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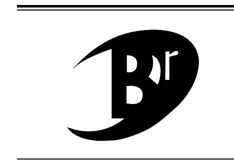

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REVIEW

Platelet function analysis

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KEYWORDS
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Platelet aggregation;
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The cone and plate(let) analyser;
Ultegra-RPFA®

Summary Since the last guidelines for BCSH platelet function testing were written in the late 1980s, many new tests have become available. Previously most platelet function tests were traditionally utilized to aid in the diagnosis and management of patients with platelet and haemostatic disorders. Most traditional tests were also largely restricted to the specialized laboratory or centre. However, nowadays there is also much renewed interest in monitoring the efficacy of anti-platelet therapy and measuring platelet hyper-function. A number of dedicated platelet function instruments have now become available that are much simpler to use and are beginning to be utilized as point of care instruments. These can now provide measurement of platelet function within whole blood without the requirement of sample processing. Some are also beginning to be incorporated into routine clinical use and can be utilized as not only as general screening tests of platelet function but to monitor anti-platelet therapy and to potentially assess both risk of bleeding and/or thrombosis. Modern flow cytometric-based platelet function analysis now also provides a wide variety of specific tests that can assess different aspects of platelet biology that are useful for diagnostic purposes. This review will highlight some of these of new tests/instruments and discuss their potential utility both within the haemostasis laboratory but also as potential point of care instruments.
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Introduction

Human platelets are small and discoid in shape, with dimensions of approximately 2.0–4.0 by 0.5 μm, and a mean volume of 7–11 fl.[1] They are the second most numerous corpuscle in the blood normally circulating at between 150–450 $\times 10^9$/l. Platelets are anucleated cells derived from megakaryocytes and typically circulate for 10 days.[1] Their shape and small size enables the platelets to be pushed to the edge of vessels, placing them in the optimum location required to constantly survey the integrity of the vasculature. Platelets are also surprisingly multifunctional and are involved in many pathophysiological processes including haemostasis and thrombosis, clot retraction, vessel constriction and repair, inflammation including promotion of atherosclerosis, host defence and even tumour growth/metastasis (Fig. 1). Although, any test(s) of platelets could therefore potentially measure any one or more of these vital processes, the majority of available tests focus only on those function(s) involved directly in haemostasis.

Upon vessel wall damage, platelets undergo a highly regulated set of functional responses including adhesion, spreading, release reactions, aggregation, exposure of a procoagulant surface, microparticle formation and clot retraction (Fig. 2). All of these platelet responses function to rapidly form a haemostatic plug that occludes the site of damage to prevent blood loss.[2,3] When there

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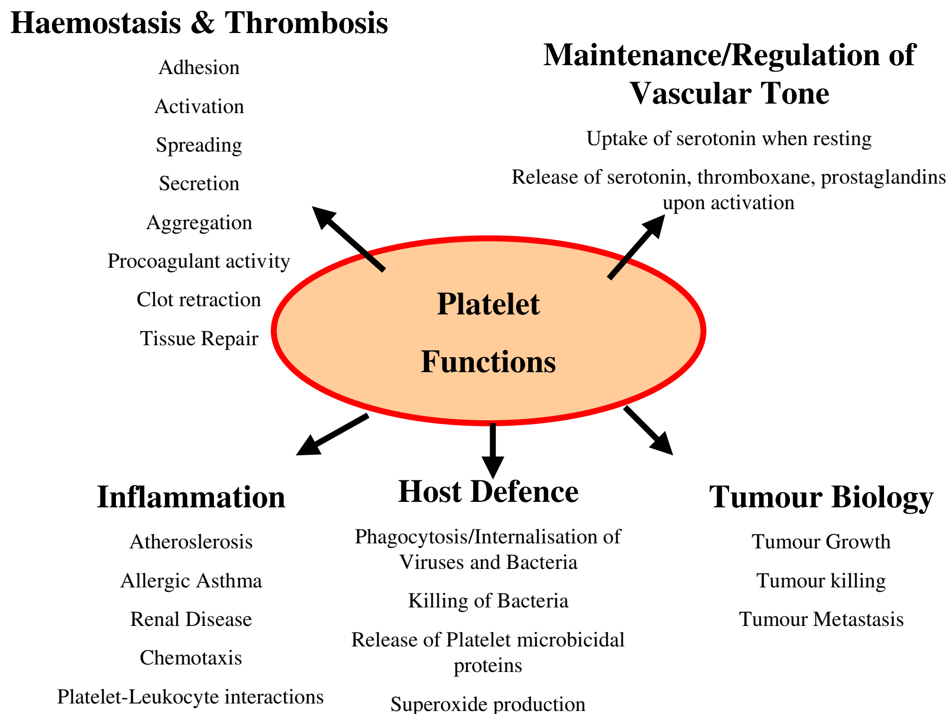

