Review Article

CURRENT UNDERSTANDING OF ANTI-HUMAN GLOBULIN REAGENTS – A REVIEW

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ABSTRACT

Anti – human globulin (AHG) reagents are commonly used in compatibility tests prior to transfusion. Cold agglutinins or irregular antibodies usually affect the accuracy in functioning of anti – human globulin reagents as they are associated with complement system. Though various applications and types of anti – human globulin reagents have been known, still there is need for clarification of influencing factors, characteristics and adequate selection prior to transfusion. In this review, basic study methods and practices in clinical settings are discussed including anti – human globulin reagents, advantages and disadvantages of various reagents in presence of complement – fixing antibodies and cold agglutinins, exploration of adequate mechanisms through which anti – human globulin reagents detection is influenced by complement system, and describe the adequate and suitable selection of anti – human globulin for the detection of antibodies which are significant clinically.

Keywords: Antibodies, complement system proteins, blood transfusion

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INTRODUCTION

Antiglobulin test (Coombs test) is of high value in diagnostics of transfusion medicine. Antiglobulin test is used for the detection of sensitized and free blood group antibodies, as well as for the investigation of etiologies of hemolytic reactions of transfusion and hemolytic disease of fetus and newborn (HDFN). [(1)] Red cell antibodies in the serum of mother with hemolytic disease of fetus and newborn were first detected with the help of antiglobulin test by Coombs, Race and Mourant. [(2)] Indirect antiglobulin test (IAT) is the test that detects antibodies in maternal serum. Indirect antiglobulin test is basically used as compatibility test prior to transfusion which include identification and screening of antibodies, phenotyping of red blood cells and cross-matching. [(3-4)]

Compatibility test as defined by Standard Terminology for Blood Transfusion Medicine (WS/T 203-2001), is the procedure for the detection of antibodies against antigens of blood group of recipient/donor in probable donor/recipient. It can also be defined as detection of presence of irregular antibodies (IgG) in blood of recipient that will cross-react with the red blood cells or screening cells of donor. Generally, these antibodies are seen in individuals with a history of blood product transfusion or pregnancy. [(5)] Objective of compatibility test prior to transfusion is to prevent antibodies production and to assure effective and safe transfusion of blood by choosing the compatible blood component with blood type of recipient so that functional activity of blood product should be maintained. [(6-7)]

The complement is essential component of the blood that interferes with antibody detection in compatibility tests prior to transfusion. In serum of healthy individuals, the complement system content is somewhat stable and is not relevant to immunosimulatory effects of external antigens. In vivo, the complement system can perform essential role in lysis of cells. In some diseases, variations in activity and content of complement system can occur, and their detection is valuable for immune status assessment and treatment. [(8)] Though, in compatibility testing prior to transfusion, detection of complement is not included except that content of complement and type of sensitization have to be detected in complement – mediated hereditary disease, diseases related to autoimmunity and disease associated with marked decreased in levels of complement. [(8)] Therefore, optimal selection of anti – human globulin reagents is necessary while procedure of compatibility testing prior to transfusion so that interference by complement can be avoided.

In this review, characteristics and type of anti – human globulin reagents, discussion regarding complement system influences and its mechanism in detecting irregular antibodies by anti – human globulin reagents are summarized. Adequate

selection of anti – human globulin reagents as per need of compatibility test is also described.

ANTI – HUMAN GLOBULIN (AHG) REAGENTS

Two types of anti – human globlin reagents are available, namely; mono-specific reagents and poly-specific reagents. Mono-specific reagents consist only of single component e.g. anti – C3d antibody or anti – IgG antibody; while poly-specific reagents include anti – complement component and anti – immunoglobulin G (anti – IgG) component. [9] From all of them, IgG antibodies are of most value in compatibility testing before transfusion; though poly-specific anti – human globlin reagents application showed various problems. [1, 10-11] Anti – human globlin reagents for red blood cell antibodies detection are described by American Association of Blood Banks (AABB) as: Though poly-specific anti – human globlin reagents have advantage to detect some Kidd antibodies, the anti – IgG reagents can prevent unanticipated reactions which are caused by in vitro binding of complement to cold – reactive antibodies. [3] Studies have demonstrated that when poly-specific anti – human globlin reagents are utilized in indirect antiglobulin test for compatibility testing prior to transfusion, components of anti – complement can easily cause unimportant positive reactions e.g. false agglutination, and significant antibodies detected by complement binding are uncommon. [10-14] This postulates that investigators should take care of positive and negative aspects of using anti – human globlin reagents with components of anti – complement.

