Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Neonatal Hyperbilirubinemia

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Slide 1: Title slide

Slide 2:
Bilirubin is an orange-yellow pigment derived from the degradation of the heme moiety of hemoproteins, particularly the hemoglobin of mature circulating erythrocytes. Hemo oxygenase is the enzyme initially responsible for the degradation of hemoglobin, producing biliverdin and carbon monoxide. Biliverdin reductase reduces the green-pigmented biliverdin to bilirubin.

Bilirubin production primarily occurs in the liver, but also can occur in the spleen and bone marrow, and as it is produced in the unconjugated form, bilirubin is highly insoluble. Bilirubin is conjugated in the liver, which allows excretion in the bile and urine. Bilirubin is mainly conjugated to glucuronic acid by uridine diphosphate glucuronosyl transferase 1A1, or UGT1A1.

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Bilirubin is photosensitive because light causes configurational and structural changes to unconjugated bilirubin. The structure of bilirubin, as established by x-ray crystallography, is a ridge-tiled configuration stabilized by six intramolecular hydrogen bonds. These six intramolecular hydrogen bonds stabilize the Z-Z structure of unconjugated bilirubin, rendering it insoluble. Rotation and/or breakage of the bonds on carbon atoms 5 and 15 lead to more open E-Z- and Z-E-bilirubin structures, which are more water soluble. The sensitivity of bilirubin to light is the basis for phototherapy in individuals with unconjugated hyperbilirubinemia. Clinical measurement of bilirubin can also be affected due to this light sensitivity.

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Four fractions of bilirubin can be revealed upon open-column chromatographic separation that does not involve deproteinization. In blood, bilirubin is bound to albumin and transported to the liver where it dissociates from albumin as unconjugated bilirubin, the first fraction listed here. It is then transported across the membrane where, once inside the hepatocyte, it is conjugated with glucuronic acid to form the second and third fractions: bilirubin monoglucuronide and diglucuronide. The fourth fraction of bilirubin is delta bilirubin, which is bilirubin that is bound covalently to albumin, but is distinct from the serum albumin- unconjugated bilirubin complex, which has a micromolar affinity for bilirubin.

Slide 5:
Bilirubin is most commonly measured by chemical methods based on the diazo reaction. In this reaction, diazotized sulfanilic acid (or the diazo reagent) reacts with bilirubin to produce azodipyrolles or azopigments. The upper panel shows the reaction for measuring direct bilirubin, which is a mixture of mono- and di-conjugated bilirubin and delta bilirubin. By adding an accelerant, such as caffeine or methanol, to the diazo reaction, as shown in the lower panel, one can facilitate the reaction of unconjugated (or indirect) bilirubin with the diazo reagent, thereby measuring the total bilirubin. The indirect (or unconjugated) bilirubin can then be calculated by subtracting the direct bilirubin from the total bilirubin.

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In addition to the diazo methods, there are several other methods for measuring bilirubin. The first of these listed here utilizes a slide-based method produced by Ortho Clinical Diagnostics on Vitros analyzers. This method relies on the use of a thin film with a mordant substance that binds to bilirubin, and can distinguish conjugated (or "Bc") from unconjugated (or "Bu") bilirubin using a spectrophotometric method. An ultrafiltration layer is added to the top of the slide to exclude hemoglobin and albumin, hence also excluding delta bilirubin. The Vitros TBIL slide should be distinguished from the BuBc slide in that it utilizes the diazo method to measure all fractions of bilirubin.

Another way to measure bilirubin is enzymatically by utilizing bilirubin oxidase to specifically oxidize mono- and di-conjugated bilirubin, while excluding unconjugated and delta bilirubin. This is a newer method and is not yet in widespread use.

Blood gas analyzers can measure total bilirubin with an oximeter, which utilizes spectrophotometry to measure different species of hemoglobin and bilirubin. The spectrum of the patient sample is compared to reference spectra to determine total bilirubin concentrations.

Transcutaneous bilirubin is a newer method that is noninvasive and is based on reflectance densitometry read at multiple wavelengths. Transcutaneous bilirubin measurement is accurate up to bilirubin levels at about 10 – 15 mg/dL.

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Hyperbilirubinemic disorders are characterized by a predomination of elevated unconjugated or conjugated bilirubin in the absence of other abnormal liver tests. Unconjugated hyperbilirubinemia poses a risk for development of acute bilirubin encephalopathy, or kernicterus, especially in infants with low birth weight.

Conjugated hyperbilirubinemias usually indicate hepatobiliary disease, such as biliary obstruction. Diseases that reduce the rate of secretion of conjugated bilirubin into the bile or the flow of bile into the intestine produce mixed or predominantly conjugated hyperbilirubinemias due to the reflux of conjugates back into the plasma.

