Letters to the Editor

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Rapid diagnosis of heparin-induced thrombocytopenia using a particle gel immunoassay in at-risk cardiac surgery patients

Sir,

Heparin-induced thrombocytopenia (HIT) is a condition characterized by a thrombocytopenia or thrombosis with a temporal relationship of 1–2 weeks after the initiation of heparin.[1] Although the diagnosis of HIT may be suspected on clinical grounds alone, the decision to discontinue heparin in a patient with a recent thrombotic event often poses a therapeutic dilemma especially when other potential causes of thrombocytopenia may be present. Therefore, laboratory conformation of the diagnosis is often desirable. Several assays such as serotonin release assay (SRA), heparin-induced platelet aggregation (HIPA), flow cytometry, and H-PF4 enzyme-linked immunosorbent assay (ELISA) have been developed to affirm or to exclude the diagnosis of HIT.[1] Each method has its own advantages and disadvantages. The tests HIPA and SRA are highly specific, but they are laborious and require selected donor platelets and extended experience. High titre immunoglobulin (IgG) antibodies correlate with clinical HIT, but ELISA is also time-consuming.

The particle gel immunoassay (PaGIA) (ID-PaGIA H/PF4, DiaMed, Cressiers/Morat, Switzerland) is a rapid assay for detection of anti-PF4/heparin antibodies. This assay uses PF4/heparin complexes bound to red, high-density polystyrene beads; after addition of patient serum or plasma, the anti-PF4/heparin antibodies bind to the antigen-coated beads. This assay has been adapted to the gel technique of the ID microtopyt. This method is available to blood banks that use a gel centrifugation technology system. We evaluated PaGIA for the rapid diagnosis of HIT in patients undergoing cardiac surgery at our hospital.

The study was conducted in the Department of Transfusion Medicine in collaboration with Department of Cardio Vascular and Thoracic Surgery at our tertiary care institute. A total of 100 adult male and non-pregnant female patients undergoing open heart cardiac surgery (valvular replacement surgery/coronary artery bypass graft/combined) were monitored for baseline, and postoperative platelet counts from day 1 to day 14. Definite thrombocytopenia was defined as more than 50% fall in the platelet count from the baseline or counts <100 x 10^9/L. Clinical T scoring was done according to Warkentin.[2] All the patients were further investigated for H-PF4 antibody using PaGIA and ELISA. Patients with clinical thrombocytopenia with the presence of H-PF4 antibody with high/intermediate T score were considered to be clinical HIT.

PaGIA was performed on serum of 42 patients with definite thrombocytopenia at the baseline level and at day 7. Out of 42 patients, 10 patients had positive results at day 7 and all were negative at the baseline level. There was a good concordance between PaGIA and ELISA for detection of HPF4 antibodies. Out of 19 ELISA positive patients, 10 were also PaGIA positive, while all the ELISA negative patient samples were also PaGIA negative. There was a good correlation of PaGIA-positive results with a high T score. Of the eight patients with high scores, six were PaGIA positive. All these six patients were also ELISA positive.

In the last decade, gel technology has replaced the conventional test tube method for various immunohematological procedures. It is also being used for the lab diagnosis of paroxysmal nocturnal hemoglobinuria, fetomaternal hemorrhage, and serological diagnosis of syphilis. We report here the use of this technology for rapid diagnosis of HIT in blood bank settings.

Our results are in agreement with other studies, in which PaGIA is compared with SRA and HIPA and showed comparable results.[3] However, it was recommended that this assay should be used in combination with a functional assay using washed platelets in order also to detect HIT antibodies against other antigens involved in HIT, such as IL-8 or neutrophil activating peptides. Alberio et al.[4] demonstrated that titration of H-PF4 antibodies using PaGIA permits better recognition of clinically relevant antibody levels than those using a qualitative test without titration. Of their 69 patients with H-PF4 antibodies, HIT was “very likely” when
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the tier was >4. The performance of PaGIA with clinical T scores in clinically suspected HIT patients was similar to other studies.\(^5\)

In our experience, PaGIA is rapid and easy to perform. It allows macroscopic evaluation of test results after a few minutes. It does not require advanced training and can be easily adapted in blood bank settings. In addition to a series of samples, individual patient samples can also be investigated effectively. Moreover, unlike SRA or HIPA it does not require freshly prepared platelets. Thus, PaGIA is a reliable tool for detection of HIT antibodies in blood bank settings as most of the advanced blood centers are already using in ID micro typing system for blood grouping and cross matching.

The high mortality associated with HIT led to an increasing demand for its laboratory exclusion in patients with various clinical conditions that mimic HIT, particularly because multimorbid patients are more likely to form PF4 antibodies. The clinical score is highly reliable to exclude HIT when clinical features are unambiguous, but a rapid assay is desirable for patients with undeterminable probability for HIT. Commercial ELISA besides are unambiguous, but a rapid assay is desirable for patients with undeterminable probability for HIT. Commercial ELISA besides confirming the diagnosis gives additional information such as prediction of thrombosis.

Rapid assays which are easy to implement in blood bank settings are now being introduced. In our experience, PaGIA is a good supplement for ELISA as a screening test in clinically suspected patients. It can also be used as a baseline test before administration of heparin.

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Platelet antibodies detection:
A limitation for Indian population

Sir,

The blood group system H is determined by \(\alpha-(1, 2)\)-fucosyltransferase genes FUT1 and FUT2\(^4\) and it is widely expressed on the human tissues and cells. The H antigen is the precursor of the A and B blood group antigens. Despite the Bombay blood group\(^6\) is characterized by devoid of the A, B and H antigens on the red blood cells (RBCs) and IgM anti-H; however, the presence of a strong IgG anti-H antibody\(^5\) is also well documented. The frequency of the Bombay phenotype is about 1 in 10,000 individuals in India; and 1 in 1,000,000 individuals in Europe.\(^6\) In 1954, Moreaux and Andre\(^7\) provided the first evidence of the ABH antigens expression on the platelets. Later in 1991, Santos and his associates\(^8\) localized the ABH antigens, which non-covalently bound to the glycoproteins (GP) I\(b\), IIb and IIIa. All platelet antibodies detection techniques available hitherto including the two cornerstones monoclonal antibody specific immobilization of platelet antigens (MAIPA) and platelet immunoflourescence test (PITF) use group O platelet panel. In other words and according to Santos et al findings,\(^8\) the O platelets have H antigen, which should be also expressed on the GP IIb/IIIa. To avoid the false positive reaction by IgG anti-H using O platelets, I would like to suggest few solutions in such cases:

1. Care should be taken in case of blood grouping of neonatal alloimmune thrombocytopenia patients and attention also to ethnic origin should be kept in mind.

2. Adsorption of anti-H antibodies to O RBCs.

3. Using of soluble peptides \(\beta_{2,3}\) and soluble GP IIb/IIIa.\(^10\)

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