Case Study

Warm Autoimmune Hemolytic Anemia and Direct Antiglobulin Testing With a False-Negative Result in a 53-Year-Old Man: The DAT Will Set You Free

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ABSTRACT

Warm autoimmune hemolytic anemia (WAIHA), the most common of the relatively uncommon autoimmune-mediated hemolytic anemias (AIHAs), is mediated by polyclonal immunoglobulin (Ig)G autoantibodies in most cases. Herein, we present a case of WAIHA involving a direct antiglobulin test (DAT) with an initially negative result. Using a modified DAT protocol, repeat testing of the same specimen material from a previously healthy 53-year-old man yielded positive results. This case demonstrates that investigation of an apparently negative DAT result plays a critical role in the differential diagnosis of patients with rapidly progressing hemolytic anemia and the reversal of that decline.

Keywords: direct antiglobulin test (DAT), warm autoimmune hemolytic anemia (WAIHA), DAT protocol, DAT negative, WAIHA diagnosis, WAIHA treatment

A 53-year-old man with no significant medical history was in his normal state of health when he noticed that his urine abruptly had become darkly colored. He experienced an episode of chills and a near-syncopal event, which led to his arrival at his local hospital for evaluation.

The local hospital determined that the patient had a hemoglobin (Hg) level of 11 g per dL, a platelet count of 118,000 per μL, total bilirubin of 10 mg per dL, direct bilirubin of 1 mg per dL, and lactate dehydrogenase (LDH) of 856 U per L. The local hospital performed the original antibody screen using tube technique with low-ionic-strength saline (LISS) enhancement. For this specific patient, his medical team also requested an antibody identification test and a direct antiglobulin test (DAT) in gel; all results were reported as negative. The peripheral smear examined at an outside facility showed normocytic and normochromic red blood cells with mild polychromasia and anisopoikilocytosis; no schistocytes were observed. On his second day of hospitalization, the hemoglobin of the patient dropped to 7.3 g per dL, and 2 units of packed red blood cells (pRBCs) were given due to his rapidly decreasing hemoglobin level. After this intervention, the hemoglobin level of the patient remained at 7.3 g per dL. The patient was reportedly administered systemic glucocorticoids, receiving 1 to 2 doses, which were discontinued when the DAT result was reported as negative. The patient was ultimately transferred to our facility, a tertiary care hospital, for evaluation of his hemolytic anemia.

The patient had no history of hematologic disease, was taking no new medications, and had no known environmental exposure. Testing at our facility (which occurred on day 0) revealed a white blood cell count (WBC) of 25.1 K per μL, an Hg level of 5.7 g per dL (Figure 1A), a total bilirubin level of 7.1 mg per dL, a direct bilirubin level of 1.3 mg per dL (Figure 1B), and an LDH level of 704 U per L (Figure 1C).

Abbreviations
Hg, hemoglobin; LDH, lactate dehydrogenase; LISS, low-ionic-strength saline; DAT, direct antiglobulin test; pRBCs, packed red blood cells; WBC, white blood cell; AHG, anti–human globulin; IVIG, intravenous immunoglobulin; CD, cluster of differentiation; AIHA, autoimmune hemolytic anemia; WAIHA, warm autoimmune hemolytic anemia

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haptoglobin level of the patient was less than 6 mg per dL, and the reticulocyte count was 0.5 M per µL. The antibody workup suggested a weak autoantibody pattern, as shown by the reactivity score of 1 or 2 out of 4, but was reported preliminarily as inconclusive because the DAT result was reported as negative (Figure 2). Our facility performs most patient typing and screening of specimens on an automated instrument, the Ortho ProVue analyzer (Ortho-Clinical Diagnostics), using gel technology, which is our primary method for antibody identification. Alternative methods used are tube technique with LISS or tube technique with no enhancement (saline). DATs are performed in tubes, first using polyclonal anti-immunoglobulin (IgG, anti-C3d anti-human globulin (AHG) and, if indicated, monoclonal anti-IgG, anti-C3b, and anti-C3d. In this case, we did not perform autocontrol procedures but performed DAT testing on saline control material, yielding a

