Blood Groups and Antibodies, Transfusion and Pregnancy

Debbie Asher
EPA Network Transfusion Laboratory Manager
To cover:

• What is a red cell antigen?
• What is a red cell antibody?
• Haemolytic Disease of the Fetus and Newborn
  – Serological testing through pregnancies
  – Finding and monitoring the ‘at risk’ pregnancies
  – Use of antenatal anti-D prophylaxis
  – Difficult interpretations and working together
  – Molecular testing – diagnostic testing v screening
  – Post delivery testing
An Antigen

• An antigen can be defined as a substance that, when introduced into the circulation of an individual lacking that antigen, can stimulate the production of a specific antibody.

• Red cell antigens
Blood Group Antigens

- Ankyrin
- Band 3
- GPA
- GPB
- MNS
- MNS
- Rh Polypeptide
- Rh Glycoprotein
- Lipid bilayer
- Spectrin tetramer
- Actin
- 4.1
- 4.2
- Diego
ABO Antigens

Example:
ABO Blood group system
A Close Up

Diagram showing carbohydrates: L-Fucose, D-Galactose, N-Acetylglucosamine, N-Acetylgalactosamine.
The D Antigen

- Most individuals are D positive or D negative.

- An individual may have a weak D antigen (previously known as $D^u$).

- An individual may have a partial D antigen (previously known as a $D^{variant}$).
RhD

**Normal D-Antigen**
- Epitope: Normal
- Antigen frequency: Normal

**D-weak**
- Epitope: Normal
- Antigen frequency: Reduced

**D-Variant**
- Epitope: Mutated
- Antigen frequency: Normal or reduced

Legend:
- Epitope
- Antigen
Inheritance

Phenotype

Genotype

Phenotype

Genotype

Phenotype

Genotype
Genetics

Phenotype

Genotype

Genotype

Phenotype

Genotype
An Antibody

• An antibody can be defined as a *serum protein* (i.e. an immunoglobulin with specific antigen binding sites) produced as a result of the introduction of a *foreign antigen*, that has the ability to combine with (and, in many cases, destroy) the cells carrying the antigen that stimulated its production.
Antibodies - IgM

Immunoglobulin M

J-chain

Carbohydrate unit
Antibodies - IgG

Immunoglobulin IgG subclasses

IgG1

IgG2

IgG3

IgG4
Red Cell Antibodies

Produced when exposed to foreign blood:

- Previous transfusion of blood/components
- Fetal maternal haemorrhage
ABO System

<table>
<thead>
<tr>
<th>Red Cells (Antigens)</th>
<th>Plasma (Antibodies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• A</td>
<td>• Anti-B</td>
</tr>
<tr>
<td>• B</td>
<td>• Anti-A</td>
</tr>
<tr>
<td>• O</td>
<td>• Anti-A,B</td>
</tr>
<tr>
<td>• AB</td>
<td>• None</td>
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Haemolytic Disease of the Fetus and Newborn (HDFN)

• Is a condition in which the lifespan of the infant’s red cells is shortened by the action of specific antibodies derived from the mother by placental transfer.

• Anaemia, jaundice, liver damage, kernicterus, IUD
Serological Testing During Pregnancy

Purpose:
- Identify RhD negative individuals so that appropriate anti-D prophylaxis can be given to prevent HDFN due to anti-D
- To identify those at risk of HDFN
- To predict the severity of the HDFN to plan treatment
Maternal Monitoring

• Booking bloods
  – ABO, D type and antibody screen
• Repeat test at 28 weeks
  – Confirm ABO and D type, repeat antibody screen
• If antibodies detected
  – Identify and monitor, regime dependent upon antibody
The Big Three

• Anti-D, anti-c and anti-K
• Test monthly up to 28 weeks
• Test every 2 weeks up to delivery
• Anti-D and anti-c are quantitated against a National Standard with results in IU/mL
• Anti-K is titrated
• Current sample is tested in parallel with previous sample to accurately identify changes in antibody level
Red Cell Immunohaematology

Hospital Transfusion Laboratory
Norfolk & Norwich University Hospital
Colney Lane
Colney
Norwich
Norfolk
NR4 7UY

Primary Requesting Clinician:
AA000001 - Head of Blood Transfusion

Patient: Sample No:

DOB: NHS No:

Date Sampled:

Date Received:

Date Reported:

Hosp Samp ID:

Change Code: 0000

EOD: 17-Oct-2016
Gestation: 10 weeks at sampling

O RhD negative

<table>
<thead>
<tr>
<th>Red Cell Antibody Results</th>
<th>Type</th>
<th>Specificity</th>
<th>Technique</th>
<th>Quantification IU/mL or Titre</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABO</td>
<td>Anti-D</td>
<td>IAT</td>
<td>Quant 4.6</td>
<td>Plasma</td>
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<tr>
<td></td>
<td>ABO</td>
<td>Anti-C</td>
<td>IAT</td>
<td></td>
<td>Plasma</td>
</tr>
</tbody>
</table>

ANTIBODY AND CLINICAL SIGNIFICANCE

There is a moderate risk of haemolytic disease of the fetus and newborn when the maternal anti-D level is between 4-15 IU/mL.

