Serology Case:
Anti-G vs Anti-D plus Anti-C

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Disclosure

• No relevant conflicts of interest
Learning Objectives

1. Serological investigation of a positive prenatal screen
2. Importance of correct interpretation of antibody titration
3. Vital importance of correct antibody identification for pregnant female
And I quote…

“Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning.”

Albert Einstein
Case Study

• 31 year old pregnant female (G5P1)
• Request for prenatal group and screen
• Pregnant for the 5th time (7 weeks gestation), referring lab reported:
  • Anti-D titre 1:8
  • Anti-C titre 1:32
# Initial Testing

<table>
<thead>
<tr>
<th>-A</th>
<th>-B</th>
<th>-D</th>
<th>Cont</th>
<th>A1C</th>
<th>BC</th>
<th>ABO/Rh</th>
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<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>O Neg</td>
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<table>
<thead>
<tr>
<th>Screening Cells</th>
<th>MTS Gel</th>
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<tr>
<td>R1R1</td>
<td>2+</td>
</tr>
<tr>
<td>R2R2</td>
<td>2+</td>
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### Initial Panel

**Serology Case**

<table>
<thead>
<tr>
<th>Supplier Lot #</th>
<th>Donor/ RhHr-Vial</th>
<th>RhHr</th>
<th>Kell</th>
<th>Duf</th>
<th>Kid</th>
<th>Low</th>
<th>P</th>
<th>MN</th>
<th>Lat</th>
<th>X</th>
<th>Additional Antigens</th>
<th>Patient’s Plasma Test Results</th>
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<tbody>
<tr>
<td>1</td>
<td>Ortho-Cl VRC177</td>
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<td>IAT 3+ 4+</td>
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<td>Ortho-Cl VRC177</td>
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<td>IAT 2+ 4+</td>
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<td>IAT 3+ 4+</td>
</tr>
</tbody>
</table>

**Evaluation:**
- IAT: Can Not Exclude D, C, Cw, V, Kpa, Jsa, Lua
- These are tentative rule-outs.
- Additional confirmatory data based on your own criteria are recommended.

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**St. Michael's**

*Inspired Care. Inspiring Science.*
Initial Testing

• Initial Panel showed pattern of anti-D + anti-C

• Patient antigen typing:
  • D neg, C neg, E neg, c pos, e pos
## Titration Result

<table>
<thead>
<tr>
<th>Reciprocal of plasma dilution</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
<th>512</th>
<th>1024</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D+C-</strong> ((R2R2))</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>D-C+</strong> ((r’r))</td>
<td>3+</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
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</tbody>
</table>

**Anti-D titre 8**  
**Anti-C titre 32**  
Unusual to have anti-D titre weaker than anti-C titre
Pregnancy History

- 1st pregnancy: ectopic in 2004, Antibody Screen Test (AST) negative, patient received RhIG

- 2nd pregnancy: term delivered in 2005, AST negative, patient received RHIG at 28 weeks and post delivery
Pregnancy History

- **3rd pregnancy**: therapeutic termination at 7 weeks gestation in July 2005, **AST negative**, patient received RhIg post procedure

- **4th pregnancy**: spontaneous miscarriage in 2008 during first trimester, **AST negative**, patient received RHIG
Pregnancy History

- Currently pregnant for the 5th time
- All 5 pregnancies with the same partner
- The Rh phenotype of the father of the fetus:
  - D+C+E-c+e+
Transfusion History

- In 2008 patient received 4 units of Rh Negative Red Blood Cells (RBC) for anemia due to hemorrhagic ovarian cyst
Is this truly anti-D?

- Patient received RhIG at appropriate intervals during each previous pregnancy
- Patient transfused only with Rh Negative RBC
- No recent RhIG
Is this truly anti-D?

- Patient transfusion and pregnancy history with unusual titre results suggested anti-G rather than anti-D plus anti-C
What is G?

• G antigen belongs to Rh blood group system
• G present on nearly all D and/or C positive rbc
• G is absent on nearly all D and C negative rbc
### What is G?

