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CHOLESTATIC ALTERATIONS IN THE CRITICALLY ILL: SOME NEW LIGHT ON AN OLD PROBLEM

Running title

CHOLESTATIC ALTERATIONS IN THE CRITICALLY ILL

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Key words: critical illness, sepsis, cholestasis, BA, bilirubin, liver

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BA	Bile acids
FXR	Farnesoid-X-Receptor
γGT	Gamma-glutamyltranspeptidase
GLDH	Glutamate dehydrogenase
ICU	Intensive Care Unit
RXR	Retinoid-X-Receptor

Abstract

Hepatic dysfunction and jaundice are traditionally viewed as late features of sepsis and other critical illnesses, and are associated with a complicated ICU stay. However, recent studies suggest that cholestatic alterations already occur early in the course of critical illnesses, perceived only as minor abnormalities in routinely used biochemical liver tests. Inflammation-induced alterations in the transport of bile acids appears to drive bile acids and bilirubin towards the systemic circulation. Ongoing bile acid synthesis with an, at least partial, loss of feedback inhibition further contributes to elevated circulating bile acids and bilirubin. To what extent these changes reflect a biochemical epiphenomenon, true illness-induced hepatic dysfunction or a beneficial and adaptive response to illness should be further investigated.

Because of the lack of specificity of standard laboratory tests, especially in the context of a complex systemic condition as critical illness, identifying true cholestatic liver dysfunction remains a great challenge. However, very high levels of cholestatic markers that are sustained in prolonged critically ill patients almost always indicate a complicated illness course and should be monitored closely. Prevention of cholestatic liver dysfunction comprises minimizing inflammation and hypoxia in the liver and preventing hyperglycemia, avoiding early use of parenteral nutrition and reducing the administration of avoidable drugs.

Future research on the effects of bile acids and on modulating underlying drivers of critical illness-induced cholestasis is warranted as this could open perspectives for a targeted diagnostic approach and ultimately for novel therapies to improve outcome.

Introduction

During sepsis and other critical illnesses, the liver is a fundamental player in the host defense mechanisms as it regulates a wide range of metabolic, endocrine and immunological processes[1,2]. However, inflammation and hypoxemia severely challenge the hepatic microcirculation and function. Abnormal liver tests, such as hyperbilirubinemia, elevated gamma glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) are reported in up to 61% of patients admitted to the ICU[3-5]. In fact, sepsis is reported as the second leading cause of jaundice in patients presenting at the emergency department[6]. Strongly elevated values, several fold the upper limit of normality, are associated with distinct hepatic pathology such as hepatic ischemia or (iatrogenic) pharmacological effects and indicative of poor outcome[7]. However, the large majority of deranged liver tests are only slightly outside the normal range. Nonetheless, also low-grade hyperbilirubinemia and mild rises in plasma concentrations of liver enzymes are associated with a more complicated ICU course, a longer duration of ICU stay and a higher risk of death[3,4,8-10]. While obstructive cholestasis due to gallstones or by inflammation-driven narrowing of the bile ducts could induce these biochemical liver test abnormalities, it is rarely the cause of hyperbilirubinemia in critical illness[11-14]. Indeed, the precise etiology of mild alterations in liver tests is not completely understood and it remains unclarified whether they clinically reflect true hepatic dysfunction or are part of an adaptive stress response and reflect severity of illness.

In this review, we summarize the state of the art knowledge, integrating some novel insights, about mild and severe cholestatic alterations in intensive care patients, discuss the epidemiology along with the relationship with outcome, the clinical diagnostic approach, the underlying (patho)physiological pathways and possible therapeutic implications.

Definitions, incidence and prognostic value of liver test alterations in critical illness

Liver test abnormalities are often found in the critically ill patient, the majority of which can be classified in two major pathological entities: "hypoxic hepatitis" and "critical illness-induced cholestasis" (Figure 1) [15]. Hypoxic hepatitis, or shock liver, presents as an early and severe rise in hepatic lysis enzymes ALT and AST, which reflects diffuse hepatic injury as a consequence of failed oxygen delivery

and ischemia of the liver, due to any cause of severe shock. Mortality of patients diagnosed with hypoxic hepatitis is directly dependent to the severity of the underlying condition, and overall high mortality rates are documented[16,17]. The typical time course shows a rapid, steep increase in plasma ALT or AST concentrations reaching at least 20x the upper limit of normality[15,17]. Hypoxic hepatitis presents in up to 10% of ICU patients, of which around at least 75% fulfill these diagnostic criteria already upon admission[16,17]. Importantly, one-third of patients suffering from hypoxic hepatitis will also develop jaundice[18].

