Targeted transfusion and anti D prophylaxis: new strategies in prevention of HDFN
Disclosure

• I have no relevant conflicts of interest to disclose with respect to this presentation
Objectives

Providing Kell negative red cells

• Outline the importance of anti Kell antibodies in HDFN
• Describe the frequency of transfusion related alloimmunisation in pregnant women
• Identify one strategy to limit anti Kell alloimmunisation in pregnancy

Fetal blood group prediction from maternal plasma

• Provide a brief overview of the available testing strategies
• Discuss management of alloimmunized pregnancies with non invasive fetal genotyping
• Describe potential changes to routine antenatal anti D prophylaxis
CASE STUDY
TR 31 yo female

• 1\textsuperscript{st} pregnancy:
  – Group B; Rh positive
  – Antibody Screen negative
  – Uncomplicated pregnancy
  – Delivery at term
  – normal birth weight female infant

• 2\textsuperscript{nd} pregnancy:
  – Group B; Rh positive
  – Antibody Screen positive
  – Anti Kell antibody identified
  – Father: Kell positive,
  – Genotyping: Heterozygous (K/k)
  – Fetal anemia with hydrops by 18 weeks
  – Fetal death following attempted IUT
Case Study Continued

• 3\textsuperscript{rd} pregnancy:
  – Group B; Rh positive
  – Antibody screen positive with anti K, anti Fya and anti Jka identified
  – Early CVS for fetal blood group genotyping: fetus genes including KEL, FYA present, JKA negative

• Therapy:
  – Plasmapheresis
  – IVIG
  – Antibody titration
  – Fetal MCA doppler monitoring for anemia

• Outcome
  – Dramatic reduction in antibody titres
  – Successful IUT beginning at 19 weeks
Case study continued...

• Fetal demise following 4th IUT
  – fetal bleeding as cause of death
  – Bone marrow examination showed adequate fetal hematopoiesis (successful therapy)
The Happy Ending

• 4\textsuperscript{th} pregnancy
  – Group B; Rh positive
  – Anti K, anti Jka, Anti Fya
  – Plasmapheresis and IVIG protocol commenced at 12 weeks; 2\textsuperscript{nd} plasmapheresis at 16 weeks to decrease titres
  – 4 successful IUT procedures starting at 20 weeks

• IVIG therapy continued weekly to 30 weeks
• healthy female infant delivered at 34 weeks gestation
Overall prevalence (Canada) 0.66% (internationally 0.42 – 0.77%)
What is it about anti Kell?

• Immunogenic antigen:
  – K was the most immunogenic antigen in a recent post transfusion cohort study with a cumulative incidence of alloimmunisation of 2.3% after 2 units transfused *Lancet Haematol* 2016; Evers et. al

• High prevalence in perinatal population
  – Australia 9.5%; Netherlands 11.6%; Canada 10%

• Clinically significant antibody in pregnancy; difficult to monitor
Conclusion: To select pregnancies with an increased risk for anti-K-mediated severe HDFN, determination of the anti-K titer early in pregnancy is sufficient to select pregnancies at increased risk for severe HDFN. The optimal cut-off value is a titer of 4.

Measurement of biological activity of the K antibodies with the ADCC test does not increase the diagnostic accuracy of laboratory monitoring.
The prevalence of anti-K in Canadian prenatal patients

Mindy Goldman, Debra Lane, Kathryn Webert, and Robert Fallis
TRANSFUSION 2015;55;1486–1491

- Anti-K was found in 397 of 390,193 patients (1.02 per 1000)
- 26 of 75 (35%) anti-K patients had received transfusions since 2001;
- (54%) had received at least one K positive RBC unit
- 3 had received all K– units;
- For 9, donor K typing was incomplete.
- 8/26 had previous pregnancies, three with K1 positive partners.
### TABLE 2. International policies for Kell matching, females less than 45 years

