"Back to Basics" Anti D Prophylaxis Laboratory and Clinical Problems

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History
Immune response
Rh D Prophylaxis
Estimation of fetal bleeds
Problems and common errors

Haemolytic Disease of the Newborn/ Fetus

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; The disease begins in intrauterine life and may result in death in utero.



Haemolytic Disease of the Newborn

BABY
During delivery, some of the baby's red cells may cross the placenta and enter the mother's circulation
Image: Construction

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Rh factor on the baby's red cells stimulates the mother's immune system to make anti-Rh antibodies

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The anti-Rh antibodies attach to the 'foreign' red cells and they are destroyed If the mother conceives another Rh-positive baby, her anti-Rh antibodies can cross the placenta and enter the baby's circulation

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The anti-Rh antibodies attach to red cells and some are destroyed...

...leaving free haemoglobin

...which is converted to bilirubin in the baby's liver ...leading to kernicterus in the newborn baby

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Prophylaxis using D immunoglobulin

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MOTHER

BABY

During delivery, some of the baby's red cells may cross the placenta and enter the mother's circulation

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The Prophylactic Anti D must be given within 72 hours of the sensitising event

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Prophylactic Anti D prevents the mother from producing her own D antibodies

History - HDN due to anti-D

1940 Landsteiner and Weiner discovered the 'Rh factor'

From K. Landsteiner and A. S. Wiener (1940). Soc. Exp. Biol. NY., 43, 223. Copyright (1940), by kind permission of A. S. Wiener and the Society for Experimental Biology, New York

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An Agglutinable Factor in Human Blood Recognized by Immune Sera for Rhesus Blood.

KARL LANDSTEINER AND ALEXANDER S. WIENER.

From the Rockefeller Institute for Medical Research and the Office of the Chief Medical Examiner of New York City.

1941 Levine et al. tested the antibody described by Landsteiner and Weiner vs. parents of HDN infants

Mothers were negative for the Rh factor and fathers positive
Maternal sensitization due to an antigen crossing placenta

1945 Coombs, Mourant and Race proved HDN caused by maternal antibodies crossing the placenta

Primary Anti-D response

Rarely detected earlier than 4 weeks after sensitisation, but usually 8-9 weeks (up to 15 weeks)
Weak IgM response, usually followed by IgG.

10-15% 'non-responders'
10-15% are very good responders

Initial sensitisation dependent on 'dose'

15% after 1mL
65-70% after 250mL

Secondary Anti-D response

A second exposure leads to a rapid rise in anti-D, 3 days onwards.
Usually IgG (IgG1 and/ or IgG3).
Increased antibody levels and avidity
Secondary responses require small 'dose' (<0.3mL)

Anti D Prophylaxis

Clarke's group from Liverpool first described the possibility of prevention using an antibody.

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From M. R. C. Working Party (1974). Brit. Med. J., ii, 75-80. Copyright (1974), by kind permission of the authors and the British Medical Association

BRITISH MEDICAL JOURNAL 13 APRIL 1974

Controlled Trial of Various Anti-D Dosages in Suppression of Rh Sensitization following Pregnancy

Report to the Medical Research Council by the Working Party on the Use of Anti-D Immunoglobulin for the Prevention of Isoimmunization of Rh-negative Women During Pregnancy

Anti D Prophylaxis

In 1971 and 1974 MRC working party reported on the success of the trials and agreed with WHO guidance of 25 micrograms (125 iu) to be issued to potential bleeds

1 april 40

From M. R. C. Working Party (1974). Brit. Med. J., *ii*, 75-80. Copyright (1974), by kind permission of the authors and the British Medical Association

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Sensitising events

Pregnant women who are D negative must be considered for prophylactic anti-D for the following potentially sensitising events: -

- Amniocentesis
- Cordocentesis
- Other in-utero therapeutic intervention/surgery (e.g. intrauterine transfusion, shunting)
- Ante partum haemorrhage (APH)
- Chorionic villus sampling
- Ectopic pregnancy
- External cephalic version
- Fall / abdominal trauma
- Intrauterine death
- Miscarriage
- Termination of pregnancy

