bjh review

The role of PFA-100® testing in the investigation and management of haemostatic defects in children and adults

Paul Harrison

Oxford Haemophilia Centre and Thrombosis Unit, Churchill Hospital, Headington, Oxford, UK

Summary

The PFA-100® provides a simple global measure of high shear-dependent platelet function, and as such is not diagnostic or specific to any disorder. Prolonged closure times must be interpreted in conjunction with a full blood count, von Willebrand factor (VWF) screen and other platelet tests. The PFA-100® may also give false negative results with relatively common platelet defects. If clinical suspicion is high, further detailed platelet function testing and VWF screening are required to exclude abnormal platelet function, even if the PFA-100® is normal. In more recent studies the PFA-100® has been used for preoperative identification and management of surgical patients with haemostatic defects and for assessing the clinical effectiveness of platelet transfusion therapy. This review highlights the up to date, evidence-based, advantages and disadvantages of the PFA-100® test in the investigation and management of haemostatic disorders in both children and adults.

Keywords: PFA-100®, platelet function testing, aggregometry, bleeding time, high shear.

Current platelet function tests are labour intensive, time consuming and require a fair degree of expertise to perform and interpret, e.g. aggregation tracings. Since the last British Committee for Standards in Haematology (BCSH) guidelines for platelet function testing were published (British Society for Haematology BCSH Haemostasis and Thrombosis Task Force, 1988), there have been considerable advances with the availability of new platelet function tests including the development of a range of global instruments/tests that attempt to simulate in vivo platelet adhesion and aggregation (Harrison, 2000, 2005; Rand *et al*, 2003). One of these tests, 'the thrombostat 4000', was developed in 1985 in an attempt to simulate high shear-dependent platelet adhesion and aggregation to collagen-coated surfaces (Kratzer & Born, 1985). In the mid-1990's, Dade-Behring (Marburg, Germany) further refined the test into a commercially available instrument called the platelet function analyser or PFA-100® (Kundu *et al*, 1995; Jilma, 2001; Francis, 2004). The instrument utilizes test cartridges that contain a collagen/ADP (CADP) or collagen/epinephrine (CEPI) coated membrane with a small central aperture (147 μm). Citrated blood is aspirated under high shear (5000–6000/s) from the sample reservoir through a capillary tube onto the membrane and blood flow is monitored through the aperture. Platelets begin to adhere and aggregate, primarily via von Willebrand factor (VWF) interactions with glycoprotein (Gp)Ib and GpIIb/IIIa, resulting in occlusion of the aperture normally between 1 and 3 min from test initiation. The instrument simply monitors the drop in flow rate with time as the aperture gradually occludes and records the final closure time (CT) and volume of blood that has been aspirated through the aperture. Maximal CTs are 5 min and the instrument will give a result >300 s if this time is exceeded. PFA-100® testing is relatively simple, rapid and easy to learn. However, there are some recently published detailed guidelines/procedures that can help to ensure optimal performance (Harrison, 2004). These include daily quality control checks, quality of anticoagulation and blood sampling, establishing local normal ranges and controlling for possible cartridge batch variation (Bock *et al*, 1999; Harrison, 2004). Various recent articles have highlighted the remarkably similar published normal ranges for both CADP and CEPI cartridges, within either 3·2% or 3·8% citrated blood, reported from many laboratories (Favaloro *et al*, 1999; Favaloro, 2001; Jilma, 2001). This adds to the growing evidence that suggests that the test is reproducible both between cartridge lots and instruments. However, despite good inter-laboratory agreement, each test centre should also ideally establish their own normal reference ranges on both cartridges utilizing normal volunteers (Harrison, 2004). Intra-sample reproducibility has been reported to be approximately 10%, which, although probably acceptable for a test of this type, may cause problems in the interpretation of values close to the upper cut-off of normal ranges (Harrison *et al*, 1999).

Variables that influence the PFA-100

The PFA-100® is a global test of high shear-dependent platelet adhesion and aggregation. The test is, therefore, sensitive to a

Correspondence: Dr Paul Harrison, Oxford Haemophilia Centre and Thrombosis Unit, Churchill Hospital, Headington, Oxford OX3 7LJ, UK. E-mail: paul.harrison@ndm.ox.ac.uk

