Quantity vs. Quality: Management of Weak and Partial D Patients

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Discussion Outline

• Understand why this topic is important
• Discuss background on D testing
• Explain “Weak D” terminology
• Differentiate Weak D Genotype and Partial D
• Understand How to Mitigate Risk of Anti-D
  • Describe who gets “Weak D” Testing
  • Review which products require selection by Rh type
  • Understand who needs Rh D negative blood
  • Discuss who needs Rh D genotyping
  • Which Genotypes need Rh D negative blood
  • Evaluate who needs RhIG
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D Antigen and Antibodies Are Particularly Important

• D is most immunogenic Rh antigen\(^1\)
  • 80% healthy D- volunteers exposed to ≥0.5 mL D+ RBC developed anti-D\(^2\)
  • 22% D- non-oncology hospital patients exposed 1-10 D+ RBCs made anti-D\(^3\)

• Alloantibodies against D are clinically significant
  • Cause severe hemolytic transfusion reactions (HTR)
  • Cause severe hemolytic disease of fetus and newborn (HDFN)\(^4\)

D Antibodies Are Preventable

- Require exposure
- RhD negative selection blood prevents antibodies
  - Easily found if in stock (RhD typing on unit label)
- RhIG administration following exposure to RhD prevents
- Prevention of anti-D → prevention of HTR, HDFN due to anti-D
So What Are Some Barriers to Preventing Anti-D?

- RhD negative blood limited
  - ~15% of Caucasians (main component of donor pool)
  - Must reserve for patients who need most

- Understanding risk of anti-D based on Rh D typing results

- Need adequate RhD typing methods

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Historical D Testing Reagents

• Polyclonal IgG
• Read result after immediate spin (IS)
  • Reagent anti-D + patient RBCs → centrifuge and examine for agglutination
• Caused agglutination with **majority** of D antigen carrying RBCs
• *But* sensitivity differed between reagents

False Negatives with Historical Anti-D Reagents

• 1946 report – blood donor RBCs agglutinated variably¹
  • Agglutinated with 20 anti-D sera
  • NO visible agglutination with 12 other anti-D sera

• 1958 Standards require “Weak D Test” to confirm D-negative in donors


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“Weak D Test”

• If no visible agglutination with anti-D at IS:
  - Incubate for 30 min., then wash to remove unbound anti-D
  - Next add IgG anti-human globulin (AHG) to RBCs
  - Incubate, centrifuge and observe for agglutination

Modern Anti-D Testing

• Overall more sensitive than historic reagents
• Most modern anti-D reagents are ‘blended’ mixture
  • Contain both IgG and IgM
    • Monoclonal IgM (specific for a single D epitope)
    • Monoclonal or polyclonal IgG
  • Often different anti-D clones/potentiating agents present
    • Impacts sensitivity from different commercial sources

False Negatives with Modern Anti-D Testing

- RBCs with normal levels of D agglutinated by IgM in test¹
- May be no or weak agglutination with
  - RBCs with depressed D antigen levels
  - RBCs lacking some D epitopes
- “Weak D” test still used for clarification

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Historic Use of Term “Weak D”

- Used inconsistently but generally applied if:
  - Variable agglutination for D antigen depending on sera used
  - “Weak D” testing required for visible agglutination with anti-D
- Previously called “Du” terminology, phased out after 1992
  - Modern, more sensitive tests showed many Du were D+
- Term “Weak D” for all weak expressions of D antigen suggested in 1992$^1$

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Modern Term: “Serologic Weak D Phenotype”

- For all *serologically* weak expressions of D antigen
  - Defined as “Weaker than expected” agglutination with anti-D
  - Most consider ≤2+ “weaker than expected”
- Modern term distinguishes serologic from molecular results
- Often incorrectly¹ referred to simply as “weak D”

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Serologic Weak D Phenotype

- Frequency estimate: 0.2-1% in U.S.¹
- “Weak D” testing still performed to clarify initial results
  - Term for test used by Standards is still “Weak D test”