Figure 1 The multifunctional platelet. Platelets are involved in many pathophysiological processes, in addition to haemostasis and thrombosis, namely maintenance of vascular tone, inflammation, host defence and tumour biology.

is a defect in any of these functions and/or platelet number, haemostasis is usually impaired and there may be an associated increased risk of bleeding. In contrast, a marked increase in platelet number or reactivity can lead to inappropriate thrombus formation. Arterial thrombi can also develop within atherosclerotic lesions resulting in stroke and myocardial infarction, two of the major causes of morbidity and mortality in the western world.[4] Anti-platelet therapy can therefore be beneficial in the treatment and prophylaxis of arterial thrombotic conditions, but must be carefully administered without increasing the risk of bleeding to an unacceptable level.[1]

The main use of platelet function tests has been traditionally to identify the potential causes of abnormal bleeding,[5] to monitor pro-haemostatic therapy in patients with a high risk of bleeding and to ensure normal platelet function either prior to or during surgery.[6,7] However, they are increasingly being utilised to monitor the efficacy of anti-platelet therapy and to potentially identify platelet hyperfunction to predict thrombosis.[6,8] For a full list of potential clinical uses of platelet function tests see Table 1.

History of platelet function testing

Platelets were first described by the remarkably early observations of Bizzozero in the late 1800s.[9] Not only did he identify platelets as distinct corpuscles within human blood but he observed them forming thrombi within damaged areas of vessel wall using real-time microscopy. Today, modern imaging methods are utilised to study in detail the same real time interactions of platelets with the vessel wall and dynamics of thrombus formation.[10]

Table 2 illustrates a list of traditional tests of platelet function including the in vivo bleeding time and platelet aggregometry.[11] In contrast to coagulation defects, where screening tests e.g. the activated partial thromboplastin time (APTT) and prothrombin time (PT) are inexpensive and fully automated, platelet function defects are more difficult to diagnose because there are no definitive screening tests. Indeed, no current or future platelet function test is likely to be a 100% sensitive, due to the large number and variety of platelet defects. The current evaluation of a potential platelet defect usually involves platelet aggregation and/or measurement of granule content/release. These tests are labour intensive, costly, time consuming and require a fair degree of expertise and experience to perform and interpret. Also additional expensive specialist tests are often required (e.g. flow cytometry and platelet nucleotides). Since the late 1980s when the last published platelet function testing guidelines were written by the BCSH,[12] a number of newer tests of platelet function have become available, including