Figure 1
Laboratory data from our patient, a 53-year-old man. Day 0 represents the first day of treatment for the patient at a tertiary care facility. Shading indicates the days after a positive direct antiglobulin test (DAT) result was reported and glucocorticoid therapy was initiated. A, Lactate dehydrogenase (LDH; solid line) and haptoglobin levels (dashed line). B, Hemoglobin (solid line) and packed red blood cell (pRBC) administration (grey square). C, Total bilirubin (solid line) and direct bilirubin (dashed line) levels. Gap from days 8 through 23 represents no laboratory tests performed during this time.
negative result (Image 1A and the left-hand side of Image 1B). The patient experienced confusion and altered mental status, with an Hg level of 5.6 g per dL, and was subsequently transfused with 6 U of pRBCs. His Hg level rose to 7.7 g per dL on day 1 before dropping rapidly to 5.1 g per dL on day 2 (Figure 1A). At this point, the Hematology service consulted the Transfusion Medicine service regarding the concern of immune-mediated hemolysis, which prompted further investigation of the DAT assay results.

**Laboratory Investigations: Initial Testing**

Routine DAT testing was performed according to guidelines listed in the AABB Technical Manual. Briefly, cells from the patient specimen were first washed 4 times in an automatic cell washer before adding 2 drops of polyclonal AHG containing anti-IgG and anti-C3d. Then, we centrifuged the test specimen and read it macroscopically and microscopically for agglutination. It was then incubated at room temperature for 5 minutes and centrifuged and read again. We confirmed the negative results with IgG-sensitized reagent RBCs. This testing yielded repeat negative results in our patient, with polyclonal AHG and monoclonal anti-IgG, anti-C3b, and anti-C3d, using complement-coated reagent RBCs as control material (Image 1A, and the right-hand side of Image 1B).

**Modified DAT Protocol Testing**

Next, we attempted to perform DAT using an unwashed 4% suspension of cells from the patient, using a method described elsewhere. The cells were tested again with polyclonal AHG, monoclonal anti-IgG and anti-C3b, anti-C3d, with 0.9% saline as the control material. The cells from the patient reacted strongly (2+) at immediate spin with the polyclonal AHG and anti-IgG (Image 1C, and the right-hand side of Image 1B).
side of Image 1A. The 5-minute room-temperature polyclonal AHG and the anti-C3b and anti-C3d results were negative. The final report from the antibody screening was changed to warm autoantibody accordingly.

Once the positive DAT was reported, the patient was immediately started on a 5-day course of intravenous glucocorticoids and intravenous immunoglobulin (IVIG). When his Hg level rose to 5.5 g per dL the following day (day 1), he was transfused with 2 units of pRBCs. His Hg level decreased to 4.4 g per dL, his LDH increased to 1273 U per L, his total bilirubin was 14.7 mg per dL, and his direct bilirubin was 4.6 mg per dL (Figure 1). His haptoglobin level remained steady at less than 6 mg/dL, and his reticulocyte count was 0.8 M per μL. The total bilirubin and direct bilirubin levels for the patient are shown in Figure 1B.

The patient was continued on glucocorticoid therapy and IVIG, and his laboratory results began to improve. By his third day of treatment, his total bilirubin level was down to 1.9 mg per dL; his direct bilirubin was 0.7 mg per dL, and his Hg improved to 6.3 g per dL. As a result, the patient was sufficiently healthy to be discharged a week after initiation of the correct therapy.

Clinical Follow-Up

The patient returned for follow-up 23 days after his initial admission to our hospital; at that time, his Hb level was 13.5 g per dL. The patient had no new symptoms and reported feeling healthy. His treatment team was considering tapering the dose of his glucocorticoids 51 days after admission, when his Hb level was reported at 14.8 g per dL (Figure 1). The patient had been doing well since his last follow-up.

Discussion

WAIHA is defined by production of autoantibodies directed against the self-antigens present on the red blood cells of patients, leading to the destruction of those cells, with the best reactive temperature at 37 °C. WAIHA totals approximately 50% to 70% of all cases of AIHA.3,4 The disorder is divided into groups based on the presence or absence of associated underlying diseases. Idiopathic/primary WAIHA is diagnosed when no underlying disease is evident. WAIHA is classified as secondary when it manifests as the complication of an underlying process or disorder. The common conditions leading to secondary WAIHA include primary immunodeficiencies such as common-variable immunodeciency, hematologic malignant neoplasms, infections, drugs, and tumors.5,6 Chronic lymphocytic leukemia and lymphomas make up approximately 50% of secondary WAIHA cases. The next-largest group of secondary WAIHA occurs due to
autoimmune diseases, systemic lupus erythematosus being the main culprit.1 WAIHA is primarily diagnosed based on DAT results. Treatment varies and depends on whether the condition is primary or secondary and on the antibody class, with IgG and IgM being the most common.7 IgA antibodies represent the minority of cases of WAIHA.8,9

