PATHCHAT

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The role of PFA-200 in evaluating platelet dysfunction

Overview of the haemostatic system and guide to investigation

Testing				
Stage	Component	Result	1st line	2nd line
Primary haemostasis	- Vessel wall and subendothelial elements - Platelets - Von Willebrand factor	Formation of a viable platelet plug	- FBC and peripheral smear - Bleeding time - PFA 200	- vWF assays - Multimer pattern - Platelet aggregometry - Glycoprotein receptor by flow cytometry - Electron microscopy of platelet ultrastructure
Secondary haemostasis	Activation of coagulation factors	Stabilisation of platelet plug by cross-linked fibrin	- PTT - PT - Fibrinogen	- Thrombin time - Inhibitor screen - Specific factors - Factor XIII - Urea clot lysis
Removal of plug by fibrinolytic system once healing is complete Increased fibrinolysis manifests as bleeding tendency – usually delayed			PTT PT Fibrinogen FDPs and D-dimer	- Plasminogen levels - Tissue plasminogen activator (TPA) - Euglobulin clot lysis - Alpha 2 antiplasmin

The focus of this update is screening for platelet dysfunction:

1. As suggested by history and the presence of platelet-related bleeding diathesis
2. Pre-op assessment of bleeding risk in patients with a positive bleeding history

NB: unselected testing of patients is not encouraged.

The following first-line tests are recommended for primary haemostasis:

1. FBC and peripheral smear for:

- platelet count, morphology size and granular content; thrombocytosis (as in myeloproliferative disorders) and thrombocytopaenia may be associated with bleeding;
- effects of bleeding on Hb;
- the presence of abnormal cells to survey for underlying haematological neoplasm; and
- changes suggesting infection and DIC.

2. Bleeding time:

In vivo test of primary haemostasis.

Principle:
- A standard incision is made on the volar surface of the forearm with the application of 40 mmHg pressure to the upper arm
- Time to cessation of bleeding is recorded

Causes of prolonged bleeding time (>8 minutes):
- Thrombocytopaenia: progressive prolongation with counts < 75
  - Contraindicated when counts are < 50 as bleeding time may be difficult to arrest.
- Qualitative defect of platelets that may be congenital as in thrombasthaenia, storage pool defects or acquired due to drugs, uraemia, paraproteins, myelodysplastic syndrome and myeloproliferative neoplasms
- Von Willebrand's Disease
- Vascular abnormality, e.g. Ehlers-Danlos Syndrome
- Occasionally severe deficiency of factor V, XI or afibrinogenaemia

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Please contact your local Ampath pathologist for more information.

The main disadvantage is that it is influenced by a number of variables, which are operator- and patient-dependent (therefore difficult to standardise):
- Sphygmomanometer pressure
- Orientation of incision
- Oedema
- Blotting technique

NB: Normal bleeding time does not exclude defect in haemostasis.

Consistent correlation with bleeding at other sites is lacking.
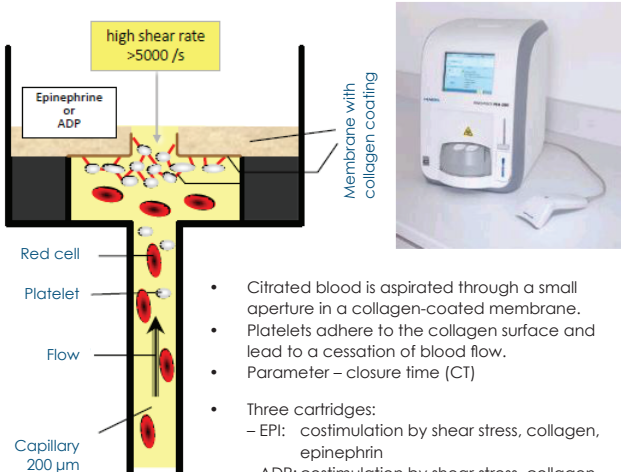
3. PFA – 200:

This test maybe seen as a cross between bleeding time and quick aggregation testing.

Principle:
- Citrated whole blood is aspirated at high shear rates through disposable cartridges.
- The cartridge contains an aperture within a membrane coated with either collagen and epinephrine (Col/EPI) or collagen and ADP (Col/ADP).
- The agonists induce platelet adhesion, activation and aggregation, resulting in occlusion of the aperture and hence cessation of blood flow.

Parameter is reported as **closure time**.

**PFA - 100/200
Principle**

- Citrated blood is aspirated through a small aperture in a collagen-coated membrane.
- Platelets adhere to the collagen surface and lead to a cessation of blood flow.
- Parameter – closure time (CT)
- Three cartridges:
  – EPI: costimulation by shear stress, collagen, epinephrin
  – ADP: costimulation by shear stress, collagen, ADP
  – P2Y: costimulation by shear stress, ADP, PGE1 and Ca++

Variables affecting results:
- Test must be performed within four hours of blood collection.
- HCT < 35% and platelet count < 150 may affect closure times.
- Samples with HCT > 50% and platelet count > 500 have not been evaluated.
- Fatty acids, lipaemia.
- Haemolysis of sample.

Advantages
- Small volume of citrated venous blood (therefore suitable for paediatric samples as well)
- Insensitive to clotting factor deficiency (not dependent on plasma fibrinogen of fibrin generation)
- Better standardisation
- No influence on result from oedema, loss of connective tissue, presence of fragile vessels (as in bleeding time performed in elderly patients)

Interpretation				
	Normal patient	**Aspirin**	**Von Willebrand's Disease**	**Glanzmann thrombasthenia**
Col/EPI	Normal 82–150 s	Prolonged	Prolonged	Prolonged
Col/ADP	Normal 62–100 s	Normal	Prolonged	Prolonged

In the event of abnormal results, further testing is indicated, especially if history supports a bleeding diathesis.

Consultation with a clinical haematologist or haemato-pathologist is strongly recommended.
- Complete review of medication and toxin history (herbal, homeopathic, occupational)
- vWF antigen, multimer analysis
- Platelet aggregometry
- Analysis of membrane glycoprotein by flow cytometry
- Electron microscopic evaluation of platelet structure

The test may be normal despite abnormal platelet function:
- In Storage Pool Disease
- Primary secretion defects
- Mild VWDx

The need for further testing must be guided by history and clinical features supportive of platelet disorder.

Availability: Testing on the PFA 200 requires a sample < four hours old. Request for the test must take into account transit time of sample to laboratory hosting the instrument.

References:

Bain, B.J., Bates, I., Laffan, M.A. & Lewis, S.M. 2012. *Dacie and Lewis Practical Haematology*, 11th edition, Churchill Livingstone.

British Committee for Standards in Haematology. 2011. *Postgraduate haematology: guidelines for laboratory investigation of inheritable platelet disorders* Aug 2011