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Physiological jaundice of the newborn within a few days of birth is common and about half of all neonates become clinically jaundiced during the first 5 days of life. Serum bilirubin is predominantly unconjugated and typically rises from 1-2 to 5-6 mg/dL within 72 hours and returns to normal in 7-10 days. Physiological jaundice of the newborn is usually a result of increased unconjugated bilirubin load due to multiple factors including RBCs with shortened life spans and bilirubin derived from ineffective erythropoiesis or non-RBC sources. It also can be due to decreased conjugation of bilirubin due to a relative lack of the UGT1A1 conjugating enzyme. This group of enzymes is mainly inactive at birth, and must be induced, which takes a few days. Physiological jaundice of the newborn can also be caused by passive reabsorption of bilirubin in the intestine due to conversion of conjugated bilirubin to unconjugated by beta-glucuronidase in intestinal contents; and inhibitors of conjugation that naturally occur in breast milk.

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Severe neonatal jaundice caused by elevated concentrations of unconjugated hyperbilirubinemia is considered pathophysiologic. The American Academy of Pediatrics (AAP) recommends that the term acute bilirubin encephalopathy be used to describe the acute manifestations of bilirubin toxicity seen in the first days to weeks after birth, and the term “kernicterus” be used for the chronic and permanent sequelae of bilirubin toxicity. Acute bilirubin encephalopathy is characterized by severe jaundice, lethargy, hypotonia, poor sucking reflexes, and vomiting. If not treated, the condition can progress to hypertonia (manifested by backward arching of the neck and trunk), fever, and high-pitched cry. The condition can further progress to apnea, coma, seizures, and even death. Infants who survive may have a severe form of athetoid cerebral palsy, hearing loss, dental dysplasia, paralysis of upward gaze, and sometimes intellectual disabilities and other handicaps.

The U.S. Kernicterus Registry identified the main epidemiologic factors for pathophysiologic hyperbilirubinemia, and they include a high preponderance of breastfed infants, male gender, early hospital discharge, and late pre-term infants.

The most common treatment for acute hyperbilirubinemia is phototherapy and changing the diet of the infant from breast milk to formula. If bilirubin levels remain high, plasmapheresis may be used to further reduce bilirubin levels.

In the first month of life, jaundice is the most common hospital readmission diagnosis. Approximately 200,000 – 325,000 infants in the U.S. undergo phototherapy each year.

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Other hyperbilirubinemia neurotoxicity risk factors include isoimmune or other hemolytic disease, such as that observed with ABO or Rh-factor incompatibility, glucose-6-phosphate dehydrogenase deficiency, or hereditary spherocytosis. Additional risk factors include family history, especially a sibling with significant hyperbilirubinemia; cephalhematoma or significant bruising indicative of bleeding; asphyxia; sepsis; acidosis; and hypoalbuminemia.

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Infants with hyperbilirubinemia most often have elevated levels of unconjugated bilirubin, but conjugated hyperbilirubinemia can also occur. Conjugated hyperbilirubinemias are characterized by elevated conjugated bilirubin levels, most commonly due to idiopathic neonatal hepatitis and biliary atresia. Idiopathic neonatal hepatitis is of unknown etiology, but possibly familial in nature with an autosomal recessive inheritance pattern. Neonates with idiopathic hepatitis initially are healthy but develop hepatosplenomegaly with pale stools, elevated serum aminotransferases, and prolonged prothrombin time. However, the prognosis is favorable and with supportive treatment, 90% of infants survive without sequelae.

Biliary atresia is a group of acquired disorders involving either the intrahepatic or extrahepatic bile ducts. In extrahepatic biliary atresia, which occurs in 1:10,000 births, the gallbladder is usually unable to be observed by imaging techniques that rely on transport of dye through the liver. Portoenterostomy, or the Kasai procedure, should be performed within 60 days after birth, with liver transplantation as the second treatment of choice. Prognosis is generally poor unless the lesion is surgically correctable. Intrahepatic biliary atresia is characterized by a paucity of intrahepatic bile ducts. Survival into adolescence is common, although growth retardation generally occurs.

Conjugated hyperbilirubinemia can also occur as a complication of parenteral nutrition, and usually occurs in premature infants with parental-associated cholestasis.

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Newborns can also have hyperbilirubinemia due to inherited disorders of bilirubin metabolism, which include Gilbert syndrome, Crigler-Najjar syndrome (types I and II), Dubin-Johnson syndrome, and Rotor syndrome. Gilbert and Crigler-Najjar syndromes involve unconjugated hyperbilirubinemia, whereas Dubin-Johnson and Rotor syndromes are characterized by mixed or predominantly conjugated hyperbilirubinemia.

