Review article

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Cholestatic liver dysfunction in critically ill children: A boon or a bane?

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Abstract

The phenomenon of "intensive care unit (ICU) jaundice" describes cholestasis that occurs in critically ill patients. This ICU jaundice occurs in 20% of critically ill subjects, as a result of non-obstructive intrahepatic disruptions. Despite its common occurrence, not many studies have explored the clinical significance behind this phenomenon. Cholestasis in the critically ill has been associated with a grim prognosis, reflected by serum bilirubin value in many prognostic markers. Not until recently, researchers have shed some light on this phenomenon, not as a mere clinical manifestation of organ dysfunction, but with a protective role in critical illness. This review highlights the physiology and pathophysiology behind cholestatic liver dysfunction in critically ill children, its possible benefit, and its harm, as well as factors affecting its severity.

Key words: Cholestatic liver dysfunction, critical illness, jaundice.

Trigger

AR, a 13-year-old boy, was admitted to the inpatient with a fever for two days. The patient had a history of medulloblastoma (received the first cycle of chemotherapy) and hydrocephalus on a ventriculoperitoneal (VP) shunt. The patient was diagnosed with sepsis due to enterocolitis. Culture results were positive for Klebsiella pneumoniae, Pseudomonas aeruginosa, and Candida albicans. Initial procalcitonin was 4.78 ng/ml increased to 9.27 ng/ml on day 10.

On day 6 patient appeared jaundiced with abdominal tenderness. The liver was palpable 6 cm below the arcus costae. Abdominal ultrasonography (USG) showed minimal ascites with edema of the abdominal wall, abdominal X-ray and computed tomography (CT) scan showed no sign of ileus. Liver function tests and bilirubin levels are illustrated in **Figure 1**. Aspartate aminotransferase (AST) and alanine transaminase (ALT) were moderately elevated, with marked elevation in bilirubin values. Immunology tests were negative for Hepatitis A, B, and C, toxoplasmosis, rubella, cytomegalovirus, herpes (TORCH), and leptospirosis. The patient was eventually diagnosed with cholestatic liver dysfunction secondary to sepsis. The kinetics of the liver function test and bilirubin are shown in **Figure 1**.

Background

Cholestasis defines a pathological condition in which bile flow is reduced or obstructed, leading to both hepatic and systemic retention, subsequently elevated bilirubin, bile acids, and various chole-

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phils, which are normally excreted via bile. (1) Cholestatic liver dysfunction (CLD) according to its etiology, may be classified as intrahepatic or extrahepatic. The former is caused by a defect in hepatocyte secretion of bile into the bile duct, while the latter is caused by any extrahepatic obstruction of the bile duct. In critical illness, cholestasis may be due to direct involvement of the hepatobiliary system, such as acute hepatitis or cholangitis. However, often it appears without such diseases. (2,3)

During the course of critical illness, cholestasis often manifests due to non-obstructive intrahepatic disruptions, subsequently building up of bilirubin and bile acids in the liver. (4) Cholestasis in critical illness, notoriously known as "intensive care unit (ICU) jaundice," occurs in up to 20% of ICU patients. (5) Despite its recurrence, CLD in the critically ill lacks a conventional definition. However, total serum bilirubin greater than 2 or 3 mg/dl (34.2-51.3 μmol/l) is often used in older children and adult populations. (4) While a cut-off of conjugated bilirubin >1.0 mg/dl (17.1 μmol/l) given total bilirubin is less than 5.0 mg/dl (85.5 μmol/l) and proportion of conjugated bilirubin of at least 20% of total bilirubin is used to diagnose neonatal cholestasis (or cholestatic hyperbilirubinemia). (6) Using this cut-off, a recent retrospective study on 179 pediatric septic patients at Cipto Mangunkusumo Hospital found a total of 15 elevated total and conjugated fractions of serum bilirubin out of 40 subjects with bilirubin examination, exclusive of those with congenital causes of cholestasis (unpublished data).

Serum bilirubin has been adapted into many prognostic markers of pediatric critical illness (**Table 1**). (7) Its value that is severity-dependent has long been correlated with worse outcomes. (8) The identified risk factors that significantly affect serum bilirubin/bile acids during critical illness are Gram-negative bacteria infection, sepsis, major surgery, major trauma, blood transfusion, use of antibiotics and other hepatotoxic medications, and parenteral nutrition. (4,5,9)

In the critically ill, CLD used to be under-looked as a mere clinical phenomenon that only indicates organ dysfunction severity. Recently, researchers have been trying to shed some light on the shades of yellow, and how it may possibly play a role in critical illness. Its significance as an adaptive response during critical illness and its possible protective role mostly remains a mystery. This review aims to provide an update on the pathophysiological and molecular mechanism behind CLD in critically ill subjects.