  doi:10.1111/j.1365-2141.2005.05511.x

large number of variables that are also known to affect platelet function *in vivo* (Kundu *et al*, 1996; Harrison *et al*, 1999). The PFA-100® is sensitive to platelet count, haematocrit, drug effects, dietary effects, major platelet receptor defects, VWF defects, release defects and granular defects (Kundu *et al*, 1996; Fressinaud *et al*, 1998; Mammen *et al*, 1998; Cattaneo *et al*, 1999a,b; Harrison *et al*, 1999; Jilma, 2001). Testing should always be performed with knowledge of the full blood count, as platelet counts and haematocrits below $<80 \times 10^9$/l or <30% respectively often result in prolongation of the CT (Kundu *et al*, 1995; Harrison *et al*, 1999; Jilma, 2001). Citrate samples have been shown to be stable for up to 5–6 h (<4 h recommended by Dade-Behring) after collection for PFA-100® testing (Harrison *et al*, 1999), but transport through a vacuum transport system is not recommended prior to analysis as this may influence the results (Dyszkiewicz-Korpanty *et al*, 2004). As the PFA-100® is a high shear system, plasma and platelet levels of VWF are very important in determining a normal CT (Fressinaud *et al*, 1998, 1999). There is a strong inverse correlation between PFA-100® CTs and plasma VWF levels. ABO blood groups, therefore, also influence the PFA-100, although CTs are usually in the normal range (Moeller *et al*, 2001). Not surprisingly, the test has, therefore, been reported to be very sensitive to all subtypes of von Willebrand disease (VWD) (except type IIN, where there is defect in the Factor VIII binding capacity of VWF but not in platelet function) (Fressinaud *et al*, 1998, 1999). Any significant defect in copy number or functionality of GpIb or GpIIb/IIIa also results in prolongation of CTs (Harrison *et al*, 1999, 2002a). Both collagen receptors Gp Ia/IIa and GPVI also seem to play a role in the direct activation and/or adhesion of platelets to the membrane (Di Paola *et al*, 1999; Best *et al*, 2003). The CEPI cartridge has also been reported to be more sensitive than the CADP cartridge at detecting storage and/or release defects in dense granular ADP and defects in thromboxane generation (e.g. caused by aspirin) (Cattaneo *et al*, 1999b; Harrison *et al*, 1999; Homoncik *et al*, 2000; Jilma, 2001). This suggests that the high concentration of ADP within the CADP cartridge is somewhat capable of largely bypassing these defects, although prolonged CADP CTs have also sometimes been reported in patients with storage pool and release defects (Harrison *et al*, 1999; Francis, 2004). In contrast, PFA-100® CTs appear largely insensitive to the absence of coagulation factors (e.g. fibrinogen, factor V, factor VIII, and factor IX), suggesting that thrombin generation and fibrin formation is insignificant during the relatively short-time to platelet plug formation under the high shear conditions in the aperture of the membrane (Harrison *et al*, 1999; Jilma, 2001). PFA-100® CTs appear to be slightly shorter in the morning (probably related to platelet hyper-functionality) and gradually increase during the day, therefore, it is unknown whether this may interfere with clinical interpretation of results, particularly those near to cut-off values (Dalby *et al*, 2000). The PFA-100® is also sensitive to many acquired variables that affect platelet function, including subtle dietary factors (e.g. cocoa resulting in an increase in CEPI and/or CADP CTs) and drug effects (e.g. aspirin and non-steroid anti-inflammatory drugs) (Jilma, 2001; Jilma-Stohlawetz *et al*, 2001; Pearson *et al*, 2002; Francis, 2004; Harrison *et al*, 2004). Abnormal PFA-100® results can, therefore, be frequently encountered in both inpatient and outpatient settings and this may be related to medications, diet and additional unknown factors. Given the transient nature of some of these factors, repeat testing is, therefore, often required to often fully interpret prolonged CTs.

The PFA-100® versus the bleeding time as a Screening Tool

Within the last 15 years it has become increasingly recognized that the *in vivo* bleeding time (BT) has a number of significant disadvantages when applied as a screening test, resulting in the decreasing popularity and usage of this test (Rodgers & Levin, 1990; Lind, 2002; Francis, 2004). In particular, the BT is insensitive to many mild platelet defects and does not necessarily correlate with, or even predict, a bleeding tendency. An accurate bleeding history is, therefore, now considered as a more valuable screening test. The important question is, therefore, whether the same limitations apply to the PFA-100®, which is often considered as an *in vitro* equivalent of the BT. There have been a number of head-to-head comparisons of the PFA-100® with the BT. Early studies have shown the PFA-100® to be more sensitive to VWD, including type I VWD than the BT (Fressinaud *et al*, 1998; Cattaneo *et al*, 1999a; Favaloro *et al*, 1999; Dean *et al*, 2000). A direct comparison of the two tests with platelet defects in an unselected population demonstrated improved sensitivity of the PFA-100® over the BT and with a high degree of agreement with platelet aggregation (86%) (Francis *et al*, 1999). A more recent study also demonstrated that the PFA-100® was more sensitive than the BT to VWD and platelet defects in a paediatric population (Cariappa *et al*, 2003). Although the overall sensitivity of the PFA-100® to VWD seems to be excellent when compared with the BT, there have been reported differing sensitivities to type I VWD (Harrison *et al*, 2002a,b; Liesner *et al*, 2004; Quiroga *et al*, 2004a). Borderline VWF levels on either side of laboratory cut-offs can sometimes give normal or abnormal CTs. This is further complicated by the important roles of platelet VWF (which may be normal or abnormal), temporal variation in VWF levels (sometimes within the normal range), collagen receptor density [which can often be lower in type I VWD (Di Paola *et al*, 1999)] and blood groups, all of which affect the clinical phenotype and PFA-100® CTs. Type I VWD, therefore, remains a diagnostic challenge. The true performance and clinical relevance of the PFA-100® in type I VWD should be shortly revealed from the results of a large European Union multicentre study (MCMDM-1VWD). In this study, a clinical bleeding score will be compared with a phenotypic assessment including the PFA-100® and BT.

In patients with Hermansky–Pudlak syndrome the BT actually seemed slightly more sensitive than the PFA-100®

although numbers studied were small (Kerenyi *et al*, 1999). A comparison of BT and PFA-100® in patients with congenital defects of platelet secretion [storage pool disease (SPD) and primary secretion defects (PSD)] showed that both tests performed similarly with low sensitivity (Cattaneo *et al*, 1999b). Given that a large number of platelet abnormalities encountered are PSD (Cattaneo, 2003), this represents a significant limitation of the PFA-100®. Perhaps the future availability of different agonists within new cartridges may help to improve the sensitivity of the PFA-100® to these type of disorders (Escolar *et al*, 2001). However, the CEPI cartridge is more sensitive than the CADP cartridge in detecting these type of disorders and has been recently shown to be significantly associated with the clinical bleeding score in 128 patients (Podda *et al*, 2005).