However, the establishment of the diagnosis of AIHA is sometimes difficult. First, the signs and symptoms of AIHA are nonspecific and common to all types of hemolytic anemia, which can be caused by many nonimmune factors. These factors include infections; oxidant agents; and other agents such as lead, copper, venomous snake or spider bite, liver disease, and some hematological disorders such as large granular lymphocyte leukemia, paroxysmal cold hemoglobinuria, and paroxysmal nocturnal hemoglobinuria. Second, the clinical syndrome observed with AIHA of the warm-antibody type varies greatly with the amount and effectiveness of the causative antibody. When the amount is small or when the antibody is inefficient at causing hemolysis, the patient may be asymptomatic, even if he or she has slight anemia. More commonly, patients who seek treatment are diagnosed as having moderate to severe anemia. Last, it is well known that patients with AIHA can have negative DAT results, or “DAT-negative” AIHA. The incidence of DAT-negative AIHA has been estimated at 3% to 11%.10 Treatment of hemolytic anemia varies and can be quite different, depending on the etiology. Whether transfusion should be provided in cases of confirmed WAIHA is highly debatable because the transfused cells will most likely share the fate of the red cells of the patient, depending on the autoantibody production. Because there is a delicate balance between clinical need and risk, transfusions should be considered only when absolutely necessary to provide relief from anemia symptoms, such as shortness of breath, and to maintain adequate oxygen transportation. The red blood cells that are transfused will not necessarily be hemolyzed any more quickly than the native cells of the patient, but overall hemolytic activity may increase because of increased red cell mass.1

Treatment of WAIHA currently includes glucocorticoids as first-line therapy. Although immunosuppression is the major therapeutic approach for AIHA, it might be futile or even contraindicated in some types of hemolytic anemias caused by infectious agents. IVIG, alemtuzumab, and other immunosuppressive drugs are also used in cases that have been refractory to glucocorticoids and splenectomy.11,12 Splenectomies are reserved for patients who do not show an acceptable response to glucocorticoids. One of the most recent treatments has been the use of rituximab, which has shown efficacy in treating WAIHA and many other autoimmune diseases. Rituximab is a monoclonal antibody directed against the cluster of differentiation (CD)20 antigen present on B cells. Rituximab administration results in the destruction of B cells via antibody-mediated cytotoxicity, and complement activation.13

In our patient, the DAT result determined the course of his treatment. Glucocorticoids were discontinued early on when the DAT was reported negative. The typical causes of DAT-negative AIHA include IgG that coats red cells are below the threshold of detection of the DAT, IgM or IgA red-cell-bound antibodies (not detectable by routine testing), or IgG that was washed off during testing because of its low affinity.1,12–15 To investigate these situations, nonroutine testing may be pursued, such as radiolabeled anti-IgG; complement-fixation antibody-consumption assay; solid-phase, column agglutination; enzyme-linked antiglobulin testing; and flow cytometric testing. However, these tests typically have low predictive value and are not standardized so they should be interpreted with caution.1 A repeat performance of DAT using the modified DAT protocol2 drastically shortened the list of potential differential diagnoses and resulted in a life-saving change in therapy, reversing the rapid decline in the course of the disorder of the patient. Trusting the veracity of data that does not fit the clinical profile can be misleading and hazardous to the health of patients. As the old saying goes, absence of evidence is not evidence of absence. The symptoms of syncope, chills, dark-colored urine, and shortness of breath, along with the laboratory values of a rapid drop in the Hb level without an obvious source of bleeding, decreased haptoglobin level, and increased LDH level help point to the correct diagnosis. It is always worthwhile to perform further investigations to verify these results, especially if doing so is as simple as performing a DAT.

AIHA is a treatable but sometimes severe, life-threatening disease that can be managed accordingly as long as certain diagnostic criteria are met. However, the diagnosis rests mainly on a positive DAT result. In our patient, the signs and symptoms were present, but this critical piece of evidence was lacking, which lead to a delay in treatment. Acting on clinical suspicion, the DAT protocol was modified, and further DAT yielded a positive result, which led to the change in treatment and the recovery of the patient. LM
References