The classical presentation of cholestatic liver dysfunction in critical illness

Unlike acute liver failure in the critically ill (secondary to liver hypoperfusion) which has an early onset during acute critical illness, CLD in critical illness usually manifests later (around one week from the onset of critical illness). (4,5) It is most commonly associated with sepsis and bacteremia. Hepatomegaly in the absence of portal hypertension is common, distinctive from end-stage liver failure. (5)

Serum bilirubin and/or bile acids progressively increase, with 70-80% of total bilirubin is conjugated. In the absence of acute liver failure (or acute liver ischemia), AST/ALT concentrations are typically normal or minimally elevated. Plasma alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) are usually elevated. Hyperglycemia is a common finding. Unspecific to CLD, serum albumin level may be lower than normal, and prothrombin time may be prolonged due to critical illness. (5)

Histopathological findings in CLD in critical illness demonstrate predominant intrahepatic cholestasis with bile cast-containing dilated bile canaliculi, and bile pigments found in hepatocytes cytoplasm and Kupffer cells. (5) Sinusoidal dilatation and increased infiltrates are common. Kupffer cells, polymorphonuclear cells, platelets, and shed endothelial cells altogether with interposing fibrin are found. This is thought to cause cell aggregations of sinusoidal linings, contributing to luminal obstruction. (3) In some areas, narrowed sinusoids secondary to centrilobular hepatocytes swelling is also found. Diffuse steatosis and Kupffer cell hyperplasia are also prominent findings. (5) Endotoxin materials containing Kupffer cells, focal necrosis of hepatocytes, and irregularity of hepatocyte membrane microvilli facing Disse's space. (3)

Normal hepatobiliary system

The liver plays a key role in bile formation and excretion. Bile acid is made from cholesterol via an extensive enzymatic cascade. Bile acid undergoes conjugation in the liver, subsequently secreted into the intestine. Here, intestinal flora metabolizes primary bile acids into secondary bile acids and their conjugates. Secondary bile acids are then reabsorbed and transported to the liver to be converted into primary bile acids. (4)

Hepatocytes are polarized cells that organize a directional exchange of macromolecules specialized to their surface. Hepatocyte lateral domains are locked by tight junctions, its basal domain interacts

with the underlying extracellular matrix at the sinusoid, while its apical domain forms bile canaliculi. (4,10)

Regulation of bile acid production, uptake, and excretion

Bile acid production and its regulation

The formation of bile acids from cholesterol is regulated by the rate-limiting enzyme cytochrome P450 family 7 subfamily A member 1 (CYP7A1), the primary target for negative feedback for inhibition of bile acid synthesis. A nuclear family receptor, the farnesoid X receptor (FXR) regulates intracellular bile acids concentration. The binding of bile acid activates FXR, leading to decreased basolateral uptake transporters and increased apical export transporters. Activation of FXR by bile acid binding also induces small heterodimer partner (SHP) expression, that inhibits CYP7A1, downregulating bile acid synthesis. Other nuclear receptors are activated by bile acids, for instance, the vitamin D receptor (VDR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR). All these receptors form heterodimers with retinoid X receptor alpha (RXRα). (4,11)

In critically ill subjects, a post-mortem biopsy found that the hepatic protein CYP7A1, which was supposedly a rate-limiting regulator, was not suppressed, suggesting the loss of feedback inhibition. The same study also found significantly decreased hepatic nuclear bile acid receptors FXR, PXR, CAR, and RXR. This further suggested a loss of feedback regulation on de novo bile acid synthesis in the hepatocyte during critical illness. (4,11,12)

Besides regulating bile acid synthesis and uptake export, the binding of bile acid to FXR activates a signaling pathway that impacts hepatic lipid homeostasis. Experimental studies have shown that in response to inflammation, caspase-1 is activated. Caspase-1 is activated by inflammasomes, a component of the innate immune system found intracellularly in both parenchymal and nonparenchymal cells of the liver. Caspase-1 is linked to lipid metabolic pathways via the activation of sterol regulatory element binding proteins (SREBPs). This gives rise to substantially increased plasma cholesterol. Cholesterol may undergo oxidation to give rise to bile acid. Hence this suggests that in response to inflammation trigger, liver inflammasomes is activated and have a downstream effect on lipid metabolism, and is associated with increased bile acid synthesis. (9,13)