A more recent study demonstrated that the PFA-100® and BT were both relatively insensitive in 148 patients with unequivocal mucocutaneous bleeding and positive family history (Quiroga *et al*, 2004a). As expected the PFA-100® performed slightly better than the BT in patients with type I VWD (sensitivity 61·5% vs. 42%) but at a much lower sensitivity than previous studies (Quiroga *et al*, 2004a). However, the BT was actually slightly better than the PFA-100® in patients with PSD (sensitivity 42% vs. 24%) (Quiroga *et al*, 2004a). The main conclusion from the study is that use of the BT and PFA-100® in the diagnostic work up of these type of patients is not completely justified and that global screening tests (e.g. BT and PFA-100®) are therefore of limited utility (Cattaneo, 2004; Quiroga *et al*, 2004a). Therefore, one could argue that a clinician faced with an individual with a well defined personal and family history of mucocutaneous bleeding should therefore not use the results of a global screening test to influence their decision whether to undertake further specific platelet function tests (Cattaneo, 2004). However, the PFA-100® does have a high sensitivity to VWD and one could equally argue that it still offers the relative comfort of a rapid screening test well before VWF and other platelet function tests can all be performed to give an accurate diagnosis (Favaloro, 2004). The availability of new rapid and reliable automated VWF antigen and VWF activity assays may somewhat help to alleviate this problem by significantly decreasing the time delays before results are obtained. However, given the high negative predictive value of the PFA-100®, the test can be urgently utilized to exclude not only VWD but also severe but rare severe platelet defects, such at Glanzmann's Thrombasthenia and Bernard Soulier (BS) disease, as these also give very prolonged CTs (Favaloro, 2004). As BS patients also present with macrothrombocytopenia, the CT is useful at discriminating this severe platelet defect from other thrombocytopenic conditions presenting with large platelets and similar counts (e.g. idiopathic thrombocytopenic purpura), as these often give normal CTs (Carcao *et al*, 2002). However, if the clinical suspicion is strong then specific tests must always still be performed even if the PFA-100® is normal (Cattaneo, 2004; Favaloro, 2004). Providing there is sufficient time to perform a comprehensive platelet function and VWF analysis then one could also argue that the PFA-100® may also be completely omitted from being used. Certain laboratories have therefore abandoned the use of the PFA-100® and the BT as a screening test because of these problems (Hajdenberg, 2004), although many laboratories still maintain the need for a global test of platelet function, mainly because clinicians historically request and like an urgent and instant test result (Quiroga *et al*, 2004b). The test may therefore be somewhat limited but still offers a rapid means to exclude the presence of VWD and the more severe forms of platelet disorders because of its high negative predictive value for these disorders (Favaloro, 2004). Of course, any false positive samples will also have to be fully tested with a panel of additional platelet function tests to eliminate any possibility of a platelet defect. Interestingly, the PFA-100® has been reported to potentially identify an unknown platelet defect(s) with consistently very prolonged CTs (>300 s) on both cartridges despite all other tests being normal in a small number of patients with well defined bleeding histories (Oxford Haemophilia Centre, unpublished observations and Doug Christie, personal communication). This suggests that the PFA-100® may identify as yet unknown defect(s) in shear-dependent platelet function.

The use of the PFA-100® for preoperative identification and management of patients with impaired haemostasis

Approximately 3–5% of patients undergoing surgery have either an acquired platelet defect, a congenital platelet defect or VWD. Could a preoperative screen with a test, such as the PFA-100®, therefore be beneficial to not only to potentially exclude them from surgery but also monitor any haemostatic therapy? A very recent large prospective study attempted to identify both primary and secondary haemostatic defects before surgery in 5649 unselected adult patients (Koscielny *et al*, 2004a). Each patient filled in a detailed questionnaire concerning bleeding history and a number of screening tests were performed including PFA-100® (CADP and CEPI cartridges), activated partial thromboplastin time, prothrombin time and platelet count. VWF and BT's were additionally performed in those patients with a positive bleeding history and/or evidence of a haemostatic defect. The bleeding history was negative in 89% ($n = 5021$) of patients and positive in the remaining 11% ($n = 628$). Of these, impaired haemostasis was verified in 40·8% ($n = 256$). Interestingly, the CEPI cartridge identified 98% ($n = 250$) of these patients (sensitivity of 91%) while the CADP cartridge identified 78%. The overall sensitivity of the CEPI cartridge was the highest (91%) compared with all other screening tests. The positive predictive value of the CEPI cartridge was 82% with a negative predictive value of 93%. Therefore, the combined use of a standardized questionnaire and PFA-100® as a screening test were capable of detecting impaired haemostasis in almost every case (Koscielny *et al*, 2004a). As part of this study, patients with impaired

primary haemostasis were then treated with desmopressin (DDAVP) (Koscielny *et al*, 2004b). Non-responders, as defined by the PFA-100®, were additionally treated with tranexamic acid or aprotinin and in the cases with VWD, VWF-containing factor VIII concentrates. The treatment of DDAVP led to correction of PFA-100® CTs of 229 of 254 patients and tranexamic acid was effective in 12 of 16, aprotinin in three of five and factor VIII concentrates in four of four patients. If all of these treatments were ineffective then conjugated oestrogens ($n = 6$) were given and finally, as a last resort, platelet transfusions were given ($n = 2$). Interestingly, the frequency of blood transfusions was no different (9·4% vs. 12·2%, $P = 0{\cdot}202$) in preoperative patients with impaired haemostasis than in patients without impaired haemostasis. In a retrospective study, the frequency of blood transfusions was significantly higher (89·3% vs. 11·3%, $P < 0{\cdot}001$) in patients without preoperative correction of impaired haemostasis *versus* patients without impaired haemostasis (Koscielny *et al*, 2004b). This shows the potential of the PFA-100® not only as a preoperative screening test but for monitoring preoperative correction with pro-haemostatic agents (Koscielny *et al*, 2004a,b). The use of the PFA-100® in this setting may therefore potentially reduce the number of unnecessary blood transfusions. The key criticisms of this study are that the control group was from a retrospective study, that there was a surprisingly low number of coagulation abnormalities (and those detected were lupus anticoagulant) and that it would have been much better to randomize the 256 patients with abnormal haemostasis, to treated and untreated, to determine if there were differences in blood transfusion usage. However, these are the largest studies to date on an unselected population of surgical patients and further work is required to clarify the role of the PFA-100® in this important area.

