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Spectrum of clinically significant anti-M antibody in patients requiring transfusion

Ashish Jain, Lakhvinder Singh, Arunpreet Kaur, Ratti Ram Sharma

ABSTRACT

Aims: The study aimed to determine the frequency and serological characteristics of anti-M antibody in patient population.

Methods: It was a retrospective study over for a period of two years (April, 2016 to March, 2018). Blood grouping was performed by tube technique. In case of incompatible cross-match, antibody screening and identification were performed using LISS Coombs' anti-human globulin (AHG) gel cards (Bio-Rad, Switzerland). Di-thiothreitol (DTT) treatment was also performed to demonstrate the existence of a potentially clinical significant IgG antibody.

Results: The frequency of anti-M antibody was 0.05% in patients (51 out of 101,364 requisitions). DTT treatment could be done in 12 samples which showed it was IgM type in 11 (91.7%) and mixture of IgM and IgG type in 1 (8.3%) case. Transfusion history was present in 21 (41.2%), absent in 13 (25.5%), and not known in 17 (33.3%) patients. M antigen phenotyping in 42 patients showed that 41/42 patients were M-negative (six of them gave a "mixed-field" agglutination due to recent transfusion of M+ PRBC unit). The 42nd patient was typed as M+ which could be again due to history of recent transfusion of PRBC.

Conclusion: Most of the anti-M antibodies were IgM type, however, serological characterization revealed the IgG type also which were found to be reactive at 37°C in AHG phase and thus clinically significant.

Keywords: Alloantibody, Anti-M, Clinically significant, M antigen

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INTRODUCTION

Following the discovery of the ABO blood group system, Landsteiner and Levine began immunizing rabbits with human red blood cells (RBCs). Among the antibodies recovered from these rabbit sera were anti-M and anti-N, both of which were reported in 1927 [1]. MNS is a highly complex blood group system consisting of 49 antigens. Anti-M is a naturally occurring immunoglobulin M (IgM) antibody and usually not clinically significant, thus, can generally be ignored in transfusion practice. When anti-M active at 37°C is encountered, IAT-cross-match compatible M-negative antigen-negative packed red blood cells (PRBCs) should be provided. Very occasionally, anti-M has been implicated as the cause of acute and delayed hemolytic transfusion reactions (HTRs), and very rarely has been responsible for severe hemolytic disease of the fetus and newborn (HDFN) [2]. We performed this retrospective study to know the spectrum of anti-M antibody in patients and determine its clinical significance.

MATERIALS AND METHODS

This was a retrospective study from the data collected over a period of two years, i.e., from 1st April, 2016 to 31st March, 2018 and included patients for whom a blood requisition was received for PRBC transfusion. The study was approved by the Institutional Ethics Committee (vide

