

occur in various disorders. The aim of this study was to assess the performances of the SPA (PLUS)[®] system for immunoglobulin testing.

Patients and methods.— Immunoglobulin concentrations in routine patients' samples were measured with turbidimetric immunoassays on SPA (PLUS)[®] (Binding Site) and with immunonephelometric immunoassays on BNII[®] (Siemens) on about 70 samples for A, G and M and on about 150, 100, and 70 samples respectively for subclasses G2, G3 and G4.

Results.— Mean concentrations for A, G and M were 2.5, 11.9 and 1.2 g/L for the SPA[®], respectively; and 2.6, 12.3 and 1.1 g/L for the BNII[®], respectively. Regression analysis between the two methods showed for A, G and M slopes of 1.10, 0.99 and 1.03 and intercepts of -0.18, -0.40 and 0.02 respectively. Bland and Altman plots revealed mean differences of 0.1, -4.3 and 5.4%. Mean concentrations for G2, G3 and G4 were 2.00, 0.86 and 0.49 g/L for the SPA[®], respectively; and 1.89, 0.46 and 0.19 for the BNII[®], respectively. Nevertheless, regression analysis between the SPA[®] and the BNII[®] for G2, G3 and G4 showed significant deviations from linearity illustrating important differences between these two methods.

Conclusions.— Our study shows a satisfactory agreement and limited bias for A, G and M testing. However, the methods were not commutable for G2, G3 and G4 testing.

doi:10.1016/j.immbio.2011.11.021

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Platelet microparticle generation assay: A valuable predictor of clinical outcome in heparin-induced thrombocytopenia diagnosis

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Background.— The release of procoagulant platelet microparticles (PMPs) is considered as the major step of type II heparin-induced thrombocytopenia (HIT). Early HIT diagnosis is essential in order to improve clinical outcomes but this is still challenging.

Objectives.— To compare PMPs generation assay (PMPGA) performance (Mullier et al. Thromb Haemost 2010) with Elisa, Light Transmission Aggregometry (LTA), ¹⁴C-Serotonin Release Assay (SRA) and clinical outcomes.

Patients and methods.— Sera or citrated-platelet-poor plasma of HIT-suspected patients ($n=72$) were first incubated (20 min; 37 °C) with citrated-whole blood from healthy donors with/without unfractionated heparin (1 or 500 IU/mL). Then, PMPs were quantified and characterized using a FACS Aria[®] flow cytometer. Elisa (PF4 Enhanced), LTA, PMPGA, SRA and clinical outcomes data were compared by Chi-Square tests and ROC Curves.

Results.— In positive HIT patient, PMPs expressing phosphatidylserine (PS+) are generated following immune complexes formation with low heparin concentration whereas PMPs rate decreases significantly in presence of high heparin concentration. However, due to technical limitations, the ratio of PMPs PS+ between low and

high heparin concentrations is a more convenient and relevant parameter than PMPs rate. The optimal cut-off ratio was determined as 2. The correlation between PMPGA and SRA was markedly more significant ($P<0.0005$, $n=57$ whose 10 positive SRA) than LTA ($P=0.0267$, $n=39$) and ELISA ($P=0.0022$, $n=58$). In comparison to SRA, sensitivity and specificity of PMPGA were 70.0 and 97.9%, respectively. Combining clinical outcome to biological testing, PMPGA sensitivity and specificity reached 100% in this pilot study.

Conclusions.— PMPGA is a rapid and reliable test reproducing ex vivo the in vivo HIT reaction. It was very well correlated to ¹⁴C-SRA performance and to HIT imputability based on clinical outcome.

doi:10.1016/j.immbio.2011.11.022

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Assessment of different methods studying the impact of carbon nanomaterials on platelet function

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Objectives.— We aimed to validate an universal, fast, accurate, reliable and relevant toxicological preclinical screening test to measure the potential impact on primary haemostasis of nanoparticles (NP) whatever their physicochemical properties.

Materials and methods.— Four types of carbon nanoparticles (Carbon Black [CB], Fullerenes [C60], Single Wall Carbon Nanotubes [SWCNT], Multi Wall Carbon Nanotubes [MWCNT]) considered as promising in medical applications were investigated. The interference of these nanoparticles on Light Transmission Aggregometry (LTA), Whole-blood Impedance Aggregometry (Multiplate[®]), Platelet Function Analyzer-100 (PFA-100[®]) and Impact-R[®] was studied before the assessment of their effect on platelet function (adhesion, activation and aggregation). Interference and functional impact were also analyzed by transmission and scanning electron microscopy.

Results.— Maximal concentrations of C60, CB, SWCNT, MWCNT that may be tested with optical methods like LTA are 500, 10, 500 and 100 µg/mL, respectively. Each nanoparticle interferes by flux obstruction with PFA-100[®] at concentration higher than 10 µg/mL. Whole-blood impedance aggregometry was not considered as a suitable method because of the interaction between negatively charged nanoparticles and the impedance measures. Impact-R[®] showed absence of interference of C60, CB, SWCNT, MWCNT up to 250, 500, 500 and 250 µg/mL, respectively. Furthermore, the addition of Bovine Serum Albumin 7.4 g/L (final concentration) to mimic human blood viscosity abolishes the interference of C60 and MWCNT. Below cut-offs without any interference, none of the nanoparticle has a significant effect on the platelet function, whatever the method used.

Conclusion.— Impact-R[®] is the most adapted test to assess the effect of manufactured NPs on platelet function.

doi:10.1016/j.immbio.2011.11.023