

Haemolytic Disease of the Fetus and Newborn

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HDFN -Background

- 1932 Diamond : ‘erythroblastosis fetalis’
- hydrops fetalis/kernicterus-part of same disorder
- characteristics
 - fetal red cell haemolysis
 - extramedullary haemopoiesis
 - nucleated red cells in excess in circulation

HDFN -Background

- Cause unknown
- 1940 Landsteiner & Weiner
 - discovery of Rh blood group system
- 1941 Levine
 - HDN due to development of anti-D in D negative woman following exposure to D positive red cells
 - antibody crosses placenta, coats D positive cells

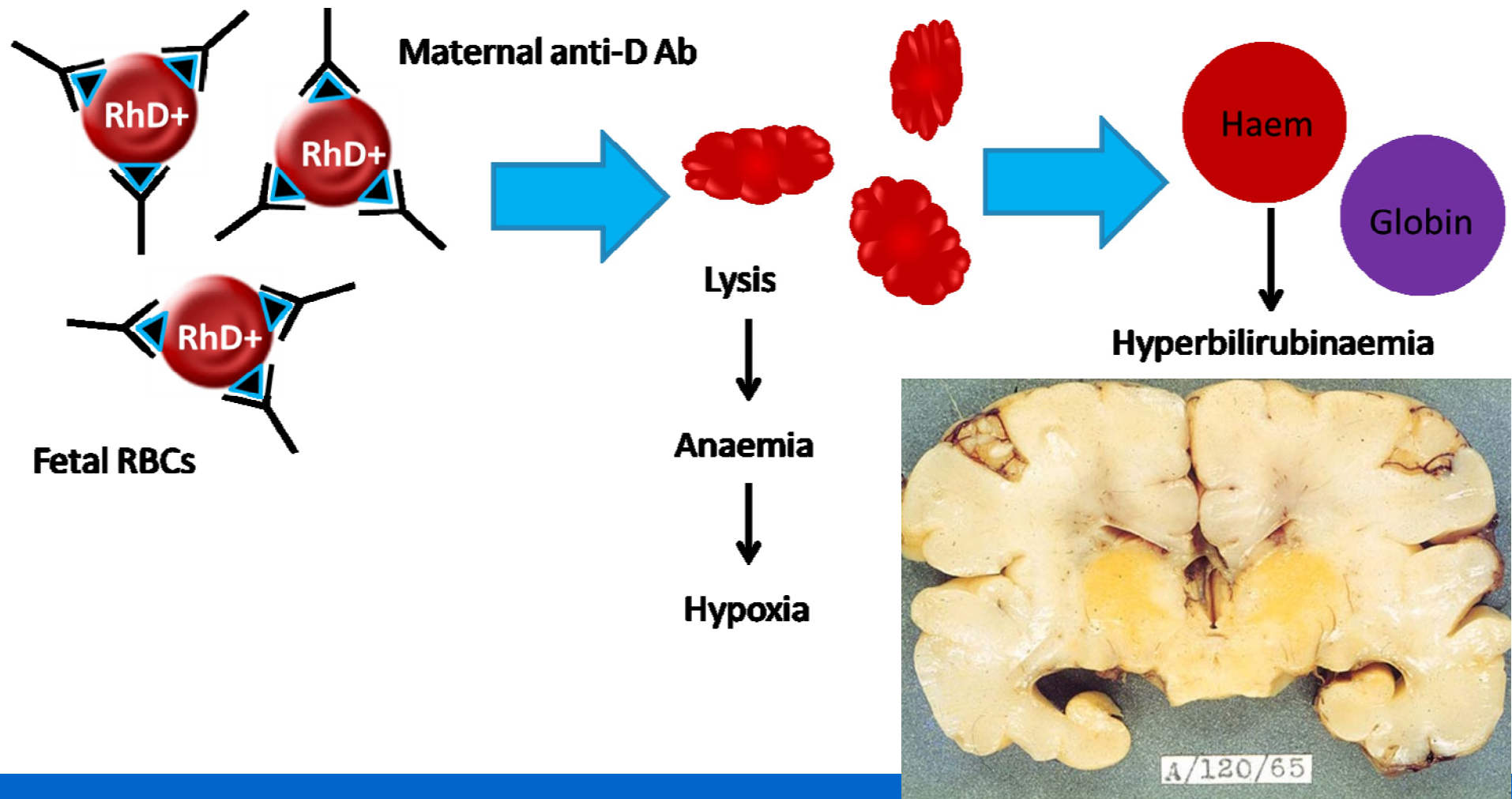
HDFN-Background

- 1948 Weiner
 - Rh alloimmunisation caused by transplacental passage of fetal red cells into maternal circulation (pregnancy/delivery)
- subsequently other antibodies implicated
 - A,B,AB
 - Rh : C,c,E,e
 - non Rh : e.g. K, Duffy, Kidd
 - most significant are D, c, K

