Neonatal Anemia and Jaundice

J. Manna
Hematopathology
2015

OVERVIEW

1. “Normal” neonatal hematology
2. Principles of Anemia
3. Principles of Jaundice
4. Tests for Assessing Newborn Anemia/Jaundice
5. Unusual cases from 2015

“Normal” Neonatal Hematology

- Newborn ≠ Small Adult
- Normal range for Hb, MCV and neutrophils different
- At birth, mostly Hb F (2 alpha and 2 gamma globins)
- By 6 months of age, there adult levels of Hb A1 (2alpha and 2 beta globins)

Terminology: Neonate = up to 4 weeks of age.
Normal Neonatal Peripheral Smear

- Mild anisopoikilocytosis
- +/- Howell-Jolly bodies
- A few nRBCs for about first 5 days post partum

Anemia

Blood Loss:

Hemolysis = Decrease RBC lifespan:

Decrease Production:

Physiologic Anemia:
  - at about 2 months of age

Common Causes of Anemia At birth

Blood Loss

Hemolysis = Decrease RBC lifespan:
Anemia At birth

Blood Loss:
- Fetal-Maternal Hemorrhage (FMH)
- Twin - Twin transfusion

Hemolysis = Decrease RBC lifespan:
- Immune - Alloimmune eg. HDN
- Hereditary - Hemoglobinopathy
- Membranopathy
- Enzymopathy
- Infection - Direct eg. malaria
- Indirect eg. DIC, toxin

Decrease Production:
- Nutritional - eg. maternal B12 deficiency
- Marrow problem

ABOUT JAUNDICE...

Definition = Excess bilirubin which manifests as 'yellow' skin and/or eyes.

Physiology = RBC 'born' in marrow, lives 120 days, and 'dies' in spleen

A. Bilirubin formed as a byproduct of RBC breakdown in spleen
B. Liver enzymes conjugate bilirubin so it is H2O soluble
C. And can be excreted via gallbladder/biliary tracts into GI system

Causes of Neonatal Jaundice =

- Physiologic
- Pathologic

Excess Unconjugated Bilirubin
Excess Conjugated Bilirubin
Neonatal JAUNDICE

Physiologic jaundice: occurs > 24 hrs post partum

Pathologic jaundice: for sure pathologic if < 24 post partum

Unconjugated bilirubin: KERNicterus

- Hemolysis
  - Crigler-Najjar syndrome – severe UDPGT defect
  - Gilbert’s syndrome – mild UDPGT defect

Conjugated bilirubin:

- Biliary tract: atresia, cysts, stones, CA
- Intrahepatic: hepatitis, hereditary (alpha 1 tryptase deficiency, trisomy 18, metabolic, etc)

OVERVIEW

1. “Normal” neonatal hematology
2. Principles of Anemia
3. Principles of Jaundice
4. Tests for Assessing Newborn Anemia/Jaundice
5. Unusual cases from 2015

LAB TESTS

Tests to help answer these questions:

2. a. Is there Hemolysis?
   b. If hemolytic anemia (HA) is present, what is the cause?
Anemia at birth

Blood Loss:
- Fetal-Maternal Hemorrhage (FMH)
- Twin – Twin transfusion

Hemolysis = Decrease RBC lifespan:
- Immune
  - Allergic/Hypersensitivity eg. HDN
- Hereditary
  - Hemoglobinopathy
  - Membranopathy
  - Enzymopathy
- Infection
  - Direct eg. malaria
  - Indirect eg. DIC, toxin

Decrease Production:
- Nutritional
  - eg. maternal B12 deficiency
- Marrow problem

LAB TESTS
- Hemolytic Tests
  - CBC
  - Reticulocyte count
  - Bilirubin
  - (LDH)
  - (Haptoglobin)
- Etiologic HA tests
  - Immune vs. Nonimmune

