Hello, is it D you’re looking for?

Red Cell Reference Laboratory – NSW/ACT

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Clinical Presentation - January 2014

- 50 y/o woman presents to Hospital 1 after a tractor roll over
  - Only clinical history is MVA 8 years prior
    - unknown prior transfusion/pregnancy

- Hospital testing on 24/01/14 demonstrated:
  - B Rh(D) Positive
    - weaker than usual reactions observed in Rh(D) typing (score 8 by CAT)
  - CDe, c-, E-, K-
  - Positive antibody screen with variation in reaction strength observed

<table>
<thead>
<tr>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Cell 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Strength</td>
<td>0</td>
<td>±</td>
</tr>
</tbody>
</table>
Clinical Presentation - January 2014

- Hospital testing on 24/01/14 cont.
  - 11 cell panel indicates probable antibody mixture due to variation in reaction strength

<table>
<thead>
<tr>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Cell 3</th>
<th>Cell 4</th>
<th>Cell 5</th>
<th>Cell 6</th>
<th>Cell 7</th>
<th>Cell 8</th>
<th>Cell 9</th>
<th>Cell 10</th>
<th>Cell 11</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Strength</td>
<td>0</td>
<td>++++</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Hospital suspects strongly reactive Anti-K with additional weak antibodies

- Sample re-collection date: 25/01/14
- Referred to RCRL for resolution of unexplained reactions
Clinical Presentation - January 2014

- RCRL testing demonstrated:
  - B Rh(D) Positive - a weakened expression of the Rh(D) antigen was noted (score 10 by IS with two different sources of anti-D)
  - Reverse group repeated with K- cells to resolve unexpected reactions
  - CDe, c-, E-; K-
  - Strongly reactive anti-K detected by saline RT, PEG-IAT and papain-IAT
  - Moderate strength anti-c detected by PEG-IAT and papain-IAT

- Request form indicated that samples were post-transfusion
  - Patient transfused on 24/01/14
  - Rh(D) investigation not pursued due to reported presence of transfused cells

- Further transfusion required - recommended R_1R_1; K- red cells
Clinical Presentation - January 2015

- The same patient presented one year later to Hospital 2 (~400km south) for ORIF of compound fracture following quad bike accident
  - Received transfusion on 22/01/15 (5 units of red cells)
  - Samples not referred to RCRL

- Approximately 1 week later, the patient presented to Hospital 3 (~100km further south)
  - Febrile and feeling unwell
  - Samples referred to RCRL
Clinical Presentation - January 2015

- Hospital 3 testing on 29/01/15 demonstrated:
  - **B Rh(D) Negative**
  - All screening cells, panel cells and auto control were reactive (score 8-10) by saline IAT and CAT-IAT
  - DAT positive (IgG specificity)

- Hospital suspecting warm AIHA
  - Samples were referred to RCRL for investigation
Clinical Presentation - January 2015

- RCRL investigations:
  - Upon receipt of the samples from Hospital 3, the patient’s historic file was retrieved
    - history of anti-c and anti-K one year prior
  - RCRL staff notified Hospital 3 of the possibility of a transfusion reaction due to the historic anti-c, and recommended postponing further transfusion (if possible) until RCR testing was complete
    - Hospital 3 prescribed IVIg and iron infusions to reduce the patient’s requirement for blood
  - RCRL staff contacted Hospital 2 to discuss their recent results (anti-D, -K)
    - Patient had been transfused 5 x Rh(D) Negative, K- red cell units:
      » 4x O \text{rr}
      » 1x O \text{r'r}
Post Transfusion Sample

- Sample from Hospital 3 (DoC 29/1/15)
- RCRL testing demonstrated:
  - B **Rh(D) Negative** with IgM anti-D reagent
  - Positive DAT - IgG (score 8) and C3d (score 1)
  - Anti-c and anti-K were detected by saline RT, RAM-IAT and papain-IAT
  - Anti-D was detected by RAM-IAT and papain-IAT
  - Eluate showed anti-D and anti-c specificity (acid glycine method)
- RCRL requested pre-transfusion sample from Hospital 2
Pre Transfusion Sample

- RCRL received the pre-transfusion sample (DoC 15/01/15) from Hospital 2
  - Positive DAT - IgG only (score 3)
  - Rh(D) Positive (suggestive of weak/partial D):
    - NOTE: Both control reagents reacted very weakly (+w) due to positive DAT
    - CDe, c-, E-, K--; Jk(a+b-); S+s-
    - Pre-transfusion sample was aging and of low volume
      - Further Rh(D) investigation not attempted
      - Recommended testing 3 months post transfusion to confirm patient’s Rh(D) type

- Unable to determine whether the anti-D was an auto or allo-antibody
  - Patient stable - Hb 78g/L
  - Recommended consultation with RCRL if further transfusion is required (as patient may require rare r’r’ units)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>IS</th>
<th>37°C</th>
<th>IAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D #1</td>
<td>0</td>
<td>+++</td>
<td>Not Tested</td>
</tr>
<tr>
<td>Anti-D #2</td>
<td>0</td>
<td>0</td>
<td>+++s</td>
</tr>
</tbody>
</table>
And then......

