Original Article

Clinical significance of antibody specificities to M, N and Lewis blood group system

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Abstract:

Context: The clinically significant antibodies are those active at 37°C and/or by the indirect antiglobulin test. Most of the published literature refers to antibodies of Lewis blood group system to be insignificant, whereas antibodies to M and N blood groups are associated with variable clinical significance. **Aims:** The aim of this study is to find the frequency and clinical significance of antibodies to M, N and Lewis blood group systems. **Settings and Design:** The study was carried out retrospectively from January 2009 to December 2012. **Materials and Methods:** Antibody screening was performed by solid phase red cell adherence (SPRCA) technique using four cell screening panel on a fully automated platform GALILEO (Immucor Inc. USA). In case of a positive antibody screen, antibody identification was performed using SPRCA (GALILEO, Immucor Inc. USA). **Results:** A total of 49,077 red cell antibody screens were performed and a total of 427 identifications of red cell antibodies were carried out. A total of 304 specific antibodies were detected: 8.22% of antibodies were of anti-M specificity and 2.96% were of anti-N specificity. Majority (84%) of anti-M and 77.78% of anti-N were of Immunoglobulin G (IgG) class reacting at 37°C. 1.31% of the antibodies were directed against Lewis system antigens of which 0.65% were anti-Lea and 0.65% were anti-Leb. Half of the Lewis system antibodies, i.e., 1 each of anti-Lea and anti-Leb were of IgG class. **Conclusion:** Our study highlights the importance of detecting the thermal amplitude of antibodies with variable clinical significance especially if both IgG and IgM types of antibodies are associated with it so as to establish their clinical significance.

Key words:

Anti-M, anti-N, clinical significance, Lewis

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Introduction

Transfusion specialists have varied definitions of a "clinically significant antibody." The clinical significance of antibodies to red cell antigens is usually judged by their capacity to shorten red cell survival by causing hemolytic transfusion reactions (HTR) or through their association with hemolytic disease of the newborn (HDN).

Several approaches such as, specificity and thermal amplitude of the antibody, 1 hr survival of ^{51}Cr-labeled incompatible red blood cells (RBCs) and functional cellular assays including monocyte monolayer assay are considered as valuable in predicting the clinical significance.[1]

In general, the clinically significant antibodies are those reactive at 37°C *in vitro* and/or those reactive in the indirect antiglobulin test (IAT) phase and are usually Immunoglobulin G (IgG) in nature. Since cellular assays and labeling studies are usually unavailable in routine laboratories, it is the historical data on the association of an antibody with HTRs and HDN, which is used to predict their clinical significance.[1]

Most of the authors refer to antibodies of Lewis blood group system to be naturally occurring, most frequently belonging to IgM class fraction and reacting at temperatures below 37°C. They are not considered to be clinically significant. Red cells compatible at 37°C regardless of the Lewis phenotype, are expected to have normal *in vivo* survival and hence, it is not considered as necessary to transfuse antigen-negative RBCs for patients with antibodies against Lewis antigens.[2]

On the other hand, antibodies to M and N blood group antigens, are associated with variable clinical significance as both IgG and IgM type of antibodies are frequently encountered. As many as 50-80% of anti-M are IgG or have an IgG component.[3] Though very occasionally, both anti-M and anti-N have been implicated as the cause of HTRs and anti-M has very rarely been implicated in severe HDN.[2]

The aim of this study was to find out the frequency of antibodies to M, N and Lewis blood group systems and to determine their clinical significance by observing their thermal amplitudes and classifying them as IgG or IgM type.

Materials and Methods

The study was conducted at the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi. We retrospectively analyzed the results of 49,077 antibody screening tests over a 4 year period from January 2009 to December 2012. Antibody screening was performed on a fully automated immunohematology analyzer (GALILEO: Immucor Inc. Norcross GA) using a four cell panel (capture R ready screen) with solid phase red cell adherence (capture) technology. The screening cell panels covered most of the clinically significant antigens with homozygous expression of the most important ones. In case of a positive antibody screen, further testing was performed to precisely characterize the irregular antibody (ies) and to determine their specificities in case of alloantibodies. Antibody identification was performed using different cell panels from Immucor Inc. by capture technique. Advanced investigations such as adsorption, elution etc. were performed whenever required. Obstetric history in case of females and other relevant clinical and transfusion records were reviewed for each case.

All anti-M and anti-N antibodies identified were confirmed by testing the serum against a panel of enzyme treated cells.

Thermal amplitude of the antibodies was determined by testing at three different temperatures: 4°C, room temperature (22 ± 2°C) and 37°C.

All data was tabulated and relevant parameters were statistically analyzed using the Pearson's 2 tailed test. $P < 0.05$ was considered to be statistically significant. The results were compared with existing literature.

Results

In the observed time interval, a total of 49,077 red cell antibody screens were performed. This included 29,917 (60.96%) males and 19,160 (30.04%) females. Antibody identification was carried out in 427 cases. A total of 304 specific antibodies were detected: 25 antibodies were of anti-M specificity, which amounted to 8.22% of the detected antibodies whereas, 9 i.e. 2.96% antibodies were of anti-N specificity. Majority of anti-M antibodies (21, 84%) were of IgG class reacting at 37°C and only 4 (16%) were cold IgM type of anti-M with their thermal amplitudes ranging between 4°C and 22°C. Amongst the antibodies of anti-N specificity, IgG class was detected in 7 (77.78%) cases, whereas IgM type was found in 2 (22.22%) cases. Of the total antibodies detected, 4 (1.31%) were directed against Lewis system antigens of which 2 (0.65%) were anti-Le^a and 2 (0.65%) were anti-Le^b. Half of the Lewis system antibodies i.e. one each of anti-Le^a and anti-Le^b were of IgG class and the other 50% were of IgM type [Table 1].

