Rh Disease: Prevention and Management

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Management of Rh disease
Isoimmunization and prevention
• Overview
• Prophylaxis
  □ Background
  □ Management details
• Isoimmunization
  □ Etiology
  □ Management details

Management of Rh disease
• Great obstetric success story
• First successful in utero therapy
• Uncommon encounter in practice
  □ Other Rh antigens (C, c, E, e)
• Primary duty is to prevent isoimmunization
• Isoimmunization management evolving
Management of Rh Disease
Genetics

- Fisher and Race 1946
  - Proposed 3 genes for 3 rhesus antigen groups
    - D, C/c, E/e
  - 1991, rhesus locus localized to short arm chromosome #1
    - 1p34-1p36
    - Only 2 genes identified: RhD and RhCE
  - RhD encodes D; absent in Rh negative
  - RhC/c and E/e inherited linked manner to RhD

- Weak D's
  - Reduced number D antigens expressed
- Partial D's
  - 'Missing' portions of D antigen
    - When exposed to RhD+ rbc's, patients can form anti-D antibodies to their missing or variant D epitopes
- RhD pseudogene

ACOG Practice Bulletin # 181; August 2017

Management of Rh disease
Genetics

- D antigen 7-10,000 mw
  - Appears early: 38-day embryo
  - Physiologic function unclear
  - "D" antigen critical
- Three RhD antigen twists
  - Weak D's
    - Reduced number D antigens expressed
  - Partial D's
    - 'Missing' portions of D antigen
    - When exposed to RhD+ rbc's, patients can form anti-D antibodies to their missing or variant D epitopes
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Management of Rh Disease
Genetics

- Weak RhD and Partial RhD in pregnancy
  - Estimated 0.96% of individuals
    - 0.3% of whites/1.7% of African descent
  - Monoclonal typing sera will label as RhD negative
  - Indirect Coombs will label as RhD positive
  - No longer recommended by AABB for prenatal testing
  - Weak RhD and Partial RhD patients now classified as RhD negative: RhD negative candidate
- Non-pregnant blood typing for donation
  - Indirect Coombs used
    - Weak RhD and Partial RhD typed as RhD positive
    - Avoid sensitizing Rh- recipients
- Bottom Line
  - Typed as RhD+ as a blood donor and RhD- when pregnant

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**RhoGam Guidelines**

**D Variants**

<table>
<thead>
<tr>
<th>RhD Blood Type</th>
<th>RhD Gene Presence</th>
<th>RhD Status</th>
<th>Rh Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amnion</td>
<td>RhD pseudogene*</td>
<td>Unknown</td>
<td>Yes</td>
</tr>
<tr>
<td>Amnion</td>
<td>RhD positive; weak or partial</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>Amnion</td>
<td>RhD positive</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>Matte</td>
<td>RhD negative</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>Product</td>
<td>RhD pseudogene*</td>
<td>Unknown</td>
<td>Yes</td>
</tr>
<tr>
<td>Product</td>
<td>Any RhD type</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>Product</td>
<td>RhD positive; weak or partial, or unknown</td>
<td>Unknown</td>
<td>No</td>
</tr>
</tbody>
</table>

* RhD pseudogene

- 69% S. African blacks; 21% African Americans
- Serologically RhD negative
  - But, entire RhD gene present on chromosome
  - Amniotic PCR testing would yield false +
  - Fetus RhD negative phenotype (serology)
  - Fetus RhD positive by genotype
- Risk of unnecessary intervention
  - Submit maternal blood with AF for fetal RhD typing to exclude presence of RhD pseudogene

**Management of Rh disease**

**Genetics**

- RhD pseudogene
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**Prevention**

- Rh negative incidence 16%
- Concept of passive antibody to prevent active isoimmunization
- Antibody mediated immune suppression
- First applied to Rh disease in early 1960's
- Half-life of RhoGAM approximately 24 days
Management of Rh disease
Prevention

- First large postpartum trial 1968
  - Yielded 10 fold decrease (1.8% v. 16%)
- 72 hour “window” due to prisoner trials
  - Protection demonstrated at 13 days
  - Some recommend administration out to 28 day
- Antepartum administration effective
  - Yielded another 10 fold decrease (0.1% v. 1.8%)
  - 300 ug RhoGAM “covers” 15cc fetal RBC’s or 30cc fetal whole blood

