Hemolytic Disease of the Fetus and Newborn: A focus on Prevention

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GHEST, 2023

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Disclosures

I have no disclosures

30 year old G2P1 at 12 weeks gestation was discovered to have an anti-D antibody, titre 1:32

Informed this is a critical titre and she may need an IUT

G1: 1 year prior

She knew she was RhD negative

Had received WinRho at 28 weeks gestation

Could not recall whether she had received it after birth

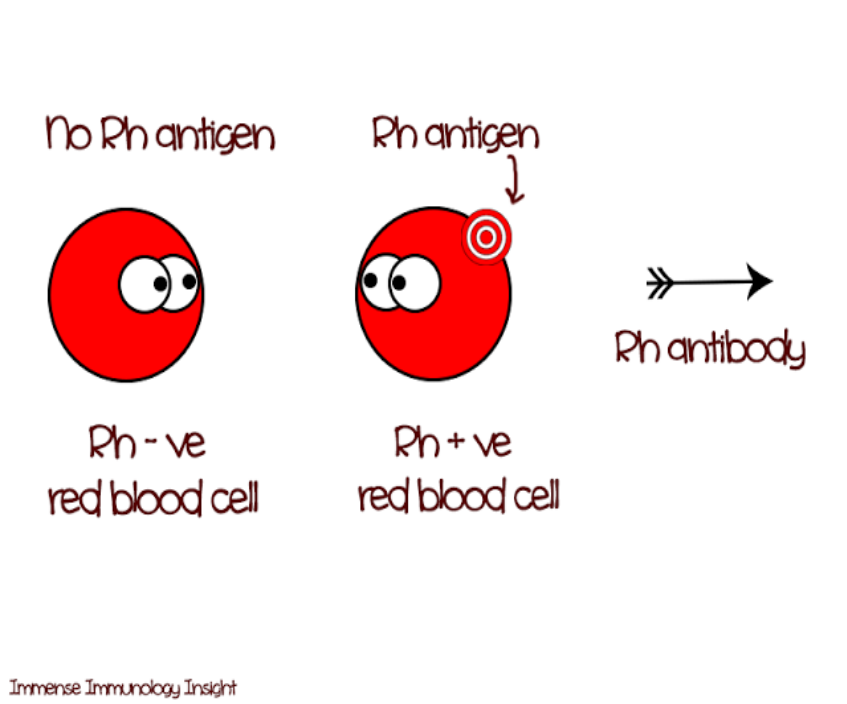
Objectives

Discuss alloimmunization during pregnancy

Identify potential gaps that can lead to alloimmunization in women of child bearing age

Discuss options to prevent alloimmunization

Alloimmunization (development of an alloantibody) occurs following exposure to foreign red cell antigens



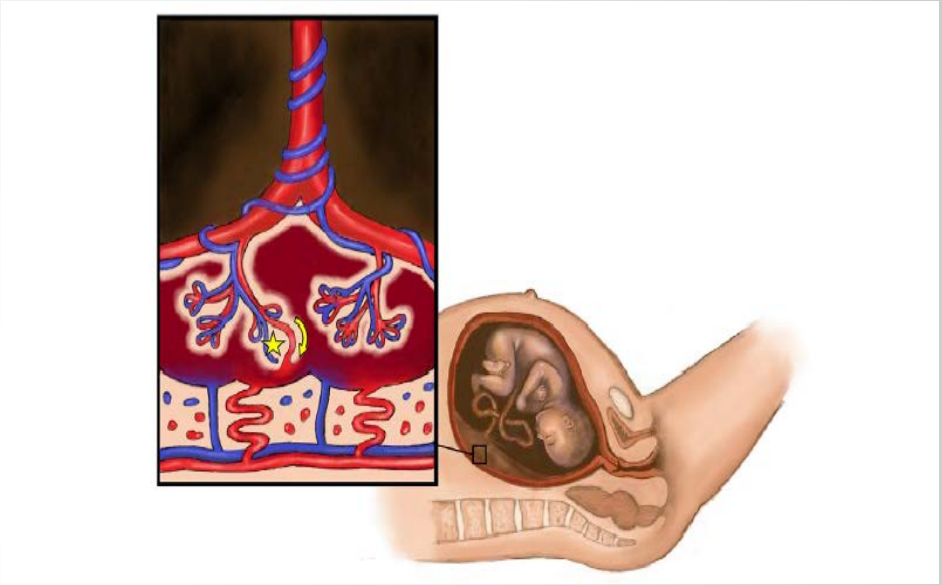
1. RBCs transfusion
2. From fetal maternal hemorrhage during pregnancy (paternal antigens)

