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Review Article

The Multifaceted Role of Bilirubin in Liver Disease: A Literature Review

Mariana M. Ramírez-Mejía[1,2], Stephany M. Castillo-Castañeda[2,3], Shreya C. Pal[2,4], Xingshun Qi[5] and Nahum Méndez-Sánchez[2,4*]

[1]Plan of Combined Studies in Medicine (PECEM-MD/PhD), Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico; [2]Liver Research Unit, Medica Sur Clinic & Foundation, Mexico City, Mexico; [3]Medical, Dental and Health Sciences Master and Doctorate Program, National Autonomous University of Mexico, Mexico City, Mexico; [4]Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico; [5]Department of Gastroenterology, General Hospital of Northern Theater Command (formerly General Hospital of Shenyang Military Area), Shenyang, Liaoning, China

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Abstract

Bilirubin, the primary breakdown product of hemoproteins, particularly hemoglobin, plays a key role in the diagnosis, prognosis, and monitoring of liver diseases. In acute liver diseases, such as acute liver failure, drug-induced liver injury, and viral hepatitis, bilirubin serves as a biomarker reflecting the extent of hepatocyte loss and liver damage. Chronic liver diseases, including alcohol-related liver disease, chronic hepatitis C virus infection, metabolic dysfunction-associated fatty liver disease, and autoimmune liver diseases, are marked by persistent liver injury and inflammation. Bilirubin levels in chronic liver diseases provide insight into liver function, disease severity, and prognosis. As a versatile biomarker, bilirubin offers valuable information on the pathophysiology of liver diseases and aids in guiding clinical decision-making regarding the treatment of liver diseases and their complications. This review aimed to explore the multifunctional role of bilirubin in liver diseases by analyzing its biological functions beyond its role as a biomarker of liver damage.

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Introduction

Bilirubin, the primary bile pigment, is a degradation product of hemoproteins, primarily hemoglobin. Hemoglobin accounts for approximately 80% of daily bilirubin production, which ranges from 250 to 400 mg.[1,2] This assessment is particularly important for the early detection, prevention, and prognosis of liver diseases .[3] Under normal physiological conditions, total bilirubin (TB) levels in the bloodstream are typically less than 1.2 mg/dL (20.5 μmol/L), with a narrow physiologic range between 0.1 to 1 mg/dL (2–17.1 μmol/L). Circulating TB exists in either unconjugated (UCB) or conjugated (CB) forms.[1,2] UCB, which is lipid-soluble, is initially produced in the spleen and bone marrow during the breakdown of hemoglobin from senescent red blood cells. It is then transported to the liver bound to albumin, where it is enzymatically converted to CB by the action of uridine diphosphate-glucuronosyltransferase (UGT1A1).[3]

The liver is the primary organ involved in bilirubin metabolism, and any alteration in liver function can significantly affect serum bilirubin levels. Consequently, elevated levels of bilirubin in the bloodstream, known as hyperbilirubinemia, can be caused by disruptions in production (e.g., increased red blood cell turnover), metabolism (e.g., altered conjugation), or transport (e.g., impaired uptake or excretion) of either CB or UCB.[4,5] Unconjugated hyperbilirubinemia usually occurs in conditions such as hemolytic anemia or ineffective erythropoiesis, where excessive bilirubin production overwhelms the liver's ability to conjugate bilirubin.[6] Additionally, genetic disorders such as Gilbert's syndrome or Crigler-Najjar syndrome, characterized by deficiencies in the enzyme UGT1A1, further impair bilirubin conjugation, leading to elevated UCB levels.[7] Conversely, conjugated hyperbilirubinemia usually reflects a defect in the hepatic excretion of bilirubin. This can be due to intrahepatic causes, such as hepatocellular injury, cholestasis, or cirrhosis, where liver cells are unable to properly excrete CB into bile.[8] Alternatively, extrahepatic causes, such as biliary obstruction due to gallstones, tumors, or strictures, may impede bile flow, leading to an accumulation of CB in the blood.[9]

Chronic liver disease can arise from various causes and, over time, can lead to progressive liver dysfunction that eventually results in cirrhosis.[10] The severity of cirrhosis is assessed by a combination of clinical and biochemical parameters, including the presence of ascites or encephalopathy, and levels of serum albumin, prothrombin, and bilirubin.[11] Although cirrhosis represents the end stage of chronic liver disease, bilirubin continues to serve as a critical marker in the progression of various liver conditions

Keywords: Bilirubin; Liver failure; Chronic liver failure; Metabolic dysfunction-associated fatty liver disease; Hepatitis; Acute-on-chronic liver failure.

***Correspondence to:** Nahum Méndez-Sánchez, Liver Research Unit, Medica Sur Clinic & Foundation 150 Puente de Piedra, Tlalpan, Mexico City 14050, Mexico. ORCID: https://orcid.org/0000-0001-5257-8048. Tel: +525-55-424-4629, Fax: +525-55-666-403, E-mail: nmendez@medicasur.org.mx or nah@unam.mx

Table 1. Scores for determining prognosis and mortality of liver diseases using bilirubin as a parameter

