



Marc Jenniskens
Lies Langouche
Yoo-Mee Vanwijngaerden
Dieter Mesotten
Greet Van den Berghe

Cholestatic liver (dys)function during sepsis and other critical illnesses

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Take-home message: During sepsis and other critical illnesses, mild hyperbilirubinemia, instead of indicating inadvertent cholestasis, may be an adaptive and beneficial response. The observed increase in circulating levels of conjugated bile acids and bilirubin may be the result of an adaptively altered bile acid production and transport back towards the systemic circulation.

Abstract Objective: In ICU patients, abnormal liver tests are common. Markers of cholestasis are associated with adverse outcome. Research has focused on the possibility that mild hyperbilirubinemia, instead of indicating inadvertent cholestasis, may be adaptive and beneficial. These new insights are reviewed and integrated in the state-of-the-art knowledge on hepatobiliary alterations during sepsis and other critical illnesses. **Data sources:** Relevant publications were searched in Medline with search terms bile, bile acids, cholestasis, critical illness, intensive care, sepsis, alone or in combination. **Data synthesis:** Studies have shown that bilirubin, but also bile acids, the main active constituents of bile, are increased in plasma of patients with critical illnesses. In particular the conjugated fractions of bilirubin and bile acids are high, indicating that during critical illness the liver is capable of converting these molecules to less toxic forms. In human liver biopsies of prolonged critically ill patients, expression of bile acid excretion pumps towards the bile canaliculi was lower, while alternative transporters towards the systemic circulation were upregulated. Remarkably, in the presence of

increased circulating bile acids, expression of enzymes controlling synthesis of bile acids was not suppressed. This suggested loss of feedback inhibition of bile acids synthesis, possibly explained by the observed cytoplasmic retention of the nuclear FXR/RXR heterodimer. As macronutrient restriction during acute critical illness, an intervention that improved outcome, was found to further increase plasma bilirubin while reducing other markers of cholestasis, a potentially protective role of hyperbilirubinemia was suggested. **Conclusion:** The increase in circulating levels of conjugated bile acids and bilirubin in response to acute sepsis/critical illnesses may not necessarily point to cholestasis as a pathophysiological entity. Instead it may be the result of an adaptively altered bile acid production and transport back towards the systemic circulation. How these changes could be beneficial for survival should be further investigated.

Keywords Liver · Cholestasis · Bile acids · Bilirubin · Critical illness · Sepsis · Parenteral nutrition · Drug-induced cholestasis

M. Jenniskens · L. Langouche · Y.-M. Vanwijngaerden · D. Mesotten · G. Van den Berghe (✉)
Clinical Division and Laboratory of Intensive Care Medicine, KU Leuven, Herestraat 49, 3000 Leuven, Belgium
e-mail: greet.vandenbergh@med.kuleuven.be
Tel.: 32-16344021

Introduction

The liver plays a central role in the systemic response to sepsis and other critical illnesses by releasing acute-phase proteins, cytokines, and coagulants and by facilitating clearance of pathogenic organisms and toxins [1]. However, alterations in biochemical liver function tests are commonly observed in critically ill patients and are interpreted as signs of hepatobiliary dysfunction and cholestasis [2–4]. Two pathophysiological conditions are typically distinguished on the basis of laboratory parameters and clinical presentation: “hypoxic hepatitis” and “cholestatic liver dysfunction” [5]. Both conditions are associated with increased morbidity, mortality, and a longer hospital length of stay [2, 6–8].

Hypoxic or ischemic hepatitis is a well-recognized condition hallmarked by diffuse hepatocellular necrosis provoked by acute cellular hypoxia because of insufficient arterial blood supply of the liver. The laboratory findings reflect the underlying pathogenesis, as a steep increase of plasma alanine transaminase (ALAT) and aspartate transaminase (ASAT) and up to 20 times the upper limit of normality indicates hepatocytic damage and release of intracellular enzymes [3]. Approximately 5–10 % of the ICU patients fulfill the diagnostic criteria for hypoxic hepatitis at ICU admission [3, 9]. Mortality of patients diagnosed with hypoxic hepatitis reflects and therefore relates to the severity of the underlying condition [3]. Acute cardiac failure can cause a mixed derangement of all liver parameters (alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), ALAT, ASAT, and bilirubin). The most important determinants for abnormalities in liver parameters in heart failure are high central venous pressure and reduced hepatic perfusion. Comparable to hypoxic hepatitis, prognostic value was related to the extent of the underlying condition [10].

