

Impact of Anti-M on Grouping and Cross Matching: The Challenges Faced in Three Cases

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ABSTRACT

Anti-M, a frequently encountered antibody of the MNS blood group system, is commonly regarded as a naturally occurring saline agglutinin that can occasionally exhibit clinically significant behaviour. Particularly when reactive at 37°C or during the antiglobulin phase, it can complicate pretransfusion testing and patient management in the blood bank. Three distinct cases (2 males, 1 female) in varying clinical scenarios, ranging from surgery to acute coronary syndrome, are described. The present cases presented discrepancies in blood grouping and compatibility testing, highlighting the challenges posed by the presence of Anti-M antibodies that react at or above room temperature and during the antiglobulin phase, thus defying the usual expectations. It is imperative to provide antigen-negative red cells and those that are compatible by an Indirect Antiglobulin Test (IAT) when Anti-M is reactive at 37°C to ensure safe transfusion. Hence, there is a need to underscore the potential clinical implications in transfusion medicine, as well as the nuances of detecting, identifying, and managing the presence of Anti-M antibodies in patients requiring transfusion.

Keywords: Antibodies, Antiglobulin test, Blood transfusion, Cold agglutinins

INTRODUCTION

The second blood group system to be recognised following the discovery of the ABO blood group system is the complex MNS system consisting of 49 antigens on two glycoproteins. The first two antigens, M and N, were named from the second and fifth letters of the word 'immune' because the corresponding antibodies were produced through immunisation of rabbits with human Red Blood Cells (RBCs) [1]. The Anti-M antibody is the most commonly encountered in this blood group system, initially described by Wolff and Johnson in 1933 [2]. It is a naturally occurring, cold-reactive saline agglutinin with an optimum temperature of 4°C, which in most cases is clinically insignificant [3]. Although this antibody is predominantly of the Immunoglobulin M (IgM) class, it can also be IgG or have an additional IgG component [4]. The Anti-M antibody can have serious clinical implications when reactive at 37°C or during the antiglobulin phase [5]. Reactivity of Anti-M is enhanced by acidification of the serum and does not agglutinate papain or ficin-treated red cells [6]. These antibodies rarely have the probability of causing haemolytic disease of the newborn, haemolytic transfusion reactions, and decreased red cell survival [4].

Case 1

A 14-year-old boy who was a known case of hypospadias since birth was admitted to the Department of Urology with complaints of intermittent burning micturition for the past three months. The patient had a previous history of surgery for the same four years back at an outside hospital for similar complaints. The patient was scheduled for hypospadias repair after an examination revealed findings of distal penile hypospadias. A request for one unit of packed RBCs was received, and his blood sample was sent to the blood bank for routine blood grouping and cross-matching. Blood grouping was performed using Column Agglutination Technology (CAT), which showed discrepant results. On forward grouping, the patient's red cells agglutinated with anti-B, but in reverse grouping, the serum agglutinated with both A1 and B cells. Further work-up by Conventional Tube Technique (CTT) showed reactivity of the patient's serum with both A1 cells, B cells, and O cells at 37°C, room temperature, and 4°C. A Direct Antiglobulin Test (DAT) was performed on red cells from an Ethylenediaminetetraacetic Acid (EDTA)

sample using polyspecific antiglobulin reagents (anti-IgG and C3d) and was found to be negative along with a negative autocontrol. Red cell Antibody Screening (ABS) carried out using a three-cell screening panel by CAT (ID-DiaCell I-II-III, Bio-Rad Laboratories, Cressier, Switzerland) was positive.

Antibody Identification (ABID) was performed using an 11-cell panel (Bio-Rad ID Micro Typing System), and Anti-M antibody was identified. The cell panel showed a 2+ agglutination reaction with homozygous M+ N- cells, and there was no agglutination reaction with heterozygous M+ N+ cells [Table/Fig-1].

The patient had no previous history of blood or blood component transfusion, as verified by the patient's parents. Crossmatching with 12 units of packed red cells was done, of which only four units were found to be compatible, and these units were M antigen negative. However, the child did not require any transfusions post-surgery as there was not much blood loss. Since there was no previous history of transfusion, the anti-M antibody in this patient's serum, which shows dosage effect, is naturally occurring and reacts at 4°C, room temperature, and 37°C, as well as at the Anti-human Globulin (AHG) phase, and is of potential clinical significance.