Letter No. INT/IEC/2021/SPL1230 dated 13.08.2021). As per the standard operating procedure of the department, an antibody screen (ABS) is performed if there is any unexpected agglutination with A1, B, or O cells in reverse (=serum) grouping of the donor or patient sample, or if an incompatibility is observed between donor–patient during cross-match with a donor PRBC unit, to detect any alloantibody. The initial blood grouping (cell grouping for ABO and RhD, and reverse grouping for ABO) for patient samples was done by tube technique [2]. An autologous control (AC) was also put up along with ABS where the patients' RBCs were tested with own serum/plasma. The ABS using a 3-cell panel (Diacell I-II-III, Bio-Rad, Switzerland) and compatibility testing were done by column agglutination technique (CAT) using polyspecific anti-human globulin (AHG) gel cards (LISS Coomb's AHG gel card, Bio-Rad, Switzerland). If ABS was positive, then the antibody identification was also done by CAT using 11-cell panel (DiaPanel, Bio-Rad, Switzerland) [3]. In situations where anti-M was identified, phenotyping for "M" antigen was also done using commercially available gel cards (Bio-Rad, Morat, Switzerland) [3]. Further characterization of anti-M antibody as IgG and/or IgM was done by treating the sera with 0.01 M di-thiothreitol (DTT) (Himedia Lab, Mumbai, India) and then repeating the ABS and identification after the DTT treatment [2]. Appropriate controls were used to validate the results. DTT treatment of serum is done to differentiate between IgM/IgG antibodies. Treatment with DTT destroys the IgM component of the antibody (reactive at room temperature or at 4°C). Reducing compounds like DTT break the sulfide bonds of immunoglobulin molecule. As IgM molecule has pentameric structure which is stabilized by interchain di-sulfide bonds that is broken by DTT, hence IgM antibodies lose their agglutinating property. For preparation of 0.01 M DTT, 0.154 gram of DTT powder was dissolved in 100 mL phosphate buffer saline (PBS) at pH 7.3. Subsequently, 1 mL of patient's serum and 1 mL of 0.01 M DTT were added in the first tube (labeled as "test"), and 1 mL of patient's serum and 1 mL of PBS at pH 7.3 in another tube (labeled as "Dilutional control"). These were then incubated at 37°C in water bath for 60 minutes. The anti-M antibody activity in each sample was tested against group O RBCs (M+N–) by titration analysis till AHG phase. A serum sample known to contain an IgM antibody was treated with 0.01 M DTT and tested in parallel. If the "test" sample had the same/higher titer as the "Dilutional control" sample then the antibody was considered as IgG type, while if it gave no reactivity at all or a reactivity with lower titer then the antibody was considered as IgM type or a mixture of both IgM and IgG. DTT treatment could not be performed in all the cases where anti-M was identified either due to time constraints with respect to providing PRBCs to patients in a timely manner or due to availability issue of additional patient's sample. In those samples, the thermal amplitude of anti-M was determined by testing at room temperature (RT), 4°C and at 37°C by an indirect antiglobulin test (IAT) in AHG phase. The AHG phase testing was done using CAT. Titer of anti-M alloantibody was also performed by tube technique using serial double dilution technique and incubating the serum/plasma with group O RBCs (M+N–), wherever adequate sample was available [2].

RESULTS

Out of the total 101,364 patient samples tested during the two-year study period, anti-M alloantibody was found in 51 (0.05%) patients. The gender distribution and other serological characteristics of anti-M alloantibody are mentioned in Table 1. The result of AC test was negative for all the samples. Among the cases with anti-M alloantibody, the age range was from 1 month to 70 years, most of them (20/51, 39.21%) being less than 10 years old. The distribution of anti-M in patients (n=51) based on diagnosis is mentioned in Table 2. In 39 patient samples, where DTT treatment could not be done, however, anti-M antibody was found to reactive at 4°C, RT as well as 37°C

Table 1: Serological characteristics of anti-M alloantibody and M phenotype in patients

	Patients (n=51)
Frequency: Number (%)	51 out of 101364 (0.05%)
Male: female	29:22
DTT treatment for characterization of anti-M (done in 12 samples)	
IgM only	11 (91.7%)
IgM + IgG	1 (8.3%)
IgG only	0
Reactivity of anti-M, where DTT treatment was not done (39 samples)	
4°C	+
RT (22°C)	+
37°C (in AHG phase)	+
Anti-M titer (done in 4 samples)	
IgM (range)	1–4
IgG (range)	1–4

Table 1: *(Continued)*

	Patients (n=51)
M antigen typing (done in 42 patients)	
M+	1 (2.4%)*
M–	35 (83.3%)
M± (mixed-field agglutination)	6 (14.3%)*

*Had a history of recent transfusion (< 3 months).
DTT: di-thiothreitol, RT: room temperature.