¹ Andrea McGonigle 2019

Serologic Weak D Phenotype

- **Includes:**
  - Weak D genotypes
  - Partial D

- “Serologic weak D phenotype” if serologic testing is:
  - Weak
  - Negative with positive weak D test

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Weak D Genotype

- Decreased quantity of D antigen
  - Do not appear to lack D epitopes
- Majority caused by point mutations in Rh
  - Mutations lead to D antigen that cannot insert/be retained in RBC membrane like normal
- 1% of Caucasians

Weak D Genotype

• >150 different named types¹

• Weak D Genotypes 1-3
  • Account for most Serologic Weak D phenotypes of European ancestry
  • NOT at risk for clinically significant anti-D

• Most samples sent for RhD genotyping are Weak D Genotypes 1-3²
  • Thus, most Serologic Weak D Phenotypes not at risk for anti-D

• Some weak D genotypes are at risk for clinically significant anti-D

Partial D

- Most commonly picked up as anti-D in Rh D positive patient
  - Majority strongly positive in RhD typing
- But can be Serologic Weak D Phenotype
Partial D

- Altered or missing D epitopes
  - At risk for formation of anti-D
- 0.5-4% of patients\(^1\)
  - 0.5% of Caucasians
  - Up to 4% of African American and Hispanics

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Side By Side Comparison: Weak D Genotype vs. Partial D

- **Serologic testing may be same → “serologic weak D phenotype”**
  - Only Rh genotyping (molecular testing) can differentiate

- **Weak D Genotype**
  - Decreased quantity of D antigen
  - Appear to have all D epitopes
  - Most are NOT at risk for anti-D

- **Partial D**
  - Altered or missing D epitopes
  - ARE at risk for formation of anti-D

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How do we mitigate risk of anti-D formation?

- Serologic “Weak D” Testing
  - Employed to prevent or detect exposure to D antigen
- Rh D Genotyping to identify patients at risk for anti-D
- Select Rh D negative blood for those in need
- Provide RhIG for those in need
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Who Gets “Weak D” Testing?

- Required to perform testing for:
  - Blood donors
  - Infants born to Rh D negative mothers
- NOT required for all transfusion recipients\(^1\)
  - Large variation in testing practice
- Helpful in women of childbearing age with D typing discrepancy

\(^1\)Standards for Blood Banks and Transfusion Services. 30\(^{th}\) Ed. Bethesda, MD: AABB; 2016. p. 36.
Donors: Consequence of False Negative D Typing

- False negative $\rightarrow$ unit labeled Rh D negative *but* D epitopes present
- Patient exposed to D $\rightarrow$ potential anti-D formation

No agglutination with anti-D at initial testing

If no weak D testing

Unit labeled as Rh D Negative

D present in Unit
Donors Who Require “Weak D” Testing

- Required for all donors with negative Rh D test

No agglutination with anti-D at initial testing

Perform weak D testing

AHG causes anti-D coated RBCs to agglutinate

Unit labeled as Rh D Positive
Infants: Consequence of False Negative D Typing

- Infants born to Rh D negative mothers
  - False negative → report infant as Rh D negative
  - Leads to incorrect presumption that mom is not candidate for RhIG
  - Presence of D epitopes in neonate blood → maternal exposure → potential anti-D

- Or -

[Diagrams showing blood reactions and immune system responses]
Which Infants get “Weak D” Testing

- Infants born to Rh D negative mothers
- With negative or weak Rh D test
  - Think of fetus as blood “donor” to mom
  - Also consider neonate as potential future patient
Who Else Gets “Weak D” Testing?

• Optional when typing a patient
• Helpful for females <50 y.o. with D typing discrepancies, e.g.:
  • Typed as Rh D positive at one institution but negative by your testing (or vice versa)
  • By report, patient Rh D positive but negative by your testing (or vice versa)
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Selecting Rh D Negative Blood for Those in Need

- Selection considered for blood products containing RBCs (RBCs & PLTs)
  - Recall PLT components contain trace RBCs\(^1,2\)
- **Not** considered for acellular products (plasma & cryoprecipitate)\(^3\)
- “Rh Negative”
  - Appears on label of cellular & acellular products
  - Conforms to clinical terminology
  - May lead to questions

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Who needs Rh D negative blood?