Bile acid uptake and excretion

The hepatocyte secretes bile acid into the bile canaliculi mediated by several protein transporters located at the apical domain of the hepatocytes. Bile acid is secreted into the bile canaliculi mainly via a bile salt export pump (BSEP), and other apical but not-specific bile acid transporters, i.e., multidrug resistance protein (MDR) and multidrug resistance-associated protein (MRP) family. A post-mortem liver biopsy revealed dramatically reduced BSEP protein expression, while the non-specific transporters MRP2 gene expression is upregulated. MDR1 and MDR3, which are non-specific bile acid transporters (also export other toxic compounds) are upregulated. This suggests that in critical illness, there is a reduction in bile acids exported to the bile canaliculi, hence an increased level of bile acids in the systemic circulation. (4,11,12,14)

Under normal conditions, basolateral protein transporters uptake bile acids from the portal circulation to be exported to bilirubin. The primary uptake transporters are the Na^+-dependent bile acid transporter (NTCP) and the less specific organic anion-transporting polypeptide (OATP). During critical illness, NTCP and OATP are downregulated. (15)

Bile acid is exported back to the portal circulation by MRP3 and MRP4 transporters. In normal conditions, only a small fraction of bile acid may escape hepatic extraction and reenter the systemic circulation as there is only a low level of MRP3 and MRP4 expression at the basolateral membrane. Recent evidence from human and animal studies revealed upregulated gene transcription and translation for MRP3 and MRP4 expressions during inflammation. Altogether this suggests trends towards decreased uptake and increased export of bile acids to the systemic circulation during critical illness. (4,11,14,15) (**Figure 2**, **Table 2**)

The role of the liver in critical illness

In critical illness, not only inflammation increases bile acid synthesis coupled with partial loss of negative feedback inhibition, but it also diverts bile acid and bilirubin towards systemic circulation, ultimately circulating bile acids and bilirubin are elevated. The liver regulates metabolic, endocrine, and immunological processes in critical illness. “ICU jaundice” or cholestatic liver dysfunction traditionally viewed as a late manifestation of severe illness (organ failure), may also be observed in the early stage of critical illness. (5,9,12) It is generally accepted that cholestatic liver dysfunction refers to cellular alteration leading to bile acid and bilirubin accumulation within the liver and systemic circulation secondary to inflammation.

There is still insufficient data to conclude whether cholestasis in critical illness only reflects liver dys-

function or may potentially be a beneficial compensatory response. It is known that systemic and local inflammation activates nonparenchymal immune cells in the liver, subsequently releasing proinflammatory cytokines. An intracellular multiprotein oligomeric complex, inflammasome, is expressed in both parenchymal and non-parenchymal liver cells. Inflammation may trigger inflammasome to activate caspase-1 and the production of interleukin (IL)-1β and IL-18. (9) Overall, these kicks start inflammatory response to a dangerous signal. Inflammasome in the liver is considered central to cell immunity against insults. A prospective analysis shows that sepsis is the second leading cause of cholestasis. Infection with Gram-negative bacteria, intra-abdominal focus, pneumonia, pyelonephritis, and endocarditis have been associated with cholestatic liver dysfunction occurrence. Inflammasomes have been shown to specifically respond to bacterial pore-forming toxins (such as the α-forming toxin of Staphylococcus aureus and pneumolysin of Pneumococci). (9,16)

In response to inflammation, reactive oxygen species (ROS) are formed, inducing liver heme oxygenase activity that results in the formation of biliverdin, iron, and carbon monoxide. Liver heme oxygenase-1 is linked to the induction of autophagy. Autophagy is deemed as a mechanism of hepatocellular stress tolerance, as well as to negatively regulate inflammasomes. (17) In addition, bilirubin can neutralize ROS. Biliverdin resulting from bilirubin oxidation can be converted back to bilirubin by bilirubin reductase. Hence this redox cycle gives rise to high antioxidative properties. (12)

The deviation of hepatobiliary transport towards systemic circulation may be beneficial in terms of energy conservation. As normal biliary transport is energy-consumptive active transport against the concentration gradient. Besides, this may confer protection to hepatocytes that are susceptible to toxic damage from intracellular bile acids and bilirubin accumulation. (4)