D preparations available

Various closes available in UK

- 250 iu, 500iu, 1250 iu, 1500 iu, 2500 iu, 5000 iu
- Standard dose is 125 iu per ml fetal cells bleed IM
- <u>Minimum</u> dose of 250 iu < 20/40
- Minimum dose of 500 iu > 20/40 (4 ml FMH)
- Some Anti D IM use only some IV and IM

The IM Anti D should be given in deltoid region to ensure that it is in muscle and not adipose. Being given in the gluteal region increases this risk **Recurrent bleeds in pregnancy**

<12 weeks ?? Required</p>

12-20 weeks 250 iu every 6 weeks

>20 weeks 500 iu very 6 weeks + FMH estimation

Anti-D Sensitisation/1000 Births 1970-86



HDN 2002

Approx 500 fetuses/year developed HDN
25-30 babies died from HDN
?? Babies lost before 28/40
Approx 15/year have major permanent development problems

Nice made recommendations to reduce this

NICE

Routine A/N prophylaxis to be offered to all non sensitised Rh negative women

To be given at 28 and 34 weeks

1 dose of 1500 iu at 28 weeks is as effective

 Any potentially sensitising events need further prophylaxis

NICE reviewed this guidance in 2008

Issues that put the success of prophylaxis at risk

SHOT Data 2005 Anti-D Immunoglobulin (n=87)

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| Type of event | Numb |
|-------------------------------------|------|
| Omission or late administration | 27 |
| Given to D positive patient | 23 |
| Given to patient with immune anti-D | 7 |
| Given to patient with weak D | 6 |
| Given to mother with D neg infant | 7 |
| Given to wrong patient | 6 |
| Expired | 9 |
| Other | 2 |
| Total cases | 87 |

2 cases of severe HDN - 1 fatal + 1 exchange tx

Types of IBCT events

| Type of event | Number (%) |
|--|------------|
| 'Wrong blood' events where a patient received a blood component intended for a different patient or of an incorrect group | 54 (14%) |
| Other pre-transfusion testing errors (excluding erroneous Hb) | 28 (7%) |
| Blood of the incorrect group given to recipients of ABO or D mismatched PBSC, bone marrow or solid organ transplant | 8 (2%) |
| Transfusion of blood of inappropriate specification or that did not meet the patient's special requirements | 108 (27%) |
| Inappropriate or unnecessary transfusions | 51 (13%) |
| 'Unsafe' transfusion where there were handling or storage errors | 74 (19%) |
| Events relating to administration of anti-D immunoglobulin | 77 (19%) |
| Total | 400 |

Table 18Cases involving anti-D Ig administration with the site(s) of contributing errors77 cases, 79 errors

| Type of event | Number |
|---|--------|
| Omission or late administration of anti-D Ig | 26 |
| Laboratory errors | 7 |
| Midwife/nurse errors | 19 |
| Anti-D Ig given to D pos patient | 19 |
| Laboratory errors (including 8 weak D groups) | 12 |
| Midwife/nurse errors | 7 |
| Anti-D Ig given to patient with immune anti-D | 13 |
| Laboratory errors | 6 |
| Midwife errors | 8 |
| Anti-D Ig given to mother of D neg infant | 9 |
| Midwife error (anti-D given before cord group done) | 1 |
| Laboratory error (4 wrong D group determinations, 2 wrong result manually entered | 8 |
| onto computer, 2 infants grouped as D neg but anti-D issued in error) | |
| Anti-D given to wrong patient (all were midwife/nurse errors) | 4 |
| Wrong dose given (1 lab error, 1 doctor error) | 2 |
| Anti-D Ig expired or out of temperature control | 2 |
| Laboratory error | 1 |
| Also midwife error | 1 |
| Clinical error in community | 1 |
| Other (laboratory errors) | 2 |
| Total cases | 77 |
| Total errors | 79 |

Anti D immunoglobulin errors

Omission or late administration of PR-D

PR-D given to D Positive women

PR-D given to patients with immune D

Laboratory errors- 47%

Guidelines available

GUIDELINE FOR BLOOD GROUPING AND ANTIBODY TESTING IN PREGNANCY

Writing group: Gooch A¹, Parker J², Wray J³, Qureshi H⁴ ¹ National Blood Service, Manchester,²Department of haematology, Derby City Hospital, Derby,

