Platelet Antibody Analysis by Three Different Tests

Nazanin Sareban,¹ Susanne Macher,¹ Camilla Drexler,¹ Ursula Posch,¹
Gerhard Lanzer,¹ and Katharina Schallmoser^{1,2,3*}

¹Department of Blood Group Serology and Transfusion Medicine, Medical University of Graz, Graz, Austria

²Department of Blood Group Serology and Transfusion Medicine, Paracelsus Medical University Hospital, Salzburg, Austria

³Spinal Cord Injury & Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University (PMU), Salzburg, Austria

Background: Platelet-reactive antibodies lead to thrombocytopenia and bleeding disorders, and diverse assays are used for their detection. In this retrospective analysis, the applicability of three different test systems was compared and antibody specificities were assessed. **Methods:** Sera of 1,234 patients were tested with an enzyme-linked immunosorbent assay (ELISA; Lifecodes PAKPLUS[®] or 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 performed. **Results:** Platelet antibodies were detected in 366 of 1,234 samples (29.7%). In 70.3% concordant negative but only in 8.4% concordant positive results were obtained with both the

methods; 185 of 1,053 in the solid-phase assay negative samples were positive in the ELISA (15.0%). In samples positive in both methods, most antibodies reacted against HLA class I antigens. Glycoprotein (GP) specific platelet antibodies, mainly against GPIIb/IIIa and GPIa/IIa, were more frequently detectable in the ELISA than in the solid-phase assay, whereas weakly positive results have to be interpreted cautiously. **Conclusion:** ELISA, solid-phase assay, and LCT showed highly divergent results. Due to several limitations, the additional analysis by the “monoclonal antibody-specific immobilization of platelet antigen” (MAIPA)—assay is highly recommended. J. Clin. Lab. Anal. 29:198–202, 2015. © 2014 Wiley Periodicals, Inc.

Key words: platelet antibody; thrombocytopenia

INTRODUCTION

The detection of platelet antibodies plays a crucial role in the diagnosis of immunologic platelet disorders such as fetal/neonatal alloimmune thrombocytopenia (FNAIT) or refractoriness to platelet transfusions, influencing further therapeutic management (1, 2). Although in suspected primary immune thrombocytopenia (ITP), the serological confirmation of glycoprotein (GP) specific antibodies is no stringent criterion for diagnosis, it may be at least helpful for the exclusion of other causes of thrombocytopenia (3).

A wide range of commercially available tests as well as in-house methods are used in clinical laboratories worldwide, whereas the identification of antibody specificities may be hampered by low assay sensitivity (4). Remarkably, the gold standard analysis for the determination of

platelet antibodies, the “monoclonal antibody immobilization of platelet antigens” assay (MAIPA), is restricted to specialized reference laboratories due to the laborious efforts involved and the consequent need for freshly prepared test platelets (5). However, Allen et al. demonstrated comparable results between commercially available antigen capture ELISAs and the MAIPA in the detection of platelet-specific alloantibodies (6).

*Correspondence to: Katharina Schallmoser, Department of Blood Group Serology and Transfusion Medicine, Paracelsus Medical University Hospital of Salzburg, Lindhofstrasse 20-22, 5020 Salzburg, Austria. E-mail: k.schallmoser@salk.at

Received 28 June 2013; Accepted 24 February 2014

DOI 10.1002/jcla.21750

Published online in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. Available Clinical Details of Patients Tested for Platelet Antibodies (*n* = 1,234)

Clinical details	Number	(%)
Thrombocytopenia	402	(32.6)
Suspected or verified malignant hematologic disease	137	(11.1)
Suspected ITP	122	(9.9)
Transfusion reaction to platelet concentrates	40	(3.2)
Suspected refractoriness to platelet transfusions	13	(1.1)
Suspected NAIT	9	(0.7)
Not specified	511	(41.4)

In our laboratory, we routinely use two commercially available test methods, an antigen capture ELISA and a solid-phase assay. For the confirmation and specification of anti-HLA class I antibodies, a complement-dependent lymphocytotoxicity test is additionally performed. In this retrospective analysis, applicability and results from the different test systems were compared and the spectrum of platelet-specific antibodies in thrombocytopenic patients was classified.