Transplacental Hemorrhage

Entry of fetal erythrocytes from the higher pressure fetal circulation into the intervillous space where they are ultimately returned to the maternal circulation

Frequency and volume depends on gestational age

Small volume in 1st and 2nd trimester unless there is trauma



Wylie B, D'Alton M, Obstet Gynecol 2010;115:1039–51

Boller MJ, AJOG 2021: 225., e1-540.e8

The incidence of fetal maternal hemorrhage in the absence of invasive procedures

1st: 56% (0.07 ml)

2nd : 63% (0.08 ml)

Volume of fetal RBCs	Percent immunized
0-0.1	3%
>0.1	14%

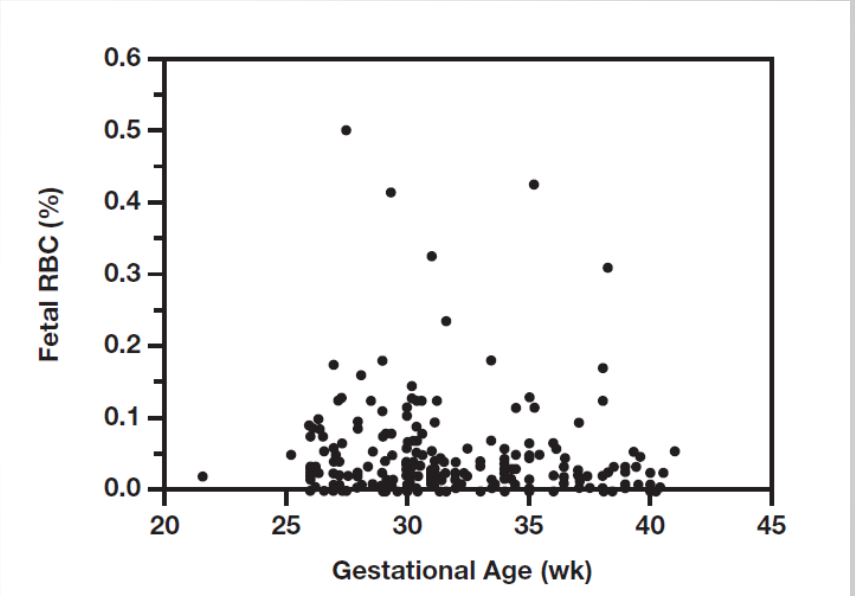
Transplacental hemorrhage is more frequent in the 3rd trimester and postpartum

Increasing size, gradual

deterioration in the placental

blood barrier in the latter weeks of

gestation



De Wit H et al. Am J Clin Pathol 2011;136:631-636. Sebring,ES, Polesky F. Transfusion I0 N 1990-Vol. 30. Zipursky, Lionels CMAJ1967:97:1245

