Anti-M is a frequently detected naturally occurring antibody that has been reported in various clinical settings and also in voluntary donors. We describe here the clinical and laboratory findings of 11 cases with anti-M detected at our center. This report is a retrospective study in which we reviewed our immunohematology laboratory records for cases involving anti-M. Both donor and patient data from a 28-month period (September 2014 to December 2016) were reviewed. During this period, 11 examples of anti-M were detected (8 patients, 1 voluntary whole blood donor, and 1 hematopoietic stem cell donor. Anti-M was also detected in one external quality assessment scheme sample received during this period. In conclusion, anti-M can be detected in various clinical settings. This antibody can be clinically significant; in the laboratory, it can present as a serologic problem such as an ABO group discrepancy or an incompatible crossmatch. After detection, management and course of action is determined by both the antibody characteristics and the clinical setting. Immunohematology 2017;33:165–169.

Key Words: anti-M, ABO group discrepancy, naturally occurring, clinically significant

The MNS blood group system was identified by Landsteiner and Levine in 1927.1 Many antibodies in this system may be naturally occurring. The most common of these is anti-M, first described by Wolff and Johnson in 1933.2

The MNS blood group system consists of 49 antigens, of which two allelic pairs (M/N and S/s) are polymorphic in most populations.3 M and N are located on glycoprotein A glycoprotein. Glycoprotein A is expressed on the surface of mature as well as developing erythrocytes.4 Makroo et al.5 reported the following prevalence rates in a northern Indian blood donor population: 34.6 percent M+N–, 54.1 percent M+N+, and 11.3 percent M–N+. Because the M antigen is destroyed by routinely available proteolytic enzymes, anti-M does not react with enzyme-treated red blood cells (RBCs).6 Anti-M is generally active below 37°C, with optimum activity at 4°C.6 Hence, these antibodies are generally ignored in transfusion practice, although they can interfere in ABO plasma grouping and cause an ABO group discrepancy. If the antibody is active at 37°C, then M–, compatible RBCs must be transfused.7 We report here anti-M cases with varied presentations detected in our laboratory.

Materials and Methods

This report is from a tertiary care hemato-oncology center in eastern India treating patients with hematologic malignancies and solid tumors (i.e., gynecologic, gastrointestinal, head and neck, and soft tissue tumors). Pretransfusion antibody detection testing and crossmatch is performed for all RBC requests. Hematopoietic stem cell transplantations are performed in patients with hematologic as well as nonhematologic disorders. The pretransplant evaluation includes ABO blood grouping, extended RBC phenotyping, antibody detection testing, direct antiglobulin test (DAT), and autocontrol in both donor and recipient. In addition, major and minor crossmatches are performed. ABO grouping is performed by a conventional tube technique (CTT) with anti-A, anti-B, and anti-D reagents (Immucor India, New Delhi, India), and plasma ABO testing is performed using in-house reagent RBCs (A, B, and O). A second anti-D reagent (Tulip Diagnostics, Goa, India) is also tested. Antibody detection testing and identification is performed by the column agglutination technique (CAT) (Biovue System; Ortho Clinical Diagnostics, Raritan, NJ) using anti-IgG glass bead cards and commercially available 3-cell panels (Surgiscreen; Ortho Clinical Diagnostics) and 11-cell panels (Resolve Panel A; Ortho Clinical Diagnostics). Whenever necessary, the same is repeated in neutral or reverse diluent cards at 4°C. DAT, autocontrol, and crossmatch are performed by CAT. Extended phenotyping is performed by CTT using commercially available monoclonal antisera from Ortho Clinical Diagnostics. Titer is performed by the double dilution method using double-dose M+ (M+N–) group O RBCs. Standard validated methods are used.8

Results

Over a period of 28 months (September 2014 to December 2016), 11 cases of anti-M were detected, comprising eight patients, one donor, one hematopoietic stem cell transplant donor, and one external quality assessment scheme (EQAS) sample containing anti-M. The patient/donor ages ranged from 2.5 to 82 years. Patient diagnoses and clinical profiles of the cases are given in Table 1.
D. Basu et al.

