Diagnostic criteria and contributors to Gilbert’s syndrome

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ABSTRACT

Hyperbilirubinemia is a well-known condition in the clinical setting; however, the causes of elevated serum bilirubin are diverse, as are the clinical ramifications of this condition. For example, diagnoses of individuals vary depending on whether they exhibit an unconjugated or conjugated hyperbilirubinemia. Diagnoses can include conditions of disordered bilirubin metabolism (Gilbert’s, Crigler-Najjar, Rotor, or Dubin-Johnson syndromes) or an acquired disease, including alcoholic/non-alcoholic fatty liver disease, hepatotropic hepatitis, cirrhosis, or hepatobiliary malignancy. Assessment of bilirubin concentrations is typically conducted as part of routine liver function testing. Mildly elevated total bilirubin with normal serum activities of liver transaminases, biliary damage markers, and red blood cell counts, however, may indicate the presence of Gilbert’s syndrome (GS), a benign condition that is present in ~5–10% of the population. In this case, mildly elevated unconjugated bilirubin in GS is strongly associated with a “reduced” prevalence of chronic diseases, particularly cardiovascular diseases (CVD) and type 2 diabetes mellitus (and associated risk factors), as well as CVD-related and all-cause mortality. These reports challenge the dogma that bilirubin is simply a potentially neurotoxic by-product of heme catabolism and emphasize the importance of understanding its potential beneficial physiologic and detrimental pathophysiologic effects, in order to appropriately consider bilirubin test results within the clinical laboratory setting. With this information, we hope to improve the understanding of disorders of bilirubin metabolism, emphasize the diagnostic importance of these conditions, and outline the potential impact GS may have on resistance to disease.

Abbreviations: ABCC2: ATP-binding cassette sub-family C member 2; DBIL: direct (conjugated) bilirubin; CN-1: Crigler-Najjar syndrome 1; CN-2: Crigler-Najjar syndrome 2; CVD: cardiovascular disease; GS: Gilbert’s syndrome; HPLC: high performance liquid chromatography; HO: heme oxygenase; IBIL: indirect (unconjugated) bilirubin; LC-MS/MS: liquid chromatography-tandem mass spectrometry; MRP2: multi-drug resistance protein-2; OATP: organic anion transporter; RBC: red blood cells; SLCO1B1: solute carrier organic anion transporter family member 1B1; TB: total bilirubin; UGT1A1: uridine glucuronosyl transferase 1A1

Introduction to heme catabolism and bilirubin metabolism

The catabolism of heme, visually apparent after traumatic tissue injury, is associated with changes in the coloration of bruising, which evolves from the black/red coloration of tissue heme accumulation and transforms to the green/blue hues of biliverdin and to the final and long-lasting yellow pigmentation of bilirubin in superficial tissue. Resident leukocytes including macrophages are believed to account for the removal of potentially deleterious heme from the extracellular milieu [1], and for the production of heme catabolites including iron, carbon monoxide, and biliverdin/bilirubin. Within a physiological context, approximately 25% of heme is sourced from ineffective erythropoiesis and from heme-containing enzymes [2], with the remaining 75% obtained from senescent red blood cells (RBC) that undergo recycling within the reticulo-endothelial system (i.e. splenic macrophages and hepatic Kupfer cells; Figure 1). Within macrophages, the catabolism of heme is first accomplished by heme oxygenase (HO), the rate limiting enzyme of heme catabolism, forming biliverdin,
which is then reduced to bilirubin by biliverdin reductase [3, 4]. This lipophilic molecule accumulates intracellularly, diffuses into the blood and binds to circulating albumin for hepatic delivery. Within the liver, the unbound fraction of bilirubin is actively and passively absorbed into hepatocytes and undergoes mono- or di-glucuronidation by uridine glucuronosyl transferase 1A1 (UGT1A1) [5], facilitating excretion through the bile canaliculus via multi-drug resistance protein-2 (MRP2). Once entering the bile, bilirubin glucuronides are initially stored within the gallbladder and are then directed into the gut with other biliary constituents, which facilitate absorption of fats and other fat-soluble compounds. Bilirubin glucuronides first undergo deconjugation by bacterial β-glucuronidases, with unconjugated pigment then being reduced and oxidized in a sequence of poorly-understood reactions that are accomplished by intestinal flora [6]. Some of these products can be reabsorbed and contribute to the circulating bilirubin pool; others are excreted via the kidney and gut and contribute to the distinctive coloration of urine and feces, respectively (Figure 1).