<table>
<thead>
<tr>
<th>Country</th>
<th>National standard</th>
<th>Practice</th>
<th>Reference/contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>No</td>
<td>Selected hospitals</td>
<td>Canadian Standards Association, Blood and blood components, CSA-Z902-10^{13}</td>
</tr>
<tr>
<td>United States</td>
<td>No</td>
<td>No</td>
<td>AABB Standards for Blood Banks and Transfusion Services, 27th edition, 2011^{14}</td>
</tr>
<tr>
<td>Australia</td>
<td>No</td>
<td>Selected hospitals</td>
<td>Personal Communication (Joanne Pink, ARCBS, August 2014)</td>
</tr>
<tr>
<td>Israel</td>
<td>No</td>
<td>No</td>
<td>Personal Communication (Vered Yahalom, Magen David Adom, Israeli National Blood Services, 08/2014)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Yes</td>
<td>K-matched or K-inconsistent age limit</td>
<td>British Committee for Standards in Haematology: Guidelines for pre-transfusion compatibility produces in blood transfusion laboratories^{15}</td>
</tr>
<tr>
<td>France</td>
<td>Yes</td>
<td>Standard practice</td>
<td>Transfusion de globules rouges homologues, recommendations, 2002 Agence Francaise de Securite Sanitaire des produits de sante^{16}</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Yes</td>
<td>Females ≤ 45 years</td>
<td>Dutch Blood Transfusion Guideline, 2011; Kamphuis et al.^{9}</td>
</tr>
<tr>
<td>Belgium</td>
<td>Yes</td>
<td>Females ≤ 45 years</td>
<td>Bonnes pratiques de transfusion à l’usage des hospitaux 2010, Conseil Superieur de la Santé^{18}</td>
</tr>
<tr>
<td>Denmark</td>
<td>No</td>
<td>Standard practice</td>
<td>Personal Communication (Karin Magnussen, University of Copenhagen, 08/2014)</td>
</tr>
<tr>
<td>Sweden</td>
<td>No</td>
<td>Done by some hospitals</td>
<td>Personal Communication (Rut Norda, University of Uppsala, August 2014)</td>
</tr>
<tr>
<td>Germany</td>
<td>Yes</td>
<td>Standard practice</td>
<td>German Hemotherapy Standards, Paul-Ehrlich Institute^{19}</td>
</tr>
</tbody>
</table>
Should we transfuse K negative red cells to female patients?

- Prophylactic use of K negative (or K matched) units is standard in many European countries.
- However *some* studies suggest little cost or clinical efficacy of Kell negative or K matched transfusions in preventing anti K related HDFN.
- With increased routine donor phenotyping may be feasible to provide K negative units to eligible patients (Canadian Experience).

Canadian Kell Audit: Background and Hypothesis

• Kell typed (and end labeled) donor units are becoming more widely available

• Hypothesis: a transfusion service with a substantial red cell inventory can provide Kell negative red cell units to Kell negative females (< 45 years) with no or minimal on-site phenotyping required
% National Antigen Test Results Negative
February 2017

![Bar chart showing the percentage of negative test results for different antigens.](chart.png)
Fiscal Year Comparisons for Kell Phenotyping

Note: Fiscal Year 2016-2017 is incomplete; April-Feb (Missing Mar 2017)
Ontario

Cumulative % of births by maternal age

98% ≤ 41
99% ≤ 42
99.5% ≤ 43
Births: 668,604

Slide courtesy of Dr. Allison Collins & Orbcon
Audit Parameters

• On a pre established start date (and without altering inventory ordering practices) the hospital transfusion service provided Kell antigen negative units to all females <45 years old, when ever possible.