Table 2: Distribution of anti-M in patients (n = 51) based on diagnosis

Diagnosis	Number (%)
Hematological disorders (including 5 patients of leukemia)	10 (19.6%)
Oncological disorders	7 (13.7%)
Trauma	7 (13.7%)
Development disorders (including 1 patient of meningomyelocele and 5 congenital heart disease)	6 (11.8%)
Infectious disorders	5 (9.8%)
Obstetrics	2 (3.9%)
Pyrexia of unknown origin	2 (3.9%)
Inflammatory disorders (lupus nephritis and ulcerative colitis)	2 (3.95)
Others: kidney disorders (3), pancreatitis (2), upper gastrointestinal bleed (2), hydrocephalus (1) and protein energy malnutrition (1)	10 (19.6%)

(in AHG phase). For all the patients in whom anti-M antibody was identified, "M" antigen negative PRBCs were given for transfusion irrespective of its thermal amplitude or the class (IgM or IgG). In addition, an "Antibody card" was also given to them mentioning the presence of anti-M antibody and that they should always receive "M" antigen negative PRBCs for all future transfusions. Out of the 51 patients with anti-M alloantibody, transfusion history was present in 21 (41.2%), absent in 13 (25.5%), and not known in 17 (33.3%) patients.

DISCUSSION

Anti-M antibody is a relatively common antibody but usually not active at 37°C, thus not clinically significant [2]. Being optimally reactive at 4°C and RT, it interferes with reverse ABO grouping and leads to ABO discrepancies [4]. When reactive at 37°C, it may lead to incompatible cross-match or falsely compatible cross-match due to "dosage" phenomenon, where donor RBC despite being M+ may not react optimally with anti-M in the patient's serum if they are also positive for the N antigen (M+N+) [2]. The patient should, nevertheless, be given M-PRBCs. In a retrospective 28-month study by Basu et al. [5], anti-M was detected in 8 patients, and in 7 of them it was a mix of IgM and IgG type with a wide thermal amplitude (4–37°C), thus, were clinically significant. In our study, at least 39 out of 51 (76.5%) patient samples having anti-M exhibited a reactivity up to 37°C, and also in 1 more case where DTT treatment demonstrated the presence of both IgM and IgG components. This reflects a proportionately higher prevalence of clinically significant anti-M alloantibody in our patient population, possibly because a large fraction (41.2%; 21 out of 51) had a history of previous transfusion. However, the anti-M titer ranged from 1 to 4 for both the IgM and IgG components, although it could be done in only 4 cases. High titer anti-M (256) of IgG type has been reported earlier from our center in antenatal women, where it led to HDFN and she required intrauterine transfusions for fetal salvage [6]. Hinchliffe et al. [7] also reported a case in which maternal anti-M containing an IgG component caused HDFN by inhibiting the growth of erythroid precursors. Kaur et al. [8] reported two cases of clinically significant anti-M antibodies in 2012: one case presented as a cross-match incompatibility and the other showed a discrepancy in blood grouping. The authors concluded that if the antibody is reactive at 37°C, the patient should receive antigen-negative red blood cells. In a recent retrospective multicenter study by Tamai et al. [9], it was observed that anti-M was identified most commonly in elective surgery and trauma patients (50%) followed by patients with malignancies including leukemia (25%). We observed that anti-M alloantibody was present in patients with a wide variety of disorders (Table 2), the most common (19.6%) being those with hematological disorders and others including those with oncological, infectious,

inflammatory, trauma, and obstetric conditions. The authors also observed that naturally occurring anti-M was common in the children of age group 1–3 years, which frequently attenuated irrespective of transfusion of M+ PRBCs. Providing M antigen negative (M–) PRBCs to the patients with anti-M is usually a challenging task keeping in view the high frequency of M antigen in our population [10], as observed in an earlier study from our center: M+N– (38.5%) and M+N+ (36.9%).

CONCLUSION

Most of the anti-M antibodies were IgM type, however, serological characterization revealed the IgG type also which were found to be reactive at 37°C in AHG phase and thus clinically significant. Hence, appropriate transfusion strategy should be adapted for the patients by providing them M-antigen negative PRBCs.

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Author Contributions

Ashish Jain – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Lakhvinder Singh – Design of the work, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Arunpreet Kaur – Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Ratti Ram Sharma – Conception of the work, Design of the work, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Authors declare no conflict of interest.

Data Availability

All relevant data are within the paper and its Supporting Information files.