The catabolism of heme and resultant formation of bilirubin in mammals and some fish represents a high-energy consuming metabolic pathway [7], the purpose of which remains a mystery. Speculation abounds regarding the physiological importance of bilirubin formation, in that it may provide a route for biliverdin elimination from the fetus [8] or that it may protect from disease [9, 10] due to its potent antioxidant properties [11]. These effects and newly-discovered associations are generally poorly appreciated within the scientific and medical community. The aim of this review is to provide a summary of bilirubin biology in humans, describe the available bilirubin tests that can differentiate forms of hyperbilirubinemia, evaluate test strengths and weaknesses, and contextualize the results gained from such analyses.

**Bilirubin biology – An evolutionary perspective**

With the evolutionary requirement of oxygen transport, heme metabolism and hence catabolism became critically important to life in oxygenated environments [12]. Heme synthesis and catabolism allowed critical adaptation in cellular respiration and metabolism in vertebrates, which required oxygen delivery and storage [13]. Naturally, these vertebrates required a means to catabolize and recycle iron-containing heme, which is confirmed by the presence of HO within tissues including but not limited to the reticuloendothelial system [14]. Heme catabolism results in biliverdin formation, which is excreted in the bile of birds and many fish [15]. However, more recently-evolved species, including mammals, obtained an additional metabolic step that resulted in the consumption of NADPH and the chemical reduction of biliverdin’s central C10 bridge to form bilirubin. Therefore, unconjugated bilirubin can twist at its C10 bridge and form a three-dimensional ridge-tile conformation. This conformation prevents bilirubin’s carboxylic acid groups from interacting with water and renders the molecule essentially water insoluble at physiological pH (solubility limit < 70 nmol/L) [16]. This rather unexpected phenomenon then required the insertion of an additional metabolic process, the conjugation of bilirubin (with glucuronic acid) and further energetically-costly active transport of conjugated bilirubin into the bile canaliculus, prior to excretion. The acquisition of multiple, energy-consuming steps in the

![Figure 1. Bile pigment metabolism – from heme to bilirubin (modified according to Wagner et al. [33]). Hemoglobin is cleaved to yield globin and heme. Heme is enzymatically converted to biliverdin by liberating iron, via oxidation of its α-methene bridge, with loss of a carbon atom (CO). This opens the porphyrin ring, forms the open-chain, linear tetrupyrrole biliverdin, which yields bilirubin after enzymatic reduction of biliverdin’s central methine bond. In the liver, bilirubin is conjugated to enable excretion into the bile, requiring the enzymes UGT1A1 (conjugation) and MRP2 (excretion into the bile). HO: heme oxygenase; BLAVR: bilirubin reductase; UGT1A1: uridine glucuronosyl transferase 1A1; MRP2: multi-drug resistance protein-2.](image-url)
The metabolic transformation of biliverdin and excretion of bilirubin suggests a physiologically-essential role for it, given that biliverdin can be excreted intact by the liver [17]. It should be noted that excretion and elimination of bilirubin from mammals are critical; without these, the accumulation of bilirubin can cause significant neurological injury. This observation suggests that, although bilirubin formation appears to be an important process, excretion of bilirubin is critical to prevent toxicity [18].

Interestingly, evidence of the continued evolution of the bilirubin metabolic and excretory pathways may exist within the human population with the vastly differing prevalence of benign, yet mildly elevated, circulating bilirubin [Gilbert’s syndrome or Gilbert-Meulengracht syndrome (GS)] in individuals of different ethnicities. For example, individuals with Eastern Asian ancestry (i.e. Chinese and Japanese) appear to have the lowest circulating bilirubin concentrations (prevalence of GS ~2%), whereas individuals originating from India, Southern Asia and the Middle East demonstrate significantly increased rates of GS, approximating ~20% (Figure 2). Caucasian ethnicity is associated with a 2–10% prevalence of GS [19–27]. Increased circulating unconjugated bilirubin is strongly associated with polymorphisms that decrease inducibility in genes controlling bilirubin conjugation (i.e. UGT1A1) and thus excretion [28,29]. With inheritance of GS occurring in an autosomal recessive manner, a prevalence of GS above 20% suggests the possibility of a survival/reproductive advantage associated with elevated bilirubin in these populations, although this has never been proven. Figure 2 provides an overview of the documented prevalence of phenotypic benign unconjugated hyperbilirubinemia (defined by an unconjugated bilirubin concentration of >17–>22 μmol/L) in various geographic regions.

