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Interference of anti-M antibody with ABO blood grouping — own experience

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Summary

Background: *Anti-M antibody alloantibody is a fairly common, naturally occurring cold-type antibody.e mostly of the IgM class but may also occur as IgG or IgM with IgG component. It is considered clinically insignificant as it reacts at temperatures below 37°C. IgM class anti-M antibody may be responsible for interference with the standard ABO blood grouping in patients.*

Material and methods: *In this study we focus on the experience of the Reference Laboratory of the Regional Blood Transfusion Center Katowice in detection, identification as well as interference of anti-M alloantibody with ABO grouping. Between January 1st 2022 and June 30th, 2022, the Reference Laboratory performed 1800 consulting and blood grouping tests. The study test pool was divided into 2 groups by age: 4 months–18 years and above 18 years of age. 137 cases of anti-M antibodyalloantibody from the MNS blood group system were detected and identified. In cases of ABO blood grouping-problems, additional procedures were applied such as blood grouping with conventional test tube technique (CTT) at 37°C, use of standard M negative O, A1, B red blood cells and treatment of sera with 2-mercaptoethanol (2-ME) agglutanation test.*

Results: *Anti-M antibody interference with ABO blood grouping was more common for individuals under 18 years of age than in the older patients. Anti-M antibody occured approximately twice as frequently in the IgM class than in the IgG/IgG + IgM class, regardless of age/study group.*

Conclusions: *Anti-M antibody was causing unexpected positive reactions of the study serum with standard red blood cells and the reason of complications in interpretation of results and delay in their the issue/release.*

Key words: anti-M alloantibody, MNS system, ABO blood grouping

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Introduction

MNS was the second blood group (following AB0 blood group) to be discovered in 1927 by Landsteiner and Levin. The ISBT (International Society of Blood Transfusion) nomenclature ascribed this blood group the MNS symbol and the 002. number. The system takes its name from the first 3 identified antigens — M, N, S. There are currently 50 antigens in the system [1–3].

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MNS blood group antigens are encoded by GYPA and GYPB genes located on chromosome 4. These genes produce: GPA (MN sialoglycoprotein also known as MIRL2) and GPB (Ss sialoglycoprotein). Both sialoglycoproteins are components of the red blood cell membrane. *GYPE* — the third gene lies adjacent to *GYPA* and *GYPB* and does not encode components of the cell membrane but participates in gene rearrangement which results in a wide variety of alleles [1, 4].

MNS blood group antigens, the sialylated glycoproteins of the RBC membrane are primarily responsible for a negatively charged surface and the repulsive electric potential between cells which prevents interaction between RBCs so that they do not succumb to agglutanation. These antigens are also receptors for cytokines and pathogenes including the malaria parasite (*Plasmodium falciparum*). Glycophorin A (GPA), the major erythrocyte sialoglycoprotein is a complement regulatory protein which protects the red blood cell from hemolysis by preventing the complement membrane attack complex termed C5b-C9 [1, 3, 4].

The first antigen of the MNS system to be discovered was antigen M identified in 1927 in serum of rabbits which were immunized with human red blood cells and produced antibody against M and N blood group antigens. The antigen is found in 78% of Caucasians and 74% of the black population [1, 4]. The names of these antigens (M and N) came from the word "immune" The common feature of both M and N antigens is their sensitivity to ficin and papain enzymes, and resistance to DTT (dithiothreitol) and acidic environment. They differ in sensitivity to trypsin: the M antigen is resistant while the N antigen is sensitive [1, 4].

The prevalence of MNS phenotypes in the Polish population is shown in Table 1 [5].