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Management of Rh disease
Prevention

- Indications for RhoGAM
  - Spontaneous/voluntary abortion
  - Threatened abortion
  - Ectopic
  - CVS/amniocentesis/cordocentesis
  - 28 weeks/postpartum
  - Antepartum hemorrhage
  - External cephalic version
  - Trauma
  - Hydatidiform mole
  - IUFD
  - ? Following postpartum tubal ligation
  - TL failure with future pregnancies
  - Avoid cross-matching issues with future transfusions

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Management of Rh disease
Prevention

- RhoGAM mechanism of action
  - Likely central inhibition
  - Rh IgG-D antigen complexes may stimulate “immune suppressor substance” that blunts immunologic response
  - Antigen blocking/deviation mechanism less likely
  - Derived from male donors who undergo repeated injections of RhD positive RBC’s
  - No reported cases viral infection
  - Scattered Hep C exposures prior to 1995

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### Management of Rh disease

#### Prevention

- Routine testing first prenatal visit
  - ABO typing
  - Rh status determination
  - Antibody screen

- Repeat antibody screen at 28 weeks
  - Low risk of isoimmunization before 28 weeks
  - Administer 300 ug RhoGAM
    - 50 ug dose not often employed clinically

#### Routine testing at time of delivery

- Maternal antibody screen
- Neonatal typing
- Routine testing for excessive fetal-maternal hemorrhage for Rh negative patients
  - Rosette test as screen
  - Kleihauer-Betke if rosette screen positive
  - Percentage of fetal cells multiplied by factor of 50 to estimate fetal-maternal hemorrhage

### Details of Screening for Fetomaternal Hemorrhage

- **Rosette test**
  - Qualitative; identifies Rh+ cells in Rh- patient
  - Exogenous anti-D antibodies are mixed with maternal blood and adhere to any Rh D+ fetal red cells
  - Rh D+ “indicator” red cells then added; form rosettes around coated fetal red cells
  - Clusters or rosettes easily identified under microscopy
  - Not appropriate when antenatal fetomaternal hemorrhage suspected; quantitative test should be pursued
**Details of Screening for Fetomaternal Hemorrhage**

- **Kleihauer-Betke test; 1957**
  - Semi-quantitative
  - Based on fetal blood having hemoglobin F
- **Smear of maternal blood obtained**
  - Dried; immersed in fixative; incubated in acid solution; stained with erythrosine B
  - Hemoglobin F-containing red cells (fetal) appear cherry red; adult red cells appear as uncolored ghost cells
  - Fetal cells counted; expressed as % of adult cells

- **Flow cytometry as an alternative**
  - Quantifies fetal cells by measuring fluorescence intensity of monoclonal antibodies to Hbg F
  - More objective; improved precision and accuracy
  - Coefficient of variation 10% (FC) v. 153% (KB)
  - Less labor intensive; 60 minutes to perform
  - Currently, only used in 4% of US labs for screening
Management of Rh disease
Prevention

Risk of isoimmunization
- Rh incompatible pregnancies
  - White 10%
  - African-American 5%
  - Asian 1%
- If paternal status unknown, risk of Rh positive fetus approximately 62%
  - But, < 20% lead to isoimmunization
  - Role of cell-free fetal DNA for fetal RhD status
    - FN rate 2.4%; FP rate 1.1%
Management of Rh disease
Isoimmunization

- Risk of isoimmunization:
  - If fetus ABO compatible; 16%
  - If fetus ABO incompatible; 1.5-2%
  - Most protective:
    - Maternal type "O"
    - Paternal type "A", "B", or "AB"
  - First trimester Spontaneous abortion; 2%
  - Second trimester vtp 4-5%

Management of Rh disease
Isoimmunization

- Requirements for isoimmunization:
  - Rh positive fetus
  - Rh negative mother
  - Maternal immunocompetence
  - Fetal-maternal hemorrhage
- First sensitized pregnancy usually results in minimal fetal/neonatal disease
- Subsequent gestations associated with worsening degrees of fetal anemia
- In general, these principles apply to other antigens
  - Kell, Kidd, Duffy

Management of Rh disease
Isoimmunization

- Erythroblastosis fetalis:
  - Maternal IgG destroys fetal rbc’s
  - Fetal anemia increases erythropoieses
  - If fetal bone marrow inadequate, liver and spleen are primary sites extramedullary erythropoieses
  - Secondary sites are fetal kidney, adrenal and intestinal mucosa
  - Hepatocellular damage decreases albumin
  - Decreased oncotic pressure results
  - Ultimately, portal hypertension develops
Management of Rh disease
Isoimmunization