• Any patient who already formed anti-D alloantibody
  • Prevents HTR
  • Male or female
• Intrauterine Transfusion
• Females <50 y.o. whom are at risk of forming anti-D
  • Rh D negative
  • Certain Weak D genotypes
  • All Partial D
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Who needs Rh D Genotyping?

- Prudent in females <50 y.o. with:
  - Unclear risk for anti-D
    - D typing discrepancies
    - Serologic weak D Phenotype
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Which Genotypes Need Rh D negative Blood?

- Weak D Genotypes other than 1, 2, 3, 4.0*, 4.1* (*or as specified in report)
- All partial D

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Who Is a Candidate for RhIG?

- Patients that have NOT formed anti-D alloantibody
- Prioritize females <50 y.o.
- Patients at risk of forming anti-D
  - Rh D negative
  - Weak D genotypes other than 1, 2, 3, 4.0*, 4.1* (*or as specified in report)
  - All Partial D
- And exposed to D antigen
  - Transfusion of Rh D+ blood (RBCs, PLTs)
  - Pregnancy with Rh D+ or Serologic Weak D Phenotype infant

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What if Rh D Genotyping Is Unavailable?

• No genotyping performed or treatment required prior to results

• If female <50 y.o. with Serologic Weak D Phenotype
  • Treat conservatively as Rh D negative
  • Results in some receiving Rh D negative blood unnecessarily
  • Avoids missing patients at risk for anti-D that cannot be distinguished without genotyping
## Summary: RhIG Candidacy in Pregnancy

<table>
<thead>
<tr>
<th>Mother’s test result</th>
<th>RhIG Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh D positive</td>
<td><strong>Not Indicated</strong></td>
</tr>
<tr>
<td>Rh D negative; no anti-D alloantibody</td>
<td>RhIG administration at:</td>
</tr>
<tr>
<td></td>
<td>- 28 weeks</td>
</tr>
<tr>
<td></td>
<td>- Delivery, if infant with Rh D antigen</td>
</tr>
<tr>
<td></td>
<td>- After event causing fetomaternal hemorrhage</td>
</tr>
<tr>
<td></td>
<td>&gt; abortion, ectopic pregnancy, abdominal trauma</td>
</tr>
<tr>
<td>Rh D negative; formed anti-D alloantibody</td>
<td><strong>Not indicated</strong></td>
</tr>
<tr>
<td>“Serologic Weak D Phenotype”; no anti-D alloantibody</td>
<td>- RhIG administration as with Rh D negative mom</td>
</tr>
<tr>
<td></td>
<td>- OR perform genotyping to determine need</td>
</tr>
<tr>
<td>“Serologic Weak D Phenotype”; formed anti-D alloantibody</td>
<td><strong>Not indicated</strong> (regardless of genotype)</td>
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## Summary: Patients Who Need RhIG After Transfusion

<table>
<thead>
<tr>
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<th>Exposure Type</th>
<th>RhIG Administration</th>
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</thead>
<tbody>
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<td>N/A</td>
<td>Not Indicated</td>
</tr>
<tr>
<td>Rh D negative; no anti-D alloantibody</td>
<td>Rh D+ Platelet</td>
<td>- RhoGam: 1 dose</td>
</tr>
</tbody>
</table>
|                                                  | Rh D+ RBCs    | - WinRho IV: 18 mcg/1 mL Rh D+ RBCs  
- Administer 600 mcg Q8 hours until total dose administered  
- mL RBC = Transfused mL * estimated packed-RBC Hct |
| Rh D negative; formed anti-D alloantibody        | N/A           | Not indicated                                                                        |
| “Serologic Weak D Phenotype”; no anti-D alloantibody | As above     | - RhIG administration as with Rh D negative  
- OR per genotyping to determine need               |
| “Serologic Weak D Phenotype”; formed anti-D alloantibody | N/A           | Not indicated (regardless of genotype)                                               |