

Tinjauan pustaka

## **HEPATIC ISCHEMIA REPERFUSION INJURY IN SEPSIS: BASIS PATHOGENIC MECHANISMS**

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### SUMMARY

Hepatic ischemia reperfusion injury is a complex patho-physiology with a number contributing factors. Ischemia insult can lead to sublethal cell injury, which is aggravated by the formation of reactive oxygen from various intracellular sources during reperfusion. In addition, formation of proinflammatory mediators and the recruitment and activation of macrophages, neutrophil and lymphocyte can further enhance the injury. Microcirculatory disturbances lead to underperfused areas in the liver and may cause ischemic injury. Hepatic IR injury involves interaction between different cell types and a variety of cellular and molecular mechanisms including kupffer cells activation, formation of ROS, release of cytokines and chemokines, neutrophil recruitment, mitochondrial permeability transition and pH paradox. There are two distinct phase of liver injury after warm ischemic reperfusion, such as early phases and followed by late phases. Clinical presentation of hepatic ischemic reperfusion injury in sepsis, including sepsis-associated cholestasis, hepatitis ischemic, cholangitis lenta and progressive sclerosing cholangitis.

### INTRODUCTION

Hepatic ischemia reperfusion injury is a phenomenon whereby cellular damage in hypoxic liver is accentuated following the restoration of oxygen delivery. Of note, injury to the liver after any type of ischemia is apparent mainly after reperfusion when oxygen supply and blood element are restore to the liver. Hepatic ischemic reperfusion injury can be categorized into warm ischemic reperfusion and cold-storage reperfusion injury.<sup>1</sup> Warm ischemic injury is common during sepsis or septic shock. Ischemia is tolerated only a period of about 1 h in normal individual and about 30 min in cirrhosis liver, and the duration of ischemia correlates with the degree of reperfusion injury. Warm ischemia leads rapidly to the death of hepatocytes.<sup>2</sup> Hepatic ischemia followed by reperfusion results in severe injuries that con-

tribute to the morbidity and mortality associated with shock.<sup>3</sup> Although substantial progress has been made in elucidating mechanisms ischemic-reperfusion injury, there still need to better understand of the pathophysiological processes.<sup>4</sup>

### HEPATIC ISCHEMIA REPERFUSION INJURY

In the hepatic ischemia reperfusion injury, controversy has emerged in recent years over whether necrotic or apoptotic cell death account for severe parenchymal injury. Some reported that the overwhelming part of parenchymal injury observed is caused by massive necrotic alterations. In contrast, that specific inhibition of apoptosis significantly prevented parenchymal injury and improved survival after prolonged periods of ischemia. The apoptotic cell death is initiated by binding

of tumor necrosis factor (TNF)-alpha to the specific receptor TNF-R1 on the cell membrane of hepatocyte in warm ischemic injury. The apoptotic signal is then transferred into the cell where different caspases are activated. This process leads to DNA fragmentation and cell death. In this process mitochondria act as amplifiers of signal, allowing even weak signals to have deleterious effects. With blocking early mechanisms of apoptosis (phase 1 and 2) can prevent parenchymal injury.<sup>2</sup>

Hepatic ischemia reperfusion injury is a complex patho-physiology with a number contributing factors. Ischemia insult can lead to sublethal cell injury, which is aggravated by the formation of reactive oxygen from various intracellular sources during reperfusion. In addition, formation of proinflammatory mediators and the recruitment and activation of macrophages, neutrophil, and lymphocyte can further enhance the injury. Microcirculatory disturbances lead to underperfused areas in the liver and may cause ischemic injury. During reperfusion, cells are killed by a combination several mechanisms including intracellular oxidant stress, exposure to external cytotoxic mediators, and prolonged ischemia. Cell death of hepatocytes and endothelial cells during reperfusion is characterized by swelling of cells and their organelles, release of cell contents, eosinophilia, karyolysis, and induction of inflammation are characteristic for necrosis, however, that most of liver cells actually die by apoptosis, that characterized by cell shrinkage, formation of apoptotic bodies with intact cell organelles and the absence of inflammation, especially during the first 3-6 h of reperfusion.<sup>4</sup>