HDFN

NHS

Blood and Transplant



HDFN -Clinical Features

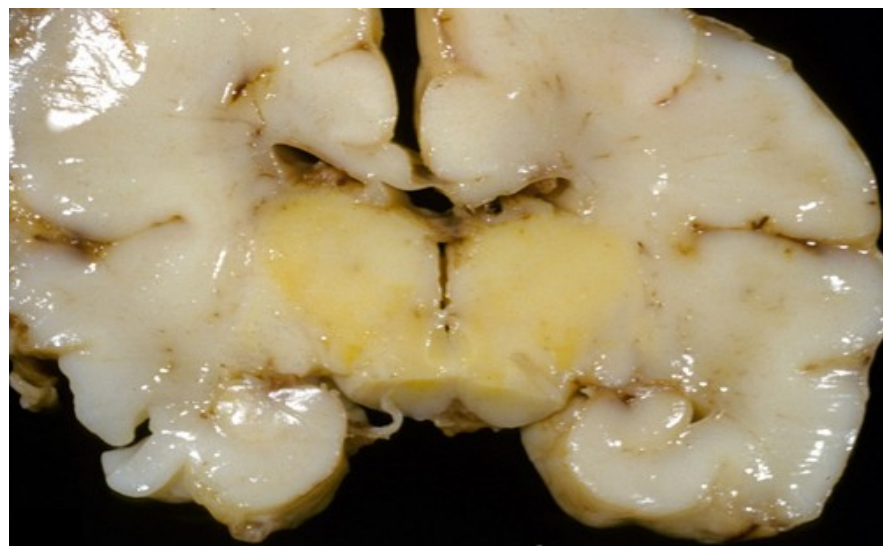
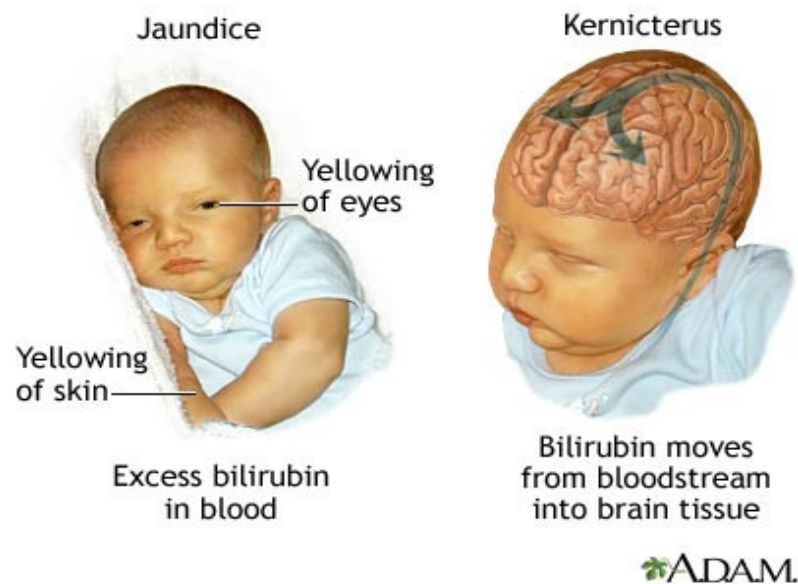
- Hydrops : primarily hepatic in origin
 - hepatosplenomegaly, portal hypertension, hypoalbuminaemia, anasarca
- Kernicterus :deposition of bilirubin in basal ganglia
 - neurosensory deafness
 - spastic choreoathetosis
 - mental retardation

Hydrops Fetalis

- Fetal oedema
- First described by Ballantyne in 1892
- But recognised for over 200 years

Kernicterus

- Yellow Kern
- Kern consists of basal ganglia, hippocampus, geniculate bodies and cranial nerve nuclei especially
- oculomotor, vestibular and cochlear



Opisthotonus

HDFN – Sensitising events

1. Transfusion of red cells to mother
2. Fetomaternal haemorrhage (FMH) during pregnancy

Incidence of alloimmunisation & HDFN



Blood and Transplant

- Before modern therapy: 1% of all pregnant women developed Rh alloimmunisation
- With anti D prophylaxis incidence now 10.2 per 10,000 live births with <10% requiring intrauterine transfusion
- Of those requiring IUT:
 - 85% anti-D
 - 10% anti-Kell (causes hypoproliferative anaemia, with less hyperbilirubinaemia)
 - 3.5% anti-c
- ABO incompatibility can occur in <1% live births associated with significant haemolysis (mother usually Group O)

Antibodies implicated in HDFN

Common	Rare	Never
Anti- D	Anti – Fy ^a	Anti – Le ^a
Anti – D+C	Anti – s	Anti – Le ^b
Anti- D+E	Anti – M	Anti – I
Anti – C	Anti – N	Anti – IH
Anti – E	Anti – S	Anti – P ₁
Anti – c	Anti - Jk ^a	
Anti – e		
Anti - K		

Causes of FMH (1)

- delivery (including CS)
- abortion
- antepartum haemorrhage (APH)
- external version of fetus
- closed abdominal injury
- ectopic pregnancy
- intrauterine death (IUD)
- stillbirth

Causes of FMH (2)

- invasive prenatal diagnosis:
 - amniocentesis
 - chorionic villus sampling (CVS)
 - fetal blood sampling (FBS)
- other intrauterine procedures
 - insertion of shunts
 - embryo reduction

HDFN - Fetomaternal haemorrhage

Events likely to be associated with large FMH:

- traumatic deliveries including Caesarean section
- manual removal of placenta
- stillbirths
- intrauterine deaths
 - abdominal trauma during 3rd trimester
 - delivery of twin pregnancy
- unexplained hydrops

HDFN - Management

- antenatal screening and monitoring
- treatment -antenatal/post natal
- prevention (anti-D HDN)

HDFN -Antenatal screening

- ABO Rh(D) group and antibody screen at booking
- If screen positive
 - antibody identification
 - antibody quantitation
 - testing protocol
 - paternal screening
 - fetal genotyping
 - post-delivery ABO/D group / Hb /DAT/ Bilirubin