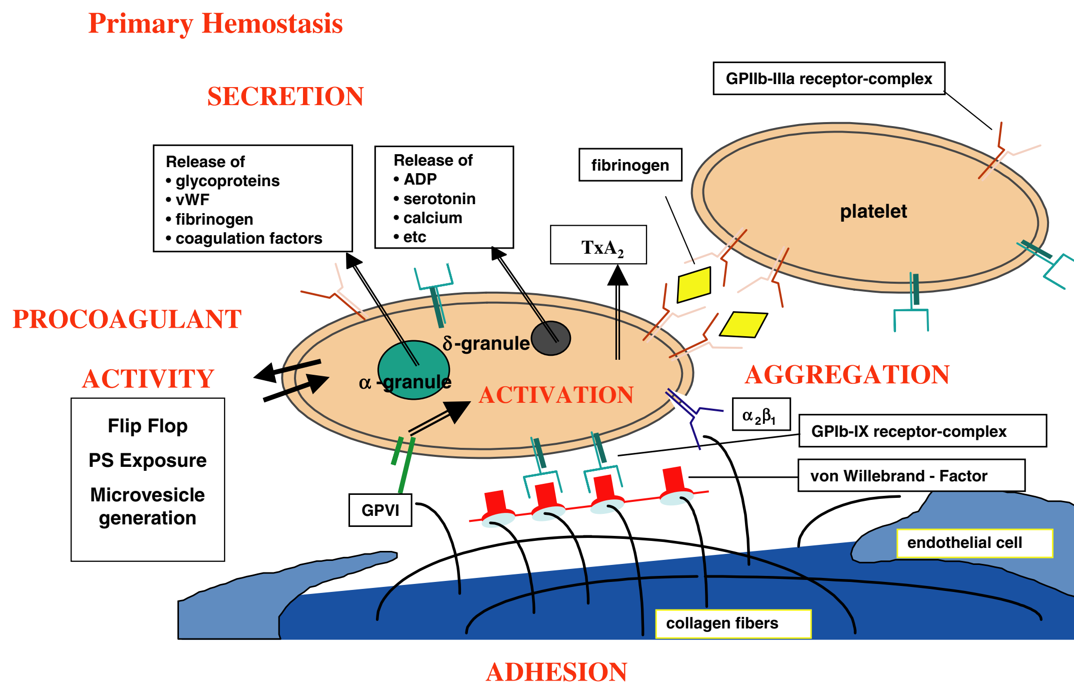

Figure 2 The multiple roles of platelets in haemostasis. Vascular wall injury results in exposure of collagen and subendothelial proteins. Initial platelet adhesion is mediated via VWF binding to the Gp Ib/IX/V complex on the platelet surface. Platelets begin to slow down and transiently adhere or roll along the vessel wall. Collagen binding to GPVI results in cellular activation resulting in firm adhesion and spreading through the activated receptors Gp IIb/IIIa and $\alpha_2\beta_1$. Platelet adhesion also results in intracellular signalling and platelet activation resulting in degranulation including release of ADP, generation of thromboxane, activation of the Gp IIb/IIIa complex and exposure of anionic phospholipid and generation of procoagulant microvesicles. These facilitate further local recruitment of platelets into the vicinity resulting in platelet aggregation mediated by fibrinogen and VWF bridging between activated Gp IIb/IIIa on adjacent cells. The exposure of anionic phospholipid provides a surface upon which platelets can support thrombin generation and fibrin formation resulting in stabilisation of the resulting haemostatic plug. (Modified with permission from Dade-Behring.)

Table 1 A list of potential clinical applications of platelet function tests.

Potential clinical utility of platelet function tests	Comments
Screening for platelet dysfunction	New tests are more reliable and sensitive than the BT?
Monitoring DDAVP therapy	Test needs to detect the influence of released VWF
Monitoring VWF replacement therapy in VWD	Some concentrates lack high MW VWF
Monitoring pro-haemostatic therapy	e.g. factor VIIa, platelet concentrates, DDAVP
Monitoring anti-platelet therapy	Increasingly used
Detecting drug resistance	e.g. aspirin and clopidogrel resistance
Platelet dysfunction in menorrhagia	High incidence of VWD and platelet dysfunction
Detection of platelet hyperfunction	Can tests predict thrombosis?
Prediction of surgical bleeding	Can tests predict bleeding reliably?
Quality control of platelet concentrates	Monitoring the platelet storage lesion
Screening platelet donors	High incidence of aspirin-like defects reported
Screening non accidental injury in children	To exclude platelet dysfunction

flow cytometry and various in vitro instruments (Table 3).[7,11,13] The principle of each test and their potential advantages/disadvantages are also listed in Table 3. The list includes a number of instruments that are prototypes but also some that have been commercialised.[14–23] Many of these are now available to the clinical laboratory, but either how to, or even whether to incorporate some of them into normal laboratory practice remains unclear. This review will focus on a number of newly available tests that are beginning to demonstrate potential as platelet function tests either within

Table 2 A list of traditional platelet function tests.

Platelet function test	Aspects of platelet function measured	Advantages	Disadvantages
Bleeding time	In vivo screening test	Physiological	Insensitive, invasive and high inter-operator CV
Aggregometry — turbidometric methods	Responsiveness to panel of agonists	Diagnostic	Labour intensive non-physiological
Aggregometry — impedance methods	Responsiveness to panel of agonists	Whole blood test	Insensitive
Aggregometry and luminescence	Combined aggregation and ADP release	More information	Semi-quantitative
Adenine nucleotides	Stored and released ADP	Sensitive	Specialised equipment
Electron microscopy	Ultrastructural evaluation of platelets e.g. dense granular defects or giant platelet disorders	Diagnostic	Specialised equipment
Electrophoretic analysis	Glycoprotein defects	Simple	Limited to major glycoproteins.
Clot retraction	Measures platelet interaction with fibrinogen/fibrin. Detects abnormalities in number, Gp IIb/IIIa, signalling and Fg	Simple	Not specific
Thromboelastography (TEG)	Global haemostasis	Predicts bleeding	Measures Clot properties only, insensitive to aspirin
Glass filterometer	High shear platelet function	Simple	Requires blood counter
Platelet release markers e.g. βTG PF4	In vivo platelet activation markers	Simple, systemic measure of platelet activation	Prone to artefact

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the experimental laboratory or as point of care instruments.

