smaller experimental apertures of 120 and 100 μm (not commercially available) in children with varying degrees of thrombocytopenia as a result of accelerated platelet destruction or decreased platelet production. Using an experimental mode, the total volume (TV; not commercially available) that flows through the aperture before occlusion was also measured.

PATIENTS AND METHODS

Controls and patients. Healthy children (5–17 years; n = 20) were screened as previously described (Carcao et al, 1998). Their platelet counts were > 150 × 10^9/l and their haematocrits were > 35%.

Patients were from the Haematology/Oncology Programme at The Hospital for Sick Children, Toronto. Those with destructive thrombocytopenia (3–17 years; n = 44) had primary immune thrombocytopenic purpura (ITP) [mean platelet count: 50 × 10^9/l (range: 3–127 × 10^9/l); mean haematocrit: 40% (range: 33–48%)] and those with underproduction thrombocytopenia (2–18 years; n = 70) had thrombocytopenia secondary to decreased platelet production [mean platelet count: 50 × 10^9/l (range: 4–148 × 10^9/l); mean haematocrit: 27% (range: 18–43%)]. The latter group consisted mainly of patients...
with malignancies receiving chemotherapy ($n = 55$); the remainder had familial thrombocytopenia or bone marrow failure states, or were in the engraftment phase following bone marrow transplantation. Patients were excluded if they had sepsis or had received a platelet transfusion within 72 h.

Informed consent was obtained for all subjects and the study was approved by the Hospital Research Ethics Board.

Methods. Blood sampling and PFA-100® testing were done as previously described (Carcao et al., 1998) with the following modifications. Six cartridge types were used ($Col/Epi – 150 \mu m$, $120 \mu m$, $100 \mu m$ apertures; $Col/ADP – 150 \mu m$, $120 \mu m$, $100 \mu m$ apertures) and, in addition to CTs, TVs were recorded. Mean CTs and TVs were significantly shorter by 10–20% with the $120 \mu m$ apertures and by 20–30% with the $100 \mu m$ apertures ($P < 0.05–0.001$) compared with the standard apertures.

In approximately 33% of patients with underproduction thrombocytopenia with platelet counts < $50 \times 10^9/l$, an indeterminate CT (i.e., CT value was less than 300 s and was preceded on the print-out by a ‘>’ symbol indicating that the test had been interrupted) was recorded with at least one cartridge type with the 150 and/or 120 $\mu m$ apertures. These patients had significantly lower haematocrits than the other patients in the group ($23.9 \pm 3.94$ vs $28.7 \pm 6.4$, means ± 1 SD; $P = 0.0002$); decreased haematocrits are associated with increased CTs (Kundu et al., 1996; Escolar et al., 1999; Harrison et al., 1999). Indeterminate CTs were excluded from analysis, but all TV measurements were utilized.

Platelet counts and haematocrits were enumerated using a Coulter MAXM (Coulter Electronics Canada, Burlington, ON, Canada). Platelet counts < $50 \times 10^9/l$ were confirmed by manual counting.

Statistical analysis. Data were analysed using Student’s $t$-test or repeated measures analysis of variance followed by post hoc testing. Differences were considered statistically significant at $P < 0.05$. Locally weighted regression plots were obtained for graphical association between CTs or TVs and platelet counts.

RESULTS

At a given platelet count, in both thrombocytopenic groups, the lowest CTs and TVs were observed with the $100 \mu m$ and the highest with the $150 \mu m$ aperture cartridges (Figs 1 and 2).

![Graphs of closure times and total volumes vs platelet counts for patients with destructive thrombocytopenia](image)

**DESTRUCTIVE THROMBOCYTOPENIA**

Fig 1. Locally weighted regression plots of closure times vs platelet counts for patients with destructive thrombocytopenia for the (A) Col/Epi and (B) Col/ADP cartridges and of total volumes vs platelet counts for the (C) Col/Epi and (D) Col/ADP cartridges with aperture diameters of 150 $\mu m$, 120 $\mu m$ and 100 $\mu m$. 

Destructive thrombocytopenia

With the Col/Epi cartridges, CTs (Fig 1A) showed little change until platelet counts decreased to $< 75 \times 10^9/l$. With lower counts, CTs were increasingly more prolonged, becoming non-closures (i.e. CT $> 300$ s) with the 150 and 120 $\mu$m apertures at platelet counts $< 30 \times 10^9/l$ and with the 100 $\mu$m aperture at platelet counts $< 10 \times 10^9/l$. With the Col/ADP cartridges, CTs (Fig 1B) showed little change until the platelet counts decreased to $< 40 \times 10^9/l$. With the 150 and 120 $\mu$m apertures, CTs then steadily increased, becoming non-closures at platelet counts $< 10 \times 10^9/l$. In contrast, only slight increases in CTs were observed for the 100 $\mu$m aperture at platelet counts $< 40 \times 10^9/l$; few non-closures were observed even at counts $< 10 \times 10^9/l$.

TV results were very similar to CT results for both cartridge types. The major differences occurred with the 100 $\mu$m apertures at low platelet counts. With the Col/Epi cartridge (Fig 1C), the TV of blood added did not flow through the aperture even at platelet counts $< 10 \times 10^9/l$ and with the Col/ADP cartridge (Fig 1D) TVs hardly increased at counts $< 40 \times 10^9/l$.

Underproduction thrombocytopenia

In these patients, a more immediate increase in closure time with decreasing platelet count was observed for both Col/Epi and Col/ADP cartridges (Fig 2A and B) than in patients with destructive thrombocytopenia (Fig 1A and B). In general, CTs were longer with all Col/ADP cartridges than those in patients with destructive thrombocytopenia over the whole range of low platelet counts. At platelet counts $< 20 \times 10^9/l$, all cartridges tended towards non-closure, with the 100 $\mu$m aperture Col/Epi cartridge producing the most closures (Fig 2A). Non-closure was observed for all three Col/ADP cartridges at very low platelet counts (Fig 2B), in contrast with the 100 $\mu$m aperture in destructive thrombocytopenia (Fig 1B).

TV results (Fig 2C and D) were very similar to CT results. Primary differences occurred with the 100 $\mu$m aperture Col/ADP cartridge (Fig 2D) at very low platelet counts, where TV of blood added generally flowed through the aperture at platelet counts $< 10 \times 10^9/l$ (compare with destructive thrombocytopenia, Fig 1D).
DISCUSSION

As measured by the PFA-100®, platelet function in destructive thrombocytopenia appears to be better than in underproduction thrombocytopenia. This is in keeping with the previous observations that platelets in ITP are more functional (e.g. Harker & Slichter, 1972).

In the context of thrombocytopenia, the standard 150 μm aperture Col/ADP cartridge may be most useful for broadly discriminating between patients with similar platelet counts (at 30–150 × 10^9/l) but varying platelet function and, thus, potentially identifying patients with platelet-related bleeding risks. At very low platelet counts (< 30 × 10^9/l), assessment of platelet function with the PFA-100® might best be determined using the Col/ADP 100 μm aperture cartridges. Further clinical studies could determine whether the PFA-100® with different aperture diameter cartridges and TV measurements along with CTs would reliably assess platelet function in thrombocytopenic patients in terms of predicting clinically severe bleeding. The potential for using the PFA-100®, with or without the enhancements described here, to assess in vitro and in vivo function of donor platelets transfused into thrombocytopenic patients is also worthy of consideration.

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REFERENCES


