Case Studies from the Trenches: Unraveling The Mystery

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Medical Director
Lions Blood Bank New Delhi
ISTM Conference Bhopal
18th November 2016
Case 1
History

- 65 years, male patient
- Diabetes, Hypertension, CKD, S Anaemia
- Haemoglobin 5.2 gm%
- On dialysis for last one year
- Historical Blood group O Positive
- H/o past transfusion
  - Two units from our blood bank ten months ago (January 2011)
  - Two units from another facility one year ago (2010)
# Type & Screen and DAT

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<th>Antibody</th>
<th>Screen</th>
<th>DAT</th>
<th>AHG</th>
<th>Poly</th>
<th>IgG</th>
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- Antibody Screen DAT AHG Poly IgG C3d
- O1 2+ 3+ 3+ 0
- O2/O3 0/0 1+ mf Auto control 2+
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## Rh Phenotype

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Interpretation

• Looks Like a simple case
• Blood group O Negative
• Antibody screen Positive
• Antibody identified Anti-C
• Patient’s phenotype C- E- c+ e+
• Cross-match compatible with O Negative PRBC

Dilemma ?
Dilemma?

• What is the correct blood group?
  O Positive (Historical) or O Negative (current)?

• If the historical blood group were incorrect and transfusion with O positive PRBC an error?

Then

• Why patient did not form anti-D?
  Antigen D is more immunogenic than C

• How to explain Positive DAT- IgG?
Review the Records

• Old records from the archives reviewed
• Group O Positive, Antibody screen Negative
• Sought fresh blood sample for repeat tests
• Repeat tests by tube and CAT- O Negative
• Cross match with O Positive units incompatible and O negative units compatible
• O negative units issued and transfusion uneventful

But Dilemma persisted
# Routine Worksheet

## Specimen Labeled

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<tr>
<th>HOSPITAL</th>
<th>DR. Poonam Shrivastava</th>
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**Date & Time Recd.**

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<th>DATE PROCESSED</th>
<th>TECH</th>
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### Rh Reagent

- O' Neg units x-matched by Rhunit

### Crossmatched Units

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### Interpretation & Comments

- Patient's grouping by CAT
- Lot No: ACC480A
- Exp - 2012-03-09

- Matching: Anti A, Anti B, Anti D
- Repeat Sample: 0
Patient an Ex army man and blood Group mentioned on the Card also “O Positive”
Antibody Screen another method

<table>
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<th>Donor</th>
<th>Rh - Hr</th>
<th>Kell</th>
<th>Duffy</th>
<th>Kidd</th>
<th>Lewis</th>
<th>P</th>
<th>MN</th>
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* indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.

An antigen designated with a 'W' represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.
**AB identification: 20 cell cell panel**

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**25-01-2017 Dr Poonam Shrivastava 13**
### ANTIBODY PANEL: Enzyme / Eluate

(CAT - IAT)

|   | D | C | E | c | e | K | Fya | Fyb | Jka | Jkb | Lea | Leb | S | s | M | N | P1 | **Enzyme** | **Eluate** |
|---|---|---|---|---|---|---|-----|-----|-----|-----|-----|---|---|---|---|---|----------|----------|
| 1 | + | + | 0 | 0 | + | 0 | 0   | +   | +   | 0   | 0   | + | + | + | 0 | 0 | 4+        | 3+        |
| 2 | + | + | 0 | 0 | + | 0 | +   | +   | 0   | 0   | + | + | + | 0 | + | 4+        | 3+        |
| 3 | + | 0 | + | + | 0 | 0 | 0   | 0   | +   | 0   | + | + | 0 | + | 0 | 4+        | 3+        |
| 4 | + | 0 | 0 | + | + | 0 | 0   | 0   | +   | 0   | 0   | 0 | 0 | + | 0 | + | 4+        | 3+        |
| 5 | 0 | + | 0 | + | + | 0 | 0   | +   | +   | +   | +   | 0 | + | + | + | + | 4+        | 3+        |
| 6 | 0 | 0 | + | + | + | 0 | 0   | +   | +   | 0   | 0   | + | + | + | + | 0 | 4+        | 3+        |
| 7 | 0 | 0 | 0 | + | + | + | 0   | +   | 0   | +   | 0   | + | + | + | + | 0 | 4+        | 3+        |
| 8 | 0 | 0 | 0 | + | + | 0 | +   | 0   | 0   | 0   | 0   | 0 | 0 | + | + | 0 | 4+        | 3+        |
| 9 | + | + | 0 | 0 | + | 0 | 0   | +   | 0   | +   | 0   | + | 0 | + | + | 4+        | 3+        |
| 10| + | 0 | + | + | + | 0 | +   | 0   | +   | 0   | +   | 0   | + | + | + | 4+        | 3+        |
| 11| + | + | 0 | 0 | + | + | 0   | +   | +   | +   | +   | 0   | + | + | + | + | 4+        | 3+        |

25-01-2017 Dr Poonam Shrivastava
Possibilities?