ETIOLOGIC H.A. LAB TESTS
- HDN (Immune) Testing:
  - Baby’s blood = Baby’s Group, DAT, Peripheral smear (PS) for spherocytes
  - Mom blood = Group/screen
- Hereditary Hemolytic Tests:
  - Enzymopathies = PS, Functional, Molecular tests
  - Membranopathies = PS, ESM via Flow Cytometry
  - Hemoglobinopathies = PS, HPLC, H-bodies, Hb Gel Electrophoresis, Molecular
- Peripheral Smear:
  - Spherocytes (HDN, HS, HPP)
  - Schistocytes (DIC, usually none)
  - Bite/blister cells (G6PD, unstable Hb)
  - Sputnik cells (PKD, usually not present)
  - Marked anisopoikilocytosis
5 Unusual 2015 Cases

1. Hyperbilirubinemia – at birth, 35 week premature boy
2. 
   A. Hyperbilirubinemia - 5 days post-partum, 29 weeks premature boy
   B. Acute anemia – 2 yo girl
3. Anemia in utero – therefore Emergency C-section at 36 weeks
4. Anemia at birth - Emergency C-section at 39 weeks for decreased fetal heart rate.

Case # 1

Hyperbilirubinemia – at birth, 35 week premature boy
- Critically high unconjugated bilirubin
- Exchange transfusion requested STAT due to possible kernicterus

Evaluating the high unconjugated bilirubin:
- occurred < 24 hrs post partum; therefore pathologic
- Hemolysis vs. inherent liver disease?
- Tests to assess possible hemolysis:
  - CBC – initially normal Hb
  - HDN tests normal: Baby = O Rh +, DAT neg.
  - Mom = O Rh +, screen neg.

- PS

"RBC Potpourri" = increased anisopoikilocytosis No sepsis
- Increased nRBCs and polychromasia No hyposia
Case # 1

Likely hemolysis, but what is the cause???

- Not immune, i.e. not HDN
- Hereditary?

Case # 1

- Hemoglobinopathies: HPLC and H-body = normal/neg
- Membranopathies: ESM via flow cytometry = normal
- Enzymopathies: G6PD normal
- Parents' CBC and PS = unremarkable
- No family history of hemolysis/gallstones
- First Nations, siblings healthy -- 4 boys and 1 girl

Case # 1

Hyperbilirubinemia – at birth, 35 week premature boy

- Critically high unconjugated bilirubin
- Double exchange transfusion due to possible kernicterus
- Hb continued drifting downwards post exchange
  - required pRBCs at about 2 weeks of age and subsequently.
- Turned to 'bird seed' tests on pre-exchange sample:
  - Macleod's phenotype: not present
  - Macleod's phenotype = lack of RBC Ag 'K' precursor
    resulting in acanthocytosis and neurological problems
Case #1

Hyperbilirubinemia – at birth, 35 week premature boy
- Critically high unconjugated bilirubin
- Double exchange transfusion due to possible kernicterus
- Requiring regular simple transfusions

- Further Tests? Little pre-transfusion sample, not fresh, 'bird seed rare tests' are send outs...
- Molecular studies for enzyme mutations – not affected by transfusions.

Diagnosis?

Pyruvate Kinase Deficiency

About PKD...Understanding the Biochemistry

Glucose

90% Glycolysis

10% HMPS
(Hexose monophosphate shunt)

Glycolysis----AHHHHHHHHH!
About PKD...

Glycolysis:

- 90% Glucose

2,3-DPG:

- 10% HMPS
- 2 NADH
- Net 2 ATP

PK:

- Pep
- Pyr

LDH:

- Lactate

About Pyruvate Kinase Deficiency...

Definition:

- Most common glycolytic enz. deficiency
- Most common cause of infant chronic hemolysis

Epidemiology:

- 51 per million in Caucasians
- More prevalent in Europe & Japan
- Autosomal Recessive, males = females

2 Genes Encode 4 Isozymes:

- R-type = RBC
- L-type = Liver
- M1 = Muscle, Brain
- M2 = WBCs, all fetal tissue

Etiology / Pathology of PKD

- Point Mutation, AR
- Pyruvate Kinase Deficiency
- Chronic HA= HNSHA

MECHANISM OF HEMOLYSIS??

