Rh Isoimmunisation

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INTRODUCTION

- Rhesus (Rh)-D negative women who deliver an Rh(D) positive baby or who are otherwise exposed to Rh(D) positive red cells are at risk of developing anti-D antibodies.
- Rh(D) positive fetuses/neonates of these mothers are at risk of developing hemolytic disease of the fetus and newborn (HDFN), which can be associated with serious morbidity or mortality.
- Implementation of programs for antenatal and postnatal anti-D immune globulin prophylaxis has led to a significant reduction in the frequency of Rh(D) alloimmunization and associated fetal/neonatal complications.
- However, Rh(D) alloimmunization with serious sequelae in offspring still occurs, particularly in low resource countries where anti-D immune globulin is not widely available.
- Where appropriate monitoring and intervention are available, HDFN can be treated successfully in most cases.

THE RHESUS SYSTEM

- Different Rh(D) phenotypes.
- **D, d, C, c, E, e, and G**
- Rh(D) negative patients may have received prophylactic anti-D immune globulin in previous pregnancies, but can still get “c” alloimmunization.
- Antibody typing

Prevalence of Rh(D)-ve blood type

- The prevalence of Rhesus antigens varies among populations.
  - Basques — 30 to 35 percent
  - Caucasians in North America and Europe — 15 percent
  - Jordan — 9.8 percent
  - African Americans — 8 percent
  - Africa — 4 to 6 percent
  - India — 5 percent
  - Native Americans and Inuit Eskimos — 1 to 2 percent
  - Japan — 0.5 percent
  - Thailand — 0.3 percent
  - China — 0.3 percent
- **Zygosity** — About 40 percent of Rh(D)-positive individuals are homozygous for the D antigen (DD); the remainder is heterozygous (Dd).

Pathogenesis

- Rh(D) antigen is expressed by 30 days of gestation.
- Only present on RBCs.
- Maternal Rh(D) alloimmunization develops as a result of maternal immune system exposure to Rh(D)-positive RBCs.
- Once anti-D IgG antibodies are produced, they can cross the placenta and opsonize fetal RBCs, which are then phagocytized by macrophages in the fetal spleen.
- Events that can cause maternal alloimmunization include:
  - Transplacental fetomaternal hemorrhage during any pregnancy
  - Injection with needles contaminated by Rh(D)-positive blood
  - Inadvertent transfusion of Rh(D)-positive blood
  - D-mismatched allogeneic hematopoietic stem cell transplantation

Transplacental fetomaternal bleeding

- Accounts for virtually all cases of maternal Rh(D) alloimmunization.
- There are reports of alloantibodies to Rh(D) antigen without identifiable maternal exposure to red cells carrying the D antigen.
- These cases may be the result of
  - Early pregnancy losses (including vanishing twins) that were not clinically recognized.
  - “Grandmother theory" has been proposed as the etiology.
- Transplacental transfer of maternal antibody leads to hemolytic disease of the fetus/newborn.
- Severe anemia leads to hydrops fetalis (two or more of the following: skin edema, ascites, pericardial effusion, pleural effusion).

Prevention of Rh(D) alloimmunization in pregnancy

Screening

- All D-negative pregnant women should undergo an antibody screen at the first prenatal visit of each pregnancy

Anti-D Immunoglobulin

- Once produced from the plasma of alloimmunized women,
- Male donors, undergo repeated injections of D+ve RBCs to develop high-titered polyclonal anti-D plasma.
    - This dwindling resource has led to a search for a synthetic anti-D immunoglobulin, but none are available for clinical use.
- Two monoclonal antibodies, BRAD-3 and BRAD-5, have been derived by immortalizing B lymphocyte cell lines from hyperimmunized donors with Epstein-Barr virus.
- A recombinant polyclonal human anti-D consisting of 25 different monoclonal antibodies has also been developed by transfecting Chinese hamster ovary cells with human genes.
- Advantages of a synthetic product over products derived from humans include
    - Greater availability and
    - Elimination of risks of pathogen transmission and adverse reactions

Sensitisation

- 16 % of D-negative women became alloimmunized after two deliveries of D-positive ABO-compatible infants.
- 1 to 2 % with routine postpartum administration of a single dose of anti-D immunoglobulin
- 0.1 to 0.3 % with the addition of routine antenatal administration in the third trimester

Dosage

- A single 300 microgram dose (1 microgram = 5 international units) contains sufficient anti-D to suppress the immune response to 15 mL of D-positive red cells (or 30 mL fetal D-positive whole blood).
- A single 50 microgram dose contains sufficient anti-D to suppress the immune response to 2.5 mL of D-positive red cells (or 5 mL fetal whole blood).

