Neonatal Hyperbilirubinemia: The Basics, Testing and Interpretation of Results

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Learning Objectives
- Describe the source of bilirubin and its basic metabolism
- Compare the tests available for measuring bilirubin
- Discuss hyperbilirubinemia and specifically the causes and treatments for neonatal hyperbilirubinemia
- Assess the utility of the Bhutani plot and the importance of using age-appropriate reference intervals

Case presentation
- 10 Month old Hispanic female
- Increasing abdominal girth
- Increasing lethargy
- GGT 92 U/L
- ALT 468 U/L
- AST 561 U/L
- NH₄⁺ 76 mmol/L
- Tbili 27.3 mg/dL (467 µmol/L)
- Dbili 22.8 mg/dL (390 µmol/L)
Bilirubin

- Product of heme degradation
- H bonds with itself to form a non-linear, cup-like structure
- Extremely non-water soluble
- Referred to as un-conjugated bilirubin
- Taken up into tissues; in blood transported by albumin

Bilirubin

Conjugated in liver by UDP-glucuronyltransferase

- Hepatocytes add one (mono-conjugated) or two (di-conjugated) glucuronic acid residues
- 90% of conjugated bilirubin is di-conjugated
- Conjugated bilirubin is water soluble
- Excreted in bile and urine

Bilirubin fractions

- \( \alpha \)-bilirubin: unconjugated
- \( \beta \)-bilirubin: mono-conjugated
- \( \gamma \)-bilirubin: di-conjugated
- \( \delta \)-bilirubin: Covalently bound to protein
  - Formed slowly from conjugated bilirubin and albumin in circulation by non-enzymatic means, (like glycated hemoglobin)
  - Generally detectable only after prolonged episode of conjugated hyperbilirubinemia
  - Clear slowly from circulation. Half-life same as albumin, 2-3 weeks.
Bilirubin Metabolism

Unconjugated bilirubin → Heme → Liver → Intestine → Conjugated bilirubin → Kidney → Urine → Systemic circulation

Bilirubin measurement history

- 1883 - Ehrlich introduced the diazo reaction
- 1916 - Van den Bergh and Muller described and coined the terms “direct” reacting ("indirect" being total minus direct bilirubin)
- 1937 - Malloy and Evelyn adapted the bilirubin procedure to the photoelectric colorimeter
- 1938 - Jendrassik and Grof described the basis of our current diazor methods – reproducible and reliable

Measurement - history

- Diazotized salts react to give “direct-reacting” or Direct Bilirubin
- Add an accelerator to the sample
  - Early on was ethanol, then caffeine, benzoate
- React with diazo-salts again to give Total Bilirubin
Diazo methods

Measurement - history

- Direct Bilirubin
  - Mono- and di-conjugates
  - Protein-bound

- Total Bilirubin
  - All bilirubin fractions

- Total – Direct = Unconjugated bilirubin
  “Indirect”

Measurement – assays

- Ortho Diagnostics Vitros dry-slide assay
  - Measures Bc and Bu – directly measures the unconjugated and conjugated
  - Use a mordant to shift maximum absorbance peak

Bilirubin Fractionation by HPLC

Transcutaneous Bilirubin measurement

Utilizes reflectance spectrometry
Skin color affects results

Assay results comparison

<table>
<thead>
<tr>
<th>HPLC Peak</th>
<th>Alpha</th>
<th>Beta</th>
<th>Gamma</th>
<th>Delta</th>
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<tbody>
<tr>
<td>Bilirubin Species</td>
<td>Unconjugated</td>
<td>Singly Conjugated</td>
<td>Doubly Conjugated</td>
<td>Bound to albumin</td>
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<tr>
<td>Total Bilirubin (TBIL)</td>
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<tr>
<td>VITROS Methods</td>
<td>Unconjugated (Bu)</td>
<td>Conjugated (Bc)</td>
<td>[Data from table]</td>
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<tr>
<td>Total Bilirubin</td>
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<tr>
<td>Diazo Methods</td>
<td>Indirect (Total - Direct)</td>
<td>Gray Zone</td>
<td>Direct Bilirubin (DBIL)</td>
<td>Total Bilirubin</td>
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<tr>
<td>Transcutaneous</td>
<td>Total Bilirubin</td>
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Adapted from: Phil Bach PhD; www.clpmag.com/graphics/mags/0206/pt01.jpg
Disorders of bilirubin metabolism