Liver disease	Score name	Year	Parameters	Cut-off points	Reference
Alcoholic Hepatitis	Maddrey Discriminant Function (MDF)	1978	Bilirubin concentrations, prothrombin time	MDF ≥ 32	10
	Glasgow Alcoholic Hepatitis Score (GAHS)	1995	Bilirubin concentrations, creatinine, PT, age	GAHS ≥ 9	11
	Lille Model	2007	Bilirubin concentrations, creatinine, age, albumin, MELD, Improvement of bilirubin	Lille score >0.45 after 7 days of corticosteroid therapy	12
	ABIC Score (Age, Bilirubin, INR, Creatinine)	2006	Bilirubin concentrations, INR, Creatinine, Age	ABIC > 6.71	13
Drug-Induced Liver Injury (DILI)	Roussel Uclaf Causality Assessment Method (RUCAM)	1993	Total bilirubin, ALT/ ALP ratio, Previous hepatotoxicity, time to onset	<0: Excluded; 1–2: Unlikely; 3–5: Possible; 6–8: Probable; >8: Highly probable	14
Acute Liver Failure (ALF)	Kings College Criteria for Non-Acetaminophen Related Acute Liver Failure	1989	INR, age, bilirubin, etiology	Not applicable	15
Chronic Liver Disease (CLD)	Model for End-Stage Liver Disease (MELD Score)	2000	Bilirubin, INR, creatinine	Higher scores indicating more severe liver dysfunction and a higher risk of mortality.	16
	MELD-Na Score	2016	Bilirubin, INR, creatinine, sodium	Higher scores indicating more severe liver dysfunction and a higher risk of mortality.	17
	MELD 3.0	2021	Bilirubin, INR, creatinine, sodium, age, albumin	Higher scores indicating more severe liver dysfunction and a higher risk of mortality.	18
	Child-Turcotte-Pugh Score	1973	Presence of ascites, bilirubin, albumin, INR, hepatic encephalopathy	Class A: 5–6 points, Class B:7–9 points, Class C: 10–15 points	19
Acute-On-Chronic	Chronic Liver Failure-Sequential Organ Failure (CLIF-SOFA)	2013	Bilirubin, creatinine, hepatic encephalopathy, mean arterial pressure, INR	Grade I: single kidney failure or single failure of the liver, coagulation, circulation, or respiration with creatinine level ranging from 1.5 to 1.9 mg/dL; Grade II: two organ failures; Grade III: three organ failures or more	20

INR, International normalized ratio; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; PT, Prothrombin time.

(Table 1).[12–23]

Beyond its role as a biomarker, bilirubin possesses significant antioxidant and immunomodulatory properties.[24] As an antioxidant, bilirubin neutralizes reactive oxygen species (ROS) and reduces oxidative stress, protecting cells from damage.[25] These antioxidant properties are particularly beneficial in conditions characterized by high oxidative stress.[26,27] Additionally, the immunomodulatory effects of bilirubin include the inhibition of proinflammatory cytokines and suppression of immune cell activation, which helps mitigate inflammation-induced liver damage.[4,28] These protective functions underscore the dual role of bilirubin in liver disease, highlighting not only its diagnostic utility but also its potential therapeutic implications.[29]

This review aimed to provide a comprehensive analysis of the current literature on bilirubin, exploring its role as both a biomarker and a multifunctional molecule in liver diseases. Furthermore, it evaluated the significance of bilirubin in clinical assessments and its potential in managing complications associated with liver conditions.

Bilirubin metabolism

The first phase of bilirubin metabolism occurs in reticuloendothelial cells, where the enzyme heme oxygenase (HO) catalyzes the breakdown of heme into biliverdin, carbon monoxide, and free iron.[30] HO is the rate-limiting enzyme in the first phase of bilirubin metabolism and exists in two main isoforms: HO-1, which is inducible and typically increases in response to stress, and HO-2, which is constitutively expressed.[31] HO-1 is highly inducible and responds to a wide range of stressors, including oxidative stress, inflammatory cytokines, growth factors, hormones, and physical stressors (e.g., ischemia/reperfusion injury and hypoxia/hyperoxia).[32,33] It is especially sensitive to prooxidant stimuli, such as ultraviolet radiation, iron-containing molecules, and heavy

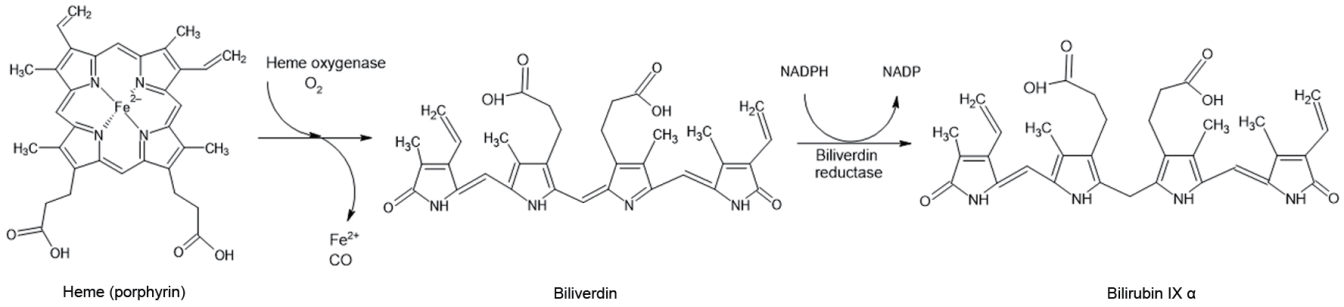

Fig. 1. Bilirubin synthesis. After the half-life of erythrocytes, the heme group of hemoglobin is degraded by the enzyme heme oxygenase into carbon monoxide, iron, and biliverdin. Subsequently, biliverdin is reduced to unconjugated bilirubin (UCB) in its natural isomeric form, IXα, by the action of biliverdin reductase. NADP, nicotinamide adenine dinucleotide phosphate.