**Bilirubin – Two sides of a coin**

In clinical practice, hyperbilirubinemia is assessed by measuring total bilirubin (TB) in blood and is sub-classified as being predominantly unconjugated bilirubin (indirect bilirubin; IBIL) or conjugated bilirubin (direct bilirubin; DBIL). When considering hyperbilirubinemia, there are usually two main perspectives. For example, pediatricians, neonatologists or hepatologists are concerned primarily about managing the potential negative effects of severe hyperbilirubinemia (or underlying diseases causing hyperbilirubinemia). Clinical symptoms are well-established, especially in neonates, and include jaundice, behavioral and neurological impairments (neurotoxicity or kernicterus), and cholestasis, conditions that have considerable risk for morbidity and mortality [30,31].

A second consideration are the potential positive, health-promoting effects of mild hyperbilirubinemia, with mildly elevated TB concentrations associated with protection from cardiovascular diseases (CVD), type 2 diabetes mellitus, some cancers, and all-cause mortality [9,10,32,33]. To distinguish between the potential detrimental and beneficial effects of TB, the current
literature indicates careful consideration of two factors: 1) the nature of the hyperbilirubinemia (i.e. whether IBIL or DBIL is elevated) and 2) the circulating concentrations. Therefore, to highlight the clinical significance of a few forms of hyperbilirubinemia, we discuss these conditions, their presentations with a direct or indirect hyperbilirubinemia, and their threshold values/concentration ranges below.

**Clinical utility of circulating bilirubin**

Bilirubin is traditionally measured to differentiate various hepatobiliary conditions/diseases. TB and DBIL (presumed to be conjugated bilirubin) levels are used to determine the underlying cause of disease in patients presenting with jaundice. For example, elevated serum TB with a normal DBIL concentration excludes biliary occlusion (cholelithiasis) and the rare condition of Dubin-Johnson syndrome, and suggests that hemolytic anemia or potentially impaired bilirubin conjugation (GS/Crigler-Najjar syndrome) may be involved. Furthermore, elevated TB and DBIL indicate underlying cholestasis or conditions influencing the excretion of conjugated pigment (Dubin-Johnson/Rotor syndrome). Additional testing is clearly warranted to conclusively rule out specific conditions including hepatitis and congenital disorders of bilirubin metabolism. However, the common use of bilirubin as a diagnostic marker for hepatobiliary disease has led to a perception that bilirubin is harmful because its accumulation is commonly associated with disease (and the presentation of jaundice). This observation is important – it should be emphasized that the accumulation of bilirubin only very rarely “causes” disease; it is more likely to reflect the presence of an underlying condition impacting upon bilirubin metabolism/excretion (Table 1).

**Causes and clinical assessment of benign hyperbilirubinemia (Gilbert’s syndrome; GS)**

GS was first described by Gilbert and Lereboulette in 1901 [34] as a chronic, yet benign, condition of jaundice, the cause of which was unrelated to hemolytic disease. This variant was first characterized within the general Caucasian population and was later described by Arias in 1962 [35]. GS has thus been diagnosed using a circulating TB concentration of >17.1 μmol/L (1 mg/dL). However, TB concentrations that are considered abnormal vary internationally. For example, Gilbert et al. [34] originally defined the condition in patients with a TB of >1 mg/dL (>17.1 μmol/L), whereas the Royal College of Pathologists of Australasia define a concentration >20 μmol/L as above the reference range [36]. The Royal College of Physicians and Surgeons of Canada indicates concentrations >22 μmol/L are outside the reference range [37]. Of critical importance for the diagnosis of GS, individuals should have blood collected after an overnight fast, should demonstrate elevated bilirubin concentrations two times over a period of six months and should have normal serum transaminases (i.e. alanine and aspartate amino transaminases) and normal markers of biliary damage/obstruction (gamma-glutamyl transpeptidase and alkaline phosphatase) [38]. Furthermore, a complete blood count (including reticulocyte count) should exclude the possibility of increased