Case 2

A 26-year-old female G2A1 at seven weeks + four days of gestation was admitted to the Obstetrics Emergency room with complaints of bleeding PV and fever for one week. The patient was planned for emergency suction and evacuation. The haemoglobin was 10.8 g/dL according to laboratory results, and the patient had no prior history of blood transfusions. One unit of packed red cells was requested. The case presented as a blood grouping discrepancy, with the forward grouping interpreted as B Rh(D) positive, while the reverse grouping showed reactivity (3+) with A1 cells, B cells, and O cells using CAT. The blood grouping was repeated by CTT, which showed the same discrepancy in reverse grouping. To resolve this ABO discrepancy, immunohaematology work-up was done. The patient's DAT and autocontrol were negative. The IAT test using pooled O cells was positive. ABS using a three-cell panel was positive. The antibody was identified as anti-M using an 11-cell panel [Table/Fig-2].

S. No.	Rh-hr	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth		Xg	Result
		D	C	E	c	e	C ^w	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg	
1	R1wR1	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	+	0	0	+	+	+	+	+	0	+	+	0	
2	R1R1	+	+	0	0	+	0	+	+	+	+	nt	nt	0	+	+	+	0	+	+	+	+	0	0	+	+	0	
3	R2R2	+	0	+	+	0	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	0	+	+	0	+	0	0	
4	r'r	0	+	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	0	0	0	+	0	+	0	0	+	2+	
5	r"r	0	0	+	+	+	0	0	+	0	+	nt	nt	+	+	+	0	0	+	+	+	+	0	+	+	+	0	
6	rr	0	0	0	+	+	0	+	+	0	+	nt	nt	+	+	0	+	0	+	+	+	0	0	+	0	+	2+	
7	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	0	0	+	+	0	+	0	+	0	+	0	
8	R0r	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	+	0	0	+	+	+	0	+	0	+	0	
9	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	0	+	0	+	+	0	+	+	0	0	
10	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	+	0	+	+	0	+	+	0	+	2+	
11	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	0	+	0	0	+	0	+	0	+	0	+	0	

[Table/Fig-1]: Antibody showing anti-M specificity with dosage. (nt: Not tested)

S. No.	Rh-hr	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth		Xg	Result
		D	C	E	c	e	C ^w	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg	
1	R ₁ wR ₁	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	0	+	0	+	0	0	+	0	+	0	+	nt	0
2	R ₁ R ₁	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	+	+	0	+	+	+	0	+	+	0	+	+	3+
3	R ₂ R ₂	+	0	+	+	0	0	0	+	0	+	nt	nt	+	0	0	+	0	+	0	+	+	0	+	0	+	0	2+
4	r'r	0	+	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	0	0	+	0	0	+	0	+	0	2+
5	r"r	0	0	+	+	+	0	0	+	0	+	nt	nt	0	+	+	+	0	+	+	+	+	0	+	+	+	0	2+
6	rr	0	0	0	+	+	0	+	+	0	+	nt	nt	+	+	+	0	0	+	+	0	+	0	+	0	+	0	
7	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	0	0	0	+	+	0	+	0	0	+	nt	3+
8	R ₀ r	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	0	0	+	+	+	+	0	+	0	+	0	3+
9	rr	0	0	0	+	+	0	0	+	+	+	nt	nt	0	+	+	0	+	0	+	+	0	0	+	0	+	+	3+
10	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	+	0	0	0	+	0	+	0	+	nt	0
11	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	0	+	+	0	+	+	0	0

[Table/Fig-2]: Case of anti-M reactive in the AHG phase. (nt: Not tested)

The discrepancy in reverse grouping was resolved by incubating B and O cells at 37°C for 30 minutes, and hence, the final blood group of the patient was found to be B Rh (D) positive.

On crossmatching with ten ABO group-specific donor units, only one unit was crossmatch compatible and M antigen negative. Thus, the anti-M found in this female patient with obstetric complications causing a discrepancy in grouping and crossmatch incompatibility, resolved at 37°C and enhanced at the AHG phase of testing, exhibits clinically significant reactivity. The unit was not transfused as there was minimal intraoperative blood loss for the patient.

Case 3

A 76-year-old male with a history of coronary artery disease and acute coronary syndrome was admitted to the Cardiology Department due to intermittent chest pain and giddiness over the past 2 days. The patient was scheduled for coronary artery bypass graft surgery, and

his haemoglobin level was 11.6 g/dL. Two months ago, the patient received one unit of packed red blood cells at an outside hospital due to shortness of breath and low haemoglobin levels.

A request for four units of packed red cells was made for reservation as part of the preoperative blood ordering protocol for elective cardiac surgeries, anticipating blood loss during the procedure. However, a discrepancy was noted during routine pretransfusion testing of the patient's blood grouping. Initially, blood grouping by the CAT method revealed A Rh (D) positive for cell grouping but panreactivity for serum grouping.

Repeat grouping using the conventional tube method also showed a discrepancy, with a 3+ reaction with B cells and a 2+ reaction with A and O cells. Additional testing found a negative direct antiglobulin test (DAT) and negative autocontrol. Antibody screening using a 3-cell panel was positive, and anti-M antibody was identified using an 11-cell panel [Table/Fig-3].