metals, which trigger its upregulation.[34]

Following heme breakdown by HO, biliverdin is reduced to UCB by the enzyme biliverdin reductase, completing the initial phase of bilirubin metabolism (Fig. 1).[1,2,27] UCB, which is hydrophobic and insoluble, is transported through the bloodstream bound to soluble complexes, primarily plasma proteins like albumin (90%) and apolipoprotein D of HDL (10%).[1,5,12,27] Once free-circulating UCB and the albumin-binding complex reach the liver, UCB dissociates from albumin. Subsequently, UCB is transported across the basolateral membrane of hepatocytes by two mechanisms: passive diffusion and receptor-mediated facilitated diffusion via the organic anion transporter polypeptide OATP1B1 (alternatively known as liver-specific transporter 1, OATPC, SLC21A6, or OATP2) and OATP1B3.[1,2,4] Out of the more than 300 known members of the OATP transporter superfamily, only 11 are found in humans.[35] Among these, OATP1B1 and OATP1B3 are liver-specific transporters located in the basolateral membrane of hepatocytes. Notably, OATP1B3 exhibits higher expression around the central vein compared to the periportal zone.[6,35]

Once UCB reaches the cytosol of the hepatocyte, it undergoes a crucial biochemical process known as glucuronidation, facilitated by the enzyme uridine-diphosphate glucuronosyltransferase (Fig. 2). Although several isoforms of uridine-diphosphate glucuronosyltransferase exist, the primary one responsible for bilirubin glucuronidation is UGT1A1.[4] This enzyme is predominantly located in the microsomes (smooth endoplasmic reticulum, rough endoplasmic reticulum), Golgi membranes, and the nuclear envelope.[36,37] UGT1A1 catalyzes the esterification of two glucuronic acid molecules to the propionic acid side chains of bilirubin. Under normal conditions, bilirubin diglucuronoside (BDG) is the predominant conjugated form produced. However, when the conjugation system is overwhelmed, a significant portion of bilirubin can be conjugated as bilirubin monoglucuronoside (BMG).[38] The conversion of bilirubin to glucuronosides is essential for its effective elimination through the biliary system.[27,39] The expression of UGT1A1 is regulated by various multifunctional nuclear receptors, including the constitutive androstane receptor, pregnane X receptor, glucocorticoid receptor, aryl hydrocarbon receptor, and hepatocyte nuclear factor 1α. This regulation occurs through the phenobarbital-responsive enhancer module.[40–42]

BDG and BMG are water-soluble conjugates that facilitate bilirubin excretion from hepatocytes. Once formed, they are actively transported across the canalicular membrane into bile via the multidrug resistance-associated protein 2 (MRP2).[1,43] MRP2 is an ATP-binding cassette transporter localized in the canalicular membrane of hepatocytes, the apical membrane of enterocytes in the duodenum and jejunum, and the renal proximal tubule epithelial cells.[44] MRP2 is the primary mechanism for the bile-independent efflux of BMG, BDG, and other organic anions into the bile canaliculus.[45] When the formation and/or uptake of BMG and BDG exceeds the transport capacity of MRP2 in the canalicular membrane, the sinusoidal efflux via MRP3 provides a pathway for compensation.[43,46] MRP3, located on the basolateral membrane of hepatocytes, redirects BMG and BDG into the bloodstream, from where they are reabsorbed into hepatocytes by OATP1B1 and OATP1B3. OATP1B1 facilitates unidirectional, high-affinity transport of BMG and BDG across the sinusoidal membrane of hepatocytes, while OATP1B3 specifically transports BMG.[47,48]

In bile, bilirubin conjugates mix with bile acids, phospholipids, and cholesterol to form mixed micelles, which are transported through the bile ducts into the intestine.[49] CB passes through the gastrointestinal tract without being absorbed by the intestinal mucosa and reaches the duodenum.[50] In the gut lumen, CB is metabolized by the gut microbiota through two main processes: 1) deconjugation of BMG and BDG molecules and 2) reduction of UCB to urobilinogen.[51] Gut bacteria hydrolyze bilirubin glucuronosides, releasing UCB, which is then reduced to urobilinogen by bacteria containing β-glucuronidases, such as *Clostridioides difficile*, *Clostridium ramosum*, *Clostridium perfringens*, and *Bacteroides fragilis.*[52] Urobilinogen can be reduced to stercobilinogen, and both urobilinogen and stercobilinogen can be oxidized to form urobilin and stercobilin, respectively. These metabolites are responsible for the characteristic colors of urine and feces.[27] Additionally, urobilinogen can be passively absorbed by the intestine into the portal system.[53]

Molecular mechanisms of bilirubin in liver diseases

Bilirubin plays a crucial role in the pathophysiology of liver diseases, serving as both a diagnostic marker and a contributor to disease processes. Elevated levels of bilirubin, whether UCB or CB, indicate various forms of liver dysfunction and provide insights into the severity and progression of liver diseases.[54] Liver dysfunction can result from various causes, such as viral hepatitis, alcoholic liver disease, metabolic dysfunction-associated steatotic liver disease (MASLD), and autoimmune liver diseases. The liver performs many essential functions, such as metabolizing nutrients, detoxifying harmful substances, and synthesizing vital proteins such as albumin and clotting factors. When the liver is damaged, these functions are impaired, leading to a cascade of physiological disorders.[2,55] The main cellular component of the liver is the hepatocyte. Injury to these cells, either through direct