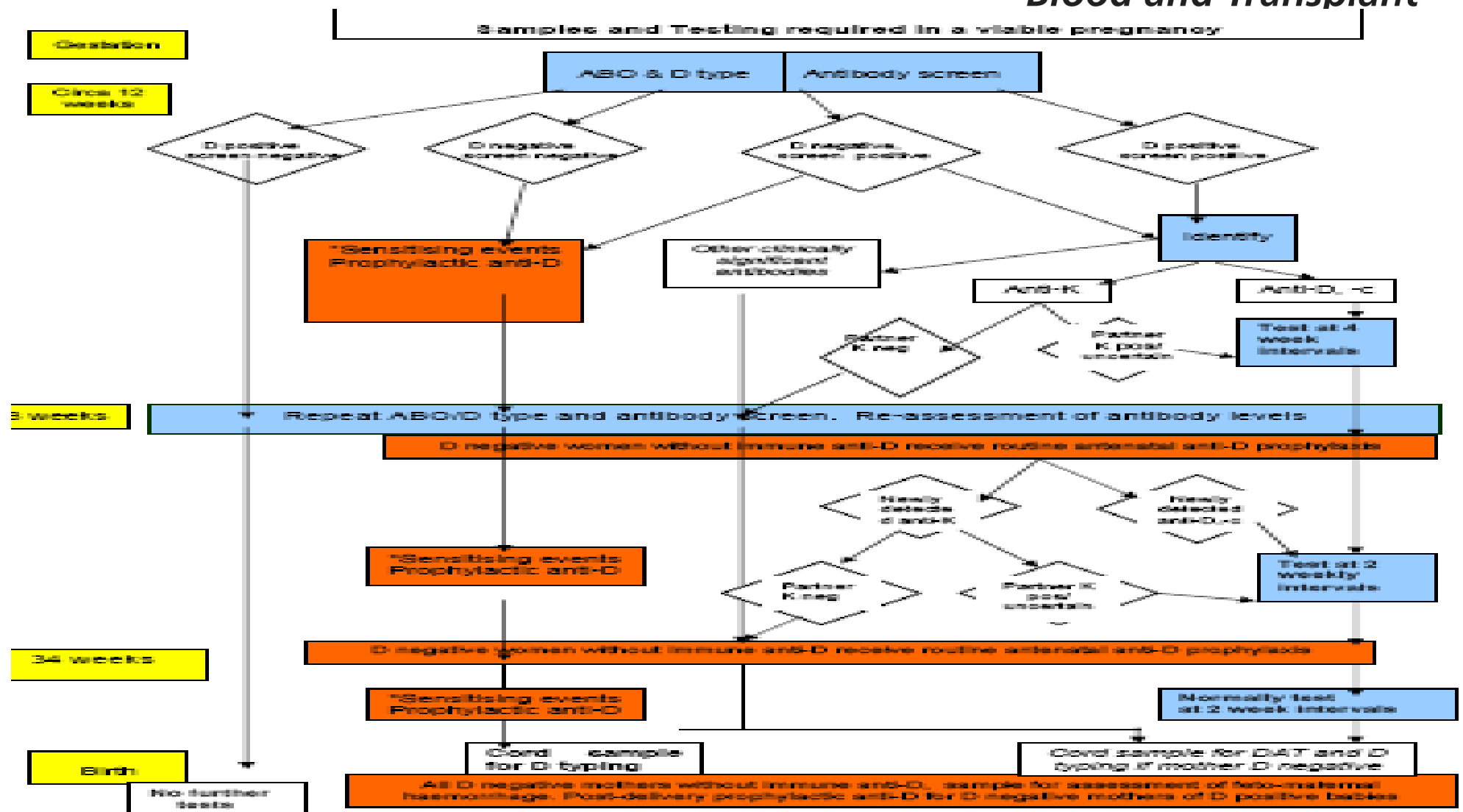
HDFN -Testing protocol



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BCSH guidelines 2006

- anti-D, c, K : 4 weekly to 28/40 then
2 weekly to gestation
- others : booking and 28 weeks



Assessing the severity of HDFN

- **Fetal Blood Typing – First Step**
 - Paternal Rh genotyping for Rh DCE / implicated antigen
 - Father heterozygous / homozygous
 - Serologic analysis → most probable genotype by haplotype tables
 - PCR analysis
- **Fetal Blood Typing – Second Step**
 - Only if fetus at risk of inheriting antigen
 - More recently from fetal DNA in maternal plasma

Assessing the severity of HDFN

- Antibody titres
- Quantitation of antibodies
- Middle Cerebral Artery Peak Systolic Velocity
- Foetal Ultrasonography
- Invasive monitoring with umbilical blood sampling
- Fetal blood typing

Assessing the severity of HDFN

- **Antibody titres**

- Assist clinicians to determine when more invasive tests needed
- Methods:
 - Saline tube techniques only
 - Gel technology to be avoided
 - Previous samples run in parallel
- Every 4 weeks till 28 weeks, then once every 2 weeks
- Critical titres 1:32
- Once critical levels reached, no further titres should be done
- Only of value in first affected pregnancy, subsequent pregnancies

Assessing the severity of HDFN



Blood and Transplant

- **Quantitation of antibodies:**
 - Anti- D
 - Measured in IU/ml
 - Done using NIBSC standard anti-D
 - Less than 4 IU/ml – HDN unlikely
 - 4- 15 IU/ml – Moderate risk of HDN
 - More than 15 IU/ml – High risk of Hydrops fetalis
 - Anti – c
 - Less than 7.5 IU/ml – Continue to monitor
 - 7.5 to 20 IU/ml – Risk of moderate HDN
 - More than 20 IU/ml – Risk of severe HDN
- others **titres** > 32 significant risk (??anti-K)

What does obstetrician want to know?

Is there an antibody?

- is it capable of causing fetal haemolysis / anaemia?
 - what antibody?
 - level?
- how frequently to test?
- refer to FMU?
early delivery?
blood for fetus/infant/mother?

Indicators of high fetal risk, ie refer to FMU

- History of non-ABO HDFN requiring transfusion, irrespective of antibody
- anti-D >10iu/ml
- anti-K
 - untransfused women
 - partner K+
- rising anti-c (>20iu)

Doppler measurement of peak velocity of systolic blood flow in the *Middle Cerebral Artery* best non-invasive test to diagnose mild-moderate fetal anaemia.