EFFECT AND MECHANISM OF IRREGULAR ANTIBODIES IN USE OF ANTI-HUMAN GLOBULIN REAGENTS

Irregular antibodies are clinically significant antibodies that react at $37^{O}C$, causing hemolytic transfusion reactions and hemolytic disease of fetus and newborn. They can also decrease the transfused red blood cells survival. [5,15] The irregular antibodies that are nonreactive at $37^{O}C$ are referred as clinical insignificant antibodies. Majority of the cold agglutinins are not clinically significant. Although, at $22^{O}C$ or even at $37^{O}C$ in some disease, few high – titer cold agglutinins show weak reactivity. These agglutinins agglutinate with red blood cells, followed by binding to complement which result in hemolysis, which in blood transfusion, cannot be ignored as interference factor. [16] So, there should be minimal interference cold agglutinins in serological testing of blood transfusion. It is recommended to detect antibodies which are clinically significant and avoid clinically insignificant antibody detection. [3] In a study from China it was observed that 14,000 out 6 million subjects consisted unexpected/irregular antibodies. [17] Majority of those antibodies were significant clinically which can cause hemolytic diseases of newborn and hemolytic transfusion reactions; whereas few of them were insignificant IgM antibodies that were nonreactive at $37^{O}C$, including anti – I, anti – P and anti – H antibodies. [15] Antibodies that are IgM in nature, are the type of agglutinins effective at complement activation due to structure of IgM pentamer as has sufficient sites for binding of both complement C1q and cell surface antigen, although only two IgG antibodies individually can attach to both C1q and antigen for complement activation. [11] Majority of the cold agglutinins that are IgM in nature, agglutinate red blood cells and cause sensitization of complement at low temperature. [9] By repeated washing or at high temperature, these IgM agglutinins detach from red blood cells, although the complement still binds to red blood cells. Therefore, in serological testing, the utilization of polyspecific anti – human globulin reagents will cause positive reaction though binding of anti – Cd3 component to complement on red blood cells but cannot cause destruction and red blood cells and hemolysis. In case where monospecific anti – IgG anti – human globulin reagents are utilized, there will be avoidance of false positive results. [4,9] Shulman et al demonstrated that 70% United States Laboratories applied monospecific anti – IgG reagents which were increased from previous use. [18]

Some irregular antibodies such as complement – fixing antibodies, can be detected only with availability of activated complement and polyspecific anti – human globulin reagents. This includes antibodies of Kidd system, anti – Jk^a and anti – Jk^b, some anti Fy^b antibodies of Duffy system and IgM – type anti – Le^a antibodies of Lewis system. [5, 19] Although, most of the time, these antibodies are clinically insignificant.

Antibodies of Kidd blood group system are uncommon alloantibodies, mostly IgG, followed by mixed IgG and IgM and rarely IgM, and include anti – Jk^a and anti – Jk^b antibodies. They are observed in mixtures of antibodies, therefore having low immunogenicity. [19] They are often not easy to detect. Elusive antibodies with the use of protease – treated red blood cells are used for the detection of weak antibodies; they cause direct agglutination of antigen – positive red blood cells having weak intensity of agglutination. [20] Due to rapid decreasing activity to a level not detectable in plasma, the missed Kidd antibodies can cause delayed hemolytic transfusion reactions, [19] indicating the significance of weak Kidd antibodies detection. In study for detection methods of Kidd system antibodies demonstrated that Coombs test is significant in rate of detection, dose effect and sensitivity in contrast to other methods. [21] In this view, antibodies of Kidd system are detected by Coombs test. From the antibodies of Kidd system, the antibodies having IgM (not IgG purely) are able to bind complement, comprising 40-50% of antibodies of Kidd system. [19] Monospecific IgG reagent detects IgG antibodies of Kidd system, while polyspecific anti – complement reagents or IgG anti – human globulin reagents can detect IgM class. Rate of detection for IgM antibodies can be affected by complement activity. Due to decreased activity of complement in preserved samples of serum, only IgM antibodies are detected in fresh serum containing complement. [19] In one study, mixing

