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A persistent severe autoimmune hemolytic anemia despite apparent direct antiglobulin test negativization

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ABSTRACT

Background and Objectives. **Not all cases of autoimmune hemolytic anemia (AIHA) are diagnosed by the direct antiglobulin test (DAT). We present and discuss a simple method of enhancing the sensitivity of the standard DAT.**

Design and Methods. **We report the case of a five-month-old child diagnosed with a severe IgG-mediated AIHA, characterized by quick DAT negativization despite clinical worsening. Warm AIHA with negative DAT, possibly due to a low affinity autoantibody, unresponsive to conventional therapy, was hypothesized.**

Results. **The DAT resulted strongly positive with anti-IgG serum using a 4°C saline for erythrocyte washing, to reduce the dissociation of the supposed low affinity autoantibody. Very intensive cytoreductive treatment was administered twice until clinical remission was obtained.**

Interpretation and Conclusions. **The clinical course of AIHA can be dissociated by the DAT. Since autoantibody-mediated hemolysis with negative DAT rarely occurs, once other causes of high reticulocyte count anemia have been ruled out, the DAT after ice-cold saline washing could be a useful and easy means of corroborating the diagnosis of AHIA, when traditional methods fail.**

Key words: autoimmune hemolytic anemia, direct antiglobulin test

The hallmark of autoimmune hemolytic anemia (AIHA) is a positive direct antiglobulin test (DAT), also called direct Coombs' test, by which IgG and/or complement (C3d) are found on the red blood cell (RBC) surface at 37°C; nevertheless it is known that a negative DAT does not rule out the diagnosis of AIHA.[1] This case report reminds clinicians and laboratory operators that performing DAT using cold saline washing can improve the DAT sensitivity, when low-affinity autoantibodies are involved. This simple approach was helpful in monitoring the peculiar clinical course of a child affected by a severe persistent AIHA, despite standard DAT negativization.

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Case report

A five-month old anemic child (hemoglobin 3.4 g/dL, hematocrit 10.2%, reticulocyte count 41%, total bilirubin level 5.10 mg/dL) with a one-week history of anorexia, pallor, jaundice and lethargy was diagnosed as having warm IgG auto-antibodies AIHA, because of a strongly positive DAT with anti-IgG serum. After no response to treatment with steroid and immunoglobulins (Figure 1) the child required transfusional support and was referred to our Department. At admission, the physical examination revealed a grade 2 systolic ejection murmur and the spleen was palpable at the left costal margin. The hemoglobin level was 5.0 g/dL, reticulocyte count 30%, total bilirubin level 1.6 mg/dL; moderate aniso- and poikilocytosis, spherocytes and some erythroblasts were observed in the blood smear. The DAT performed by standard tube test,[2] using anti-IgG serum, unexpectedly resulted negative in our hands; the DAT with anti-IgA, monomeric IgM, C3, C4 and C3d serum, as well as the indirect antiglobulin test (IAT) were also negative. Other possible causes of high reticulocyte count anemia, such as bleeding and most common erythrocyte enzyme defects associated with hemolytic anemia (G6PD and pyruvate kinase deficiency) were ruled out. Defects of the erythrocyte membrane could not be excluded, but the probability was considered very low because the red cell morphology was not striking and the parents were hematologically normal. Therefore, the existence of a low affinity autoantibody, undetectable by a standard DAT, was hypothesized and more sensitive methods were considered to reveal it. A simple technique described by Garratty *et al.* was adopted,[3] differing from a standard tube DAT only by the RBC washing, performed by means of 4°C frozen saline. The *cold-wash DAT* resulted strongly positive with anti-IgG serum. In order to exclude that a cold autoantibody was involved, cold-washed RBC were also tested in parallel with 6%

