Editorial

Significance of antiglobulin test in clinical diagnosis

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Introduction

The antiglobulin test, also known as the Coombs' test, is a useful tool in clinical medicine, particularly in hematology and transfusion sciences. The antiglobulin test demonstrates the presence of immunoglobulins (antibodies), complements, or both attached to red blood cell (RBC) membranes *in vivo* or *in vitro*. Antiglobulin reagent is used to produce a visible agglutination reaction. Antibodies and/or complements that are bound to the surface of RBCs within the patient's body or *in vivo* sensitization can be investigated by the direct antiglobulin test (DAT). Conversely, the indirect antiglobulin test (IAT) is utilized for the detection of immunoglobulin or complement components circulating in the patient's serum. Therefore, the IAT is applicable for antibody detection, antigen typing, and compatibility testing. As a standard antiglobulin test, several methods are employed, such as the conventional tube technique (CTT), column agglutination technique (CAT) or gel test, and solid phase assay. However, the sensitivity of these methods varies. Thus, more sensitive immunoassays and flow cytometry may be applied as alternatives for antiglobulin testing if required.[1,2]

Clinical diagnosis using DAT

The DAT is one of the diagnostic tests in patients with the presence of hemolysis. Several scenarios such as autoimmune hemolytic anemia (AIHA), hemolytic disease of the fetus and newborn (HDFN), and transfusion-related hemolysis might cause a positive DAT. In patients with hemolytic anemia, the predictive value of a positive DAT is 83%, whereas in patients deprived of hemolytic condition, it is only about 1.4%.[3] All red blood cells appear to have small amounts of IgG and complement. In healthy individuals, IgG and C3d molecules per red cell might range from 5 to 90 and 5 to 40, respectively.[4,5] However, these amounts are below the threshold of detection in routine testing. The amount of IgG from 100 to 500 molecules and C3d ranging between 400 and 1,100 molecules per red cell can be detected by DAT. Positive DAT is reported in blood donors at approximately 1:1,000 up to 1:14,000 and found in patients at 1% to 15%.[6] Donors with positive DAT have not only been shown to be involved in systemic lupus erythematosus (SLE), but surprisingly, it has a significantly increased risk of cancer, particularly hematologic malignancies, even during a short follow-up period.[7,8] Several types of AIHA, including warm autoimmune hemolytic anemia, cold agglutinin syndrome, mixed- or combined-type AIHA, and paroxysmal cold hemoglobinuria can result in a positive DAT. However, sometimes immune hemolytic anemia can occur when no immunoglobulin is detectable by routine methods. Negative DAT in these cases may be due to several explanations, for instance, low amount or low affinity of IgG coated on RBCs. In addition, AIHA induced by IgA or IgM autoantibodies may be missing from detection by commercial antiglobulin reagents. Because reagents generally used in routine laboratories contain only anti-IgG and anti-C3d. Therefore, more sensitive methods may be required for these conditions. Even though DAT is not a diagnostic test and can be positive in other pathologies, it rarely yields negative results when the patient has AIHA. Additionally, intake of some drugs can result in drug-induced immune hemolytic anemia (DIIHA) that can lead to the production of antibodies against RBCs and subsequently induce a positive DAT. Drugs associated with positive DAT include penicillin

and its derivative, methyldopa, quinidine, rifampicin, etc. A high dose of penicillin can cause the immune system to produce antibodies that bind to the drug on the surface of RBCs.[9] Methyldopa can lead to the production of autoantibodies against RBCs.[10] Quinidine can form immune complexes that attach to RBCs and result in hemolysis.[11] In the presence of rifampicin, IgM is capable of fixing complement to normal RBCs which subsequently induces a positive DAT.[12] However, the patient's medical history, pregnancy, blood transfusion, and prescription are necessary for a clinical diagnosis, which cannot be made solely based on the results of the DAT. Furthermore, in cases of hemolytic disease of the fetus and newborn (HDFN), DAT can be used to study maternal antibodies (IgG) that cross the placenta and bind to the infant's RBCs. DAT can detect the presence of donor RBC-specific alloantibodies in patients undergoing hemolytic transfusion reactions (HTR) as well.