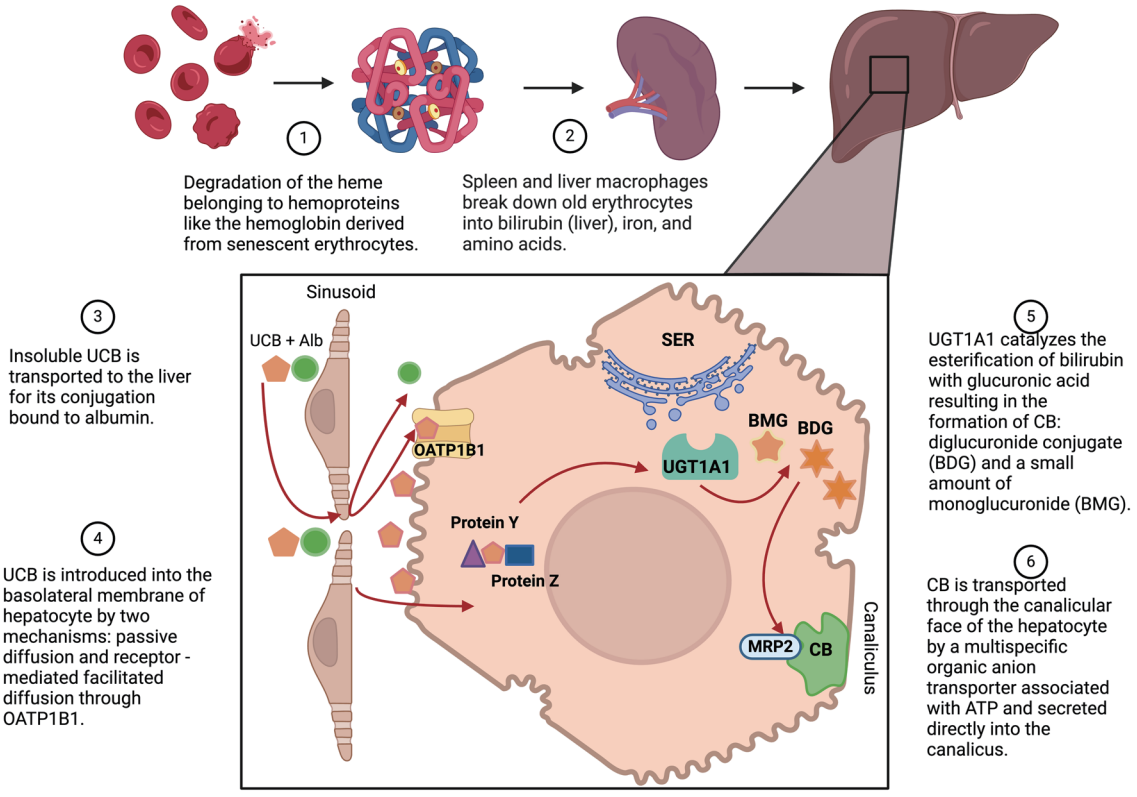

Fig. 2. Bilirubin metabolism. After about 100–120 days, (1) erythrocytes are removed from circulation to the spleen or liver, where they are broken down into globulins, heme, and iron. (2) In the liver, heme is catalyzed by heme oxygenase (HMOX) and biliverdin reductase (BVR) to form biliverdin and then unconjugated bilirubin (UCB), which primarily circulates bound to albumin. (3) The UCB passes through the sinusoids without albumin and (4) enters the hepatocyte by binding to a transporter protein (OATP1B1) or via passive diffusion, crossing the cell membrane. Inside the cell, it binds to Y and Z proteins and then to ligandin (not shown) for transport to the smooth endoplasmic reticulum (SER). (5) In the SER, bilirubin is conjugated to glucuronic acid by UDP-glucuronosyltransferase 1 (UGT1A1), producing monoglucuronides (BMG) and diglucuronides (BDG) of bilirubin. Finally, (6) conjugated bilirubin (CB) is secreted into the canaliculus by the adenosine triphosphate–binding cassette transporter protein (MRP2).

toxic effects (such as drug-induced liver injury) or immune-mediated mechanisms (such as viral hepatitis), results in the release of intracellular contents, including bilirubin, into the bloodstream, leading to elevated serum bilirubin levels that reflect the extent of hepatocyte damage.[6,56]

Altered metabolism

In liver diseases, normal bilirubin metabolism is often disturbed. This can occur at multiple stages: from excessive bilirubin production to defects in its conjugation by UGT1A1 and problems with the secretion of CB into the bile.[57] In certain liver conditions, such as cirrhosis and chronic liver disease, increased bilirubin production may occur due to accelerated red blood cell breakdown (hemolysis) or ineffective erythropoiesis.[58] This excessive bilirubin production can overwhelm the hepatocytes' ability to absorb and conjugate UCB, leading to its accumulation in the bloodstream and causing jaundice.[59]