• Availability of Kell negative ABO/Rh appropriate units tracked for 3 months
Participating hospitals

- Moncton NB
- Edmonton AB (CBS and AHS)
- BC Children’s and Women’s Hospital, Vancouver BC
- Victoria (VGH and RJH) and Nanaimo BC
- Winnipeg and Brandon MB
Results

- Total number of donor units transfused to study population: 1950 (939 patients; 1197 transfusion episodes)
- Number of Kell negative units available in inventory: 1861
- Number of group O substitutions: 181
- Percentage of phenotyped Kell negative units available: 95%
- Where Kell negative were not available:
  - Neonatal patient with additional red cell attributes required – 10%
  - Patient with unexpected antibodies – 7%
  - Group B or AB – 21%
  - Edmonton Hospitals (56% of the transfusions but 80% of cases where Kell negative not available)
Site by site comparison

Kell negative available

- Moncton Hospital: 100%
- Winnipeg and Brandon: 89%
- Edmonton CBS: 96%
- Edmonton Hospitals: 71%
- BC Children's & Women's Hospital: 100%
- Vancouver Island Health Authority: 100%
Summary

• Providing Kell negative red cells to female patients of child bearing potential is feasible in most cases
• Distribution of Kell negative units to hospitals is “uneven”
• Hemovigilance reports indicate a decline of 50% in K immunization of pregnant women in some European countries where this practice is in place

Solheim BG, Provision of K- blood to women not more than 50 years of age. Transfusion 2015 (55) 469
Predicting fetal red cell antigens by non invasive perintatal testing

FETAL DNA IN MATERNAL PLASMA
Recommended Prenatal Testing & Timing

• Mothers – @ first prenatal visit – ABO Rh and ab screen
• Mothers – @ 26 - 28 weeks (before RhIg)
  • **D negative mums with no anti D receive RhIg**
  • **All benefit from ABO re check and assessment for non D antibodies**
• Mothers – Ab present – Ab identification - regular follow up titration
  • Every 4 wks to 3rd trimester, then every 2 wks to delivery
  • More often if rise in titre or clinical concern
Post Natal *targeted* anti D prophylaxis

D Neg mother, maternal clinically significant antibody, or signs of HDFN in the baby

- cord blood ABO & Rh group;
- antigen typing;
- DAT

Fetal Bleed Screen

- Rh Neg mothers
- D (or wk D) Pos baby

Quantitation of fetal bleed

**RhIg administered**

- D negative mums with no immune anti D and
- D positive neonate

*Canadian Blood Services*

*it's in you to give*
RhIg Recommendations

• If Rh negative with no anti D at first prenatal visit...
  – Then repeat screen and administer RhIg at 28 weeks gestation
• If Rh negative or Rh unknown, cord testing should be performed at delivery...
  – RhIg administered to all D–ve women who deliver a D +ve baby
  – Maternal antibody screen positive for anti D?
    • Could it be passive anti D from previous RhIg prophylaxis
RhIg

- Prophylaxis also indicated for D–ve women *without* an anti D who have:
  - Spontaneous or therapeutic abortion
  - Threatened abortion
  - Abdominal trauma
  - Obstetrical manipulations
  - Amniocentesis/CVS
  - Molar pregnancy
  - Transfusion of D+ve blood components
The alloimmunized Pregnancy: Antibody ID and titration

• Is the antibody clinically significant?
  – IgG
  – Antigen expressed on fetal red cells

• Antibody Titration
  – Quantifies the antibody
  – Not a predictor of HDFN severity
  – A screening test to signal when MCA-Dopplers should begin
  – When titre reaches critical clinical monitoring begins
His gathering of Rh disease researchers in Hamilton in 1977, Dr. Alvin Zipursky is third from left. Fifth from left is Dr. Jack Bowman and beside him, holding a cane, is Bruce Chown. The three led Canadian efforts to develop a vaccine.

http://www.pressreader.com/canada/toronto-star/20160220/284623191540985
HDFN prevention – a short history

- 1941 – Levine et al associated maternal anti D with HDN
- 1965 – passive immunization by anti D shown to prevent sensitization in D negative volunteers; Clarke et al and Freda et al
- Clinical trials in Rh negative pregnant women; development of Rh immune globulin; and RhIg licensed for postpartum use in 1967 -1968 (Bowman and Chown; Zipursky)
 Successful Prevention of Experimental Rh Sensitization in Man with an Anti-Rh Gamma-Globulin Antibody Preparation: A Preliminary Report

