International validation of recombinant B3 integrin-coupled beads to detect HPAL-A antibodies in fetomaternal alloimmune thrombocytopenia

Navarrete C.
PhD, FRCPath. Histocompatibility & Immunogenetics (H&I) Laboratories, National Health Service Blood & Transplant (NHSBT) & Division of Infection and Immunity, University College London, Reino Unido.
Email: cristina.navarrete@nhsbt.nhs.uk

ABSTRACT

Fetomaternal alloimmune thrombocytopenia (FMAIT) is caused by maternal alloantibodies against paternally inherited human platelet antigens (HPA) on fetal platelets. The reference method for HPA antibody detection is the monoclonal antibody immobilisation of platelet antigens (MAIPA) assay but it requires access to a panel of HPA-typed platelets and validated glycoprotein-capture monoclonal antibodies (MoAbs). Recombinant β3 integrins, displaying the bi-allelic HPA-1 epitopes (rHPA-1a and 1b) encoded by the single nucleotide polymorphism rs5918 and coupled to fluorescently labelled beads, were used to detect HPA-1a alloantibodies implicated in FMAIT. An international clinical validation of this newly developed assay was performed using 51 control samples and 529 samples from 497 suspected FMAIT cases. Control samples gave a mean concordance of 94% across all laboratories compared to the reference MAIPA results. Testing for the presence or absence of HPA-1 antibodies in the FMAIT cases by the rHPA-1 beads gave a 96% concordance with local historical MAIPA results. Of the 19 discrepant samples, 12 were from confirmed FMAIT cases with the remaining seven from possible FMAIT cases. The rHPA-1 bead assay is a rapid three hour assay for the detection of HPA-1 antibodies. Rapid detection of HPA-1a antibodies provides prompt confirmation of diagnosis in suspected cases of FMAIT and allows for evidence-based use of HPA selected donor platelets for treatment.
INTRODUCTION

Human Platelet Antigen (HPA) incompatibility can lead to the development of clinically significant alloantibodies in pregnancy, transfusion and transplantation. The alloimmune response against β3-Leucine33 (HPA-1a) in HPA-1b1b pregnant women leads to the formation of clinically significant HPA-1a antibodies which can cause severe fetomaternal alloimmune thrombocytopenia (FMAIT) with platelet counts <20 x 10^9/L occurring in 1 in 1200 term newborns. Such severe thrombocytopenia may result in intracranial haemorrhage in the perinatal period and hence transfusion of HPA-1b1b platelets is the preferred treatment to increase the platelet count and reduce the risk of bleeding, which can cause life-long disability. Current tests for detecting HPA antibodies such as the platelet immunofluorescence test (PIFT), the monoclonal antibody immobilisation of platelet antigens (MAIPA) assay and commercial ELISA-based tests are relatively laborious and require up to 24 hours before results are available. Moreover, both the PIFT and MAIPA assays require HPA-typed platelets and the results of the former test may be confounded by the presence of co-existing HLA class I antibodies. The MAIPA assay does provide HPA-specific results but it requires careful selection of monoclonal antibody (MoAb) reagents is required to achieve optimal sensitivity in detecting clinically relevant HPA-1a antibodies. As a consequence, several other assay platforms have been developed for HPA antibody detection. More recently other assay platforms have been developed for HPA antibody detection including the use of recombinant proteins. In this study we have used recombinant β3 integrins, displaying the bi-allelic HPA-1 epitopes (rHPA-1a and 1b) encoded by the single nucleotide polymorphism rs5918 and coupled to fluorescently labelled beads, to detect HPA-1a alloantibodies implicated in FMAIT.

MATERIAL AND METHODS

An international clinical validation of this newly developed assay was performed using 51 control samples and 529 samples from 497 suspected FMAIT cases. Soluble recombinant calmodulin (CaM)-tagged β3 integrin fragments, ΔβA-Leu33 (rHPA-1a) and ΔβA-Pro33 (rHPA-1b), as well as recombinant GPVI-hD1D2 (rGPVI) were expressed in Drosophila Schneider (S2) cells and purified. These recombinant proteins were biotinylated and coupled to xMAP
LumAvidin beads Biotinylated β3 integrins were then coupled to beads at 800 ng/10^5 beads with incubation for 1 hr and HPA antibody detection was performed. Binding of human IgG antibodies to the beads was detected following incubation with R-PE-conjugated anti-human with the beads and acquisition on a Luminex 100 fluoroanalyser. The Mean Fluorescence Intensity (MFI) values from 100 events of each bead population was measured and analysed. The rHPA-1 coupled beads and assay reagents were prepared centrally at laboratory for distribution to 6 testing laboratories along with a protocol for performing the rHPA-1 bead assay. The MFI data from each laboratory was collated for analysis.

Fifty-one blinded quality assurance (QA) samples were tested by six laboratories to standardise the test and generate a training set of results. Five laboratories retrieved FMAIT samples, previously tested by the monoclonal antibody immobilisation of platelet antigens (MAIPA) assay, from their local archives for testing with the rHPA-1 beads. The results of 498 independent FMAIT cases were evaluated using an algorithm developed with the training set of results to classify the samples.

**MAIN RESULTS**

Control samples gave a mean concordance of 94% across all laboratories compared to their reference MAIPA results. Testing for the presence or absence of HPA-1 antibodies in the FMAIT cases by the rHPA-1 beads gave a 97% concordance with local historical MAIPA results. Of the 19 discrepant samples, 12 were from confirmed FMAIT cases with the remaining seven from possible FMAIT cases.

**CONCLUSIONS**

The rHPA-1 bead assay is a rapid three-hour assay for the sensitive detection of HPA-1 antibodies. Rapid detection of HPA-1a antibodies could provide a prompt confirmation of suspected cases of FMAIT and allows for evidence-based use of HPA-1a 1b platelets.
RECOMMENDATIONS

With the addition of other recombinant HPAs, the Luminex multiplex technique would be extremely useful in enabling the prompt diagnosis and management of alloimmune platelet disorders. Furthermore, large scale screening for HPA antibodies becomes feasible with an affordable and simple-to-use bead assay as described here. Such an assay for HPA antibody detection could be used in prospective screening for FMAIT, routine screening in patients refractory for ABO and D matched platelets and the testing of female apheresis donors to make transfusions of platelets or fresh frozen plasma safer by preventing thrombocytopenia in the recipient by passive transfer of HPA antibodies.

REFERENCES