The PFA-100® can also be potentially used to monitor the effectiveness of platelet transfusion therapy. In a recent study, platelet function testing using the PFA-100® provided a better indication of outcome than did the gold standard, the post-transfusion corrected count increment (CCI) (Salama *et al*, 2004). Post-transfusion outcome from 35 transfusions in 31 patients were divided into two groups: those that resulted in shortening (>40 s) or normalization of the CT (group A, $n = 17$) and those that had no change or prolongation of the CT (group B, $n = 18$) and both were compared with pretransfusion values. In group A, nine patients had bleeding as indication for transfusion and six had improvement in their control of haemorrhage. In contrast, in group B, 7 patients had bleeding as indication for transfusion and none showed cessation of hemorrhagic symptoms (Salama *et al*, 2004). Further studies are required to determine whether not transfusing platelets into patients with normal PFA-100® CTs is not detrimental. The PFA-100® may, therefore, prove to be useful at supporting platelet and blood transfusion decisions and improve the management of the hospital blood bank platelet inventory (Koscielny *et al*, 2004b; Salama *et al*, 2004).

The use of the PFA-100® in a paediatric setting

The evaluation of platelet function in children can be difficult. The 'gold standard' diagnostic tests of platelet function remain platelet aggregation and nucleotide measurements. These tests are usually only performed in well-equipped coagulation laboratories and are time consuming and often technically challenging. They also usually require a minimum of about 10–20 ml of blood, which can often be a significant problem in young infants. The BT is very difficult to perform in young infants because the test involves laceration of the forearm and the stress on parents, patients and clinical staff can be substantial as they attempt to restrain the child during the test (Cariappa *et al*, 2003). Standard template devices that have been used for BT measurement in Adults have been modified in for use in children and neonates so that the size of the incision is decreased (Feusner, 1980; Andrew *et al*, 1989). BTs have been reported to be longer in children than adults but shorter in newborn infants (Andrew *et al*, 1990; Sanders *et al*, 1990). This also raises the ethical issue of performing normal ranges for the BT in healthy children. However, all of the current criticisms of the BT remain (see above) (Rodgers & Levin, 1990; Lind, 2002; Francis, 2004), and moreover repeat and even single testing is often unacceptable to children and their families. Non-invasive tests, which utilize minimal quantities of blood, are therefore potentially very useful in this setting. Only a total of 1·6 ml of citrated blood is required to perform a PFA-100® test with both CADP and CEPI cartridges. PFA-100® normal ranges for children beyond the neonatal period have also been reported to be very similar to adults (Carcao *et al*, 1998; Jilma, 2001). However, blood from term neonates has been shown to exhibit shorter CTs than both older children and adults (Kottke-Marchant *et al*, 1999; Israels *et al*, 2001). The shorter CTs appear to correlate with raised Haematocrits and increased VWF ristocetin cofactor activity due to the presence of ultra large VWF (Israels *et al*, 2001; Boudewijns *et al*, 2003). As normal ranges in older children are similar to adults, then the PFA-100® can be a very useful rapid test to exclude VWD and severe platelet defects, such as Glanzmann's Thrombasthenia and BS disease. Three separate studies have evaluated the utility of the PFA-100® in a paediatric setting (Carcao *et al*, 1998; Dean *et al*, 2000; Cariappa *et al*, 2003). All concluded that the test is potentially useful in screening for VWD and is more sensitive than the BT. One study suggested that if PFA-100® testing was performed twice and the results were in the normal range on both occasions, then a full VWD work up is not required (Dean *et al*, 2000). Children with Haemophilia A and B were also shown to give normal PFA-100® results, confirming that the test is independent of coagulation (Carcao *et al*, 1998; Rand *et al*, 1998; Dean *et al*, 2000). More recent evidence suggests that the test is not completely sensitive for the detection of non-severe platelet defects in children, including some patients with type I VWD, dense granular defects, PSD (Liesner *et al*, 2004) and Hermansky Pudlak syndrome (Harrison *et al*, 2002b), results that parallel similar studies in adults

(Harrison *et al*, 2002a; Quiroga *et al*, 2004a). PFA-100® testing of paediatric samples may therefore provide some important information for the exclusion of VWD and severe platelet defects, especially where the availability of blood may be a limiting factor. The test may also help to exclude these disorders in cases of non-accidental injury (Liesner *et al*, 2004), although exclusion of other platelet defects with formal platelet function testing may also be necessary.