- Peak serum levels are achieved faster after intravenous than intramuscular injection,
- Half-life is approximately around 24 days.
- In most patients, a low antibody titer (≤4) can be detected in maternal serum for several weeks after administration.
- Persistence of the antibody can result in a positive direct antiglobulin test in the newborn but does not have adverse clinical effects.

Antepartum Prophylaxis

- Two schools:
  - A single dose of 300 micrograms (1500 iu) of anti-D immunoglobulin is administered at approximately 28 weeks of gestation.
  - Two-dose regimen, 100 micrograms (500 iu) of anti-D at 28 and 34 weeks of gestation.
- ICT; anti-body screening should be repeated before any dosing.

Prophylaxis after antepartum events

- Miscarriage or induced abortion .
- Invasive prenatal diagnostic or therapeutic procedures
- Blunt abdominal trauma and external cephalic version
- Ectopic pregnancy
- Fetal death in the second or third trimester
  - Fetal demise, not delivery, is the sensitizing event as fetal demise may be caused by massive fetomaternal hemorrhage or occult abruption.
- Antepartum hemorrhage in the second or third trimester
- Hydatidiform mole
  - A complete mole does not contain fetal red cells,
  - Fetal red cells are present in partial molar pregnancies.
  - Sometimes it is initially difficult to determine whether the patient had a molar pregnancy or a missed abortion or a partial mole with fetal absorption.

Testing for fetomaternal hemorrhage

- Routinely testing all D-negative women for excessive fetomaternal bleeding at the time of delivery to ensure that they receive an adequate dose of anti-D immunoglobulin.
- The incidence of fetomaternal hemorrhage
  - > 20 to 30 mL at delivery is estimated to be approximately 1 in 200 to 300 deliveries, which is at the limit of effective prophylaxis from a single 300 microgram dose of anti-D immunoglobulin.
  - >80 mL is estimated to occur in 1 in 1000 deliveries
  - >150 mL 1 in 5000 deliveries.
- *Rosette test*; a qualitative, yet sensitive test for fetomaternal hemorrhage.
  - A standard dose of anti-D immunoglobulin is given to patients with a negative test.
- *Kleihauer-Betke* test or *flow cytometry* : Quantitative

No prophylaxis if

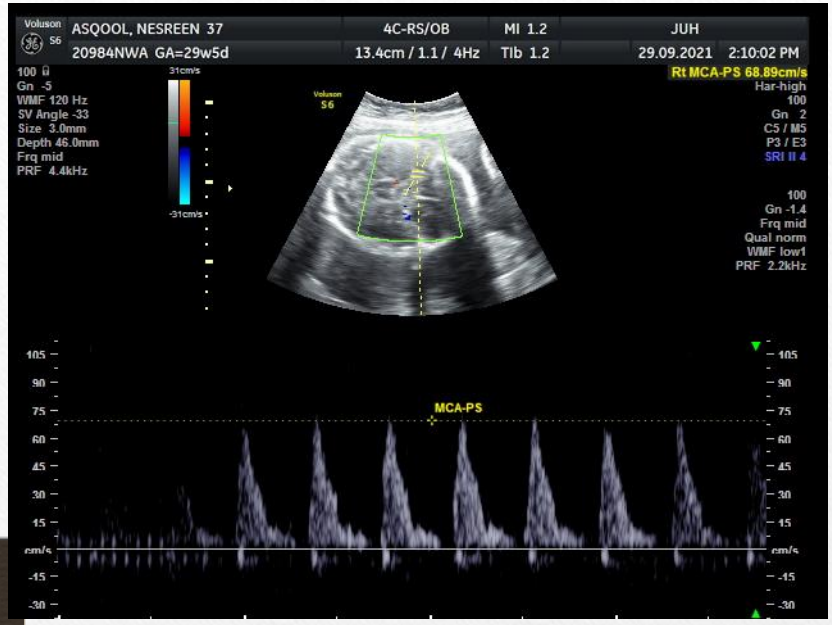
- The biologic father of the baby is known **with certainty** to be D-negative
- Cell-free DNA (cfDNA) results on maternal plasma suggest that the fetus is D-negative.
- Fetal blood sampling confirmed the fetus to be Rh – ve.
- Amniocentesis/CVS