of K_2 EDTA 0.1 mol/L to sample of serum led to complement inactivation completely, resulted in very low detection rate of IgM. [5] Additionally, rate of detection for IgM is also affected by complement – binding ability of antibody, which normally need availability of Mg^{2+} and/or Ca^{2+}. Therefore, the major cause of detection failure for such antibodies is the use of anticoagulants chelated with Mg^{2+} and/or Ca^{2+}. Although, antibodies of Kidd system are not commonly detected in complement – dependent manner. Qualitative detection of antibodies related to Kidd system was carried out in one study, which showed binding to complement in only 27.9% cases, and anti – Jk^a antibodies which were IgM in nature could not bind to complement on further testing, indicating that extreme dependance on polyspecific anti – human globulin reagents in old methods and strategies for detection of antibodies related to Kidd system should be reconsidered. [20]

Natural IgM antibodies are commonest type of anti – Le^a antibody. Lewis system antibodies are rare cause of hemolytic transfusion reactions in routine practice, as most of antibodies of Lewis system are not clinically significant and do not react at 37^OC. Additionally, in donor plasma, the soluble red blood cells antigens of Lewis system reduce in quantity via Lewis system red blood cell antibodies neutralization of recipient, and on the surface of transfused red blood cells, Le^a antigens will diffuse into plasma of recipient, which result in reduced antigenicity. [5] In study from European population, it was suggested that antibodies of Lewis system which showed activity while in vitro testing below 37^OC could not unduly devastate their transfused antigen – specific red blood cells in vivo; therefore, patients with antibodies of Lewis system could be transfused blood compatible for crossmatch at 37^OC. [19] Some data showed that titers of Lewis antibody may be higher in population of Southeast Asia, and can bind the complement for induction of in vivo hemolysis. [19] However, antibodies of Lewis system rarely cause hemolytic transfusion reactions. Furthermore, complement can be activated by binding of red blood cells to specific IgM – type Lewis antibodies to form a coating of complement rather than coating of immunoglobulin on red blood cells surface. [5] Therefore, in compatibility testing, the utilization of polyspecific anti – human globulin reagents with components of complement tend to produce agglutination which is clinically not significant and interferes with test results. A microcolumn agglutination card is used in China for crossmatching and screening of antibody. By this method, the shedding problem of antibody from red blood cells can be avoided after procedure of washing and is also helpful for detection of reactive antibodies of Lewis system at 37^OC. Though, antibodies of Lewis system show improved reaction conditions at 4^OC or room temperature, techniques using low temperature incubation can also be implemented as required in procedures of identification and screening of antibody. [4]

In clinical practice, the Lewis system anti – Fy^b antibody is rare, is of IgG type and can be identified by Coombs test easily. Anti – Fy^b which is complement fixing antibody is ever rarer. They are found only in serum of Fy(b-) phenotype patients, and they can occasionally cause mild hemolytic disease of newborn. Anti – Fy^b antibody is mostly found in serum along with other alloantibodies and facilitate weaker immune response in comparison of anti – Fy^a antibodies and cause hemolytic transfusion reactions rarely. [5, 22]

EFFECT OF COMPLEMENT BINDING TO RED BLOOD CELLS IN UTILIZATION OF AHG REAGENTS

Binding of complement to red blood cell membrane takes place both in vitro and in vivo through following mechanisms: first, there is specific binding of antibodies to membrane of red blood cell to create antigen – antibody complexes which further start complement activation for adherence to membrane of red blood cell; second, non – specific immune complexes to red blood cell antigen are available in plasma and initiate non -specific complement components activation for adhesion to membrane of red blood cell. When immune complex is dissociated, only activated complement is yet attached to membrane of red blood cell. The mechanism of attachment of complement to membrane of red blood cell that do not involve specific antigen – antibody reactions is called as complement coating or innocent bystander. [5] In relation to above given mechanisms, continuous complement C1-C9 activation will prompt destruction of red blood cells and hemolysis; although, there will be no hemolysis if activation of complement only advances to C3 or C4, as C3 attached to the membrane of red blood cell is not bioactive C3b, rather it is C3d, which only attaches decisively to C3 and C4 receptors on red blood cells. Such effect is mostly seen in antibodies with low affinities, autoantibodies, cold antibodies, or antibodies that are dependent on drugs, trying to detach from membrane of red blood cell after reacting with red blood cells and leaving only components of complements that are resolutely bound to membrane of red blood cells. [23-26] In such cases, pre-transfusion compatibility test with use of polyspecific anti – human globulin reagents can show positive results for agglutination of red blood cells, although the utilization of monospecific reagents efficiently prevents detection of clinically non-significant positive results. [27-28]