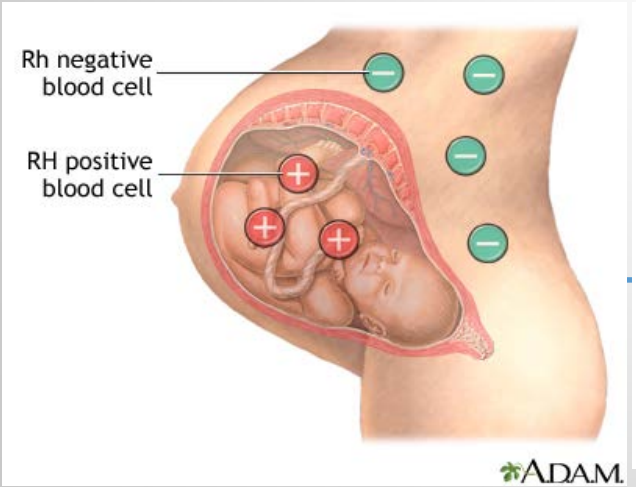
The result is

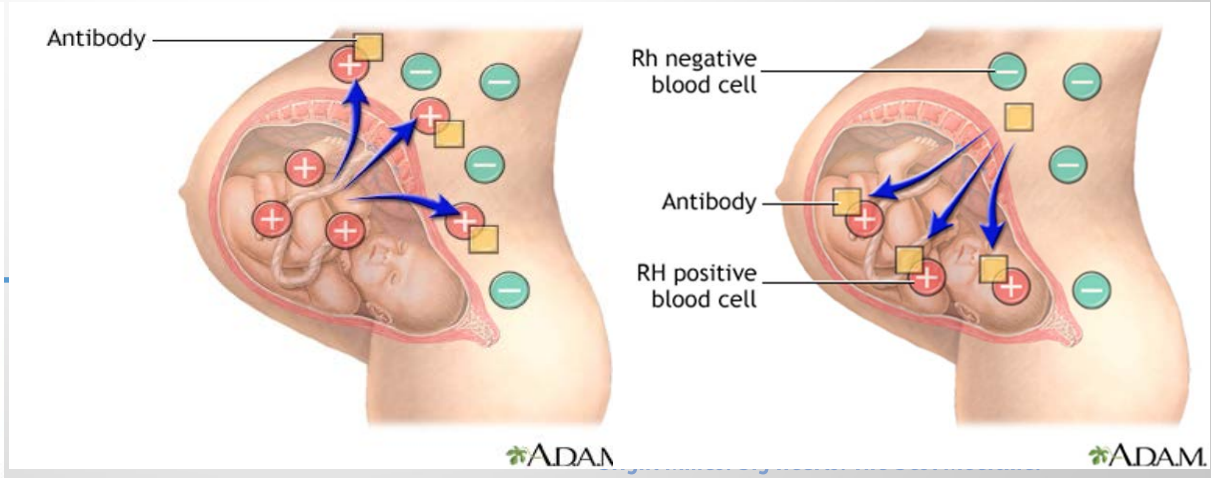
Destruction of fetal or neonatal red cells secondary to maternal IgG alloantibodies

Antigen exposure

Primary IgM antibody and does not cross the placenta

Subsequent exposure: IgG antibody that crosses the placenta





http://www.nlm.nih.gov/medlineplus/ency/presentations/100217_3.htm

Spectrum of HDFN

No disease

No fetal destruction of RBCs

Mild disease

Hemolytic disease of the newborn

Severe disease

Hemolytic disease of the fetus requiring intrauterine transfusion

Fetal hydrops

Antigen	Severe Disease in Alloimmunized Pregnancies*	Neonatal anemia
D	20%	May be prolonged up to 4 months Prolonged following IUT and IVIG
K	11-50%	
c	17-26%	
E	1%-18%	
Duffy	14-16% (Duffy a> b)	