Anti-M alloantibody is most often naturally occuring IgM antibody reacting optimally at 4°C in a direct agglutination test in NaCl. Naturally occurring anti-M antibody is mostly of the IgM class, however, an IgG component can also be present along with IgM. Most often, it is inactive at 37°C and in transfusion practice it is then considered as clinically insignificant. On the other hand, anti-M alloantibody reactive at 37°C in the indirect antiglobulin test is considered clinically significant, and only M negative RBCs are selected for transfusion. This antibody rarely causes severe hemolytic post-transfusion reactions or hemolytic disease of the fetus and the newborn (HDFN). It is nonreactive in enzyme assays and shows a 'dosage effect' (stronger reaction with homozygous MM phenotype standard cells than with heterozygous MN phenotype cells) [1, 3, 4]. The detection frequency for naturally occurring anti-M antibody is 1/2500 healthy donors and higher for sick children and adults with bacterial infections [1, 4]. The antibody may also appear during pregnancy. It often happens that NN phenotype pregnant women produce anti-M alloantibody although the fetus has no M antygen [1, 3, 4]. Although the specific mechanisms of natural anti-M formation are yet to be elucidated, it is believed that (like for ABO and other naturally occurring IgM antibody) gut microflora, pathogens, and food-associated proteins are the immunogenic stimuli for antibody formation mediated by the innate immune system [6].

Table 1. The prevalence of MNS phenotypes in the Polish population [5]

Phenotype	%
MMSS	8.4
MMSs	14.4
MMss	9.7
MNSS	6.1
MNSs	21.7
MNss	22.7
NNSS	0.7
NNSs	4.6
NNss	11.6

Anti-M antibody may cause discrepancy in standard blood grouping because of its nature and the optimum temperature of reaction (4°C to room temperature). IgM anti-M alloantibody, just like anti-A and anti-B isoagglutinins, are complete antibody. In blood grouping by microcolumn techniques, it can react with A1, B reference cells positive with the M antigen which may lead to unexpected positive reactions with the test serum (Fig. 1). In O group patients, the presence of anti-M usually does not cause problems in determining the blood group, because positive reactions with reference blood cells, both A1 and B, are in general expected, so both specificity of isoagglutinins only mask each other. Using tube technique, unexpected positive reactions can occur with group O, A1 and B reference cells positive with M antigen from the MNS system [1, 3, 4, 7].

Material and methods of ABO blood grouping

The primary technique used for ABO blood grouping and immune alloantibody detection is

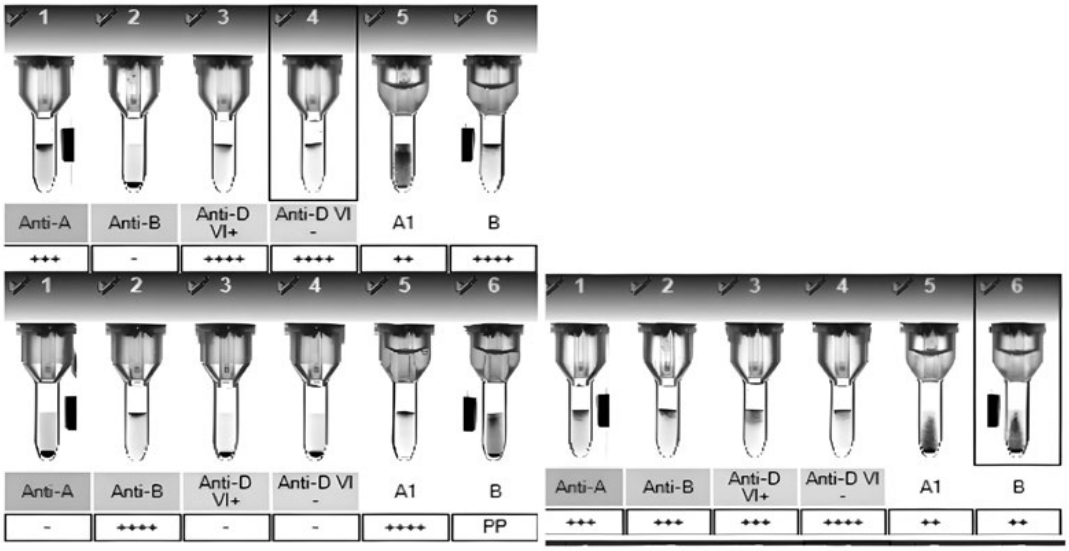

Figure 1. Examples of blood grouping discrepancies in patients caused by anti-M alloantibody

