HDFN TECHNICAL CASE #4, ANSWERS

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1. What is the probable identity of this antibody? Is any further workup needed to prove it? Why were the additional 3 gel test panel cells run after the initial 11 cell panel?

   The antibody in this patient is alloanti-M. Standard criteria for antibody identification are met (3 M-neg cell samples reactive, 3 M-pos cells reactive, other common, clinically significant antibodies ruled out, patient is M-neg) so no further workup is needed for identification. The additional cells were needed to rule out anti-C, -K, -Fy^a, and –S which had not been ruled out in the initial panel.

2. What was the cause of the ABO discrepancy? How was this resolved?

   The patient's "forward" or cell group was A, but the reverse/serum group was O, presumably because the patient's anti-M was reacting with the group A reverse typing cells. These are pooled cells from multiple donors so many would be expected to express the M antigen. The discrepancy was resolved by repeating the test with ficin-treated RBCs, since ficin destroys the M antigen, but not A and B antigens. The autocontrol was necessary for this testing because if the patient had a cold-reactive autoantibody, it might agglutinate the reverse typing cells leaving the impression of a group O reverse type.

3. What is the indirect antiglobulin test (IAT) titer of this antibody? Why was the titer repeated?

   Our laboratory specifies that the endpoint of a titer is the highest dilution showing macroscopic agglutination, namely a weak positive (w+) reaction. Therefore the titer is 64. The initial titration was repeated because it was not clear that the endpoint had been reached.

4. The tube IAT was initially negative (see 37^o and IAT reactions, tube test panel #06776), but in the titration the antibody reacted in the IAT? Can you explain this discrepancy?

   The titration results demonstrate the "pro-zone" phenomenon, in which an antibody fails to agglutinate RBCs bearing the corresponding antigen unless the serum or plasma is diluted. The serum/cell ratio at which most blood group antibodies agglutinate RBCs in the various test phases of the "saline-IAT" has been established empirically, typically 2 to 4 volumes (drops) of serum/plasma to 1 volume (drop) of a 3-5% suspension of RBCs. However, occasional antisera appear to have too high a concentration of antibody to yield agglutination by this method.

   Our laboratory would not typically perform a titration on a sample of anti-M that failed to react at 37^{o}C or in the saline/tube IAT in a pre-warmed test. However, the technologist felt that the large difference in reaction strength between the gel and tube IAT warranted further investigation. Because of the pro-zone the pre-warmed test gave an erroneous result suggesting that there was no reactivity at 37^o and in the IAT. Experience matters!

5. Are you concerned that this pregnancy might be affected by hemolytic disease of the fetus and newborn? If so, what would you do to investigate this possibility?

   We frequently see cold-reactive anti-M in pregnant women that is of no significance to the fetus. Anti-M antisera that fail to react at 37^{o}C or in the IAT are presumed to be largely IgM which does not cross the placenta, and has little hemolytic potential in the individual forming the antibody. However, there are a few reports of anti-M causing severe HDFN, and high titer reactivity in the saline/IAT raises the possibility of an IgG component to this patient's antibody. This can be investigated by treating the serum with a sulfhydryl reducing agent such as dithiothreitol (DTT) which disrupts the IgM molecule such that it can no longer agglutinate RBCs. Thus, if DTT treatment of an antiserum eliminates its ability to agglutinate RBCs, the antibody is demonstrated to be IgM.

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6. What is your conclusion regarding the likelihood of this antibody to produce HDFN? Why is the titer of the dilution control interpreted as "32" rather than "16"?

   No IgG component of this patient's anti-M is detectable in the DTT-treated plasma, so it does not appear to have the potential to cross the placenta and cause HDFN.

   The patient's serum in the dilution control has already been diluted by a factor of 2 by the DTT solution, so all actual dilutions are double. The titer of 32 is not regarded as different from the original titer; the difference between 32 and 64 is within the error of the method. Note that the antibody is still showing the pro-zone phenomenon.

7. Would you recommend any other testing during this pregnancy related to this problem?

   It is theoretically possible that ongoing maternal stimulation by fetal maternal hemorrhages could cause the patient's immune system to start making IgG anti-M. To detect this rare occurrence we recommended that samples be submitted for a repeat study in the first half of third trimester, but these were never received.

8. What do the DTT control studies demonstrate?

   DTT is a relatively unstable compound when exposed to the oxygen in air, so control studies are necessary to demonstrate that it has not lost function. The loss of anti-B reactivity in the patient's serum demonstrates that the DTT can still inactivate IgM agglutinins. Maintenance of reactivity when a volume of saline equal to the DTT is added demonstrates that this effect is not simply due to dilution (dilution control).

9. Would you make any other recommendations regarding testing or management during this pregnancy?

   Nothing has changed significantly; it still looks like the patient's antibody is entirely IgM. We again recommended a repeat study in the third trimester but did not receive additional samples.