- PKD
- ATP
- Na/K Pump
- Cell Lysis
- Increased Volume
- Splenic Sequestration / Destruction
PKD Lab Tests

1. Peripheral Smear:
   - No characteristic morphology - although ‘Sputnik cells’ described but often not present.

2. Enzyme Function Tests:
   - Depletion of NADH

3. Molecular Testing:
   - More complex than G6PD due to 2 genes encoding 4 isozymes

Enzyme Function Tests

PEP + ADP  →  PK  →  Pyruvate + ATP
Pyruvate + NADH + H →  LDH →  Lactate + NAD

Measures consumption of added NADH

1. Screening Test = UV Fluorescence of NADH
2. Diagnostic Test = Spectrophotometrically measure light absorption of NADH @ 340nm

What are 3 reasons for false-negative results from a PKD screen??

1. Increased reticulocytes
2. Leukocytosis
3. pRBC Transfusion
5 Unusual 2015 Cases

1. Hyperbilirubinemia – at birth, 35 week premature boy

2. A. Hyperbilirubinemia - 5 days post-partum, 29 weeks premature boy
   B. Acute anemia– 2 yo girl

3. Anemia in-utero – therefore Emergency C-section at 36 weeks

4. Anemia at birth - Emergency C-section at 39 weeks for decreased fetal heart rate.

Case #2a

- Hyperbilirubinemia - 5 days post-partum, 29 weeks premature boy
  - Critically high unconjugated bilirubin (~ 360)
  - Phototherapy helped

Evaluating the high unconjugated bilirubin:

- occurred > 24 hrs post partum; therefore
  - physiologic or pathologic

- Bili quite high for usual physiologic jaundice – assess for hemolysis:
  - CBC – normal Hb at birth, acute anemia at 12 days of age
  - HDN tests normal: Baby = A Rh+, DAT neg.
  - Mom = A Rh+, screen neg.
  - PS at 5 days old = unremarkable
  - PS at 12 days old =

Bite Cell

Blister Cell
Case #2a

- Hyperbilirubinemia - 5 days post-partum, 29 weeks premature boy
  - Critically high unconjugated bilirubin (~ 360)
  - Phototherapy helped
  - Bite/blister cells
  - East Indian ethnicity

- No evidence of membranopathy or hemoglobinopathy
- Enzyme function tests completed since there was no transfusion

Diagnosis?

G6PD Deficiency

About G6PD...

Glucose $\rightarrow$ G6P $\rightarrow$ HMPS $\rightarrow$ G6PD

Glycolysis

2,3-DPG

PK

Pep

Pyr

LDH

Lactate

90%

10%

G6PD acts as a “sweeper” of damaging oxidative radicals

The Hexose Monophosphate Shunt

G6PD

Glucose $\rightarrow$ Glucose 6 Phosphate $\rightarrow$ 6 phosphogluconate

Hexokinase

G6PD

GSH

GSSG

Glutathione reductase

Glycolysis

Ribose-5-P
### About G6PD Deficiency

**Definition:**
- Most common enzyme deficiency
- Usually asymptomatic to mild
- Usually triggered episodic hemolysis

**Prevalence:**

**Types:**

### Epidemiology:

- Mutation is relatively common
- ~400 million affected worldwide
- Affects 1/10 Africans, also common in Asia and the Mediterranean
- X-linked recessive, Males > Females

### Selective Advantage to G6PDD?

**Resistance to Malaria**

[Image of a mosquito]
What are other disorders that may confer resistance to malaria?

- Hemoglobinopathies---Sickle Cell Trait
- Thalassemias
- Membranopathies---SAO
- No Duffy a/b antigen on RBC

About G6PD Deficiency...