Application of IAT

The IAT is used to detect free or unbound form alloantibodies or autoantibodies against particular antigens on RBCs present in the patient's serum. It is regularly performed before blood transfusions and during pregnancy. By ensuring that the donor and recipient's blood components are compatible, it is inevitable to use the IAT to minimize the risk of post-transfusion reactions. The IAT is also used in prenatal testing to identify antibodies from mothers that can cross the placenta and harm the fetus. Additionally, it is employed to screen unexpected antibodies that can cause reactions in recipients. Confirmation of weak D and Rh negative also requests an IAT step. Occasionally, there is an unavoidable factor that interferes with the IAT, for example, daratumumab (DARA). DARA is a monoclonal antibody that is used primarily for the treatment of multiple myeloma (MM). Patients receiving DARA showed pan-agglutination reactions, which led to false positives in IAT and misinterpretation. Several approaches have been suggested to eradicate drug interference and treatment with dithiothreitol (DTT) is one such strategy. Patient's RBCs or the reagent RBCs treated with DTT can prevent false-positive reactions due to DARA. Besides, the DAT of RBCs obtained from DARA-treated MM patients showed a weak positive or negative reaction.[13,14] This medicine can interfere with DAT because it targets CD38 which is expressed on the surface of many cells, including RBCs and plasma cells.[15]

Conclusion

The antiglobulin test is a crucial tool in the diagnosis of hemolytic conditions and in ensuring blood safety in transfusions. The DAT is an indispensable diagnostic device for identifying immune-related hemolysis. A positive result indicates that the patient's red blood cells are being targeted by the immune system, which may require further investigation and treatment. Certain factors, such as recent transfusions or drug interactions, can influence the test, leading to false positive or negative results. Aside from routine testing before a blood transfusion, the IAT is also used to manage pregnant women whose neonates have a risk of HDFN. Therefore, a positive IAT indicates the presence of antibodies or complements that could lead to immune-mediated hemolytic reactions, which require cautious management to avoid adverse outcomes.

References

1. *Hannon JL. Management of blood donors and blood donations from individuals found to have a positive direct antiglobulin test. Transfus Med Rev. 2012;26:142-52.*
2. *Lin JS, Hao TC, Lyou JY, Chen YJ, Liu HM, Tzeng CH, et al. Clinical application of a flow cytometric direct antiglobulin test. Transfusion. 2009;49:1335-46.*
3. *Kaplan HS, Garratty G. Predictive value of direct antiglobulin test results. Diagn Med. 1985;8:29-32.*
4. *Garratty G. The significance of IgG on the red cell surface. Transfus Med Rev. 1987;1:47-57.*
5. *Freedman J. The significance of complement on the red cell surface. Transfus Med Rev. 1987;1:58-70.*
6. *Gorst DW, RawLinson VI, Merry AH, Stratton F. Positive direct antiglobulin test in normal individuals. Vox Sang. 1980;38:99-105.*

7. *Rottenbery Y, Yahalom V, Shimar E, Barchana M, Adler B, Paltiel O. Blood donors with positive direct antiglobulin test are at risk for cancer. Transfusion. 2009;49:838-42.*
8. *Bohnen RF, Ultmann JE, Gorman JG, Farhangi M, Scudder J. The direct Coombs' test: its clinical significance: study in a large university hospital. Ann Intern Med. 1968;68:19-32.*
9. *Garratty G. Drug-induced immune hemolytic anemia. Hematology Am Soc Hematol Educ Program. 2009;2009:73-9.*
10. *Murphy WG, Kelton JG. Methyldopa-induced autoantibodies against red blood cells. Blood Rev. 1988;2:36-42.*
11. *Johnson ST, Fueger JT, Gottschall JL. One center's experience: the serology and drugs associated with drug-induced immune hemolytic anemia--a new paradigm. Transfusion. 2007;47:697-702.*
12. *Blajchman MA, Lowry RC, Pettit JE, Stradling P. Rifampicin-induced immune thrombocytopenia. Br Med J. 1970;3:24-6.*
13. *Oostendorp M, Lammerts van Bueren JJ, Doshi P, Khan I, Ahmadi T, Parren PW, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. Transfusion. 2015;55(6 Pt 2):1555-62.*
14. *Hosokawa M, Kashiwagi H, Nakayama K, Sakuragi M, Nakao M, Morikawa T, et al. Distinct effects of daratumumab on indirect and direct antiglobulin tests: a new method employing 0.01 mol/L dithiothreitol for negating the daratumumab interference with preserving K antigenicity (Osaka method). Transfusion. 2018;58:3003-13.*
15. *Plesner T, Arkenau HT, Gimsing P, Krejcik J, Lemech C, Minnema MC, et al. Phase 1/2 study of daratumumab, lenalidomide, and dexamethasone for relapsed multiple myeloma. Blood. 2016;128:1821-8.*