Most of damage after hepatic ischemia occur during reperfusion. Ischemia reperfusion injury is characterized by inflammation and vascular occlusion during reperfusion periods. The degree of liver damage that occurs as a primary injury during ischemia, could be aggravated by secondary damage that occurs during reperfusion. Inflammatory event that occurs during reperfusion leads to disruption of the integrity of the

vascular endothelium and sinusoids, platelet aggregation, immunocyte activation (monocyte/macrophages, kupffer cells, neutrophil), chemokine and cytokine secretion, and complement activation. Induction of chemokines has been suggestive as a possible contributory factors in ischemic reperfusion injury-induced inflammation. Certain chemokines act as activator of neutrophil and monocyte diapedesis in the early stage of reperfusion injury. Chemokines may trigger the expression of adhesion molecule (p-selectin, ICAM). CXC chemokines production from kupffer cells decreases the degree of liver damage during ischemic reperfusion. Has been thought that T cells are recruited in late stage (48-72h) but not in the early stage (24h) reperfusion injury, but small number of T-lymphocyte that participate in early ischemic reperfusion injury.<sup>5</sup>

The liver injury induced by ischemia-reperfusion has two distinct phases.

The initial phases (early phases), of this response characterized by activation of kupffer cells and their production of reactive oxygen species (ROS), leading to mild hepatocellular injury. The generation of oxidant during this phase is also thought activate redox-sensitive transcription factors such as NF-kB and activator protein-1, which control of expression of pro-inflammatory mediators, such as TNF-alpha. The expression of TNF-alpha followed by later phases (late phases) of the liver injury characterized by the induction of second mediators, including neutrophil-attracting CXC chemokines and endothelial adhesion molecules, which facilitate the adhesion and transmigration of neutrophils from the vascular space into the hepatic parenchyma. Accumulated neutrophil release oxidant and proteases that directly injure hepatocyte and endothelial cells and may also obstruct hepatic sinusoids, resulting hepatic hypoperfusion.<sup>6</sup>

Acute primary apoptosis during early reperfusion is crucial to the initiation of reperfusion-induced inflammation, but TNF-alpha as a central of inflammatory

mediators was shown to contribute to late apoptosis of hepatic ischemic injury. TNF-alpha is also an inducer of the acute phase response (APR) including alpha1 acid glycoprotein (AGP) and alpha1-antitrypsin (AAT). These two major acute phase proteins exhibit various anti-inflammatory effects and have been shown to prevent hepatocyte apoptosis.<sup>7</sup>

Hepatic steatosis is a major risk factor in ischemic reperfusion, steatosis had increased lipid peroxidation and hepatic injury, however steatosis can decrease survival after 60 minutes ischemia. Several hypotheses have been suggested to explain the decreased tolerance of steatotic liver to ischemic reperfusion injury, include increased 1) lipid peroxidation, 2) neutrophil infiltration, 3) microcirculatory alteration, 4) release of proinflammatory such as TNF-alpha. Ischemic precondition is an endogenous protective mechanism by which brief periods of vascular occlusion, confer protection against subsequent sustained ischemic reperfusion. Despite intensive investigations, the underlying mechanisms remain to be elucidated.<sup>8</sup>

### **Resident cells intrahepatic activation during hepatic ischemia reperfusion injury**

An excess of inflammatory response is clearly recognized as a key mechanism of injury during ischemic reperfusion. In the early phases of hepatic ischemic reperfusion injury shown activation of Kupffer cells, mitochondria and endothelial cells, than in the late phases shown recruitment of neutrophil and T cells lymphocytes. With consequences release of oxidant stress. Proinflammatory cytokines, protease, nitric oxide, carbon oxide in early reperfusion and release of adhesion molecule (ICAM-1, P-selectin) in the late reperfusion.<sup>4</sup>

When hepatic ischemia reperfusion injury happens, a series of metabolic and structural and functional disorders of hepatic tissue cells would occur, which directly influence the prognosis of patients.