More frequently observed in the ICU department are mild liver test abnormalities with predominantly cholestatic features, which is currently labeled “critical illness-induced cholestasis” (Figure 1). Cholestasis is clinically defined as decreased or absent bile-flow towards the duodenum, caused by either impaired bile formation or an inability to excrete bile through the biliary system. This manifestation is traditionally considered a late event in sepsis and multi-organ failure, although recent findings indicate that such signs can also be observed in the more early stages of severe illness[3,8,19]. Whereas hypoxic hepatitis evidently relates to patients with severe shock and liver hypoxia, critical illness-induced cholestasis appears to be hallmarked by inflammation-driven cellular alterations resulting in a transient accumulation of bile acids (BA) and bilirubin within the liver and in the systemic circulation. In daily clinical practice, the most often used diagnostic criterion for critical illness-induced cholestasis is a total plasma bilirubin concentration higher than 2 or 3 mg/dl (34-41mmol/L) often combined with a maximally two or threefold increase in ALP and γ-GT levels. Accordingly, up to 20% of ICU patients fulfill these diagnostic criteria of critical illness-induced cholestasis at admission[3,8,19]. However, cholestatic alterations may be more prevalent, considering that most patients display milder derangements of cholestatic markers that do not exceed these cutoff levels[20]. The occurrence of critical illness-induced cholestasis has been related to the use of parenteral nutrition, antibiotics or other medications and to mechanical ventilation with high levels of PEEP[8,21,22]. Other typical risk factors for onset of critical illness-induced cholestasis are sepsis with gram-negative bacteria, major surgery, severe trauma or other severe shock states[14,15]. In addition, pre-existing (subclinical) presence of alcoholic, viral or toxin-induced hepatitis might further accentuate liver test abnormalities during critical illness[23].

Diagnostic tools for critical illness-induced liver dysfunction and cholestasis

The liver plays a pivotal role in key processes of metabolism, synthesis of acute phase proteins and coagulation components and excretion of toxic compounds. However, no single biomarker currently reflects all these functional activities (Table 1). In current daily practice, bilirubin is the most widely used biomarker in the assessment of liver function and has been incorporated in several organ dysfunction scores[24,25]. In addition, elevated circulating levels of ALT, AST, ALP and γ-GT can further indicate liver injury and cholestasis[5,8]. However, because of the lack of specificity of these standard laboratory tests, identifying true (cholestatic) liver dysfunction remains a great challenge[8].

Bilirubin is the routinely used marker in the assessment of excretory dysfunction. However, the reliability of hyperbilirubinemia as a marker of cholestasis in critically ill patients may be questioned, since multiple ICU-associated factors can influence levels of circulating bilirubin, such as changes in red blood cell turnover and blood transfusions[26]. Furthermore, bile excretion is a secretory process that depends largely on the osmotic drive induced by BA[27,28]. If bile-flow is hampered as a consequence of retained BA, bilirubin will also accumulate, but essentially reflects a biochemical epiphenomenon. Emerging evidence indicates that elevated BA levels reflect critical illness-induced cholestasis with a higher sensitivity and specificity, independently from bilirubin[29]. Furthermore, BA could potentially be useful as disease severity marker. Indeed, in a cohort of critically ill patients, total bile acids predicted 28-day mortality independently of severity of illness, gender, age and serum bilirubin concentration[30]. Moreover, molecular alterations in intrahepatic BA regulation appear to precede biochemical laboratory abnormalities[31]. However, BA isoforms including the conjugated fractions are difficult to measure and not readily available in clinical practice.

Increased γ-GT and ALP are very sensitive indicators for the diagnosis of liver and bile duct diseases[32]. Their synthesis and systemic release are observed during cholestasis with increased intra-biliary BA[33]. Increased levels of γ-GT and ALP are observed in 20-30% of ICU patients at admission and decrease thereafter[8,34]. Surprisingly, elevated levels of γ-GT and ALP are not associated with complicated clinical events, such as mechanical ventilation, hypoxia, higher APACHE II scores, or antibiotic use[8]. Furthermore, both markers have a high sensitivity but low specificity, making them weak indicators when used for the individual assessment of hepatic dysfunction in a single patient[32].