Cholestatic liver dysfunction (CLD) is traditionally defined as impaired bile formation and flow, which are caused by extrahepatic obstruction of the bile duct or by an inability of the hepatocyte to secrete bile into the bile duct (intrahepatic cholestasis). Obstructive cholestasis due to acute mechanical blockage of the canalicular system or by inflammation-driven narrowing of the bile ducts can be easily and reliably diagnosed by ultrasonography, but is seldom the cause of what is referred to as “CLD” during critical illness [11, 12]. In contrast, what is labeled CLD in critically ill patients is most often the result of non-obstructive intrahepatic alterations that may lead to accumulation of bilirubin and bile acids in the liver. In contrast with hypoxic hepatitis, clear diagnostic criteria for this type of CLD during critical illness are lacking. In fact, the criterion most often used in clinical practice is a serum total bilirubin greater than 2 or 3 mg/dl [13]. Besides the use of bilirubin as a marker, increased ALP and GGT are also used to indicate CLD, with used

thresholds around 2–3 times the upper limit of normality. Up to 20 % of ICU patients develop CLD according to these diagnostic criteria, of which the majority already fulfills them upon admission to the ICU [2, 4, 9]. Risk factors are sepsis with gram-negative bacteria, but also major surgery, trauma with systemic inflammation response syndrome (SIRS), and other severe shock states [7, 14]. The occurrence of CLD during critical illness is also associated with the use of parenteral nutrition, antibiotics, and other medications [15, 16].

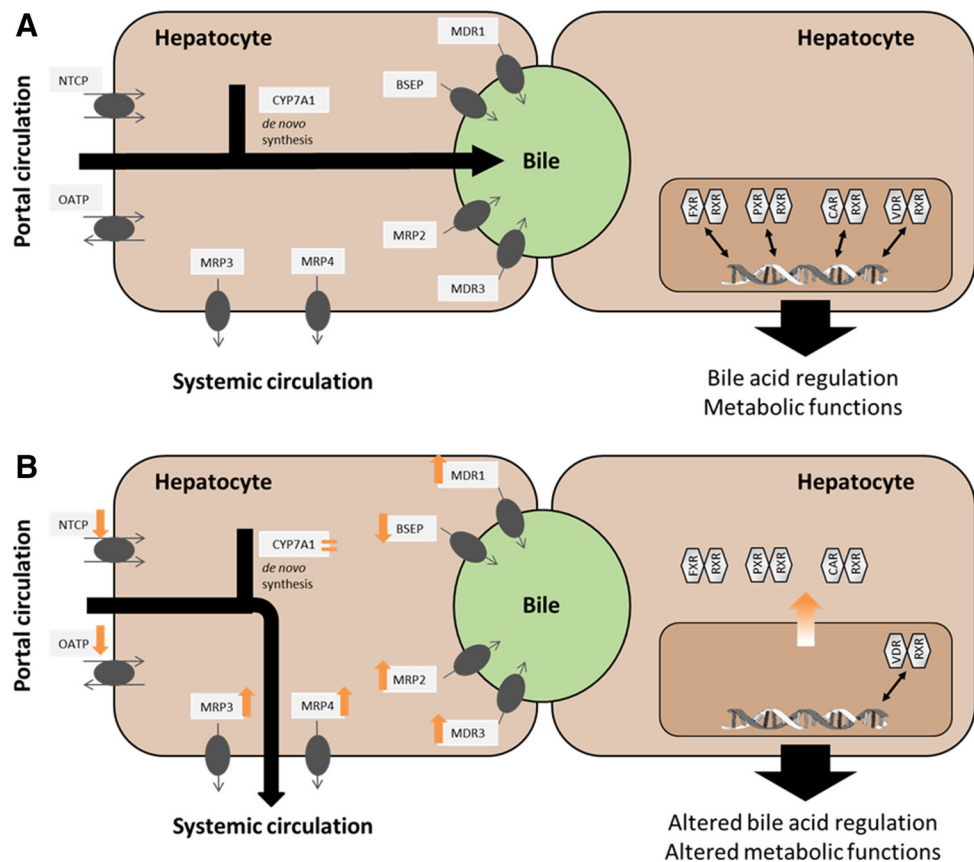
Although increased amounts of bile acids and bilirubin within the hepatocyte are presumed to be the culprit in inducing the clinical presentation of CLD, the underlying molecular and biochemical pathways are poorly characterized. Furthermore, whether hyperbilirubinemia during critical illness truly reflects cholestasis is not clear. Overt hyperbilirubinemia in the prolonged phase of critical illness may not be the same as a mild increase of circulating bilirubin, which is almost always observed during the acute phase of critical illnesses. In addition, many other factors can affect bilirubin such as hemolysis, blood transfusion, and (viral) infections of the liver. Furthermore, proof of causality in the link between hyperbilirubinemia and worse outcomes is still lacking. Hyperbilirubinemia might also be merely a biochemical epiphenomenon, indicative of severe hepatobiliary dysfunction, or it could reflect a transient and adaptive response during critical illness. Better insights into the pathophysiological and molecular aspects of hepatobiliary alterations during critical illness are clearly needed. In addition, the role of bile acids in the CLD that is present during critical illness has largely been overlooked, despite their known pivotal role in bile formation and in the metabolism of cholesterol and lipids [17].

The aim of this article is to review recent novel insights into the hepatobiliary transport and regulation of bilirubin and bile acids during critical illness and integrate these in the state-of-the art knowledge on this topic.