- Prior obstetric history important
  - Fetal demise
  - Neonatal transfusion
- Evaluate paternal antigen status/zygosity
  - Historically, linkage analysis used
  - Quantitative PCR better tool
  - Cell-free fetal DNA for fetal RhD detection evolving
    - Reverse transcriptase PCR amplify specific RhD exons
    - If RhD positive, fetus at risk for anemia
    - If RhD negative, must confirm fetal DNA via SNPs analysis

Algorithm for determining the results of cell-free fetal DNA testing to determine the fetal RhD status. SNP, single-nucleotide polymorphism.

Management and Prevention of Red Cell Alloimmunization in Pregnancy: Obstetrics & Gynecology

- Typing by PCR with AF accurate/reliable
- Earlier management Rh disease with fewer invasive procedures in RhD negative fetus
- Rare discrepancies seen with 4 different sets of oligonucleotide primers (1.5%)
  - Sensitivity/specificity 98.7%/100%
  - Positive/negative predictive value 100%/96.9%
- Submit paternal and maternal blood sample with amniotic fluid when testing fetal Rbc antigen status
  - Evaluate for RhD pseudogene

ACOG Practice Bulletin #75 Reaffirmed 2016
Management of Rh disease

Isoimmunization

- “Critical” titer of 1:16
- Varies from 1:8-1:32
- At critical titer, additional testing required
- First affected pregnancy only
- Titers less reliable for Kell isoimmunization

Evolution of surveillance tools

- Amniocentesis
  - Essentially historical
- Cordocentesis
  - For IUVT
- MCA PSV Doppler interrogation

Management of Rh disease

Amniocentesis

- Fetal hydrops not consistently evident until fetal hemoglobin < 5 g/dL.
- Increased AFV, increased placental thickness, pleural/pericardial effusions, ascites and subcutaneous edema

ACOG Practice Bulletin #75 Released 2016
Management of Rh disease
MCA Doppler

- Non-invasive MCA PSV Doppler interrogation
- Start as early as 16-18 weeks; repeat 1-2 weeks
- Adjust for gestational age (perinatology.com)
- Not as useful after second IUIVT

Management of Rh Disease
Doppler MCA Velocimetry

Management of Rh disease

Cordocentesis

 Advantages include
- Direct vs. indirect evaluation
- Fetal hemoglobin and antigen status
- IUIVT superior to IUIPT
  - IUIPT at < 22 weeks for severe, recurrent disease
- Monitor post-transfusion fetal hemoglobin
 Disadvantages include
- Procedure-related loss of 1-2%
- Exacerbation of maternal isoimmunization

Management of Rh disease

Cordocentesis/IUIVT

- RBC’s typically “O”, RhD negative
  - CMV negative
  - Packed to hct of 75-85%
  - Irradiated to prevent graft-vs-host reaction
- Maternal blood is alternative
  - Decreased risk of sensitization to new antigens
  - Fresh unit can be routinely acquired
  - Repeated maternal donations produce maternal reticulocytosis, enhances lifespan of donor cells
  - Additional folate and iron supplementation required

Management of Rh disease

Prognosis

- Consistently excellent results reported
- Prior to RhoGAM
  - PNM 15/10,000 births
- Current era
  - Attributable PNM 0.54/10,000 births
- Survival rates excellent
  - Severely affected infants 72-96%
  - IUIVT (non-hydropic) 90% ±
  - IUIVT (hydropic) 82%
    - Increased risk CP (2.1%), developmental delay (3.1%)
Management of Rh disease

Adjunctive therapies

- Limited benefit
  - Serial plasmaphereses
  - Oral RhD-positive red cell stroma to desensitize
  - Promethazine to decrease phagocytosis by r.e.s.