S. No.	Rh-hr	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth		Xg	Result
		D	C	E	c	e	C ^w	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg	
1	R1wR1	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	0	+	0	+	+	+	0	+	+	0	+	+	4+
2	R1R1	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	+	+	0	+	+	+	0	+	+	+	+	+	4+
3	R2R2	+	0	+	+	0	0	0	+	0	+	nt	nt	0	+	+	0	0	0	+	+	0	+	+	0	+	+	4+
4	r'r	0	+	0	+	+	0	0	+	0	+	nt	nt	+	+	0	+	0	+	0	0	+	0	+	0	+	+	0
5	r"r	0	0	+	+	+	0	0	+	0	+	nt	nt	+	+	+	0	0	0	+	+	0	+	+	0	+	+	4+
6	rr	0	0	0	+	+	0	+	+	0	+	nt	nt	+	0	0	+	0	+	+	0	+	+	+	+	+	0	
7	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	+	+	+	+	0	0	0	+	+	0	+	+	0	0	
8	R ₀ r	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	+	0	0	+	+	0	0	+	0	+	+	4+
9	rr	0	0	0	+	+	0	0	+	+	+	nt	nt	+	+	+	+	0	+	0	+	+	0	+	0	+	0	4+

10	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	0	+	+	0	+	+	0	0	+	+	0
11	rr	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	0	0	+	+	+	0	+	0	0	+	+	4+

[Table/Fig-3]: A case of alloimmune anti-M.
(nt: Not tested)

The discrepancy in reverse grouping was resolved by incubating the A, B, and O cells at 37°C for 30 minutes, confirming the patient's blood group as A Rh (D) positive. When crossmatching with seven donor units, only two units were found to be Coombs crossmatch compatible, both being M antigen negative. The anti-M antibody detected in the patient is of alloimmune origin and is reactive at the antiglobulin phase, likely developed as a result of the packed red cell transfusion the patient received two months prior.

DISCUSSION

Pretransfusion testing refers to a set of procedures required before blood is issued to ensure compatibility. The goal is to select blood or its components in a way that ensures adequate survival when transfused and prevents damage to the donor's red cells. ABO grouping and compatibility testing are vital steps that must be performed to ensure compatibility between the donor unit and recipient plasma.

Although there are ways to prevent cold alloantibodies from interfering with pretransfusion test findings, they nevertheless cause unexpected reactions in ABO typing and create problems for blood group serologists trying to crossmatch [1]. Anti-Le^a, -Le^b, -M, -N, and -P are common cold antibodies in the blood bank that bind their target antigen best at levels below body temperature (37°C). One relatively common antibody among these is anti-M, as it is naturally occurring, found in the sera of persons who have not been exposed to human erythrocytes, and hence generally ignored in transfusion practice. Anti-M mostly reacts below 37°C [5], but in Case 1, the anti-M detected reacts at 37°C and in the AHG phase. However, in this case, it is naturally occurring as revealed by the patient's transfusion history. Hence, the importance of these antibodies cannot be left unattended because of their potential to cause haemolytic transfusion reactions and haemolytic disease of the newborn. Therefore, an appropriate M antigen-negative, IAT crossmatch-compatible red cell unit was selected for transfusion in Case 1.

Naturally occurring anti-M antibodies are more commonly found in children than in adults [3]. The findings in Case 1 are similar to those by Yousuf R et al., who described a similar case of a naturally occurring, clinically significant anti-M reactive at 37°C and AHG phase causing a discrepancy in ABO grouping in a two-year-old child [7]. Since the anti-M in this case showed a dosage effect and heterozygous units were crossmatch-compatible, caution was taken, and an M-antigen-negative unit was reserve instead of heterozygous compatible units in order to prevent the haemolytic transfusion reaction that might occur in a delayed manner.

Despite the claim that this anti-M antibody is a common cold saline agglutinin, these antibodies would not frequently be found if room-temperature incubation is eliminated from compatibility testing and screening for antibodies [8]. Therefore, anti-M cannot be considered a negligible factor in blood banking serology but should be interpreted cautiously, especially when reactive at the AHG phase and at 37°C.

Case 2 describes a 26-year-old female with obstetric complications in whom anti-M antibody was detected during emergency pretransfusion testing. This case highlights the importance of rigorous Immunohaematological work-up to resolve discrepancies in blood grouping and ensure compatible transfusion under emergent conditions.