the automated microcolumn technique using BIO-RAD's IH-500 analyzer and dedicated standard cells. Apart from this automated microcolumn technique, other methods and techniques such as manual microcolumn technique from BIO-RAD and the test tube technique have also been used for blood grouping, alloantibody detection and identification (as described in the available legislation [8]).

In this paper we analyse test results from the first half of 2022 related to detection and identification of anti-M alloantibody as well as to blood grouping difficulties caused by its presence. A total of 1 800 anti-M examination and blood grouping tests were performed. These tests were divided into two groups based on age: 4 months–18 years (73 patients) and over 18 years of age (1727 patients). Samples were collected in outpatient collection centers and hospital wards. In 137 cases anti-M antibodyalloantibody was detected and identified. Red blood cells of all patients with anti-M alloantibody were determined as M negative. In all patients with blood group O determined by microcolumn techniques, there were no problems with interpretation of blood grouping reactions, despite the presence of anti-M alloantibody in their serum; this group of patients was analysed separately.

How we solved problems related to blood grouping discrepancies

The following options are available for unexpected positive reactions of serum A1 and B standard blood cells in the microcolumn technique:

— **Test tube technique:** blood grouping in tubes at 37°C; at this temperature IgM class anti-M antibody is non reactive, so normal reactions with reference blood cells were observed;

— **2-ME (2-Mercaptoethanol) solution:** differentiation between IgG and IgM antibody based on decomposition of IgM antibody [9]. 2-ME solution was added to serum at 1:1 ratio and incubated at 37°C for 2 hours. This is a very effective method for IgM anti-M antibody, but it must be kept in mind that the 2-ME solution decomposes serum anti-A and anti-B isoagglutinins. Blood grouping following the use of 2-ME, was performed with the tube technique at room temperature;

— **Blood grouping with tube technique and M antigen negative O, A1, B standard cells produced by RCKiK in Katowice.** The use of these standard cells allowed to obtain normal reactions of the test serum with O, A1, B cells (according to Landsteiner's rule).

Results

The study group aged 4 months–18 years included 73 patients; blood grouping as well as antibody detection and identification was performed in all of them. Anti-M alloantibody was identified in 25 patients. In 13/25 cases, blood grouping problems occurred when the automated method was applied. In 12 patients, ABO blood group was correctly determined and interpreted with no additional procedures; in 5 cases the O blood type was determined (Fig. 2).

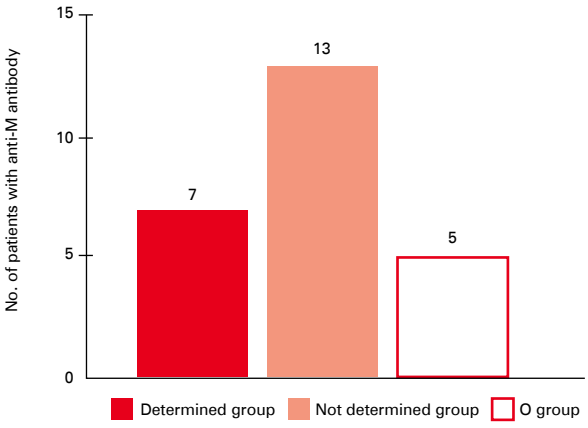

Figure 2. Blood grouping (4 months to 18 years)

