AIHA CASE #9, ANSWERS

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Indian Immunohematology Initiative

1. Do you agree with this diagnosis? Are you comfortable stopping there?

No! The technologist has demonstrated that there is a cold autoantibody reactive at 15°C, but that does not prove that the reactions seen in gel are DUE to the cold autoantibody. The latter is an assumption.

2. Why might there be a discrepancy between the initial and repeat DAT results? (Hint: check the DAT procedure or the manufacturer's package insert.) (Hint #2: it was a busy morning.) Why was the evening shift supervisor prompted to repeat this test?

The anti-IgG component of the AHG may react best if read immediately, so a DAT by the tube method should be mixed, spun, and read IMMEDIATELY. This is demonstrated in the repeat DAT in which we see that the agglutination disappeared after a 5 minute incubation. With time the IgG antibody bound to the RBCs presumably elutes off in some cases, causing a false negative result. Given the indication that there was IgG bound to the patient's RBCs an eluate was prepared which contained a panagglutinin, presumably warm autoantibody.

The 5" incubation is performed in order to increase the sensitivity of the anti-complement component of the AHG. In this case the reaction remained negative after incubation.

The evening shift supervisor repeated the test because he did not think that demonstration of a cold autoantibody explained the gel reactions and perceived a discrepancy between the panel and DAT results.

3. What antibody(ies) is(are) present? What assumption made by the initial technologist led to the erroneous result? What does the warm autoadsorption show? Why did the autoadsorbed plasma react by the gel method but not by the LISS/tube or PEG tube methods? Can we make use of this observation?

The technologist who initially worked on the problem made the assumption that the plasma reactions with the gel method were explained by the cold autoantibody they had demonstrated. This could have been the case as the gel technique is relatively sensitive to cold autoantibodies. However, it is also sensitive to warm auto-antibodies, and in order to ascribe the positive reactions in gel to a cold autoantibody, there should have been explicit demonstration that the reactions were eliminated by adsorption, either cold auto-adsorption or with rabbit erythrocyte stroma ("REST"). In fact REST adsorption did not eliminate the reactions in gel so they were not due to cold autoantibody. And as discussed, after reinterpretation of the DAT warm autoantibody was recovered in an eluate.

The supervisor then performed a warm autoadsorption. This eliminated the positive reactions with screening cells using LISS and PEG indirect antiglobulin tests, but the adsorbed plasma still reacted in gel. This difference just shows us that gel is a more sensitive technique than the others for detecting a weak warm autoantibody. We can take advantage of this fact by routinely testing autoadsorbed sera by these techniques that are less sensitive to residual autoantibody but perfectly acceptable for ruling out an underlying alloantibody. Up to 3 or 4 adsorptions can be done if there are enough patient cells, but autoadsorptions take time and effort which might be better spent on other tasks. If we can eliminate the need to adsorb the plasma again so much the better. And not that in this case the patient sample had been used up!