Apoptosis is a way of cell death is significant for maintaining normal cell development and stabilization, and it is closely related to initiation and development of clinical manifestation, and also participates in hepatic reperfusion injury. Mitochondria as one of organelle of cells play an important role in providing energy, adjusting osmotic pressure, calcium balance, pH value and cell signaling. Mitochondria confirm their function by production of ATP and ROS which are known as signals regulating gene expression and triggering cell death. The changes of structure and function of mitochondria was shown in hepatic ischemic reperfusion injury.

In hepatic ischemic reperfusion injury, several of stress factors can activate the apoptotic pathway mediated by mitochondria, which leads to increase the apoptosis, decrease of total number of parenchyma cells, and thus hepatic tissue and liver function are severely damaged. Mitochondria apoptotic pathway participates in hepatic reperfusion injury, and is a ring-point in these complicated mechanisms.<sup>9</sup>

Kupffer cells, the resident macrophages of the liver, are activated after ischaemia/reperfusion stress, and have been recognized to produce ROS, proinflammatory cytokines, chemokines, and other mediators. Pharmacological inactivation of Kupffer cells has been reported to suppress hepatic ischaemia/reperfusion injury, suggesting pathogenetic involvement of these macrophages. A transcription factor, nuclear factor kB (NFkB), has been determined to be crucial in the cascade bringing about Kupffer cell activation. ROS produced by Kupffer cells have been proposed to activate DNA binding activity of NF-kB in these cells. Thus inactivation of NF-kB in Kupffer cells might be of clinical value in blocking injurious events in total hepatic ischaemia/reperfusion. In hepatic ischaemia/reperfusion injury, activated liver macrophages (Kupffer cells) are dominantly regulated by a transcription factor, nuclear factor kB (NF-kB), with respect to expression of inflammatory cytokines, acute phase response proteins, and cell adhesion molecules.<sup>10</sup>

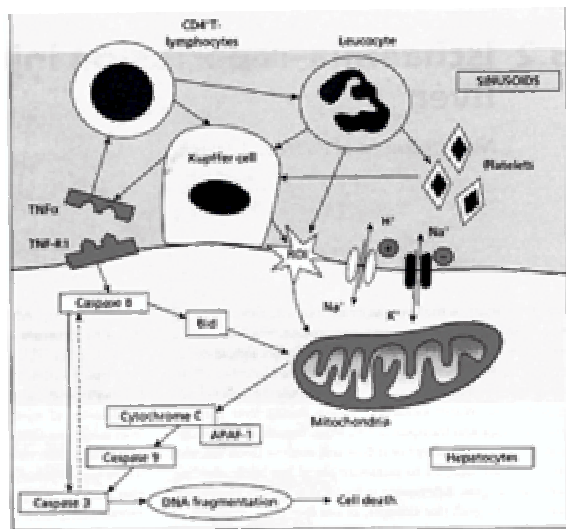


Fig 1. Mechanisms of warm ischemic injury. Major pathways include TNF- alpha mediated apoptosis, dysregulation of ion distribution, and the generation of reactive oxygen intermediates (ROI)<sup>4</sup>

The NO radical is generated in the liver by constitutively expressed eNOS or iNOS. However eNOS is only expressed in sinusoidal endothelial cells, and iNOS can be transcriptionally up-regulated in endothelial cells, hepatocyte and other liver cells types.