(false +ves in cardiac anomalies and 'non-allo immune anaemia')

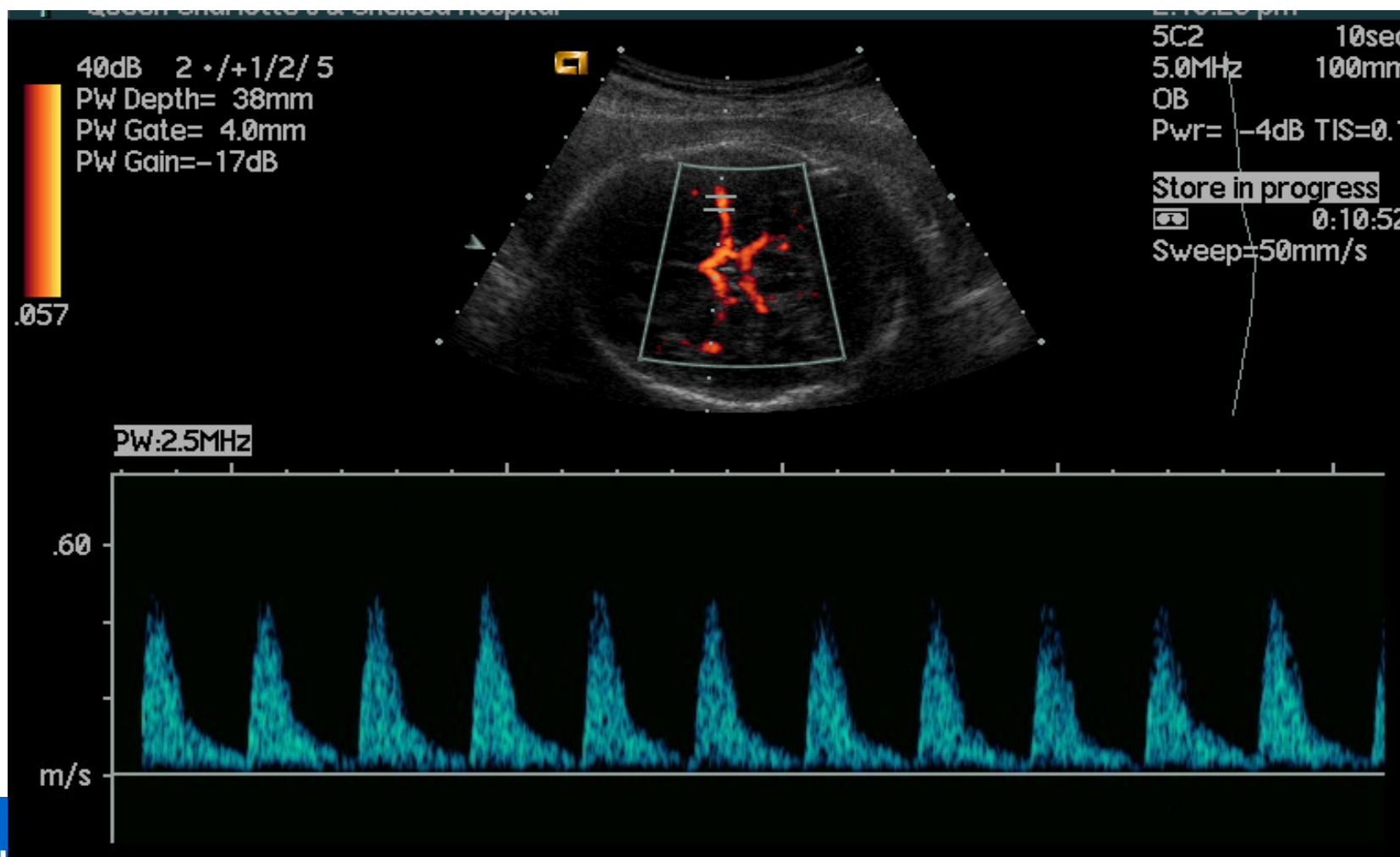
Mari et al NEJM 2000, 342:9-14

Assessing the severity of HDFN

- **Middle cerebral artery peak systolic velocity & Fetal ultrasonography**
 - Accurate test for detecting fetal anaemia
 - Non invasive – no fetal risk of miscarriage / preterm labour
 - Reciprocal relationship between fetal Hb & Velocity of cerebral flow
 - Can be used for all alloimmunised pregnancies
 - 100% sensitivity for moderate / severe anaemia-
Threshold value of peak systolic x 1.5 multiples of the median