The release of ALT and AST in the circulation is largely dependent on cellular necrosis and evidently related to severe hypoxia (Figure 1). However, levels may be mildly elevated during critical illness, as inflammatory signals increase cellular permeability and could promote enzyme leakage. The enzyme glutamate dehydrogenase (GLDH) is predominantly localized in the centrolobular region, which is more susceptible to hypoxia, and may potentially point to acute liver injury with more accuracy[35]. However, the use of GLDH in the context of critical illness has not been systematically investigated.

Next to static laboratory measurements, dynamic tests such as indocyanine green can be used to assess excretory function of the liver. Indocyanine green is an organic anion that is exclusively eliminated by the liver. In a recent study, dynamic measurement of hepatic biotransformation and excretion was investigated in critically ill animals using the clearance rate of indocyanine green[29]. In these critically ill mice, dye was rapidly cleared from the circulation and taken up by liver cells, but the appearance in bile was delayed, indicating that defective cellular function primarily involves excretion of transformed molecules from the hepatocyte into the biliary canaliculi rather than the uptake in liver cells[29]. Furthermore, in a retrospective analysis from 336 critically ill patients, plasma disappearance rate of indocyanine green correlated with mortality with comparable sensitivity and specificity as the SAPS and APACHE II scores[36]. Moreover, in patients with hypoxic hepatitis, dynamics of plasma disappearance rate of indocyanine green over time demonstrated that plasma clearance did not increase over time in patients who died within 28-days on the ICU, whereas a clear improvement was observed in patients who survived[37]. Noteworthy, plasma disappearance rate of indocyanine green is greatly depending on liver perfusion and impaired clearance rates may reflect both liver hypoperfusion and reduced excretory function[38]. Furthermore, redistribution in extravascular tissues, protein binding and complex pharmacokinetics further complicate the interpretation of the plasma disappearance rate of indocyanine green[39]. Hence, indocyanine green should be used with caution in the complex clinical context of critical illness[39].

Outside the context of critical illness, hepatic synthesis capacity can be estimated by quantifying serum concentrations of proteins that are exclusively produced by the liver, such as albumin. Albumin is the most abundant circulating protein and accounts for up to 50% of hepatic protein synthesis. However, serum protein concentrations do not directly reflect protein synthesis, as breakdown and distribution to the

extravascular compartment also play a role. During critical illness, low albumin can reflect blood losses, hemodilution, and extravascular albumin leakage, rather than hepatic synthesis dysfunction[40,41]. Also, availability of coagulation factors, indirectly estimated via measuring standardized prothrombin time (INR), is used to assess the synthetic capacity of the liver. The INR is used to calculate the Model for End stage Liver Disease-score (MELD), in turn used to prioritize end stage liver disease patients for liver transplant25. However, in the context of critical illness, other factors, such as bleeding, hemodilution, disseminated intravascular coagulation and the administration of coagulation factors or fresh frozen plasma, can alter the INR[41]. It is clear that neither albumin nor INR are preferred markers to test synthetic function of the liver in critically ill patients. However, observational data suggest that the hepatocellular synthesis performance could be estimated with use of serum cholinesterase[42,43]. Indeed, serum cholinesterase activity is lower than normal in patients with burns and septic shock[44] and correlates with the severity of the injury[42]. However, because normal values span over a wide range, clear diagnostic cutoffs for cholinesterase activity to diagnose liver dysfunction in critical illness are lacking. Furthermore, healthy volunteers showed both increased and decreased cholinesterase activity after an endotoxin challenge[45]. Possibly, in the context of critical illness, cholinesterase activity reflects the systemic cholinergic status rather than the synthetic capacity of the liver[42,45].

