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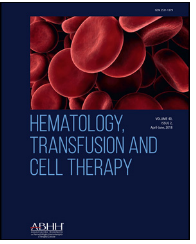

Original article

Platelet antibodies identification: comparison between two laboratory tests

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ABSTRACT

Introduction: Platelet antibody identification is indispensable for diagnosing the human platelet antigen (HPA) or human leukocyte antigen (HLA) immunization, mostly because it can restrict the compatibility and results of transfusions. Correct detection of these antibodies is of utmost importance for the diagnosis and treatment.

Method: We present 16 platelet alloimmunization results, comparing two tests with different technologies: the MAIPA (monoclonal antibody immobilization of platelet antigens), as a reference technique, and a bead-based assay, the Pak-Lx.

Results: Eleven samples (68.75%) showed agreement in both techniques. Two tests were false negatives in the Pak-Lx: a pan-reactivity in GPIIbIIIa and an anti-HPA-9b. On the other hand, the Pak-Lx was more sensitive to detect a decreasing anti-HPA-5b. The Pak-Lx found an anti-HPA-2b positive, but with a low median fluorescent intensity (MFI), suggesting a false-positive result. Moreover, in one case, the MAIPA was negative for a positive Pak-Lx HLA.

Conclusion: Antibody platelet diagnosis can sometimes be challenging. The methods seemed similar, the Pak-Lx being faster and simpler than the MAIPA, and they can be complementary to solve clinical issues.

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Introduction

Despite being anucleate, platelets express HLA (human leukocyte antigen) class I, being HLA-C with no clinical importance. The HPAs (human platelet antigens) are membrane glycoproteins, which can induce alloantibody development due to their polymorphisms.[1]

Previous transfusions, pregnancies or transplantation are causes of alloimmunization. Alloantibodies are a clinical problem, as they can be responsible for decreased transfusion efficiency, adverse outcomes and increased healthcare costs.[2,3] The improvement of their correct detection is of utmost importance for the diagnosis, especially in refractory patients that need a successful platelet increment. Identification of platelet alloantibodies is also indispensable

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for diagnosing fetal/neonatal alloimmune thrombocytopenia (FNAIT) and post-transfusion purpura (PTP). Over the years, tests progressed to more sensitive and specific assays.[4] Solid-phase methods, using the bead-based assay have become established as the "gold standard" testing for anti-HLA antibody detection in solid organ transplants.[5] More recently, this kind of technology has been successfully applied to detect the anti-HPA/anti-HLA antibodies to solve platelet issues.[6]

Historically, in 1978 Von Dem Borne et al.[7] described the platelet suspension immunofluorescence test (PSIFT), with nonspecific fluorescence being overcome by the fixation of the cells with paraformaldehyde, and it was a reference test for years. Since 1987, monoclonal antibodies against major platelet membrane constituents (glycoproteins IIb/IIIa and Ib) have been available.[8] And, nowadays, there are at least four techniques to detect HPA antibodies: the platelet immunofluorescence test (PIFT), the monoclonal antibody immobilization of platelet antigens (MAIPA) assay, the solid-phase assay and a variety of the enzyme-linked immunosorbent assay (ELISA)-based techniques. Moreover, it is also possible to achieve an indirect prediction of the presence of HPA antibodies using molecular typing.[9]

Among these tests, the MAIPA is considered the reference method. However, the research of alloantibody anti-HPA-1, 2, 3, 4, 5, anti-HLA and anti-GPIV discovered the use of new technologies, such as the Luminex platform. One advantage is that the bead-based assays are faster and simpler than other methods[10] and, even when interferences occur, especially with the anti-HLA, some techniques can be applied, such as treatment with adsorption, dithiothreitol (DTT), ethylenediaminetetraacetic acid, heat inactivation, or dilution.[11]

A study recently analyzed the sensitivity and specificity of in-house and commercially available methods for detecting alloantibodies against human platelet antigens and found variable interlaboratory results, contrasting with genotyping assays that show high consistency and accuracy.[12]

Considering practical issues in a blood bank, we present 16 platelet alloimmunization results comparing two tests with different technologies: the Pak-Lx and the gold standard, the MAIPA. We hope to contribute to a better understanding of the sensitivity of these tests.

Methods

The Local Ethics Committee approved this project under the Ethical Process Number 51560715.2.0000.0071.