On the other side, defects in the conjugation of UCB by the UGT1A1 enzyme may be due to genetic variations, as seen in Gilbert's syndrome and Crigler-Najjar syndrome (CN), or acquired causes.[60] In CN type 1 (CN1), there is a complete absence of UGT1A1 activity due to genetic lesions in the UGT1A1 gene, causing a severe form of unconjugated hyperbilirubinemia and a high risk of developing kernicterus, a potentially fatal neurological condition.[61] CN1 is typically caused by genetic changes that result in premature truncation or substitution of critical amino acid residues, which completely eliminate the enzyme's function.[62] In CN type 2 (CN2), UGT1A1 activity is partially deficient rather than completely absent, resulting in milder elevations of UCB compared to CN1. CN2 is due to the substitution of individual amino acid residues that markedly reduce, but do not abolish, the catalytic activity of the enzyme. Both types of the syndrome follow an autosomal recessive inheritance pattern.[62] In contrast, Gilbert's syndrome is a much milder condition caused by a partial deficiency of UGT1A1 activity, linked to a specific genetic variation known as the UGT1A1*28 polymorphism (A(TA)7TAA promoter sequence, rs8175347).[63] This is the most common inherited cause of unconjugated hyperbilirubinemia and follows an autosomal recessive inheritance pattern, similar to CN1 and CN2. However, in Gilbert's syndrome, UGT1A1 activity is only slightly reduced, resulting in intermittent, mild elevations of UCB, usually triggered by factors such as fasting, stress, illness, or strenuous exercise.[64] In addition to genetic disorders, conditions such as advanced hepatitis or cirrhosis may cause a slight reduction in bilirubin conjugation capacity. However, in these cases, conjugation is usually better preserved than other aspects of bilirubin metabolism.[3] Moreover, several drugs, such as pregnanediol, novobiocin, chloramphenicol, gentamicin, and atazanavir, can induce unconjugated hyperbilirubinemia by inhibiting UGT1A1 activity.[65]

Finally, CB secretion problems in bile can lead to conjugated hyperbilirubinemia. As previously discussed, this step in

bilirubin metabolism involves the active transport of CB from hepatocytes into bile canaliculi via specific transporters.[1,43] In cholestatic diseases, such as primary biliary cholangitis and primary sclerosing cholangitis, inflammation or scarring of the bile ducts obstructs bile flow, resulting in the retention of CB and bile acids in the liver. This not only causes jaundice but also risks hepatocyte damage due to the toxic accumulation of bile acids.[66,67] Genetic disorders, such as Dubin-Johnson syndrome, also illustrate how defects in CB secretion can occur at the transporter level. In this disease, genetic changes in the ABCC2 gene (encoding the MRP2 transporter) prevent the efficient excretion of CB into the bile, leading to the accumulation of CB in hepatocytes and its subsequent release into the bloodstream via MRP3.[68] Rotor syndrome is another genetic disorder affecting CB secretion, although it differs from Dubin-Johnson syndrome in its underlying mechanism. In Rotor syndrome, both CB and UCB accumulate in the bloodstream due to defects in hepatic uptake and storage of bilirubin, resulting from genetic variations affecting the OATP1B1 and OATP1B3 transporters.[7,69] Beyond genetic conditions, it has been observed that in patients with advanced liver disease, especially of cholestatic origin, there is an upregulation of MRP3 and downregulation of MRP2. This could represent an adaptation to protect hepatocytes from the accumulation of toxic biliary components, including bilirubin.[70] Furthermore, the upregulation of MRP3 expression may be related to the inflammatory response triggered in chronic liver diseases.[71] Nevertheless, the mechanisms behind this association are not yet fully understood.

Molecular associated pathways

Studies have highlighted bilirubin's antioxidant, anti-inflammatory, and cytoprotective properties.[72–75] The antioxidant properties of bilirubin are closely associated with the biliverdin–bilirubin antioxidant cycle. This cycle, driven by HO-1 and biliverdin reductase, neutralizes ROS and maintains cellular redox balance.[76] Bilirubin contains an extensive system of conjugated double bonds and a pair of reactive hydrogen atoms, which are key to its antioxidant properties. It has been proposed that these hydrogen atoms participate in antioxidant activity by donating to free radicals, thereby neutralizing them and preventing oxidative damage.[77] In contrast, biliverdin possesses only the conjugated double bond system, and its antioxidant capacity probably comes from the formation of resonance-stabilized structures that can absorb and disperse oxidative energy.[77] This regenerative antioxidant cycling between biliverdin and bilirubin provides a unique advantage: unlike many antioxidants that are consumed in the process of neutralizing ROS, bilirubin, through its cycling with biliverdin, can continue to exert protective effects.[78] Furthermore, bilirubin inhibits nicotinamide adenine dinucleotide phosphate oxidase activity and acts synergistically with other antioxidants to decrease lipid peroxidation.[25,79,80]

Additionally, bilirubin influences lipid metabolism by reducing lipogenesis and promoting fatty acid oxidation through peroxisome proliferator-activated receptors.[81,82] In glucose metabolism, bilirubin inhibits gluconeogenic enzymes, increases insulin sensitivity by enhancing insulin signaling, and reduces inflammatory mediators.[83,84] Moreover, the anti-inflammatory effects of bilirubin are significant in conditions where chronic inflammation is prevalent. It reduces proinflammatory cytokines, suppresses immune cell activation, and inhibits the NF-κB pathway, thereby mitigating inflammation-induced liver damage.[28] Finally, bilirubin modulates the activity of nuclear receptors such as the aryl hydrocarbon receptor, constitutive androstane receptor, and pregnane X receptor. These receptors are involved in detoxification and metabolic regulation, influencing the expression of genes associated with lipid and glucose metabolism, and overall energy homeostasis.[85]

