Variations in platelet antibody testing due to different assays

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Introduction: Platelet reactive antibodies against glycoproteins (GP), HLA class I antigens or human platelet antigens (HPA) may lead to significant thrombocytopenia and bleeding disorders in various clinical syndromes. A wide range of different assays are used for their detection in clinical laboratories. In this retrospective analysis the suitability of three different test systems was examined and the spectrum of antibody specificities was assessed.

Materials and methods: From 2008 to 2011 serum samples of 1,250 thrombocytopenic patients with suspected autoimmune thrombocytopenia (ITP), malignant hematologic diseases, transfusion reaction to platelet concentrates, refractoriness to platelet transfusions and suspected neonatal alloimmune thrombocytopenia (NAIT) were tested for platelet antibodies with an enzyme-linked immunosorbent assay (ELISA; Lifecodes PAKPLUS® and PAK 12®, Gen-Probe) and a solid-phase assay (Capture-P Ready Screen®, Immucor Inc.). In cases of suspected anti-HLA class I antibodies a specific lymphocytotoxicity test (LCT, Bio-Rad®) was additionally performed.

Results: In total, 382/1,250 samples were positive in the ELISA or in the solid-phase assay (30.6%). In 69.4% we obtained concordant negative but only in 8.6% concordant positive results with both methods implying that 197 samples of 1,065 in the solid-phase assay negative samples were positive in the ELISA (18.5%). The most frequent specificities of antibodies, only detectable in the ELISA were GP IIb/IIIa (36/197; 18.3%), GP Ia/IIa (28/197; 14.2%) and combined GP IIb/IIIa and GP Ia/IIa (45/197; 22.8%). In the group of samples tested positive by both methods the majority of antibodies reacted against HLA class I antigens (42/107; 39.3%) followed by GP IIb/IIIa and GP Ia/IIa (45/197; 22.8%). In the group of samples tested positive by both methods the majority of antibodies reacted against HLA class I antigens (42/107; 39.3%) followed by GP IIb/IIIa and GP Ia/IIa (45/197; 22.8%). The frequency of HPA-specific antibodies was comparable in both groups (7/197; 3.6% and 4/107; 3.7%). All other specificities were rare.

Conclusion: In contrast to HLA-class I and HPA antibodies, GP-specific platelet antibodies were more frequently detectable in the ELISA than in the solid phase assay. Therefore, suitability of the different methods seems to depend on suspected clinical
diagnoses. In our laboratory the ELISA and LCT are the first line screening tests, the solid-phase assay is used as additional test to confirm HPA antibodies. However, in cases of uncertain results analysis by the ‘monoclonal antibody-specific immobilization of platelet antigen’ (MAIPA) – assay is highly recommended.