MATERIALS AND METHODS

Patients

From 2008 to 2011, serum samples from 1,234 patients (median 58.2 years, range 0.7–99 years; 573 female/661 male) were sent to our laboratory for analysis of HLA- or platelet-specific antibodies. Samples were tested immediately or frozen at -20°C until use. The results of different assays were compared retrospectively; the study was approved by the local ethics committee of the Medical University of Graz (protocol number 24–512, ex 11/12). As far as available, details concerning clinical circumstances were considered. The causes for antibody testing provided by clinicians are listed in Table 1, the most frequent one being thrombocytopenia without further details (402/1,234; 32.6%). Unfortunately, clinical details were completely lacking for 511 patients (41.4%).

Testing for Platelet Antibodies

All samples were analyzed by ELISA (Lifecodes PAKPLUS® [*n* = 1,067] or PAK12® [*n* = 167], Gen-Probe, Waukesha, WI, US), by a solid-phase assay (Capture-P Ready Screen®, Immucor Inc., Norcross, GA, US), and in specified cases by a specific lymphocytotoxicity test (LCT, Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany) according to the manufacturer's instructions.

In the ELISA, wells are coated with purified or recombinant platelet antigens binding specific IgG, IgA, and IgM antibodies against GPIIb/IIIa, GPIa/IIa, GPIb/IX, GPIV (no more available in the PAK12 ELISA), human platelet antigen (HPA) 1a/b, HPA 2a/b, HPA 3a/b, HPA 4a/b, HPA 5a/b, and HLA class I antigens. Following the manufacturer's instructions, a result was considered positive when the OD value was equal or higher than the double mean OD value of the negative control.

In contrast, the solid-phase assay is a screening test able to detect IgG antibodies against certain HLA class I antigens and human platelet antigens by providing dried, immobilized platelet membranes from 13 blood group O donors with varying antigen structures.

The LCT was performed in cases of clinically suspected or, by the described assays detected, HLA class I antibodies. This test kit consists of microtiter plates containing 56 cavities prepared with frozen lymphocytes from HLA-typed donors. The interpretation was conducted by calculating the panel reactivity antibody score (% PRA) and analyzing the antibody specificity.

Statistical Analysis

Variations in the gender distribution of patients with detected platelet antibodies were tested using a χ^2 test statistic with 1 degree of freedom; a *P*-value < 0.05 was considered significant.

RESULTS

A total of 70.3% (868/1,234) of samples were concordantly negative, 21.3% (263/1,234) were reactive either in the solid-phase assay or in the ELISA, and 8.4% (103/1,234) were positive in both assays. The results of positive samples were concordant only in 28.1% (103/366), reflecting the high variability of platelet antibody testing dependent on the type of assay.

Table 2 shows the frequency of platelet-specific antibodies in the ELISA depending on positive or negative solid-phase assay testing. The most frequent specificities of antibodies, only detectable in the ELISA, were GPIIb/IIIa (36/185; 19.5%), GPIa/IIa (28/185; 15.1%), and combined GPIIb/IIIa and GPIa/IIa (45/185; 24.3%). In the group of samples tested positive by both methods, the majority of the antibodies reacted against HLA class I antigens (42/103; 40.8%) followed by GPIIb/IIIa (10/103; 9.7%). The frequency of HPA-specific antibodies was comparable in both groups (7/185; 3.8% and 4/103; 3.9%). All other specificities were rare. In our study, female patients showed an equal incidence for GP-specific platelet antibodies as male patients (53.8%, *P* > 0.05), but were significantly more frequently immunized against

TABLE 2. Frequency of Solitary and Combined Platelet-Specific Antibodies in the ELISA^a Depending on Positive or Negative Testing in the Solid-Phase Assay

