Serologic Characteristics and Clinical Significance of Miltenberger Antibodies among Chinese Patients in Hong Kong

Hong Kong Red Cross Blood Transfusion Service, Department of Medicine, Chinese University of Hong Kong, and Department of Obstetrics and Gynecology, and Prenatal Diagnostic and Counselling Department, Tuen Yuk Hospital, Hong Kong

The MiIII phenotype (GP.Mur) has a high incidence among people in South-East Asia: 9.7% in Thais [1], 7.3% in Taiwanese Chinese [2] and 6.28% in Chinese blood donors in Hong Kong [3]. Although there have been 5 cases of transfusion reactions (TR), a case of delayed haemolytic transfusion reaction (DHTTR) and 2 cases of haemolytic disease of the newborn (HDN) including a case of hydrops fetalis due to anti-‘Mi’ [4], there has still been some controversy with respect to the clinical significance of these antibodies [2, 5]. To address the issue further, a collaborative study on hospital in-patients and a survey on antenatal patients to screen for anti-‘Mi’ were undertaken. Results are summarized in table 1.

Anti-‘Mi’ was the most common atypical allo-antibody in both in-patients and pregnant women, being detected in 0.34 and 0.46% of them, and accounting for 31 and 48% of the atypical antibodies, respectively.

About 40% of the anti-‘Mi’ detected in in-patients were non-reactive at room temperature and therefore would not be picked up in the ‘immediate-spin’ phase of the ‘type and screen’ procedure. All of the 5 anti-‘Mi’ implicated in TR belonged to this category.

Of all the samples that had been IgG subclassed, none was composed of solely IgG2 or IgG4.

Only 4 of the 24 pregnant women with anti-‘Mi’ (16.7%) had a GP.Mur (MiIII) husband, indicating that most of the anti-‘Mi’ were naturally occurring. Three of the 24 anti-‘Mi’ gave a positive monocyte monolayer assay (MMA) result: only 1 from a patient with a GP.Mur husband. These results showed that some of the naturally occurring anti-‘Mi’ had the capability of mediating red cell destruction.

All of the anti-‘Mi’ implicated in TR and HDN were quite potent and gave strongly positive results in the MMA. The anti-‘Mi’ causing DHTTR had a negative MMA, but it was composed solely of IgG3.

Recommendation
In view of these updated data, we would recommend the following criteria for the local Chinese population:
(1) for hospitals using a ‘type and screen’ policy or, in particular, a computer cross-match procedure, GP.Mur (MiIII) cells should be included in the screening panel; (2) routine screening for anti-‘Mi’ in antenatal patients is not indicated; however, the work-up of neonatal jaundice or suspected cases of HDN should include the exclusion of anti-‘Mi’; whenever such antibodies are implicated, monitoring in a subsequent pregnancy is warranted, as a case of hydrops fetalis and a case of HDN have been encountered; (3) MMA has a significant prognostic value as a positive result was obtained in all of the 5 cases of haemolytic TR and the 2 cases of HDN.

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Table 1. Summary of laboratory findings to date on the anti-‘Mi’ detected among Hong Kong Chinese patients

<table>
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<th>Samples from</th>
<th>Anti-‘Mi’ incidence</th>
<th>MMA</th>
<th>Other atypical antibodies</th>
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| Collaborative studies  
(n = 20,423;  
9/1994–12/1996) | 0.34% positive  
(= 31% of atypical antibodies detected) | 3/4 potent anti-‘Mi’ strongly positive  
16 tested:  
IgG3: 9; IgG1+3: 3  
IgG1: 3; IgG1+2: 1 | anti-E: 24.2%; Lewis antibodies: 21.2%;  
anti-P1: 9.9%; anti-D: 7%; anti-c: 3.95%;  
others: 2.85% |
| Antenatal survey  
(n = 5,215;  
9/1995–12/1996) | 0.46% + positive  
(= 48% of atypical antibodies detected) | 1/4 cases with a GP.Mur husband and 2/20 with a GP.Mur-negative husband were positive  
4 tested:  
IgG3: 2; IgG1+3: 2 | Rh antibodies: 28%;  
Lewis antibodies: 16%; anti-M: 6%;  
Bg antibodies: 2% |
| TR | HTR: 5  
all positive  
not tested  
IgG1+3: 2  
IgG3 | none detected in all samples |
| HDN | HTR: 5  
all positive  
not tested  
IgG1+3: 2  
IgG3 | none detected in all samples |
| DHTTR  
negative  
not tested  
IgG1+3: 2  
IgG3 | none detected in all samples |

HTR = Haemolytic TR.
In a recent edition of Vox Sanguinis, Llopis et al. [1] reported a technique using boric acid cell fixation buffer to immobilize red blood cells in wells of microplates for use in the identification of red blood cell antibodies and subsequently for ABO and Rh typing [2]. We have adapted this technique for the immobilization of platelets in microplates and assessed the suitability for platelet antibody screening. This method was compared to the solid-phase red cell adherence (SPRCA) method of Lown and Ivey [3], using antithrombocyte globulin (ATG) for platelet immobilization.

Briefly, platelet-rich plasma was isolated from citrated whole-blood samples and added to boric acid cell fixation buffer [1] to give a final platelet concentration of 50–60×10³/µl. The monolayer was formed by dispensing 50 µl of diluted platelets into methanol-treated microwell strips (NUNC, Roskilde, Denmark) followed by centrifugation at 400 g for 3 min and incubation at room temperature for 15 min. Non-adherent platelets were removed by washing with phosphate-buffered saline. One hundred microlitres of 1.9% glycine and 50 µl of serum or plasma were added to the platelet-coated wells and incubated at 37°C for 15 min. The supernatant was removed and the wells washed with phosphate-buffered saline. Fifty microlitres of human anti-IgG, anti-C3d globulin reagent (Epiclone; CSL, Melbourne, Australia) was added to each well together with 50 µl of 0.6% IgG-sensitized antiglobulin control cells (CSL) and centrifuged at 90 g for 2 min.

Forty-four sera, containing known platelet antibodies directed against HLA, HPA-1a, HPA-3a, and HPA-5a antigens as well as quinine-dependent antibodies, were tested. Thirty-eight sera from bone marrow transplant recipients who presented for routine platelet antibody screening were examined (table 1). HLA and HPA-1a antibodies were also titrated (table 2).

References