Normal hepatobiliary transport

The formation and excretion of bile is one of the key functions of the liver (Fig. 1a). Bile consists mainly of bile acids that are known for their role in dietary lipid absorption in the small intestine [18]. Bile synthesis and secretion is also important for the removal of excess cholesterol, bilirubin, and excretion of endogenous toxic compounds and xenobiotics. In the hepatocyte, primary bile acids are synthesized from their precursor molecule cholesterol by an extensive enzymatic cascade [18]. The rate-limiting enzyme CYP7A1 is regulated by multiple mechanisms and serves as the primary target in the negative feedback inhibition controlling the synthesis of bile

Fig. 1 Hepatobiliary transport system in **a normal conditions** and **b during critical illness**. During the course of critical illness the specific uptake transporter NTCP and less specific OATP transporters are downregulated. Also the specific export pump BSEP is downregulated. MRP3 and MRP4, pumps mediating alternative export to the **systemic circulation, are upregulated**. Simultaneously, nuclear receptors are no longer located in the nucleus of the hepatocyte and are unable to bind to their respective response elements. *NTCP* Na⁺-taurocholate co-transporting polypeptide, *OATP* organic anion transporting polypeptide, *BSEP* bile salt export pump, *MRP* multidrug resistance-associated protein, *FXR* farnesoid X receptor, *RXR* retinoid X receptor alpha, *PXR* pregnane X receptor, *VDR* vitamin D receptor



acids [18]. The **two primary bile acids** produced by the liver are **cholic acid (CA)** and **chenodeoxycholic acid (CDCA)**. The majority of CA and CDCA are **conjugated** in the **liver** with **glycine** or **taurine**, which enhances their physiological function. After secretion in the **intestinal lumen**, primary **bile acids** are **transformed** by the **bacterial flora** of the gut into the secondary bile acids **deoxycholic acid (DCA)** and **lithocholic acid (LCA)** and their respective conjugates (Fig. 2). **Secondary bile acids** are **absorbed** in the intestinal lumen and **transported** to the **liver**, where they are **reconverted** to their **primary** form.

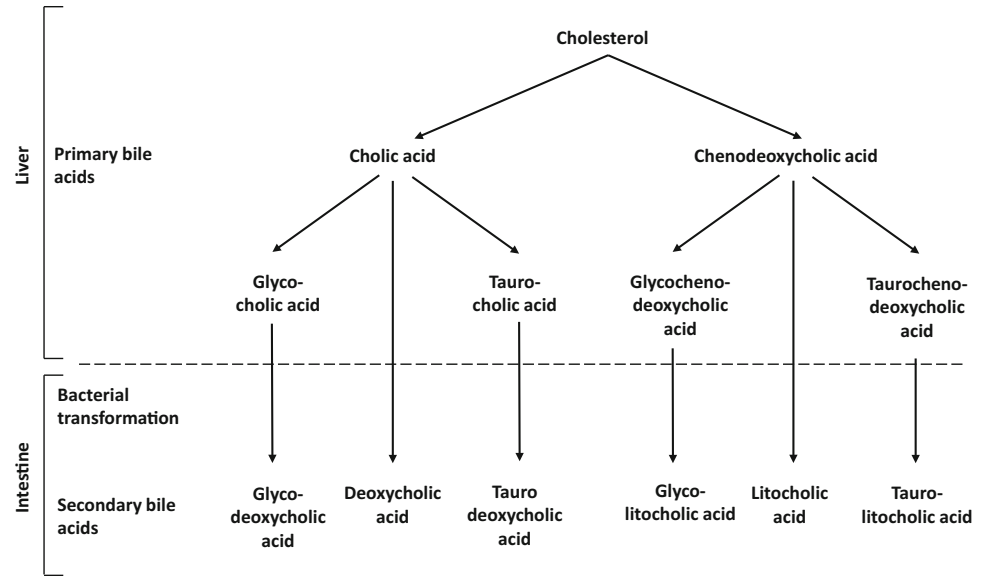
Bile acid uptake and export transporters

The hepatocyte is a strongly polarized cell type that features specific hepatobiliary transporters for the uptake and excretion of biliary constituents from the blood compartment into the bile compartment (Table 1). Cell polarity is maintained through the presence of tight junctions and the cytoskeleton to create distinctive basolateral and apical cell membranes. **Hepatic excretion of bile acids to the bile canaliculi is the rate-limiting factor** in bile salt dependent bile flow. The apical excretion is mainly mediated by the bile salt export pump (**BSEP**) [19]. Other apical but less

specific bile acid transporters are the multidrug resistance protein (MDR) and multidrug resistance-associated protein (MRP) family. MRP2 transports amphipathic conjugates (including bilirubin and bile acids), whereas MDR1 and MDR3 excrete cytotoxic agents and xenobiotics (including bile acids) [20]. Excreted bile acids are delivered to the small intestine and transported through the portal circulation back to the liver; this overall process is known as the **enterohepatic cycle**. Reuptake of bile acids from the blood into the hepatocyte is mainly mediated by the high affinity Na⁺ dependent bile acid transporter (NTCP) and the less specific organic anion transporting polypeptide (OATP). Aside from these influx systems, the basolateral membrane expresses the export transporters MRP3 and MRP4 [21, 22]. In normal conditions, only low levels of these export transporters are expressed at the basolateral membrane of hepatocytes through which a small fraction of bile acids in the portal circulation escapes the hepatic extraction and can re-enter the systemic circulation. From the systemic circulation, bile acids can also be directly excreted by the kidneys, although **highly efficient reabsorption minimizes urinary excretion, even under cholestatic conditions** [23].

Fig. 2 Bile acid differentiation.

The primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) are formed by the hepatocyte from their precursor molecule cholesterol. In the liver, these primary bile acids are conjugated with glycine or taurine, which improves their physiological function and decreases their toxicity. In the intestinal lumen, these primary bile acids are converted by the gut microbiome to secondary bile acids, which are efficiently reabsorbed and recycled in the enterohepatic cycle

**Table 1** Overview of the hepatobiliary transporters which are altered during critical illness

Protein name	Localization	Uptake/export	Function	Expression during critical illness [45, 51]
NTCP	Basolateral	Uptake	Primary uptake transporter of bile acids from portal circulation	Downregulated
OATP	Basolateral	Uptake	Multispecific uptake transporter of organic anions (including bilirubin) and bile acids from portal circulation	Downregulated
BSEP	Apical	Export	Primary export transporter of bile acids to the canalicular system	Downregulated
MRP2	Apical	Export	Multispecific export transporter of organic anions (including bilirubin) and bile acids from portal circulation	Upregulated
MRP3	Basolateral	Export	Multispecific export of organic anions to systemic circulation	Upregulated
MRP4	Basolateral	Export	Multispecific export of organic anions to systemic circulation	Upregulated
MDR1	Apical	Export	Excretion of organic cations, xenobiotics, and cytotoxic agents	Upregulated
MDR3	Apical	Export	Excretion of organic cations, xenobiotics, and cytotoxic agents	Upregulated

Regulation of bile acid homeostasis

The homeostasis of bile acids is regulated through a group of nuclear hormone receptors, a family of intracellular, ligand-dependent transcription factors [24]. In the nuclear receptor family, the farnesoid X receptor (FXR) has the highest affinity for bile acids. FXR activation leads to reduced expression of basolateral uptake transporters and stimulation of apical export transporters of bile acids, and it therefore has a self-regulating role by interfering with the intracellular concentration of its ligand. Binding of bile acids to FXR in hepatocytes also induces the expression of a small heterodimer partner (SHP) that inhibits the key enzyme CYP7A1 in bile synthesis [25, 26]. Furthermore, SHP inhibits target genes of other nuclear receptors including the expression of bile acid

transporters [27]. Other nuclear receptors with bile acid affinity are the vitamin D receptor (VDR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR) [28, 29]. All bile acid-sensing nuclear receptors operate in a heterodimer form with the obligatory partner retinoid X receptor alpha (RXR α) (Fig. 1a).

Because of the highly efficient reuptake system in the enterohepatic organs only a small amount of bile acids is present in the systemic circulation, although circulatory bile acids can increase 4–6 times after a meal [30]. The ingestion of meals regulates daily variation in serum bile acids concentration, although regulation by the circadian clock might also play a role [31]. These factors result in a diurnal rhythm that allows bile acids to serve as systemic metabolic signaling molecules to peripheral tissues. Indeed, circulatory bile acids have an additional important

metabolic regulatory role besides their function in **dietary lipid absorption** and bile acid homeostasis [32–36]. These peripheral effects are mainly mediated by the nuclear receptor FXR and via the G-coupled TGR-5 receptor [32, 34, 36].

Hepatobiliary transport during critical illness

Pathophysiological manifestations of critical illness-associated CLD

Histologically, cholestatic abnormalities in the liver during critical illness are characterized by **hepatocellular bilirubinostasis**, sinusoidal dilatation, an increased number of infiltrating cells in the sinusoid, and **Kupffer cell hyperplasia** [37]. The **increase in cell size from Kupffer cells** in combination with infiltrating polymorphonuclear cells, eosinocytes, and platelets induces aggregate formation which can lead to **obstruction of the sinusoidal lumen** [37]. The resulting hypoperfusion of the sinusoid can be suspected by the presence of a dilated space of Disse. The centrilobular region is typically most affected, as it is a metabolically active region that hosts the p450 enzymes, while it has only little oxygen available [38]. In severe illness, apoptosis and necrosis of liver parenchyma can be observed [37, 39]. Besides hepatocellular bilirubinostasis, signs of **bilirubinostasis can also be found in the canalicular and ductular bile ducts** [40]. This phenomenon is driven by **loss of contractile activity in actin and myosin microfilaments**, which play a **key role in the transport of bile acids through the canalicular route** [38, 41]. The impairment of polymerization and depolymerization of microfilaments results in the loss of canalicular membrane contraction and leads to **bile ducts paralysis, distension, and bile secretory failure** [37]. Development of such **canalicular changes have been observed in septic shock patients and after long-term treatment in the ICU and are associated with severe morbidity** [42, 43]. These histological findings can be accompanied by radiological abnormalities and **biliary sludge**, although **ultrasonographic changes are also observed in patients without suspected biliary abnormalities** [11, 12]. However, as explained above, presence of CLD during critical illness is mostly **suspected clinically by elevated plasma bilirubin**, as liver biopsies that would be required to confirm true cholestasis are hardly ever available for this indication given the invasiveness of this procedure.

