Are U there?

Suzanne Cho
Diagnostic Services – Donor Testing
Canadian Blood Services
2014-10-03
MNS Biochemistry

- MNS is complex blood group system
  - 46 antigens
  - Glycophorin A, (GPA)
  - Glycophorin B, (GPB)

- Chromosome: 4q31.21

- Gene Name: GYPA (M/N), GYPB (S/s)
### U Biochemistry

<table>
<thead>
<tr>
<th></th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ficin/papain</td>
<td>Resistant</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Resistant</td>
</tr>
<tr>
<td>DTT</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

**Diagram:**

- GPB
- 20 Leucine
- 21 Serine
- 22 Threonine
- 23 Threonine
- 24 Glutamic acid

- Red cell membrane
- *Amino acids 1-19 are cleaved*
U Frequency

- Described in 1953, “almost universal distribution” of the antigen
- Expressed on cord RBCs
- S-s- phenotype ~ 0.1% in Whites, ~ 1.2% in African descent
  - Up to 35% among Pygmies of Congo
- S-s- phenotype encoded by two molecular backgrounds in those of African descent
- U- and U+var phenotypes
U-

- The S-s-U- phenotype results from a large deletion in *GYPB* gene
  - homozygous for *GYPB* deletion
  - Complete lack of GPB expression

- Can make anti-U
  - Associated with HTR and HDFN
  - Can bind to U\textsuperscript{var} red cells
## Anti-U Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Anti-U</th>
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<tbody>
<tr>
<td>Ig Class</td>
<td>IgG</td>
</tr>
<tr>
<td>Reaction Phase</td>
<td>IAT</td>
</tr>
<tr>
<td>Transfusion Reaction</td>
<td>Mild to Severe</td>
</tr>
<tr>
<td>HDFN</td>
<td>Mild to Severe</td>
</tr>
<tr>
<td>Auto-antibody</td>
<td>Yes (WAIHA)</td>
</tr>
<tr>
<td>Note</td>
<td>Heterogeneous</td>
</tr>
</tbody>
</table>

U+var

- S-s-U+var phenotype associated with the expression of GPB variants
- Result in decreased U expression
- Requires adsorption/elution for detection
- Among the S-s- individuals 50% are U+var and 50% are U-
  - Describes hybrid gene with He antigen
Molecular Basis of $U^{\text{var}}$

- **U variant:** $GYPB^*$ point mutations, homozygous for 1 mutation or heterozygous for both

  - $GYPB^*S_{\text{null}}$ (IVS5+5t) – mutation in intron 5
  - $GYPB^*S_{\text{null}}$ (230T) – mutation in exon 5
Case Report 1

활동

27-year old woman from Republic of Niger

Medical history:
- Sickle cell disease
- History of red cell transfusions
- gravida 2, para 0

Admitted in 30th week of gestation: anemia due to hemolytic crisis
- Hb: 89g/L (120-160g/L)
- LDH: 397 U/L (50-150U/L)

Case Report 1 Cont’d

- Blood Group: O Positive
- Phenotype: D+C-E+c+e+; K-k+
- Antibody Screen: Negative
- Management therapy:
  - Folic acid
  - Vitamin B12
Pre-Op Care

- 1 unit of PRBC transfused (T&S compatible)
  - O Positive
  - D+C-E+c+e+; K-k+

- Continued treatment with folic acid and vitamin B12
Cord Sample Findings

- Baby boy blood group: O positive
- DAT: Negative
- Phenotype: D+C-E+c+e+
Post-partum Findings

- Hb: 57 g/L 10 days post delivery
- 3 cell Antibody Screen: positive (4+ score)
- DAT: negative
- Cross-match: incompatible with all units tested
Subsequent Testing

- Tested mother’s serum against:
  - Ortho Panel 37C : 4+ panreactive
  - Ortho Panel 37C, ficin treated: 4+ panreactive
  - Ortho Panel 20C: 2+ panreactive

- Extended Phenotype:
  - Mother: Fy(a-b-); S-s-U-
  - Baby boy: Fy(a-b-); S-s+U+
Subsequent Testing (Cont’d)