Increased circulatory bile acids and bilirubin is beneficial as it has antioxidative and cytoprotective roles. An experimental animal study on lipopolysaccharides (LPS)-induced sepsis demonstrates that rats treated with bilirubin had milder hepatic damage mediated by inhibition of hepatic inducible nitric oxide synthase (iNOS) expression, lower cytokine at transcriptional levels (mRNA), decreased inflammatory cell infiltrations and apoptosis rate, and improved overall survival. (18) Furthermore, a study in critically ill subjects demonstrates that circulating bile acid levels are correlated to reduced cortisol breakdown secondary to lowered cortisol-metabolizing enzymes. Hence elevated plasma cortisol in vital organs and tissues regulates inflammation and metabolic stress response. (12)

Transiently increased bile acid also has metabolic benefits. Bile acid activates a pathway that increases energy expenditure and prevents insulin resistance. It is also shown to increase insulin secretion by inducing glucagon-like peptide-1 production. Glucose control is beneficial for the outcome of critical illness. (9,12)

On the other side of the coin, elevated bile acids are not without their negative effects on the critically ill. Elevated circulating bile acid reflects the accumulation of drugs and other xenobiotics. A study in an animal model of critical illness demonstrated decreased hepatic excretory function, leading to drug accumulation that directly damaged hepatocytes, inhibited canalicular transporters, and interfered with nuclear receptors. It was shown that drugs (mainly, drugs with biliary excretion) contributed to the development of ICU jaundice. (12)

Also, bile acid is vital in maintaining intestinal integrity. This alteration of bile transport may subsequently disrupt the gut microbiome and promote bacterial translocation. This may lead to sustained endotoxemia that complicates the course of critical illness. Furthermore, the intestinal absence of bile acids may cause malabsorption of lipids and vitamins. (12)

In the critically ill, parenteral nutrition and administration of lipids compared to enteral nutrition have been shown to contribute to CLD. However, it was found that fasting during critical illness is linked to increased conjugated bilirubin and higher expression of MRP3 exporter, and lower FXR expression. (9) A large clinical randomized control study (EPaNIC) on the effect of early versus late parenteral nutrition on critical illness outcome showed that delayed parenteral nutrition (more than one week) was associated with more favorable outcomes, despite higher plasma bilirubin levels. Upon administration of parenteral nutrition in the following week, the plasma bilirubin level equalizes to the control group. (19) This finding suggests that during critical illness, hyperbilirubinemia may not purely reflect cholestasis. Furthermore, interesting finding on the concurrent higher plasma bilirubin and better clinical outcome in the intervention group suggests the possible protective mechanism behind CLD in critical illness.

Conflicts of interest and source of funding

The authors have no conflict of interest to declare.

Table 1. Bilirubin value in prognostic scorings of critically ill children

pSOFA	Score	0	1	2	3	4
	Bilirubin	<1.2 mg/dl	1.2-1.9 mg/dl	2.0-5.9 mg/dl	6.0-11.9 mg/dl	≥12.0 mg/dl
PRISM III	Score	6				
	Total bilirubin for >1 month age	>3.5 mg/dl				
P-MODS	Score	0	1	2	3	4
	Bilirubin	<0.5 mg/dl	0.5-2.0 mg/dl	2.0-5.0 mg/dl	5.0-10.0 mg/dl	>10 mg/dl

Legend: pSOFA=pediatric sequential organ failure assessment; PRISM III=pediatric risk of mortality score III; P-MODS=pediatric multiple organ dysfunction scores.

