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

- 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
  - RhD pseudogene

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; Rhogen candidates
- 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
RhoGam Guidelines

D Variants

<table>
<thead>
<tr>
<th>RhD Blood Type</th>
<th>RhD Pseudogene?</th>
<th>Maternal</th>
<th>Fetal or Newborn</th>
<th>Rh-D Immune Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anergic</td>
<td>RhD positive</td>
<td>RhD antigen, weak partial, or colonies D</td>
<td>Unknown</td>
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<tr>
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<td>Reducian</td>
<td>RhA negative</td>
<td>Any RhD type</td>
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- 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
  - 69% S. African blacks; 21% African Americans
  - Serologically RhD negative
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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 days
- 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

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

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
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

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

- Kleihauer-Betke
  - Labor intensive; 10,000 cells must be counted
  - Turnaround time variable; technician dependent
  - May underestimate amount of hemorrhage
    - Cells fail to stain; decreasing Hgb F concentration
    - Overestimate if maternal blood contains hemoglobin F
  - Increases in pregnancy; peaks mid-gestation
    - SC anemia; beta thalassemia; hereditary persistence

- Flow cytometry as an alternative
  - Quantifies fetal cells by measuring fluorescence intensity of monoclonal antibodies to Hbg F
    - Fluorescence intensity fetal Hbg F-containing cells greater than adult Hgb F-containing cells
  - Also measures red cell size/distinguish from adult
    - 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

Management of Rh disease
Isoimmunization

- 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%

ACOG Practice Bulletin # 181; August 2017

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

ACOG Practice Bulletin #75 Reaffirmed
2016

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

ACOG Practice Bulletin #75 Reaffirmed
2016
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 of Rh disease
Fetal RhD typing by PCR

- 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
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
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

Algorithm for clinic...

Management and Prevention of Red Cell Alloimmunization in Pregnancy: A Systematic Review
Moise, Kenneth J. Jr; Argoti, Pedro S.
doi: 10.1097/AOG.0b013e31826d7dc1
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
- Procedure-related loss of 1-2%
- Exacerbation of maternal isoimmunization

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

Non-Immune Hydrops

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Non-Immune Hydrops

NIH

- Fetal Hydrops not due to blood group incompatibility
- 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>17-35%</td>
</tr>
<tr>
<td>Chromosomal</td>
<td>7-16%</td>
</tr>
<tr>
<td>Hematologic</td>
<td>6-12%</td>
</tr>
<tr>
<td>Metabolic</td>
<td>3-5%</td>
</tr>
<tr>
<td>Thoracic</td>
<td>0%</td>
</tr>
<tr>
<td>Twin-to-twin transfusion</td>
<td>3-10%</td>
</tr>
<tr>
<td>Urinary tract abnormalities</td>
<td>2-3%</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0.5-4%</td>
</tr>
<tr>
<td>Lymphatic dysplasia</td>
<td>3-4%</td>
</tr>
<tr>
<td>Tumors, including chorangioma</td>
<td>2-3%</td>
</tr>
<tr>
<td>Renal dysplasia</td>
<td>3-4%</td>
</tr>
<tr>
<td>Syndromic</td>
<td>3-4%</td>
</tr>
<tr>
<td>Idiopathic fetal ascites</td>
<td>2-2%</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3-10%</td>
</tr>
<tr>
<td>Unknown</td>
<td>15-25%</td>
</tr>
</tbody>
</table>

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

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

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

Figure 2
Non-Immune Hydrops

Prognosis

- Prognosis generally poor
  - 95% mortality if structural anomaly present
- Therapy dependent on etiology
  - Anti-Arrhythmic medications
  - Transfusion
- Best prognosis groups (70% survival)
  - Tachyarrhythmias
  - Hematologic disorders
  - Hydro/cholethorax 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, atrial fibrillation</td>
<td>Maternal transplacental administration of antiarrhythmic medications</td>
<td>Treatment with antiarrhythmic medication unless gestational age is close to term or 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 blood sampling followed by intrauterine transfusion, unless maternal or obstetrical contraindication to therapy</td>
</tr>
<tr>
<td>Fetal hydrothorax, chylothorax, or large pleural effusion associated with bronchopulmonary sequestration</td>
<td>Fetal needle drainage of effusion or thoracoamniotic shunt; if gestational age is advanced, consider drainage at delivery</td>
<td>Fetal needle drainage of effusion or thoracoamniotic shunt; if gestational age is advanced, needle drainage prior to delivery</td>
</tr>
<tr>
<td>Fetal CPAM</td>
<td>Macrocystic type: fetal needle drainage of effusion or thoracoamniotic shunt; microcystic type: maternal corticosteroid administration (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, or maternal corticosteroid administration if large microcystic CPAM has resulted in NIHF</td>
</tr>
<tr>
<td>TTTS or TAPS</td>
<td>Laser ablation of placental anastomoses or selective termination</td>
<td>Consideration of fetal intervention or laser ablation of placental anastomoses for TTTS or TAPS that has resulted in NIHF</td>
</tr>
</tbody>
</table>

Non-Immune Hydrops

ANY QUESTIONS?