*Requiring intrauterine transfusion or neonatal exchange transfusion

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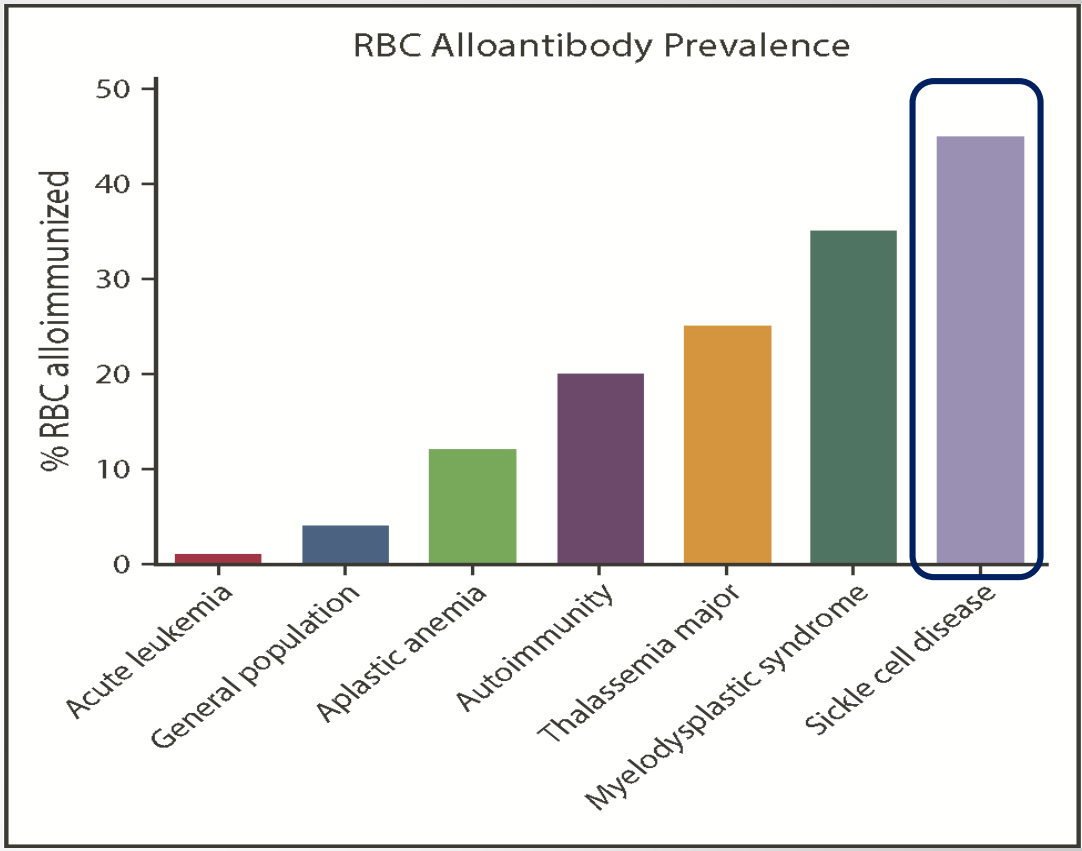
Hackney DN et al. ObstetGyn 2004;103:24-30, *J.M. Koelewijn, Transfusion* 2008;48:941-952. Grant SR. BJOG 2000;107: 481-485, Joy SD et al. Obstet Gynecol 2005;105:24–8

The ability to develop an alloantibody depends on the **immune responsiveness of the individual**

Depends on disease state



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Tormey CA, Hendrickson J. Blood 2019; 133:1821-1830,

If an alloantibody is detected…

The father's phenotype for the antigen and zygosity are determined

The zygosity for the father's antigens can be determined for most antigens by phenotyping

Zygosity for D is presumed based on phenotyping Rh antigens

If the father is heterozygous/unknown, cell free DNA from maternal plasma can determine the D status of the fetus 18 weeks onward

If the fetus is potentially or affected…..

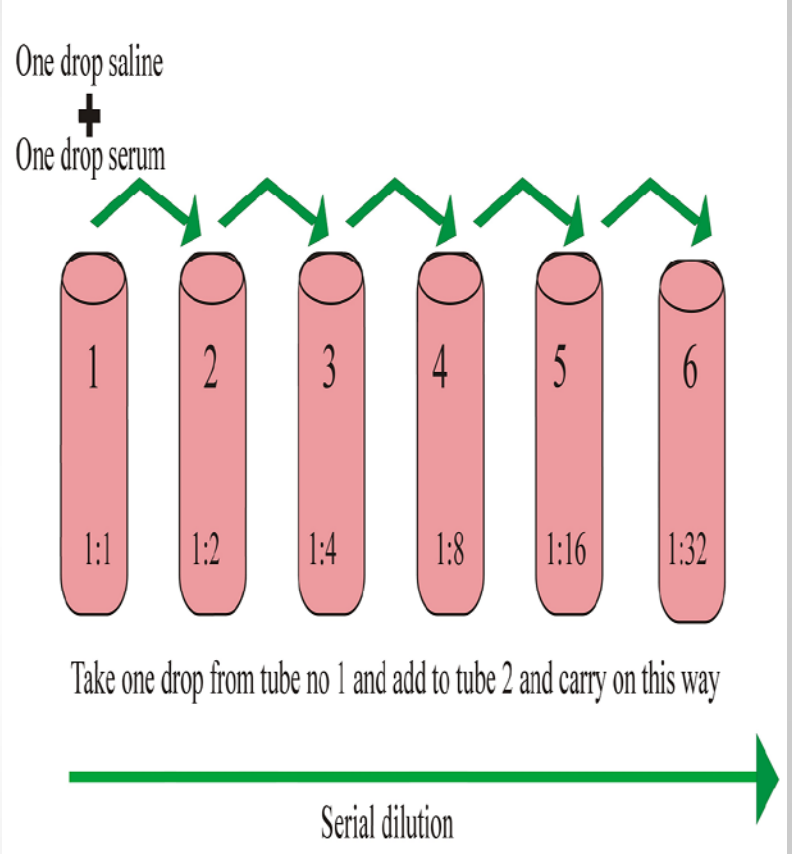
Serial antibody titres: a method to determine strength of the antibody