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Gilbert syndrome is characterized by mild, chronic, unconjugated hyperbilirubinemia. Serum bilirubin levels are generally <3 mg/dL, and may fluctuate between 1.5 and 3 mg/dL. Bilirubin concentrations can increase with illness and fasting. Gilbert syndrome is autosomal recessive and can be caused by reduced expression of UGT1A1, commonly due to variation in the promoter region TATAA box. Most individuals with Gilbert have 7 copies of the TA repeat, whereas a minority of individuals have 8 copies of the TA repeat. In Asia, Gilbert's syndrome has sometimes been found to be caused by a common point mutation in exon 1 of the UGT1A1 gene, which is a missense mutation resulting in a substitution of Arginine for Glycine at residue 71, also known as the *6 allele. Gilbert syndrome occurs at a frequency of about 3-7% in U.S. and Europe.

Individuals with Gilbert syndrome have hepatic UGT1A1 activity reduced to approximately 30% of normal. Gilbert syndrome is thought to be a relatively benign condition with icterus commonly being the only presenting factor. No treatment is needed for Gilbert syndrome, but patients must take caution when taking certain medications that are metabolized by glucuronidation, such as acetaminophen. Also, it should be noted that individuals with reduced UGT1A1 activity are at risk for severe neutropenia with the chemotherapeutic agent irinotecan.

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Crigler-Najjar syndrome is a group of genetic disorders that also presents with high unconjugated bilirubin levels, but the levels are higher than what is observed with Gilbert syndrome. Crigler Najjar syndrome Type I is due to a complete (or near complete) absence of UDP-glucuronosyltransferase 1A1 and is manifested by very high levels of unconjugated bilirubin. In the past, patients with CN Type I generally suffered from severe brain damage caused by kernicterus and died within the first year or two of life. With the advent of phototherapy and intermittent plasmapheresis, survival until puberty is not uncommon. However, bilirubin encephalopathy generally develops around the time of adolescence, when phototherapy becomes less effective due to thickening of the skin. Orthotopic liver transplantation or auxillary transplantation of a single liver lobe has resulted in long-term survival in several cases and is considered the only definitive treatment for CN Type I.

CN Type II is characterized by a partial deficiency in UDP-glucuronosyltransferase and is manifested by high levels of unconjugated bilirubin, but not as severely elevated as CN Type I. Individuals with this disorder usually respond favorably to phenobarbital treatment and can be expected to live a normal life. Both CN types I and II are rare autosomal recessive disorders caused by mutations in one of the 5 exons of the UGT1A1 gene. In CN Type II, at least one of the mutations is a missense mutation, resulting in an amino acid change, which leads to a marked reduction, but not total loss, of bilirubin-UGT activity. Phenobarbital is effective in CN Type II because it can induce the residual bilirubin-UGT activity.

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Dubin-Johnson syndrome is a rare disorder characterized by mild, predominantly conjugated hyperbilirubinemia. Serum bilirubin is generally between 2-5 mg/dL, but can be as high as 20-25 mg/dL, and bilirubinuria is frequently observed. Individuals with Dubin-Johnson syndrome present with jaundice but otherwise have a normal physical exam. They also have normal liver function, but interestingly have livers that are pigmented black or dark-brown, due to deposition of a melanin-like pigment in the hepatocytes. Increased urinary excretion of coproporphyrin isomer I is also observed in these individuals. Some individuals with DJS may experience increased bilirubin levels upon intake of steroid hormones, such as oral contraceptives, and during pregnancy. Dubin-Johnson has an autosomal recessive mode of inheritance and is due to mutations in the CMOAT or ABCC2 gene, which encodes for an organic anion transporter protein.

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Rotor syndrome is a benign inherited disorder characterized by the accumulation of both conjugated and unconjugated plasma bilirubin. It was previously thought that Dubin-Johnson and Rotor syndromes were variants of a single disorder, but it is now know that they are distinct entities. Unlike Dubin-Johnson syndrome, Rotor syndrome is not characterized by abnormal hepatic pigmentation. Additionally, Rotor syndrome is characterized by a marked increase in total urinary coproporphyrin excretion, which is distinct from what is observed with the more specific increase of coproporphyrin isomer I in Dubin-Johnson syndrome. It is theorized that the underlying mechanism for Rotor syndrome is impaired hepatocellular storage of conjugated bilirubin. The genetic cause for Rotor syndrome is unknown at this time.

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Current guidelines for management of hyperbilirubinemia in the term and late preterm infant were set by the American Academy of Pediatrics in 2004 and the Canadian Pediatric Society in 2007. Both guidelines follow similar recommendations of performing total serum bilirubin or transcutaneous bilirubin measurements and assessment of clinical risk factors to evaluate the risk of subsequent hyperbilirubinemia. A bilirubin risk zone can be determined for the infant and then further action can be made, including discharging the infant if in a low risk zone, following-up with additional bilirubin measurements if in a high-intermediate risk zone, and evaluating for phototherapy or possibly exchange transfusion if in a high risk zone.

It is important to recognize that bilirubin encephalopathy is reversible in the initial stages and that it is thought that many cases of kernicterus could have been prevented. Thus, prompt intervention to reduce bilirubin levels in the brain by such measures as phototherapy and even exchange transfusion can prevent progression to the irreversible stage.