Laboratory Diagnosis of Platelet Disorders.

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The diagnostic evaluation of platelet disorders is complex, poorly standardized and time consuming. This coupled with the wide spectrum of a known range of disorders some of which are very rare, presents a significant challenge to even the best diagnostic laboratory. Global tests of platelet function are often used as first line screening tests during the laboratory investigation of individuals with suspected haemostatic defects. Since global tests of platelet function do not enable specific diagnosis of platelet disorders, they are normally performed as the first part of a two step screening strategy which requires further testing with more specialised assays of platelet function (e.g. Light transmission aggregometry (LTA), Whole Blood Aggregometry (WBA), Lumiaggregometry, Multiple electrode aggregometry (MEA, Multiplate®), nucleotides, flow cytometry, electron microscopy, molecular genetics etc) to confirm or refute the diagnosis.

The most commonly proposed rationale for testing global platelet function as a first line investigation is that normal test results may exclude a diagnosis of platelet function disorder so that further specialised testing can be avoided. For this reason, global platelet function tests are usually initially performed at the same time as global assays of coagulation pathway function (prothrombin time (PT) and activated partial thromboplastin time (aPTT), von Willebrand Factor screening tests (VWF:Ag, VWF:RCo and Factor VIII:C)) and measurement of platelet number.

The most widely performed tests for screening platelet function disorders are currently the template bleeding time (BT) and the closure time (CT) within the Platelet Function Analyser (PFA-100®, Siemens Healthcare Diagnostics). Measurement of platelet number and size using automated cell counters and blood film analysis are highly sensitive and specific for numerical platelet function disorders and are therefore valuable to perform early in the course of investigation of patients with abnormal bleeding. The utility of BT and PFA-100® CT in this setting is however less clear for patients with mild symptomatic bleeding, because these tests have low sensitivity for mild platelet function defects. This means that demonstration of normal BT or PFA-100® CT cannot reliably rule out an underlying platelet function defect (or VWD) and therefore the effectiveness of these tests as screening investigations is limited. Since abnormal BT or PFA-100® also have poor specificity for platelet function further definitive investigation of both platelet function and VWD are also required in all patients who show abnormalities. In practice therefore, further specialised investigation using LTA and other tests is required in all patients with a history of mild abnormal bleeding irrespective of the results of the BT and PFA-100® CT tests. The BT and PFA-100® CT have higher sensitivity for GT, BSS and moderate to severe forms of VWD and so normal test results in a patient with severe bleeding symptoms will reliably exclude these disorders. Since the PFA-100® is minimally invasive, requires small quantities of blood and offers rapid assay results, this investigation is to be considered optional when clinical need demands preliminary diagnosis of severe platelet function defects or severe VWD before definitive diagnostic assays (e.g. LTA, MEA etc) can be performed. However, since the BT procedure is invasive and has poor reproducibility, this test is no longer recommended even in this exceptional circumstance.

Light transmission aggregometry (LTA) was invented in the early 1960's and is still regarded as the gold standard for platelet function testing. Despite its widespread use, the test is complex, poorly standardized and there are wide variations in laboratory practice. LTA is a time consuming and technically challenging technique that is affected by many pre-analytical

and analytical variables. For example LTA can be significantly affected by exposure of patients to non-prescription drugs and dietary factors that affect platelet function. LTA may be unreliable if the test subject is thrombocytopenic and there is poor standardization in the practice of normalising or adjusting platelet counts in PRP (e.g. to 200 x 10^9/L) and in the range of concentrations and choice of agonists. The sensitivity of LTA to secretion defects using standard agonist concentrations is also suboptimal and it is important that either stored and/or released nucleotides are also measured by alternative methods (e.g. lumiaggregometry) and by use of an increased range of agonist concentrations. Finally the objective comparison of LTA results to healthy donor reference ranges is not widely practiced and the diagnostic criteria for most mild platelet function disorders are not established. Some of these issues have recently been addressed by a number of different organisations (e.g. CLSI, BCSH and ISTH platelet physiology SSC) and emerging new guidelines and initiatives should help to improve the standardization and practice of this important diagnostic test. WBA and MEA provide a simpler and more standardized whole blood approach to measuring platelet function in response to classical agonists and the latter test is becoming increasingly popular in Europe. Other platelet function tests include flow cytometry which can be used to quantify platelet surface expression of glycoproteins (e.g αIIbβ3 and GpIb-IX-V) to diagnose GT and BSS especially where the volume of blood may be limiting (e.g. in small children). Other flow cytometry assays are available (e.g. measurement of granular secretion) and techniques such as shear dependent assays of platelet adhesion and electron microscopy of platelet ultrastructure and molecular genetics are widely used in research laboratories but are not often available to many clinical laboratories. Further research on the diagnostic utility of the different methods for the screening and diagnosis of platelet defects is therefore of importance and should help to define a more evidence based approach to the accurate diagnosis of bleeding defects.