Quality control, blood sampling and anticoagulation

Platelet function testing presents many challenges in ensuring that accurate and meaningful results are obtained. Firstly, unlike with coagulation tests, there are no widely available internal or external quality control materials available. Most assays are performed on fresh blood and so many laboratories either establish normal ranges using control volunteer blood and/or assay known normal samples in parallel to ensure that each test/reagent is viable. Many platelet function tests such as aggregometry remain poorly standardised. Different laboratories often use panels of different agonists often at different ranges of concentrations. Normal platelet function is also largely calcium dependent, so anticoagulation of the blood sample through calcium chelation can immediately present a problem. Most laboratories utilise tests that require citrated blood samples within a narrow time window (<2 h). Although this is convenient within a coagulation laboratory as citrate tubes are also used for clotting tests, the quality, handling, temperature and age of the blood sample can also cause significant artefacts in platelet analysis. Platelets are inherently prone to artefactual activation but also to desensitisation. It is important that these are minimised during phlebotomy, anticoagulation, sample transit and handling within the laboratory. There are a number of published guidelines that can help to minimise platelet activation and platelet aggregation.[24–26] These include using a light tourniquet, a needle of at least 21 gauge, a non-traumatic venipuncture with smooth blood flow, discarding the first few ml of blood drawn, using polypropylene or siliconized glass tubes/syringes, ensuring immediate gentle mixing with anticoagulant, minimising delays from

Table 3 A list of new platelet function tests.

Platelet function test	Aspects of platelet function measured	Advantages	Disadvantages
Clot signature analyser (CSA®)	Global haemostasis. High shear platelet function	Measures global haemostatic function	Cost of cartridges
Cone and plate(let) analyser (CPA) or IMPACT ® device	High shear platelet adhesion/aggregation	Small volume of blood, physiological	Little widespread experience
Flow cytometry	Platelet glycoproteins, activation markers, Platelet function	Whole blood test, flexible, wide variety of tests	Specialised operator expensive equipment
Ichor — plateletworks®	Platelet counting pre and post activation	Simple point of care instrument	Indirect test measuring count after aggregation
Hemostasis analysis system®	Platelet contractile force, clot clastic modulus and thrombin generation time	Rapid and simple	Measures mainly clot properties
Hemostasus® Device	Platelet procoagulant activity	Simple	Insensitive to aspirin and Gp Ib function
Laser platelet aggregometer (PA-200)	Platelet micro-aggregates	Sensitive, study hyperfunction	Little widespread experience
Platelet function analyser (PFA-100®)	High shear adhesion/aggregation	Rapid and simple screening, In vitro bleeding time, potential point of care instrument	Inflexible
Ultegra (RPFA®) — rapid platelet function analyser	Efficacy of anti-platelet therapy e.g. Gp IIb/IIIa, aspirin, clopidogrel	Simple test point of care instrument	Inflexible, cannot be used to detect platelet defects or VWD
Thrombotic status analyser (TSA)	High shear platelet function	Simple	Little widespread experience
Gorog thrombosis test	High shear platelet function and global haemostasis	Simple	Little widespread experience
Platelet-Stat	Platelet adhesion to a collagen membrane with a cut	Simple	Prototype
Sensitive proteomic analysis	Potentially measures total protein content	Increasing sensitivity	Normal proteome yet to be fully defined, loss of some membrane proteins during analysis. Requires pure preparations
Platelet genome analysis	Measures total mRNA content by microarray technology	Potentially measures message from all MK/platelet synthesized proteins	Platelet genome yet to be fully defined. Requires pure preparations. Instability of mRNA preparations.

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sampling to analysis, keeping all tubes at room temperature, checking that the blood tube is not over or under-filled and avoiding unnecessary manipulation of the sample.[24,25] These measures are most often utilised when performing the sensitive technique of measuring platelet activation by flow cytometry, but one could argue that they should also be implemented when performing any platelet function testing. Many potential problems with samples are commonly overlooked and this may cause significant problems and artefacts when testing is performed. Therefore ensuring sample quality is of utmost importance before any platelet function testing is performed.

Initial approach to diagnosing platelet dysfunction

When investigating a suspected bleeding disorder, it is essential to obtain a detailed clinical and family history and perform a physical examination before choosing the laboratory tests to be undertaken.[3,27] In particular a recent drug history is critically important and patients should be advised to discontinue where possible any drug that could interfere with platelet function. Some drugs may cause a transient haemostatic defect, so repeat or deferred testing is often necessary. Other acquired defects in platelet function can also be associated with other clinical conditions.[28]

There are various characteristic clinical features that can be used to distinguish a platelet or primary haemostatic disorder from a coagulation or secondary haemostatic defect.[27] Primary haemostatic disorders are largely characterised by immediate bleeding after injury often with a disproportionate amount of bleeding compared to the degree of trauma. Delayed bleeding is more characteristic of a coagulation type defect. A mucocutaneous bleeding pattern often with petechiae, epistaxis and menorrhagia are more common in patients with a primary haemostatic disorder. Deep tissue bleeding, haemarthroses and intramuscular haematomas are more common in coagulation disorders.[27,29] The laboratory evaluation of platelet dysfunction should always include some basic clotting tests (e.g. the APTT and PT) to exclude any potential coagulation defects that may be the primary or additional cause(s) of bleeding.