NO is potent vasodilator, which diffuse freely cross membrane and acts intracellularly by activation guanylate cyclase. In response to vasoconstrictors, NO can induced vasodilatation at the level of sinusoid as well as at presinusoidal sites. In addition to vasodilatory effect, NO reacts with superoxide to form the potent oxidant peroxynitrite. It has been assumed that eNOS-derived NO is responsible for maintaining liver blood flow and excessive NO formation by iNOS may lead to peroxynitrite-induced injury as well as systemic effects. Microcirculatory disturbances and nonperfused sinusoids are well recognized phenomena that contribute to reperfusion injury after hepatic ischemia. Endothelins have been identified as the most potent vasoconstrictors generated during reperfusion, and NO are produced

to effectively counteract the enhanced vasoconstrictive state during reperfusion.<sup>4</sup>

The injurious effect of I/R presents with a spectrum of clinical manifestations ranging from asymptomatic elevation of liver enzymes to acute liver failure and death.

It has been suggested that the response of the hepatic endothelium to I/R plays a key role in the development of injury. eNOS-derived NO may protect the liver from hepatic I/R-induced injury may related to its inhibition of platelet aggregation and adhesion as well as attenuation of endothelium-leukocyte interactions, all of which may be beneficial to reduce hepatic I/R injury. TNF- $\alpha$  has been shown to play a critical role in inflammatory responses during hepatic I/R injury. It was also found that eNOS-derived NO may protect animals after hepatic I/R by protecting the liver from the injurious effects of TNF- $\alpha$ .<sup>11</sup>

The process of leukocyte recruitment in the liver is quite different to other organ due to architecture specialties of hepatic microcirculation, such as dual blood supply, low pressure vascular system, fenestrated endothelium, and lack of basal membrane in sinusoids. Expression pattern of adhesion molecule is different. The hepatic sinusoids lack of expression of selectin, however the post sinusoids venule, selectins mediate initial leukocyte rolling, while beta2-integrins responsible for subsequent leukocyte adherence to the endothelium. In the sinusoids, leukocyte accumulation is not preceded by rolling, does not require selectins, but mediated by expressed endothelial intracellular adhesion molecule-1 and vascular adhesion protein-1.<sup>12</sup>

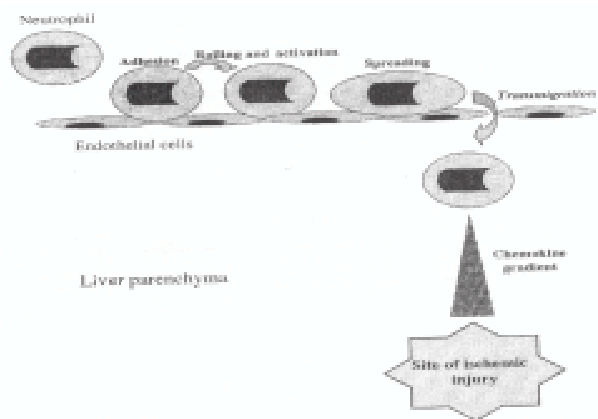


Fig.2. Mechanisms of neutrophil adhesion and transmigration. Initial interaction between neutrophil and endothelium , including adhesion and rolling, are mediated by selectins. Chemokine gradients in the liver parenchyma direct transmigrated neutrophils to the site of injury<sup>4</sup>

Ischemic/ reperfusion injury occur when blood flow to the organs or tissue is interrupted for some periods and subsequently reestablished. Subsequent event involves adhesion and infiltration of polymorphonuclear leukocyte (PMN) as an early step. Subsequently PMN may elicit tissue damage by diverse processes from direct elaboration of cytotoxic mediators. PMN adhesion is accompanied and followed by a complex sequence of hemodynamic event. Pressure and flow may vary dramatically in injury liver. Haemodynamic in the hepatic microcirculation are also influence by PMN adhesion to the sinusoidal lining. L-selectin receptors play a role in propagating adhesion and PMN plugging in the microvasculature through interaction between previously adherent and flowing PMN. Platelet and other blood substances may also adhere to stationary PMN, producing partial or complete plugging that leads to reduction or cessation of blood flow.<sup>13</sup>