- Two basic types of hyperbilirubinemia
  - **Unconjugated** Hyperbilirubinemia (UHB)
    - Three main causes
    - Extremely serious if very high or continuous
  - **Conjugated** Hyperbilirubinemia (CHB)
    - Two main causes
    - Less serious – water-soluble bilirubin can be excreted

**Unconjugated Hyperbilirubinemia causes**

1. Conditions which **Heme → Bili conversion**
   - Increased hemolysis
   - Rapid turnover of increased RBC mass (newborn)
2. Conditions which **Bili delivery to liver** or **Bili uptake by the hepatocytes**
   - Right-sided congestive heart failure
3. Conditions which **conjugation**
   - Neonatal jaundice
   - Hepatocellular dysfunction
   - Inborn errors of bilirubin metabolism (Gilbert’s syndrome; Crigler-Najjar -partial or complete enzyme deficiencies)

**Conjugated Hyperbilirubinemia causes**

1. Conditions which **conjugated Bili secretion into canaliculi**
   - Hepatocellular disease (Hepatitis, Cholestasis)
   - Dubin-Johnson and Rotor’s syndromes
2. Conditions which **conjugated Bili drainage from liver**
   - Extrahepatic obstruction
   - Stones, carcinoma, stricture, atresia
   - Intrahepatic obstruction
   - Primary biliary cirrhosis, Bile duct paucity, tumors
Neonatal hyperbilirubinemia

- **Unconjugated** - ‘neonatal jaundice’
  - Immature liver enzymes
    - Shortened RBC life and increased production
    - Breast-feeding - α-glucuronidase in breast milk unconjugates bilirubin and allows it to be re-absorbed (affects about 30% of breast-fed infants)

Treatment –
- Phenobarb
- Phototherapy (UV light)
- Nurse (Sr. J Ward) in Essex noted infants by the window less jaundiced than farther into the room – Dr. (RJ Cremer) listened to her

NHB sequelae

- Unconjugated hyperbilirubinemia
  - Kernicterus
    - Bilirubin deposition in brain tissue (basal ganglia & brainstem nuclei)
    - Neurological damage
    - Early symptoms – vomiting, lethargy, poor feeding
    - Later seizures & death (70%)
    - Survivors – severely brain damaged
Neonatal hyperbilirubinemia

- American Academy of Pediatrics (AAP) guidelines
  - Measure TSB or TcB if jaundiced in the first 24 hours.
  - Interpret bilirubin levels according to the infant’s age in hours.
  - Perform risk assessment prior to discharge.

- AAP guidelines – Risk factors
  - TSB or TcB >75%
  - Jaundice <24hr or before discharge
  - ABO with + DAT or other hemolytic disease (G6PD)
  - Gestation <39wk
  - Previous sibling jaundiced
  - Cephalhematoma or bruising (vacuum)
  - Exclusive breastfeeding
  - East Asian
  - Male
  - Discharge <72hr

Bhutani plot

Neonatal hyperbilirubinemia

- AAP guidelines – Risk factors

Bhutani plot

Importance of age-related (pediatric) reference intervals

Bhutani plot

Determine if NHB is conjugated or unconjugated!
Neonatal hyperbilirubinemia

- Conjugated –
  - Idiopathic neonatal hepatitis
  - Unknown etiology – possibly familial
  - Jaundice appears after 2 weeks of age
  - Liver and spleen become enlarged
  - LFTs ↑; PT ↑
  - Cholestasis present
  - Treatment supportive with 90% survival without sequelae
  - Biliary atresia

Case Presentation

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  - Failed Kasai procedure

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Case

Liver transplant