Critically ill patients also often develop post-hepatic biliary abnormalities. Most frequently, biliary sludge is observed during critical illness, with a prevalence of approximately 50-60% in patients requiring more than 5 days of intensive care[19]. True mechanical biliary obstruction can be easily and reliably assessed with use of abdominal sonography. However, mechanical biliary obstruction is rarely the cause of deranged cholestatic liver parameters in the context of critical illness, which argues against a routine sonographic screening for calculous cholesystitis[11,13]. However, decreased blood supply via the hepatic artery can impair the microcirculation of the gallbladder wall and lead to acalculous cholecystitis, inflammation and necrosis. This rare but severe condition is associated with a complicated ICU stay and can be lethal[12]. The use of ultrasound to detect such acalculous cholecystitis is only of value in combination with a high level of clinical suspicion[46]. Also the use of CT imaging for acalculous cholecystitis has previously shown to be too aspecific, as up to 96% of patients displayed gallbladder abnormalities[47]. Importantly, persisting cholestatic serum abnormalities may indicate the development of

163 secondary cholestatic pathologies, such as secondary sclerosing cholangitis, in turn associated with poor
164 outcome[48,49]. For patients who continue to reveal cholestatic serum abnormalities, also after clinical
165 improvement, further clinical follow up with biliary imaging is advisable.

166 As to date, no specific liver test for critical illness-induced cholestasis is available, it remains a
167 'diagnosis of exclusion'. In absence of a recognized hypoxic or pharmacotoxic insult, obstruction or pre-
168 existing liver disease, abnormal liver tests more likely indicate critical illness-induced cholestasis.
169
170 **Underlying pathophysiological pathways of critical illness-induced cholestasis**
171 Critical illness-induced cholestasis appears to be the result of functional intracellular alterations in
172 BA homeostasis, with changes observed in intrahepatic BA synthesis, feedback-regulation, conjugation
173 and BA transportation[31,50]. BA are actively produced in the liver from its precursor cholesterol through an
174 extensive enzymatic cascade[51]. Key proteins in BA production are CYP7A1 and CYP27A1, the rate
175 limiting enzymes of the normal and alternative synthesis pathway respectively[51,52]. BA can suppress their
176 own synthesis through binding with its regulating nuclear receptors Farnesoid-X-Receptor (FXR) and the
177 Retinoid-X-Receptor (RXR). Upon binding with BA, FXR-RXR dimers translocate to the hepatocyte
178 nucleus and regulate DNA transcription of Small Heterodimer Partner (SHP), which inhibits CYP7A1 and
179 CYP27A1[52]. In an observational study of non-surviving ICU patients, circulating BA levels were 11-fold
180 increased as compared with elective surgery patients, but in the liver biopsies, protein expression of
181 CYP7A1 was not suppressed. Furthermore, hepatocytic nuclear content of the key sensors and
182 transcriptional regulators of BA synthesis and intrahepatic transport, FXR and RXR, was remarkably low,
183 suggesting an at least partial loss of feedback-inhibition in high risk critically ill patients[4].
184 Bilirubin and BA are normally conjugated within the hepatocyte to detoxify and convert these
185 molecules to less toxic forms[51,52]. While in a lethal animal model of sepsis, a BA pattern indicative of a
186 conjugation defect was documented[29], data from non-surviving ICU patients suggested that conjugation
187 was unaffected by critical illness[4]. In an observational study of 280 critically ill patients, conjugation of
188 primary BA even increased over time in ICU[20]
189 Hepato-biliary BA transporters are the final critical determinant of BA homeostasis and control
190 intrahepatic and circulating levels of BA[27,28]. In normal hepato-biliary transport, BA enter the hepatocyte

from the portal circulation via Na+-Taurocholate Cotransporting Polypeptide (NCTP) at the basolateral membrane. In addition, several multispecific anion transporters (OATPs) facilitate transport of BA, bilirubin and other hormones and compounds. The canalicular membrane transporter bile salt export pump (BSEP) regulates the excretion of BA into the bile duct lumen. The multispecific export transporters multidrug resistance-associated protein 2 (MRP2) and multidrug resistance proteins (MDR1, 2, 3) mediate excretion of BA, bilirubin and various xenobiotic compounds. Alternatively, BA can be excreted into the systemic circulation via multidrug resistance-associated protein 3 and 4 (MRP3, MRP4). In liver biopsies harvested from critically ill patients who died in the ICU, expression of hepatic BA transporters was altered in comparison with liver biopsies from patients with elective non liver related surgery, suggesting a diverted export of bilirubin and BA to the systemic circulation[53]. Gene expression of hepatic basolateral uptake transporters NTCP and OATP's was suppressed, as well as apical exporter BSEP gene expression. Interestingly, export pumps MRP3 and MRP4 were strongly upregulated, which may serve as alternative escape routes from hepatocytes into the sinusoidal blood[4]. Inevitably, this postmortem study was restricted to the most severe phenotype of ICU patients. It is not clear whether such reversal in BA transport is a general phenomenon occurring in all types of critical illnesses. However, also acute animal models of sepsis have shown changes in the maximal transport velocity of BA and organic anions along with downregulation in gene and protein levels of BA transporters[29,54].