Bile acid transport during critical illness

The majority of the current insights into the alterations that occur in the hepatocellular bile acid transport during critical illness are based on **acute animal models**

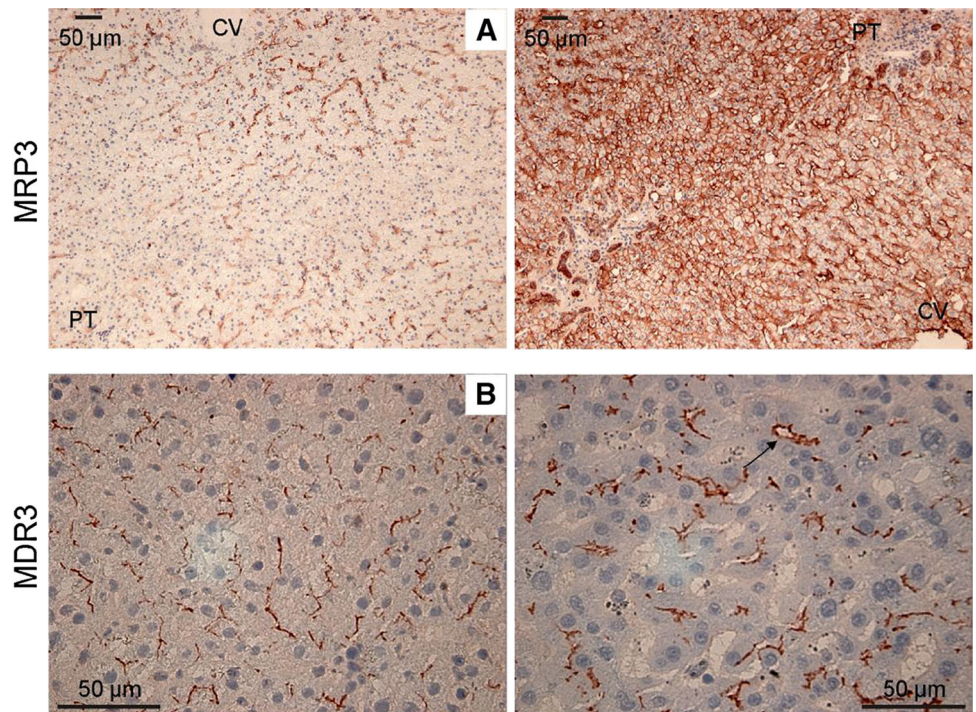
challenged with pro-inflammatory agents such as LPS or cytokines. However, also limited but quite valuable data are available from studies of human patients (Fig. 1b).

In endotoxemic and septic animal studies and in critically ill humans, gene expression and protein levels of the basolateral uptake pump NTCP are markedly downregulated, likely due to suppression of essential transcription factors by pro-inflammatory cytokines [44–46]. The OATP uptake transporters are also downregulated by inflammation-induced mechanisms [45–47]. In rat endotoxemia and in LPS-treated human liver slices, a suppressed expression of the export pumps BSEP and MRP2 protein levels has been observed [48, 49]. Also in biopsies harvested immediately postmortem from critically ill patients, BSEP protein expression was dramatically reduced, whereas upregulated MRP2 gene expression was reported [45]. MDR1 and MDR3, which are involved in the export of toxic compounds to the bile canaliculi, are upregulated during critical illness in humans [45]. The most prominent changes observed in both animal and human studies are the increase in the basolateral export pumps MRP3 and MRP4 at both the transcriptional and translational level [45, 48, 50] (Fig. 3). Normally MRP3 and MRP4 are expressed at very low levels in hepatocytes, but upregulation is observed during inflammation and longstanding cholestasis, presumably shifting transport of bile acids back to the blood compartment. In **human liver biopsies, MRP3 correlated strongly with histological bilirubinostasis and serum bilirubin and conjugated bile acids, suggesting that MRP3 upregulation may be a compensatory reaction to cholestasis** [51].

Retrospective analysis of **preagonal serum from ICU non-survivors** demonstrated **elevated bile acid levels** (11-fold) and **elevated bilirubin** (eightfold) levels [45]. Predominantly the **conjugated forms of both bilirubin and bile acids were increased**, which implies that the liver was **still capable of converting these toxic compounds to their less toxic conjugated forms** [45]. **Despite the increased circulating levels of bile acids, hepatic protein levels of CYP7A1, which is the rate-limiting enzyme for bile acid synthesis, was not suppressed in liver biopsies from these patients** [45]. The absence of a suppressed CYP7A1 protein expression in the face of quite elevated plasma bile acids **suggests that the normal feedback inhibition is suppressed during critical illness, with ongoing synthesis despite increased bile acid availability.**

The observed alterations in plasma bile acid levels and in the **expression of hepatobiliary transporters in liver biopsies harvested immediately postmortem** from human patients coincided with a remarkable decrease in the hepatocytic nuclear amount of the nuclear bile acid receptors FXR, PXR, CAR, and their obligatory partner RXR [45] (Fig. 1b). Again this is compatible with at least a **partial loss of bile acid sensing and feedback regulation of de novo bile acids production that occurs during the**

Fig. 3 Representative liver sections for MRP3 and MDR3. *Left panel* control patients, *right panel* ICU patients. **a** Normal basolateral MRP3 staining showing clear centrilobular and midzonal activity (*left panel*). Markedly upregulated panlobular honeycomb MRP3 staining pattern (*right panel*). **b** Normal pattern of a fine canalicular linear MDR3 staining (*left panel*). Strong double-stranded pattern of MDR3 staining around multiple dilated canaliculi (*right panel*). MRP multidrug resistance-associated protein, MDR multidrug resistance protein, CV centrilobular vein, PT portal tract. Reproduced with permission from [45]



course of critical illness. In vitro and in vivo models suggest that nuclear export of these receptors is regulated through c-Jun N-terminal kinases which can be activated by inflammatory mediators [52].