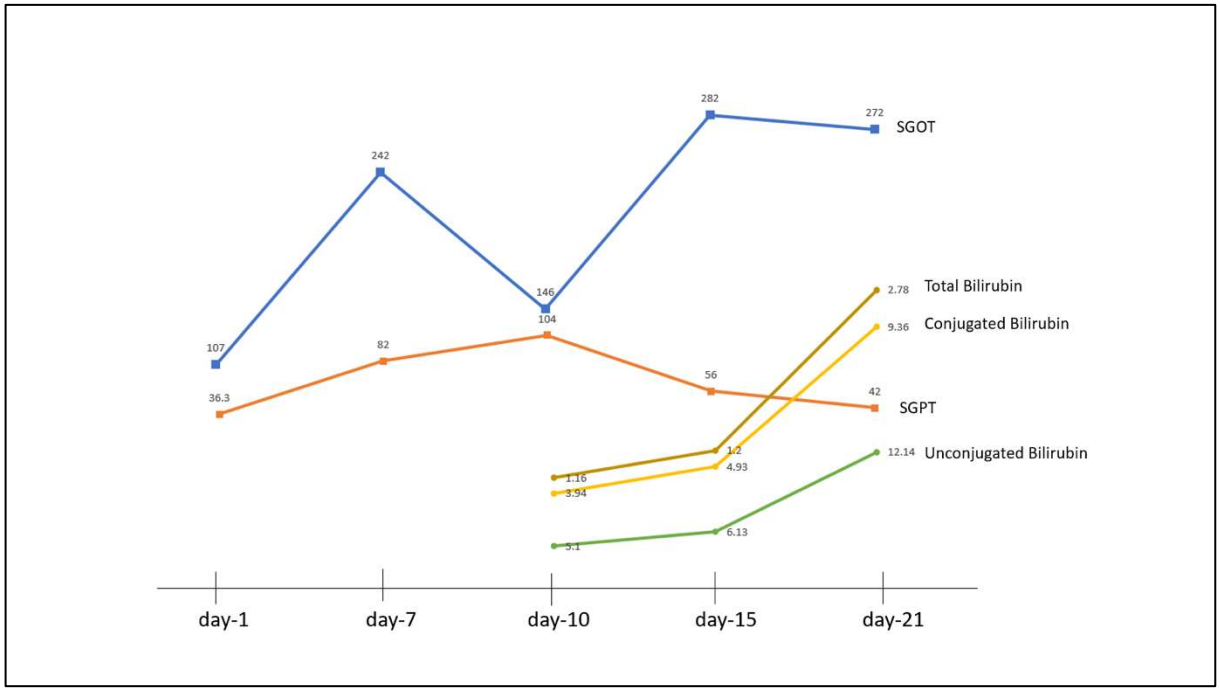
Table 2. Alteration of specific proteins during critical illness contributing to cholestasis

Protein	Location	Function in bile acid synthesis	Alteration during critical illness
CYP7A1	Endoplasmic reticulum	Synthesis of bile acid and regulation of cholesterol	Loss of inhibition
SREBP	Endoplasmic reticulum	Biosynthesis of cholesterol, uptake of free fatty acid	Activated
NTCP	Basolateral of hepatocyte	Primary uptake transporter of bile acids from portal circulation	Downregulated
OATP	Basolateral of hepatocyte	Organic anions (including bilirubin) uptake transporter from portal circulation	Downregulated
BSEP	Apical of hepatocyte	Primary export transporter of bile acids to the bile canaliculi	Downregulated
MRP2	Apical of hepatocyte	Organic anions (including bilirubin) and bile acid export transporter from portal circulation	Upregulated
MRP3	Basolateral of hepatocyte	Organic anions (including bilirubin) export transporter to the systemic circulation	Upregulated
MRP4	Basolateral of hepatocyte	Organic anions (including bilirubin) export transporter to the systemic circulation	Upregulated
MDR1	Apical of hepatocyte	Excretory transporter for organic cations, xenobiotics, and cytotoxic agents	Upregulated
MDR3	Apical of hepatocyte	Excretory transporter for organic cations, xenobiotics, and cytotoxic agents	Upregulated

Adapted and modified from Jenniskens M. et al. (4)

Legend: SREBP=sterol regulatory element binding proteins; NTCP=Na^+-taurocholate cotransporting polypeptide; OATP=organic anion-transporting polypeptide; BSEP=bile salt export pump; MRP=multidrug resistance-associated protein; MDR=multidrug resistance protein.

Figure 1. Kinetics of liver enzymes and bilirubin levels



Legend: SGOT=serum glutamic oxaloacetic transaminase; SGPT=serum glutamic pyruvic transaminase.