In vitro and acute models of sepsis suggest cytokines as possible drivers of cholestatic alterations in critical illness. Indeed, cell culture models showed similar nuclear export of RXR after administration of IL-1β and TNFα[55]. In addition, in acute models of cytokine-induced sepsis, signs of diverted transport were observed[56,57]. However, there was no association between markers of inflammation and markers of cholestasis in liver biopsies from ICU-nonsurvivors, suggesting that inflammation may not be the main contributor to cholestatic phenotype in prolonged critical illness[4]. In addition, disturbances in the microcirculation of the biliary system could play a part in the development of hyperbilirubinemia in critical illness. Ischemic cholangiopathy, secondary to severely impaired biliary blood supply, can contribute to bile stasis and the formation of biliary casts, and can promote the development of clinical jaundice[58,59].

Clinical implications of critical illness-induced cholestasis

The current novel insights from studies on bilirubin and BA synthesis and transport in the critically ill, and their regulation by nuclear receptors do not suffice to conclude whether they represent a "failure" of liver functions or instead reflect a beneficial compensatory response within the hepatocyte in response to critical illness. Thus, whether the typical mild cholestatic alterations observed in most if not all ICU patients reflect an adaptive response or indicate impaired organ function is still unclear. The diverted hepato-biliary transport could indeed reflect an adaptive escape mechanism for the hepatocyte to reduce the high energy demanding transport of bile components against the concentration gradient into the biliary system. Alternatively, in order to increase favorable effects of systemic bilirubin and BA signaling, ongoing BA production and reversal of intrahepatic transport towards the circulation might also reflect an adaptive and beneficial process. Indeed, via a positive feedforward mechanism, even small amounts of bilirubin may exert known antioxidative and cytoprotective roles[60]. Bilirubin has shown to be protective in various animal models, including sepsis[60-63]. In an animal model of LPS-induced sepsis, bilirubin treated rats showed attenuated hepatic organ damage and had improved survival, at least partially mediated by inhibited hepatic iNOS expression[63]. In addition to bilirubin, metabolic and endocrine effects of BA could play a protective role in the hypermetabolic and inflammatory response of acute critical illness. BA are currently being investigated as promising new drug targets for metabolic diseases as they play a role in glucose, cholesterol, triglyceride and energy homeostasis[64-67]. Furthermore, animals with reduced BA signaling are more susceptible to liver injury in LPS-induced sepsis, and show higher cytokine mRNA levels, more pronounced inflammatory infiltrates and an increased rate of apoptotic cells[68]. In addition, injection of (conjugated or unconjugated) BA have shown to increase circulating cortisol levels in rats by reducing the expression and activity of cortisol metabolizing enzymes[69]. A positive correlation between the rise in BA and plasma cortisol has also been described in critically ill patients, suggesting that the reduced breakdown of cortisol, recently documented as a universal phenomenon in the critically ill patient, may be at least partially explained by increased circulating levels of BA in response to critical illness. BA could thereby contribute to elevated plasma cortisol concentrations and higher cortisol availability in those vital organs and tissues that express the cortisol-metabolizing enzymes, thus playing a key role in the inflammatory and metabolic stress response[70].

In contrast, since BA play a vital role in intestinal integrity and reduce bile-flow in the intestine, absence of flow may promote translocation of the gut microbiome and elevate levels of LPS in the portal circulation[71-73]. This might result in sustained inflammatory signals as is often observed in prolonged critical illness[71,72]. In addition, prolonged lack of BA in the intestine can cause malabsorption of lipids and vitamins.

In addition, beneficial effects of other cholestatic markers, such as ALP, have been suggested. ALP has shown to elicit favorable effects in animal experiments and in two small phase II clinical trials of critically ill patients suffering from acute kidney injury[74-76]. Administration of recombinant ALP improved markers of kidney function and inhibited the upregulation of renal inducible NO synthase, leading to subsequently reduced NO metabolite production[75]. Furthermore, experimental data from animal studies suggest that ALP is able to detoxify LPS and may provide a new strategy to attenuate LPS-induced diseases[77,78]. In addition, next to the detoxification of LPS, presence of ALP prevented bacterial invasion across the intestinal mucosal barrier in an ischemia/reperfusion mouse model[79].

