ABSTRACT FORM ECAT SYMPOSIUM 8 – 9 NOVEMBER 2018

Name:

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Title:

Platelet function testing: the view from the bench

Abstract:

The role of platelets within the haemostatic mechanism has gained increased significance following recent advances in characterisation of platelet function within vasculature, inflammation, tumour growth and artherosclerosis. Diagnosis of platelet function disorders has traditionally been the preserve of large reference laboratories potentially leading to mis/under-diagnosis, particularly of milder forms. The costs associated with platelet testing are considerable leading to the review and consolidation of our practise to increase efficiency. Our aim was to streamline testing from referral and pre-analytical process, through the testing regimen, to post analytical analysis and consequent diagnosis.

Referrals frequently arise following prolonged platelet function assay (PFA) closure times. Our experience suggests that a comprehensive clinical history and testing for von Willebrands disease (VWD) is recommended prior to proceeding to full platelet testing. Candidates deemed suitable for enhanced platelet assay are provided with an information leaflet outlining the test process and pre-analytical questionnaire to be completed prior to appointment. This helps to limit occasions where platelet testing is postponed due to aspirin or other anti-platelet agent ingestion.

Platelet testing guidelines stipulate that testing must be completed within 4 hours of collection. Large sample volumes (20-30mL whole blood) are further required rendering the process unsuitable for infants/young children. For optimal process, we aim to co-ordinate sample collection from three patients and a parallel sample from a volunteer with no known history of bleeding disorder to provide 'presumed normal' quality control material. A pre-analytical questionnaire is also completed by the volunteer control. Analysis of control sample confirms the correct preparation and function of platelet agonists during testing.

Samples are promptly transported to the laboratory where whole blood is retained for ATP release testing and remaining sample processed to generate platelet rich (PRP) and platelet poor (PPP) plasma for light transmission aggregometry (LTRA) studies. Platelet agonists, specific for ADP, thrombin (PAR-1), epinephrine (adrenergic), thromboxane (TXA2) and collagen receptors are added to PRP preparations to assess aggregation extent and profile. Arachidonic acid is added to assess TXA2 generation and consequent aggregation whilst ristocetin is employed to directly examine GPIb-IX-V binding to von Willebrand factor (VWF).

The Chrono-log® lumi-agregometer is used to assess ATP release in response to collagen in fresh whole blood. This test may identify storage pool release defects. In addition each patient sample is prepared for platelet nucleotide studies and frozen for future batch testing to confirm a storage pool defect should the aggregation and ATP release results suggest this.

Analysis of platelet membrane glycoprotein expression by flow cytometry analysis is also available in our centre. Although not routinely employed, it has the distinct advantage of requiring small volumes of whole blood. Platelet glycoprotein analysis is useful to confirm the absence of a surface receptor following abnormal aggregometry results but is most commonly employed where a platelet disorder is suspected in infants and young children where platelet aggregometry is unsuitable.

To further enhance efficiency of platelet testing we have recently explored moving to an automated system employing the Sysmex CS2x00i® coagulation analyser which will further optimise our sample throughput.

The application of quality control to platelet function testing has been a particularly difficult challenge faced by us in an increasingly regulated environment. The use of volunteer donor sample in parallel with the patients provided a 'normal' quality control for the reagents and testing environment, however the requirement for a routine abnormal QC proved a more difficult challenge. Addition of inhibitory reagents, prostaglandin E1, aspirin, GPIIb/IIIa inhibitor and EDTA to normal PRP was evaluated and produced expected abnormal profiles. The routine addition of EDTA to a control sample followed by testing with a nominated agonist is now undertaken to facilitate abnormal QC. As ristocetin is unaffected by EDTA, a separate validation with abnormal QC is undertaken following batch change. Donor PRP is mixed with

genetically confirmed Type III VWD plasma to confirm specificity of ristocetin response.

Platelet results are discussed with clinicians at the weekly multidisciplinary team meeting giving an opportunity to highlight any unusual wave forms and/or unexpected biphasic profiles in addition to aggregation extent following agonist addition. Diagnosis may then be made on the basis of both scientific and clinical input.