A perplexing case of foetal anaemia – a case of good luck and good practice

Lina Holmquist
Pathology Queensland
The Townsville Hospital Laboratory
On the afternoon of the 22/04/15 a 33 year old woman presented to Women’s and Children’s Clinic (WCC).

Blood was collected for the following tests:

- A clinical note was provided
- All results essentially normal
- Except...
Blood Group & Antibody Screen

- Group card showed mixed field (MF) in forward group (Anti-B well).

<table>
<thead>
<tr>
<th>BLOOD GROUP</th>
<th>Anti A</th>
<th>Anti B</th>
<th>Anti D</th>
<th>Cont</th>
<th>A1 Cell</th>
<th>B Cell</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>MF</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>AB Rh(D) POSITIVE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AB SCR</th>
<th>Cell I</th>
<th>Cell II</th>
<th>Cell III</th>
<th>Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Sample Expiry: 16:13 29-Apr-15  ID: klh13  Location No:  

- Patient’s group: AB Rh(D) positive
- Very small quantity of negative cells in the Anti-B well
• Routine Group & Ab screens performed on Biorad IH-1000

• Most common causes of mixed field:
  • Red cell transfusion with another group
  • Bone marrow transplant
  • Rare chimerism (twin-twin exchange of haemopoietic stem cells)
Mixed Field reactions

• ? Transfusion history – none with us.

• The two local private laboratories both had a recent group, but no mixed fields, and no transfusions.

• One lab had a gestational age, from which we calculated that the patient was now in her 33rd week of pregnancy.

• So why the mixed field??

• Going back to the request form…
Clinical notes matter!

• Clinical note “? fetal anaemia”

• Could baby be bleeding into mum? i.e foeto-maternal haemorrhage (FMH)
  – are the B-negative cells actually foetal?

• Seemingly a routine group & antibody screen (sample had not been marked as urgent), it had been performed in due time with other routine samples.

• The urgency of the matter was becoming clear, however, if we were actually seeing foetal cells!

→ Kleihauer stain to confirm FMH hypothesis
Patient’s Kleihauer slide

- Phoned clinician immediately
- Evidence of large foeto-maternal haemorrhage
- Estimated ?50ml bleed
- Term baby ~200ml blood
Kleihauer count

**FETO-MATERNAL HAEMORRHAGE SCREEN**

Kleihauer : 118.8 mL foetal RBCs

2475 Pos Cells in approx. 50,000 RBCs

30 Fields Counted

Anti-D required : vials

Maternal Blood Gp : AB Rh(D) POSITIVE

- 3 counts performed: 90ml, 120 ml, 140 ml -> reported middle count
- Referred to RBH for flow cytometry - at this stage purely academic
- Notified clinician – emergency caesarian planned
- Decision based on abnormal foetal heart sounds together with Kleihauer
### Cord blood at 23.20

<table>
<thead>
<tr>
<th>BLOOD GROUP</th>
<th>Anti A</th>
<th>Anti B</th>
<th>Anti AB</th>
<th>Anti D</th>
<th>D cont</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>A Rh(D) POSITIVE</td>
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</table>

<table>
<thead>
<tr>
<th>DIRECT AHG</th>
<th>Poly</th>
<th>Direct AHG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>Negative</td>
</tr>
</tbody>
</table>

- Fits pattern on mum’s group card:
- NICU Point of Care (POC) Radiometer blood gas analyser