Importantly, the clinical impact of an altered hepatic transport on compounds normally excreted by the hepatocyte, such as toxins, drugs and other metabolic substances remains uncertain. The exogenous administration of antibiotics and other drugs can accumulate due to decreased bile excretion. Furthermore, certain drugs could amplify the underlying cholestatic alterations as several drugs routinely used in the ICU are themselves associated with the occurrence of cholestasis[29,80].

Therapeutic implications

Because critical illness-induced cholestasis is primarily a result of functional alterations at the hepatocyte and bile duct level, it is usually reversible and there is no clear argument for attempting to treat this condition. Up until today, there is in fact no treatment available for critical illness-induced cholestasis, but the general management of the ICU patient focusses on avoiding additional inflammatory stimuli and hypoxia in the liver microenvironment. Prevention however appears possible to a certain extent. Indeed, optimizing supportive care and reducing risk factors that could provoke severe cholestasis, such as the excessive use of antibiotics and other drugs, preventing hyperglycemia and not using parenteral nutrition

during the first week of critical illness have shown to prevent cholestatic features observed during critical illness[19,81,82].

Adequately optimizing hemodynamic perfusion is an essential step in preventing liver dysfunction[83,84]. Importantly, experimental data suggest that some catecholamines can also exacerbate hepatic dysfunction observed during sepsis by altering the biotransformation of drugs[85]. As hepatic biotransformation and biliary export are the primary mechanisms for clearance of most drugs and toxins, routinely used drugs in intensive care medicine can accumulate in the critically ill patient[80,86]. Compounds that need to be metabolized in the liver and those that need to be excreted via the bile are known to have hepatic adverse effects[87]. Commonly used drugs used in daily critical care practice, such as the majority of antibiotics, anesthetics and sedatives therefore form an additional threat for the hepatocyte. Although insights in individualized therapy are increasing rapidly, there are currently no commonly used strategies to adjust doses for decreased biliary excretion in the critically ill.

Lowering blood glucose to healthy fasting ranges, thereby avoiding hyperglycemia, in adult critically ill patients also reduced the occurrence of cholestasis and biliary sludge, as revealed by 2 randomized controlled trials[19,81,82]. Also the impact of early initiation of parenteral nutrition, as compared with accepting a large macronutrient deficit for one week in the ICU when only using enteral feeds, on the occurrence of cholestasis has been investigated. For hemodynamically stable patients with a functioning gastrointestinal tract, early enteral feeding is currently the recommended standard of care. Enteral nutrition promotes the intestinal reuptake of BA and recovery of the enterohepatic cycle[88]. Furthermore, restoration of the enterohepatic cycle by enteral nutrition improves gut oxygenation and barrier function, and may reduce microbial translocation and inflammatory signaling in the liver[89,90]. The use of enteral nutrition compared to parenteral nutrition has been associated with fewer infections and reduced occurrence of metabolic complications in patients with liver disease and after liver transplantation[91]. However, enteral feeding is often not well tolerated by ICU patients. A large randomized controlled trial assessed the impact of early versus late initiation of parenteral nutrition in adults and found an important effect on liver function markers[92]. Indeed, this study showed that not using early parenteral nutrition not only shortened ICU and hospital length of stay and reduced the number of new infections, it also improved laboratory markers of hepatobiliary damage (maximum levels of ALT, γ-GT and ALP)[92,93] and lowered the

incidence of biliary sludge[20]. Surprisingly, levels of bilirubin were slightly but significantly higher in the group not receiving early parenteral nutrition[20]. Similar results were observed in the pediatric equivalent of this study[93]. Underlying mechanisms of the beneficial effects of delaying parenteral nutrition have been investigated in a prolonged critical illness rabbit model[53]. In this study, withholding parenteral nutrition reduced markers of hepatocyte injury and further accentuated the reversed BA transport towards the systemic circulation, suggesting that fasting by itself could partially initiate some "cholestatic" alterations during critical illness[53].

