Original Research Paper Immunohaematology

Anti-M: Report of a case review of literature.

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ABSTRACT Anti-M is a relatively common naturally occurring antibody reacting optimally at 4°C and weakly or nonreactive at 37°C. It is usually clinically insignificant but can be active at 37°C because of thermal amplitude of IgM component or presence of IgG component. It can cause or delayed hemolytic transfusion reactions or hemolytic disease of newborn. At our center we have received a case of anti-M antibodies presenting as crossmatch incompatibility.

KEYWORDS : Anti-M, immunizing, naturally occurring, antenatal.

Introduction:
The presence of irregular antibodies in healthy voluntary blood donors is uncommon but the prevalence of red blood cell (RBC) alloantibodies among general, hospital-based patients is around 1% .[1] Most anti-M are not active at 37^{0}C and generally are ignored in transfusion practise[1]. Sometimes, however, they can be clinically significant and become a problem in the immunohaematology laboratory.

Case Study:
A 75 year old male, was admitted to hospital with complaint of chest pain and unstable angina. On coronary angiography, it was found to be Triple Vessel Disease and Coronary angiography, it was found to be Triple Vessel Disease and Coronary Artery Bypass was planned. Demand for 4 units of Packed Red Blood Cells was received in the blood bank. The blood group of patient was a Rh 'D' Positive. 10 units were cross matched with column agglutination technology and found to be incompatible. Direct agglutination test (DAT) was found negative with negative auto control. Indirect agglutination test (IAT) was positive (4+) by column agglutination technology (ID micro typing System). Antibody screening was done using Low Ionic Strength Solution (LISS) indirect Agglutination Test screening with 3 cell panel showed reaction in AHG phase and at room temperature. Antibody identification was done using II cell panels (Bio-Rad, ID Micro Typing system) which identified anti-M with dosage effect. An extended phenotype of patient showed antigen M negative.

Determination of blood group was done by gel technique (Ortho biovue system) reverse grouping for patients, (Bio-Rad, ID Micro typing system) Forward grouping showed the blood group as A positive while reverse grouping showed it as O positive. For further confirmation we repeated the gel technique with a new sample and fresh cards, but the results were identical.

First, we used A1 lectin to rule out the possibility of a subgroup of A. To rule out the possibility of cold antibodies, the reverse grouping was repeated using the tube method at 4^{0}C, room temperature (RT), and after incubation at 37 ^{0} C. The reactions in all of the thermal ranges with A1 cells showed +3 to +4 strength, indicating the possibility of a clinically significant antibody. Next, we performed an antibody screen (Ortho biovue system) along with autocontrol. The reaction was strongly positive (+4) in all 3 test cells. The autocontrol was negative, ruling out the possibility of an autoantibody. We then ran an 11-cell ID panel (Bio-Vue). After crossmatching out, the antibodies that could not be ruled out were anti-M, anti-E, anti-K,anti-Jk^b, and anti-Lu –^{a}antibodies. The pattern and strength of the reaction was indicative of anti-M antibody but we ran a papainized 11-cell ID panel to further rule out other uncrossed antibodies (Bio-Vue). There were negative reactions in all 11 papainized cells.

To confirm the presence of antibodies, the following criteria were used:
1. The 3-cell rule (3 cells positive with M antigen showing a + 4 reaction and 3 cells negative with M antigen showing a negative reaction)
2. Patient is negative for M antigen
3. The enzyme treatment panel completely abolished the reactions.
4. Cell number 2,6 and 8 show +2 to +3 reactions due to a dose effect.

Using these criteria, we confirmed the presence of anti-M antibody.

Discussion:
Anti M is a naturally occurring saline agglutinin that was first identified by Wolff and Johnson in 1933. [1.] It is the most commonly encountered antibody of the MNS system. Most example of anti M are reactive temperature below 37 ^{0}C, with an optimum temperature of 4°C, and are considered to be clinically insignificant. However, rarely, the antibody agglutinates red cells at 37 ^{0}C or at the antiglobulin phase of testing and can lead to hemolytic transfusion reactions and hemolytic disease of the new born (HDN) [2]

There are approximately 30 recognized blood group system, some of the which are clinically very important.[3] Of these, the ABO and Rh system are well understood. The MNS system (International Society of Blood Transfusion number 002) is a highly complex system that has 46 antigens [4.] The common antigens in this system are M,N,S, and s.[1] The prevalence of naturally occurring anti-M antibody detectable with saline-suspended cells at room temperature in blood donors who donate routinely is 1:2500 with M+N - cells and 1:5000 with M+N + cells [5]. Antibodies to M are generally not considered clinically significant because most react at low temperatures. Although it is not an uncommon antibody, studies about anti-M antibodies are few. The largest series reported was from Severance Hospital, South Korea, were 20 patients with naturally occurring anti-M antibodies were examined over 7 years. [6]

Similar to Razzaki et al [2] the nature of the antibody, whether IgM, IgG, or IGM with an IgG component, could not be determined in our case but because the reaction was seen in a wide thermal range the antibody may be an IgM with an IgG component. In the literature, 50-80% of these antibodies are considered IgG or with IgG component.