Together, these findings suggest that the so-called cholestasis, with high plasma bilirubin and bile acids, in acute critical illness is brought about by alterations in the hepatobiliary transport system. Critical illness induces an immediate increase in conjugated bile acids and bilirubin in the circulation, apparently through a reversal of the normal bile acid transport back into the blood. Furthermore, the hepatocyte of a critically ill patient appears to switch off the nuclear bile acid sensors. Whether this is a maladaptive or an adaptive response cannot be concluded from these data and should be further investigated.

linked to ICU jaundice [14]. Animal models of sepsis confirmed that endotoxin and inflammatory cytokines can alter the expression of the transporters for bile acids and bilirubin. Translocation of bacteria and endotoxin due to failure of the gut barrier might further enhance the altered hepatobiliary transport. Furthermore, reduced bile flow in the intestine has been suggested to augment this translocation, which might play a role in sustaining endotoxemia during the course of systemic inflammation [55]. However, similar alterations in bile acid transporter expression have been observed in an animal model of severe burn injury [51]. Furthermore, non-septic critically ill patients also display elevated plasma bilirubin levels, often already upon admission to the ICU [45, 56]. These observations suggest a more general underlying trigger as the main cause of the altered bilirubin and bile acid transport (Fig. 4).

Pathologies/interventions that affect CLD during critical illness

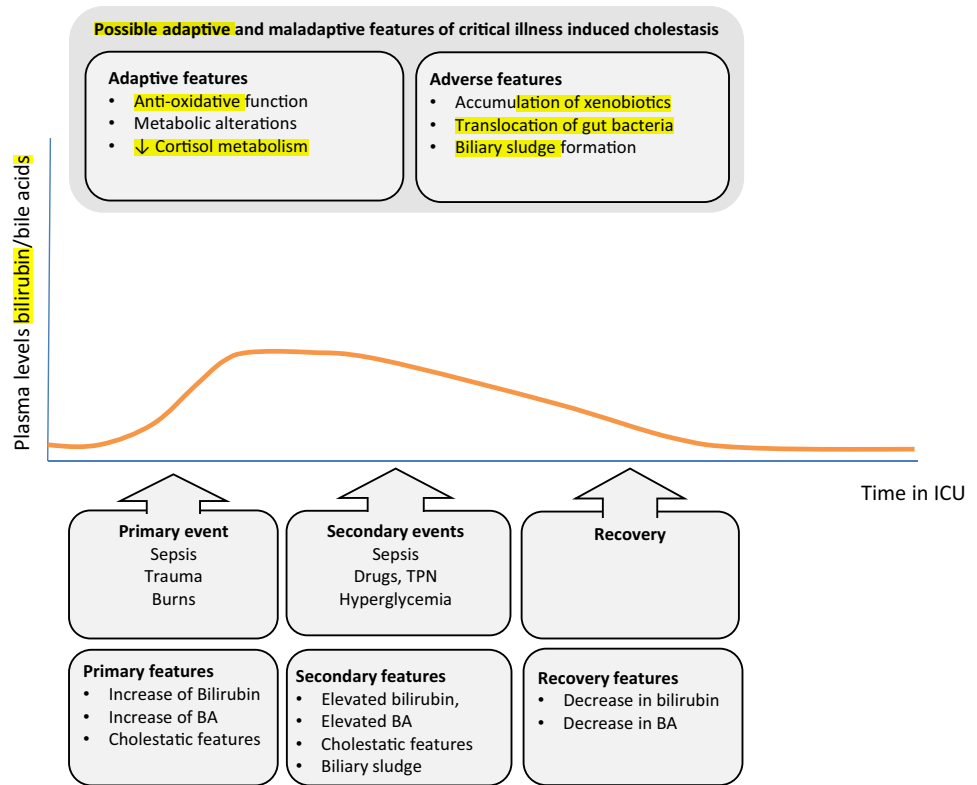
Sepsis

Sepsis and extrahepatic bacterial infection with inflammation are often accompanied by a rise in cholestatic markers [53]. A prospective analysis showed that sepsis is the second leading cause of cholestasis in clinically jaundiced patients [54]. Cholestasis in sepsis is linked predominantly to infections by gram-negative bacteria, especially with an intra-abdominal focus. In addition, pneumonia, pyelonephritis, and endocarditis have been

Parenteral nutrition

Parenteral nutrition is often administered during the course of critical illness when patients cannot be fed enough macronutrients via the enteral route. However, lack of enteral feeding and the administration of lipids through parenteral nutrition are thought to contribute to the development of CLD during critical illness [57]. The impact of parenteral nutrition on the development of CLD during critical illness has been assessed in a third-degree burn rabbit model [51]. In this model, markers of liver damage were significantly lower in the group of animals that were fasted as compared with those that received