Saline IAT

Antibody titre: greatest dilution with a agglutination reaction

In a primigravida for D, c antigens

titres every 4 wks in the 1st and 2nd trimester or every 2 wks if there is a rise and in the 3rd trimester



Cells used for antibody titration

R2R2 for D-uniform expression of D

R2R2 for c, E or combinations

K, heterozygous

Avoid Bg+ cells

Titration done twice by 2 technologists-concordance

Run subsequent samples in parallel

Critical titre: Refer to a high risk centre

Critical Titre: A threshold or cut-off value

Implies an increased risk for severe HDFN

1st pregnancy

When the alloantibody titre (tube) **>1:16,** D, c, E

At any **alloantibody titre for K** as titres may not correlate with the severity of anemia: K alloantibodies also suppresses fetal erythropoiesis

Subsequent alloimmunized pregnancy where the first had HDFN

Once pregnancy is confirmed

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De Has M et al. Vox Sang(2015) 109, 99–113

Tube vs. Gel

Using the mean value as a correlation between the tube and gel technique, gel titre >tube titre

- Anti-D: 2 fold
- Anti-c: 2.7 fold
- Anti-K: similar

Could the development of the antibody have been prevented?

Red cell transfusion is the most common cause of alloimmunization despite paternal exposure for non RhD antigens

	Prevalence risk factor in control group (%)	All cases (n = 900; n controls = 625)	Cases with antigen-positive partner (n = 527; n controls = 625)
General risk factors*			
Parity			
0	43.5	1	1
1	39.0	1.3 (1.0–1.7)	2.6 (1.9–3.6)
2	14.6	1.4 (1.0–2.0)	3.1 (2.1–4.5)
>2	2.9	3.2 (1.8–5.8)	6.2 (3.3–11.6)
RBC transfusion	5.1	16.7 (11.4–24.6)	11.6 (7.7–17.4)
Major surgery	12.6	1.4 (1.1–1.8)	1.4 (1.0–1.9)
Haematological disease	1.9	2.1 (1.0–4.2)	2.5 (1.1–5.5)

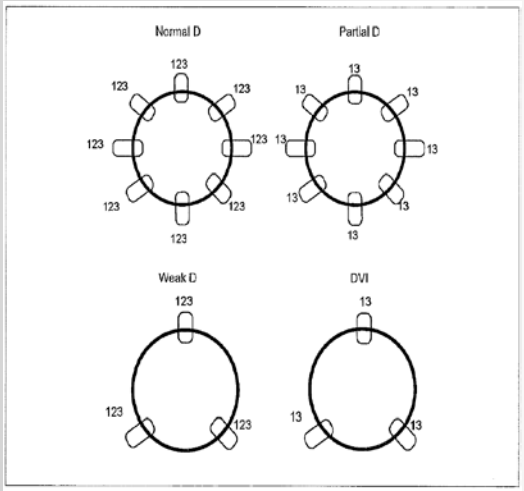
83% of the K-sensitized women had a history of RBC transfusion

50% with anti-c antibody, had received RBC transfusion

Phenotyped/genotyped RBCs for individuals with sickle cell disease/thalassemia

Koelewijn JM et al. BJOG 2009;116:655–664

Reducing the risk of alloimmunization: Identifying D variants by genotyping



D variants

0.2 – 1.0% Caucasian

1.7% black

Partial D: 5 – 10% of D variants

Genotyping advised for pregnancies with serological weak D phenotype= reactivity of RBCs with anti-D reagent ≤2+ but moderate/strong agglutination with AHG (a weak D test) to detect partial D

Flegel W et al. JOGC 2007;29: 746

Sandler SG et al. BJH 2017 ; 179(1): 10–19.