- May 2015 - BloodNet order submitted by Hospital 2 requesting
  - 2 x rare r’r’ red cell units to support surgery (femur repair)

- As the patient was now >3 months post transfusion, RCRL requested samples for Rh(D) phenotyping, genotyping and antibody investigation. RCRL testing demonstrated:
  - Rh(D) status inconclusive serologically (as control was similar strength)
  - Positive DAT - IgG (score 5)
  - Anti-D, anti-c and anti-K detected
  - Eluate showed a preference for Rh(D) positive cells
  - Anti-D was removed by auto adsorption
Auto vs Allo Anti-D

- Geoff Daniels recommends when referring to Weak D Types that the anti-D:
  
  “should only be dismissed as auto-anti-D if there were a positive DAT, anti-D could be eluted from the patient’s red cells, and/or the patient’s red cells adsorb the anti-D from autologous plasma” *


- This patient exhibits all 3 of these characteristics, therefore the anti-D was strongly suspected to be an auto-antibody

- Whilst awaiting RHD genotyping results, RCRL called in rare r’r’ donors for this patient’s upcoming surgery
  - 2 x r’r’ red cell units were issued to Hospital 2 for crossmatch
The Outcome

- Genotyping results from RCRL in Brisbane:
  - Weak D type 3
  - *RHD* zygosity testing showed only one *RHD* gene detected

- The patient therefore appears to be $R_1r'$ (CDe/Cde)
  - It is noted that the C antigen in trans position is associated with weakened expression of the Rh(D) antigen (Ceppellini effect), which may further impact on Rh(D) antigen expression in this patient

- The 2 units of $r'r'$ red cells were transfused following surgery
  - A third unit of blood was requested by hospital haematologists post-op as the patient’s Hb dropped to 63 g/L however it was decided not to transfuse.
D Variants (weak and partial D)

- Reduced expression of the Rh(D) antigen occurs in <1% of Caucasians

- Weak D
  - Single point mutations in intracellular or transmembrane regions of the RhD protein
  - Affect the efficiency of insertion and therefore quantity of RhD protein in red cell membrane

- Partial D
  - \textit{RHD}/\textit{RHCE} hybrid genes
  - Single point mutations in extracellular RhD protein segments
  - Multiple missense mutations dispersed across the \textit{RHD} gene
RHD alleles with single missense mutations

- Amino acid substitutions causing weak D types are shown in black (intracellular or transmembrane); those relating to partial D are shown in grey (extracellular).

D Variants

- More than 200 different D variants have been described

- D variants can be separated into four different allele clusters
  - Eurasian
  - African DAU
  - African weak D type 4
  - African DIVa
Weak D

- Weak D types 1-84 have been reported, with several subtypes
- Weak D types 1, 2 and 3 are the most common
  - Together they account for approximately 95% of all weak D’s
  - Great variation in frequency can be observed based on geography

**Distribution of the molecular weak D types in three different regions**

<table>
<thead>
<tr>
<th></th>
<th>Tyrol (Austria)</th>
<th>Northern Germany</th>
<th>South-western Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak D Type 1</td>
<td>43 (33%)</td>
<td>169 (65%)</td>
<td>95 (60%)</td>
</tr>
<tr>
<td>Weak D Type 2</td>
<td>10 (8%)</td>
<td>44 (17%)</td>
<td>43 (27%)</td>
</tr>
<tr>
<td>Weak D Type 3</td>
<td>65 (50%)</td>
<td>45 (17%)</td>
<td>7 (4%)</td>
</tr>
<tr>
<td>All other types</td>
<td>12 (9%)</td>
<td>2 (1%)</td>
<td>14 (9%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>130</td>
<td>260</td>
<td>159</td>
</tr>
</tbody>
</table>

Weak D Antigen Density

Table 3. RhD antigen densities of the prevalent weak D types

<table>
<thead>
<tr>
<th>Weak D Type</th>
<th>Median</th>
<th>Mean†</th>
<th>Range</th>
<th>Phenotype</th>
<th>Samples Tested (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>759</td>
<td>795</td>
<td>533-1283</td>
<td>CcDee</td>
<td>25</td>
</tr>
<tr>
<td>Type 2</td>
<td>674</td>
<td>625</td>
<td>446-818</td>
<td>ccDEe</td>
<td>24</td>
</tr>
<tr>
<td>Type 3</td>
<td>1948</td>
<td>1926</td>
<td>1333-2650</td>
<td>CcDee</td>
<td>11</td>
</tr>
<tr>
<td>Type 4</td>
<td>1872</td>
<td>1919</td>
<td>1687-2406</td>
<td>ccDee</td>
<td>7</td>
</tr>
<tr>
<td>Type 5</td>
<td>316</td>
<td>311</td>
<td>218-386</td>
<td>ccDEe</td>
<td>6</td>
</tr>
</tbody>
</table>

