Interpretation of a Positive Direct Antiglobulin Test

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Case History

A 71-year-old Caucasian woman was transferred to our hospital from a smaller hospital. Because the patient was actively bleeding from the gastrointestinal tract, the emergency room physician requested four units of blood crossmatched stat.

The test results obtained on the patient’s admission specimen are given in Fig. 1.

An ether eluate prepared by using the modified method of Rubin did not react with a panel of group O cells in any phase. In spite of the negative antibody screen, an enzyme panel and an albumin to antiglobulin panel were done on the patient’s serum, but gave negative reactions with all cells in all phases.

The attending physician stated that the patient had received seven units of blood at the other hospital five days prior to transfer, “some of which were O-positive.” The patient’s medication history was given as Digoxin, Lasix, Tylenol and vitamin K.

Questions

1. What is the most likely cause of the positive DAT in this patient?
2. What further testing should be done in the blood bank to resolve this problem?
3. Why is the D test result a distinctly mixed field, while the DAT result is a questionably mixed field?
4. Why is it important to determine the exact cause of the positive DAT in this case?

Answers

1. Initially we suspected that the patient was producing anti-D in response to the transfusion of O Rh-positive blood. The O Rh-positive donor cells were still in the circulation, and all the anti-D was bound to these cells making the direct antiglobulin test (DAT) positive. The patient’s D test result was a distinctly mixed field, indicating the presence of O Rh-positive donor cells. If the patient was undergoing a delayed hemolytic transfusion reaction, the DAT result should have been a distinctly mixed field, and the eluate of the patient’s red cells should have demonstrated anti-D activity. The patient should have had clinical or laboratory signs of delayed hemolytic transfusion reaction or autoimmune hemolytic anemia. However, none of these findings existed. In addition, none of the medications that the patient was reportedly taking could explain the positive DAT due to IgG.

We decided to repeat the direct and indirect antiglobulin tests
38% to 75% of cephalothin-treated patients. 

2. Since no explanation for the laboratory findings had developed, we carefully reviewed the patient's chart. We discovered that until two days before transfer to our hospital she had received Keflin, two grams every six hours, for a total of eight days. Keflin (cephalothin) has been reported to cause a positive DAT and hemolytic anemia on a dosage as little as two grams per day, with the DAT remaining positive for 10 weeks after the drug was discontinued. A positive DAT may develop within 24-48 hours of beginning treatment. This phenomenon has been observed in 38% to 75% of cephalothin-treated patients.

Cephalothin coats all normal red cells both in vivo and in vitro. A positive DAT may develop by any one of the following three mechanisms:

a) The red cell membrane is modified by the drug so that the cell binds normal serum proteins, such as IgG and complement, nonimmunologically. As the antibodies involved are not specific for normal red cells or Keflin-coated red cells, the eluate from the patient's red cells does not react with either normal or Keflin-coated red cells.

b) Specific anti-Keflin antibody is stimulated in response to the drug. This IgG antibody attaches to the Keflin-coated cells resulting in a positive DAT. The patient's serum and red cell eluate will react with Keflin-coated cells but not with uncoated cells.

c) Preexisting anti-penicillin antibodies in the patient may crossreact with the Keflin-coated cells resulting in a positive DAT. The patient's red cell eluate and serum react with both Keflin- and penicillin-coated cells but not with uncoated cells.

To investigate the possibility that the positive DAT in this patient was due to Keflin, serial dilutions of her serum and red cell eluate were tested with the Keflin-coated cells according to the method of Garratty and Petz. The patient's serum reacted with the Keflin-coated cells to a dilution of 1:300 and the eluate reacted strongly with the coated cells. Both serum and eluate were not reactive with the uncoated cells. Thus, the presence of anti-Keflin antibody in the patient's serum and on her red cells was confirmed.

3. This case illustrates that one should not be misled in the investigation of a positive DAT by incomplete information. In this instance, the O Rh-negative patient had received O Rh-positive blood, so it was logical to attribute the positive DAT to this fact alone. It is interesting to note that several technologists interpreted the Du test result as a distinctly mixed field by microscopic examination, but the DAT result as a questionably mixed field. In the Du test, reagent anti-D reacted in vitro with the previously transfused O Rh-positive donor cells and produced a distinctly mixed field pattern. The "questionably" mixed field pattern of the DAT was probably due to the presence of Keflin-coated and uncoated cells in the circulation at the same time. At the time the patient's specimen was being tested in our laboratory, Keflin therapy had been discontinued and all the available drug had coated the red cells. New cells being produced by the bone marrow and the donor cells that were subsequently transfused were not coated by the drug. Even the coated cells probably had a small number of Keflin molecules bound onto the cell surface.

Anti-Keflin antibody can only bind to Keflin-coated cells, and there was a high concentration of this antibody in the patient's circulation as demonstrated by our titration studies. An excess of this antibody, combined with the fact that there were very few antigenic sites available, produced the "questionably" mixed field pattern in the DAT.

4. In this patient it was important to recognize the actual cause of the positive DAT, since it meant distinguishing between a delayed hemolytic transfusion reaction and a drug-induced positive DAT.

Another important point illustrated by this case is that the history obtained from the nursing station or the attending physician over the telephone may not be complete. In questionable cases, the blood bank technologist or the pathologist should interview the patient or review the patient's chart to obtain complete information.

References