Rh Isoimmunisation

Dr Oqba Al-Kuran, FRCOG

Consultant Maternal & Fetal Medicine
associate Professor

Faculty of Medicine

University of Jordan

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.

pregnant first produce IgM antibodies which don't cross the placenta --> first pregnancy is not affected.
then IgG (after 6 months) --> subsequent pregnancies are affected.

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

the phenotypes other than D are less associated with complications

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. (of fetal age) , and 6 weeks of gestational age.
- 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

hemolysis and anemia doesn't occur before 18-20 weeks,when the reticuloendothelial system of the fetus develops.

Transplacental fetomaternal bleeding

- Accounts for virtually all cases of maternal Rh(D) alloimmunization.

thus anti D prophylaxis should be given to all Rh negative pregnants

- 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

not all Rh negative pregnants become sensitized even if exposed to Rh(+) fetal blood, why?
if the fetus blood is ABO incompatible with the maternal, antibodies to A/B antigens will eliminate the fetal RBCs before sensitization to Rh antigen occurs.
only 7-10% become sensitized in the first pregnancy, 16% in the second pregnancy (when fetus is ABO compatible)

Sensitisation

- 16 % of D-negative women became alloimmunized after two deliveries of D-positive ABO-compatible infants. without receiving anti-D
- 1 to 2 % with routine postpartum administration of a single dose of anti-D immunoglobulin within 72 hours of birth
- 0.1 to 0.3 % with the addition of routine antenatal administration in the third trimester at week 28 (decrease the risk because sensitization may occur during pregnancy)

at week 28 because half life of Anti-D is 6 weeks

Dosage

/1500 IU

- 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).

kleiheur-Betke test is used to measure the amount of fetal blood in the maternal circulation (fetal RBCs are resistant to KOH). if high --> need the larger dose.

- Peak serum levels are achieved faster after intravenous than intramuscular injection,
- Half-life is approximately around 24 days. it stays up to 6 weeks
- 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.

indirect coombs test

- ICT; anti-body screening should be repeated before any dosing. positive: either she received the Anti-D within the last 6 weeks or she is sensitized

antibody screening is done for all pregnants even if Rh positive
if positive indirect coombs test in Rh (+) pregnant: indicate that there are other antigens than D.

next step: perform antibody pannel to determine the antigen

Prophylaxis after antepartum events

- Miscarriage or induced abortion .
- Invasive prenatal diagnostic or therapeutic procedures amniocentesis, CVS, fetal blood sampling
- Blunt abdominal trauma and external cephalic version
- Ectopic pregnancy
- Fetal death in the second or third trimester given at the time of death not at the time of delivery
  - 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 only for partial. clinically it's given for all (histopathology takes time)
  - 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 if genetics reveiled Rh negative