- 2 additional Immucor panels (LISS 37C and Saline 20C) :
  - Fy(a-b-); S+s-
  - Fy(a-b-); S-s+
  - Fy(a-b-); S-s-U-He-

- All cells tested positive EXCEPT:
  - Fy(a-b-); S-s-U-He-
Case Report 2

- Perinatal patient in Edmonton, A.B.
- Medical history:
  - gravida 6, para 5
  - Unremarkable delivery in 2012
- History of Anti-U
- Phenotype: D+C-E+c+e+; K-; M-N+S-s-; Fy(a-b-); Jk(a+b+)
- EDD: 2014-09-21
Case Report 2 – Aug sample

- Blood Group: A Positive
- DAT: Negative
- Antibody Investigation: Anti-U
  - All cells tested positive EXCEPT:
    - S-s-U- (Immucor Panel)
    - Unable to exclude anti-C, anti-K, and anti-Fya
- Anti-U Titre: 8
**Patient Summary:**

Blood Group: A Positive  
Known Antibodies: Anti-U  
Phenotype: C- E+ c+ e+ M- N+ S- k- K fya- fyb- Jka+ Jkb+  
EDD: 2014-09-21  

<table>
<thead>
<tr>
<th>Test Performed</th>
<th>Results</th>
<th>Date Collected: 2014-08-27</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO/Rh</td>
<td>A Positive</td>
<td></td>
</tr>
<tr>
<td>Antibody Screen</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Direct Antiglobulin Test</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Antibody Investigation</td>
<td>Anti-U</td>
<td></td>
</tr>
<tr>
<td>Anti-U has been implicated in HDFN.</td>
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Antibody Titre  
Slight change in antibody titre not significant. It is most likely due to the inherent variability of titre method.

**Remarks:**

Anti-U is an antibody to a high prevalence red cell antigen. Allow maximum notice of possible transfusion. Consultation with transfusion medicine physician is recommended. Follow-up sample requested every two weeks.

Unable to exclude antibodies to the following blood group antigens: C, K, and Fya (no suitable cells available). Anti-C, -K and -Fya have been implicated in HDFN.
Father

- Blood Group: A Positive
- Phenotype: C- S- s+ K- Fya-
- Remarks:
  - No licensed source of U antisera available.
  - Father’s cells tested as U positive against mother’s plasma (as source of anti-U)
  - Zygosity cannot be determined
  - HDFN due to anti-U is a concern
Case Report 2 Sept sample

- Blood Group: A Positive
- DAT: Negative
- Antibody Investigation: Anti-U
  - All cells tested positive EXCEPT:
    - S-s-U- (Immucor Panel)
    - Unable to exclude anti-C and anti-K
- Anti-U Titre: 8
Case Report 2 - Cord findings

- Delivered on 2014-09-05
- Baby Boy Blood Group: A Positive
- Baby Boy DAT: Negative
- No blood required for Mom
U-Typed Units

- Request handled by Medical
- Specific donors contacted to donate (liquid units)
- ePROGESA search to identify U- donors (frozen units)
U-Typed Units

- Two ePROGESÁ Test Codes:
  - U – 4525
  - $U_{var}$ – 5380

- Previous U typing done a number of years ago

- Process used for testing might be unknown
U-Typed Units

- Commercial reagent for testing donor/patient red call samples for the U-Antigen is not available.
- NIRL has ‘unlicensed reagent’. Frozen patient/donor sample previously identified with an Anti-U.
- Genotype Testing new standard for U phenotype, available at NIRL
U-Typed Units

- Issue how to handle donors entered in ePROGESAA as U-/Uvar+
  - Many of these donors have not donated recently
- Decision to delete all U- test results from ePROGESAA
- Distribution Department to discard all associated frozen Red Blood Cell components
Conclusion

- Case reports: diagnostic challenge solved, transfusion problem remains
- Switch paradigms as technology and knowledge evolve
- Need to characterize S-s- patients as U- or U\textsuperscript{var} by molecular testing
- Donors previously determined to be U- by serological testing reevaluated using molecular testing
Questions