*Estimated antigen density based on the geometric mean of the number of epitopes detected with 6 IgG anti-D (BS221, BS227, BS228, BS229, BS231, H41).
†Geometric mean of the antigen densities of the different samples.

Weak D

- The most common weak D types 1, 2 and 3 are not associated with allo-anti-D immunisation
  - It is therefore generally accepted in the literature that weak D types 1, 2 and 3 can be treated as Rh(D) positive for transfusion and do not require administration of Rh(D) Immunoglobulin in pregnancy
  - However, unless genotyping is performed we can not be certain that a patient is a weak D type 1, 2 or 3

- There is increasing momentum globally to introduce genotyping for all weak D patients in an effort to reduce unnecessary usage of Rh(D) Immunoglobulin and depletion of the Rh(D) negative blood supply
Back to our patient…

- Weak D type 3
  - First published in 1999
  - Single amino acid substitution 8 C>G
  - Ser to Cys at codon 3
  - Typically associated with CDe haplotype
  - May require IAT method for detection

- No alloanti-D has ever been documented
Discussion points

- History of anti-c not known to Hospital 2
  - Whilst all hospitals involved now share a common computer system, this patient’s records had not been migrated over to the new system
  - Hospital 1’s previous computer system contained a transcription error in the patient’s file - anti-C was entered rather than anti-c

- Why does the patient’s Rh(D) expression change over time?

- What blood would you recommend for this patient?
## Patient Test Result Timeline

<table>
<thead>
<tr>
<th>Sample Collection Date</th>
<th>Laboratory</th>
<th>Rh(D) phenotype</th>
<th>DAT</th>
<th>Antibodies Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>24/01/2014</td>
<td>Hospital 1</td>
<td>Positive (score 8)</td>
<td>Negative</td>
<td>Sent to RCRL</td>
</tr>
<tr>
<td>25/01/2014</td>
<td>RCRL</td>
<td>Positive (score 10)</td>
<td>Negative</td>
<td>Anti-c Anti-K</td>
</tr>
</tbody>
</table>

**Patient transfused Rh(D) positive blood**

<table>
<thead>
<tr>
<th>Sample Collection Date</th>
<th>Laboratory</th>
<th>Rh(D) phenotype</th>
<th>DAT</th>
<th>Antibodies Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/01/2015</td>
<td>Hospital 2</td>
<td>Negative</td>
<td>Positive (IgG score 8)</td>
<td>Anti-D Anti-K</td>
</tr>
<tr>
<td>15/01/2015</td>
<td>RCRL</td>
<td>Negative (Immediate Spin) Positive IAT (score 10)</td>
<td>Positive (IgG score 3)</td>
<td>Insufficient sample</td>
</tr>
</tbody>
</table>

**Patient transfused c positive blood**

<table>
<thead>
<tr>
<th>Sample Collection Date</th>
<th>Laboratory</th>
<th>Rh(D) phenotype</th>
<th>DAT</th>
<th>Antibodies Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>29/01/2015</td>
<td>Hospital 3</td>
<td>Negative</td>
<td>Positive (IgG score 8)</td>
<td>Panagglutination Sent to RCRL</td>
</tr>
<tr>
<td>29/01/2015</td>
<td>RCRL</td>
<td>Negative</td>
<td>Positive (IgG score 8 C3d score 1)</td>
<td>Plasma: Anti-c Anti-D Anti-K Eluate: Anti-c Anti-D</td>
</tr>
</tbody>
</table>

**21/05/2015 (>3 months post transfusion)**

<table>
<thead>
<tr>
<th>Sample Collection Date</th>
<th>Laboratory</th>
<th>Rh(D) phenotype</th>
<th>DAT</th>
<th>Antibodies Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>21/05/2015</td>
<td>RCRL</td>
<td>Negative (Immediate Spin) Inconclusive IAT (control cells similar strength)</td>
<td>Positive (IgG score 5)</td>
<td>Plasma: Anti-c Anti-D Anti-K Eluate: Preference for Rh(D) positive cells Auto adsorption removed anti-D</td>
</tr>
</tbody>
</table>

Genotype: Weak D type 3 One RHD gene detected

*Control cells weakly positive due to positive DAT*