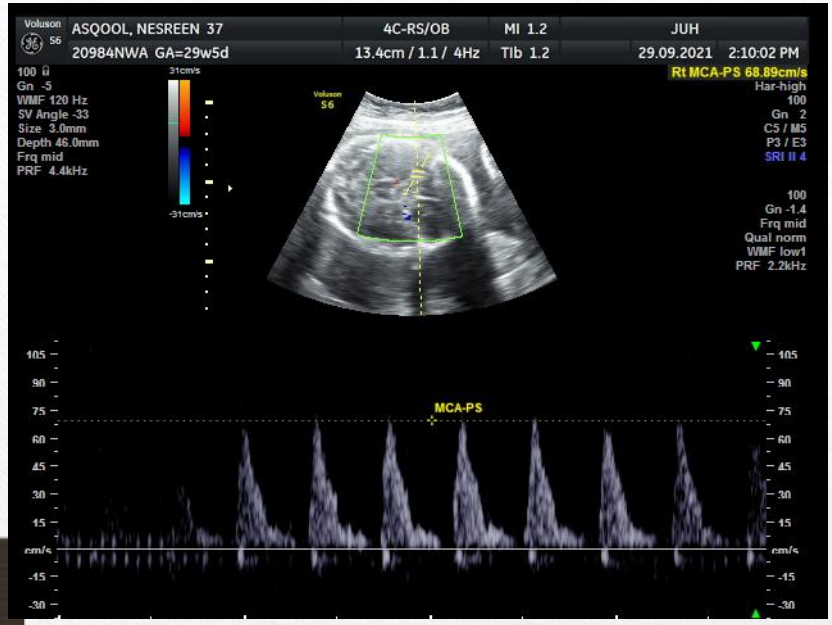
DIAGNOSIS

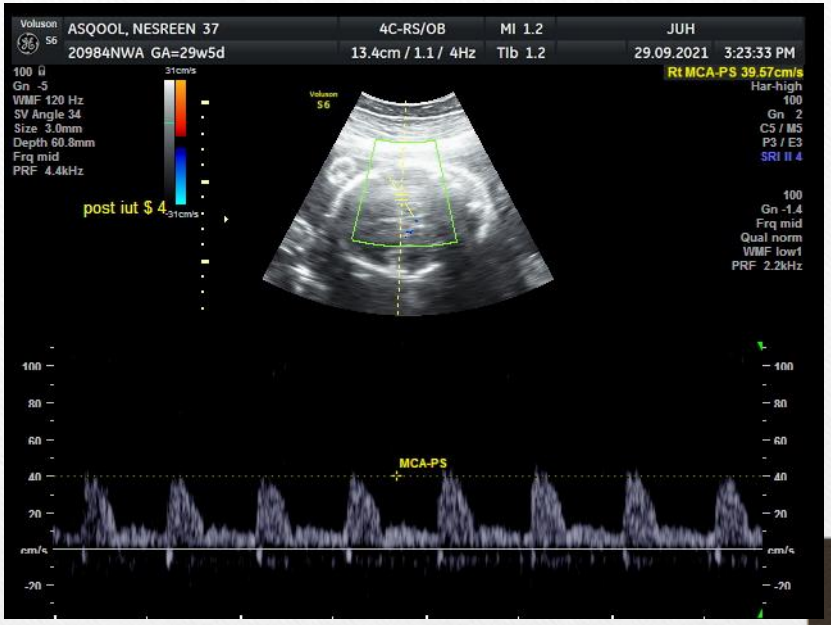
- The diagnosis of Rh(D) alloimmunization is based upon detection of anti-Rh(D) antibody in maternal serum.
- ICT positive
- Anti-body identification