In line with these beneficial effects that may prevent the development of MASLD, it has been observed that individuals with MASLD tend to have low bilirubin concentrations.[86] This observation suggests a possible inverse relationship between bilirubin concentrations and the incidence of MASLD, indicating that higher bilirubin concentrations may be associated with a lower risk of developing MASLD.[87] It has been proposed that bilirubin metabolism is dysregulated in MASLD patients, likely due to increased oxidative stress.[86]

Neurotoxicity of bilirubin

Bilirubin neurotoxicity is a significant concern in the pathophysiology of liver diseases, especially in conditions associated with severe hyperbilirubinemia. UCB, in particular, can cross the blood-brain barrier and accumulate in neural tissues, leading to bilirubin encephalopathy.[88] This condition, characterized by neurological impairment, occurs when bilirubin levels overwhelm the brain's capacity to detoxify and eliminate this pigment.[89] Studies have shown that bilirubin-induced neurotoxicity results from its ability to interfere with various cellular functions, including neurotransmitter synthesis, release, and uptake, as well as mitochondrial function and energy metabolism.[90,91] Bilirubin can alter mitochondrial function, reducing ATP production and increasing oxidative stress in neurons. Bilirubin-induced oxidative stress is a major contributor to neurotoxicity, as UCB accumulation in neuronal tissues generates ROS, which damages cellular components such as lipids, proteins, and DNA. This oxidative damage can trigger cell death pathways, including apoptosis and necrosis.[92,93] Additionally, bilirubin can induce an inflammatory response in the brain. Activation of microglia, the resident immune cells of the central nervous system, leads to the production of proinflammatory cytokines, further aggravating neuronal damage. This inflammation can amplify the neurotoxic effects of bilirubin.[94,95] Moreover, bilirubin inhibits glutamate uptake by astrocytes, leading to excessive glutamate accumulation in the synaptic cleft. This phenomenon, known as excitotoxicity, leads to overactivation of glutamate receptors in neurons, causing calcium overload and subsequent neuronal injury and death.[91,96]

Hepatic encephalopathy is a severe neurotoxic complication of liver disease, arising from the liver's inability to detoxify harmful substances that subsequently accumulate in the bloodstream and impair central nervous system function.[97,98] Elevated bilirubin levels have been shown to exacerbate oxidative stress and contribute to neuronal dysfunction, as evidenced by bilirubin's inhibition of glutamate uptake in astrocytes, which can lead to excitotoxicity and neuronal cell death.[96] Nevertheless, the exact role of bilirubin in the pathogenesis of hepatic encephalopathy remains unclear and warrants further investigation.

Gut-liver axis

It has been demonstrated that gut microbiota composition can affect bilirubin metabolism, highlighting the importance of a balanced microbiota for normal bilirubin catabolism.[99] A study by Walker *et al*. showed that a high-fat diet alters gut microbiota and increases urobilinoid levels in obese mice, suggesting a role for bilirubin in lipid metabolism and obesity.[100] Further investigations have revealed that specific bacterial populations in the gut are responsible for the deconjugation and reduction of bilirubin. For instance, bacteria such as *Clostridium ramosum*, *Clostridium perfringens*,

