Special article

DAT positive: related factors and management

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Introduction

Direct antiglobulin test (DAT) is a method used to demonstrate the coating of red blood cells with immunoglobulin and/or complement on the red blood cell surface in-vivo found in both patients and blood donors.[1] A DAT is originally performed using a polyspecific antihuman globulin (AHG) reagent or Coombs serum, which contains anti-IgG and anti-C3d to facilitate the visualization of agglutination reactions. If positive, monospecific anti-IgG and monospecific anti-C3d are subsequently used to distinguish the specific antibody bound to the red blood cell surface.[1] The DAT can be performed using various techniques, including the conventional tube technique (CTT), column agglutination technique (CAT), and solid-phase assays, which are methods used in routine antiglobulin testing but have different sensitivities.[1,2] Additionally, radioimmunoassay and flow cytometry, which are used in research, have much higher sensitivities especially for IgG antibody detected.[3,4] DAT is sometimes positive in hematologically normal individuals. In some situations, false positive reactions can occur, which in most cases may be due to incorrect processing and interpretation of the test.[3]

DAT-positive in patients

DAT-positive in patients can be caused by many reasons, including alloimmune-mediated hemolytic transfusion reaction, hemolytic disease of the fetus and newborn (HDFN) and autoantibodies.

1. Alloimmune-mediated hemolytic transfusion reaction occurs due to the formation of alloantibodies after receiving blood transfusion. It can happen rapidly, within 2 to 3 days after the transfusion and can cause hemolytic transfusion reaction (HTR).[5] These alloantibodies in the patients with or without hemolysis will bind to antigens on the surface of transfused red blood cells which is detected as DAT positive.[6]

2. Hemolytic disease of the fetus and newborn (HDFN) includes IgG alloantibodies from the mother that can cross the placenta and react with antigens on the surface of the infant's red blood cells, causing hemolysis of fetal red blood cells. The most common type of HDFN is due to ABO incompatibility, which occurs in approximately 15% to 25% of births but is usually not severe. A study by Abbas found that 19.6% of cases were DAT positive[7], similar to other studies that found group O mothers have a greater chance of having a DAT positive infant.[8-10]

3. Autoantibodies are detected in patients whose immune systems produce autoantibodies against antigens on their own red blood cells. When a reaction occurs between the autoantibodies and these red blood cell antigens, it results in a shorter lifespan of the red blood cells leading to a condition called autoimmune hemolytic anemia (AIHA), which DAT positive can be detected in most cases. The causes of AIHA can be from various infections including bacterial and viral infections such as HIV and mycoplasma hypogammaglobulinemia, ovarian tumors, and chronic inflammatory bowel disease (ulcerative colitis), which can occur due to the immune system in the body function abnormally. In patients with chronic myeloid leukemia treated with interferon-α, the immune system may function abnormally and unable to control the production of autoantibodies, leading to AIHA.[11-13] Additionally, there are reports that infection with coronavirus disease 2019 or COVID-19 is also asso-

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ciated with DAT positivity.[14-17] However, no association could be demonstrated between DAT reactivity and the medications administered during hospitalization, and also considered the possibility that DAT reactivity occurs as a consequence of antibodies directed to viral proteins bound to the red blood cell membrane.[18] Certain drugs or drug-induced immune-mediated hemolysis include drugs in the penicillin group and derivatives, cephalosporins such as cefotetan and ceftriaxone, methyldopa, β-lactamase inhibitors, and quinidine. Antibodies to drugs can be classified into two types: drug-independent (autoantibodies) and direct drug antibodies such as penicillin-type or immune complex type. Pierce and colleagues found that patients receiving penicillin develop penicillin-induced hemolytic anemia within 7 to 14 days after receiving the drug.[19] In a study by Sarkar RS., signs and symptoms appeared within 30 to 45 days after penicillin administration, and the onset was slow but severe.[20]