AHG REAGENTS SELECTION ACCORDING TO PURPOSIVE TEST

Detection of reactive antibodies at 37^OC is the main aim of antibody screening, through reaction of serum samples of recipient with reagent red blood cells of recognized blood groups. After the detection of antibodies, next step should include antibody identification test for detection of their specificity. [4] Both plasma and serum samples can be utilized for identification and screening of antibody;

although if target of detection is antibody that needs confirmation by complement activation e.g., antibodies of Kidd system, samples from serum and reagents of polyspecific antigens are advocated. [4] In testing for identification and screening of irregular antibody, few IgMs which are reactive at room temperature such as anti – IH, anti – I, anti – Le^a and anti – Le^b antibodies, are assumed negligible and insignificant antibodies clinically, so there is there is no requirement for provision of respective antigen – negative red blood cells to recipient during transfusion of blood. [15] For avoidance of insignificant positive results by insignificant antibodies, use of monospecific anti – IgG reagents should be considered for identification and screening of irregular antibodies. Additionally, to detect immune antibodies in cold agglutinin disease or cold autoimmune hemolytic anemia, patient plasma and screening cells are incubated at 37^OC for the exclusion of screening cells that are coated with components of complement; mostly C3d, which can be activated by cold IgMs. The monospecific anti – IgG reagent use rather than multispecific anti – human globulin reagents is supportive. [3] For the assurance that screening of antibody does not overlook detection and so cannot exclude interference factors apart from immune antibodies, it is recommended that various types of anti – human globulin reagents can be utilized for detection simultaneously. [14, 27] If monospecific anti – IgG reagents and polyspecific anti – human globulin reagents show negative and positive results respectively, the samples of serum should be incubated for 30 minutes at 56^OC for inactivation of complement prior to identification and screening of antibody. If hemolytic transfusion reaction or agglutination is not observed in serum samples in polybrene, anti – C3d medium or polyspecific anti – human globulin reagents, this can suggest the absence of alloantibodies that are clinically significant in patient serum. [27]

The basic objective of crossmatch is to test if there are specific antigen – antibody reactions between recipient and donor blood. Beside crossmatch test performed in saline medium, a crossmatch test should also be carried out in AHG medium for detection of IgG – type alloantibodies, which have same procedure as identification and screening of antibody. [4] In patients with certain diseases including autoimmune diseases, viral infection, malignant lymphoma, infectious mononucleosis and mycoplasma pneumonia, there is increased cold agglutinin titer. [29-31] These cold agglutinins can cause agglutination of red blood cells and complement adherence, results in hemolysis at low temperature; although when there is rise in ambient temperature, there is detachment of antibodies from red blood cells while complement resides on red blood cells surface, giving false – positive crossmatch reactions. [14, 32-33] Due to presence of coated C3d complement on red blood cells of patient, which give rise to positive results in direct anti – human globulin tests, at 37^OC, red blood cells should be washed with saline for removal of cold agglutinin IgM – type. The use of monospecific anti – IgG reagent can also avoid the complement interference in crossmatch test. The temperature of crossmatch should be controlled strictly at 37^OC for the detection of IgG alloantibodies to remove cold agglutinin interference. When AHG test is used for crossmatching, the serum of recipient and red blood cells of donor should be mixed and incubated for 30 minutes at 37^OC prior to detection. If serum is used instead of plasma, the use of polyspecific anti – human globulin reagents should be encouraged.

CONCLUSION

In pre – transfusion compatibility testing with the indirect antiglobulin test method, the anti – human globulin reagents selection is associated with purpose of blood transfusion test. False – positive reactions caused by cold antibodies which are clinically not significant, can be avoided by utilization of monospecific anti – IgG reagents; although polyspecific reagents can avoid missed identification of antibodies which fix the complement. If monospecific anti – IgG anti – human globulin reagents give negative results in compatibility testing for detection of clinically significant alloantibodies, polyspecific anti – human globulin reagents can detect them rarely. Although, irregular antibodies detection by use of monospecific anti – IgG anti – human globulin reagents can significantly prevent interference of complement sensitization in vitro and so enhance the accuracy.

CONFLICT OF INTEREST

None

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Rab Nawaz Sathio	Drafting and methodology, data interpretation Analysis and interpretation of data for work & Data Collection