Anti-M antibody is an uncommon cause of hemolytic disease of the newborn. It is recommended that, if the maternal blood has anti-M antibody, which is IgG in nature and optimally reactive at 37 ^{0} C, then the father's blood must be checked for the presence of the M antigen.[7] If the father's blood has the M antigen, the fetus may be

risk. There are not enough studies and guidelines in the literature outlining what titers should be considered clinically significant. After delivery, the infant's MN antigen status should be determined.[7]

Similar to Razzaki et al [2] we also noted the clinically significant difference in reaction strength with a double dose of M as compared to a single dose. All cells with a double dose showed a+4 reaction, whereas single-dose cells (cell numbers 2,6 and 8), showed +2 to +3 reactions. Column Agglutination technology plays an important role in diagnosis of IgG antibody with a better Turn over time (TAT) also have a better interpretation and objectivity.

Despite the introduction of methods to avoid interference with the results of pretransfusion testing, cold alloantibodies still yield unexpected reactions in ABO/Rh typing and create a nuisance for blood group serologists. Majority of these antibodies including anti M antibodies are IgM antibodies that are not active at 37 ^{0}C and the discrepancy can be resolved by performing the testing at strict warm temperature.[8]

Most of the anti –M antibodies are non-complement activating IgM antibodies, but occasionally IgG component may co-exist and cause complement activation. Freedman et al, in 1980 described the complement activating nature of anti-M by testing with 125 1-labeled anti C3d.[9] Combs et al, in 1991 described an automatic - causing hemolysis in vitro by activating complement in conventional tube testing at low ionic strength.[1] At least 15 cases of patients with autoanti-M have been reported and reviewed by Sacher et al. [6] Variable degree of Hemolytic disease of new born ranging from exchange transfusion to intrauterine death hence been reported in association with anti-M antibodies[11] Detection of anti-M antibodies during antenatal screening in the second commonest non –Rh antibody after anti-Kell [12].

Clinically insignificant anti-M that reacts strongly at room temperature and not at all at 37 ^{0}C could be misinterpreted as 37 ^{0}C reactive if the reactants in the test tube are permitted to cool after centrifugation prior to evaluating hemmagglutination reactions. Furthermore, certain anti-M antibodies with high titer and high affinity such as anti-M may react strongly at room temperature and cause hemmagglutination to carry through 37 ^{0}C and the antiglobulin test phase. Therefore to rule out the possible reactivity observed at the AHG phase is due to the presence of IgG and not binding of IgM from the room temperature test phase, testing should be done in strict warm conditions.[9]

MN antibodies are often pH dependent, IgM anti-M has an optimum pH of 6.5 and are mostly inactive at pH 7.5, and below pH 6.5, they become non-specific. Another feature of this antibody is its failure to react with ficin or papain premodified cells. Proteolytic enzymes, such as ficin or papain, cleave membrane sialoglycoproteins at well defined sites. Reactivity of anti-M is abolished by commonly used enzyme techniques[13] Rarely anti-M has been implicated in immediate and delayed hemolytic transfusion reactions which are supported by the results of 51 Cr survival tests and monocyte phagocytosis assays.[14]These examples demonstrate that anti-M can at times be of clinical importance and interpretation of test results should be done with caution.

Conclusion:

Anti M is considered a naturally occurring antibody that is usually active at temperature below 37 ^{0}C and is thus of no clinical significance. This antibody, if present in an individual, can lead to a discrepancy between forward and reverse ABO grouping and thus creates diagnostic difficulties for blood bank staff.

Based on the rare significance of these antibodies, it is extremely important to carefully interpret the results of blood grouping and pretransfusion testing in laboratories and it is wise not to release any discrepant results without resolution and confirmation.

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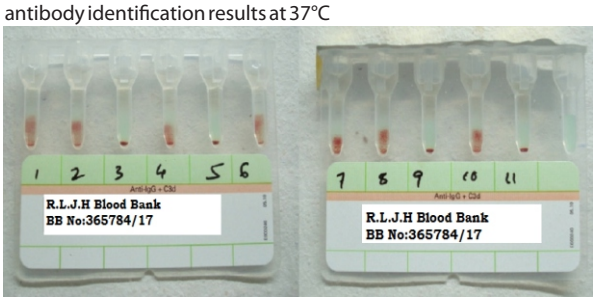
Figure 1: LISS Coomb's gel card showing three-cell panel antibody screening results at 37°C

Figure 2: LISS Coomb's gel card showing one to 11-cell panel antibody identification results at 37°C



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