- O Negative / Partial D with Allo anti-D and anti-C
  Or
- AIHA with Auto anti-D and Allo anti-C
  Or
- Mimicking auto antibody with apparent D and C specificity

- The auto antibody nature can be demonstrated by autologous and allogenic adsorption studies
  X Sample QNS for auto adsorption
  ✓ Allo adsorption
**Alloadsorption**

<table>
<thead>
<tr>
<th></th>
<th>Alloloadsorbed Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1R1</td>
<td>D+ C+ c- E-e+</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>R2R2</td>
<td>D+ C- c+ E+ e-</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>rr</td>
<td>D- C- c + E- e+</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

**Interpretation**

- The antibodies are totally adsorbed by D+ C+ and D-C- cells.
- This is quite unlike the behaviour of allo anti-D and -C which would be adsorbed only by D+ and C+ cells.
- **Warm autoantibodies with apparent specificity anti-D and anti-C**
Lessons learnt

• Auto antibodies at times have patterns of reactivity that suggests the presence of alloantibodies
• Autoantibodies may appear as mimicking alloantibodies
• Apparent specificity for simple Rh antigen (D,C,c,E,e) is occasionally seen specially in saline or LISS IATs
• AIHA is associated with suppression (sometimes total) or alteration of antigen expression. Antigens affected are Rh, Kell, Jk^a, En^a etc (Marion E. Reid; Blood group antigens and antibodies,201)
• Mimicking antibodies, whether auto- or alloantibodies, are there to confuse us and delay the issuance of compatible blood components.
Case 2
History

• 32 years, female patient,
• Diagnosis - Severe Anemia with MTP
  Incomplete abortion, bleeding pv 15 days
• Married, three children, LCB 8 years, all normal deliveries
• No history of prior blood transfusion
• Hb 4.4 gm%
• Compatible Blood not found in two blood banks
• Referred to Lions from a far away village of Delhi for urgent requirement of three units PRBCs at 2.30 am
Tests at Lions

• Blood Group- (Tube Forward and reverse) A Positive
• Antibody Screen (CAT LISS IAT) Positive 3/3, all 4+
• Antibody identification 11 cell panel (CAT LISS IAT) Positive 11/11 all 4+
• Auto control (CAT,LISS IAT) Positive 4+
• Cross match 3 units (CAT LISS IAT) Incompatible all 4+
Antibody Screen and Cross match
15/10/15
Sudha
Gel - Specific Antibody
Antibody Identification
Possibilities considered

• Antibody against high frequency antigen
• Autoimmune Hemolytic Anemia
Direct Antiglobulin Test

- **CAT** Poly specific AHG
  - Negative

- **Tube test Saline AHG**
  - Poly  Negative
  - IgG  Negative
  - C3d  Negative
Interpretation

- In view of Positive antibody screen
  Incompatible cross match,
  Positive auto control and
  Negative DAT

- Possibilities considered were
  - Antibody to ingredient in enhancement medium constituent
  - Enhancement dependent autoantibody

- Decided to repeat testing in another medium
Repeat Antibody Screen–Tube AHG IgG

Antibody Screen–
(Tube saline  AHG IgG)  Negative 3/3
Auto control (Tube saline  AHG IgG)  Negative

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Antibody Screen - Pooled cells by SPRCA Neo

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample ID</th>
<th>ABO</th>
<th>RH</th>
<th>RHC-A-B AB-D1 D2 A1 B</th>
<th>PHENO RH</th>
<th>KELL</th>
<th>IAT</th>
<th>Weak D</th>
<th>Operator</th>
<th>Done on</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>A</td>
<td>RH+</td>
<td>-3-344-3</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td>SONALI</td>
<td>2015/10/15</td>
<td></td>
</tr>
</tbody>
</table>

Antibody Screen - Pooled cells by SPRCA Neo, Negative
Repeat Antibody Screen-IgG Cards