Once the clinician suspects a platelet defect, it is always important to exclude thrombocytopenia as a primary cause of bleeding by performing a full blood count often in conjunction with a blood-film examination, particularly if abnormal platelet flags (e.g. count, MPV, distribution) are given on the Haematology counter. The blood film can also give valuable information about platelets including their size, granule content and number, although one must be also aware of potential artefacts (e.g. pseudothrombocytopenia caused by platelet satelletism or cold reacting agglutinins). For a classification of inherited thrombocytopenias there is an excellent recent review.[30] The most common bleeding diathesis is von Willebrand's disease (VWD) and is characterised by either qualitative and/or quantitative defects of VWF resulting in defective platelet adhesion.[31] VWD also gives very similar bleeding symptoms to platelet dysfunction. As it is more common, evaluation of VWF levels/functionality should also be included in the initial evaluation/screening of a potential platelet disorder.[29]

Hereditary disorders of platelet function have been recently re-classified.[32] They can be broadly categorized into (1) abnormalities of the platelet receptors for adhesive proteins; (2) abnormalities of the platelet receptors for soluble agonists; (3) abnormalities of the platelet granules; (4) abnormalities of the signal transduction pathways; (5) abnormalities of the membrane phospholipids; and (6) miscellaneous abnormalities of platelet function. The detailed classification of platelet defects is beyond the scope of this review and the reader is referred to some excellent recent reviews. [27,29,30,32,33]

The bleeding time

The bleeding time (BT), developed by Duke in 1910,[34] was the first in vivo test of platelet function and was still regarded as the most useful test of platelet function until the early 1990s.[12] The bleeding time is the time taken for bleeding to stop after an incision is made into the skin, usually into the anterior surface of forearm (Fig. 3). The test has been refined and standardised particularly with the use of a sphygmomanometer cuff and a spring-loaded template device to make standard sized cuts within the skin. Normal times are usually between 2 and 10 min, whereas severe platelet defects can result in a BT $>$30 min. However despite its apparent simplicity, the test is poorly reproducible, invasive, insensitive and time

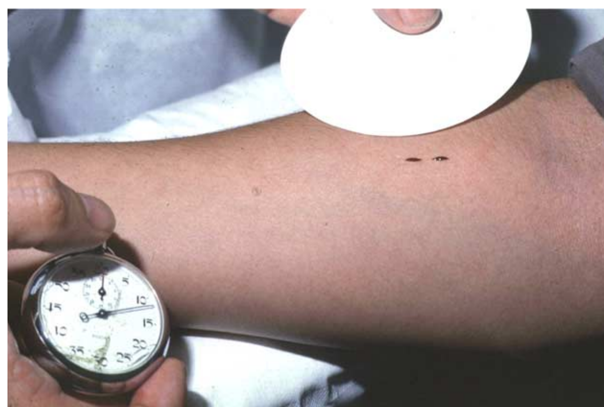

Figure 3 The in vivo bleeding time performed using a Simplate II device. 2 horizontal cuts are made into the skin, excess blood removed using a filter paper and the time recorded until bleeding stops. (reproduced with kind permission of Professor Sam Machin, University College Hospital, London).

consuming.[35] In particular the BT does not seem to correlate with the bleeding tendency within individual patients and it is widely considered that an accurate bleeding history is a more valuable screening test. Recent data suggests that the BT is not predictive of either MI or ischaemic stroke.[36] The clear advantage of the BT is that it does study natural haemostasis and the role played by the vessel wall in this process. The test does not require expensive equipment or a laboratory and is not prone to variables associated with blood sampling and anticoagulation.

Platelet aggregation

Platelet aggregometry was developed in the early 1960s and soon became regarded as the "gold standard" of platelet function testing.[37] This is still the most widely used test for identifying and diagnosing platelet function defects and can be performed within commercially available multichannel aggregometers. Whole blood aggregometers using impedance technology are also available and are sometimes combined with luminometry to simultaneously measure dense granular ADP release. However, in most laboratories, citrated blood is normally centrifuged to obtain platelet rich plasma (PRP), which is stirred within a cuvette incubated at 37 °C between light source and a detector. Upon addition of various concentrations of a panel of agonists (e.g. collagen, ADP, thrombin, ristocetin, adrenaline etc), the platelets aggregate and light transmission increases. Classical platelet responses to each agonist can then be monitored including lag phase, shape change and primary and secondary aggregation. Parameters measured include the rate (slope) of aggregation and the maximal amplitude (%) or percentage of aggregation after a fixed period of time. There is no doubt that aggregation will remain an important clinical test within the specialised laboratory as many platelet disorders are easily diagnosed. But the clinical significance of mildly abnormal aggregation to weak agonists remains undefined. Furthermore, aggregometers test platelets under relatively low shear conditions and in free solution within PRP, conditions that do not accurately simulate primary haemostasis. Also it is logistically impossible to perform platelet aggregation on all patients with suspected platelet defects and, in general, it is advisable that tests are performed within 2 h of blood sampling.

