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Hemolytic Disease of the Fetus and Newborn

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Hemolytic disease of the fetus and newborn (HDFN)

- Definition
- Pathophysiology
- Diagnosis
- Management
  - Prenatal
  - Postnatal

HDFN

- Pre-existing maternal RBC alloantibodies target paternally inherited fetal RBC antigen(s).

- Maternal IgG antibody transports across the placenta into the fetal circulation where it binds to the corresponding RBC antigen, causing destruction of antigen positive fetal RBCs by macrophages in the fetal spleen.

Pathophysiology of HDFN

- Mother is negative for an RBC antigen.
- Mother exposed to foreign RBC antigen through prior pregnancy or transfusion.
- Mother forms IgG alloantibody to this antigen.
- Fetus inherits antigen of interest from father.
- Maternal IgG antibody crosses the placenta to target fetal RBC antigen.
- Antibody coated RBCs are destroyed by macrophages in the fetal spleen.

Pathophysiology of HDFN

- Fetus responds by increasing erythropoiesis, releasing immature RBCs into the circulation prematurely (erythroblastosis fetalis).
- As anemia worsens, erythropoiesis occurs in fetal liver and spleen, causing organomegaly and portal hypertension.
- Decrease in albumin leads to reduced plasma colloid oncotic pressure, generalized edema, ascites and effusions known as “hydrops fetalis”.
- Untreated, hydrops fetalis can lead to death from high output cardiac failure as early as 18-20 weeks gestation.

Diagnosis of HDFN

First prenatal visit:

- Determine maternal ABO and Rh(D) type
- Maternal antibody screen

Diagnosis of HDFN

Antibody screen:

- In the example below, the patient's serum reacts with screening cell II (SCII) (3+), but not screening cell I (SCI).

		D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	
SCI	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0
SCII	R2R2	+	0	+	+	0	+	+	+	+	+	+	+	0	+	+	3+

Diagnosis of HDFN

If antibody screen is positive:

- Identify the antibody (IgG class)
- Baseline titer should be performed

If titer is not "critical":

- Repeat at 2-4 week intervals beginning at 18-20 weeks gestation until critical titer reached.
- Previous titer is stored frozen and repeated in parallel with current specimen.

Diagnosis of HDFN

Most common HDFN is ABO.

- Generally mild
- Usually Group O mother and A baby

Next most common are anti-D, anti-K and anti-c.

- Anti-D HDFN drastically reduced incidence since creation of Rh immune globulin (RhIG) in the 1960's.

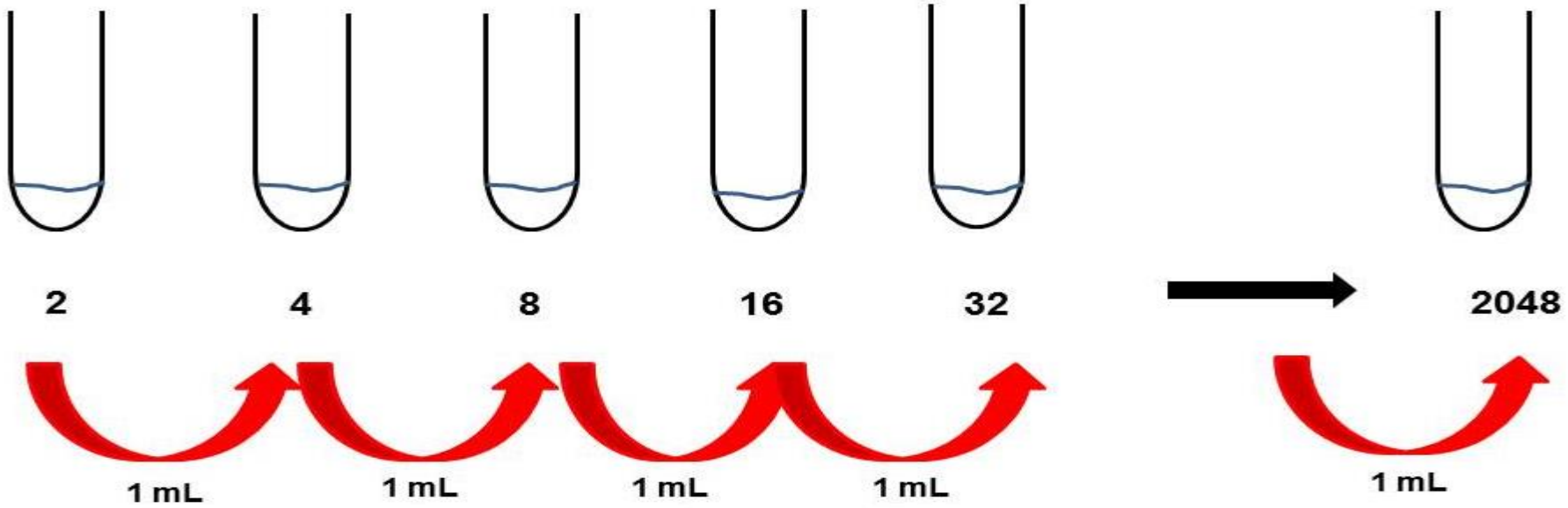
Diagnosis of HDFN

Titers

- Reported as the integer of the greatest tube dilution with a positive agglutination reaction.
- May vary between labs so titers should be done where patient is receiving care.
- Change in more than 1 dilution is considered significant.

