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Case Report

# DAT positivity in blood donors: A perplexing scenario

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ABSTRACT

A blood request was received for 70 year male patient suffering from Chronic Obstructive Pulmonary Disease with anemia. One unit was found incompatible in AHG phase. Patient's antibody screen, indirect antiglobulin test (IAT), direct antiglobulin test (DAT) and auto control was negative. DAT of donor unit was positive with anti IgG gel card and negative with C3d reagent along with positive auto control. Donor was 30 year male with no history of blood transfusion and medication and had no evidence of hemolysis. Donors with positive DAT should be deferred, notified and referred to physician but further studies are required.



## 1. Introduction

The direct antiglobulin test (DAT) is a simple test used to determine if red cells have been coated in vivo with immunoglobulin, complement, or both. Clinically significant in vivo causes of a positive DAT include autoimmune hemolytic anemias (AIHA) either due to warm- or cold-reactive antibodies, drug-induced positive DAT with or without hemolytic anemia, hemolytic transfusion reactions, hemolytic disease of the fetus/newborn, autoimmune disorders such as systemic lupus erythematosis (SLE), and certain malignant disorders [1]. In 1965, Weiner was the first to describe the occurrence of a positive DAT in hematologically healthy individuals in the United Kingdom and found an overall incidence of 1:5000 in his study [2]. We hereby report a case of a DAT positive donor with no clinical and laboratory evidence of hemolysis.

## 2. Case report with results

A request for two units of packed red blood cells (PRBCs) was received for a 70 year male patient suffering from chronic obstructive pulmonary disease (COPD) with severe anemia. The patient's blood group was found to be A Rh D positive on forward and reverse grouping. Two units of A Rh D positive PRBCs were crossmatched using AHG phase tube technique. One unit (unit I) was compatible while the other (unit II) was found incompatible in AHG phase (Table 1).

As per departmental policy, the blood group of blood unit II was reconfirmed and found to be A Rh D positive. Further, the compatibility of the unit in saline phase cross match also ruled out any ABO incompatibilty and cold reacting antibodies. The patient's antibody screen (ID Dia Cell I–II–III screening panel, Diamed, Gmbh Switzerland), indirect antiglobulin test (IAT), DAT (using polyspecific AHG) and auto control was negative by microcolumn gel technique which ruled out the presence of auto and alloantibodies in the patient's serum. After that, we proceeded with DAT and auto control of blood unit II which was found positive using the microcolumn gel technique. Antibody screen and identification panel from the plasma of the same PRBCs unit was pan reactive with same strength of reaction. Components of the donation were retrieved and quarantined. The donor questionnaire form was reviewed. The donor was a young 30 year male with no history of blood transfusion and medication in recent past. Donor was contacted telephonically for a repeat fresh blood sample. The donor gave a past history of hearing loss in

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**Table 1**
Details of units cross matched.

| S. no. | Blood bag no. | Blood group | Saline | Albumin | AHG | Compatible |
|---|---|---|---|---|---|---|
| 1 | Unit I | A Rh D positive | Neg | Neg | Neg | Yes |
| 2 | Unit II | A Rh D positive | Neg | Neg | 3+ | No |

right ear due to chronic suppurative otitis media and currently was not undergoing any medical treatment. He gave family history of unexplained liver damage and history of Glucose 6-phosphate dehydrogenase (G6PD) enzyme deficiency in his father. No other significant history pertaining to in vivo hemolysis was elicited from the donor. Repeat EDTA and clotted blood samples were taken. Complete hemogram along with liver function tests and LDH levels were within normal limits. The peripheral blood smear showed predominantly normocytes with no evidence of hemolysis. A test for G6PD deficiency was also negative.

DAT, IAT and auto control was repeated with fresh blood sample using microcolumn gel technique and was found positive. Repeat IAT was done using the donor's plasma obtained after thawing FFP of the donor and was positive.

Further, the DAT was repeated with AHG monospecific gel card for IgG and monospecific C3d reagent and was found to be positive with the anti IgG gel card and negative with C3d reagent.

## 3. Discussion

A positive DAT may or may not be associated with immune mediated hemolysis. The predictive value of a positive DAT is 83% in a patient with hemolytic anemia, but only 1.4% in a patient without hemolytic anemia [3]. Small amounts of IgG and complement appear to be present on all red cells. Healthy individuals can have 5–90 IgG molecules/red cell [4] and 5–40 C3d molecules/red cell [5]; these levels are usually below the threshold of detection in routine testing. Depending on the technique and reagents used, the DAT can detect 100–500 molecules of IgG/red cell and 400–1100 molecules of C3d/ red cell. Positive DATs are reported in 1:1000 up to 1:14,000 blood donors and 1–15% of hospitalised patients [6]. These large differences in incidence probably relate to the different techniques used for performing the test.

Most healthy individuals with a positive DAT do not show clinical or laboratory evidence of hemolysis and the strength of the DAT is not necessarily an indicator of the presence or severity of hemolysis. Issitt and Anstee [7] reported that, of blood donors with a positive DAT and IgG coating the RBCs, 5–10% will develop AIHA, 20–25% will become DAT negative over time, and 60–70% will remain DAT positive but hematologically normal. Similarly, our donor also did not show any laboratory and clinical evidence of hemolysis. Of individuals with positive DATs, Garratty [4] found that about two thirds of individuals had IgG coating the RBCs, of which about half had IgG only and the other half IgG plus complement. The remaining one third of individuals had complement only coating the RBCs. In our case, the donor's red cells were coated with IgG and negative for complement using IgG specific gel card and monospecific C3d AHG respectively.

Evidence indicates that there is no immediate harm to a transfusion recipient in receiving RBCs from a donor with a positive DAT if crossmatching can be done successfully. In fact, facilities using an immediate spin or a computerized cross-match would not detect this abnormality before transfusion, and so these units (including some with a strongly positive DAT) are unknowingly transfused from time to time in most facilities [8]. Based on published data and clinical experience, there is little reason to suspect that RBCs weakly coated with IgG will have a decreased posttransfusion survival [9]. Similarly in our case, the blood unit was compatible at saline phase cross match and incompatible at AHG phase using tube technique.

Substantial literature is available regarding the diagnosis and management of patients with positive DAT. On the contrary, there are no clear cut guidelines with respect to management of DAT positive donors and utilisation of blood units with positive DAT. DAT positive donors are usually detected either at the time of blood donor antibody screening or cross match. It can be missed if only saline phase cross match is performed. However, no case has been reported till date highlighting the adverse consequences after transfusion of DAT positive blood. Nevertheless, saline phase cross match should be performed only in life saving cases and strongly discouraged in routine situations as these donors are otherwise perfectly healthy and go undetected. Rottenberg et al. [10] found significantly increased risk of cancer, especially hematological malignancies, among blood donors with positive DAT and suggested that DAT positivity may predate the clinical detection of cancer by months to years which further reinforces our concern. In our case, we called the donor for a follow up after 3 months and asked to refrain from donation till that time.

There are no clear cut guidelines and established policy for deferral of DAT positive donors and referral of such donors to physician. A prudent approach would be to encourage AHG phase testing for all situations as donor antibody screening of all blood donors is not feasible and cost effective in resource constrained developing countries. Donors with a positive DAT should be deferred, notified and referred to physician for further medical counseling and follow up. Reinstatement of such donors could occur if the DAT becomes negative at a predetermined interval after the initial DAT positive result, and the donor meets other donor eligibility criteria but further studies are required.

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