30 year old G2P1 at 12 weeks gestation was discovered to have an anti-D antibody, titre 1:4

G1: 1 year prior

She knew was RhD negative, had received WinRho at 28 weeks gestation but could not recall whether she had received it after birth

She has not been transfused

Prophylactic RhIG has reduced alloimmunization from 16% - ~ 0.2-1%

Allo anti-D remains the #1 antibody for the need of IUT

300 micrograms to all D-negative women at 26-28 weeks' gestation,

120-300 micrograms within 72 hours of delivery (for fetuses that are D+ or D unknown) **+**

Assessment for FMH for additional RhIg

300 micrograms suppresses the response of up to 15 ml of RBCs

Preventative when there is an increased risk of FMH

Early Pregnancy Loss and Termination
• Anti-D 120–300 μg after spontaneous/induced abortion
• Antibody screening prior to anti-D after abortion
• Ectopic pregnancy: 120–300 μg Rh immune globulin
• Molar pregnancy: 120–300 μg Rh immune globulin

Invasive Fetal Procedures
• Amniocentesis: 300 μg Rh immune globulin
• CVS: 120–300 μg Rh immune globulin
• Cordocentesis: 300 μg Rh immune globulin
APH, Abdominal Trauma, ECV, FMH
• Quantitative FMH testing
• Anti-D 120–300 μg following placental trauma

SOGC JOGC 2018;40(1):e1–e10
BSH. Transfusion Medicine, 2007, 17, 252–262

ASSESSMENT OF FMH

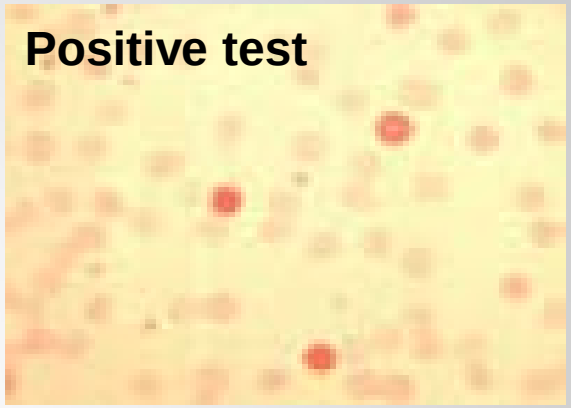
The Betke Kleihauer acid elution test detects Hemoglobin F

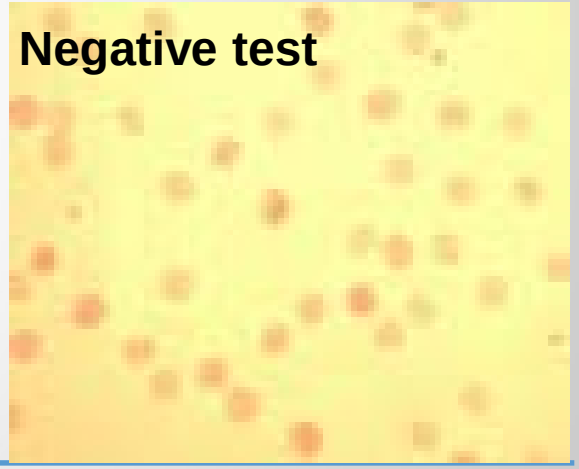
Fetus: Hemoglobin F is the predominant hemoglobin of the fetus, is more acid-resistant than other hemoglobins (fetal cells appear as dark reddish/pink cells)

Adult: Hemoglobin F usually < 1% in adults, concentrated in F cells

Predominant HbA is denatured by acid and eluted=ghost cells

ml bleed=% fetal cells (#fetal/#counted) x 5L (BV)





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RhIg dosing for pregnancies with weight >70 kg

Current dosing based on TBV of 5000 ml (70 kg)

Patients > 100 kg potentially under-dosed

Intramuscular injection may not be adequately accessed-consider iv

Pham HP et al. Arch Pathol Lab Med. 2015;139:1084. Woo E, Kaushal M. Arch Pathol Lab Med. 2017;14:17.