Definition:
- Most common enzyme deficiency
- Usually asymptomatic to mild
- Usually triggered episodic hemolysis

Prevalence:
- Mutation is relatively common
- ~400 million affected worldwide
- Affects 1/10 Africans, also common in Asia and the Mediterranean
- X-linked recessive, Males > Females

Types:
- Normal: B  G6PD
  A + G6PD = 20 % Africans
- Abnormal: A-
  = 11% Africans
  Mediterranean = Severe
  Canton = Severe

Etiology / Pathology / Clinical Aspects of G6PD

Point Mutation, X-Linked recessive
- G6PD due to decreased stability
- RBC susceptibility to oxidative damage

Neonatal Jaundice
- Minimal to no hemolysis
- Due to combined:
  G6PD + immature liver enzymes

Precipitated HA
- Drugs
- Infection
- Food
- Diabetic ketoacidosis

Chronic HA = HNSHA
- Very Rare
Precipitated HA in G6PDD

- Drugs: primaquine, dapsone, sulfa, nitrofurantoin, methylene blue
- Infection: pneumonia, typhoid, rocky mountain spotted fever
- Food: fava beans, unripe peach, red suya
- Diabetic Ketoacidosis: ??

Increased Oxidants + Decreased Ability to “mop’’

Damage to:
1. Membrane → Bite / Blister cells → I/V + E/V HA
2. Hb → Heinz Bodies → E/V HA

G6PDD Lab Tests

1. Peripheral Smear:
   - Bite/Blister cells

2. Supravital Stain: Heinz bodies

3. Enzyme Function:
   - Detect NADPH formation
   - Screening and Diagnostic Function Tests

4. Molecular

Enzyme Function Tests

\[ \text{G6P + NADP} \xrightarrow{\text{G6PD}} \text{6-PG + NADPH + H} \]
\[ \text{NADPH + GSSG} \xrightarrow{\text{GR}} \text{NADP + GSH} \]

Measures production (+/- consumption) of NADPH

1. Screening Test = UV fluorescence of NADPH
2. Diagnostic Test = Spectrophotometrically measure light absorption of NADPH @ 340 nm
What are 3 reasons for false-negative results from a G6PD screen?

1. **Increased reticulocytes** — contain normal levels of G6PD in G6PD A – variant

2. **pRBC Transfusion**

3. **Heterozygote woman** – ie. a ‘carrier’ of the G6PD mutation

**5 Unusual 2015 Cases**

1. Hyperbilirubinemia – at birth, 35 week premature boy

2. A. Hyperbilirubinemia - 5 days post-partum, 29 weeks premature boy
   B. Acute anemia – 2 yo girl

3. Anemia in-utero – therefore Emergency C-section at 36 weeks

4. Anemia at birth - Emergency C-section at 39 weeks for decreased fetal heart rate.
Case #2b

- Acute anemia in a 2 yo African girl
  - in ER for infection/fever
  - Hb = 80
  - Anemia resolved on its own

Evaluating the acute anemia:

- Common pediatric causes = nutritional deficiencies and infection
- Uncommon pediatric causes = non-traumatic blood loss, hemolysis

Blood loss, nutritional deficiencies and malaria excluded
- CBC = normocytic anemia, mild neutrophilia and monocytosis
- Retic count = normal
- PS = possible spherocytes
- Hemolysis? -- RBC changes, increased LDH but normal bili
- No membranopathy
  - one deletion alpha thalassemia detected -- red herring?
  - Enzyme function studies confirmed.....

G6PD deficiency
Case #2b

- Because G6PD is X-linked, usually females are asymptomatic carriers
- How can females become symptomatic as in this case?

1. Turner's syndrome, XO
2. Homozygous mutation
3. Unfavourable Lyonization: i.e. more of the normal, unmutated X chromosomes are inactivated at the embryonic stage
4. Uniparental disomy

5 Unusual 2015 Cases

1. Hyperbilirubinemia – at birth, 35 week premature boy
2. A. Hyperbilirubinemia - 5 days post-partum, 29 week premature boy
   B. Acute anemia – 2 yo girl
3. Anemia in-utero – therefore Emergency C-section at 36 weeks
4. Anemia at birth - Emergency C-section at 39 weeks for decreased fetal heart rate.