Because of the many disadvantages of the BT and aggregometry, several alternative automated technologies have been developed which attempt to simulate haemostasis in vitro.[11] Many of these are listed with Table 3. A number of these tests are discussed in further detail below.

The platelet function analyser — PFA-100®

The PFA-100® is a relatively simple bench top instrument that simulates high shear platelet function within disposable test cartridges (Fig. 4).[17,38,39] Citrated blood is aspirated under constant negative pressure from the sample reservoir through a capillary and a microscopic aperture (147 μm) cut into a membrane. The membrane is coated with either collagen/epinephrine (CEPI) or collagen/ADP (CADP). The presence of these platelet activators and the high shear rates (5000—6000 s^{-1}) under the standardised conditions result in platelet adhesion, activation and aggregation resulting in formation of a platelet plug within the aperture. Platelet function is thus measured as a function of the time it takes to occlude the aperture. The test is simple to perform, rapid (with maximal closure times (CT) of 300 s) and can test relatively small volumes (0.8 ml/cartridge) of citrated blood up to 4 h from

Figure 4 The PFA-100® device (reproduced with permission from Dade-Behring).

sampling. However, as with any other laboratory tests of platelet function, there are recommended PFA-100® good practice guidelines that are required to maintain optimal performance. These include daily instrument QC checks, ensuring the quality of blood sampling, anticoagulation and checking for cartridge batch to batch variation. Various reports have shown that the test is reliable with near identical normal ranges reported from many different laboratories.[38,40] However, it is recommended that each laboratory should establish their own reference range using normal blood samples taken into identical citrate anticoagulant that is utilised within the user's institution i.e. either 3.8% (0.129 M) or 3.2% (0.105 M) buffered trisodium citrate. Typical normal ranges obtained with 3.2% trisodium citrate are 55—112 s for CADP and 79—164 s for CEPI (Oxford Haemophilia Centre, 2002 normal range). Although widespread experience is increasing (>170 Medline articles, February 2004), how exactly the test should be incorporated into normal laboratory practice remains to be fully defined. The PFA-100® is sensitive to many variables that influence platelet function including abnormalities in platelet number, haematocrit, drug and dietary effects, platelet receptor defects, VWF defects, release and granular defects.[38] A full blood count should therefore always be performed to exclude thrombocytopenia or anaemia. The test is largely insensitive to coagulation type defects including haemophilia A and B, afibrinogenemia and defects in factors V, VII, XI and XII. Comparison with the bleeding time reveals that the PFA-100® is more sensitive, [39,41] especially to VWD, including Type I VWD.[42] Overall these results suggest that the test could be used as a screening tool that could be incorporated into a panel of existing tests.[40,43] Certainly our own studies demonstrate that the instrument has a high negative predictive value of > 90% in 740 normal and abnormal samples tested. Given a normal reading, then the sample can be eliminated from further detailed analysis e.g. aggregation studies. However, it should be noted that the test is not always sensitive to all platelet function defects and will give false negative results for example in patients with Storage Pool Disease, Primary Secretion Defects, Hermansky Pudlak syndrome, mild Type I VWD and Factor V Quebec. Diagnosis of these disorders would therefore be missed if relying upon the PFA-100® alone. Also in patients tested with apparently normal platelet function the instrument has been shown to give occasional false positives which then may have to be fully worked up by additional tests to exclude a defect. In this context, ingestion of aspirin is a particular problem. Abnormal CT's are therefore not diagnostic. As the test is sensitive to several acquired variables (e.g. dietary and drug effects) that influence platelet function, borderline CT's either side of the upper normal ranges can also be difficult to interpret, given that the reported CV's for of a normal sample have been reported as 10%. It is important that repeat or deferred tests are performed if drug ingestion is strongly suspected.