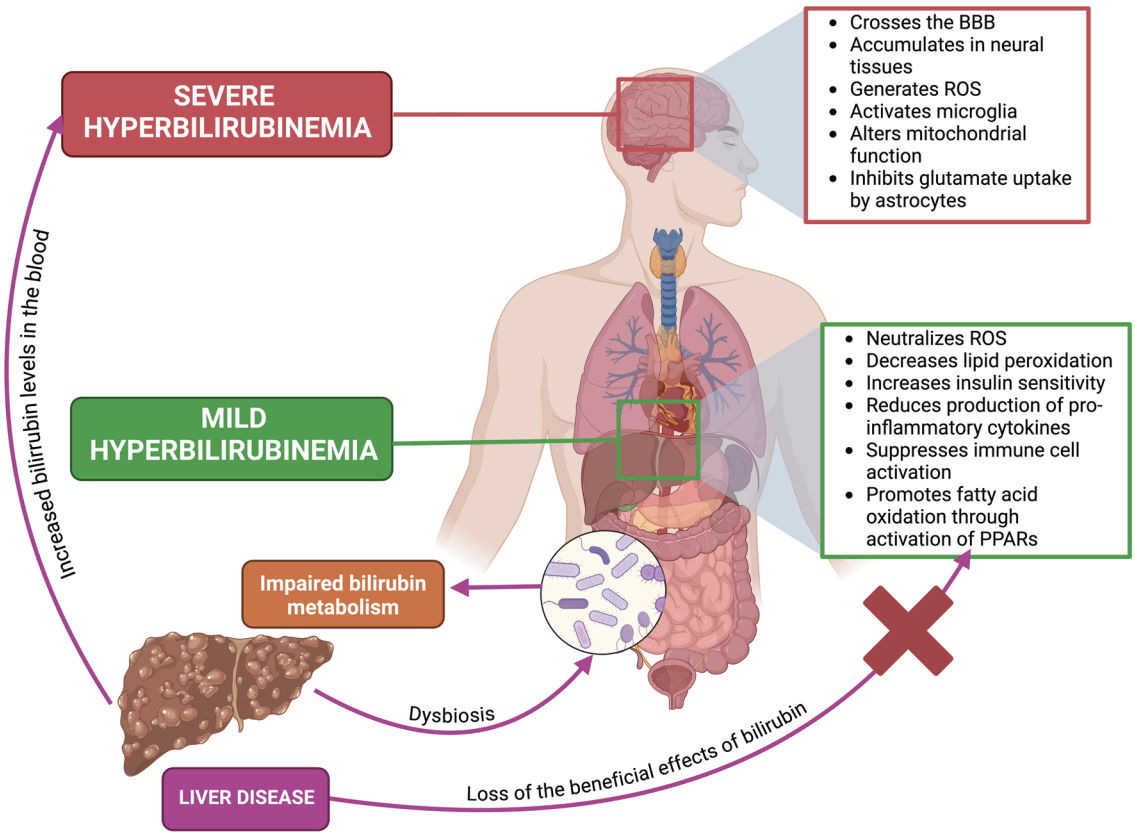

Fig. 3. The dual roles of bilirubin in liver disease. In cases of severe hyperbilirubinemia, elevated bilirubin levels cross the blood-brain barrier (BBB) and accumulate in neural tissues. This accumulation leads to the generation of reactive oxygen species (ROS), activation of microglia, alteration of mitochondrial function, and inhibition of glutamate uptake by astrocytes. These processes result in neurotoxic effects such as neuronal damage and excitotoxicity. Conversely, in mild hyperbilirubinemia, bilirubin exerts protective effects. It neutralizes ROS, decreases lipid peroxidation, increases insulin sensitivity, reduces the production of pro-inflammatory cytokines, suppresses immune cell activation, and promotes fatty acid oxidation through the activation of peroxisome proliferator-activated receptors (PPARs). These actions contribute to its antioxidant, anti-inflammatory, and metabolic regulatory roles. The transition from mild to severe hyperbilirubinemia underscores the importance of maintaining bilirubin within a physiological range to harness its protective effects while avoiding neurotoxicity.

Clostridium difficile, and *Bacteroides fragilis* play critical roles in transforming bilirubin into urobilinogen and other metabolites.[101] Recent research has advanced our understanding of this process by identifying BilR, an intestinal microbial enzyme responsible for the reduction of bilirubin to urobilinogen, highlighting the critical role of microbial metabolism in maintaining bilirubin homeostasis within the gut-liver axis. This enzyme is predominantly encoded by *Firmicutes* species in the gut microbiome and is considered vital in preventing the accumulation of UCB, which might otherwise be reabsorbed into the bloodstream. Dysregulation of this microbial reduction process can result in elevated serum bilirubin levels.[102] While the protective roles of bilirubin and its metabolites are recognized, many mechanisms and functions within the gut-liver axis remain unknown, highlighting the need for further research.

Bilirubin is an integral part of the pathophysiology of liver diseases, serving as both a diagnostic marker and an active participant in pathological processes (Fig. 3). Its role in pathways associated with metabolism, anti-inflammatory responses, regulation of apoptosis and autophagy, and interaction with nuclear receptors highlights its multifaceted nature. Despite its beneficial effects, dysregulation of bilirubin metabolism can lead to neurotoxicity, particularly in conditions of severe hyperbilirubinemia. Understanding the complex molecular mechanisms of bilirubin in liver diseases provides crucial information on its diagnostic and therapeutic potential, underscoring the need for further research to fully exploit its clinical benefits while mitigating the associated risks.[4]

Clinical implications

Hyperbilirubinemia, particularly seen in genetic conditions such as Gilbert's syndrome, CN syndrome, Dubin-Johnson syndrome, and Rotor syndrome, holds significant clinical implications.[103] In CN, clinical attention is focused on preventing neurological damage caused by extremely high levels of UCB. Early and aggressive treatment, such as phototherapy or liver transplantation, is essential to prevent complications such as kernicterus.[104] In contrast, Dubin-Johnson syndrome and Rotor syndrome, characterized by conjugated hyperbilirubinemia, do not usually pose serious health risks beyond jaundice and, in some cases, fatigue and abdominal pain. In most cases, hyperbilirubinemia is an incidental finding.[105] On the other hand, the mild hyperbilirubinemia observed in Gilbert's syndrome has been associated with cardiovascular and metabolic benefits.[84] Studies have shown that individuals with Gilbert's syndrome may have a reduced risk of cardiovascular disease due to bilirubin's role as a potent antioxidant, protecting against oxidative stress and low-density lipoprotein oxidation, which is a key factor in the development of atherosclerosis.[106,107] Additionally, the protective

role of bilirubin extends to metabolic disorders. Elevated levels of UCB have been linked to a reduced risk of developing metabolic syndrome, MASLD, and diabetes.[108,109] Interestingly, studies have demonstrated that both acute and chronic oral administration of zinc sulfate significantly reduces serum UCB levels in individuals with Gilbert's syndrome, likely by inhibiting the normal enterohepatic circulation of UCB.[110,111] This suggests potential therapeutic strategies for managing UCB levels in cases where reductions are clinically desired.