The risk of haemolytic disease of the fetus and newborn may increase if gestation proceeds beyond term.

CLINICAL ADVICE

Refer to / continue to monitor by a fetal medicine specialist.

REPEAT SAMPLING

Please send further sample as soon as possible for further anti-D investigation.

FURTHER ACTIONS

At delivery a cord DAT should be performed and, if positive, the baby’s Hb and bilirubin monitored.

BLOOD SELECTION

Select ABO compatible D- C- E- K compatible red cell units for crossmatching by IAT.

OTHER

Guidelines recommend a current paternal phenotype is performed as it may provide useful information.

This case was discussed with Vanessa on 06/04/2016 at 16:02.
The Others

- Tested at booking and 28 weeks
- In general a titre of >32 may possibly cause HDFN
- A steep increase in titre between the two samples is worrying and may lead to further monitoring
**Red Cell Immunohaematology**

**NHSBT Report 2**

**Red Cell Phenotype:** M-

**Red Cell Antibody Results**

<table>
<thead>
<tr>
<th>Type</th>
<th>Specificity</th>
<th>Technique</th>
<th>Quantification IU/mL or Titre</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>Anti-M</td>
<td>Bio-Rad IAT</td>
<td></td>
<td>Plasma</td>
</tr>
</tbody>
</table>

**ANTIBODY AND CLINICAL SIGNIFICANCE**

The risk of haemolytic disease of the fetus and newborn is low as the titre is less than 32.

**CLINICAL ADVICE**

This woman should be in the care of a hospital obstetric unit.

**REPEAT SAMPLING**

If gestation is less than 28 weeks, guidelines recommend repeat testing at 28 weeks gestation.

**FURTHER ACTIONS**

At delivery a cord CAT should be performed and, if positive, the baby’s Hb and bilirubin monitored.

**BLOOD SELECTION**

Select ABO, D and K compatible M- red cell units for crossmatching by IAT.

An antibody card for this patient is provided.
Paternal Testing

• Determining paternal phenotype and likelihood of fetal genotype may be useful.

• Misidentification of the father needs to be acknowledged.
Fetal blood group genotyping in alloimmunised pregnancies

Useful when:
• Clinically significant antibody present
• History of HDFN
• Father’s antigen status is unknown or he expresses the corresponding antigen

Issues:
• False negative rate – not truly known (need good feedback ie testing of post delivery samples)
• Samples must not be sent too early in pregnancy
Timing and Frequency of Antibody Screening in Pregnancy (RCOG Greentop guideline 65, 2014)

At booking
All pregnant women
ABO + RhD* typing
Antibody screen

Clinically significant antibody screen positive (see 6.2)

- Anti-D, -c or -K antibodies**
  - Test monthly until 28 weeks
  - See Appendix 3

- From 28 weeks:
  - Test 2 weekly until delivery
  - See Appendix 3

No clinically significant antibodies

All other clinically significant antibodies (see 6.2)
- Consider paternal/fetal genotyping for corresponding antigen(s) (see 6.4)

Repeat testing at 28 weeks

Repeat antibody screen at 28 weeks

Cord blood for:
DAT, Hb, bilirubin

No antibodies
No further action

Clinically significant antibodies
Management Algorithm for Pregnancies Complicated by anti-D, anti-K or anti-c
(RCOG Greentop guideline 65, 2014)
Preventing HDFN

• Prevent production of red cell antibodies in females of child-bearing potential
  – conservative transfusion regimes
  – transfuse D negative blood to D negative females of child bearing potential
  – and K negative blood to females of child bearing potential

• Give anti-D prophylaxis
Prophylaxis Regime

Following a sensitising event:
• <20 weeks gestation 250iu
• >20 weeks gestation at least 500iu followed by a test to measure the size of the FMH

Routine antenatal anti-D prophylaxis:
• 1500iu at 28 weeks or
• 2x500iu at 28 and 34 weeks

Following delivery of a D positive baby:
• At least 500iu followed by a test to measure the size of the FMH
Difficult Interpretations