Use of the PFA-100® in monitoring haemostatic therapy

The PFA-100® seems to have utility for rapidly monitoring responses to DDAVP therapy (Cattaneo *et al*, 1999a; Fressinaud *et al*, 1999; Favaloro *et al*, 2001). As the test is sensitive to VWF level, DDAVP responses can be monitored because of the rapid release of the endothelial stored pool of endogenous high molecular weight (MW) VWF, resulting in a transient increase in the level of plasma VWF levels and increased proportion of high MW multimers. Patients with normal VWF and with type I VWD, therefore, usually respond very well to DDAVP, with shortening and/or normalization of CTs (Cattaneo *et al*, 1999a; Fressinaud *et al*, 1999; Favaloro *et al*, 2001). However, patients with some forms of type I and type IIA VWD were shown not to respond in their CTs as well, despite apparent correction of plasma VWF levels, presumably because of the role of intraplatelet VWF in platelet plug formation and/or the absence of high MW multimers.

Table I. A list of Clinical Applications of the PFA-100 test. The major advantages and limitations of the test are shown for each individual application.

Clinical application	Advantage	Limitation
(1) Replacing the BT as a screening tool	More sensitive and reliable than the BT. Rapid results	Results not diagnostic or specific
	Easy test to perform	Inflexible fixed system, only two cartridges currently available
	Non-invasive – requires only 0·8 ml of blood per cartridge	Requires buffered citrate samples
	Samples can be tested <4 h old	Vacuum transport system for samples may affect test results
	High sensitivity to VWD and severe platelet defects	Low sensitivity to SPD, HPS and PSD. Some false negatives occur in type I VWD
	CVs of normal samples <10%	Interpretation of results near cut-offs. CVs are higher in abnormals
	Physiological test of high shear dependent platelet function	No measure of vascular wall function
	High negative predictive value	False and true positives have to be diagnosed with a panel of tests. Also, if clinical suspicion is high and PFA is normal then other tests are still required
	Sensitive to acquired platelet defects – drug and dietary effects	Abnormal CTs can be frequently encountered. Retesting often required
(2) Monitoring pro-haemostatic therapy	Sensitive to DDAVP and release of endothelial VWF into the blood	Sensitive to platelet VWF and high MW VWF. CTs do not always correct with VWF concentrates
	Can monitor platelet transfusions	Often sensitive to platelet count
	Coagulation-independent	Insensitive to Factor VIIa
(3) Monitoring anti-platelet therapy	Aspirin and NSAIDs usually prolong the CEPI CT	Often insensitive to clopidogrel
(4) Detecting and managing preoperative haemostatic defects	Can be used as a point-of-care test without a specialized laboratory	Not diagnostic
(5) Screening women with menorrhagia	High incidence of VWD and platelet dysfunction. High negative predictive value	Not diagnostic
(6) Screening platelet donors	Detection of both permanent and transiently acquired defects, e.g. aspirin. Donors can be eliminated or deferred	Not diagnostic. No proof yet that transient drug/diet effects influence the haemostatic potential of concentrates
(7) Quality control of platelet concentrates	Monitor storage lesion	Requires addition of RBC and plasma to the concentrates for testing

BT, bleeding time; VWD, von Willebrand disease; PSD, primary secretion defects; HPS, Hermansky–Pudlak syndrome; SPD, storage pool disease; CV, coefficient of variation; CT, closure time; PFA, platelet function analysis; DDAVP, desmopressin; VWF, von Willebrand factor; MW, molecular weight; NSAIDs, non-steroid anti-inflammatory drugs; CEPI, collagen/epinephrine; RBC, red blood cells.

The PFA-100® has also been utilized to monitor responses to VWF concentrate infusion in patients with DDAVP non-responsive type III and type IIA VWD. Disappointingly CTs tend not be normalized, probably because the concentrates are deficient in the highest MW multimers and/or that platelet VWF is absent in type III VWD (Cattaneo *et al*, 1999a; Fressinaud *et al*, 1999). The test therefore appears to have limited utility for monitoring VWF concentrate therapy. It will be interesting to determine whether future development and availability of fully multimeric VWF concentrates (e.g. recombinant VWF) may result in normalization of CTs postinfusion.

High dose factor VIIa infusion is now becoming increasingly useful for the treatment of platelet disorders. In our experience, factor VIIa does not influence CTs on the PFA-100® in children with platelet disorders (Almeida *et al*, 2003). Theoretically, as factor VIIa is increasing the amount of thrombin generation on the platelet surface, it is conceivable that the PFA-100® should be insensitive to this clotting factor, as the test is largely coagulation independent. It is likely that the test may not be useful for monitoring factor VIIa therapy. Koscielny *et al* (2004b) also studied the utility of the PFA-100® in monitoring pro-haemostatic therapy in patients with impaired haemostasis. Interestingly, they concluded that the PFA-100® could be useful for monitoring not only DDAVP, but transexamic acid, aprotinin, oestrogen, platelet transfusion and even factor VIII concentrates (containing VWF) (Koscielny *et al*, 2004b).

What can the PFA-100 be used for in clinical practice?

The PFA-100 provides us with a unique test of high shear-dependent platelet function. Widespread experience with the test is increasing, with greater than 200 publications on Medline as of January 2005. There is no doubt that the test is superior to the BT in the detection of VWD and a number of platelet defects and therefore can provide a potentially useful screening tool for the diagnosis and work up of both paediatric and adult haemostatic disorders. Table I summarizes the potential utilities of the PFA-100 in clinical practice and highlights the advantages and disadvantages of the test. The test however, is not diagnostic and although abnormal CTs are an indication of platelet dysfunction, they are non-specific to any disorder, without knowledge of other variables that influence the test, e.g. VWF, full blood count and other platelet function tests. Although early comparisons with the BT were favourable, more recent evidence suggests that screening tests, such as the BT and PFA-100®, have limited value in the diagnosis of patients presenting with mucocutaneous bleeding. This is because the PFA-100® appears to have equivalent low sensitivity compared with the BT in the detection of PSDs and SPD, conditions which are often frequently encountered. Clinicians should always perform a full range of platelet function tests where clinical suspicion is strong and where exclusion of a platelet defect is essential, even if the PFA-100® CADP and CEPI CTs are normal. However, the test does offer a high negative predictive value for excluding VWD and rare forms of severe platelet defects (e.g. Glanzmann's Thrombasthenia and Bernard Soulier syndrome). The PFA-100® may also be useful as a preoperative screening tool to identify and manage patients with impaired primary haemostasis. The test appears particularly useful for monitoring responses to DDAVP therapy. The PFA-100®, although of limited value as a screening tool, can still offer clinicians and laboratories the comfort of a rapid test result well before other laboratory results become available. Although further studies are required to help define the exact utilities of the PFA-100®, the test does represent a significant advance over the BT and provides a rapid, simple and reliable measure of platelet function.