MCA Peak Systolic Velocity

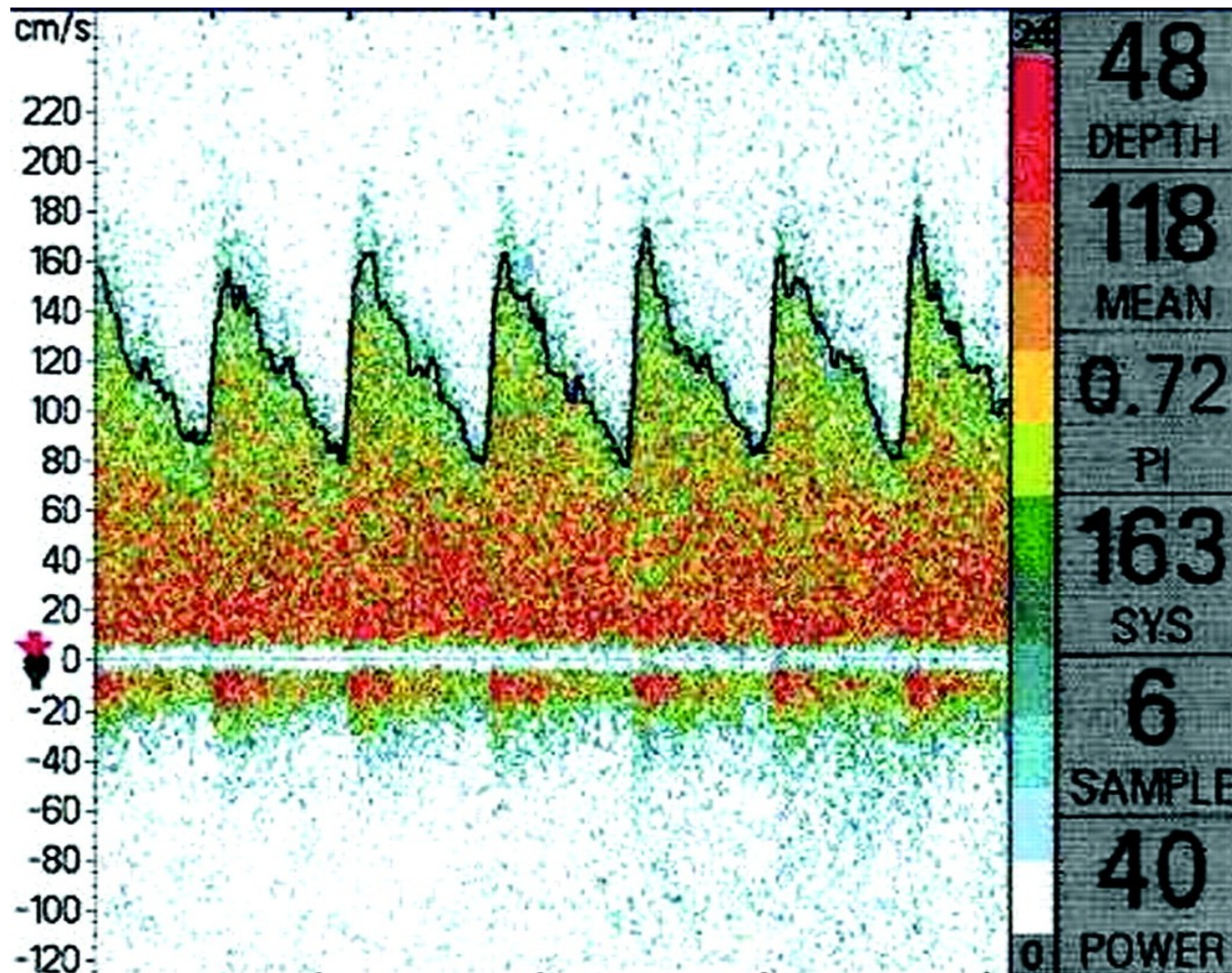
Blood and Transplant



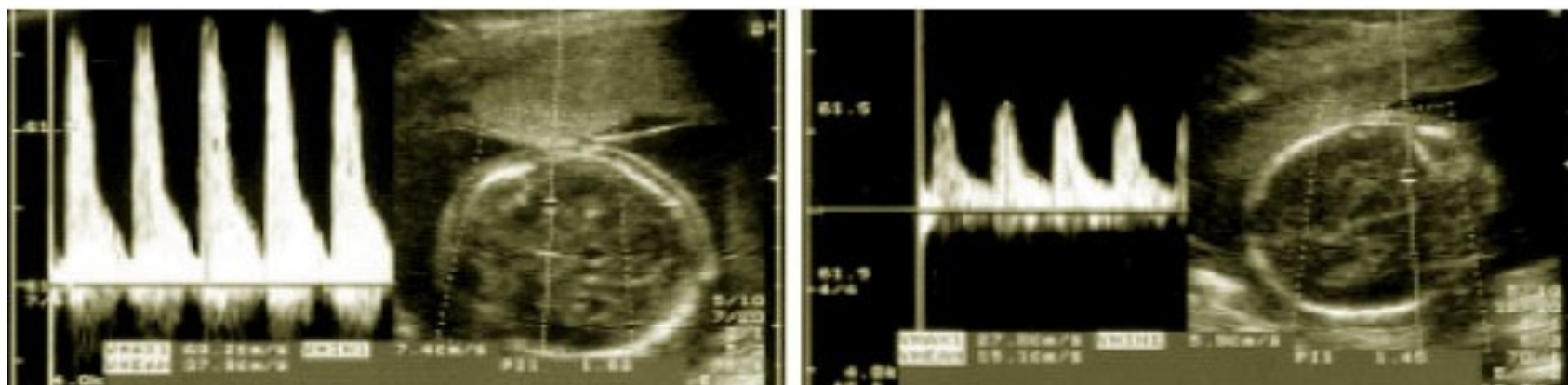
--Typical transcranial Doppler sonographic recording from middle cerebral artery

NHS

Blood and Transplant



McCarville, M. B. et al. Am. J. Roentgenol. 2004;183:1117-1122



Flow velocity wave form in the fetal middle cerebral artery in a severely anaemic fetus at 22 weeks (left) and in a normal fetus (right). In fetal anaemia, blood velocity is increased

Tests used by obstetricians to predict fetal anaemia

Ultrasound

- placental thickness
- umbilical vein diameter
- liver length
- spleen perimeter

Doppler

- measure fetal blood flow

- useful to assess fetal maturity
- does not identify early fetal disease - changes visible only once hydrops has occurred
- weak correlation with fetal Hct/Hb

- detects early fetal anaemia

Should women with antibodies be delivered early?