VINCENT J. FRED, JOHN G. GORMAN, WILLIAM POLLACK

From the Departments of Obstetrics and Gynecology and Pathology, Columbia University, College of Physicians and Surgeons, New York City, and the Ortho Research Foundation, Raritan, New Jersey

**Abstract**

The results on the use of γG-immunoglobulin to Rh factor for the prevention of active immunization of Rh-negative mothers at risk appear most promising. One hundred and seven mothers in the clinical trial have been followed for periods of about 6 months to 1½ years after delivery. Of these, 48 were treated mothers who received 5 ml γG-immunoglobulin to Rh, and 59 were untreated mothers. Of the 48 treated mothers none are actively immunized; seven of the 59 control mothers have become actively immunized to Rh.
Antenatal prophylaxis of Rh isoimmunization: 28-weeks' gestation service program

J.M. Bowman, MD; J.M. Pollock

Two (0.13%) of 1586 Rh-negative primigravidae or multigravidae treated similarly in all previous pregnancies, who were given a single injection of Rh immune globulin (300 μg) at 28 weeks' gestation and subsequently were delivered of Rh-positive babies, had demonstrable Rh isoimmunization at the time of delivery. The remaining 1584 (who were treated again after delivery) had no evidence of Rh isoimmunization at delivery and none of the 512 screened at 6 months after delivery appeared to be immunized. If the 28th-week injection had not been protective, one would have expected 14 of the 1584 to have been demonstrably Rh isoimmunized and evidence of Rh isoimmunization to have persisted in 6 of the 512 observed 6 months after delivery.

Six of 719 Rh-negative multigravidae who had not received Rh immune globulin after previous pregnancies or had been treated only after delivery showed evidence of Rh isoimmunization despite a single injection of Rh immune globulin at 28 weeks in a subsequent pregnancy. In three of the six the cause was most likely "sensitization" due to previous exposure to Rh-positive blood or an unreported Rh-positive pregnancy. In 3 of the remaining 716 (0.42%) there may have been true failure of antenatal Rh prophylaxis administered at the 28th week. One would have expected this figure to be 12 of 716 if antenatal Rh prophylaxis at 28 weeks' gestation was totally unsuccessful.

It is concluded that a single intramuscular injection of Rh immune globulin, 300 μg, is 98% effective in preventing Rh isoimmunization during pregnancy in Rh-negative primigravidae and in multigravidae treated antenatally in all previous pregnancies, and is 75% effective in preventing Rh isoimmunization in Rh-negative multigravidae untreated during previous pregnancies. The majority of failures are due to Rh isoimmunization during pregnancy prior to antenatal prophylaxis at 28 weeks.

Sur 1586 primigravidae et multigravidae Rh négatif ayant été traitées de la même façon aux grossesses précédentes, qui ont reçu une seule injection d'immunoglobe Rh (300 μg) à la 28e semaine de la grossesse et qui subéquemment on donné naissance à un bébé Rh positif. 2 (0.13%) ont démontré une isoimmunisation Rh au moment de cette injection et doivent être considérées comme des échecs de "logique" en ce qui a trait à la prévention prénatale. Les 1584 autres femmes (qui ont été traitées encore après l'accouchement) n'en ont montré aucun signe d'isoimmunisation Rh lors de l'accouchement et aucun des 512 testés systématiquement 6 mois après l'accouchement n'a semblé être immunisé. Si l'injection à la 28e semaine n'aurait pas protégé, on se serait attendu à ce que 14 de ces 1584 patients montrent une isoimmunisation Rh et à ce qu'il y aurait persistence des signes d'isoimmunisation Rh chez 6 des 512 patientes observées 6 mois après l'accouchement.