Immunohematologic Features of Anti-M in Patients

An ABO blood group discrepancy was noted in six of the eight patients, where anti-M interfered in the reverse grouping as detected by an extra reaction with O reagent RBCs. In five of these patients, this discrepancy was resolved by repeating the reverse group with a 15-minute incubation at 37°C. In one patient, the discrepancy was resolved by using M– reagent RBCs in reverse grouping.

Specificity of the antibody was determined in seven patients by antibody detection testing and identification at 37°C. In one patient, anti-M was detected at 4°C. A dosage effect of anti-M, showing stronger agglutination strength with double-dose M+ reagent RBCs, was observed in four patients.

Antibody detection testing using serum treated with 0.01 M dithiothreitol (DTT) (Himedia Laboratories, Mumbai, India) was performed on samples from five patients. In all these patients, the agglutination strength decreased but did not disappear, suggesting the presence of both IgG and IgM components in the antibody. In two patients, DTT treatment of the serum could not be performed, and the antibody detection testing was not repeated by a prewarmed test. Hence, whether this finding was a false reaction at 37°C due to high affinity IgM anti-M or a reaction due to an IgG component of anti-M was not determined.

Titration of the anti-M was performed in four cases and was <1 (no reaction in neat plasma) at 37°C in all these cases. Because the test was performed by CTT, it is possible that titration by CAT might have shown different results, since CAT is more sensitive. Phenotyping for M was performed on samples from all eight patients; seven were found to be M– and one was M+. Of the seven M– patients, six had no known history of blood transfusion; it thus appears that, in these patients, the anti-M was naturally occurring. In the seventh patient (who received RBC transfusion earlier [outside our hospital]), the anti-M could not be characterized as immune-stimulated or naturally occurring because the donor of the transfused RBCs was unavailable for M antigen testing. One patient with anti-M (acute lymphoblastic leukemia [ALL] case) was phenotyped as M+ with a positive DAT and autocontrol. This was thus a case of autoanti-M. The immunohematologic profile of these patients is summarized in Table 2.

Table 1. Clinical profile of the cases

<table>
<thead>
<tr>
<th>Case number</th>
<th>Donor/patient</th>
<th>Age (years)/gender</th>
<th>ABO group/D type</th>
<th>Diagnosis</th>
<th>History of previous blood transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole blood donor</td>
<td>24/F</td>
<td>O+</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Stem cell donor</td>
<td>3/F</td>
<td>B+</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Patient</td>
<td>3/F</td>
<td>O+</td>
<td>Pre B ALL</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Patient</td>
<td>2.5/F</td>
<td>A+</td>
<td>Pre B ALL</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Patient</td>
<td>5/M</td>
<td>O+</td>
<td>T ALL</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Patient</td>
<td>63/M</td>
<td>A+</td>
<td>Squamous cell carcinoma of buccal mucosa</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Patient</td>
<td>24/F</td>
<td>O+</td>
<td>Carcinoma breast</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Patient</td>
<td>66/M</td>
<td>O+</td>
<td>Multiple myeloma</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Patient</td>
<td>82/F</td>
<td>A+</td>
<td>Squamous cell carcinoma of cervix</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Patient</td>
<td>72/M</td>
<td>A+</td>
<td>Seminoma testis</td>
<td>No</td>
</tr>
</tbody>
</table>

NA = not applicable; Pre B ALL = Precursor B cell acute lymphoblastic leukemia; T ALL = T cell acute lymphoblastic leukemia.

Table 2. Serologic profile of anti-M in patients

<table>
<thead>
<tr>
<th>Case number</th>
<th>Presentation</th>
<th>DAT</th>
<th>Class of antibody</th>
<th>Thermal amplitude</th>
<th>Titer</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Antibody detection testing</td>
<td>Positive</td>
<td>IgM ± IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 16 RT: 4</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>4</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>5</td>
<td>ABO group discrepancy</td>
<td>Positive</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>6</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 4</td>
<td>No transfusion</td>
</tr>
<tr>
<td>7</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM ± IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>8</td>
<td>Antibody detection testing</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 8</td>
<td>M– RBC transfusion</td>
</tr>
<tr>
<td>9</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>4°C: 4</td>
<td>No transfusion</td>
</tr>
<tr>
<td>10</td>
<td>ABO group discrepancy</td>
<td>Negative</td>
<td>IgM</td>
<td>4°C, RT</td>
<td>NP</td>
<td>No transfusion</td>
</tr>
</tbody>
</table>