<table>
<thead>
<tr>
<th>Arterial</th>
<th>Temp.</th>
<th>37.0</th>
<th>Degree C</th>
<th>Na</th>
<th>133 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway</td>
<td>Corr pH</td>
<td>7.27</td>
<td></td>
<td>K</td>
<td>4.6 mmol/L</td>
</tr>
<tr>
<td>FIO2</td>
<td>0.21</td>
<td></td>
<td></td>
<td>Cl</td>
<td>108 mmol/L</td>
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<tr>
<td>pH</td>
<td>7.27</td>
<td></td>
<td></td>
<td>Anion Gap</td>
<td>7 mmol/L</td>
</tr>
<tr>
<td>pCO2</td>
<td>41</td>
<td>mmHg</td>
<td></td>
<td>Creatinine</td>
<td>umol/L</td>
</tr>
<tr>
<td>pO2</td>
<td>30</td>
<td>mmHg</td>
<td>Oxy Hb</td>
<td>Ca (Ionised)</td>
<td>1.40 H mmol/L</td>
</tr>
<tr>
<td>O2 Sat.</td>
<td>69</td>
<td>L %</td>
<td>Carboxy H</td>
<td>Glu</td>
<td>5.9 mmol/L</td>
</tr>
<tr>
<td>p50</td>
<td>22.3</td>
<td>mmHg</td>
<td>Met Hb</td>
<td>Lact</td>
<td>6.2 H mmol/L</td>
</tr>
<tr>
<td>HC03-</td>
<td>18</td>
<td>mmol/L</td>
<td>Sulph Hb</td>
<td>Bili (Total)</td>
<td>12 umol/L</td>
</tr>
<tr>
<td>ABE</td>
<td>-7.4</td>
<td>L mmol/L</td>
<td></td>
<td>Fetal Hb</td>
<td>%</td>
</tr>
</tbody>
</table>

Comp. Val. Yes

COMMENT:
Total Haemoglobin results may be due to the specimen not being properly remixed prior to analysis (particularly with delay in analysis).

• Normal Hb for 33 week baby is 150 g/L
FBC 00.10 23/04/15

Diff: Manual Specimen: Blood DBRDW, NER, Flg, HBL, MCVH, WCH, L5, BH, PL
Hgb: 39*C WBC: 8.8 L IM Ratio 0.04
PLT: 135*L : 51.2*H IT Ratio 0.03
RBC: 0.68*L HCT: 0.10*L
MCV: 146*H MCH: 57.4*H
RDW: 22.9*H MCHC: 394*C

Neut (57 %): 5.03 Meta (0 %): 0.00 AbnLy (0 %): 0.00
Lymph (32 %): 2.82 L Mye (2 %): 0.18 H ProLy (0 %): 0.00
Mono (8 %): 0.71 Prom (0 %): 0.00 Plasm (0 %): 0.00
Eosin (1 %): 0.09 L Blast (0 %): 0.00 Other (0 %): 0.00
Baso (0 %): 0.00 AbnIm (0 %): 0.00 NRC 481 H /100 WBC
Band (0 %): 0.00 AtyLy (0 %): 0.00 NRBC /100 WBC

SusFlg WBC AS, RBC AD, IG?, NRBC?, BLA?, TURB?, PLT CL

00:10 23-Apr-15 Haematology review: Very polychromatic film with large number of nucleated red cells and reticulocytes seen. No evidence of fragments on film. Film suggestive of severe bone marrow stress. Patient has been discussed with treating team. Reviewed by Dr Renee Squires - Haematology Registrar. Reviewed by Dr. Caroline McNamara.
FBC 02/06/15

Diff: Manual  Specimen: Blood  *Flg,L5,
Hgb :  98  WBC : 10.7  IM Ratio 0.08
PLT :  371  : 10.7  IT Ratio 0.08
RBC :  3.29  HCT : 0.28
MCV :  87  MCH : 29.8 L
RDW : 16.8 H  MCHC : 344

Press shift-insert to view reference ranges
Neut ( 11 %): 1.17 L  Meta ( 0 %): 0.00  AbnLy ( %):
Lymph ( 79 %): 8.48  Mye ( 0 %): 0.00  ProLy ( %):
Mono ( 5 %): 0.58  Prom ( 0 %): 0.00  Plasm ( %):
Eosin ( 4 %): 0.39  Blast ( 0 %): 0.00  Other ( %):
Baso ( 0 %): 0.00  AbnIm ( %):  NRC 0 /100 WBC
Band ( 1 %): 0.10  AtyLy ( %):  NRBC /100 WBC
In hindsight

• We were thanked by treating Obstetrician for picking up the unsuspected FMH (no bleeding or trauma reported)

• Patient referred to hospital by GP due to
  • abnormal ultrasound scan & reduced foetal movement

• At WCC: connected to cardiotopograph (CTG) for monitoring – foetal heart tones were non-reassuring

• Kleihauer result was final evidence needed to make decision for immediate delivery, any delay would increase risk of still birth

• Placental histology normal, no viral infection

• Cause of FMH remains unknown – in hindsight patient recalled that she had bumped her belly against the kitchen counter…
What can we learn?

