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Laboratory Evaluation of Positive Antibody Screen in an Rh Negative Patient: Passive or True Anti-D: A Case Study Approach

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Presentation:
The patient is a 30 year old female G3P1A1. She is O negative and 8 weeks pregnant with her 3rd child. The obstetrician orders an ABO/Rh and antibody screen; she types O negative with anti-D identified in her plasma. The laboratory is not sure if the anti-D identified is passive due to RhIG administration or immune anti-D.

History and Laboratory Evaluation:
The patient has a history of miscarriage at 10 weeks of gestation in 2007, and at that time a micro dose of RhIG 50ug was given. In 2012, the patient delivered a child on July 20. Her antibody screen was positive at the time of the delivery and passive anti-D was identified. The cord blood workup of her child revealed the baby was O positive and the patient required another RhIG work up. The postpartum sample was tested by the laboratory. The fetal screen test was negative, so she only received one standard dose of 300ug of RhIG, IM injection 24 hours after the delivery and was discharged without complications.

Present diagnosis:
On November 15, the same patient presented to her obstetrician with suspicions she could be pregnant. The obstetrician ordered a quantification HCG and the pregnancy was confirmed. The physician also ordered an ABO/Rh and antibody screen. Results begin on Page 4.

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Anti-A	Anti-B	Anti-D	Dctrl	A1Cells	Bcells	Interpretation
0	0	0	0	4+	4+	0 Negative

Screen Cells	Result (Tango - automated)
I (R2Ro)	2+
II ~~(rr)~~ R1r	W+
III (rr)	0
Interpretation: positive	

Antibody workup shows the presence of anti-D. The physician calls the Blood Bank to speak with the supervisor. He asks the Blood Bank if his patient has a true anti-D and if he should be worried. The Blood Bank supervisor tells the physician more testing must be performed to safely conclude the antibody found in the patient's plasma is still the product of a previous RhIG administration[1]. The physician asks to be notified as soon as the laboratory reaches a conclusion.

The laboratory repeats the antibody screen using the tube method, the result are below:

Screen Cells	IS	37C	AHG
1 (R2Ro)	0	0	1+
II (R1r)	0	0	0
III (rr)	0	0	0
Interpretation: positive			

Results after treating the patient's plasma with dithiothreitol (DTT):

Screen Cells	IS	37C	AHG
I (R2Ro)	0	0	1+
II (R1r)	0	0	0
III (rr)	0	0	0
Interpretation: postitive			

The laboratory suspects RhIG administration is the reason for the patient's consecutive positive antibody screen, however the Blood Bank performed a titer to confirm:

The patient's titer results for anti-D are below:

Titer (tube method) R1R2 cell	Reaction (AHG - LISS) Specimen date: 11/15/12
1:1	1+
1:2	1+
1:4	W+
1:8	0
1:16	0
1:32	0
1:64	0
1:128	0
1:256	0
1:512	0
1:1024	0
1:2048	0
1:4096	0

The Laboratory calls the physician and requests a new sample to be drawn 8 weeks from the previous sample, so the titers could be compared:

Titer (tube method) R1R1 cell	Reaction (AHG-LISS) Sample Date 11/15/12 (8 weeks gestation)	Reaction (AHG-LISS) Sample date 01/15/13 (16 weeks gestation)
1:1	1+	W+
1:2	1+	0
1:4	W+	0
1:8	0	0
1:16	0	0
1:32	0	0
1:64	0	0
1:128	0	0
1:256	0	0
1:512	0	0
1:1024	0	0
1:2048	0	0
1:4096	0	0

The laboratory also decides to treat the patient's sample with DTT and repeat the antibody screen; the results were:

Screen Cells	Sample 09/02/13 (AHG Phase Only)	Treated sample from 09/02/13
I	1+	1+
II	0	0
III	0	0

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Evaluation *From Page 5*

RhIG is known to be entirely IgG whereas active immune anti-D is part IgM. Treatment of a patient's plasma with 2-mercaptoethanol (2-ME) or DTT would partially or totally inactivate the antibody if true anti-D was present. DTT treatment would not have any effect if the positive antibody screen was caused by RhIG administration.[1] It is known that titers of RhIG usually do not exceed 1:4 and the half life is usually 25 to 31 days[1,2]. This half life may vary depending on the patient's immune response and may be present in maternal circulation for up to 6 months. Another important part of the identification of this antibody is that active immune anti-D usually reacts strongly at immediate spin tube method. The 3-4+ strength reaction is carried from the saline IS phase through 37^{O}C and AHG. Passive anti-D, due to IgG characteristics, usually does not react at IS and 37^{O}C by the tube method and repeating the patient's antibody screen using the tube method is helpful if the initial screen was performed using highly sensitive methods such as gel, solid phase or automation.

In 2013, a study was performed by Tiblad et al, where Rh negative women had a non-invasive fetal RHD screening performed by DNA extraction and PCR, to determine the fetus genotype regarding its Rh status. During the study, if an Rh negative mother is confirmed to be carrying an Rh positive fetus, she would receive RhIG 300ug at 28 weeks gestation[1,2,5]. However, if the Rh negative mother was confirmed to be carrying an Rh negative fetus, she would not receive RHIG at 28 weeks.

In United States, the American Academy of Obstetrician's current guidelines recommend one dose of RhIG be administered at 28 weeks of gestation regardless the fetus Rh status. According to the study performed in Sweden, (5, 6) Rh negative mothers carrying a Rh negative fetus would not receive 28 weeks RhIG.

It is believed that before RhIG was discovered in 1968, the risk of sensitization of Rh negative mothers to produce anti-D when pregnant with a Rh positive fetus was 16%. The risk decreases to 1-2% when RhIG is administered up to 72 hours after delivery and to 0.1% if the mother also receives an additional 300ug dose RhIG at 28 weeks of gestation, but many countries only administer RhIG postpartum.[3,4]

In this particular case study, determining the Rh status of the fetus during the second pregnancy by the non-invasive method using a maternal EDTA blood sample would also have helped to conclude if the anti-D identified in the maternal circulation in the first trimester of the pregnancy could be residual passive anti-D, because the fetus from the second pregnancy could very well be Rh negative.

References:

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