The PFA-100® appears superior to the bleeding time and is therefore recommended as a replacement screening test.[38,39,41,44] Many additional larger studies are required to assess whether the PFA-100® can also reliably predict either thrombotic or bleeding complications in different patient groups especially in patients who appear to be non-responsive or resistant to aspirin therapy.[45–50]

The cone and plate(let) analyser

Another test that has recently been developed is based upon the adhesion of platelets to the extracellular matrix using whole blood exposed to high shear. The original The cone and plate(let) analyser (CPA) device tested whole blood platelet adhesion and aggregation on a plate coated with extracellular matrix (ECM).[19,51] The CPA has now been developed into a fully automated commercial instrument called the IMPACT® (Diamed, Switzerland) (see Fig. 5) and now utilises polystyrene plates instead of the ECM. This modification facilitated the commercialisation of the test. The test is now fully automated, simple to operate, uses a very small quantity of whole blood (0.12 ml) and displays results in 6 min.[19] The instrument contains a microscope and performs staining and image analysis of the platelets adhering and aggregating upon the plate under an applied shear rate of 1800 s^{-1}. The software enables storage of the images of each analysis and records a number of parameters including surface coverage, average size and a distribution histogram of the adhered platelets.[19] Comparative analysis of the polystyrene and ECM plates shows a good correlation both in normals and VWD. No platelet adhesion occurs on plastic plates in samples from patients with either Glanzmann's thrombasthenia or afibrinogenemia.[19] The adhesion of platelets to the plate is absolutely dependent upon plasma VWF, fibrinogen binding to the plastic surface, the platelet glycoproteins Gp Ib and Gp IIb/IIIa, and platelet activation events. Recent data suggests that the instrument is a reliable tool for the diagnosis of platelet defects. The instrument has only just become commercially available so widespread experience is limited.

Figure 5 The Diamed IMPACT® device (reproduced with permission from Diamed).

The ultegra rapid platelet function assay — RPFA®

The Ultegra-RPFA® (Accumetrics, Inc, San Diego, California) is a turbidimetric based optical detection system (see Fig. 6) that measures platelet induced aggregation as an increase in light transmittance.[23,52] This modified platelet aggregometry device was originally developed as a near patient testing instrument in order to provide a simple and rapid functional means of monitoring anti-Gp IIb/IIIa therapy with various anti-platelet drugs (e.g. abciximab).[52] The disposable cartridges contain fibrinogen-coated beads and a platelet activator. The instrument simply measures changes in light transmission automatically and thus the rate of platelet/bead aggregation. The test appears to correlate well with conventional platelet aggregometry.[53] This represents a significant advance as the test can be performed reliably within different institutions/clinics without requiring either transport of sample, blood handling or processing, time delay or specialised personnel to perform the test. The test has recently been adapted to potentially measure the effectiveness

Figure 6 The Ultegra-RPFA® device (reproduced with permission from Accumetrics).

of either aspirin or clopidogrel therapy within modified cartridges.[54,55]

Hemostasis analysis system®

Hemodyne have developed and refined a Hemostasis Analysis System® (HAS) over the last 7 years (Fig. 7). This instrument is based upon the original technique developed by Carr.[21] The HAS measures platelet contractile force (PCF®), clot elastic modulus (CEM®) and thrombin generation time (TGT®) in a sample (700 μl) of clotting whole blood. PCF® is the force generated by platelets during clot retraction. In this instrument a small sample of whole blood is trapped between two parallel surfaces. Clotting is initiated by addition of a variety of clotting agents. During clot formation a downward force is imposed from above and the degree of deformation is directly measured by a displacement transducer. From this measurement, elastic modulus is calculated. As the clot forms, the platelets within the clot attempt to shrink the clot in the process known as clot retraction. The forces produce pull on the movable upper plate and the subsequent deflection is detected by the displacement transducer. The elastic modulus serves as a calibration constant for conversion of the displacement signal to force. A software package continually makes the calculations and plots clot elastic modulus (CEM®) and platelet contractile force (PCF®) as a function of time. CEM® is a complex parameter that is sensitive to changes in clot structure, fibrinogen concentration, the rate of fibrin production and red cell flexibility. PCF® is thrombin dependent function of platelets. It is

Figure 7 The platelet analysis system® (reproduced with permission from Hemodyne).

Figure 8 The Ichor and plateletworks system (reproduced with permission from Helena Laboratories).

sensitive to the rate of thrombin production, the presence of thrombin inhibitors, and the degree of Gp IIb/IIIa exposure. The thrombin generation time (TGT®) provides a measurement of the time between calcium addition and initiation of the PCF® during clot formation.[56] The TGT® is thus sensitive to clotting factor deficiencies. The TGT® has been demonstrated to be shorter and the PCF® to be increased in thrombophilic states but also in coronary artery disease[57] and diabetes.[58] Treatment of either haemophilic or thrombophilic conditions therefore results in normalisation of the TGT®.[59] Therefore the HAS allows rapid assessment of global haemostasis and can be used to monitor both procoagulant and anticoagulant medications. The instrument may therefore not only be useful within the Haemostasis Laboratory but also as a point of care instrument within surgical departments, intensive care units and cardiology departments.