- Polyspecific CAT Cards used have Dextran besides Glass beads and Buffer

- IgG cards contain PEG

  Antibody Screen using IgG cards was Positive 2+
Cross match

• Patient’s Rh Phenotype  C+ c- E- e+ K –
• Cross match (Tube saline  AHG IgG)
  - 3 units A positive Rh Kell matched PRBCS  cross matched
  - All units found compatible
• Case discussed with the treating gynecologist
• Blood issued with instructions
• Transfusion uneventful
Lessons learnt

• Antibodies to reagent components and additives can cause positive results in antibody detection, identification and cross match
• Implicated ingredients include –parabens (in some LISS additives), sodium caprylate (in some albumins), and thimerosal (in some LISS/saline preparations)
• Most of anomalous reactions are in vitro phenomenon and have no clinical significance in transfusion therapy
• But cause lab problems and delay in transfusion
• DAT is savior
Case 3
History

• 36 years female
• 3\textsuperscript{rd} gravida, three months pregnant, one living child from first pregnancy. The second pregnancy had to be terminated in second trimester due to severe pre-eclampsia.
• Blood Group O positive and ICT 2+ Positive detected on routine screening
• Referred to Lions for antibody Identification
Tests at Lions

• Blood Group  O Positive
• Antibody Screen  (CAT Polyspecific)
  All 3 cells Negative
• Antibody Identification (CAT Polyspecific)
  All 11 cells Negative
• Positive ICT in hospital was done by Gel cards Biorad
• Possibility - ? Antibody against low frequency antigen

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• If antibody in the serum of a pregnant woman is suspected of reacting with a low prevalence antigen, testing the father’s red cells with maternal plasma (if ABO compatible) can provide the possibility of incompatibility with the fetus and can make identifying the antibody unnecessary.

*(Ref: AABB Technical manual sixteenth edition -491)*
**Phenotype: Husband and Wife**

Cross match between husband’s red cells and maternal plasma compatible

<table>
<thead>
<tr>
<th></th>
<th>ABO Rh</th>
<th>C</th>
<th>c</th>
<th>E</th>
<th>e</th>
<th>K</th>
<th>Jka</th>
<th>Jkb</th>
<th>S</th>
<th>s</th>
<th>M</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wife</td>
<td>O Pos</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>Husb and</td>
<td>O Pos</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>1+</td>
<td>3+</td>
<td>3+</td>
<td>1+</td>
</tr>
</tbody>
</table>
Direct Antiglobulin Test (DAT)

- **CAT** using Poly specific AHG Cards    Positive 4+
- **Tube test Saline AHG**
  - Polyspecific Positive 4+
  - IgG Positive 4+
  - C3d Negative
- **Autocontrol**
  - CAT Positive 4+
  - Tube AHG IgG 4+
## Repeat Antibody Screening & Elution

<table>
<thead>
<tr>
<th></th>
<th>CAT (Poly AHG)</th>
<th>Tube (Poly AHG)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All 3 cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>Negative</td>
<td>1+</td>
</tr>
<tr>
<td>Plasma</td>
<td>Negative</td>
<td>1+mf</td>
</tr>
<tr>
<td>Eluate</td>
<td>Positive 4+</td>
<td>W+</td>
</tr>
</tbody>
</table>

- **IS**
  - Serum: 1+
  - Plasma: 1+mf
- **37**
  - Serum: 1+
  - Plasma: 2+mf
- **AHG**
  - Serum: 2+
  - Plasma: 2+mf
  - Eluate: W+
Impression

- It looks like warm autoantibody IgG
- But no signs and symptoms of hemolysis
- Hb 13.1g%,
- LFT, KFT and all investigations Normal Limits
- What could be the cause of Positive DAT and autocontrol?
Review history

• Called back the patient and took detailed history
• She had been taking Tab Aldomet for last 2 years. Drug was started when she developed hypertension during 2\textsuperscript{nd} pregnancy at 5 months gestation and continued even after termination of pregnancy
• Positive DAT and Autocontrol could be due to drug Aldomet
Follow up

- Discussed the case with the Gyne
- Referred to hematologist. Aldomet was immediately changed to other anti hypertensive. BP was maintained 110/70 throughout the pregnancy.
- Delivered a full term normal, healthy baby
- Still on anti hypertensive
- Has not come back for DAT test in spite of repeated reminders and requests
- Lesson Learnt-
  
  Clue to solve the problem is hidden in the History
About Aldomet (Alpha Methyldopa)