Acknowledgements

Dr Paul Harrison is a consultant for Sysmex UK and was a consultant with Dade-Behring until 30 November 2004. The author would like to thank Dr David Keeling for carefully reading this manuscript.

References

Almeida, A.M., Khair, K., Hann, I. & Liesner, R. (2003) The use of recombinant factor VIIa in children with inherited platelet function disorders. *British Journal of Haematology*, **121,** 477–481.

Andrew, M., Castle, V., Mitchell, L. & Paes, B. (1989) Modified bleeding time in the infant. *American Journal of Hematology*, **30,** 190–191.

Andrew, M., Paes, B., Bowker, J. & Vegh, P. (1990) Evaluation of an automated bleeding time device in the newborn. *American Journal of Hematology*, **35,** 275–277.

Best, D., Senis, Y.A., Jarvis, G.E., Eagleton, H.J., Roberts, D.J., Saito, T., Jung, S.M., Moroi, M., Harrison, P., Green, F.R. & Watson, S.P. (2003) GPVI levels in platelets: relationship to platelet function at high shear. *Blood*, **102,** 2811–2818.

Bock, M., De Haan, J., Beck, K.H., Gutensohn, K., Hertfelder, H.J., Karger, R., Heim, M.U., Beeser, H., Weber, D. & Kretschmer, V. (1999) Standardization of the PFA-100(R) platelet function test in 105 mmol/l buffered citrate: effect of gender, smoking, and oral contraceptives. *British Journal of Haematology*, **106,** 898–904.

Boudewijns, M., Raes, M., Peeters, V., Mewis, A., Cartuyvels, R., Magerman, K. & Rummens, J.L. (2003) Evaluation of platelet function on cord blood in 80 healthy term neonates using the Platelet Function Analyser (PFA-100); shorter in vitro bleeding times in neonates than adults. *European Journal of Pediatrics*, **162,** 212–213.

British Society for Haematology BCSH Haemostasis and Thrombosis Task Force (1988) Guidelines on platelet function testing. *Journal of Clinical Pathology*, **41,** 1322–1330.

Carcao, M.D., Blanchette, V.S., Dean, J.A., He, L., Kern, M.A., Stain, A.M., Sparling, C.R., Stephens, D., Ryan, G., Freedman, J. & Rand, M.L. (1998) The Platelet Function Analyzer (PFA-100): a novel in-vitro system for evaluation of primary haemostasis in children. *British Journal of Haematology*, **101,** 70–73.

Carcao, M.D., Blanchette, V.S., Stephens, D., He, L., Wakefield, C.D., Butchart, S., Christie, D.J. & Rand, M.L. (2002) Assessment of

thrombocytopenic disorders using the Platelet Function Analyzer (PFA-100). *British Journal of Haematology*, **117,** 961–964.

Cariappa, R., Wilhite, T.R., Parvin, C.A. & Luchtman-Jones, L. (2003) Comparison of PFA-100 and bleeding time testing in pediatric patients with suspected hemorrhagic problems. *Journal of Pediatric Hematology/oncology*, **25,** 474–479.

Cattaneo, M. (2003) Inherited platelet-based bleeding disorders. *Journal of Thrombosis and Haemostasis*, **1,** 1628–1636.

Cattaneo, M. (2004) Are the bleeding time and PFA-100 useful in the initial screening of patients with mucocutaneous bleedings of hereditary nature? *Journal of Thrombosis and Haemostasis*, **2,** 890–891.

Cattaneo, M., Federici, A.B., Lecchi, A., Agati, B., Lombardi, R., Stabile, F. & Bucciarelli, P. (1999a) Evaluation of the PFA-100 system in the diagnosis and therapeutic monitoring of patients with von Willebrand disease. *Thrombosis and Haemostasis*, **82,** 35–39.

Cattaneo, M., Lecchi, A., Agati, B., Lombardi, R. & Zighetti, M.L. (1999b) Evaluation of platelet function with the PFA-100 system in patients with congenital defects of platelet secretion. *Thrombosis Research*, **96,** 213–217.

Dalby, M.C., Davidson, S.J., Burman, J.F. & Davies, S.W. (2000) Diurnal variation in platelet aggregation iwth the PFA-100 platelet function analyser. *Platelets*, **11,** 320–324.

Dean, J.A., Blanchette, V.S., Carcao, M.D., Stain, A.M., Sparling, C.R., Siekmann, J., Turecek, P.L., Lillicrap, D. & Rand, M.L. (2000) von Willebrand disease in a pediatric-based population–comparison of type 1 diagnostic criteria and use of the PFA-100 and a von Willebrand factor/collagen-binding assay. *Thrombosis and Haemostasis*, **84,** 401–409.