DIAGNOSIS

- The diagnosis of Rh(D) alloimmunization is based upon detection of anti-Rh(D) antibody in maternal serum.
- ICT positive
- Anti-body identification

Management

- Determining the fetal Rh(D) type
- Assess risk of fetal anemia
  - Following maternal anti-D titers, 15 international units/mL is the critical value
  - Ultrasound assessment of fetal middle cerebral artery peak systolic velocity.
  - Amniotic fluid bilirubin levels (obsulete)
  - Fetal blood sampling (not routine)
- Severe fetal anemia near term is treated by delivery for neonatal treatment;
- Intrauterine fetal transfusions are performed.
  - Intravascular
  - Intraperitoneal
- Serial combined maternal plasmapheresis
- Intravenous immune globulin therapy is a promising approach for decreasing the severity of fetal disease, but is investigational.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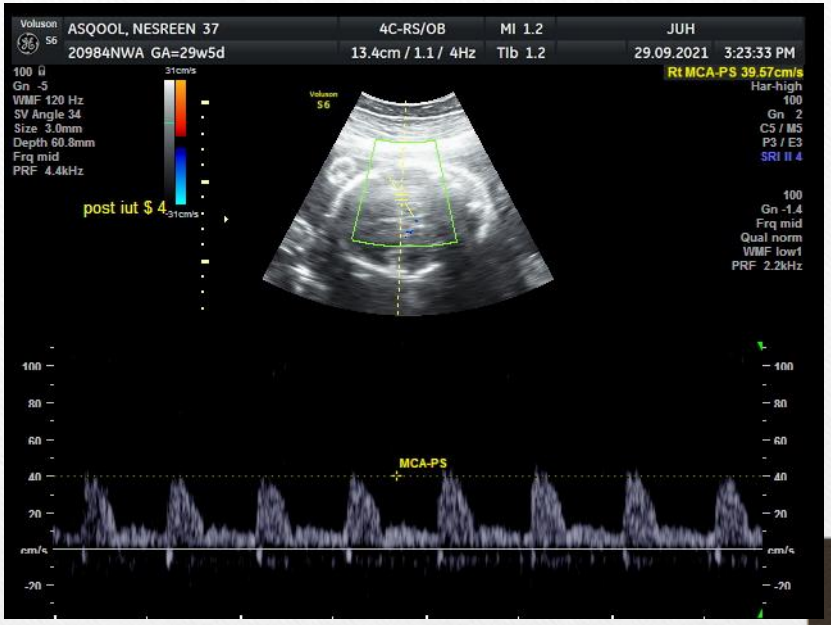
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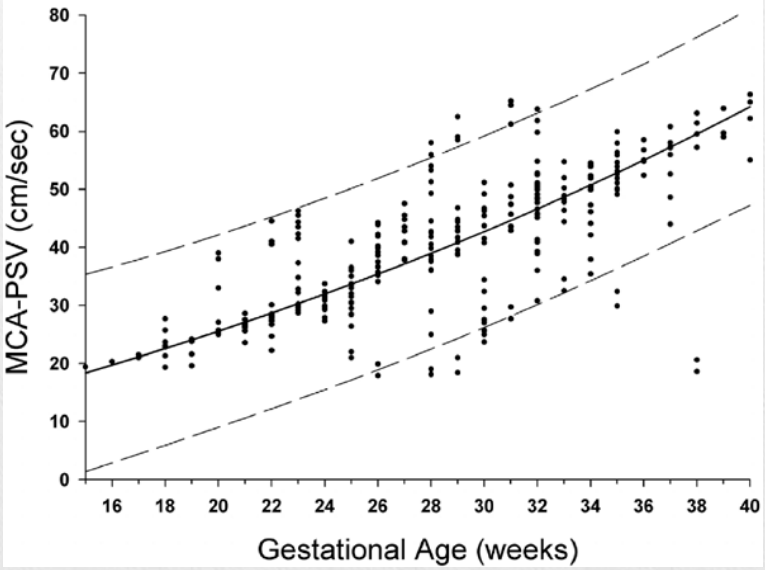
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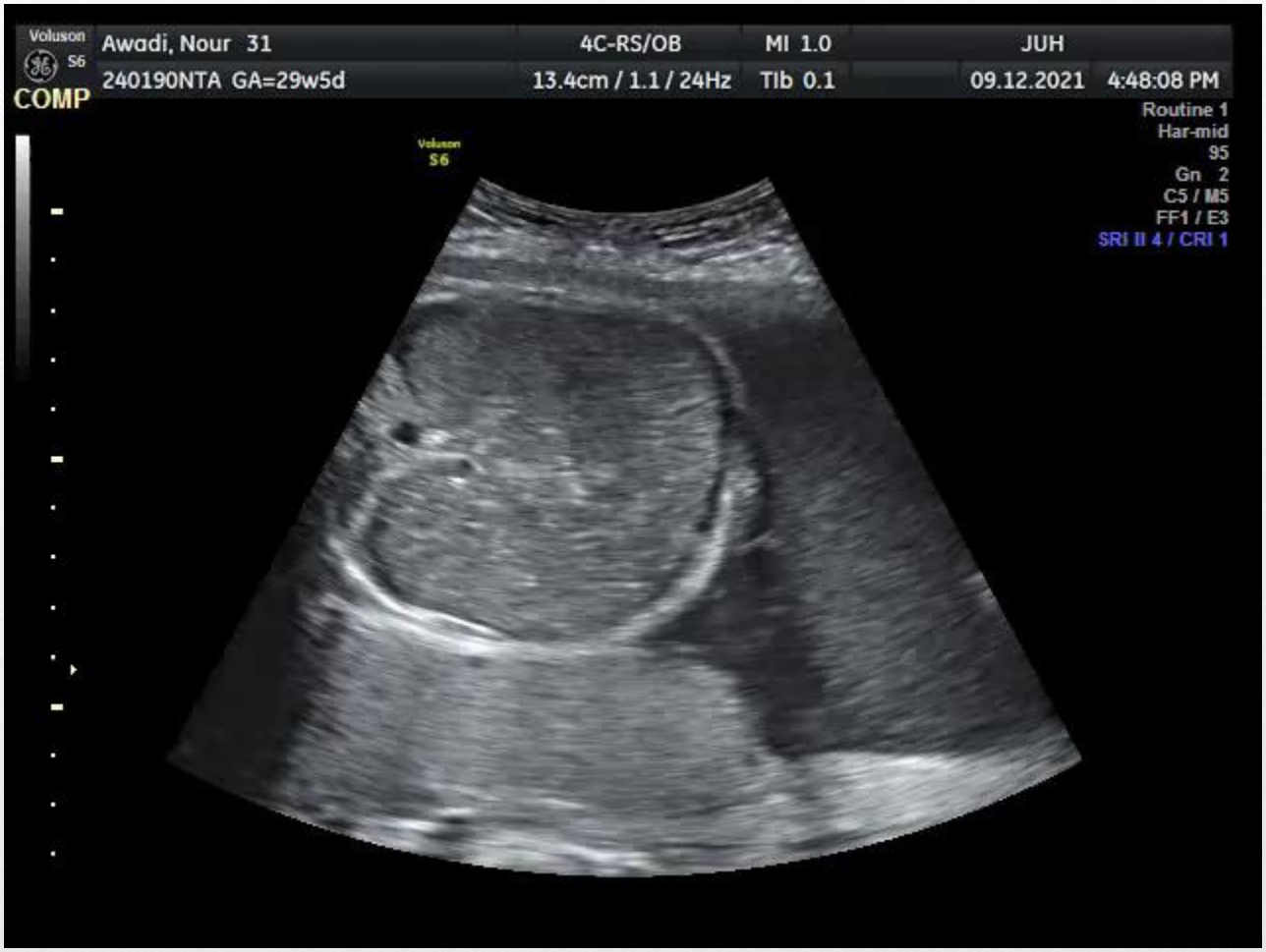
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