<table>
<thead>
<tr>
<th>Phenotypes with G present</th>
<th>Phenotypes with G absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>D+C+ (R1, RZ)</td>
<td>D-C- (r, r”)</td>
</tr>
<tr>
<td>D+C- (R2, R0)</td>
<td></td>
</tr>
<tr>
<td>D-C+ (r’, r’)</td>
<td></td>
</tr>
</tbody>
</table>
What is anti-G?

- Anti-G looks like combination of anti-D & anti-C
- Anti-G can be stimulated by pregnancy or transfusion
- Anti-G can cause:
  - None to severe transfusion reaction
  - None to severe HDN (usually mild)
What is anti-G?

Donor

Patient

Anti-G (and/or anti-C)

http://www.bbguy.org/education/gandanti-G.asp
What is anti-G?

Donor

Anti-G (and/or anti-D)

http://www.bbguy.org/education/gandanti-G.asp
Confirmation of anti-G

- Specimen sent to reference lab for confirmation
- Results confirmed anti-G and possible anti-C
- Anti-D was NOT identified
Confirmation of anti-G

- Reference Lab results:
  - Patient plasma reacted with two cells that are D-C-G+
  - After alloadsorption 3x with r’r red cells, the alloadsorbed plasma was non-reactive, suggesting there is no anti-D
Confirmation of no presence of anti-D

Patient Sample

http://www.bbguy.org/education/gandanti-G.asp
Confirmation of presence of anti-D

Patient Sample

http://www.bbguy.org/education/gandanti-G.asp
Case Study Continued

- Anti-D was not identified, therefore the patient remains a candidate for RhIG
- RhIG was issued at 29 weeks
- Titre was monitored until 29 weeks when it reached level of 64, at which point fetal monitoring was recommended
Case Study Continued

• Patient delivered a full term male baby with Hgb of 216 g/L, bilirubin level of 98 µmol/L (normal range <130 µmol/L)
• Total bilirubin peaked on day 2, level 161 µmol/L
### Cord Blood Test Results

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO &amp; Rh</td>
<td>O Positive</td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td>4+</td>
<td></td>
</tr>
<tr>
<td>Eluate</td>
<td>Reactive with: D+C- cells, D-,C+ cells,</td>
<td>Anti-D (presumably due to RhIG) and anti-G eluted from cord cells.</td>
</tr>
<tr>
<td>Baby’s Rh phenotype</td>
<td>D+C-E-c+e+</td>
<td>Probable Genotype: R_0r</td>
</tr>
<tr>
<td>Mother’s Rh phenotype</td>
<td>D-C-E-c+e+</td>
<td>Genotype: rr</td>
</tr>
<tr>
<td>Father’s Rh phenotype</td>
<td>D+C+E-c+e+</td>
<td>Probable Genotype: R_0r’</td>
</tr>
</tbody>
</table>
Case Study Continued

- In spite of 4+ DAT, baby’s hemoglobin remained normal & baby did clinically well
  - HDFN caused by anti-G is usually less severe than with anti-D

- Infant was discharged on day 4
Case Study Continued

• Patient received post delivery RhIG

• Patient given Antibody Card
  • Blood Group, Antibodies Identified
  • Our hospital’s Transfusion Medicine contact info

• Letter sent to Primary Care Physician
  • Blood Group, Antibodies Identified
Case Study Continued

- Anti-G most likely developed secondary to transfusion of Rh Negative G+ (D-C+) units
Discussion

- Rh negative females of childbearing age should ideally receive Rh negative, C negative RBC to prevent allo immunization to G antigen.

- Every pregnant female with a pattern of anti-D and anti-C should be tested for the presence of anti-G.
Discussion

- Suspect possible anti-G, if titre with D+C-cells \( \leq \) titre with D-C+ cells, patient never been transfused with Rh positive blood product and has always received RhIG

- Pregnant patient with anti-G remains a candidate for RhIG
Final Remarks

• **For transfusion:**
  - Differentiation of anti-D, -C and -G is not necessary, just transfuse D-C- RBC

• **In pregnancy:**
  - The presence of anti-G and/or anti–C versus the presence of anti-D is vital for decision to administer RhIG and to reduce the risk of HDFN
And I quote…

“The only source of knowledge is experience”

Albert Einstein
Thank you