<table>
<thead>
<tr>
<th>Molecular mechanism</th>
<th>Gilbert’s syndrome (GS)/Crigler-Najjar (CN) syndrome</th>
<th>Dubin Johnson syndrome</th>
<th>Rotor syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected plasma concentration of unconjugated bilirubin</td>
<td>GS: 17–85–100 μmol/L</td>
<td>50–100 μmol/L</td>
<td>Normal</td>
</tr>
<tr>
<td>Histology</td>
<td>CN: 100–800 μmol/L</td>
<td>to 400 μmol/L</td>
<td>Normal</td>
</tr>
<tr>
<td>Urine</td>
<td>Normal</td>
<td>Normal urine coproporphyrin</td>
<td>Normal</td>
</tr>
<tr>
<td>Aminotransferases</td>
<td>Normal</td>
<td>Normal urine coproporphyrin</td>
<td>2–5-fold increased excretion of coproporphyrin</td>
</tr>
<tr>
<td>Hepatobiliary transport</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Pruritus</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Benign (GS)/potentially fatal (CN)</td>
<td>Benign</td>
<td>Benign</td>
</tr>
<tr>
<td>Precipitating factors</td>
<td>Fasting/reduced gastrointestinal motility, onset of pubescence.</td>
<td>Oral contraceptives, pregnancy, intercurrent illness</td>
<td>Oral contraceptives</td>
</tr>
<tr>
<td>Hematology</td>
<td>Limited evidence of increased RBC count/total hemoglobin.</td>
<td>Not documented</td>
<td>Not documented</td>
</tr>
</tbody>
</table>

**Table 1. Diagnostic and clinical features of hyperbilirubinemia syndromes (modified according to Strassburg [67]).**
RBC production/destruction and a blood smear should rule out anomalies in RBC structure [39].

Jaundice is occasionally observed in GS individuals and maybe noted at times of stress, illness, and fasting [39]. The presentation of jaundice typically develops at bilirubin concentrations exceeding 40–45 μmol/L and can concern some GS individuals who may demonstrate concentrations up to 85 μmol/L. Although IBIL can be neurotoxic at very high concentrations (i.e. >300 μmol/L), TB concentrations in GS are not sufficiently elevated to cause neurological symptoms. If TB concentrations of >85 μmol/L are observed, additional investigations probing the underlying cause of hyperbilirubinemia are warranted to rule out hemolytic disease and rare conditions of bilirubin metabolism (i.e. Crigler-Najjar Syndrome type 2). In the instance that patients return a TB concentration of <85 μmol/L and would like to confirm the cause of their GS, genotyping for one of many polymorphisms in the UGT1A gene (e.g. UGT1A1*28 variant) are available [40], though this is not necessary for a diagnosis of GS. Such genotyping simply assists in identifying the cause of underlying hyperbilirubinemia. The activity of UGT1A1 in comparison to serum concentrations of IBIL and the definitions of hyperbilirubinemia are summarized in Figure 3.

**Contributing factors to hyperbilirubinemia**

Circulating bilirubin concentrations increase in the days after birth as a consequence of increased heme catabolism and exchange of fetal for adult hemoglobin in RBCs. This turnover of heme leads to the generation of a physiological jaundice in the first two weeks of life in most term neonates [41]. Indeed, severe jaundice can be observed in the instance of erythroblastosis fetalis, or Rh incompatibility, and requires phototherapy [42]. Furthermore, in adults, increased red cell mass, and therefore hemoglobin turnover, is also associated with increased bilirubin concentrations. Some evidence of this has been observed in individuals with GS [43]; in this study, more than 800 individuals (males and females) with GS demonstrated increased hemoglobin, RBC count and hematocrit (typically, increases of 2–3%), compared to age- and gender-matched controls. Importantly, no difference in red cell volume was noted and all patients were otherwise healthy. Another cross-sectional study demonstrated increased total hemoglobin and RBC counts in GS vs. randomly-selected controls [44], which was supported by a smaller case-control study [45].