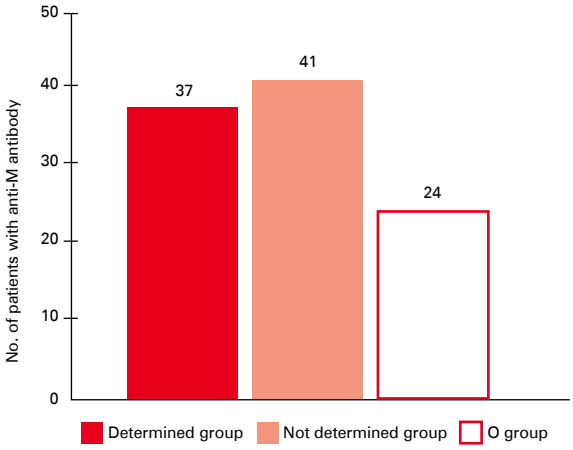

Figure 4. Blood grouping (> 18 years)

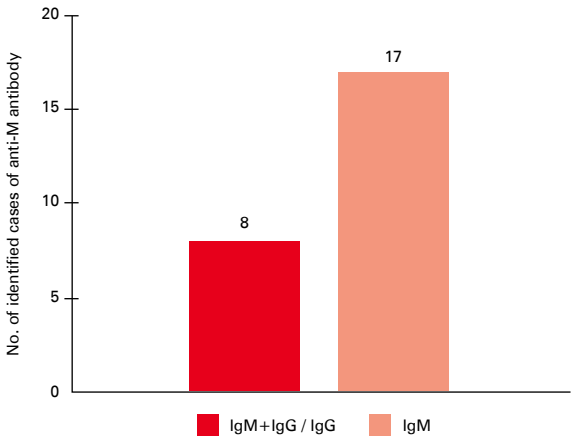

Figure 3. Anti-M antibody class detection (in 4 month to 18 year old patients)

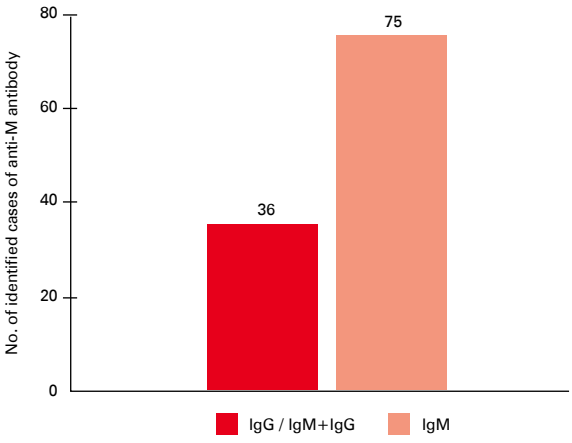

Figure 5. Anti-M antibody classes in the study group > 18 years of age

In 17 of the 25 anti-M antibody cases, the reaction in the indirect antiglobulin test (IAT) was negative in sera treated with 2-ME (2-mercaptoethanol) reagent or in IAT with anti-IgG monoclonal reagent, which indicated IgM class of detected anti-M. The remaining 8 cases were IgG or IgG + IgM (Fig. 3).

The age group > 18 included 1727 patients and all were subject to antibody detection and/or identification. Anti-M antibody was identified in 111 cases; in 102 of them blood grouping was performedantibody. In 41 cases there occured problems with blood grouping using automated microcolumn technique. In 61 cases, blood grouping was correctly determined and interpreted with no additional procedures required; in 24 cases O blood group was determined (Fig. 4).

75 of the 111 detected anti-M alloantibody belonged to the IgM class; 9 were negative in the IAT (reactive only in saline tube test at room temperature); 6 were negative in IAT with anti-IgG monoclonal reagent; 60 cases were negative in IAT with the 2-ME reagent. The remaining 36 results were of the IgG or IgG + IgM class (Fig. 5).

Discussion

Anti-M antibody is a are fairly common, natural cold-type antibody. It is usually of IgM class, but may also occur as IgG or IgM class antibody with an IgG component. The clinical significance of allo

anti-M depends on the temperature at which it reacts, its class and origin. Patients with identified allo anti-M require blood transfusion with M antigen negative compatible RBCs. Naturally occurring anti-M antibody is more common in children than in adults [10].