Di Paola, J., Federici, A.B., Mannucci, P.M., Canciani, M.T., Kritzik, M., Kunicki, T.J. & Nugent, D. (1999) Low platelet alpha2beta1 levels in type I von Willebrand disease correlate with impaired platelet function in a high shear stress system. *Blood*, **93,** 3578–3582.

Dyszkiewicz-Korpanty, A., Quinton, R., Yassine, J. & Sarode, R. (2004) The effect of a pneumatic tube transport system on PFA-100 trade mark closure time and whole blood platelet aggregation. *Journal of Thrombosis and Haemostasis*, **2,** 354–356.

Escolar, G., Pino, M., Reverter, J.C., Diaz-Ricart, M., Pujol, N., Maragall, S., Galan, A.M., Zurbano, M.J. & Ordinas, A. (2001) Precise Differentiation Between Storage Pool Disease and Aspirin Defects using the PFA-100 with a combination of collagen and collagen-arachidonic acid cartridges. *Thrombosis and Haemostasis*, **86,** 395 (abstract).

Favaloro, E.J. (2001) Utility of the PFA-100 for assessing bleeding disorders and monitoring therapy: a review of analytical variables, benefits and limitations. *Haemophilia*, **7,** 170–179.

Favaloro, E.J. (2004) Template bleeding time and PFA-100 have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: comparative study of 148 patients – a rebuttal. *Journal of Thrombosis and Haemostasis*, **2,** 2280–2282.

Favaloro, E.J., Facey, D. & Henniker, A. (1999) Use of a novel platelet function analyzer (PFA-100) with high sensitivity to disturbances in von Willebrand factor to screen for von Willebrand's disease and other disorders. *American Journal of Hematology*, **62,** 165–174.

Favaloro, E.J., Kershaw, G., Bukuya, M., Hertzberg, M. & Koutts, J. (2001) Laboratory diagnosis of von Willebrand disorder (vWD) and monitoring of DDAVP therapy: efficacy of the PFA-100 and vWF:CBA as combined diagnostic strategies. *Haemophilia*, **7,** 180–189.

Feusner, J.H. (1980) Normal and abnormal bleeding times in neonates and young children utilizing a fully standardized template technic. *American Journal of Clinical Pathology*, **74,** 73–77.

Francis, J.L. (2004) Platelet Function Analyzer (PFA-100). In: *Platelets* (ed. by A.D. Michelson), pp. 325–335. Academic Press, San Diego, CA.

Francis, J.L., Francis, D., Larson, L., Helms, E. & Garcia, M. (1999) Can the platelet function analyser (PFA-100) substitute for the bleeding time in routine clinical practice. *Platelets*, **10,** 132–136.

Fressinaud, E., Veyradier, A., Truchaud, F., Martin, I., Boyer-Neumann, C., Trossaert, M. & Meyer, D. (1998) Screening for von Willebrand disease with a new analyzer using high shear stress: a study of 60 cases. *Blood*, **91,** 1325–1331.

Fressinaud, E., Veyradier, A., Sigaud, M., Boyer-Neumann, C., Le Boterff, C. & Meyer, D. (1999) Therapeutic monitoring of von Willebrand disease: interest and limits of a platelet function analyser at high shear rates. *British Journal of Haematology*, **106,** 777–783.

Hajdenberg, J. (2004) Template bleeding time and PFA-100 have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: comparative study of 148 patients – a rebuttal. *Journal of Thrombosis and Haemostasis*, **2,** 2282–2283.

Harrison, P. (2000) Progress in the assessment of platelet function. *British Journal of Haematology*, **111,** 733–744.

Harrison, P. (2004) In vitro measurement of high-shear platelet adhesion and aggregation by the PFA-100. *Methods in Molecular Biology*, **272,** 215–223.

Harrison, P. (2005) Platelet function analysis. *Blood Reviews*, **19,** 111–123.

Harrison, P., Robinson, M.S., Mackie, I.J., Joseph, J., McDonald, S.J., Liesner, R., Savidge, G.F., Pasi, J. & Machin, S.J. (1999) Performance of the platelet function analyser PFA-100 in testing abnormalities of primary haemostasis. *Blood Coagulation and Fibrinolysis*, **10,** 25–31.

Harrison, C., Khair, K., Baxter, B., Russell-Eggitt, I., Hann, I. & Liesner, R. (2002b) Hermansky-Pudlak syndrome: infrequent bleeding and first report of Turkish and Pakistani kindreds. *Archives of Disease in Childhood*, **86,** 297–301.

Harrison, P., Robinson, M., Liesner, R., Khair, K., Cohen, H., Mackie, I. & Machin, S. (2002a) The PFA-100: a potential rapid screening tool for the assessment of platelet dysfunction. *Clinical and Laboratory Haematology*, **24,** 225–232.

Harrison, P., Segal, H., Furtado, C., Verjee, S., Sukhu, K. & Murphy, M.F. (2004) High incidence of defective high-shear platelet function among platelet donors. *Transfusion*, **44,** 764–770.

Homoncik, M., Jilma, B., Hergovich, N., Stohlawetz, P., Panzer, S. & Speiser, W. (2000) Monitoring of aspirin (ASA) pharmacodynamics with the platelet function analyzer PFA-100. *Thrombosis and Haemostasis*, **83,** 316–321.

Israels, S.J., Cheang, T., McMillan-Ward, E.M. & Cheang, M. (2001) Evaluation of primary hemostasis in neonates with a new in vitro platelet function analyzer. *Journal of Pediatrics*, **138,** 116–119.

Jilma, B. (2001) Platelet function analyzer (PFA-100): a tool to quantify congenital or acquired platelet dysfunction. *Journal of Laboratory and Clinical Medicine*, **138,** 152–163.