Case #3

Anemia in-utero – therefore Emergency C-section at 36 weeks
- at birth, Hb critically low at 96

Evaluating the Anemia:
- Common causes in neonate = blood loss and hemolysis
- Kleihaur-Betke test normal = blood loss unlikely
- Hemolysis? Initial tests = CBC, bilirubin, PS
Anemia in utero – therefore Emergency C-section at 36 weeks
- at birth, Hb critically low at 96

Evaluating the Anemia:
- Common causes in neonate = blood loss and hemolysis
- Kleihau-Betke test normal = blood loss unlikely
- Hemolysis?  -Hb = 96, increasing bilirubin
  -PS = spherocytes + increased polychromasia & nRBCs for age
- Most common cause of neonatal hemolysis?

Immune Hemolysis = HDN (hemolytic disease of the newborn)
Case # 3

Anemia in-utero – therefore Emergency C-section at 36 weeks
- at birth, Hb critically low at 96

Evaluating the Anemia:
- Hemolysis?
  - Hb = 96, increasing bilirubin
  - PS = spherocytes + increased polychromasia & nRBCs for age
- HDN tests:
  - Baby = O Rh pos, DAT is strongly positive, PS = see above
  - Mom = B Rh neg, screen (IDAT) shows allo-anti- D and anti-E present.
- Treatment = IVIG + exchange transfusion

Case # 3

Anemia in-utero – therefore Emergency C-section at 36 weeks
- at birth, Hb critically low at 96

Evaluating the Anemia:
- HDN tests:
  - Baby = O Rh pos, DAT is strongly positive, PS = spherocytes + nRBCs/poly
  - Mom = B Rh neg, screen (IDAT) shows allo-anti- D and anti-E present.
- Mom is G2P1, had RhIg in previous pregnancy
- This pregnancy ------
  - Anti-D and anti-E titers monitored
  - Monitored baby via doppler US of MCA to assess for anemia
  - Paternal tests showed D+ and E+ phenotype

About HDN:
Etiology & Pathophysiology

Fetal Ag pos RBC into maternal blood
Ag neg Mom produces alloantibody
+/- Amnestic response upon SUBSEQUENT pregnancy
IgG alloantibody crosses placenta & binds to Ag
Extravascular hemolysis results in fetus
• Mom = RBC antigen negative
• Fetus = RBC antigen positive

• Most common = ABO, IgG ---- 1 / 150
• Most potent = Rh (anti-D) ---- 1 / 1000
• Other less common
  But potentially significant =
  - Kell, Kidd, Duffy, C,c,E,e...

<table>
<thead>
<tr>
<th>ABO</th>
<th>Rh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most common: 1/150</td>
<td>Less common: 1/1000</td>
</tr>
<tr>
<td>Asymptomatic to mild</td>
<td>Symptomatic</td>
</tr>
<tr>
<td>3 to 5 dys post-partum</td>
<td>In-utero or ex-utero</td>
</tr>
<tr>
<td>Not amnestic</td>
<td>Amnestic</td>
</tr>
</tbody>
</table>

- Prevention of Rh HDN = Rh Ig given to Rh neg mom
  - HDN better understood in the 50's
  - Rh Ig became more routine in the 70's
  - Rh Ig significantly decreased Rh HDN in the 80's and after
About HDN: ABO vs Rh HDN

- ABO HDN = typically asymptomatic to mild due to:
  1. ...gent not fully expressed at birth
  2. Antibody neutralized by A & B Ag in fluid and tissues

- ABO HDN can be severe in Africans, Asians, Arabians, Hispanics:
  - In 2014, African-Canadian newborn with severe anti-B HDN
  - exchange transfusion and IVIG
  - Rare case studies describing severe anti-B HDN specifically in the African population

HDN Testing:

- If "HDN testing" or DAT requested, then the following tests are done together:
  - Baby's blood = Group, DAT, peripheral smear
  - Mom's blood = Group and Screen

- If mom has known allo-antibodies, then CBS results summarized:
  - Mom's group, phenotype
  - Antibody titers
  - Paternal phenotype

5 Unusual 2015 Cases

1. Hyperbilirubinemia – at birth, 35 week premature boy
2. A. Hyperbilirubinemia - 5 days post-partum, 29 weeks premature boy
   B. Acute anemia – 2 yo girl
3. Anemia in-utero – therefore Emergency C-section at 36 weeks
4. Anemia at birth - Emergency C-section at 39 weeks for decreased fetal heart rate.
Case # 4

Anemia at birth - Emergency C-section at 39 weeks for decreased fetal heart rate.