Conclusions

Hepatic dysfunction and jaundice are traditionally viewed as late features of sepsis and as part of multi-organ failure. However, recent studies suggest that minor "cholestatic" alterations occur already early in the course of critical illness, but these would not be considered as a clinically relevant problem according to advised thresholds for normality of standard laboratory tests. Inflammation- and hypoxia-driven alterations in the synthesis and transport of BA and bilirubin explain these early and often mild alterations. Elevated values of routinely used laboratory tests appear to be brought about by altered BA and organic anion transport machineries at the basolateral and biliary pole that appear to drive bile components towards the systemic circulation. To what extent these changes reflect a biochemical epiphenomenon, true illness-induced hepatic dysfunction or a beneficial and adaptive response to illness should be further investigated. It is known that BA and bilirubin play important roles in various metabolic and inflammatory pathways and mild elevation of these compounds could attenuate the severe burden of critical illness. Possibly, in the context of a complex systemic condition as critical illness, routinely used liver tests may not necessarily represent similar processes than in patients with primary liver disease. However, very high levels of cholestatic markers that are sustained in prolonged critically ill patients almost always indicate a complicated illness course and should be monitored more closely. The fascinating possibility of beneficial effects of increased BA availability during critical illness opens perspectives for future research in a targeted diagnostic approach and, ultimately, in the development of novel therapies aimed to improve outcome.

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561 **Table 1: Diagnostic tools for critical illness induced cholestasis**

Parameter	Description and rationale	Advantage/disadvantage as diagnostic marker for ICU cholestasis
Total Bilirubin	Catabolic end-product of senescent red blood cells. Elevated levels indicate high secretion, intrahepatic dysfunction or post-hepatic obstruction	+ Partially indicates hepatic excretory function + Correlates with ICU outcome - Biliary excretion dependent on BA-flow - Differentiation in pre/intra/post hepatic origin difficult
Direct Bilirubin	Conjugated isoform of bilirubin. In addition to total bilirubin, elevated levels indicate a maintained conjugation function	+ Indicates detoxification/conjugation function + Partially indicates hepatic excretory function - Biliary excretion dependent on BA flow - Differentiation in pre/intra/post hepatic origin difficult
Bile acids (BA)	Intrahepatic synthesized drivers of bile-flow. Elevated levels indicate high secretion, intrahepatic dysfunction or post-hepatic obstruction	+ Indicates ongoing synthesis and impaired feedback + Indicates excretory function - Differentiation in pre/intra/post hepatic origin difficult - Conjugated/unconjugated fraction difficult to measure
Aspartate Transaminase (AST)	Intracellular enzyme mainly located in liver, heart, skeletal muscle, kidney, brain. Serum activity of AST increases after hypoxia with liver parenchymal cell loss	+ Indicates parenchymal cell loss - Parenchymal cell loss not primary present - Does not indicate liver (dys)function
Alanine Transaminase (ALT)	Intracellular enzyme mainly located in liver. Serum activity of ALT increases after hypoxia with liver parenchymal cell loss	+ Indicates parenchymal cell loss - Parenchymal cell loss not primary present - Does not indicate liver (dys)function
Gamma-glutamyl transpeptidase (γ-GT)	Intracellular enzyme mainly located in liver, bile ducts, kidney and bone. Cholestasis enhances ALP synthesis and release to the circulation	+ High sensitivity - Low specificity
Alkaline phosphatase (ALP)	Intracellular enzyme mainly located in liver, bile ducts, kidney and bone. Biliary 'stasis' enhances ALP synthesis and release to the circulation	+ High sensitivity - Low specificity - Also increased in bone/skeletal disease
glutamate dehydrogenase (GLDH)	Intracellular enzyme mainly located in the (oxygen poor) region of the liver. Serum activity of GLDH increases earlier after hypoxia with liver parenchymal cell loss	+ May detect early hypoxic injury with higher accuracy - Does not indicate (dys)liver function - Use in ICU patients not well investigated
Indocyanine green plasma disappearance rate	Cyanine dye that is removed from the circulation exclusively by the liver. Decreased levels indicate impaired splanchic blood flow or excretory function	+ Partially indicates excretory function + Correlates with mortality and severity of illness - Confounded by hepatic blood flow
Cholinesterase activity	Intrahepatic synthesized esterase that lyses choline-based esters. Decreased levels indicate impaired hepatic synthetic capacity	+ Might indicate lower synthetic function of the liver - Use in ICU patients not well investigated - No clear diagnostic criteria - Confounded by drugs