This study included samples from patients previously diagnosed with platelet refractoriness (PR) (CCI 1 h < 5000, in two previous ABO-compatible transfusions), fetal neonatal alloimmune thrombocytopenia (FNAIT) (newborn platelets $<50 \times 10^9$/L) or post-transfusion purpura (PTP) (abrupt onset of thrombocytopenia 5 to 10 days after a transfusion). All of them were tested by the MAIPA, in the routine laboratory workflow, and retested by the Pak-Lx (Immucor). To solve discrepant results, when these occurred, the MAIPA was repeated.

The Pak-Lx assay was performed according to manufacturer recommendations, and a positive result was defined by the ratio cutoff calculated by the software "Match it!". Briefly, beads were incubated with serum samples at room temperature (21 °C to 24 °C) and washed to remove unbound antibodies. In sequence, an anti-human IgG antibody conjugated with phycoerythrin was added. The sample mixture was analyzed on the Luminex 100.

The procedure for the MAIPA was based on Kiefel et al., 1987[8] and the antibodies were the clones CD109, β2 microglobulin. (B1G6), glycoprotein IaIIa. (Gi9, CD49b), glycoprotein IbIX. (SZ2, CD42b), glycoprotein IIbIIIa. (P2, CD41). The optical density (OD) was calculated using the mean of two results, and samples were considered positive when the OD > 0.4.

Statistical analysis was performed using the Microsoft Excel 2010 and GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California, USA.

Results

Sixteen samples were analyzed, 15 (93.7%) were female and only one (6.3%) was male. The median age was 38 (5 to 89) years. Clinically, 6 were PRs, 7 FNAITs, two platelet autoantibodies possibly from autoimmune diseases and one PTP. Eleven samples (68.75%) showed agreement in both techniques. In one case, a PR, the MAIPA sensitivity did not detect the anti-HLA antibody (median fluorescent intensity (MFI) = 1032). In another case, the Pak-Lx assay was negative (MFI maximum in the HPA 1a-3a-4a = 131), but the MAIPA showed pan-reactivity in the GPIIbIIIa (the MAIPA OD: 1aa/3aa = 0.799; 1bb/3aa = 0.653; 1aa/3bb = 0.690). In another PR, only the Pak-Lx detected an HPA-5b antibody (MFI = 1706). In one case, there was an anti-HPA-9b, detected only by the MAIPA. Finally, in one PTP, in addition to an anti-HLA identification from both techniques, an anti-HPA-2b was not detected by the MAIPA (MFI = 555). Table 1 describes the results.

As the anti-HLA was the most common antibody identified in 10 samples (62.5%), Figure 1 shows a linear regression between the MAIPA and Pak-Lx for this antibody. The Pearson correlation showed $r = 0.59$, p-value = 0.016, 95% CI = 0.1306–0.8392.

Discussion

We compared two different technologies. The Pak-Lx offers the detection of the alloantibodies anti-HPA-1, 2, 3, 4, 5, anti-HLA and the anti-GPIV. Most of the cases were in agreement in both tests. Porcelijn et al., 2014,[6] found a 93% concordance between the tests, but this study did not use the MAIPA to compare the anti-HLA. Our data identified only one HLA discordant result not detected by the MAIPA. The Pearson correlation and Figure 1 showed a statistically significant and positive relationship between the tests, although Porcelijn et al., 2014,[6] found a better result when analyzing HPA-specific antibodies ($R^2 = 0.6997$).

There were two false negatives in the Pak-Lx (12.5%). In one case, the MAIPA showed a broadly reactive GPIIbIIIa. Clinically, the case was a FNAIT and the crossmatch between the mother and the father was positive, suggesting a possible new antibody. The other one was an anti-HPA-9b not detected by the Pak-Lx. The beads panel did not include the HPA-9 antigen epitopes, which explains why only the MAIPA detected it. This is an alloantibody considered problematic

Table 1 – Description of MAIPA and Pak-Lx results.

	MAIPA *versus* Pak-Lx results		
Concordant results (*n*,%)		11 (68.75)	
Discordant results (*n*, %)		5 (31.25)	
Case 12	MAIPA	β2 microglobulin	Neg
	Pak-Lx	bead HLA	Pos (MFI = 1032)
Case 13	MAIPA	GpIIbIIIa	Panreactivity
	Pak-Lx	GpIIbIIIa	Neg
Case 14	MAIPA	GPIaIIa	Neg
	Pak-Lx	bead HPA-5b	Pos (MFI = 1706)
Case 15	MAIPA	GpIIbIIIa (HPA-9b)	Pos
	Pak-Lx	bead HPA-9b not available	Neg
Case 16	MAIPA	GPIbIX	Neg
	Pak-Lx	bead HPA-2b	Pos (MFI = 555)

Pos: positive; Neg: negative; MFI: median fluorescent intensity; GPIIbIIIa: glycoprotein IIbIIIa; GPIaIIa: glycoprotein IaIIa; GPIbIX: glycoprotein IbIX.