- Induces drug independent autoantibodies that are serologically indistinguishable from those of WAIHA
- Red cells coated with IgG
- Eluate as well as the serum react with virtually all cells tested, in the absence of drug
- Blood group specificity has been demonstrated at times
- Proof that drug causes autoantibody production is difficult to obtain.
- Sufficient evidence would include demonstration that autoantibody production began after drug administration, resolution of the process after withdrawal of the drug and recurrence of autoantibody if drug is re-administered
About identifying antibodies

• No substitute for actual laboratory experience to become proficient in Antibody Identification
• Efficiently choosing and performing the available tests appropriately and analyse the clues to solve serological puzzle is its own reward
• Must maintain records
• Finding a compatible cross-match is a wonderful feeling
I keep six honest serving men
(they taught me all I knew)
Their names are What and Why and When and How and Where and Who!

Rudyard Kipling
Case of a Neonate

The Mirage
History

• Blood samples of a mother and her 5 day old neonate were referred to us for cross matching an appropriate blood unit.
• Mother’s Blood Group - B Negative
• Baby’s Blood Group - B Negative
• Mother’s Indirect Antiglobulin Test (IAT) Positive
• Baby’s Direct Antiglobulin Test (DAT) and antibody screening Positive.
History (cont)

- The baby had one exchange transfusion with O negative whole blood
- followed by transfusion of one unit B Negative red blood cells (PRBCs).
- Hb 7 gm/dl and S.bilirubin 25 mg/dl,
- Baby needed one more exchange transfusion
- But this time the cross-match was incompatible.
Investigations at Lions

**Mother:**
- Mother’s Blood Group **B Negative**
- Antibody screen Positive 4+ (2/3 cells)
- Antibody identification - Anti-D and anti-C
- Rh phenotype **C - c+ E - e+ K-**

**Baby: (tests not valid as post exchange)**
- Baby’s blood group **B Negative**
- DAT positive 4+
- Antibody screen Positive
- Antibody identification - Only Anti-D
- Rh phenotype **C+ c+ E- e+ K-**

**Father:**
- Father’s Blood Group **A Positive**
- Rh Phenotype as **C+ c- E- e+ K-**

25-01-2017
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Antibody Screen : Mother & Baby
Cross-match

- Compatible with O Rh D Negative C Negative units which were issued,
- Exchange transfusion successful and baby improved.

The Dilemma: Cause of severe haemolysis in B Negative baby of B Negative Mother?
Anti-G : Anti-C

- Titres of mother’s serum:
  a. Anti-D titre using R2R2 cells was 8192.
  b. Anti-C titer using r’r cell was 256.

- If it were anti-G, titre with r’r would have been greater than titre with R2R2 RBCs.

- Thus, the possibility of anti-G was ruled out.
Antibody identified

• Only anti-D in Baby’s Antibody identification panel
• Eluate revealed only anti-D
  D+ C- cells were agglutinated but
  D- C+ cells were not agglutinated
• Thus Anti-C ruled out
• Antibody identified- Anti-D
Baby’s Eluate : Antibody Screen
Eluate - Select Cells
Rh type of Baby – Pos or Neg?

- Presence of anti-D and clinically proven haemolysis raised questions about baby’s Rh D typing result – B Negative
- Baby’s pre-exchange sample from the parent blood bank was requested.
- It showed positive reaction (2+) with anti-D anti sera in tube testing. This confirmed that the baby was actually Rh D Positive.
- The weak reaction with anti-D could be due to prozone phenomenon as the titre of anti-D is very high (8192)
Conclusion

- This case also highlights the problems faced by Rh Neg Mothers.
- Facility for correct ICT testing and antenatal RhIg prophylaxis for prevention of HDFN not effective
- Rh D testing by slide method may be misleading.
- Tube testing is the gold standard.
Correct D testing Very important for RH Ig

Test for D antigen must by using two Anti-Ds, tube test

Case report
28 years, 8 months pregnant, G2P1 came for IAT as blood group B negative
Test in BB- B Positive, IAT Negative

Patient advised to bring her records
2001- B Neg outside lab
2002- B Neg
2003- FTND in some other outside Hosp, Anti-D given
2007- Currently 2nd pregnancy, B Neg in outside Hosp,
      Came to ESI in last trimester
      Tube test using 2 anti-Ds confirmed her to be B Pos

Slide test revealed 1+ agglutination after 30 seconds

25-01-2017 Dr Poonam Shrivastava
Thank you