Identification of Anti-TSEN in Antenatal Patient – Case Study

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Red Cell Reference Laboratory - QLD
Case Study

- Patient 34 year old female
- Presented for routine antenatal screen at approximately 5 weeks.
- Clinical history patient:
  - On Thyroxine
  - No previous transfusions
  - 4th pregnancy, 2 miscarriages, 1 child

- Testing at the pathology lab
  - ABO group was performed
  - 3-Cell Antibody Screen performed

- Results:

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>RhD</th>
<th>Antibody Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
<td>Cell 2</td>
<td>Cell 3</td>
</tr>
<tr>
<td>A</td>
<td>Positive</td>
<td>0</td>
</tr>
</tbody>
</table>
Further Testing at the Pathology Lab

- Antibody Identification using the 11 Cell Panel.

<table>
<thead>
<tr>
<th>Cell Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Panel B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Panel C (enzyme)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Results showed there was no antibodies detected.
- The original 3-cell screen was repeated with a different batch.

<table>
<thead>
<tr>
<th>Antibody Screen (new Batch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>
Samples Referred to Red Cell Reference

- Samples referred to the Red Cell Reference in QLD
- Request was for an ABID with a query to low incidence antigen with a 3+ reaction in one cell.
- Due to the small volume of sample received a full antibody panel was not performed.
- The screening cells were requested to enable testing and identification.
- Testing commenced in the lab:…….
Testing at the Red Cell Reference Lab

- Firstly a ABO group and full extended phenotype was performed on the patient’s cells.

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>RhD</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_1</td>
<td>Positive</td>
<td>C+E-c+e+ K- Fya+Fyb+ Jka+Jkb- M+N- S+s-</td>
</tr>
</tbody>
</table>

- The patient’s plasma was tested against screening cell 3 and the auto cells.

<table>
<thead>
<tr>
<th>Method</th>
<th>Cell 3</th>
<th>Auto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline RT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saline 37°C</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IAT + PEG</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme IAT</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Further Testing at ARCBS

- Cell 3 (reactive cell) and the patient’s red cells were chemically treated using:
  - 0.2M DTT (Dithiothreitol)
  - Trypsin
  - α-Chymotrypsin

<table>
<thead>
<tr>
<th>Chemical</th>
<th>IAT-PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell 3</td>
</tr>
<tr>
<td>0.2M DTT (Dithiothreitol)</td>
<td>12</td>
</tr>
<tr>
<td>Trypsin</td>
<td>10</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>0</td>
</tr>
</tbody>
</table>

- Reaction is destroyed in Papain and α-Chymotrypsin only.
Where to next…

- More samples were requested from the patient and her partner.
- Patient’s plasma tested against rare cells with glycophorin low incidence antigens in saline and IAT.

<table>
<thead>
<tr>
<th></th>
<th>Vw+</th>
<th>Hut+</th>
<th>Mur+</th>
<th>Hop+</th>
<th>Hil+</th>
<th>TSEN+</th>
<th>Bun+</th>
<th>A₁ cell</th>
<th>Cell 3</th>
<th>Cell 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline RT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IAT + PEG</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

- Antibody is Anti-TSEN

- Patient’s cells and Cell 3 typed for Mia, Vw, Mur, Mut, Mill, and Hil,
- Both the Cell 3 and the patient were negative for all these antigens
- No reliable monospecific anti-TSEN available for typing.
Partner’s samples

- Partner’s samples arrive and tested…
- Partner’s red cells typed as
  - Group O RhD Positive
  - C-E+c+e+, K-, Fy(a+b+), Jk(a-b+), M-N+S+s+
  - Mia(-), Vw(-), MUT(-), Mur(-), Mill(-), Hil(-)

- Partner’s red cells were compatible with the patient’s plasma in IAT tests.

  - So where has the Anti-TSEN come from?
Sequencing...

- Sequencing to confirm the TSEN negative phenotype of both samples.
- Sequencing was performed using the TruSight™ One sequencing panel.

- Patient’s samples:
  - Predicted Genotype GYP*A*01/*01 and GYP*B*03/*03.
  - Predicted phenotype M+ N-, S+s-, TSEN neg
  - A novel hybrid was suggested, but would not affect the phenotype.

- Partner’s Samples:
  - Predicted Genotype: GYP*A*02/*02 and GYP*B*03/*04
  - Predicted phenotype: M-N+, S+s+, TSEN neg
TSEN Antigen - Background

- TSEN Antigen (MNS33) is a low prevalence MSN blood group antigen.
- Named in 1992 and the occurrence is <0.01% in most populations

- Located at the junction of glycophorin A (GPA) to glycophorin B (GPB) in several hybrid glycophorin molecules.

- Associated with hybrid GP.JL (Mi.XI) and GP.Hop (Mi.IV)

Reference: Four Examples of Anti-TSEN and Three Examples of TSEN-Positive Erythrocytes, Storry, J.R etal; VoxSang 2000;79:175-179
TSEN Antigen

- TSEN antigen occurs in Europeans, Southern Chinese, Taiwanese and in Hispanics.

- TSEN is usually found due to discrepant S typing or by detection of an antibody to a low prevalence antigen.

- TSEN is expressed when S antigen is present.

- 5 examples of Anti-TSEN have been reported.
  - 1 reported case of HDFN in 2003 and no Transfusion Reactions
Conclusions

- Patient’s plasma contained Anti-TSEN antibody.

- Partner was TSEN negative, and had no other glycophorin related Miltenberger antigens.

- Patient has a potential novel hybrid allele and further Long Range PCR is required to investigate further.

- Anti-TSEN has been known to cause HDFN in pregnancy.

- Patient will need to be monitored during the pregnancy, although the partner is TSEN negative and compatible with the antibody.
Questions

- Where did the anti-TSEN come from?
- Could the Anti-TSEN be naturally occurring?
- What further testing could be done?
- Samples to be collected from the baby and siblings would be useful for further testing.

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