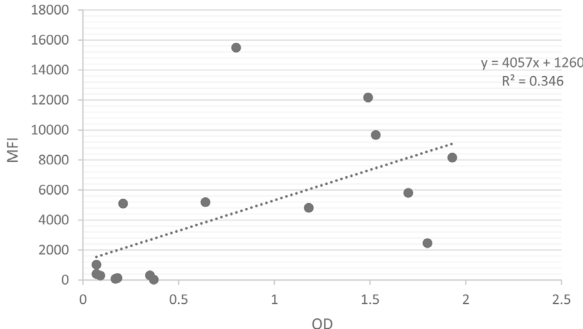

Figure 1 – HLA-specific antibodies correlation between MAIPA and Pak-Lx.
This figure shows the correlation between the MFI (median fluorescent intensity) from HLA beads of the Pak-Lx and the OD (optical density) detected from the β2 microglobulin of platelet suspension used in the MAIPA.

in the diagnosis, in part because of the presence of the cell type-specific glycans situated near the polymorphic amino acid that form the epitope. Because conventional techniques can fail, new approaches are proposed, including the use of bioengineering.[13]

The Pak-Lx found an anti-HPA-2b positive, but with a low MFI (555), suggesting a false-positive result. The MAIPA showed a false negative for detecting an HPA-5b antibody and the sensitivity of the test justifies it. Interestingly, one year before, the same patient had a previous MAIPA detecting an anti-HPA-5b (OD = 1.01). This probably suggests a better sensitivity of the Pak-Lx to decreasing titles. Moreover, Rockenbauer et al., 2015,[14] tested one sample with the anti-HPA-5b and found that the beads basic assay-specific platelet antibody assay (SASPA) and the Pak-Lx technology were more sensitive than the MAIPA.

Sareban et al., 2015,[15] comparing 1234 samples with three different tests (the ELISA, a solid-phase assay and a specific lymphocytotoxicity test) found divergent results, suggesting difficulties for proper diagnosis of platelet antibodies. Therefore, platelet serology is in constant development and we agree with Porcelijn et al., 2020[9] that there is variation in antigen recognition and that some challenging clinical issues need more than one assay. One example of this are the rare HPAs in the Chinese population for which beads could not cover the antibody detection[16] and it is crucial also for a mixed population, such as the Brazilian.

Despite being the standard method, the MAIPA technique needs validation, clones, and positive controls, which can vary among laboratories. The choice of capture monoclonal antibodies in the MAIPA assay can influence the results, especially against the HPA-15.[12] Moreover, it can depend on HPA-typified platelets and sometimes repetitions. The Pak-Lx seemed somewhat feasible, more sensitive than the MAIPA, and less laborious. The Pak-Lx is a sensitive and specific multiplex bead-based assay for detecting human platelet antibodies; nevertheless, it includes a limited panel of manufactured HPA antigen epitopes. The MAIPA, despite having a laborious technique, uses platelet suspension with all its *in vivo* characteristics.

Lewin et al., 2020,[12] included 29 laboratories from 17 countries and found that some specificities were of a high consistency in detection (at least 89% for the anti-HPA-1b, anti-HPA-2b and anti-GPIV), in contrast with the anti-HPA-15b and anti-HPA-3a (less than 20% of reporting laboratories).

Our study has a small sample; however, it is the first to report and compare these techniques in a Brazilian population, which has a history of five centuries of miscegenation resulting in an HPA and HLA diversity. This kind of information can contribute to the improvement of the techniques, including beads-panel construction.

Conclusion

Our data showed a statistically significant and positive relationship between the tests. Only 5 (31.25%) tests were discordant, being two false negatives in the Pak-Lx (12.5%), justified by the non-available beads in the panel. The MAIPA showed false negative to one anti-HLA antibody and an HPA anti-5b. In one case, the Pak-Lx detected an HPA-2b, with a low

MFI value. The two techniques seemed to be similar and can be complementary to solve some clinical issues.

Conflicts of interest

The authors declare no conflicts of interest.

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