- clinical decision
- previous outcome
- MCA velocity/ultrasound
- severity of HDFN vs risk of prematurity

At Delivery:

- when any IAT-reactive antibody is present in pregnancy, a cord DAT must be done ASAP
- if DAT is +ve, check Hb and bilirubin to diagnose HDN (NB. RAADP)
- if DAT is -ve, no risk of HDN (except ABO)

Management of Alloimmunisation during pregnancy

- Intrauterine red cell transfusions
- IVIgG
- Premature delivery

Intrauterine Red Cell Transfusion

- Performed by direct infusion of allogeneic blood into:
 - Umbilical cord
 - Intrahepatic portion of hepatic vein
 - Intraperitoneally

Intrauterine Red Cell Transfusion



Blood and Transplant

- Red cells selected are :
 - Group O Rh D Neg (unless anti c), Kell Neg, High Titre Neg
 - Hct 0.7-0.85
 - CMV Neg, irradiated
 - < 72 hrs old (<24 hours following irradiation)
 - Hb S-Neg
 - Negative for the offending antigen
 - Crossmatch-compatible with maternal plasma

Intrauterine Red Cell Transfusion

- Maternal blood used in case of rare antibodies
- IUT is only possible from 20 weeks gestation
- IUT also useful for –
 - PRCA from Parvovirus B19
 - Fetomaternal haemorrhage
 - Twin-to-twin transfusion

Intrauterine Red Cell Transfusion



Blood and Transplant

- Amount of blood to be transfused –
$$\frac{\text{Desired PCV} - \text{Fetal PCV}}{\text{Donor PCV} - \text{Desired PCV}} \times \text{Fetoplacental blood volume}$$
- Final Hct ~ 40%
- Transfusions every 14 days until 35 weeks

Intrauterine Red Cell Transfusion - outcomes

- Fetal outcomes superior with IUT:
 - Mortality before Hydrops – 2 to 8%
 - Mortality after Hydrops – 22 to 30%
- IUT suppress erythropoiesis causing hypoproliferative anaemia in the neonatal period – may need top ups
- Despite severe fetal haemolytic disease, normal developmental outcomes can be expected from children treated with IUT

Intrauterine Red Cell Transfusion - outcomes



Blood and Transplant

- Van Kamp et al; AJOG, 2005:
 - 254 foetus with 740 IUTs treated in between 1988 – 2001
 - Death from procedure related complications – 1.6%
 - Overall procedure-related complication rate 3.1%:
 - 0.1% PROM
 - 0.3% Infection
 - 2.0% Emergency Ceasarean Section
 - 0.9% Fetal death

Intravenous Immunoglobulin

- Prenatal use –
 - Efficacy unknown
 - No RCT
 - Only Case reports & Case series
- Postnatal use – 1g/kg within 12 hours
 - Effective in reducing the hospital stay
 - Reducing the duration of phototherapy
 - Need for exchange transfusion

Premature delivery

- Early delivery after 35 weeks performed in all severe cases of HDFN
- Have blood on standby for exchange:
 - Group O Rh D Neg (unless anti c), Kell Neg, High Titre Neg
 - Hct 0.5-0.6
 - CMV Neg, irradiated
 - < 72 hrs old (<24 hours following irradiation)
 - Hb S-Neg
 - Negative for the offending antigen

Management of Alloimmunisation after delivery

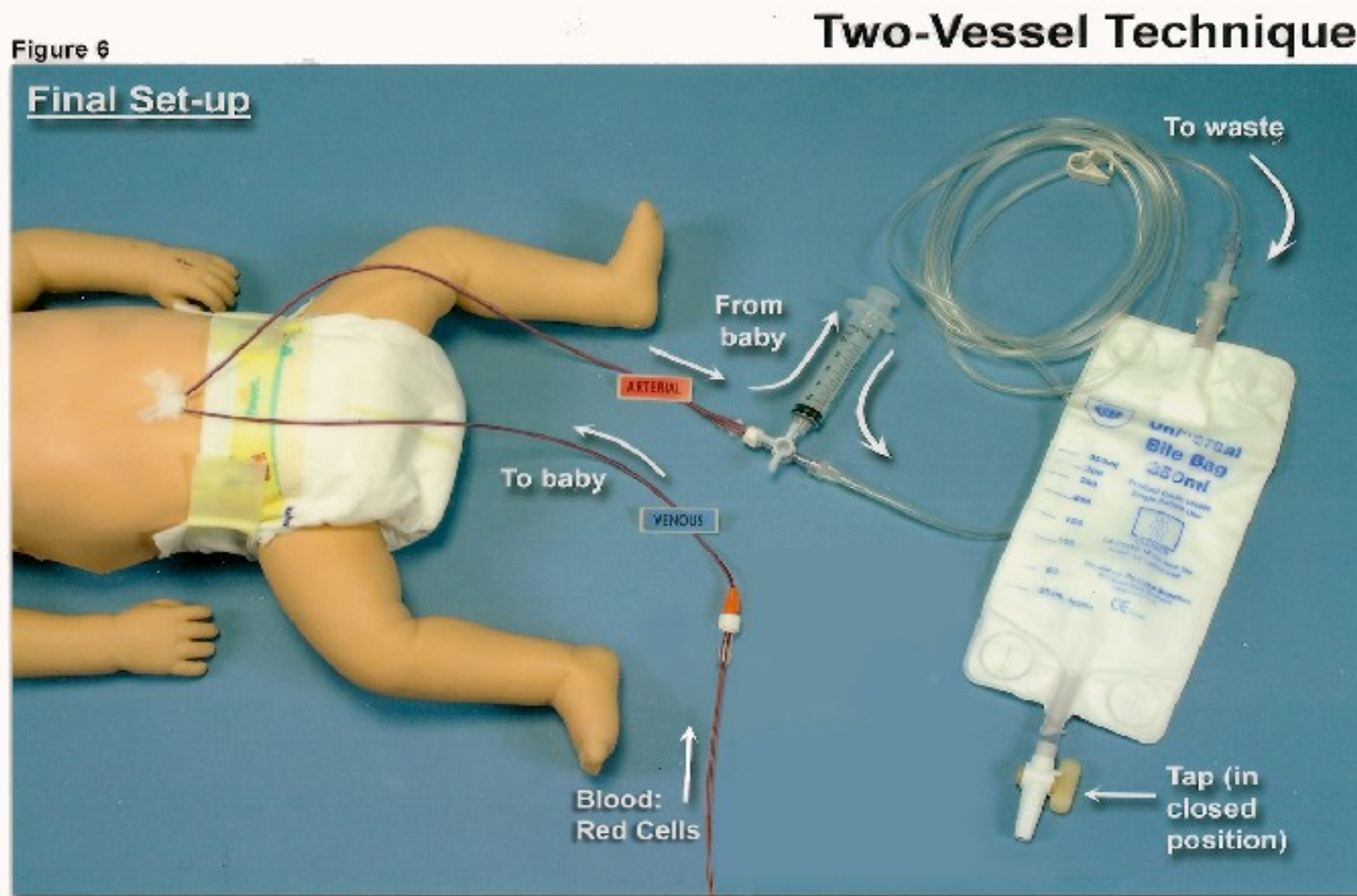
- Samples after delivery
- Exchange transfusions
- Phototherapy
- Top – Up transfusions