- At birth, Hb markedly decreased at 40!

Evaluating the Anemia:

- Common causes in neonate = blood loss and hemolysis
- Kleihaur-Betke test normal, flow cytometry of HbF was normal
- Hemolysis? - Bilirubin not increased!
- Retic count increased
- Peripheral smear

Baby also had

- cardiomegaly
- hepatomegaly
- absent spleen
- no overt hydrops fetalis (swelling)
- normal 21 week ultrasound

-Mom =

- 42 yo
- Normal pregnancy
- one child, previous normal pregnancy/delivery
When things don't make sense...

- Clerical error? --- No
- Atypical presentation of a common disorder (Anemia)?
  - Eg. atypical hemolysis
- Very rare disorder?

---

Case # 4

Anemia at birth - Emergency C-section at 39 weeks for decreased fetal heart rate.

- At birth, Hb markedly decreased at 40 and erythroblastosis!
- Cardiomegaly, hepatomegaly, absent spleen

Evaluating the Anemia:

- Common causes in neonate = blood loss and hemolysis
  - Kleihauer-Betke test normal, flow cytometry of HbF was normal
- Hemolysis?
  - bilirubin not increased!
  - PS = increased polychromasia & nRBCs for age
- Etiologic tests for hemolysis:
  - not HDN, baby = A pos, DAT neg, mom = A pos, screen neg
  - No H-bodies, therefore alpha thalassemia major very unlikely
  - Excluded hemoglobinopathies, membranopathies, & enzymopathies

When things don't make sense...

- Clerical error? --- No
- Atypical presentation of a common disorder (Anemia)?
  - Eg. atypical hemolysis --- No
  - Eg. Blood loss but not into maternal circulation?
- Very rare disorder?
  - A syndrome including marrow pathology?
  - how would erythroblastosis still occur in this setting?

- Need placental examination
- Bone marrow biopsy

---
Case # 4

- Autopsy declined (baby tragically died)
- Placental examination showed ...

Gross Placental Examination

Diagnosis = Fetal blood loss into intraplacental choriocarcinoma

Common cause of anemia secondary to an extremely rare disease
About Intraplacental Choriocarcinoma

Definition = extremely rare cancer of the placental, chorionic cells

Epidemiology:
- Very rare – 0.04% of all gestational trophoblastic diseases (GTDs).
- Most common GTD = hydatidiform mole

Risks:
- Occurs in older (> 35 yo) or very young women
- No increased risk of subsequent pregnancies developing this cancer

Pathophysiology & Clinical:
- Aggressive cancer that clinically manifests in the third trimester, at birth or just after birth.
- Marked fetal/neonatal anemia due to blood loss into placental malignancy.

Diagnosis:
- Placental examination – subtle cancer, so can be missed.
- Clinical information crucial.

Management:
- Usually emergency C-section due to fetal distress
- Supportive therapy for baby, eg. pRBCs
- Assess for metastasis in baby and mom: hCG levels
  - and depending on hCG, radiologic scanning may be required.
  - If metastasis is present, then chemotherapy is initiated.

Case #4:
- Baby unfortunately died (from heart failure secondary to the severe anemia)
- Mother’s hCG normalized, no metastasis therefore no chemotherapy was required
Acknowledgments

BCCH, Hematopathology Department
- Thank you to the hematopathologists and lab technologists re: discussions and conducting G6PD diagnostic testing.

Hamilton Laboratory Staff
- Thanks for conducting diagnostic molecular PK testing

Victoria, Anatomic Pathology Department
- Thank you to Dr. N. Van der Westhuizen and lab technologists for discussions, articles, images and diagnosing intraplacental choriocarcinoma

Victoria, Hematopathology Department
- Thank you to special hematology, core lab and transfusion medicine for their collaboration

References


