Rhesus System: Picking up the Pieces

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ASCLS-MT Spring Conference
Missoula, MT
April 23, 2015

Objectives
At the end of the session, participants will be able to:

- compare/contrast Weak D/ Partial D (altered) phenotypes
- Differentiate the Partial D vs Weak D State
- Compare different manufacturer clones and impact in RH testing
- Understand the AABB Taskforce Recommendations on Molecular Testing for Weak D

A little history…..

1939: Levine/Stetson – Erythroblastosis fetalis
1940: Landsteiner/Weiner - serum from rabbits immunized w/ RBCs of Rhesus monkey
1946: Letters to Editor - Nature 131: 25-26 (06 July 1946) - doi:10.1038/131025a0
A New Rh Allelomorph
F. Stratton

RhD and RhD variants
“While the agglutination of this blood sample by certain anti-D sera was strong, using others it was weak, with only a few cells being clumped, and some gave completely negative results.”

1948/1950: Race, Renton, Stratton – IAT required to detect D⁺
1951/1953: Anti-D found in D⁺ person – first clue that partial antigens existed
1958: Standards, 1st edition
   Donor RBCs: testing for D⁺ required
   Transfusion recipients’ RBCs: anti-D serum was “sufficient”

   Addressed RhIg for first time
   Test mother’s RBCs same as a blood donor’s

1981 ACOG: RhD-negative women “whether D⁺-positive or D⁺-negative” were candidates for RhIg
1981 ACOG: “A woman who is genetically D⁺ positive is Rh-positive and RhIg is unnecessary.”

1992: D⁺ is renamed weak D
1992 ACOG: “A woman with a weak D is Rh-positive and should not received RhIg.”
1999 ACOG: Ditto

Requirements weak D test on donors
Requirements weak D test on babies born to RhD-neg moms
Weak D test for transfusion recipients/OB’s unnecessary

Standards, 29th ed
Many epitopes
"Conventional" D pos – all epitopes expressed
Prevalence:
- Caucasians – 85%
- Sub-Saharan Africa – 95%
- Eastern Asia - >99.5%
Serologic weak D – decreased level of ag expression
- weaker than expected (< 2+) reactivity with multiple examples of monoclonal anti-D
- Generally, not at risk of forming anti-D

Partial D – changes in, or absence of, epitopes
- absence of reactivity with one or more examples of monoclonal anti-D
- At risk of forming anti-D

Variation in D Ag Expression

Quantitative variant
All epitopes present
Changes in transmembrane or cytoplasmic region
Prevalence in Caucasians = 0.2 – 1%
80+ Weak D types (Types -1,-2,-3 ~90%)
Not (usually) associated with alloanti-D development ★

Alloimmunization has been observed with other weak D types:
- type 4.2
- DAR
- type 11
- type 15
- type 21
- type 57
**Partial D Phenotypes**

Qualitative variant
RhD protein missing D epitopes
Changes predicted to be in external loop
In U.S., most persons expressing partial D phenotype are of African ancestry
Alloanti-D can be made against missing epitopes

**RhD category VI (DVI)**

Clinically most important partial D
- Transfusion recipients – categorize as D-neg
- Donors – categorize as D-pos

**Normal vs Weak vs Partial**

- Presents as RhD-negative by conventional typing methods
- Adsorption/elution studies required for detection serologically
- Averages 22-36 RhD antigen sites/cell
- More common among persons of Asian ancestry
- Between 10-33% of Japanese, Chinese, and Korean red cell samples shown to be D neg serologically, found to be DEL
- RhD alloimmunization has occurred when RhD-negative/DEL-positive RBCs were transfused to RhD-negative recipients
"Heterogeneous antibodies are not optimal as reagents for use in serologic testing because they can vary in concentration, serologic properties, and epitope recognition and can contain other antibodies of unwanted specificity."

"The ideal serum for serologic testing is a concentrated suspension of highly specific, well-characterized, uniformly reactive, immunoglobulin molecules."

**Polyclonal vs Monoclonal Abs**

**Polyclonal**
- Mixture of antibodies
- Directed against different epitopes
- Affinity for Ag varies
- Supply limited

**Monoclonal**
- One antibody type made from one progenitor cell line
- Directed against specific epitope
- Consistent affinity for Ag
- Limitless supply

**Monoclonal - One Epitope**

>200 RHD alleles characterized by mutations that lead to qualitative and/or quantitative changes in serologic expression of the D antigen.