Early evidence of increased RBC turnover also exists in GS. For example, Berk et al. [46] demonstrated significantly reduced RBC survival in GS individuals vs. controls. Cartei et al. [47] later published that as circulating IBIL increased, RBC survival time progressively decreased in GS individuals. It should be noted that no signs of overt hemolysis (i.e. siderosis of bone marrow/liver) or abnormal iron metabolism were noted and RBC and bone marrow morphology remained normal [48]. Furthermore, in individuals with GS, increased heme catabolism (derived from RBC breakdown) is supported by increased resting carboxyhemoglobin concentrations [45] and increased carbon monoxide production rates.
[48], both of which are accentuated during fasting in hyperbilirubinemic individuals. These data suggest that both increased heme catabolism and reduced bilirubin excretion underlie the condition of benign unconjugated hyperbilirubinemia. It should be noted, however, that evidence also exists to indicate no significant association between circulating IBIL and RBC survival [49] (i.e. decreased RBC survival occurred only in ~42% of GS individuals), which might reflect significant heterogeneity in RBC survival within the population. More recent reports demonstrate that bilirubin has a strong affinity for RBC phospholipids and can interfere with membrane composition and dynamics [50–52]. Furthermore, increasing bilirubin levels in plasma can induce mild hemolysis at high physiological concentrations [53]. Cumulatively, these data suggest that mildly elevated IBIL has the potential to disrupt RBC membrane structure and may decrease RBC survival in humans. The impact of increased RBC turnover remains unknown; however, it is expected to increase heme catabolism, HO activity, and subsequent liberation of carbon monoxide, iron release, and bilirubin production [35], which may further contribute to elevating bilirubin in GS. It should be noted that increased bilirubin concentrations are also observed in individuals with mitral regurgitation [54], which is a likely consequence of increased RBC destruction due to shearing of RBCs upon transit through the heart.

Absorption of bilirubin from the gut

Gastrointestinal transit time clearly influences circulating bilirubin concentrations; increased transit time (i.e. reduced gastric motility during fasting) allows the absorption of deconjugated bilirubin glucuronides (IBIL) via passive diffusion and is accompanied by a 1.5- to 2-fold increase in circulating bilirubin [39]). Interestingly, some evidence suggests delayed gastric emptying in GS [55], which supports the possibility that gastrointestinal motility could influence bilirubin concentrations or that bilirubin could influence gastrointestinal motility.

Hepatic processing of bilirubin

The hepatic processing of bilirubin is the most important determinant of circulating bilirubin concentrations in otherwise healthy individuals. For example, the enzyme responsible for bilirubin conjugation and therefore excretion (UGT1A1) is believed to account for ~45% of the variance in circulating bilirubin concentrations [56]. Not surprisingly, endogenous and exogenous molecules that inhibit this enzyme significantly influence circulating concentrations. Important physiological inhibitors of UGT1A1 include the cholesterol-based hormones, estrogen, and testosterone [57]. These in vitro observations agree well with clinical observations that the onset of puberty, when the concentrations of reproductive hormones increase, is often associated with diagnosis of GS [58]. These data also suggest that circulating bilirubin concentrations should change over the menstrual cycle and that they should decrease upon menopause, possibly contributing to age-related decreases in bilirubin and, potentially, susceptibility to CVD. Evidence to support this has not yet been published; however, these are important ideas for future laboratory studies. Interestingly, a recent report indicates that concentrations of thyroid hormones (thyroxine and triiodothyronine) are increased in individuals with greater bilirubin concentrations [59]. The fact that thyroid hormones also inhibit the transcription of UGT1A1 provides an additional explanation for increased bilirubin in GS individuals and decreased bilirubin in patients with metabolic syndrome, who typically exhibit reduced thyroid hormone concentrations.