Anti-M antibody is clinically significant because it may cause hemolytic reactions in patients transfused with M antigen positive blood. It may also increase the risk of hemolytic disease of the fetus and newborn. Placental transfer of maternal IgG antibody to the fetus and thus the destruction of M-positive erythroid precursor cells may cause fetal anemia and reticulocytopenia. This leads to inhibition of erythropoiesis. The mechanism of HDFN induction by anti-M alloantibody resembles that of anti-K alloantibody from the Kell system [3, 10].

In the period under analysis (January 2022–June 2022), allo-anti-M was detected and identified in 137 patients; this accounts for 7.6% of the 1 800 assays performed. Macroo et al. report the frequency of anti-M antibody detection at a level of 8.22%,[11] while Shah et al. estimate the level of detected allo anti-M in the study patients at 13.98% [12] In Dutch and Japanese patients allo anti-M detection is estimated at 10.3% and 7.2% respectively [6].

IgM anti-M alloantibody may cause discrepancies in the standard pattern of reactions during ABO blood grouping [6]. In the study samples of children and adults this alloantibody was one of the likely reasons of discrepancy in ABO blood grouping. In the first study group (4 months–18 years), anti-M antibody accounted for 34.25% of the total numer of detected antibody; 68% were of the IgM class. Discrepancies in blood grouping were recorded in 52% of children identified with anti-M alloantibody; in 28% of children the blood group was determined with no additional procedures required; in 20% the O blood group was determined.

In the study group > 18 years of age, allo-anti-M was detected in 6.5% of cases; 67.6% were identified as IgM class. Within the group of adult patients with anti-M antibody, problems with ABO blood grouping with automated microcolumn technique were reported in 40.19% of cases, in 36.27% blood grouping was performed with no additional procedures and in 23.52% the O blood group was determined.

Discrepancies in blood grouping were solved by introducing additional procedures such as: blood grouping with tube technique at 37°C, use of M antigen negative O, A1, B standard cells, treatment of test serum with 2-ME reagent. In all samples the reaction of standard cells with serum/plasma was normal.

A similar course of action was described by Tondon et al. They described the case of a 15-year-old patient with anti-M alloantibody, who presented unexpected positive serum reactions to O, A1, B reference cells. To obtain a correct pattern of reaction, the test was repeated at 37°C and then the blood group was confirmed with M antigen negative A1, B reference cells [13]. Ferdowsi et al. also described cases of ABO blood grouping complicated by the presence of anti-M antibody. M antigen negative A1, B blood cells were used to obtain correct reactions with reference cells [14].

Another solution for such discrepancies was suggested by Mathur et al. who described the case of a 32-year-old blood donor with positive serum reactions to O, A1, B cells and to a panel of red blood cells for alloantibody detection. The alloantibody was determined as IgM + IgG anti-M alloantibody. Blood grouping was repeated with microcolumn technique and A1, B papainated standard cells. Correct blood grouping followed. The M antigen is sensitive to papain which degrades sialoglycoproteins at the site of MNS antigen expression and so anti-M antibody fails to react with papain premodified cell panel [15]. Khalid et al. also described a case of a 58-year-old patient with anti-M alloantibody that interfered with ABO blood grouping. When mixed with the patient's serum the A1and B reagent red blood cells agglutinated in the microcolumn technique. Normal reactions were obtained by adsorbing the serum with MM phenotype red blood cells at room temperature. Following anti-M alloantibody adsorbtion, the patient's blood type was correctly determined [16].

Conclusions

Anti-M antibody is more of a problem for blood grouping in patients below 18 years than in older patients (blood type O is an exception). IgM anti-M antibody is about twice as frequent as IgG/IgG+IgM, regardless of the age of the study group. It is responsible for unexpected positive serum reactions with reference blood cells and is a source of problems related to interpretation and delay of blood group results.

Conflict of interest: none declared

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