Antibody specificity	ELISA pos/solid-phase assay pos 103/181		ELISA pos/solid-phase assay neg 185/1,053	
	Number	(%)	Number	(%)
GPIIb/IIIa	10	(9.7)	36	(19.5)
GPIIb/IIIa + GPIa/IIa	3	(2.9)	45	(24.3)
GPIIb/IIIa + GPIa/IIa + GPIV	—	—	3	(1.6)
GPIIb/IIIa + GPIa/IIa + HLA-I	3	(2.9)	2	(1.1)
GPIIb/IIIa + GPIa/IIa + GPIb/IX	—	—	2	(1.1)
GPIIb/IIIa + GPIa/IIa + GPIV + HLA-I	1	(1.0)	3	(1.6)
GPIIb/IIIa + GPIV	—	—	1	(0.5)
GPIIb/IIIa + GPIV + HLA-I	—	—	1	(0.5)
GPIIb/IIIa + GPIV + GPIb/IX + HLA-I	1	(1.0)	1	(0.5)
GPIIb/IIIa + GPIb/IX	—	—	1	(0.5)
GPIIb/IIIa + HLA-I	8	(7.8)	2	(1.1)
GPIa/IIa	6	(5.8)	28	(15.1)
GPIa/IIa + GPIV	1	(1.0)	—	—
GPIa/IIa + GPIb/IX	—	—	2	(1.1)
GPIa/IIa + GPIb/IX + GPIV + HLA-I	—	—	2	(1.1)
GPIa/IIa + HLA-I	7	(6.8)	1	(0.5)
HLA-I	42	(40.8)	17	(9.2)
HLA-I + GPIb/IX	1	(1.0)	—	—
HLA-I + GPIV	4	(3.9)	2	(1.1)
HLA-I + HPA 1a	3	(2.9)	—	—
HLA-I + HPA 5b	1	(1.0)	—	—
GPIV	—	—	2	(1.1)
GPIb/IX	2	(1.9)	3	(1.6)
HPA 1a	2	(1.9)	—	—
HPA 1b	—	—	1	(0.5)
HPA 5b	2	(1.9)	6	(3.2)
Pan-reactive	6	(5.8)	24	(13.0)

^aResults of the ELISA were defined positive according to the cutoff provided by the company.

HLA class I antigens and HPA (86.1% and 100%, respectively; $P < 0.001$).

Referring to the cutoff as defined by the manufacturer's instructions, 30 sera showed positive results for all antigens in the ELISA (27 in the PAKPLUS and three in the PAK12) whereas only six were also positive in the solid-phase assay. Fifteen of these samples had only weakly positive results reflected by an OD ratio (OD value/cutoff) < 3 . Ten samples had only one highly positive, one sample had two, and three samples had three highly positive results. Only one sample had OD ratios > 3 for all antigens. There was no correlation with the results in the solid-phase assay. The weakly positive results were retrospectively considered rather unspecific or false positive.

One of our reasons for using the solid-phase assay as a screening test was its supposed high sensitivity for detecting anti-HLA I antibodies. In 78 sera, we observed positive reactions only in this assay. For the analysis and specification of complement-dependent anti-HLA class I antibodies, the LCT was performed in 61 of these sam-

ples: only three of these samples were tested positive and no specific HLA class I antibody could be verified.

In the ELISA, 101 sera were positive for anti-HLA class I antibodies, from which 71 (70.3%) were also reactive in the solid-phase assay. Of the remaining 30 samples tested negative by solid-phase assay, 26 were examined additionally by the LCT. In 14 of 26 sera (53.8%), complement activating HLA antibodies were detected; in 20 of 89 in the ELISA anti-HLA positive samples (23.1%) a specificity could be determined. These results show that the ELISA detects significantly more anti-HLA class I antibodies confirmed by the LCT than the solid-phase assay as a screening test does.

Of 122 samples tested for serological confirmation of suspected ITP, in 13.9% (17/122) anti-GP antibodies were detected. Hereby the most frequent specificity was GPIIb/IIIa (64.7%, 11/17), followed by GPIa/IIa (17.6%, 3/17), GPIb/IX (5.9%, 1/17), GPIV (5.9%, 1/17), and the combination of GPIa/IIa with GPIIb/IIIa and GPIV (5.9%, 1/17). Of course a diagnosis of ITP was

restricted due to the serum-only detection of antibodies with our available test methods.

The frequency of anti-GPIV in 22 of 288 (7.6%) in the ELISA-positive patients was surprisingly high for patients of Caucasian ancestry, by retrospectively calculating the OD ratios no sample was >3 , indicating again false-positive results.