Fig. 4 Course and features of **critical illness-associated cholestasis**. The **orange line** represents plasma levels of bilirubin and bile acids during the course of critical illness. A steep rise in the acute phase of critical illness coincides with the burst of inflammatory cytokines as a consequence of sepsis, trauma, or severe burn injury. Additional factors that can affect markers of cholestasis are persistent or new sepsis episodes, drugs, parenteral nutrition, and hyperglycemia. In the recovery phase, bilirubin levels and bile acid are assumed to decrease to baseline levels again. *BA* bile acids, *TPN* total parenteral nutrition, *ICU* intensive care unit



parental nutrition [51]. Also, in this animal model, fasting during the course of illness induced a shift from unconjugated to conjugated circulating bile acids, in a higher hepatic expression of the efflux transporter MRP3 and a lower expression of FXR [51]. Markers of hepatobiliary function were also studied in a large clinical randomized controlled study of 4640 patients on the effect of early versus late initiation of parenteral nutrition on the outcome of critical illness (EPaNIC) [58]. This study showed that delaying the initiation of parental nutrition to beyond the first week of critical illness was associated with fewer infections, less organ failure, and significantly shorter duration of intensive care dependency and hospital stay [58, 59]. Biochemical markers of hepatocytic damage (maximum levels ALT) and of cholestasis (GGT, ALP) were lower in the group that did not get parenteral nutrition during the first week. In contrast, plasma bilirubin was significantly higher in this group throughout the 7-day intervention window, but it became equal to the group receiving parenteral nutrition as soon as parenteral nutrition was started in the fasting group beyond that first week [60]. These findings clearly suggest that hyperbilirubinemia during critical illness does not necessarily reflect true cholestasis. Given that those higher levels of bilirubin occurred in the group that experienced fewer complications and had better outcomes may also suggest an adaptive nature of the stress-induced rise in plasma bilirubin.

Hyperglycemia

In two randomized controlled trials assessing the impact of blood glucose control on outcome of critical illness in adults, in a context of early parenteral nutrition, an effect of preventing hyperglycemia on hepatobiliary function has been shown. Lowering blood glucose levels with insulin reduced biliary sludge and cholestasis in prolonged critical illness [9, 60–62].

Drug-induced cholestasis

Results from animal models also demonstrated that critical illness induces hepatic biotransformation and suppresses excretory function, which may account for the accumulation of not only bile acids and bilirubin but also exogenous xenobiotics [63, 64]. Drugs administered as part of the intensive care of ICU patients could therefore reflect a toxic threat to the hepatocytes. Accumulation of drugs can directly cause hepatocyte damage, but also exerts toxic accumulation of bile acids, bilirubin, and toxins by inhibition of canalicular transporters and interference with nuclear receptors [65]. Canalicular transporters form the rate-limiting step in clearance of lipophilic drugs and their metabolites by excretion along with bile. Drugs with biliary excretion contribute to the development of jaundice [66]. However, the majority of

drugs are standard care in daily ICU practice. **Cholestatic dysfunction is described for basically all antibiotics. Anesthetics and sedatives** form another major group of commonly used drugs in the ICU **associated with cholestasis**. Currently, except for some specific toxins, there are no commonly used strategies available to eliminate this burden, besides discontinuation of potentially harmful drugs. Although extracorporeal liver support systems are known to reduce circulating bile acids, bilirubin, and toxins, this is not routine practice in ICU medicine.

Critical illness-induced CLD: beneficial or maladaptive?

One could **speculate** that the **reversal of the hepatobiliary transport back to the blood** could reflect an **escape to cope with the inability of the hepatocyte to excrete bile acids** against the **concentration gradient** into the **bile ducts**, which would thus be a **protective mechanism of the hepatocyte to prevent toxicity of intracellular bile acids and bilirubin**. Alternatively, ongoing bile acid production with a **reversal of normal flow into the circulation** can reflect an **adaptive process** in order to **increase systemic effects of circulating bile acids and bilirubin**. Indeed, **bilirubin may attenuate organ damage by improving endothelial function and protecting against oxidative stress and high glucose levels** [67–71]. Knockout mice lacking heme oxygenase, which is the **rate-limiting factor in bilirubin production**, were shown to have a **higher mortality and more organ damage** after induction of LPS than wild-type mice [71]. In an animal model of LPS-induced sepsis, **bilirubin-treated rats** had significantly **lower** expression of markers of **hepatocyte damage** and showed **improved survival** [70]. The **protective role of bilirubin** was shown to be, at least in part, **mediated by inhibiting hepatic iNOS expression** [70]. Another investigated mechanism in which bilirubin reduced cellular damage is **redox cycling**. **Bilirubin can react** with reactive oxygen species (ROS), which **neutralizes the toxicity and oxidizes bilirubin to biliverdin**. Biliverdin can be converted back to bilirubin through biliverdin reductase. By **repeating this cycle, the antioxidative and cytoprotective effect of small amounts of bilirubin is greatly amplified** [69]. In addition, **bile acids** were shown to **reduce cortisol-metabolizing enzymes** in rats [72]. An association between bile acids and cortisol was later also described in critically ill patients, suggesting that an **increased availability of bile acids** during critical illness may **explain the reduced breakdown of cortisol**, thereby contributing to **elevated plasma cortisol concentrations** and higher cortisol availability in vital organs and tissues and thus playing