Figure 2. Hepatobiliary transport system

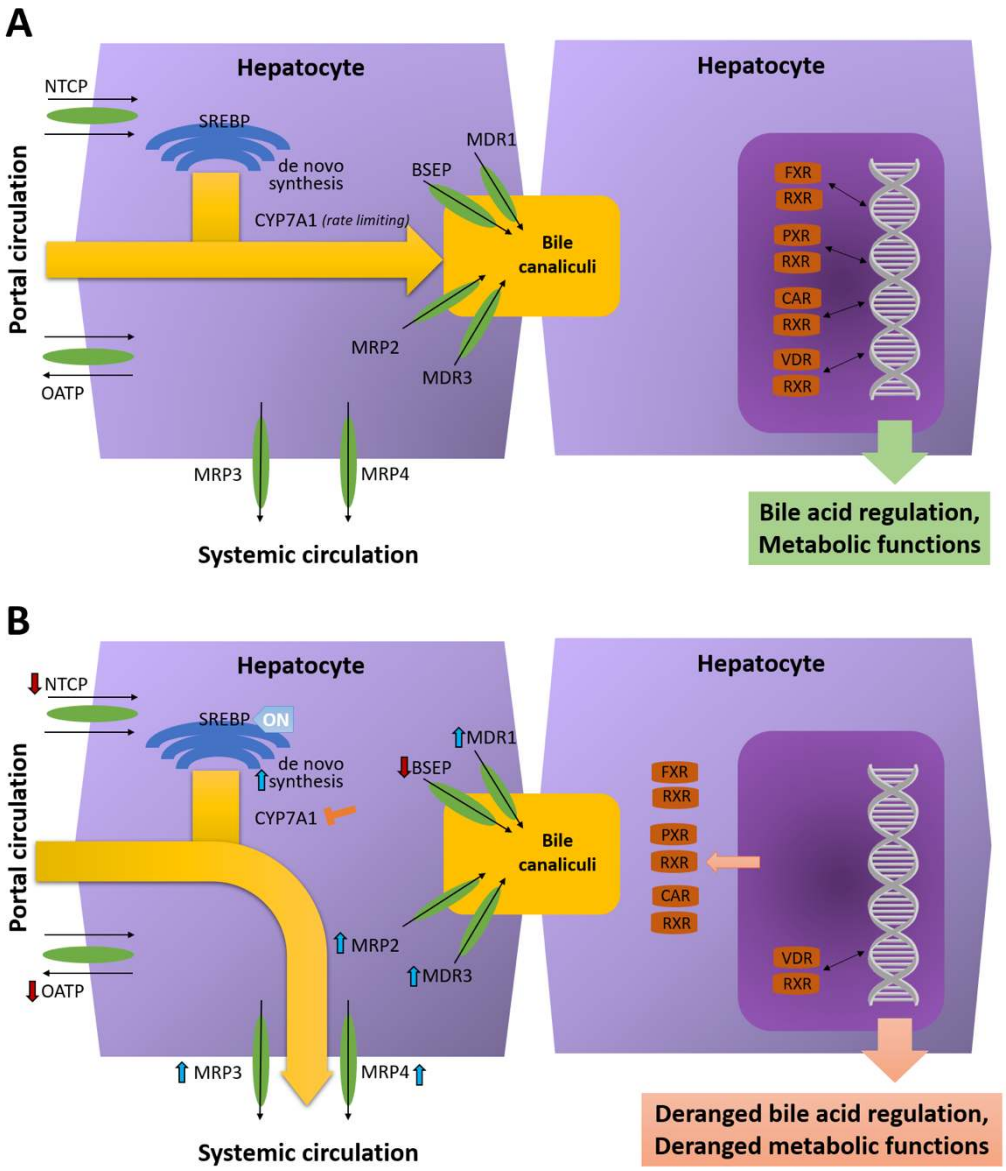


Figure adapted and modified from Jenniskens M. et al. (4)

Legend: NTCP=Na^+-taurocholate cotransporting polypeptide; OATP=organic anion-transporting polypeptide; SREBP=sterol regulatory element binding proteins; CYP7A1=cytochrome P450 family 7 subfamily A member 1; MDR=multidrug resistance protein; BSEP=bile salt export pump; MRP=multidrug resistance-associated protein; FXR=farnesoid X receptor; RXR=retinoid X receptor alpha; PXR=pregnane X receptor; CAR=constitutive androstane receptor; VDR=vitamin D receptor.

A. Hepatobiliary transport under normal conditions. B. Alteration of hepatobiliary transport, regulation of bile acid synthesis, and metabolic function during critical illness. Downregulated uptake transporters (NTCP and OATP), downregulated exporters to bile canaliculi (BSEP) coupled with upregulated export transporters (MRP3 and MRP4) to the systemic circulation. Increased bile acid synthesis due to activation of SREBP and loss of CYP7A1 inhibition. Altered regulation due to displacement of nuclear receptors (FXR, PXR, and CAR).

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