Drug-induced immune hemolytic anemia (DIIHA) can occurs after treatment with piperacillin/tazobactam or ceftriaxone for about 7 to 13 days.[21,22] The symptoms include acute and severe anemia, which may be accompanied by hemoglobinuria, nausea, chest pain, back or joint pain, excessive sweating, headache, fatigue, and numbness. Laboratory findings often show elevated lactate dehydrogenase (LDH) a marker of tissue damage as well as increased levels of total and indirect bilirubin. Piperacillin/tazobactam stimulates the occurrence of hemolytic anemia through an immune mechanism. All reported cases showed positive direct antiglobulin test (DAT) results on red blood cells and symptoms of acute anemia. The treatment administered was blood transfusion and discontinuation of piperacillin/tazobactam.[21] The occurrence of drug-induced immune hemolytic anemia (DIIHA) is explained by three mechanisms: haptenic mechanism, immune complex mechanism and true autoantibody formation.[23] The haptenic mechanism occurs when the drug binds to proteins on red blood cells and stimulates the body to produce antibodies against the drug and those proteins. Then, the antibodies bind to the red blood cells and activate the complement system, leading to the destruction of red blood cells. In this case, red blood cells are destroyed when the drug is present in the bloodstream. Drugs in this group include penicillin and cephalosporin. The immune complex mechanism occurs when some medications stimulate the body to produce antibodies against certain proteins in the plasma. When immune complexes form, these complexes may activate complement in the plasma or precipitate on red blood cells. The destruction of red blood cells is a result of the body's reticuloendothelial system (RES) attempting to eliminate the formed immune complexes. In this case, red blood cells are destroyed when the medication is present in the bloodstream. Medications in this group include quinidine, quinine, isoniazid, sulfonamides, sulfonylureas, thiazides and others. True autoantibody formation occurs when the drug stimulates the body to produce antibodies against proteins on red blood cells directly, leading to the destruction of red blood cells even when the drug is no longer in the bloodstream. Drugs in this category include methyldopa, levodopa, cefazolin, procainamide, and NSAIDs, such as mefenamic acid, diclofenac and ibuprofen etc.[24,25]

DAT-positive in blood donors

DAT positive in blood donors who are generally healthy individuals without any abnormal symptoms and not anemic, thus passing the blood donor screening criteria and being able to donate blood. DAT positive in blood donors is mostly of unknown cause, which may be due to reasons other than DAT positivity from immunoglobulin or complement.[15] Furthermore, the choice of methods with good sensitivity, especially the column agglutination technique (CAT), which can effectively detect IgG antibodies, results in some donated blood having false-positive results, with reports of occurrence at a rate of 0.008%.[26,27] DAT positive in blood donors shows that antibodies of the IgG type and/or IgG combined

with complement are attached to the red blood cell surface. Only 1 in 3 cases has complement alone, and mostly do not show symptoms or laboratory evidence indicating hemolysis. The strength of the DAT itself is not significant or indicative of the severity of hemolysis.[28] The study of Puri V reports two cases of healthy DAT positive donors with laboratory evidence of hemolysis and indicates that blood donors with symptoms of AIHA are found in only 1 in a million.[29] The risk of healthy donors with positive DAT in the absence of any underlying clinical symptoms progressing to clinically significant disease is an increased risk of cancer, especially hematological malignancies. Among blood donors who are DAT positive, it is suggested that DAT positivity may precede the clinical detection of cancer by several months.[29] Blood donors with DAT positive for IgG had 3-10% who developed AIHA, and 20-25% became DAT negative. Another 60-70% remained DAT positive but showed no hematological abnormalities.[23] In Thailand, a study of DAT positive blood donors among 918 Thai individual[31] found that 11 cases (1.2%) had abnormal autoimmune disease markers, including rheumatoid factor (RF) levels higher than 15 IU/mL, anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-Tg), and antinuclear antibody (ANA) titer, of which anti-thyroglobulin and ANA are important biomarkers for multiple systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), and may be associated with an increased risk of developing rheumatoid arthritis (RA).[32] Additionally, 2 cases had a reactive VDRL test. There are 152 cases (23.2%) that have other factors that might cause a positive DAT, including 13.2% with a history of medication, 3.9% with a history of diseases and infections, 5.3% with a history of allergies and hives, 6.6% with a history of vaccination, 11.8% with dietary supplement intake, 2.0% at risk of chemical exposure, and 57.2% with a history of medication for diseases involving allergies, vaccination, dietary supplement intake, and herbal use, as well as chemical exposure from work. In some cases of DAT positive donors, it may be due to drug administration because in Thailand, most people like to buy and take the unnecessary medicines by themselves especially penicillin which is found in many drug stores and can cause DAT positive in healthy people including blood donors.

The impact of DAT positive to standard cells production

Currently, DAT positivity in blood donors is a significant problem in blood services, especially in the production of standard cells, such as screening cells used to detect antibodies to other blood groups in addition to anti-A and anti-B (antibody screening). Additionally, the production of panel cells used for antibody identification also have the same problem. In standard cell production, red blood cells from blood donors of blood group O with non-reactive infectious markers such as HIV, HCV, HBV, and VDRL must be selected and tested for specific antigens, especially rare antigens such as S(+), Fy(a-b+), Mi(a+), and Di(a+). Some antigens are very rare in Thai people, such as K (+). Donated red blood cells to be used for producing antibody screening cells must have complementary antigenic profiles and complete antigen-antibody profiles of clinical importance, as well as homozygous antigens for multiple clinically important blood group antigens.[33] The donated blood to be prepared as standard cells must have a negative DAT tested with gel automation, gel manual and conventional tube test. Every unit of blood must have a negative DAT result for all three methods. If a DAT positive result is found, it is necessary to find another blood donor to replace it, which is quite difficult if the donor with a positive DAT has rare antigens such as K(+), Fy(a-), or s(-) and Jk^a or Jk^b is homozygous. When it cannot be replaced, it is necessary to select donated blood from other blood donors, which may cause the standard cells of each production batch to not meet the standards. Moreover, screening cells in some blood bank hospital which change the concentration of reagent, leading to a large number of positive antibody screening results. The varying methods and batches of gel cards utilized

could be the cause of this DAT positive outcome. At present, the NBC, TRCS recommends to use of 3% standard cells for conventional tube technique. If other techniques are used or the standard cells are prepared at a different concentration, method validation must be performed before use.