Leukocytes are implicated in the pathology of hepatic ischemia reperfusion injury. Because activated leukocyte release variety of inflammatory mediators, including cytokines, neutrophil proteases, reactive oxy-

gen species, all of which can damage adjacent endothelial cells, they have been thought to play a role in tissue injury.<sup>14</sup>

## MECHANISM OF HEPATIC ISCHEMIC REPERFUSION INJURY IN SEPSIS

Septic shock is often preceded by prolonged hemodynamic instability that results in hypoperfusion of a variety of vascular beds. Extended hypoperfusion produces ischemic injury as a result of a reduction in tissue oxygen and other cellular nutrients. Simultaneously, accumulation of metabolic by-products and gradual loss of cellular homeostasis occur. Resuscitation from a low-flow state has been postulated to cause a second phase of injury through activation of phagocytes and endothelial cells. Phagocyte and endothelial cell activation leads to a proadhesive state. The adhesion of neutrophils to endothelium establishes a microenvironment between adherent cells that can allow inflammatory molecules to overcome anti-inflammatory mechanisms. In addition, neutrophils (PMNs) can adhere to one another, forming microvascular plugs that can alter microcirculatory perfusion and further augment local ischemia. Neutrophil-endothelial adherence is recognized as a pivotal step in the pathogenesis of extravascular inflammation, local tissue injury, and ultimately, functional organ failure.<sup>15</sup>

Hepatic ischemia followed by reperfusion results in severe injuries that contribute to the morbidity and mortality associated with shock. It has been generally accepted that reactive oxygen species (ROS) contribute to hepatic ischemia/reperfusion injury and such injury has been demonstrated to occur in a biphasic pattern involving initial- and subsequent-phase responses. Activated Kupffer cells generating an increasing amount of ROS mainly mediate the initial phase of injury. These cells are activated during ischemia and are further stimulated by complement activation during reperfusion. The

initial responses of the ischemia/reperfusion injury trigger the infiltration of neutrophils into postischemic liver. The recruitment of neutrophils results from a complex series of ischemia-induced cellular responses in the liver and changes in the vasculature that serve to alter the adherent characteristics of the neutrophils. These include the increased expression of adhesion molecules, such as intercellular adhesion molecule-1.

ICAM-1 is expressed on endothelial cells and plays a key role in the potent adhesion of neutrophils and their transendothelial migration. The accumulation of neutrophils in the liver is reported to take place mainly between 30- and 60-min postreperfusion, but they do not spontaneously release ROS in the vasculature. The burst of neutrophils adhering to a biological surface, such as endothelial cells or extracellular matrix proteins, is characterized by a lag-phase of 30 to 90 min between the adherence of activated neutrophils and the subsequent long-lasting ROS formation. Thus, activated neutrophils play a central role in the later phase of hepatic injury by releasing ROS.<sup>3</sup>

The precise mechanisms of ischemia reperfusion injury have not been elucidated. Substantial evidence exists regarding the participation of diverse factors. This included (i) loss of calcium homeostasis, (ii) reactive oxygen and nitrogen species generation, (iii) changes in microcirculation (iv) kupffer cells activation and (v) complement activation.

Hepatic ischemic reperfusion injury occurs from biphasic responses involving the initial phase and subsequent phase. The event according in the initial phase is believed to be characterized by the production of reactive oxygen species (ROS). Whereas the subsequent phase is typified by the accumulation of inflammatory cells (neutrophil), the activated neutrophil play a pivotal role in the later phase of hepatic ischemia reperfusion injury.