Seven of 13 sera of patients suspected as refractory for platelet transfusion were negative, in six samples HLA class I antibodies were detected by the ELISA. Five of these sera were tested by LCT and confirmed positive (one polyspecific result with PRA 80%, one serum with anti-A3, and three sera with antibodies against multiple antigens: anti-A1,-A3,-A11,-B7,-B8,-B55,-B56; anti-B7,-B27,-B55,-B56,-B60,-B61; and anti-A2,-A23,-A24,-A28,-B44,-B57,-B58). Five of the six ELISA-positive sera were also positive in the solid-phase assay, without identifiable pattern.

In 40 sera from patients with nonhemolytic transfusion reactions within the scope of platelet substitution, only once we could identify anti-HLA A1, reactive in all three test methods. We also observed only one anti-HPA 1b and in two sera anti-GP antibodies (once GPIa/IIa and once combined GPIa/IIa and GPIIb/IIIa). Thirty-six samples (90%) were negative, reflecting the lack of association of platelet antibodies with nonhemolytic transfusion reactions.

Due to clinically suspected FNAIT, nine maternal sera were sent to our laboratory. In two patients only HLA class I but no HPA-specific antibodies were detected with both the ELISA and solid-phase assay, seven sera were tested negative. Only in three cases it was possible to get additional blood samples from the newborn or the father for HPA genotyping, whereas all three maternal sera were tested negative. In two cases anti-HPA-15b could not be excluded due to the limitations of the ELISA, in one case HPA constellation and negative testing excluded a common alloantibody.

DISCUSSION

In this retrospective analysis we compared the results of an antigen capture ELISA, a solid-phase assay, and an LCT, and assessed its qualification in detecting platelet-specific antibodies in 1,234 patients. As clinical data were not available retrospectively, our interpretation is of course limited.

The use of the ELISA for the detection of platelet-specific alloantibodies is supported by the Platelet Immunology Quality Assurance Exercise, organized by the National Institute for Biological Standards and Control (6). This evaluation demonstrated the equal quality of the commercially available ELISA kits in the detection of platelet-specific alloantibodies when compared to the in-

house assay of MAIPA. Similar to previous reports (7,8), the difficulties in detection and identification of anti-HPA 3a antibodies were emphasized.

Remarkably, in our lab the results of various test methods diverged widely, reflecting the difficulties for a sound diagnosis of platelet antibodies. The fact that in total 30 sera were tested pan-reactive leads to the speculation that preexisting human anti-mouse antibodies may cause such "unspecific" results as reported by Leach et al. (9), but we were not able to retest. Also for anti-GPIV antibodies, we had to observe that the cutoff recommended by the manufacturers of the ELISA was set too low leading to putative false-positive results. Anyhow, the next generation of ELISA, the PAK12, does not contain the GPIV antigen anymore due to low significance.

The solid-phase assay has formerly been used as a daily available screening test, as it is easy and fast to perform. However, in this analysis the assay has proven ineligible for the detection of specific cytotoxic HLA class I antibodies. In our previous experience, only in a case of NAIT caused by anti-HPA-3a antibodies this assay has been shown to be more sensitive compared to other test systems such as the MAIPA assay (7,10), and is therefore retained as backup test in the lab for additional clarification of suspected NAIT.

The incidence of antiplatelet antibodies in ITP patients varies from 45 to 80% in the literature (3,11) and precise determination is still challenging. In recent evidence-based guidelines (12), its analysis is not required for the evaluation of patients with suspected ITP, because, among other reasons, of low sensitivity of the available assays. Nevertheless, in cases of therapy-resistant thrombocytopenia, antiplatelet antibodies are still requested (3). Disadvantageously, most commercially available tests are not capable of detecting platelet-bound antibodies. Thus the low incidence of positive results for GP-specific antibodies in 13.9% of the sera with the suspected diagnosis of ITP could be interpreted as expected. Therefore, our results may confirm the reported inferiority of detecting free platelet antibodies in patients with ITP, compared to assays detecting platelet-bound antibodies (13). Concerning the specificities of ITP-associated platelet antibodies, anti-GPIb/IX (5.9%) was less frequently detected than reported in the literature (19–74%) (14–16), presumably indicating either the failure of platelet-bound antibodies or in contrast the existence of non-ITP in the patients.