Probable RHD Genotype: RHD*weak D type 11/RHD*01N.01
Predicted phenotype: weak D+ C+ E+ c+ e+
Comments: Weak partial 11 RBCs can be associated with a DEL or weak D phenotype depending on the phase of inheritance of an RHCE*Ce allele. Given the RH genotype of this individual, it is not possible to determine whether the RHD variant allele is linked to the RHCE*Ce or RHCE*ce allele.
A 65-year-old African American woman presents to the hospital with anemia. The patient is a blood donor and has been previously tested as Rh positive. Routine blood typing prior to transfusion reveals an Rh negative phenotype. A week D test is performed and found to be positive. The sample is sent for Rh genotyping and the results show a partial D phenotype (Dm).

The patient should be managed as:

A. RhD positive and requires RhD positive Red Blood Cells for future transfusions.
B. RhD negative and requires RhD positive Red Blood Cells for future transfusions.
C. RhD partial D and requires RhD positive Red Blood Cells for future transfusions.
D. RhD partial D and requires RhD negative Red Blood Cells for future transfusions.

Correct Answer: D

Reference:

RhD-negative and requires RhD partial D Red Blood Cells for future transfusions.

With your answer choices, you will be able to answer this question again. Please read this page at any time to see an updated version of the % Responses.

Explanation:
- A partial D phenotype is an Rh variant that causes an amino acid substitution in the transmembrane or intracellular segments and expresses a reduced amount of D antigen (generally, less than 50% D antigen per RBC). A partial D, in contrast, typically occurs with certain amino acid substitutions on the extra-erythrocyte surface. Individuals with partial D phenotype may form anti-D when transfused with normal RhD positive blood due to the absence of critical D epitopes. For this reason, individuals with partial D alleles are managed as RhD negative as blood donors, but require RhD positive red blood cells as recipients.

Reference:
The Survey Says…….

1. Does your institution routinely perform an antiglobulin test for weak D on patients who test negative with anti-D on direct agglutination (ie, without AHG)?

<table>
<thead>
<tr>
<th>Year</th>
<th>1999</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>58.2</td>
<td>19.8</td>
</tr>
</tbody>
</table>

The Survey Says…….

2. If a patient tests positive with an antiglobulin test for weak D after testing negative with anti-D on direct agglutination (ie, without AHG), how do you report the patient's Rh type?

<table>
<thead>
<tr>
<th>Year</th>
<th>1999</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh positive</td>
<td>50.7</td>
<td>46.9</td>
</tr>
</tbody>
</table>

The Survey Says…….

3. If a patient is found/know to be a weak D phenotype, your policy would dictate transfusion with:

<table>
<thead>
<tr>
<th>Blood Component</th>
<th>Year</th>
<th>1999</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh-negative</td>
<td></td>
<td>43.5</td>
<td>49.2</td>
</tr>
</tbody>
</table>

The Survey Says…….

<table>
<thead>
<tr>
<th>No. of Patients or Donors Identified</th>
<th>% of Transfusion Services in 1999</th>
<th>% of Transfusion Services in 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>68.1</td>
<td>74.6</td>
</tr>
<tr>
<td>1</td>
<td>10.9</td>
<td>10.6</td>
</tr>
<tr>
<td>2</td>
<td>6.4</td>
<td>5.9</td>
</tr>
<tr>
<td>3</td>
<td>3.7</td>
<td>2.9</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>&gt;5</td>
<td>7.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>

• RhD alloimmunization has decreased
• Dichotomy in RhD typing has increased
Potential Benefits of *RHD* Genotyping

**Fiscal**
- ~$80/300μg vial
  - $200 X 24,700 = $4,940,000

**Safety**
- potential risk for transmission of infection
  - alloimmunized RhD-neg volunteer

**Ethical**
- unnecessary administration of a blood product
**Recommendations**

*RhD* genotyping be performed when
- discordant RhD typing results are encountered and/or
- when a serologic weak D phenotype detected in patients, including pregnant women, newborns, and potential transfusion recipients.

**Anticipated benefit**
- fewer unnecessary injections of RhIG
- increased availability of RhD-negative RBCs
New AABB Recommendations

- AABB 2014 TASKFORCE on D Testing
- 2 Year Recommendation
- Molecular Type all RH negative OB/prenatal patients