DAT = direct antiglobulin test; RT = room temperature; RBC = red blood cell; NP = not performed.
Immunohematologic Features of Anti-M in Donors

Anti-M was found in one whole blood donor and one hematopoietic stem cell donor. In the whole blood donor, a blood group discrepancy was noted and was resolved when the reverse group was repeated with prewarmed serum. The anti-M was also detected at 4°C and not at 37°C, suggesting possibly only an IgM component. The donor’s RBCs tested as M–.

In the stem cell donor, anti-M was detected during the pretransplant evaluation. Although both the donor and recipient were group B, D+, the minor crossmatch was incompatible. Antibody identification confirmed anti-M reactive at 37°C, showing a dosage effect. Presence of IgG along with IgM was confirmed by DTT treatment of the serum. The RBCs were phenotyped as M–. Neither this donor nor the whole blood donor had history of blood transfusion, suggesting that the anti-M in both donors was naturally occurring. The immunohematologic profile of these donors is summarized in Table 3.

EQAS Sample

During the study period, anti-M was identified in one EQAS sample. This antibody was detected at 37°C and showed dosage. The agglutination strength increased when the testing was repeated at 4°C. DTT treatment of the plasma or antibody titration studies could not be performed nor was a transfusion history supplied with the sample.

Clinical Outcome of Anti-M in Patients and Donors

Among the patients with anti-M, five patients required RBC transfusion. As in most of the cases, the IgG component was present along with IgM; hence, the anti-M was considered to be clinically significant, and these five patients received M– RBC units. The patient with ALL who had autoanti-M had clinical as well as laboratory features of hemolysis. The unconjugated bilirubin was increased at 1.5 mg/dL (normal 0–1.1 mg/dL), total bilirubin was 1.8 mg/dL (normal 0.2–1.3 mg/dL), reticulocyte count was 4% (normal 0.5–2.5%), and lactate dehydrogenase was 4039 U/L (normal 313–618 U/L). This patient received injection dexamethasone along with RBC transfusion and responded well, confirmed by an increase in hemoglobin increment from 4.5 to 9.4 g/dL.

The anti-M in the hematopoietic stem cell donor was detected during pretransplant evaluation. Incompatibility between donor’s plasma and patient’s RBCs was detected, and anti-M was then identified. Because the stem cell donor was 3 years of age, a marrow harvest was undertaken. RBC loss in the stem cell donor required transfusion of 1 RBC unit during the harvest. Because the donor had anti-M, group-specific, M–, crossmatch-compatible RBCs were transfused. To further complicate the matter, the stem cell recipient was M+. Because the donor anti-M was clinically significant, the marrow harvest product was plasma-depleted before transfusion to reduce chances of a hemolytic transfusion reaction. Hence, once anti-M is detected, further management of the patient depends on the specific clinical setting.

The donor unit, which had anti-M in the plasma, was separated into RBCs, platelets, and plasma components. The plasma was sent for fractionation, platelet concentrate was transfused to an M– patient, and the RBC unit was transfused to an ABO group-specific patient.

Discussion

In patients, anti-M may be detected in various medical as well as surgical settings. Das et al.10 reported three cases of anti-M, all of which were detected during pre-transfusion testing before surgery. In the present study, anti-M was detected in our medical and surgical oncology patients at the time of blood grouping and antibody detection testing. Our population consisted of medical and surgical oncology patients.