Quality FMH assessment in Ontario of 14 participants described potential for inadequate RhIg dosing

FMH of 30 mL: 32% participants reported results that could have lead to inadequate prophylaxis

FMH of 45 mL: 21% reported results that could have lead to inadequate prophylaxis

FMH of > 10 ml: 19% proficiency tests reported results that could have lead to inadequate prophylaxis

Lafferty J et al. Am J Clin Pathol 2003;119:72-77

Flow cytometry has better accuracy: can differentiate fetal cells from adult F cells

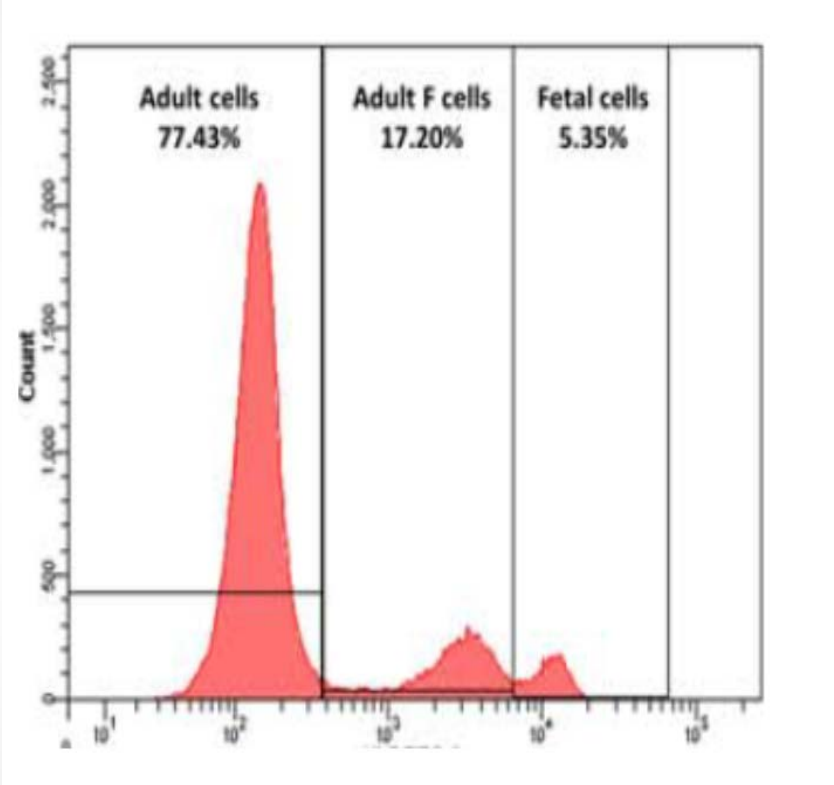
F cells contain 20-25% HbF in an adult

F cells increases during pregnancy

Can be as high as 7% by 32 wks

F cells increased with hemoglobin disorders

Better accuracy in estimating volume of FMH



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Othman J et al. Cytometry B Clin Cytom 2018;94(4):695–8

British Society of Hematology guidelines: Send for flow cytometry when volume >2 ml

The recommended time to give RhIg is within 72 hours

Most large centers have flow cytometry

https://b-s-h.org.uk/guidelines/guidelines/the-estimation-of-fetomaternal-haemorrhage

Other gaps

Identifying D negative pregnancies in obstetric clinics to ensure all receive RhIg

Clinic or chart documentation

Continue to administer RhIg if anti-D may be secondary to RhIg administration and not alloimmunization

Take home points

HDFN results from antibody mediated destruction of fetal/neonatal red cells by IgG alloantibodies resulting in fetal/neonatal anemia

Manifestations range from neonatal hyperbilirubinemia to fetal hydrops

D, K and c are the most common antigens requiring intrauterine transfusion

Prevention includes restrictive transfusion strategies, K neg RBCs for women of child bearing age, identifying D variants, identification and follow up of RhD negative pregnancies to ensure adequate dosing of RhIg is administered, RhIg for antepartum hemorrhage

Thank you. Questions?

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