Figure 1. Titration procedure

1) Start by adding 1 mL of isotonic saline to each empty master dilution tube (2 through 2048).
2) Next add 1 mL patient serum to tube #2.
3) Mix and then take 1 mL of tube #2 contents and add to tube #4 and so on etc.



4) Next, transfer 2 drops of each master dilution tube into corresponding testing tubes.
5) Add 1 drop of reagent RBC suspension to each labeled dilution tube and mix by gentle shaking.
6) Incubate all tubes for 1 hour at 37C and then wash 3-4 times with isotonic saline.
7) Add 2 drops of anti-IgG to each tube and centrifuge.
8) Starting with tube 2048 read the agglutination towards tube 2 and grade.
9) Add 1 drop of Coombs control cells to each negative and w+ tube and centrifuge. Rxns must be 2+ or greater for test to be valid.

Figure courtesy of Dr. K O'Brien.

Image courtesy of Dr. K O'Brien.

Titers

For partners with a prior child with HDFN:

- Titers are inadequate for surveillance of fetal anemia

Critical antibody titer:

- The level below which HDFN and hydrops fetalis are unlikely and no invasive procedures are needed.
- Critical titer for anti-D is 16 in AHG phase (Technical Manual, 18th ed).
- Critical titer for all other IgG antibodies (except for anti-K) are extrapolated from critical titer for anti-D.

Anti-K is an exception

Kell HDFN

- Kell system antigens are present on early RBC precursors.
- Even a low maternal anti-K titer can result in erythropoietic failure and severe fetal anemia.
- No safe titer for anti-K in pregnancy (*ACOG Practice Bulletin No. 75*).

Management - Prenatal

Phenotype the father if paternity is assured.

- For example, in the case of maternal anti-E, phenotype the father for the E-antigen and, if positive, the e-antigen to determine zygosity.
- Father could be EE, Ee or ee, putting fetus at 100%, 50% or 0% risk of HDFN from anti-E if paternity is assured.
- Cannot determine zygosity for D-antigen through serology.

If paternal phenotype is heterozygous or unknown, fetal genotyping can potentially be performed.

- Amniocentesis
- Maternal serum

Management - Prenatal

Transcranial middle cerebral artery (MCA) Doppler ultrasonography

- Moderate to severe anemia predicted by peak systolic velocity above 1.5 times the median for gestational age.
- Sensitivity of 100% and false positive rate of 12%.
- Correct technique is critical; should only be performed by those with adequate training and clinical expertise.

Amniocentesis with bilirubin measurement using spectral analysis at 450 nm (Δ OD_{450})

- Plotting Δ OD_{450} on either Liley graph in late second and third trimesters or Queenan curve for earlier gestational age (19-25 weeks).
- Not appropriate for anti-K HDFN.
- Largely replaced by less invasive MCA Doppler.

Intrauterine transfusion

If MCA Dopplers are concerning:

- Cordocentesis to obtain fetal hematocrit (HCT).
- Goal of IUT is a post-procedure fetal HCT of 40-45%.
- Group O, Rh(D) negative RBCs that are negative for antigen of interest.
  - Irradiated, CMV-safe, fresh (< 7 days), hemoglobin S negative, maternally crossmatched RBCs
- Umbilical vein is most common site for transfusion.
- Performed as early as 18-20 weeks gestation.
- 1-2% risk of fetal mortality.

Intrauterine transfusion

Volume of blood to be transfused:

- Determine the fetal and placental total blood volume (TBV) by multiplying the ultrasound est. fetal weight (grams) by 0.14 mL/g
- Multiply the TBV by the difference in post-transfusion (desired) and pre-transfusion hematocrit (HCT) (ex, 0.40-0.15 = 0.25)
- Divide the amount above by the HCT of RBC unit (eg, 0.85)

Example: Estimated fetal weight of 1000 g with pretransfusion HCT of 15%:

[(1000g) x (0.14 mL/g) x (0.40-0.15)]/85 = 41.2 mL

Technical Manual 18th ed. Bethesda (MD):AABB Press 2014; page 564.

Management - Postnatal

Close monitoring of the bilirubin level required.

- Blue-green light phototherapy and IVIG to prevent kernicterus.
- Double volume exchange transfusion may be needed in infants unresponsive to phototherapy and IVIG.
  - Group O, Rh(D) negative RBCs negative for target antigen that are ideally < 7 days old.
  - Remove group O plasma and replace with AB plasma (dilute to HCT of 40-50%).
  - CMV safe, irradiated, hemoglobin S negative.

Conclusions

- HDFN occurs when a mother has an RBC alloantibody to a paternally inherited fetal RBC antigen.
- Erythroblastosis fetalis, hydrops fetalis, and death from high output cardiac failure are potential outcomes of HDFN.
- An antibody screen and identification performed during early pregnancy can identify at-risk patients and establish a baseline titer.
- Titers performed every 2-4 weeks starting at 18-20 weeks gestation are used for monitoring until a critical titer is attained.

Conclusions

- Transcranial middle cerebral artery Doppler ultrasonography or less commonly amniocentesis with bilirubin measurements are useful for assessing for fetal anemia once a critical titer is reached.

- Intrauterine transfusions may be performed when non-serologic tests are suspicious for moderate to severe fetal anemia beginning at 18-20 weeks gestation.

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