Sur 719 multigravidae Rh négatif qui n'ont pas reçu d'immunoglobe Rh lors de leurs grossesses antérieures, de l'isoimmunisation Rh en dépôt de l'injection d'immunoglobe Rh à la 28e semaine d'une grossesse subéquemment. Chez trois des six patients qui causent la plus probable cause d'isoimmunisation Rh prénatale, un seul de ces résultats a été observé. Chez deux des 716 autres femmes (0.42%) il peut y avoir un échec réel du traitement prénatal anténatal Rh administré à la 28e semaine. On aurait pu s'attendre à un chiffre de 12 sur 716 si la prévention prénatale à la 28e semaine de la gestation était complètement sans succès.

On conclut qu'une seule injection intramusculaire de 300 μg d'immunoglobuline globulaire Rh est efficace à 98% dans la prévention de l'isoimmunisation Rh durant la grossesse chez les primigravides Rh négatif et chez les multigravidae Rh négatif qui ont été traitées avant la naissance durant chacune de leurs grossesses précédentes; elle est efficace à 75% chez les multigravidae Rh négatif qui n'ont pas été traitées durant leurs grossesses précédentes. La majorité des échecs est due à une isoimmunisation Rh durant la grossesse et avant de recevoir le traitement prénatal à la 28e semaine.

As a result of the evidence of the occurrence of Rh isoimmunization during pregnancy and its successful prevention by antepartum intramuscular administration of approximately 300 μg of Rh immune globulin (RhD25) immune globulin, Connaught Laboratories, Toronto) at 33 and 34 weeks' gestation, a service program of antenatal Rh prophylaxis was begun in Manitoba July 1, 1978. Reasons of
Some RhIg is unnecessary

In Canada all D negative women receive antenatal anti D prophylaxis:

- at 28 weeks gestation,
- at any potentially sensitizing event

However 40% of D negative women carry a D negative fetus

Alberta Study: 10,393 D negative pregnancies annually; an estimated 4072 doses of RhIg would be “saved” if we knew whether the fetus was D positive or negative

When a critical Titre antibody is present:

Obstetrical consultation and follow up

- Paternal phenotype may be assessed for relevant antigen
- Middle Cerebral Artery Doppler ultrasound
  - On average – 9 per pregnancy
- Anemia diagnosis and possible fetal blood sampling and transfusion
free fetal DNA

10% of all cell free DNA in maternal plasma is fetal

Detectable from 4 weeks GA

Major source is placental

We can determine fetal blood group genotype (along with genotype of many other antigens) by using this DNA from mum's plasma

Non-invasive fetal blood group genotyping

- detect fetal RH D, c, E, Kell status using maternal blood sampling
- Use results to guide prenatal management
How does this work?

• Fetal DNA extracted from maternal plasma
• Primers added and relevant exons amplified
• The pattern of exon amplification predicts whether the fetus will be D positive or negative (or K1 positive or negative, or c or E...)
• Maternal genotype may also be determined
<table>
<thead>
<tr>
<th></th>
<th>Antibodies of critical or rising titre</th>
<th>These are the clinically significant antibodies that require frequent clinical follow up and anemia screening through ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Anti-D, Anti C, anti c, anti E, anti e and anti-K</td>
<td>These antibodies are the ones for which genotyping of fetal DNA is available routinely from maternal plasma</td>
</tr>
<tr>
<td>3</td>
<td>Samples from mothers where the father is heterozygous for the implicated antigen</td>
<td>A heterozygous paternal type means that the fetus may or may not have the antigen (50% chance), and therefore may or may not require clinical follow up</td>
</tr>
<tr>
<td>4</td>
<td>Samples from mothers where the father is unknown or father’s sample is not available.</td>
<td>Unable to determine the risk to the fetus.</td>
</tr>
</tbody>
</table>
Figure 2  Common D-negative RHD variants. Asterisks in the RHD gene denote sites used for PCR amplification by most groups. Bands indicate the RHD specific nucleotides. Bands in the RHD pseudogene denote the missense mutations in exons 4 and 5, and the nonsense mutation in exon 6. Shaded exons in RHD-CE-Ds represent exons derived from RHCE gene.
... if fetus is antigen negative

- No RhIg prophylaxis
- No RhIg for antenatal bleeding events or procedures
- No HDN monitoring in alloimmunized pregnancies

... if fetus is antigen positive

- No cord testing at delivery (Just give RhIg)
- Follow up care is needed throughout pregnancy for alloimmunized women
Is it accurate?