¹ National Blood Service, Manchester,²Department of haematology, Derby City Hospital, Derby, ³University of Salford, Salford, Greater Manchester, ⁴Depratment of Haematology, University Hospitals of Leicester, Leicester, UK

08/06/2006

Guidelines for the use of prophylactic anti-D immunoglobulin

British Committee for Standards in Haematology

Writing group: **Parker J¹, Wray J², Gooch A³, Robson S⁴, Qureshi H⁵** ¹Department of haematology, Derby City Hospital, Derby, ² University of Salford, Salford, Greater Manchester, ³ National Blood Service, Manchester, ⁴School of Surgical and Reproductive Sciences, University of Newcastle upon Tyne, ⁵Depratment of Haematology, University Hospitals of Leicester, Leicester, UK

Guidelines available

2009

Guidelines for the Estimation of Fetomaternal Haemorrhage

Working Party of the British Committee for Standards in Haematology, Transfusion Taskforce. Writing group: E Austin1, S Bates2, M de Silva3, D Howarth4, A Lubenko5, M Rowley6, M Scott7, E Thomas8, J White9, M Williams6

Estimation of FMH

UK FMH guidelines 2009

Sample requirements
Serology
Techniques
Calculation
Follow-up and confirmation of result
Quality assurance

Methods

Acid Elution - Kleihauer

 Good for screening and initial assessment

- Very subjective
- Needs attention to detail

Flow cytometry

- **Expensive**
- Used as reference method
- Appears more accurate
- Method to confirm volume of FMH
- Not available to everyone quickly

Reporting Results

Reason for the sample Sensitising event, post delivery, other reason Result of FMH in mL fetal cells Avoid 'positive' and 'negative' Advice about anti-D immunoglobulin required (standard dose, additional dose) Advice regarding follow-up samples to check for clearance Communication and reporting of results very important

False Positives

Care required with AE

Hb F increases in pregnancy but not normally a problem

Can be raised in some genetic disorders

Do Flow estimation if in doubt

Sample requirements

Timing of samples taken

Dangers of mislabelling

Cord and Maternal group to be done

If same group- MCV

 Alkali denaturation test (APT)

Audit

To see if all D negative women have had a FMH test at the appropriate time and that, if fetal cells are detected, appropriate action is taken

Compare the results of confirmatory tests with the initial quantification

Audit the follow-up procedures in the small number of women requiring additional anti-D

Problems

When is immune Anti D prophylactic Anti D Does PRD affect the Kleihauer results How do we assess a DCT + if PRD given High Hb F what do we do for screening Right sample in bottle look at CG data Effective communication Electronic issue in PRD patients

Future

Postpartum anti-D: can we safely reduce the dose?

Bradley M Augustson, Elizabeth A Fong, Dianne E Grey, Janine I Davies and Wendy N Erber

- Australian paper (Bradley et al MJA22006;1184((12):611-613)
- Retrospective study>5000 D- women FMH results by flow
- Proposing reduction of post natal dose to 250iu anti-D

Conclusions: This large retrospective audit shows that a currently available dose of 250 IU (50 mg) of anti-D would have been sufficient for 98.5% of the 5148 Rh D-negative women. On the basis of this evidence, a reduction in the recommended routine postpartum dose of anti-D from 625 IU to 250 IU when flow cytometric quantitation for FMH is available should be considered. Adopting such a strategy would ensure the ongoing provision of a valuable human blood product currently in limited supply.

MJA 2006; 184: 611-613



Fetal genotyping for all pregnancies where mother is D negative

40% D negative mothers will not need RAADP

Future ?not need anti-D!

Potential therapy using Rh peptides administered as nasal spray
Mucosal tolerance

Activation of immune regulation
Prevent or switch off immune response

Could be used as prophylaxis and to treat women already sensitised
Progressing to clinical trials

Research at University of Aberdeen -Scotland

Laboratory

Right sample

Correct fetal cell count

Appropriate dose of Anti D

Given in appropriate timescale

Give appropriate advice