Understanding the role of bilirubin in acute and chronic liver diseases has important clinical implications. In acute liver diseases, such as acute liver failure, rapid elevation of bilirubin levels reflects severe hepatocellular injury and decreased liver function.[112] Monitoring bilirubin concentrations is crucial for diagnosing acute liver failure, assessing its severity, and guiding therapeutic decisions. Elevated bilirubin levels are integrated into prognostic scoring systems such as the Model for End-Stage Liver Disease (MELD) score and the Maddrey Discriminant Function (Maddrey score), underscoring their importance in predicting patient prognosis and guiding therapeutic decisions.[10] The MELD score is widely used to assess the severity of chronic liver disease and prioritize patients for liver transplantation. It incorporates serum bilirubin levels, along with creatinine and the international normalized ratio of prothrombin time, to calculate a score that predicts the three-month mortality risk.[21] The Maddrey score is used specifically to assess the prognosis of patients with alcoholic hepatitis, calculated using the patient's prothrombin time and serum bilirubin levels.[13] Both the MELD and Maddrey scores illustrate the enduring importance of bilirubin as a biomarker in liver disease, highlighting its reliability and relevance in assessing liver function, guiding therapeutic decisions, and predicting patient outcomes.[113] In chronic liver diseases, such as cirrhosis, hepatitis, and alcohol-related liver diseases, bilirubin concentrations are essential indicators of liver function and disease progression. Elevated bilirubin levels indicate advanced liver dysfunction and are associated with complications such as hepatic encephalopathy.[114,115]

Bilirubin is also a critical biomarker in the management of drug-induced liver injury, as higher bilirubin concentrations at the onset and peak of drug-induced liver injury are associated with more severe liver injury and worse outcomes.[116] In conditions such as metabolic dysfunction-associated fatty liver disease and autoimmune liver diseases, bilirubin provides valuable information on liver function, disease severity, and progression. Interestingly, slightly elevated bilirubin concentrations may have a protective effect against metabolic syndrome and metabolic dysfunction-associated fatty liver disease, suggesting that higher bilirubin levels, particularly indirect bilirubin, may reduce the risk of developing these diseases.[86,87,117] Overall, bilirubin is a critical biomarker in several liver diseases, guiding clinical decision-making, assessing treatment efficacy, and predicting patient outcomes. Our recent study on molecular species of bilirubin in acute-on-chronic liver failure (ACLF) has further elucidated the clinical significance of bilirubin. We observed that individuals with ACLF exhibit significantly higher levels of bilirubin diglucuronide and bilirubin monoglucuronide compared to healthy subjects and those with compensated cirrhosis. In addition, we identified distinct bilirubin species profiles that correlate with the severity and prognosis of ACLF.[118]

Furthermore, hyperbilirubinemia in genetic disorders underscores the importance of understanding bilirubin metabolism for accurate diagnosis and management.

How to assess hyperbilirubinemia?

Direct hyperbilirubinemia is defined as a CB concentration above 1.0 mg/dL (conjugated fraction >50% of TB). Indirect hyperbilirubinemia is considered when UCB comprises >85% of TB.[119,120] While unconjugated hyperbilirubinemia can be clinically ambiguous, the presence of conjugated hyperbilirubinemia always suggests liver disease.[55,121] Although we have mentioned the importance of bilirubin concentrations in various scales for stratifying disease severity, it is important to note that bilirubin concentrations vary widely across different populations. Ethnicity, age, race, and other potentially confounding variables can make the currently used physiological ranges inaccurate, as noted by Vitek *et al*.[122] Nevertheless, analyzing bilirubin levels in different clinical entities might still provide an indicator of expected ranges, which could be used as diagnostic support after adjusting for confounding factors. Furthermore, the variations in bilirubin concentrations in disease states are greater than what can be attributed to these confounding factors (in contrast to concentrations in healthy individuals). Even if used as a reference interval rather than as a decision value, bilirubin remains a vital biomarker. Understanding its variability in different diseases is fundamental as an initial step.[122]

Future directions

Looking ahead, several promising avenues of research and clinical application could significantly improve our understanding and management of the dual role of bilirubin in liver disease.[123] One promising field is the development of targeted therapies that can selectively enhance the beneficial effects of bilirubin, such as its antioxidant and anti-inflammatory properties, while mitigating its neurotoxic risks. This could include the use of bilirubin analogues or compounds that promote endogenous bilirubin production and stability.[29,124] In addition, integrating a holistic approach that combines dietary modifications, lifestyle changes, and pharmacological treatments could improve the treatment of liver diseases.[125] For example, dietary interventions that support a healthy gut microbiota could improve bilirubin metabolism and its protective functions.[123] Interdisciplinary research efforts that bring together experts in hepatology, neurology, pharmacology, and microbiology will be essential to fully understand the complex mechanisms through which bilirubin operates and to develop comprehensive treatment strategies.

Conclusions

Although bilirubin has traditionally been defined as a waste product, its role in liver metabolism is undisputed. The measurement of bilirubin and its fractions in serum is crucial for diagnosis, prognosis, and treatment evaluation, as its concentration is directly related to liver function. Therefore, liver damage can be detected quickly. As shown in various studies presented in this review, bilirubin concentrations can vary from normal to very high, leading to serious consequences such as organ failure and death. Based on this review, it is important to study specific bilirubin molecular species in different diseases, given that total bilirubin concentrations (without understanding the ratio of its species) do not show clear differences among pathological entities. Continuing to study bilirubin concentrations in various diseases is essential to identify critical cutpoints, develop better treatments, and identify patients who may benefit from early liver transplantation or a life-support system.

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Conflict of interest

XQ has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2023, NMS has been an Associate Editor of *Journal of Clinical and Translational Hepatology* since 2021. The other authors have no conflict of interests related to this publication.

Author contributions

Concept and design (NMS, SMCC), drafting of the manuscript (SMCC, SXP, MMRM, XQ), critical revision of the manuscript for important intellectual content (NMS, SMCC, MMRM), and supervision (NMS). All authors have made significant contributions to this study and have approved the final manuscript. All authors have approved the final version and publication of the manuscript.

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