Literature reports delve into the ambit of anti-M and showcase the apparent role of a transfusion medicine expert during dire circumstances when blood transfusion is required immediately and

ample time is not provided to find crossmatch-compatible blood [9]. In such circumstances, having a well-established blood donor registry with O typed blood donors of regional origin can significantly reduce the burden of any blood centre and ensure that the appropriate blood is provided to the appropriate patient in a timely way [9]. In each of these situations, an immunohaematology card can also be provided, outlining the nature and type of the antibody, and informing the patient of its importance and need during future blood transfusion requirements [10].

Although usually not active at 37°C, anti-M antibodies cannot be ignored when they react at 37°C. In such cases, anti-M may have an IgG component, either along with IgM or may be exclusively IgG. Dithiothreitol (DTT) is used to differentiate between IgM and IgG antibodies by dissolving IgM antibody disulfide bonds and inactivating them, allowing the identification of unaffected IgG antibodies. DTT treatment of the test serum or plasma can aid in antibody investigation, where there may be a mixture of IgM and IgG antibodies. It is important to accurately determine the specificity of the anti-M antibody, as it can influence clinical outcomes [11].

In cases 2 and 3 described above, the anti-M antibody causing ABO grouping discrepancy was resolved by incubating reverse grouping at 37°C for 30 minutes but enhanced at the AHG phase and caused crossmatch incompatibility, suggesting that the antibody may be of the IgM+IgG class. Further testing with DTT is necessary. Similar findings were observed in case reports by Navkudkar A et al., and Tondan et al., who described a case of immunising anti-M with IgG+IgM component showing dosage effect and reacting at both 37°C and AHG phase causing problems in blood grouping and cross-match compatibility [4, 11].

A study by Das R et al., reported two cases in which one was a naturally occurring anti-M of IgM type solely reactive below 37°C and another case of naturally occurring anti-M of IgM+IgG type reactive at 37°C [3]. Meanwhile, Shah SP et al., reported 13 cases of anti-M antibodies that were biphasic in nature (reactive at room temperature and at 37°C), and "M" antigen-negative blood was transfused to these patients [12]. Naturally occurring anti-M antibodies reacting at high thermal amplitudes causing serological problems in the interpretation of ABO blood grouping and crossmatch incompatibility have been reported by Raturi M et al., Chandak SA, and Ranjan V, [9,13,14].

From naturally occurring clinically insignificant anti-M complicating ABO grouping reported by Khalid S et al., [5], to clinically significant anti-M antibodies in case reports described by Kaur G et al., Navkudkar A et al., and Tondon R et al., show that anti-M antibodies can have variable presentations [2,4,11]. Moreover, a literature review by Ferdowsi S et al., confirmed this characteristic feature of anti-M, while also reporting three cases of naturally occurring, clinically significant anti-M causing ABO discrepancy [10]. Hence, diligent care should be taken to find suitable blood for transfusion in patients with clinically significant anti-M antibodies.

In addition, anti-M antibodies have been detected in antenatal mothers, as in the study by Philip J et al., presenting as crossmatch incompatibility and blood grouping discrepancies [15]. A rare case report by Crispin P et al., delineates a case of passively transferred maternal anti-M of low thermal amplitude and low titre, causing delayed haemolytic disease of the newborn with laboratory features resembling cold agglutinin disease and requiring transfusion [16]. A case of neonatal red cell aplasia with a significant decrease in erythroid cell proliferation in culture involving anti-M antibodies (IgM+IgG type) has been reported [17]. Sacher RA et al., reported and reviewed cases of auto-anti-M antibodies in their series [18].

The study by Jain A et al., highlights the spectrum of anti-M antibody in patients and its clinical significance [19]. The majority of anti-M antibodies were of the IgM type; however, serological characterisation also identified the IgG type, which was demonstrated to be clinically significant as it was reactive at 37°C in the AHG phase. A previous transfusion history was present in a large fraction of patients, and M antigen-negative PRBCs, irrespective of their thermal amplitude or class (IgM or IgG), were transfused.

The M antigen phenotype prevalence in the Indian blood donor population is 87.2% [20], which means that only 13% of donors lack the antigen, thus making it difficult to provide an antigen-negative unit in clinically significant cases. In this study, for all cases, approximately 7-12 units were crossmatched, of which 1-4 units were compatible on average.

CONCLUSION(S)

In conclusion, the enigma of anti-M antibodies in blood banking ranges from being a common naturally occurring cold agglutinin to a clinically significant one. The detection of anti-M antibodies reactive at 37°C and during the antiglobulin phase highlights their potential clinical significance, challenging the conventional understanding and management of these antibodies in transfusion practice. The present study emphasises the necessity for comprehensive and meticulous pretransfusion testing and careful interpretation in the presence of anti-M antibodies to ensure transfusion safety. The findings advocate for the provision of M antigen-negative and IAT compatible red cells in cases where anti-M is reactive at 37°C, portraying the diverse and clinically relevant spectrum of anti-M antibody presentations in transfusion medicine.

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