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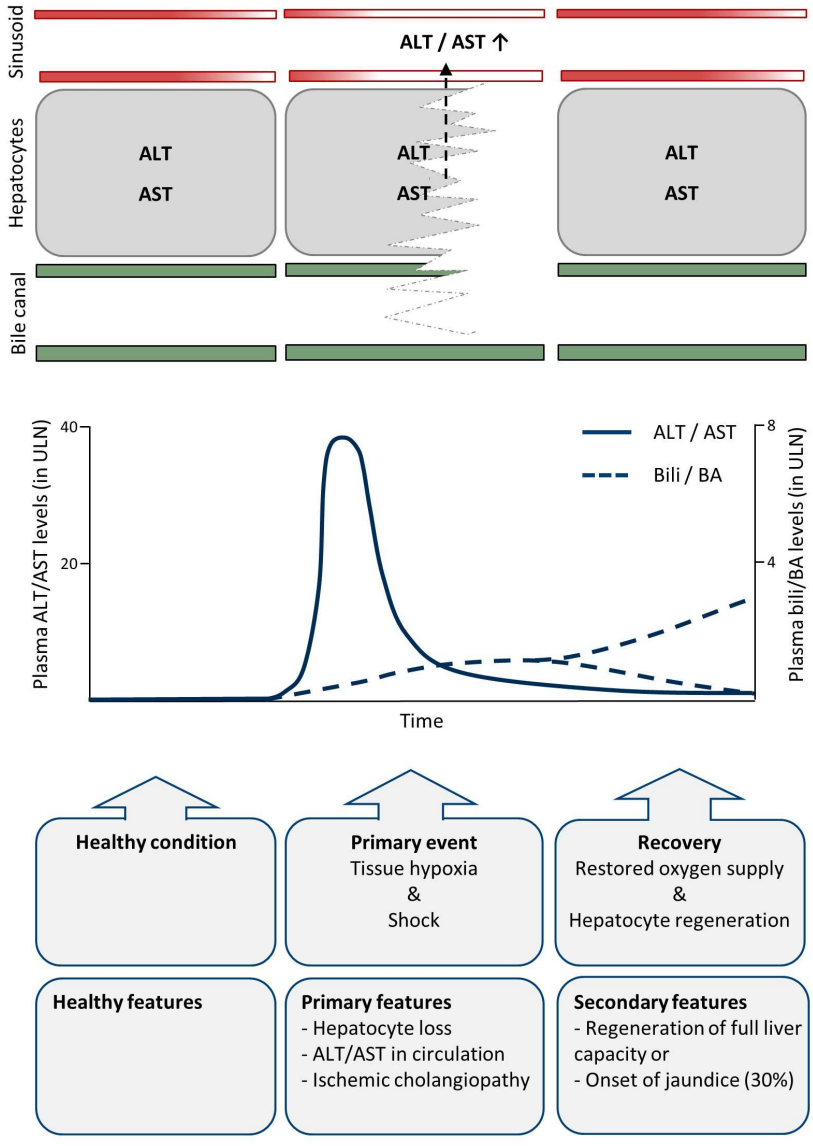
564 **Figure 1: Schematic overview of hepatic alterations and serum markers in hypoxic hepatitis and**
565 **critical illness induced cholestasis.** A: In hypoxic hepatitis, oxygen supply to the liver is impaired,
566 resulting in cellular necrosis of hepatocytes. Accordingly, ALT and AST, and to a lesser degree ALP and
567 γ-GT are released into the circulation. Excretory function measured by bilirubin and BA may be mildly
568 impaired. In the recovery phase, oxygen supply to the liver is restored and hepatocytes regenerate. In
569 30% of patients, clinical jaundice develops after hypoxic hepatitis. B: During ICU cholestasis, intrahepatic
570 alterations in the liver transport machinery results in higher circulating bilirubin and BA. Biliary stasis
571 promotes release of ALP and γ-GT from cholangiocytes. Mild elevation of ALT and AST may also be
572 present. Abbreviations: Bili: Bilirubin, BA: bile acids, ALT: alanine transaminase, AST: aspartate
573 transaminase, ALP: alkaline phosphatase, γ-GT: gamma glutamyl transpeptidase, ULN: Upper limit of
574 normality.

A. Hypoxic Hepatitis



B. Critical illness induced cholestasis