In addition to UGT1A1, variation in the organic anion transporter (OATP) 2 gene may result in severe hyperbilirubinemia in neonates [47,48]. Variations of 388 G > A (Asp130Asn, rs2306283), 521 T > C (Val174Ala, rs4149056), 463 C > A (Pro155Thr, rs11045819) of the solute carrier organic anion transporter family member 1B1 (SLCO1B1) gene, which encodes the hepatic solute carrier organic anion transporter 1B1, a putative bilirubin transporter, may predispose individuals to hyperbilirubinemia by limiting hepatic bilirubin uptake [60,61]. Furthermore, in a genome wide association study, two polymorphisms of the SLCO1B3 gene (rs17680137 C > G and rs2117032 C > T) had strong associations with serum bilirubin and contributed to mild idiopathic unconjugated hyperbilirubinemia in healthy adults [62,63].

Transepithelial transport of bilirubin from blood to the gut

Bilirubin is also transported from the blood to the gut in animals with diminished hepatic UGT1A1 activity, suggesting that this route of bilirubin secretion is important in regulating the circulating bilirubin pool. The transepithelial (non-biliary) route of bilirubin disposal has been augmented in jaundiced neonates and Gunn rat using strategies that also reduce serum cholesterol and include oral orlistat, polyethylene glycol, and agar administration [64–66]. The mechanism by which either cholesterol or IBIL enters the intestinal lumen trans-intestinally remains unknown; however it is likely to diffuse passively [17].
Differential diagnoses of disordered bilirubin metabolism

A relatively small number of differential diagnoses exists for congenital disorders of bilirubin metabolism. Diagnosis of these conditions rely somewhat upon blood biochemistries for TB and DBIL, with IBIL being calculated by subtraction of DBIL from TB concentrations. It is important to note that TB-detecting reagents are believed to react completely with all forms of bilirubin, both DBIL and plasma-albumin-associated IBIL. However, quantification using direct-reacting bilirubin reagents assumes that only DBIL (water soluble and non-albumin bound) reacts, due to the lack of a surfactant or another solubilizing reagent in the kit. The main conditions that require testing include Crigler-Najjar, Dubin-Johnson and Rotor syndromes, which are typified by circulating TB concentrations of 50–100 μmol/L (3–6 mg/dL) [67] or above. GS is associated with TB concentrations >17.1 to 85–100 μmol/L (1–5 mg/dL). A critical requirement for distinguishing between some of these conditions is the nature of the hyperbilirubinemia.

Dubin-Johnson syndrome

Dubin-Johnson syndrome is associated with multidrug resistance-associated protein 2 (MRP2) deficiency, which limits active transport of DBIL into the bile. DBIL therefore refluxes from the liver back into the circulation and causes increased TB and DBIL in the blood. In addition, the patients would likely demonstrate jaundice, produce dark urine, and demonstrate liver histology with course granulation within hepatocytes [68].

Crigler-Najjar syndromes

Crigler-Najjar syndromes 1 (CN-1) and 2 (CN-2) are typically diagnosed soon after birth and are associated with pronounced levels of bilirubin (IBIL 100–855 μmol/L [6–50 mg/dL]) with relatively little DBIL. Extremely elevated TB concentrations are caused by near-complete absence of UGT1A1 activity in the liver, which is responsible for conjugating bilirubin to glucuronic acid prior to bilirubin elimination [69]. Similarly, GS is typified by elevated TB but not DBIL, due to ~60–70% reduction in UGT1A1 activity.

Sometimes difficulties exist in distinguishing between GS and CN-2 even after the neonatal period because of considerable overlap in the serum IBIL concentration between the two syndromes; genotyping may be required to identify the cause. Bilirubin is glucuronidated by UGT1A1, with CN-1 caused by complete loss of UGT1A1 activity. The cDNA of UGT1A1 was cloned in 1991 [70] and a large number of UGT1A1 mutations have been found in patients with CN-1, CN-2, and GS [67]. Most patients with CN-2 have homozygous missense mutations or compound heterozygous mutations that reduce enzyme activity to less than 10% of normal [71].

Gilbert’s syndrome (GS)

Common polymorphic mutations in GS include a TA-insertion mutation in the TATA box [A(TA)7TAA] (UGT1A1*28) and c.0.211 G > A (p.G71R) in exon 1 (UGT1A1*6) [72,73]; A(TA)7TAA is also shown as c.-53TA [8] according to the Human Genome Variation Society nomenclature [74]. UGT1A1*60 also contributes to the elevation of serum bilirubin but does not cause GS. However, in Caucasian and African populations, almost all GS individuals are homozygotes for UGT1A1*28 [72,75]. In Japanese, Chinese, and Korean populations, an additional mutation, UGT1A1*6, exists, which causes GS in the homozygous state [76,77].