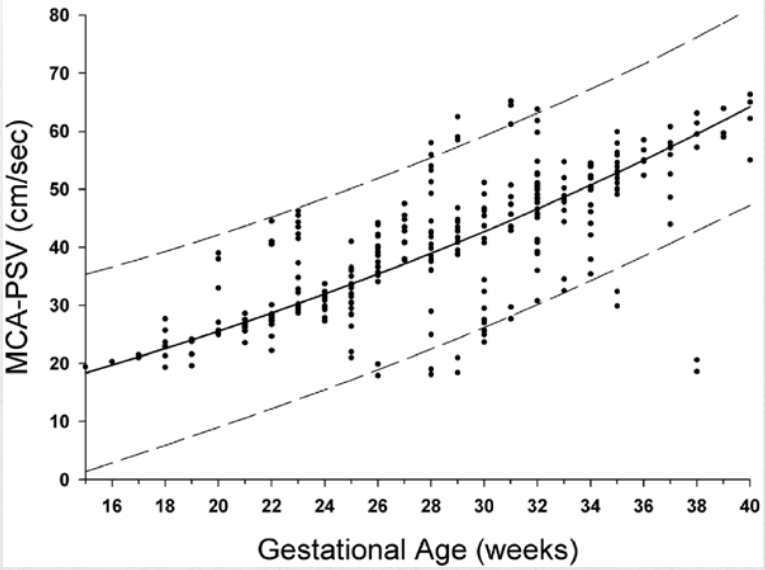
Management

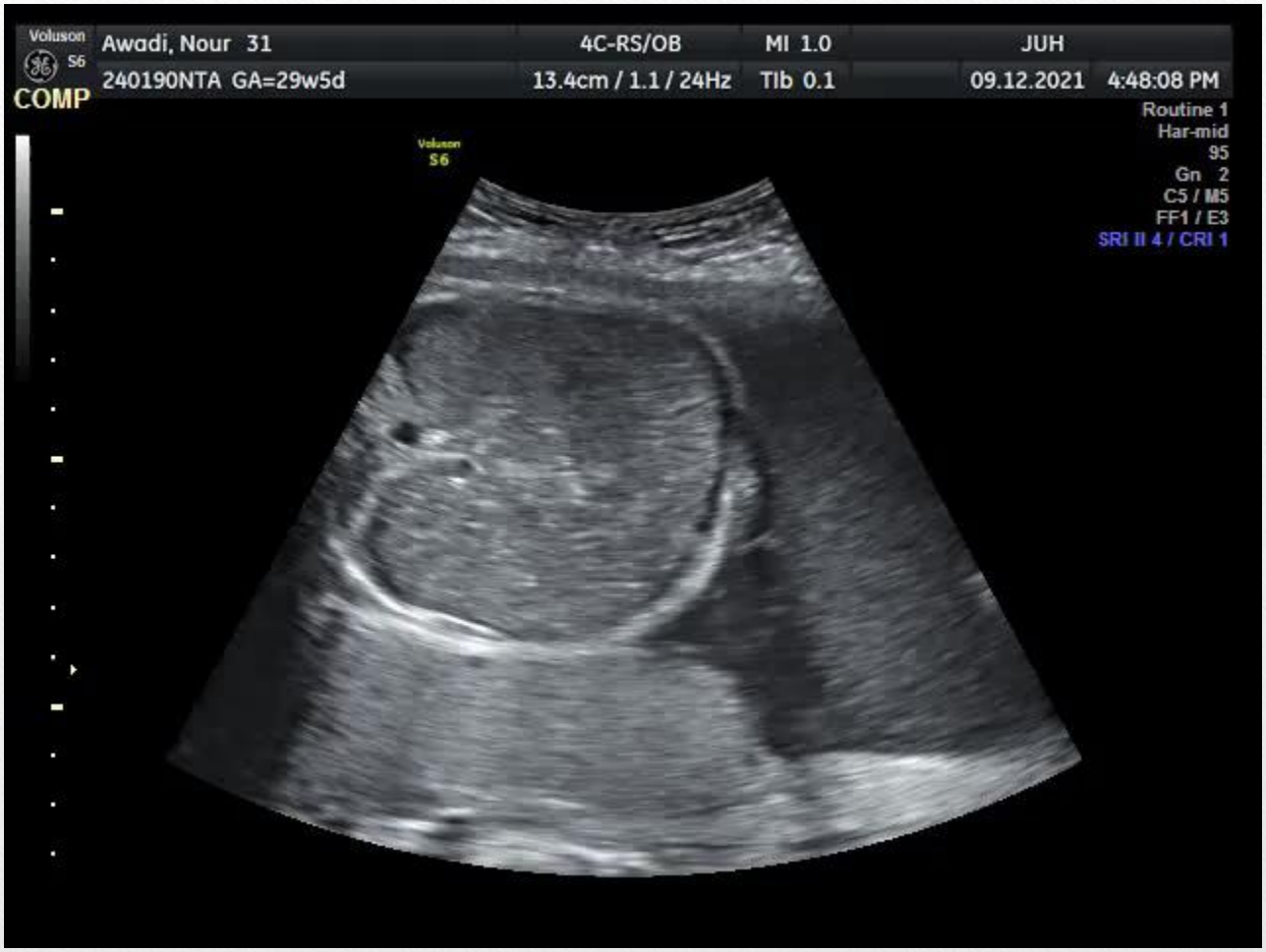
if positive Anti-D antibodies -->

- Determining the fetal Rh(D) type if Rh (+) -->
- Assess risk of fetal anemia
  - Following maternal anti-D titers, 15 international units/mL is the critical value the higher the titer the more risk
  - Ultrasound assessment of fetal middle cerebral artery peak systolic velocity. high velocity flow indicates anemia
  - Amniotic fluid bilirubin levels (obsulete)
  - Fetal blood sampling (not routine) not done
- Severe fetal anemia near term is treated by delivery for neonatal treatment; if >34 weeks
- Intrauterine fetal transfusions are performed. if <34 weeks
  - Intravascular in the umbilical vein (concentrated blood (Hct 80%))
  - Intraperitoneal by absorption
- Serial combined maternal plasmapheresis not effective
- Intravenous immune globulin therapy is a promising approach for decreasing the severity of fetal disease, but is investigational.


ASQOOL, NESREEN 37
4C-RS/OB
MI 1.2
JUH
20984NWA GA=29w5d
13.4cm / 1.1 / 4Hz
TIb 1.2
29.09.2021
2:10:02 PM
Rt MCA-PS 68.89cm/s
MCA-PS


ASQOOL, NESREEN 37
4C-RS/OB
MI 1.2
JUH
20984NWA GA=29w5d
13.4cm / 1.1 / 4Hz
TIb 1.2
29.09.2021
3:23:33 PM
Rt MCA-PS 39.57cm/s
post iut $ 4
MCA-PS


MCA-PSV (cm/sec)
Gestational Age (weeks)
0
10
20
30
40
50
60
70
80
16
18
20
22
24
26
28
30
32
34
36
38
40


Voluson
S6
COMP
Awadi, Nour 31
240190NTA GA=29w5d
4C-RS/OB
13.4cm / 1.1 / 24Hz
MI 1.0
TIb 0.1
JUH
09.12.2021
4:48:08 PM
Routine 1
Har-mid
95
Gn 2
C5 / M5
FF1 / E3
SRI II 4 / CRI 1
Voluson
S6