Midwives:
• Maintain a clear record of prophylactic anti-D given: dose and date.
• Inform laboratory ie must be clear on request forms
• Vital to take 28 week samples for group and antibody screen BEFORE giving routine prophylaxis

Laboratory:
• Identify and quantitate antibody
• Statement on likely significance with respect to HDFN
• Give advice on further anti-D prophylaxis based on history provided and results obtained
• Request further samples at stated times to monitor the level of antibody
Guideline for blood grouping and red cell antibody testing in pregnancy (Transfusion Medicine, 2016, 26, 246–263)

Fig. 3. Managing pregnancies when anti-D has been detected in a woman’s plasma for the first time after a dose of anti-D Ig prophylaxis. *Levels of anti-D >0.4 IU/mL,” are assumed to be immune in origin and managed accordingly unless a large dose of anti-D Ig has been given (>3000 IU) or the sample was taken within hours of an intravenous dose of anti-D immunoglobulin, in which case it could be passive, and anti-D Ig prophylaxis should also continue.
**Red Cell Immunohaematology**

**Blood and Transplant**

**Patient:**

**Sample No.:**

**NHSBT No.:**

**Date Sampled:** 12-Jun-2013

**Date Received:** 14-Jun-2013 05:19:00

**Date Reported:** 20-Jun-2013

**Hospital Address:**

**Red Cell Antibody Results**

<table>
<thead>
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<th>Type</th>
<th>Specificity</th>
<th>Technique</th>
<th>Quantification IU/mL or Titr</th>
<th>Sample Type</th>
</tr>
</thead>
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<td>Anti-D</td>
<td>IAT</td>
<td>Quant &lt;0.1</td>
<td>Plasma</td>
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**EDD:** 30-Jun-2018

**Gestation:** 37 weeks at sampling

**Primary Requesting Clinician:**

**Request form states 1500IU anti-D Ig was given on 23/01/2013 and 17/03/2013.**

**ANTIBODY AND CLINICAL SIGNIFICANCE**

From information provided the anti-D detected is probably due to recent anti-D prophylaxis.

**CLINICAL ADVICE**

Continue antenatal and post-natal anti-D prophylaxis.

**REPEAT SAMPLING**

If anti-D was NOT detected prior to administration of routine prophylaxis at 28 weeks gestation, no further samples are required. If this cannot be confirmed, ESHG Guidelines recommend repeat testing every 3 weeks until delivery.

**FURTHER ACTIONS**

If maternal sampling was continued at delivery, if the baby is typed as D positive, a cord DAT should be performed and if positive, the baby's 1st unit bled/unmatched.

**BLOOD SELECTION**

Select ABO and K compatible D+ or E+ red cell units for crossmatching by IAT.

**OTHER**

An antibody card has not been supplied.

**Consider referral the sample for fetal DNA RHD genotyping. If genotyping confirms fetus is D negative, no further samples would be required for testing.**
Midwives:

- If immune (allo) anti-D is present do NOT give prophylactic/passive anti-D
  - failed to prevent anti-D formation
  - must not give an unnecessary blood product

- If interpretation of results is in doubt give anti-D as
  - may prevent HDFN
  - anti-D is a blood product with a good safety record

- If further samples are requested send them
  - could miss catching an immune anti-D that is increasing to a dangerous level.
High throughput non-invasive prenatal testing for fetal RHD – Fetal RHD screening test

https://www.nice.org.uk/guidance/dg25

• A non-invasive fetal DNA test to predict fetal D group from 11+2 weeks gestation
• The test offers >99.9% negative predictive value ie fewer than 1:1000 babies will be falsely predicted to be D negative
• The test prevents unnecessary administration of anti-D. Currently 40% of women receive this human blood product when they don’t need
• A cost effective option to guide antenatal prophylaxis with anti-D
• Reduces the need for laboratory tests ie quantitations, FMH estimation
• It is available from an NHS laboratory ie IBGRL, Bristol
  – Results available electronically on Sp-ICE
  – No transport costs
  – Information leaflets available
High throughput non-invasive prenatal testing for fetal RHD - Restrictions

• This is NOT a diagnostic test for the fetal RhD status of women who have produced immune anti-D
• This test has been designed to minimise false RhD negative results but in approx. 2% of tests the results will be incorrectly predicted to be RhD positive ie 2% of RhD negative women will receive anti-D Ig unnecessarily compared to the 40% who currently do.
### Post Delivery

<table>
<thead>
<tr>
<th>Maternal D type</th>
<th>D+</th>
<th>D+</th>
<th>D-</th>
<th>D-</th>
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<tbody>
<tr>
<td>Antibody status</td>
<td>None detected</td>
<td>Antibody detected</td>
<td>None detected</td>
<td>Antibody detected</td>
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