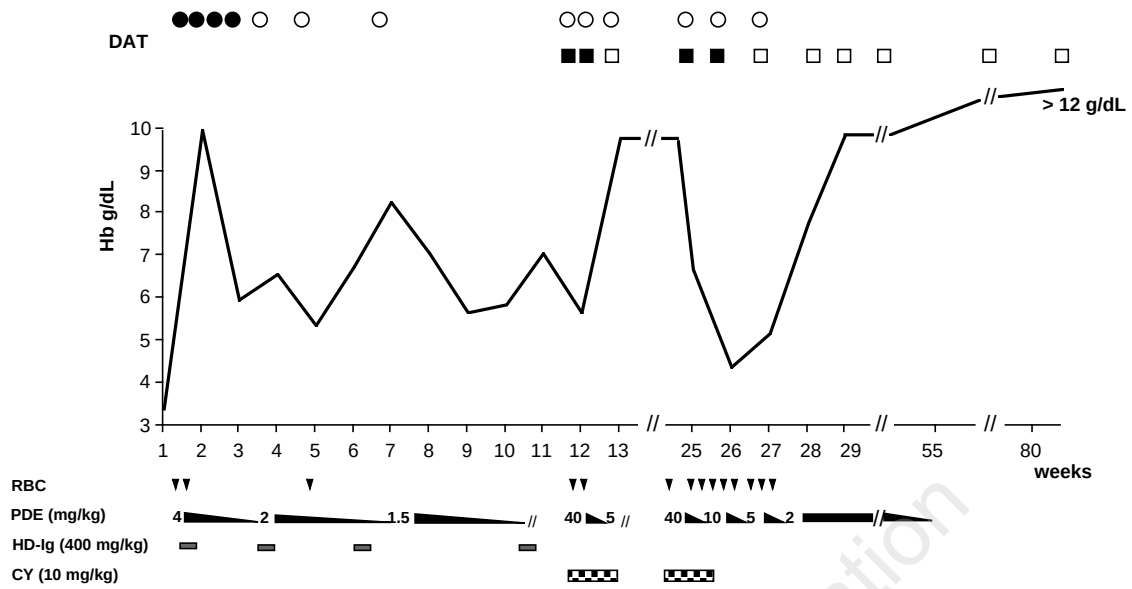

Figure 1. Clinical course depicting hemoglobin level, transfusional support, DAT, type of treatment.
RBC (▼) = red blood cells transfusion. PDE = corticosteroid as prednisone dose equivalent. HD-Ig = high-dose immunoglobulins. CY = cyclophosphamide. ● ○ = positive, negative DAT performed by standard technique. ■ □ positive, negative DAT after RBC washing with 4°C normal saline.

albumin and agglutination was absent.

More intensive treatment was adopted with cyclophosphamide (10 mg/kg/day for ten days) associated with a very high-dose of steroid (methylprednisolone 40 mg/kg/day for 5 days, which was then tapered down over twenty days to 2 mg/kg/day).[4] After this treatment the child progressively improved, the hemoglobin level increased and the *cold-wash DAT* became negative.

The child remained in a good clinical condition and continued steroid therapy for about 3 months, until the AIHA relapsed: again the DAT resulted positive only when the above-mentioned technique was used. The child needed transfusional support and high-dose cyclophosphamide associated with methylprednisolone was again administered. The patient progressively improved with his hemoglobin level rising to 11 g/dL. The maintainance therapy consisted of methylprednisolone 0.1 mg/kg every other day. Fifteen months after the onset and seven months after the last RBC transfusion, the steroid was discontinued. The child is now in a good clinical condition with a negative DAT and has been off steroid treatment for 18 months.

Discussion

We reported the case of a five-month old child affected by a severe form of idiopathic AIHA, unresponsive to conventional therapy with steroids and high-dose immunoglobulins which required two cycles of high-dose steroid and cyclophosphamide to dominate.[1,4,5] A possible usual panreactive IgG class autoantibody, determined at the onset by a strongly positive standard DAT, might have been cleared by conventional treatment. During our observation period the standard DAT constantly resulted negative, while other tests aimed at demonstrating other high reticulocyte count anemias were negative.[1,6] What was most striking in our child was the dissociation between the worsening clinical course and the DAT negativization. Most pediatric AIHAs present with a positive DAT, which reveals IgG on the RBC surface at 37°C.[1] Although DAT positivity is considered the hallmark of AIHA, the incidence of a negative DAT in patients with AIHA is reported to be between 2 and 4%.[7] A possible explanation for these findings is that the number of IgG molecules per red cell necessary for accelerated *in vivo* destruction is sometimes lower than the number necessary to yield a positive DAT.[8] In other patients with negative DAT, but with clinical and hematologic features typical of AIHA, IgA autoantibodies or monomeric IgM may be involved.[1] Nevertheless sometimes low affinity antibodies may be involved and can dissociate from the RBC surface, when DAT is performed under standard conditions.[3] In standard DAT RBCs are repeatedly washed in large volumes of room temperature saline solution, which possibly separates a low affinity antibody from RBC surface; thus, RBCs that are strongly sensitized with IgG *in vivo* may appear to have little or no IgG on the RBCs when tested *in vitro*. Garratty found that washing the patient's RBCs with ice cold saline, preferably

in a refrigerated centrifuge, helps to keep low-affinity IgG bound to the RBCs.[7] Simultaneous RBCs testing with 6% albumin other than IgG is recommended to avoid false positive results due to clinically insignificant cold autoagglutinins. Among other available techniques adopted to increase DAT sensitivity[8] the *cold-wash DAT* was chosen because it is extremely simple, easy to interpret and requires neither specific training nor sophisticated equipment.

In our patient the low affinity antibody production was not suppressed by conventional therapy, but eventually decreased after very intensive cytoreductive immunosuppression.

Since autoantibody-mediated hemolysis with negative or weak DAT rarely occurs, it is useful to remind clinicians and laboratory operators that, once other causes of high reticulocyte count anemia have been ruled out, the extremely simple but not widely acknowledged method of ice-cold RBC washing, described by Garratty[7] and reported in immunohematology manuals,[9] could be a useful means of corroborating the diagnosis of AHIA when traditional methods fail.

Contributions and Acknowledgments

AB and EB were responsible for the conception and design of the report, mainly with regard to the clinical aspects, and also wrote the paper. GA and FR prompted the laboratory approach for the detection of low affinity autoantibody and substantially contributed to the analysis of the data and the interpretation of the biological process involved in this autoimmune hemolytic anemia. MJ and BN followed the patient's clinical course and approved the final version of the paper before submission.

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Disclosures

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