Exchange transfusion

- Indicated if:
 - cord Hb <8 g/dL
 - cord bilirubin > 100 μ mol/L, and
 - rapidly rising bilirubin
- Double volume exchange removes 85 - 90% of infant's cells
- Use plasma-reduced blood (Hct 0.5 - 0.6)
- Check Hb 2-weekly until 3 months may have hypoproliferative anaemia

Exchange transfusion: Two-vessel technique



Top-up transfusion

- 10 - 15 ml/kg
- Use multi-doses from paedipack if possible
- SAG-M acceptable
- CMV neg
- HT anti-A/B neg
- Extended Ab screen neg
- Sickle neg
- Irradiated if previous IUT

ABO HDN

- Usually group O mother with Group A (or B) baby
- IgG anti A (or B)
- Expression of A and B antigens much weaker on neonatal cells – haemolysis often not significant
- DAT may or may not be positive
- Hyperbilirubinaemia more common than anaemia

Intrauterine transfusion required for antibodies other than anti-D, c, K

Aim

- We wanted in the initial analysis to identify how often fetal treatment was required for non-D, c or K antibodies

Method

- A form was sent to hospitals around the time of delivery, for women found by RCI Labs in England, to have antibodies with a titre ≥ 16 . Information requested included any need for intrauterine transfusion (IUT) phototherapy, exchange or top-up transfusion, or death
- Data from 2006-11 were analysed. Whilst NHSBT tests most antenatal women in England with antibodies, we also cross-checked against IUT blood issued in 2010 and 2011, to ensure no cases of HDF were missed

- Dear Colleague
 - **Re: Collection of data on Haemolytic Disease of the Newborn**
 - Thank you for supporting the National Health Service Blood and Transplant (NHSBT) programme of data collection on the outcome of pregnancies of women with red cell antibodies. By recording the data collected in this exercise we hope to accumulate a body of evidence for the effect of specific antibodies of known strength on the fetus/newborn. We hope that you will be willing to help by completing the attached questionnaire.
 - Any information you can provide will be useful, and we hope that you will be able to complete the forms as fully as possible. If necessary, please contact the appropriate clinician or midwife so that all the relevant clinical information can be captured.
 - Please return the completed form in the envelope provided, to the address below.
 - Data Protection
 - Patients are informed that we collect information about their baby in the patient information leaflet entitled 'Blood Groups and Red Cell Antibodies in Pregnancy' produced by the NBS and distributed to all hospital clinics and GPs for whom we provide a screening service.
 - Please be assured that all patient data will be held securely, and in accordance with the patient's rights, under the Data Protection Act (1998).
 - Thank you in anticipation of your help. If you have any queries please contact your local NHSBT Red Cell Immunohaematology laboratory or myself.
-
- Liz Pepperell
 - RCI Project Scientist
 - NHSBT Cambridge
 - Long Road
 - Cambridge
 - CB2 0PT
-
- Tel. 01223 588132
 - e-mail: liz.pepperell@nhsbt.nhs.uk

- This data is being collected by the NBS in order to establish the outcomes of pregnancies with antibodies. Expectant mothers are informed via the antenatal information leaflet 'Blood Groups and Red Cell Antibodies in Pregnancy' and NBS antibody card. Data Protection issues are covered by these documents and the Service Level Agreement between the NBS and your Trust.
- Mother's details: [to be completed by NBS]
- Surname: Forename[s]:
- DoB: Hospital/GP?
- NHS Number: Hospital Reference No:
- NBS Ref No: E.DD.
- Maternal antibody specificity
- **Father** Rh phenotype: Other relevant positive antigens

- :
- **Clinical information - to be answered by:**
- **Transfusion Laboratory &/or Paediatrician &/or midwife :**
- Date of delivery: **Please give details**
- Was this baby affected by HDN? **Yes / No**
- Investigations e.g. MCA Doppler **Yes / No**
- Early delivery due to antibody level / HDN **Yes / No**
- Intrauterine death **Yes / No**
- Still birth **Yes / No**
- Intrauterine transfusion required **Yes / No**
- Live birth – exchange transfusion required **Yes / No**
- Live birth – top up transfusion required **Yes / No**
- Live birth – phototherapy required **Yes / No**
- **What was final outcome?**
- Perinatal/postnatal death **Yes / No**
- Residual morbidity **Yes / No**
- Alive and well **Yes / No**
- **Section completed by:** **Position:** **Date:**
- [please print]
-

- **Laboratory information on baby:**
- Blood group [ABO + RhD]
- Relevant positive antigen(s) Cord DAT score
- Haemoglobin at delivery Bilirubin at delivery
- Lowest haemoglobin Maximum serum bilirubin
- **Records of transfusion - please complete details (dates/volumes) below:**
- Intrauterine transfusion
- Exchange transfusion
- Top up transfusion
- **Completed by:** **Position:**
Date:
- [please print]
-