- More promising results
  - Maternal intravenous immune globulin
    - Expensive
  - Maternal immunomodulation holds promise

Management of Rh disease

Isoimmunization

Neontal Alloimmune

Thrombocytopenia
NAIT PATHOGENESIS

- NAIT is platelet equivalent of hemolytic (Rh) disease
- Develops as result of maternal alloimmunization to fetal platelet antigens with transplacental transfer of platelet-specific antibody and fetal platelet destruction
- Incidence of 1/1000-3000 live births
- Unlike RBC alloimmunization, first pregnancies can be affected
- Mother always negative for target antigen with heterozygous fetus

NAIT Clinical Presentation

- Presents in uncomplicated pregnancy with normal maternal platelets
- Neonate then born with profound thrombocytopenia
- Differential diagnosis
  - Sepsis, disseminated intravascular coagulation (DIC), IUGR
- Clinical findings
  - Petechiae/ecchymosis
  - Visceral hemorrhage, circumcision bleeding, intracranial hemorrhage
  - ICH occurs in 15% of neonates with platelets < 50k
  - ICH occurs can occur in utero; may be diagnosed by US
  - May see intraventricular, periventricular, or parenchymal hemorrhage
- Fetal thrombocytopenia due to HPA-1a tends to be severe, occurs as early as 20 wks
  - Platelet count can decrease 10 x 10⁹/L per week
- Uncertain if disease process inexorably worse with next pregnancy

NAIT Relevant Antigens

- Several polymorphic diallelic antigen systems lead to NAIT
- Confusing, because multiple naming conventions in past
  - Now, termed Human Platelet Antigens (HPA)
  - Numbers identify specific antigen groups
  - Alleles designated as "a" or "b"
  - 15 officially recognized platelet specific antigens
  - Most severe cases associated with HPA-1a
  - Formerly known as PIA1 and Zwa
NAIT Evaluation

- BloodCenter of Wisconsin most reliable lab for consultation
- Determination of maternal/paternal HPA type and zygosity
  - Confirmation of maternal antiplatelet antibodies with specificity for paternal (or fetal-neonatal) platelets and incompatible antigen
  - Fetal platelet antigen typing from amniocytes or cfDNA (if paternal heterozygote)
- No reliable indirect method to assess fetal platelet count
- Maternal antiplatelet antibody titer correlates poorly with disease severity
- Prior sibling's clinical course does not reliably predict
- Umbilical blood sampling only accurate means to assess fetal platelets
  - Associated with 10-15% rate of emergency CS

NAIT Management

- Risk assessment
  - Standard: presence of HPA antibody but no prior intracranial bleed
  - High: HPA antibody with intracranial bleed in prior child > 28 weeks
  - Very high: HPA antibody with intracranial bleed/IUFD prior child < 28 weeks
- Start treatment between 16-20 weeks depending upon risk
  - IVIG 1 gm/kg maternal bw administered weekly
  - May add prednisone 1 mg/kg starting at 24 weeks based on risk
- Platelet transfusions (IUIVT) only for rescue therapy
  - Allogeneic or washed maternal platelets
- Fetal platelet sampling only after 32 weeks if TOL being considered
- Route of delivery counseling important
  - Recommend CS at 38 weeks/prior to labor for high-risk groups

Non-Immune Hydrops

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Non-Immune Hydrops

NIH

- Fetal Hydrops not due to blood group incompatibility
- Greek for water
- Presence of 2 or more abnormal fluid collections in the fetus
- Ascites, pleural or pericardial effusions, skin edema (> 5 mm)
- Placental thickening (> 4 cm), polyhydramnios
- Prevalence of 1:2500-1:3500 births

Etiologies of NIFH

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>17.3%</td>
</tr>
<tr>
<td>Chromosomal</td>
<td>7.1%</td>
</tr>
<tr>
<td>Hematologic</td>
<td>6.1%</td>
</tr>
<tr>
<td>Infectious</td>
<td>5.7%</td>
</tr>
<tr>
<td>Thoracic</td>
<td>0%</td>
</tr>
<tr>
<td>Twin-twin transfusion</td>
<td>3.15%</td>
</tr>
<tr>
<td>Urinary tract abnormalities</td>
<td>2.3%</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>6.3-6%</td>
</tr>
<tr>
<td>Lymphatic dysplasia</td>
<td>3.4%</td>
</tr>
<tr>
<td>Tumors, including chorioangiomas</td>
<td>3.3%</td>
</tr>
<tr>
<td>Retinal dysplasia</td>
<td>3.4%</td>
</tr>
<tr>
<td>Syndromes</td>
<td>3.4%</td>
</tr>
<tr>
<td>Idiopathic malformations</td>
<td>1.2%</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3-10%</td>
</tr>
<tr>
<td>Unknown</td>
<td>15-25%</td>
</tr>
</tbody>
</table>