Generally, anti-M is IgM in nature, optimally reactive below 22°C, and most often causes a blood grouping discrepancy. It may also be detected incidentally during pretransfusion evaluation. If there is no known stimulating event (i.e., RBC transfusion, pregnancy, or other exposure to foreign RBCs), the antibodies are classified as naturally occurring. Autoanti-M has also been reported in the literature. Anti-M is more commonly observed in children.6 In the present study, however, there were only four individuals younger than 10

### Table 3. Serologic profile of anti-M in donors

<table>
<thead>
<tr>
<th>Case number</th>
<th>Presentation</th>
<th>DAT</th>
<th>Class of antibody</th>
<th>Thermal amplitude</th>
<th>Titer</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABO group discrepancy</td>
<td>NP</td>
<td>IgM</td>
<td>4°C and RT</td>
<td>NP</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>Minor crossmatch incompatible</td>
<td>Negative</td>
<td>IgM + IgG</td>
<td>4°C, RT, and 37°C</td>
<td>NP</td>
<td>M– RBC transfusion</td>
</tr>
</tbody>
</table>

DAT = direct antiglobulin test; NP = not performed; RT = room temperature; NA = not applicable; RBC = red blood cell.
years of age (three patients and one stem cell donor). Mathur et al.\(^{11}\) described one case of anti-M in a voluntary blood donor where the antibody had a thermal amplitude of 22°C to 37°C but showed no reaction at 4°C. This finding is in contrast to our finding from the whole blood donor, where the anti-M was reactive at room temperature with optimum activity at 4°C. Sacher et al.\(^{12}\) described 17 cases of autoanti-M, of which four had symptoms of cold agglutinin disease, such as Raynaud’s phenomenon, livedo reticularis, and acrocyanosis, and two had mild hemolysis; the rest were asymptomatic despite having a significant titer of the autoanti-M.

Patients with anti-M have been described by Tandon et al.\(^{13}\) and Kaur et al.\(^{14}\) These antibodies were identified as IgM, causing ABO grouping discrepancy, with optimum reactivity below 37°C. Anti-M with both IgM and IgG components and wide thermal amplitude (4°C–37°C) were also described. Hence, those authors suggest that when evaluating for the IgG component of anti-M, strict prewarmed conditions must be maintained during antibody identification to avoid interference by high-affinity, high-titer IgM.

Anti-M, which is commonly IgM in nature, can cause complement activation. In 1991, Combs et al.\(^{15}\) described an autoanti-M causing hemolysis in vitro by activating complement in CTT at low-ionic strength.

Naturally occurring anti-M with only an IgG component has been reported very rarely.\(^{6}\) Anti-M shows a dosage effect, that is, it shows a stronger reaction with RBCs with double-dose expression of M (M+N−) than with RBCs with single-dose expression (M+N+). For this reason, the prevalence of naturally occurring anti-M in blood donors was shown to be 1 in 2500 when testing with M+N− RBCs and 1 in 5000 when using M+N+ RBCs.\(^{7}\) In our series, a dosage phenomenon was noted in 6 of the 11 cases described. Anti-M has also demonstrated increased reactivity when tested with serum that has been adjusted to a pH of 6.5.\(^{3}\) Beattie and Zuelzer\(^{16}\) described two anti-M examples that were identified after acidification of the serum.

Delayed hemolytic transfusion reaction is very rare because of alloanti-M. Sancho et al.\(^{17}\), Alperin et al.\(^{18}\), and Furlong and Monaghan\(^{19}\) reported cases of alloanti-M that were undetectable during pretransfusion evaluation but were subsequently detected 5–15 days posttransfusion during investigation of posttransfusion hemolysis. Anti-M has also been implicated in hemolytic disease of the fetus and newborn (HDFN). The clinical effects in HDFN as described in the literature range from severe HDFN with intrauterine death\(^{20}\) to cases where the fetus is unaffected.\(^{21}\) Like anti-K, anti-M has been reported to cause suppression of erythroid precursors, leading to RBC aplasia and prolonged anemia of the newborn.\(^{22,23}\) Hence, anti-M in various clinical settings presents differently and demands different management.

In conclusion, anti-M can have varied clinical presentations and can interfere during blood grouping and crossmatching. To determine the clinical significance of anti-M, elaborate methods such as antibody titration, the determination of antibody class, and thermal amplitude testing may be necessary. In the case of a transfusion requirement for a patient with anti-M, the specific clinical setting along with the antibody characteristics would determine the course of action to be taken. This report also highlights the fact that, in all pretransplant evaluations, a minor crossmatch or antibody screening of the donor must be performed to ensure that a donor antibody is not missed. Lastly, in a stem cell donor, the harvest may require modification to avoid any adverse transfusion reaction in the recipient.

**References**


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