• (what we already know) Clinical notes matter!!
  o “?foetal anaemia” gave us all this information:
    1) patient was pregnant!
    2) why she was having blood tests
    3) unlikely she had been transfused recently
    4) source for group discrepant cells!

• All mixed field results need to be explained
  o Investigate transfusion history – public AND private
  o Do not leave for the next day – it could be significant!
  o Consider all possible causes – if ANTENATAL: think NEONATAL!

• What cells are you grouping??
Mode of sampling

• Group & Antibody screen EDTA samples are centrifuged to separate cells and plasma.
• Neonatal cells are larger & heavier than adult cells – will be concentrated at bottom of tube.
• Manual testing usually samples from top. Analyser samples from bottom…

CELLS FROM TOP

CELLS FROM BOTTOM
• Bub got lucky!
  o Mum and bub’s blood groups were different
  o Analyser performed blood group and screen test (sampling from bottom)
  o Ironically, bleed was large enough to be seen

• Good practice made the difference
  o Clinician gave us information about clinical scenario
  o The mixed field result was followed up immediately
  o Laboratory initiated further investigations

• This case highlights the importance of critical evaluation of pathology results in the context of clinical information.

• Medical laboratory scientists DO impact on patient outcomes!
Questions?
Discussion points

• Why did we not see a mixed field on the reverse group?
Kleihauer Stain

- Normally performed post-delivery on Rhesus negative women to assess degree of sensitisation for administration of Anti-D.
- Also for ?placental abruption or abdominal trauma for ?FMH
- Smears made from FBC EDTA sample, stained together with a positive and negative control.
- Stain detects HbF, present in foetal cells, by resistance to acid elution: remaining Hb stained with eosin.
Positive and negative controls

• Positive control: cord cells (CSL) in normal male blood (Hb ~115) (Mimic Hb of pregnant woman but exclude possibility of foetal cells being present.)
• Negative control: same male blood
### Histopathology Report

**Biopsy No:** TN15P3246

**HISTORY**
Placenta.
Antepartum haemorrhage. Foetal bleed.

**MACROSCOPIC**
**Placenta:** This pot contains a singleton placenta which is up to 17.1cm across and has central insertion of 31.2cm of cord, the cord being tightly coiled to the left side. This placenta is light pink on section. (dw. 23.04.2015)

**MICROSCOPIC**
Section shows three vessels in the umbilical cord, this cord having no evidence of either funisitis or of embryonal remnants. The membranes have no evidence of chorioamnionitis. The chorionic villi are mildly immature. No evidence of villitis is evident. Calcification is not seen in the placenta.

**SUMMARY**
**Placenta:** No evidence of infection.
<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV IgG (EIA)</td>
<td>Non reactive</td>
<td>&lt; 4 AU/mL</td>
</tr>
<tr>
<td>CMV IgM (EIA)</td>
<td>Non reactive</td>
<td>0.08 TV</td>
</tr>
<tr>
<td>Parvovirus B19 IgG (EIA)</td>
<td>Non reactive</td>
<td></td>
</tr>
<tr>
<td>Parvovirus B19 IgM (EIA)</td>
<td>Non reactive</td>
<td></td>
</tr>
<tr>
<td>Toxo gondii IgG EIA</td>
<td>Non reactive</td>
<td>1 IU/mL</td>
</tr>
<tr>
<td>Toxo gondii IgM EIA</td>
<td>Non reactive</td>
<td>0.06 TV</td>
</tr>
</tbody>
</table>