Etiologies of NIFH

AJOG February 2015
Non-Immune Hydrops
Etiology

- Primary myocardial failure
  - Arrhythmia
  - Severe anemia
  - Cardiac malformation
  - Myocarditis
  - TTTS
- High-output cardiac failure
  - Severe anemia
  - A-V shunt

Non-Immune Hydrops
Etiology

- Decreased plasma oncotic pressure
  - Decreased albumin production
    - Hepatitis, congenital cirrhosis
  - Increased albumin excretion
    - Congenital nephrotic syndrome
- Increased capillary permeability
  - Anoxia
  - Congenital infection
  - Placental edema

Non-Immune Hydrops
Etiology

- Obstruction of venous return
  - Neoplasm
  - Space-occupying lesions
- Obstruction of lymphatic return
  - Cystic hygroma
  - Mass effects
Non-Immune Hydrops Diagnosis

- Crucial for determining prognosis
- Maternal evaluation
  - Blood typing and antibody screen
  - CBC and indices
  - Kleihauer-Betke
  - TORCH/SP screen
  - Maternal medical screen including medications

- Non-invasive fetal evaluation
  - Ultrasound, echocardiography, MCA doppler
  - Invasive fetal evaluation
    - Amniocentesis
      - Karyotype/CGH micro-array, AF culture and PCR (CMV, toxo), metabolic survey
    - Cordocentesis
      - Karyotype/CGH micro-array, metabolic survey, infectious disease screen, CBC, hemoglobin chain analysis, immunoglobulins
Non-Immune Hydrops

Prognosis

- Prognosis generally poor
  - 95% mortality if structural anomaly present
- Therapy dependent upon etiology
  - Transfusion
  - Anti-Arrhythmic medications
- Best prognosis groups (70% survival)
  - Tachyarrhythmias
  - Hematologic disorders
  - Hydro/chylo-thorax groups

Therapy for Selected Etiologies of NIFH

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Therapy</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac tachyarrhythmia, supraventricular tachycardia, atrial flutter, or atrial fibrillation</td>
<td>Maternal transplacental administration of antiarrhythmic medication(s)</td>
<td>Treatment with antiarrhythmic medication unless gestational age is close to term or there is maternal or obstetrical contraindication to therapy</td>
</tr>
<tr>
<td>Fetal anemia secondary to parvovirus infection or fetomaternal hemorrhage</td>
<td>Fetal blood sampling followed by intrauterine transfusion</td>
<td>Fetal intrauterine transfusion if anemia is confirmed, unless pregnancy is at an advanced gestational age and risks associated with delivery are considered to be less than those associated with procedure</td>
</tr>
<tr>
<td>Fetal hydrothorax, chylothorax, or large pleural effusion associated with bronchopulmonary sequestration</td>
<td>Fetal needle drainage of effusion or placement of thoracoamniotic shunt; if gestational age is advanced, needle drainage prior to delivery in selected cases</td>
<td>Consider drainage of large unilateral pleural effusion(s) resulting in NIHF, or, if gestational age is advanced, consideration of needle drainage prior to delivery</td>
</tr>
<tr>
<td>Fetal CPAM</td>
<td>Macrocystic type: fetal needle drainage of effusion or placement of thoracoamniotic shunt; microcystic type: maternal administration of betamethasone 12.5 mg IM q24 h × 2 doses or dexamethasone 6.25 mg IM q12 h × 4 doses</td>
<td>Consider drainage of large macrocystic CPAM that has resulted in NIHF; if large microcystic CPAM has resulted in NIHF, we suggest that management options include maternal corticosteroid administration</td>
</tr>
<tr>
<td>TTTS or TAPS</td>
<td>Laser ablation of placental anastomoses or selective termination</td>
<td>Consideration of transabdominal placement of percutaneous radiofrequency ablation in TTTS or TAPS that has resulted in NIHF</td>
</tr>
<tr>
<td>Twin-reversed arterial perfusion sequence</td>
<td>Referral for consideration of percutaneous radiofrequency ablation</td>
<td>Follow for correlation of transabdominal placement of percutaneous radiofrequency ablation and maternal or fetal distress</td>
</tr>
</tbody>
</table>

Non-Immune Hydrops

ANY QUESTIONS?