Plateletworks®

Platelets within whole blood, when stimulated by agonists forms aggregates with a reduction in the number of free platelets. By counting the number of platelets in a control sample (usually EDTA) and within a sample that has been activated with a classical platelet agonist it is possible to assess the degree of platelet aggregation that can occur in a given sample. The Plateletworks® aggregation kits (Helena Biosciences) are simply based upon comparing platelet counts within a baseline EDTA tube and after activation/aggregation with one two agonists ADP or collagen. The user can also choose to measure the platelet counts within these tubes using the Ichor® Full Blood Counter (see Fig. 8) available from the same company (Helena Biosciences). The test has several advantages in that only 1 ml of blood is required for each tube, results are obtained in under 5 min and no sample preparation is required. Previous data suggests that these type of tests correlate well with conventional platelet aggregometry.[60] The test can also be used to monitor anti-platelet therapy.[61] Plateletworks® thus potentially offers both the platelet count and function as a point of care test for use within an acute care environment.

Flow cytometry

There is no doubt that one of the biggest advances in platelet function analysis has been the application of flow cytometry.[25,62] This technique however, requires access to an expensive instrument and specialised training to perform. A number of largely research applications continue to evolve into clinically useful tests particularly as many of the reagents, antibodies and dyes are now commercially available.[11] A list of currently available diagnostic flow cytometry assays are shown in Table 4.[26] The most commonly used routine tests

Table 4 A list of clinically useful platelet function tests available by flow cytometry.

Flow cytometric platelet function test	Examples
Diagnosis of platelet disorders	Glanzmann's thrombasthenia, Bernard Soulier syndrome, Storage Pool Disease, HIT, Scott syndrome
Quantification of receptor density	Platelet receptor defects. Influence of receptor polymorphisms.
Detection of activated platelets	CD62p, CD63, CD40L, Gp IIb/IIIa conformation, PS exposure, platelet—leukocyte conjugates, microparticles, platelet aggregates
Monitoring platelet responses to agonists	Using classical agonists in combination with activation markers
Monitoring anti-platelet drugs	GpIIb/IIIa antagonists, Clopidogrel, aspirin
Platelet production in thrombocytopenia	Reticulated platelets
Accurate platelet counting	New reference method — PLT/RBC ratio
Platelet associated IgG	Immune thrombocytopenia, detection of alloantibodies
Platelet survival	Biotinylation studies
Signal transduction	Calcium measurement, intracellular phosphorylation

are the quantification of glycoprotein receptor density (i.e. diagnosis of deficiencies in platelet glycoproteins e.g. Glanzmann's thrombasthenia and Bernard Soulier disease).[63,64] However, tests have also been devised to measure dense granules (using mepacrine uptake and release),[65–67] microparticle formation and exposure of anionic phospholipids (procoagulant activity).[68,69] These are beginning to prove to be very useful in the diagnosis of Storage Pool defects and Scott syndrome and related disorders. Most laboratories now prefer to analyse platelets within whole blood. Only small quantities of blood are required and, providing the venipuncture and analysis technique(s) are standardised, platelets can be analysed in their circulating state. Many laboratories have measured a variety of different platelet activation markers and shown that they are elevated in various clinical conditions associated with platelet activation.[26,62] However, it is also possible to measure platelet activation in response to classical agonists in vitro. For recommendations and protocols how to perform flow cytometric analysis, the reader is referred to recent review articles.[24–26]

Conclusions

The last guidelines for platelet function testing were written in the late 1980s.[12] Given the advances in this field and the introduction of potentially useful additions to our existing portfolio of platelet function tests, work is currently in progress to rewrite these guidelines. Nevertheless, the incorporation of new platelet function tests into the routine laboratory has already begun and a number of new approaches have been suggested.[5,29,40] Flow cytometric-based platelet function tests now also provide a rich variety of specific tests that are beginning to prove very useful at diagnosing various defects.[26] The development of reliable, sophisticated but simple to use whole blood tests that attempt to simulate in vivo haemostasis provides the ability to screen samples rapidly before applying our existing portfolio of tests.[6,11] Certainly the general consensus is that the in vivo bleeding time should be replaced. Many of the simpler platelet function tests could also be potentially utilised as point of care instruments for assessing bleeding risk and monitoring anti-platelet therapy. Platelet function testing is therefore become increasingly utilised outside of the specialised laboratory. Although this represents an important advance, validation, reliability and quality control testing of these tests will also become an increasingly important issue. It is highly likely that in the near future important developments in the platelet genome[70–72] and proteome[73–75] will be made that will lead to resulting in many exciting advances in the field such as platelet-specific microarrays, which may also have significant impact upon the diagnosis and management of patients with either haemostatic or thrombotic defects. In conclusion, many new platelet function tests have recently and will continue to become available. Many are now beginning to prove to be useful additions to our existing portfolio of tests.

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