In conclusion, the three commercial assays are feasible only for detecting soluble platelet antibodies, whereas we observed highly divergent results. Particularly the ELISA's suitability is restricted to some extent as the detection of anti-HPA-15 antibodies relevant for the diagnosis of NAIT is not possible. Additionally, in our experience the cutoff as defined by the company seems to be too

low for some antigens reflected by partially unreasonable results. In cases as suspected ITP or critical FNAIT antibody analysis should be extended by the MAIPA assay to detect a broader spectrum of particularly platelet-bound antibodies.

ACKNOWLEDGMENTS

The authors thank Maria Luise Stubenrauch for excellent technical assistance and Monica Farrell for proof-reading the manuscript.

CONFLICT OF INTEREST

The author and all coauthors confirm that they have no relevant conflicts of interest to declare.

REFERENCES

1. Norton A, Allen DL, Murphy MF. Review: Platelet alloantigens and antibodies and their clinical significance. *Immunohematology* 2004;20:89–102.
2. Hod E, Schwartz J. Platelet transfusion refractoriness. *Br J Haematol* 2008;142:348–360.
3. Matzdorff A, Giagounidis A, Greinacher A, et al. [Diagnosis and therapy of autoimmune thrombocytopenia. Recommendations of a joint Expert Group of DGHO, DGTI, DTH]. *Onkologie* 2010;33(Suppl 3):2–20.
4. Wu GG, Kaplan C, Curtis BR, Pearson HA. Report on the 14th International Society of Blood Transfusion Platelet Immunology Workshop. *Vox Sang* 2010;99:375–381.
5. Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C. Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): A new tool for the identification of platelet-reactive antibodies. *Blood* 1987;70:1722–1726.
6. Allen D, Ouwehand WH, de Haas M, Kekomaki R, Kaplan C, Metcalfe P. Interlaboratory variation in the detection of HPA-specific alloantibodies and in molecular HPA typing. *Vox Sang* 2007;93:316–324.
7. Harrison CR, Curtis BR, McFarland JG, Huff RW, Aster RH. Severe neonatal alloimmune thrombocytopenia caused by antibodies to human platelet antigen 3a (Baka) detectable only in whole platelet assays. *Transfusion* 2003;43:1398–1402.
8. Bessos H, Wilson DW, Metcalfe P, Allen D, Urbaniak SJ. Report on the 12th International Society of Blood Transfusion platelet Immunology Workshop. *Vox Sang* 2005;89:105–113.
9. Leach MF, Aubuchon JP. False reactivity in GTI Pak Plus ELISA kits due to the presence of anti-mouse antibody in patients' samples. *Immunohematology* 2003;19:112–116.
10. Schallmoser K, Kutschera J, Macher S, et al. Delayed detectability of anti-HPA-3a by the MAIPA assay in a severe neonatal alloimmune thrombocytopenia, but successful transfusion of incompatible donor platelets: A case report. *Vox Sang* 2006;91:181–183.
11. Raife TJ, Olson JD, Lentz SR. Platelet antibody testing in idiopathic thrombocytopenic purpura. *Blood* 1997;89:1112–1114.
12. Neunert C, Lim W, Crowther M, Cohen A, Solberg L, Jr., Crowther MA. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood* 2011;117:4190–4207.
13. Berchtold P, Muller D, Beardsley D, et al. International study to compare antigen-specific methods used for the measurement of antiplatelet autoantibodies. *Br J Haematol* 1997;96:477–483.
14. Kiefel V, Santoso S, Kaufmann E, Mueller-Eckhardt C. Autoantibodies against platelet glycoprotein Ib/IX: A frequent finding in autoimmune thrombocytopenic purpura. *Br J Haematol* 1991;79:256–262.
15. Davoren A, Bussell J, Curtis BR, Moghaddam M, Aster RH, McFarland JG. Prospective evaluation of a new platelet glycoprotein (GP)-specific assay (PakAuto) in the diagnosis of autoimmune thrombocytopenia (AITP). *Am J Hematol* 2005;78:193–197.
16. Najaoui A, Bakchoul T, Stoy J, et al. Autoantibody-mediated complement activation on platelets is a common finding in patients with immune thrombocytopenic purpura (ITP). *Eur J Haematol* 2012;88:167–174.