Table 1: Frequency and distribution of MN and Lewis system antibodies

Antibody	Total (%)	IgG (%)	IgM (%)
Anti-M	25 (8.22)	21 (84)	4 (16)
Anti-N	9 (2.96)	7 (77.7)	2 (22.22)
Anti-Le^a	2 (0.65)	1 (50)	1 (50)
Anti-Le^b	2 (0.65)	1 (50)	1 (50)

IgG: Immunoglobulin G; IgM: Immunoglobulin M

History of one or more episodes of blood transfusion was elicited in 9 out of 25 patients with anti-M antibody and 6 patients gave significant obstetric history as well. However, the relation did not reach statistical significance. Similarly, no significant correlation was observed between history of transfusion or pregnancy and the presence of anti-N antibody. The number of patients with Lewis antibodies was very small to determine any statistical correlations.

Discussion

Though anti-M is a frequently encountered antibody of the MNSs blood group system, anti-N is relatively rare. They are not considered to be clinically significant and are very occasionally associated with HTR or HDN.[2] In our study, 8.22% of detected antibodies were of anti-M specificity and 2.96% were anti-N. Various authors report the prevalence of anti-M to be ranging from 3.6% to 13.8%,[4-7] whereas frequency of anti-N is reported to be in the range of 0.87-1.47%.[6,8] Our study reported majority (84%) of anti-M and 77.78% of anti-N to be of IgG class or possessing an IgG component reacting at 37°C and hence, potentially clinically significant. In a study by Mladenovic,[6] majority of their anti-M antibodies were of the warm IgG type, whereas, amongst the anti-N it is the IgM class that predominated. Other similar cases of clinically significant anti-M and anti-N have been reported in the literature.[9-18] Most of the authors confer that whenever M or N antibodies active at 37°C are encountered, antigen-negative or red cells compatible by an IAT should be provided.[19] In the specifications[20] outlining Red Cell Imunohematology (RCI) clinical policy for the supply of blood for transfusion to National Health Service-Blood and Transplant (NHSBT) they recommend that for anti-M reacting at 37°C, M antigen negative blood be provided, whereas for anti-N of similar nature, provision of red cells compatible by IAT at 37°C suffices.

Most Lewis antibodies are naturally occurring IgM,[2] though, some may have an IgG component.[21-23] Rarely, they may be of pure IgG isotype.[24] Usually, purported to be naturally occurring, some Lewis antibodies may be stimulated by RBC transfusions.[25]

In our study, the prevalence of Lewis antibodies was 1.31% of which 2 (0.65%) were anti-Le^a and 2 (0.65%) were anti-Le^b. Others have reported the prevalence of anti-Le^a to be in the range of 3.68-24.6%[6,7,26,27] and that of anti-Le^b to be between 1.21 and 14.3%,[6,7,27,28] which is relatively high as compared to our data.

Half of the Lewis system antibodies detected by us i.e. one each of anti-Le^a and anti-Le^b were of IgG isotype making them potentially clinically significant. Mladenovic,[6] on the other hand have reported majority of anti-Le^a (71 of 76 detected) and very few anti-Le^b (7 of 25 detected) to be of IgG type.

Lewis antibodies are rarely implicated in HTRs as most Lewis antibodies are not purported to be active at 37°C, transfused RBCs lose their Lewis antigens into the recipient's plasma and there is neutralization of Lewis antibodies in the recipient by Lewis substance in donor plasma.[3] Amongst the Lewis antibodies, anti-Le^a is more frequently associated with acute HTRs[29-32] than is anti-Le^b.[33,34] Cases of delayed HTRs have also been reported.[35,36]

Lewis antibodies are rarely implicated in HDN. It is attributed more to poor expression of Lewis antigens on fetal cells rather than the frequently cited high incidence of IgM type of Lewis antibodies.[22] However, both anti-Le^a and anti-Le^b have been implicated in cases of mild HDN.[37,38]

Transfusion services vary in their selection of RBC units for patients with Lewis antibodies. If clinically significant antibodies are detected, some prefer transfusion of Lewis antigen negative blood whereas, most consider transfusion of blood compatible by IAT at 37°C to be safe.[19,20,39]

Antibodies against MN and Lewis blood group antigens with their thermal amplitudes in the range of 22-30°C gain special importance in certain conditions of induced hypothermia. These antibodies with a higher thermal range, which would otherwise be termed clinically insignificant, will induce *in vivo* hemolysis in patients with lowered core body temperature, which is now a common practice in various surgeries such as neuro surgeries, cardiac surgeries etc.[40] Therefore, the thermal amplitude of the antibody must always be determined and if judged to be clinically significant, corresponding antigen negative blood must be provided.

Conclusion

Our study highlights the importance of detecting the thermal amplitude of antibodies with variable clinical significance. Since a large majority of anti-M and anti-N antibodies and 50% of both Lewis antibodies are of warm reacting IgG type (clinically significant) it is imperative to provide corresponding antigen negative blood whenever these antibodies are identified, whereas for antibodies with lower thermal amplitude, patients can safely be transfused with Anti Human Globulin (AHG) compatible blood under warm conditions.

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