Table 1. Results from prenatal RHD screening

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Samples (n)</th>
<th>Gestational weeks (median)</th>
<th>RHD exons</th>
<th>Sensitivity (%)</th>
<th>False-negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>van der Schoot et al. (^5^6)</td>
<td>2006</td>
<td>1257</td>
<td>30</td>
<td>7</td>
<td>99.6</td>
<td>3</td>
</tr>
<tr>
<td>Finning et al. (^3^6)</td>
<td>2008</td>
<td>1869</td>
<td>8-38 (28)</td>
<td>5, 7</td>
<td>99.7</td>
<td>3</td>
</tr>
<tr>
<td>Müller et al. (^5^7)</td>
<td>2008</td>
<td>1022</td>
<td>6-32 (25)</td>
<td>5, 7</td>
<td>99.7</td>
<td>2</td>
</tr>
<tr>
<td>Daniels et al. (^5^0)</td>
<td>2012</td>
<td>4876</td>
<td>7-24</td>
<td>5, 7</td>
<td>&gt;99.2(^a)</td>
<td>19</td>
</tr>
<tr>
<td><strong>Routine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clausen et al. (^1^4)</td>
<td>2012</td>
<td>2312</td>
<td>25</td>
<td>5, 7; 7, 10; or 5, 10</td>
<td>99.9</td>
<td>2</td>
</tr>
<tr>
<td>Wikman et al. (^1^7)</td>
<td>2012</td>
<td>3291</td>
<td>8-40</td>
<td>4</td>
<td>98.9(^b)</td>
<td>23</td>
</tr>
<tr>
<td>De Haas et al. (^1^6)</td>
<td>2012</td>
<td>6941</td>
<td>27</td>
<td>5, 7</td>
<td>&gt;99.6(^c)</td>
<td>NA(^d)</td>
</tr>
</tbody>
</table>

\(^a\)Estimated from their reported data; the sensitivity reached >99.8% at 11 weeks of gestation, with only three false-negative results in 4011 samples.

\(^b\)The sensitivity reached 99.3% at 10 weeks of gestation.

\(^c\)Estimated from reported false-negative results <0.25%.

\(^d\)NA, not available.

Clausen, F. Integration of noninvasive prenatal prediction of fetal blood group into clinical prenatal care. Prenatal Diagnosis 2014, 34: 409
Costs

It depends…

• # of exons amplified and testing algorithm;
• gestational age of testing;
• cessation of cord testing;
• Use for targeted antenatal RhIg or just in alloimmunized pregnant women

High throughput automated testing may reduce costs

Transport to centralized laboratories (specimen tracking, packaging transportation) may increase costs

Initial capital costs for automation; decreased follow up costs for RhIg
D alloimmunization risk & Cost effectiveness

- Varies according to study
- Population based cohort study predicts lower costs and fewer immunisations *Neovius, Tiblad, et al. BJOG 2015*
Summary

Targeted Rh prophylaxis feasible with no significant increase in risk of sensitization

Reduction of 40% of RhIg doses

Many international studies indicate cost neutrality; though some suggest increased cost and increased alloimmunization rates

Is it ethical to continue with routine prophylaxis when tests to determine need are available?


Routine administration of Anti-D: the ethical case for offering pregnant women fetal RHD genotyping and a review of policy and practice

Julie Kent, Anne-Maree Farrell and Peter Soothill

Summary
In short we have argued that, on ethical grounds, there is a strong case for reviewing policy and practice relating to the routine administration of prophylactic antenatal Anti-D Ig. By making fetal RHD genotyping more widely available, women would be better informed about whether or not they need this blood product.