a key role in the stress response [73]. Potentially, a metabolic effect of bile acids on energy metabolism could play a protective role to reduce the hypermetabolic response of acute critical illness. Indeed, **bile acids can regulate glucose, cholesterol, triglyceride, and energy homeostasis** and are **promising novel drug targets** in the **treatment for metabolic diseases** [33–36]. Activation of **bile acid signaling pathways increases energy expenditure and prevents development of insulin resistance** [74]. In addition, **bile acids** were shown to **increase insulin secretion** by inducing the production of glucagon-like peptide-1 [75]. **Mildly elevated bilirubin and/or bile acids** could theoretically also have a **protective role in advanced liver disease**. However, in these patients hyperbilirubinemia is present over a much longer time frame than observed during critical illness and the **severity** appears to **reflect the extent** of the **underlying pathogenesis** [76].

However, the **alterations observed in the hepatocytes during critical illness** not only increase levels of bilirubin and bile acids. **Drugs and other xenobiotics** that are administered in daily ICU practice can **easily accumulate** as a **result of decreased bile excretion**. **Reduced bile flow** in the **intestine** has also been shown to **exert translocation of the gut microbiome** and could **play a role in sustained release of endotoxin in the systemic circulation** [55]. A prolonged **lack of bile acids** in the intestine can cause **malabsorption of lipids and vitamins**. In addition, sustained molecular changes in bile acid signaling pathways can be harmful. Evidence suggests that **bile acids and FXR signaling regulate cell growth and inhibit tumor genesis**. Animals lacking FXR or its downstream target SHP develop **hepatocellular carcinoma** [77, 78]. Furthermore, high levels of conjugated bilirubin in mice induce erythrocyte cell death which could contribute to **anemia** [79].

Besides supportive care and interventions to prevent liver damage, **no treatment options** are currently available **for CLD**. Reducing the load of **inflammatory signaling molecules to the liver** microenvironment in order to **decrease the need for an adaptive response** may be desirable. The latter can be achieved by reducing risk factors that provoke CLD, such as **prevention of hyperglycemia** and **avoiding the administration of parenteral nutrition during acute critical illness** [9, 60]. Opioids have been suggested to **attenuate the inflammatory response and sequential liver injury**, but this is still being **investigated in vitro and in vivo**, and thus clinical evidence is currently lacking [80, 81]. Ursodeoxycholic acid (UDCA) is used in **various cholestatic liver diseases** and **improves clinical and/or laboratory parameters** [82]. There is, however, currently **no evidence** to **support the use of UDCA in critically ill patients**.

The **possible protective role of mild hyperbilirubinemia during critical illness** **questions the relevance** of assessing

Table 2 Liver evaluation in commonly used ICU prediction and organ assessment scores

Scoring system (range)	Collection of data	Selection of variables and their weights	Used feature for liver evaluation	Classification cutoffs	Weight
Apache IV (0–286)	Admission	Multiple logistic regression	Bilirubin (mg/dl)	<1.9	0
				2.0–2.9	5
				3.0–4.9	6
				5.0–7.9	8
				>8.0	16
SAPS II (0–163)	Admission	Multiple logistic regression	Bilirubin (mg/dl)	<4.0	0
				4.0–5.9	4
				>6.0	9
SOFA (0–24)	Daily (worst in 24 h)	Expert opinion	Bilirubin (mg/dl)	<1.2	0
				1.2–1.9	1
				2.0–5.9	2
				6.0–11.9	3
				>12.0	4
MODS (0–24)	Daily (morning)	Literature review and multiple logistic regression	Bilirubin (mg/dl)	<1.2	0
				1.2–3.5	1
				3.5–7.0	2
				7.0–14.0	3
				>14.0	4

liver dysfunction in ICU patients exclusively via laboratory parameters and of prognosticating via organ dysfunction scores that include bilirubin (Table 2) [13, 83, 84].

Conclusion

Alterations of biochemical cholestasis markers during the course of critical illness reflect a multifactorial process involving hepatic biotransformation and altered transporter expression. The current evidence is insufficient to conclude on whether these alterations reflect true cholestasis or only indicate a biochemical epiphenomenon. Whether the hepatobiliary alterations must be interpreted as maladaptive or adaptive changes also needs to be further elucidated. Bilirubin might therefore not be the ideal marker to assess liver dysfunction as it is currently used in organ assessment scores. Only an interventional study, in which circulating bilirubin levels can be actively and directly manipulated, can answer the questions whether the mild hyperbilirubinemia

that is uniformly present during acute critical illness is a beneficial response that protects against adverse outcomes. Also, in order to explore possible specific therapeutic targets, the molecular pathways involved in the pathophysiology of the different aspects of critical illness-induced CLD need to be further elucidated. The novel insight that alterations in nuclear receptors may play a key role during critical illness opens perspectives for future research and to ultimately develop better targeted strategies to improve outcome.

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Compliance with ethical standards

Conflicts of interest The authors have no conflicts of interest to disclose.

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