Rotor syndrome

Interestingly, Rotor syndrome is associated with elevated TB and DBIL; it is a consequence of reduced DBIL transport into hepatocytes, which is caused by reduced expression of organic anion transporters OATP1B1 or OATP1B3 in the basolateral membrane of hepatocytes [78]. An increased DBIL suggests either Rotor or Dubin-Johnson syndrome. Rotor syndrome can be differentiated from Dubin-Johnson syndrome by observing delayed plasma clearance of bromsulphthalein, increased urinary excretion of coproporphyrins, and a lack of granular hepatocyte pigmentation [67].

Measurement of bilirubin

The concentration of many bile pigments, including bilirubin, have been measured in various biological tissues and fluids including the brain, urine, pericardial fluid, feces, blood, the bile, testis, heart, spleen, kidney, the visceral fat, and peripheral blood mononuclear cells [79–89]. Radioimmunoassays have been used to assess brain and cerebrospinal fluid bilirubin levels after intravenous administration of [14C]-UCB (IBIL) to Gunn rats and guinea pigs [90,91]. Bilirubin and its oxidation products have also been determined by enzyme-linked immunosorbent assays using an anti-bilirubin antibody [92]; this approach has been used for bilirubin quantification in cerebrospinal fluid of Alzheimer’s disease patients [93] and in the intestinal mucosa from rats.
challenged with endotoxin [94]. The same antibody was used for immuno-histochemical determination of bilirubin in foam cells from rabbit atherosclerotic lesions [95]. These methods are, however, not generally accessible because of the lack of commercial availability of radio-labeled bilirubin and/or anti-bilirubin antibody.

In the past 50 years, the methods used to measure circulating bilirubin have progressed from using reactive dyes (including sulfanilic acid), as described by van den Bergh in the 1920s, [96] to slides that utilize enzyme-linked spectrophotometry (bilirubin oxidase) [97] to high-performance liquid chromatography (HPLC) [86,98–101] and noninvasive transcutaneous bilirubinometry [102].

Currently, the diazo dye method [96] is the most widely-used assay in clinical laboratories and quantifies the bilirubin content in blood and urine using a diazotized sulfanilic acid solution. The assay detects DBIL (mostly bilirubin glucuronides) or TB (inclusive of unconjugated, albumin bound and bilirubin glucuronides) when solubilizing agents are added. The van den Bergh reaction (diazo test) is widely used because of its low cost, its simplistic design, and its ability to quantify clinically-relevant levels of unconjugated and physiologically conjugated bilirubins [103]. Although the diazo method has these benefits, HPLC analysis is gaining in popularity because it can differentiate between many different bile pigments. These compounds include IBIL and its isomers (IIIx, IXx, and XIIIx), bilirubin mono, or diglucuronide, and biliverdin [81,104]. HPLC analysis is particularly well-suited within the research setting because of its low detection levels. Currently many different HPLC assays for bile pigments have been published [17,86]. More recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have also been developed [105].

Conclusions

Hyperbilirubinemia has a broad variety of underlying causes that require diagnostic workup to identify conditions that could cause severe hepatobiliary injury or that potentially have no adverse effects. Among the benign conditions is GS, which is associated with impaired bilirubin glucuronidation. Individuals with an increased number of TA repeats in the gene promoter for UGT1A1 (usually increased number of TA repeats in the gene promoter impaired bilirubin glucuronidation. Individuals with an IBIL concentration > 17.1 μmol/L (>1 mg/dL). GS is highly prevalent in the general population and surprisingly is associated with a reduced prevalence of CVD, diabetes, some cancers, and all-cause mortality.

Therefore, an accurate diagnosis based on clinical/analytical data, which are summarized within this paper, is needed. Further, considering the potentially-protective association of IBIL with all-cause mortality in GS individuals, we hope that a renewed appreciation of benign hyperbilirubinemia can be achieved. This may encourage the development of interventions that can mildly increase circulating concentrations of bilirubin, particularly in persons with low TB concentrations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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