Number of Pregnancies England + Wales

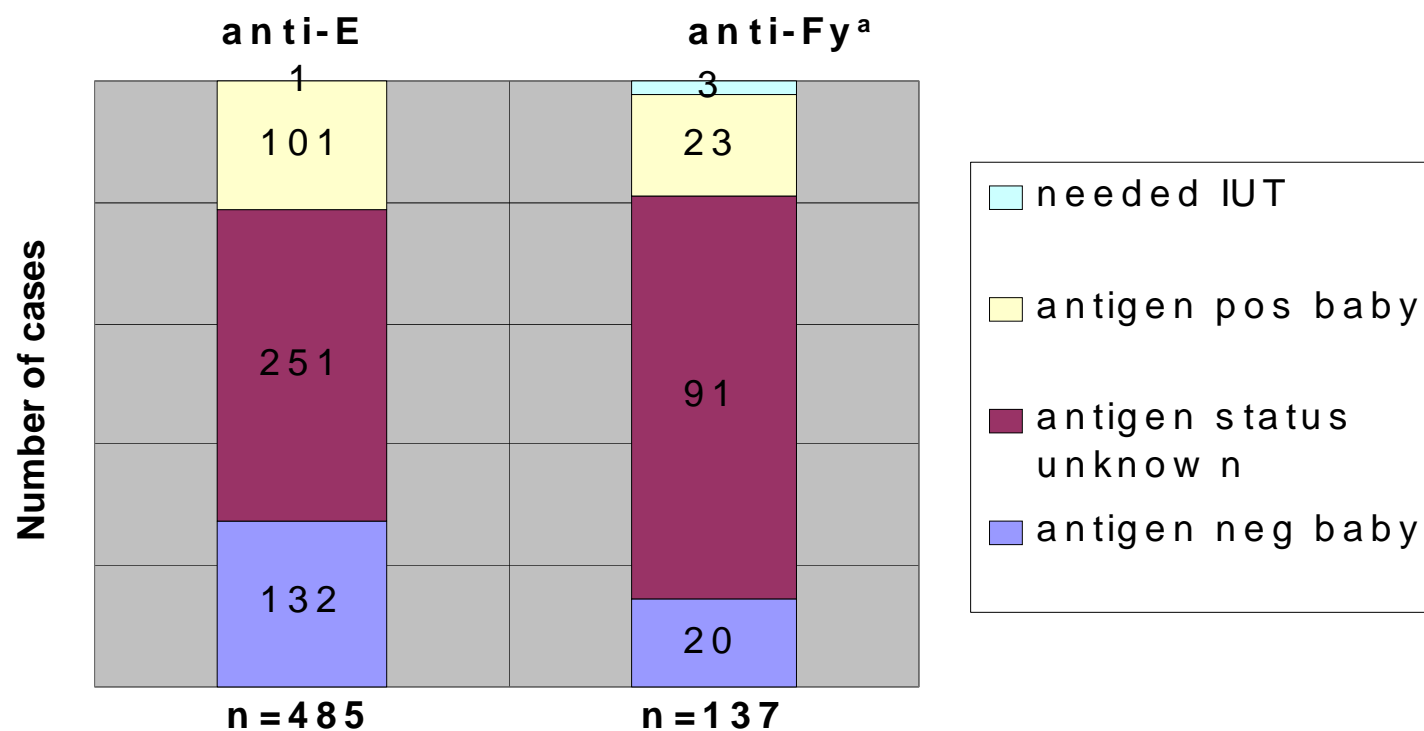
Year

•2006-7	633,000
•2007-8	653,000
•2008-9	656,000
•2009-10	656,000
•2010-11	672,000
•2011-12	673,000

Results

- Replies were returned on 3787 cases (67% response rate).
- Total number of cases with red cell antibodies, but excluding anti-D, c or K = 1454 (figure 2[f1]).
- Only 4 required IUT: 1 with anti E titre of 512; 3 with anti-Fya titre ≥ 512 [p2] .
- Of 485 cases of anti-E alone, 132 (27%) babies were E neg; 102 (21%) were E pos and 251 were unknown (but as 30% of population are expected to be E pos, about 75 of these may be E pos). So, $1/177 = 0.6\%$ needed IUT (see figure 3).
- Of 137 cases with anti-Fya alone, 20 (15%) babies were Fya neg; 26 (19%) were pos and 91 were unknown (but as 60% of population are Fya pos, about 54 of these may be Fya pos). So, $3/80 = 4\%$ needed IUT.
- There were no deaths due to HDFN.
- Of note, while IUT was required in 4 cases with non-D, c or K antibodies, the number of cases requiring IUT for anti-D = 73; anti-c = 10 and anti-K = 8.

Figure 3 - Number of women with antibodies and number of IUTs



Conclusions

- While predictors of HDFN include a previous history of HDFN, antibody titre >32 or a rising titre
- Practice in the frequency of monitoring non-D, c or K antibodies after 28 weeks varies from doing nothing until testing cord blood at delivery (for Hb and bilirubin to monitor for haemolysis), to fortnightly monitoring of antibody titres +/- MCA Doppler monitoring for fetal anaemia.
- This has associated implications for resources involved and the inconvenience to mothers of extra hospital attendances and potential for the 5% false positive rate of MCA Doppler testing to result in unnecessary fetal blood sampling \pm IUT, with a 1-2% rate of miscarriage.

Recommendations

- In light of our findings, together with previous literature, discussion is needed with the Royal College of Obstetricians and Gynaecologists to rationalise monitoring for optimal patient care, without overuse of resources. Monitoring could be tailored differently for some antibodies.