Management of DAT positive donors and red cell components of the National Blood Centre, Thai Red Cross Society (NBC, TRCS)

In the past, the NBC, TRCS had a policy of accepting DAT positive donated blood from the hospital blood bank and re-testing the blood sample using the conventional tube technique (CTT) and the column agglutination technique (CAT). If the test result is DAT negative it will be returned to the hospital but when the hospital blood bank uses it; it may still be found to be DAT positive. This positive result depends on the reagents, method and equipment used for testing. Currently, there are many varieties, especially the use of automatic detectors, which differ in both technique and method of detection, including the gel media contained in each column, which also differs from company to company. This also causes the variation of the test results. However, when the hospital detects a DAT positive blood unit, the doctors usually do not transfuse this unit to the patient and will return to the NBC, TRCS and all Regional Blood Centre, TRCS (RBC, TRCS). from 2020 to 2024, the NBC, TRCS has received DAT positive donated blood from blood banks of various hospitals with 126 units in 2020 increasing to 190 units in 2021, 438 units in 2022, 470 units in 2023, and 447 units in 2024, respectively. Some hospitals follow the recommendation of the NBC, TRCS to use AHG (monospecific anti-IgG) for crossmatching. When the result is compatible, the donated blood unit will be used for transfusion; therefore, the blood unit will not be returned to the NBC, TRCS. This is another way to reduce the waste of donated blood. Most importantly, for rare blood types such as para-Bombay, if the DAT is positive, crossmatching can be performed using anti-IgG AHG. If compatibility is confirmed, the blood may be safely transfused to the patient. In cases of other very rare blood types or in patient with multiple antibodies whom no compatible blood can be found, the hospital can consult the NBC TRCS physician to determine whether a blood unit can be safely accepted for transfusion.

However, the guidelines for the blood transfusion services in the UK[35] recommend that the Direct Antiglobulin Test (DAT) positive donations may be detected incidentally during laboratory testing, such as positive result in the autologous/reference control during ABO/RhD blood grouping, a positive antibody screen and detection of anomalies during red cell phenotyping. Non-red cell components may be prepared and issued from DAT positive red cell donations. Red cell units may be prepared and issued from DAT positive red cell donations provided that the ABO and RhD groups are confirmed and red cell antibodies have been excluded through the mandatory antibody screening. Donors found incidentally to have a positive DAT during donation testing may continue to be accepted as blood donors, provided they continue to pass the health screening questionnaire and maintain a normal hemoglobin level. While the Standards for Blood Banks and Transfusion Services of the NBC, TRCS recommend temporary donors with a DAT positive result. In some blood units, a DAT positive result may be identified during weak D testing. In such cases, non-red cell components from the affected unit may be issued. The red cell component, should only be issued if the unit is considered rare and essential for the patient. The decision must be made on a case-by-case basis by the attending physician. For donors with a first-time DAT positive result, a deferral period of one year should be applied. After this period, the donor should be retested. If the result remains positive, permanent deferral is required. If the result is negative, the donor may re-entry for blood donation. However, if a subsequent donation gives a DAT positive result again, another one-year deferral is

necessary. If the donor returns for another donation, the aforementioned criteria should be applied. In cases involving rare blood types, donation may be allowed if necessary for patient care. If a hospital detects a DAT positive result in donated blood unit due to crossmatching problems, the same criteria shall apply to the donor's eligibility for future donations.[36]

Conclusion

In summary, DAT positive can be detected in both patients and blood donors. In patients, the conclusion of DAT requires patient information, including medical history, medication history, pregnancy history, autoimmune hemolytic anemia, clinical data, and laboratory diagnostic results. In blood donors, the cause of the disease is often unknown but may be associated with autoimmune disease. Therefore, the finding is useful to the donors themselves. Even though doctors do not give DAT positive donated blood to patients, the NBC, TRCS recommend that it can be given to patients with cross-matched compatibility using monospecific anti-IgG to reduce the loss of donated blood that may have only false-positive reactions due to method sensitivity that is gradually increasing. For a very rare blood type with a positive DAT, the doctor should consider the necessity to use this unit.

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