Jilma-Stohlawetz, P., Hergovich, N., Homoncik, M., Dzirlo, L., Horvath, M., Janisiw, M., Panzer, S. & Jilma, B. (2001) Impaired platelet function among platelet donors. *Thrombosis and Haemostasis*, **86,** 880–886.

Kerenyi, A., Schlammadinger, A., Ajzner, E., Szegedi, I., Kiss, C., Pap, Z., Boda, Z. & Muszbek, L. (1999) Comparison of PFA-100 closure time and template bleeding time of patients with inherited disorders

causing defective platelet function. *Thrombosis Research*, **96,** 487–492.

Koscielny, J., Ziemer, S., Radtke, H., Schmutzler, M., Pruss, A., Sinha, P., Salama, A., Kiesewetter, H. & Latza, R. (2004a) A practical concept for preoperative identification of patients with impaired primary hemostasis. *Clinical Applications of Thrombosis and Hemostasis*, **10,** 195–204.

Koscielny, J., von Tempelhoff, G.F., Ziemer, S., Radtke, H., Schmutzler, M., Sinha, P., Salama, A., Kiesewetter, H. & Latza, R. (2004b) A practical concept for preoperative management of patients with impaired primary hemostasis. *Clinical Applications of Thrombosis and Hemostasis*, **10,** 155–166.

Kottke-Marchant, K., Powers, J.B., Brooks, L., Kundu, S. & Christie, D.J. (1999) The effect of antiplatelet drugs, heparin, and pre-analytical variables on platelet function detected by the platelet function analyzer (PFA-100). *Clinical Applications of Thrombosis and Hemostasis*, **5,** 122–130.

Kratzer, M.A. & Born, G.V. (1985) Simulation of primary haemostasis in vitro. *Haemostasis*, **15,** 357–362.

Kundu, S.K., Heilmann, E.J., Sio, R., Garcia, C., Davidson, R.M. & Ostgaard, R.A. (1995) Description of an in vitro platelet function analyzer – PFA-100. *Seminars in Thrombosis and Hemostasis*, **21** (Suppl. 2)**,** 106–112.

Kundu, S.K., Heilmann, E.J., Sio, R., Garcia, C. & Ostgaard, R.A. (1996) Characterization of an in vitro platelet function analyzer – PFA-100. *Clinical Applications of Thrombosis and Hemostasis*, **2,** 241–249.

Liesner, R., Hann, I. & Khair, K. (2004) Non-accidental injury and the haematologist: the causes and investigation of easy bruising. *Blood Coagulation and Fibrinolysis*, **15** (Suppl. 1)**,** S41–S48.

Lind, S.E. (2002) The bleeding time. In: *Platelets* (ed. by A.D. Michelson), pp. 283–289. Academic Press, San Diego, CA.

Mammen, E.F., Comp, P.C., Gosselin, R., Greenberg, C., Hoots, W.K., Kessler, C.M., Larkin, E.C., Liles, D. & Nugent, D.J. (1998) PFA-100 system: a new method for assessment of platelet dysfunction. *Seminars in Thrombosis and Hemostasis*, **24,** 195–202.

Moeller, A., Weippert-Kretschmer, M., Prinz, H. & Kretschmer, V. (2001) Influence of ABO blood groups on primary hemostasis. *Transfusion*, **41,** 56–60.

Pearson, D.A., Paglieroni, T.G., Rein, D., Wun, T., Schramm, D.D., Wang, J.F., Holt, R.R., Gosselin, R., Schmitz, H.H. & Keen, C.L. (2002) The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thrombosis Research*, **106,** 191–197.

Podda, G., Bucciarelli, P., Lussana, F. & Cattaneo, M. (2005) Are the PFA-100 Closure Time and the Skin Bleeding Time useful in the Diagnostic work-up of Patients with Bleeding Diathesis? Correlation with the Severity of Bleeding Symptoms and sensitivity for known defects of primary haemostasis. *Blood*, **104,** 3037 (abstract).

Quiroga, T., Goycoolea, M., Munoz, B., Morales, M., Aranda, E., Panes, O., Pereira, J. & Mezzano, D. (2004a) Template bleeding time and PFA-100 have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: comparative study in 148 patients. *Journal of Thrombosis and Hemostasis*, **2,** 892–898.

Quiroga, T., Pereira, J. & Mezzano, D. (2004b) Template bleeding time and PFA-100 have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: comparative study in 148 patients – a reply to rebuttals. *Journal of Thrombosis and Hemostasis*, **2,** 2283–2285.

Rand, M.L., Carcao, M.D. & Blanchette, V.S. (1998) Use of the PFA-100 in the assessment of primary, platelet-related hemostasis in a pediatric setting. *Seminars in Thrombosis and Hemostasis*, **24,** 523–529.

Rand, M.L., Leung, R. & Packham, M.A. (2003) Platelet function assays. *Transfus Apheresis Science*, **28,** 307–317.

Rodgers, R.P. & Levin, J. (1990) A critical reappraisal of the bleeding time. *Seminars in Thrombosis and Hemostasis*, **16,** 1–20.

Salama, M.E., Raman, S., Drew, M.J., Abdel-Raheem, M. & Mahmood, M.N. (2004) Platelet function testing to assess effectiveness of platelet transfusion therapy. *Transfus Apheresis Sciences*, **30,** 93–100.

Sanders, J.M., Holtkamp, C.A. & Buchanan, G.R. (1990) The bleeding time may be longer in children than in adults. *American Journal of Pediatric Hematology andOncology*, **12,** 314–318.