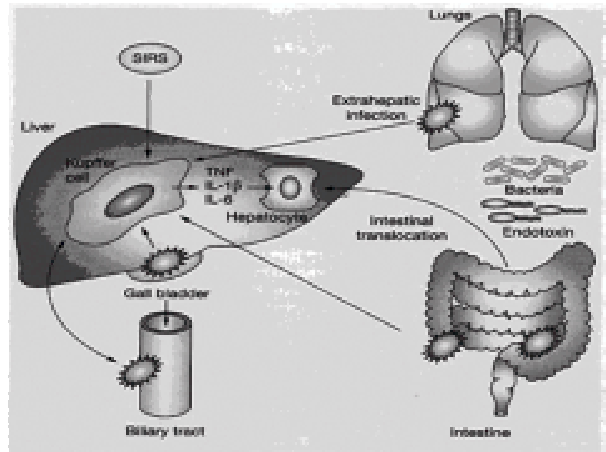


Fig.3. Hepatic cytokine production in sepsis and extrahepatic bacterial infections. Gram negative bacteria release endotoxin , which induced production of cytokines (TNF, IL-1, IL-6) by macrophages resident in the liver (Kupffer cells). Gut-barrier failure during sepsis can induced translocation of endotoxin from the intestinal lumen into the portal blood<sup>9</sup>

Hepatic IR injury caused significant increase in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which were accompanied by elevation of malondialdehyde (MDA) concentration and reduced SOD activity in the liver. These results suggested that liver injury was mediated by ROS. Where, ALT levels were significantly increased after reperfusion. Another finding, that high counts of aerobic and low counts of anaerobic bacterial population in IR injury. Failure of intestinal barrier function often occurs in many clinical condition, including sepsis, resulting in intestinal permeability and subsequent translocation of bacteria or/and endotoxin from gut. It is clear that increased gut permeability bacteria with or without endotoxin translocation plays a key role in the development of severe complications such as systemic inflammatory response syndrome (SIRS), sepsis, multiple organ dysfunction and multiple organ failure.<sup>16</sup>

## CLINICAL PRESENTATION OF SEPSIS-INDUCED LIVER INJURY

The mechanisms and clinical presentation of sepsis-associated liver injury vary according to the severity to the severity of the bacterial infection. Proinflammatory cytokines and nitric oxide cause cholestasis by impairing hepatocellular and ductal bile formation. Ischemic liver injury can also be found in patients with septic shock. The liver is being increasingly recognized as critically important in the pathogenesis of sepsis, because of major roles in bacterial scavenging and inactivation of bacterial products, as well as in the production and clearance of inflammatory mediators.

Early hepatic dysfunction in patients with sepsis is mainly related to shock and liver hypoperfusion, whereas the hepatic dysfunction with predominantly cholestatic features that occurs later in the time course of sepsis is caused by a secondary burst of inflammatory mediators. Mostly in those infected patient with gram negative bacteria, but also in SIRS. In addition, gut barrier failure during sepsis, shock, total parenteral nutrition can induce translocation of endotoxin (lipopolysaccharide) from the intestinal lumen into the portal blood. The common pathophysiological denominator of sepsis-associated cholestasis is the induction of pro-inflammatory mediators, with results in impaired bile secretion. The viral infection can also characterized by inflammation-induced cholestasis. Cholestasis in sepsis is generally reversible, it is caused by functional alteration at the hepatocellular and/or bile duct level. Whereas cholestasis as an indicator of liver dysfunction rarely progresses to true liver failure with coagulation defects and hepatic encephalopathy.<sup>17,18</sup>

### SEPSIS-ASSOCIATED CHOLESTASIS

The reduced canalicular secretion of bile acids and other organic anions during endotoxemia, is caused reduction in the expression of Mrp2. Simultaneous in-

creased expression of other export proteins (such as Mrp1 and Mdr1) is mediated by NF- $\kappa$ B, and in hepatocytes, might confer resistance to cytokine-induced metabolic stress. In the early phase of LPS-induced cholestasis in particular is characterized by the reversible and rapid retrieval of the transporter protein Mrp2 from the canalicular membrane.

The sepsis-associated liver injury varies according to the severity of the bacterial infection, whereas functional hepatocellular cholestasis predominates in patients with ischemic liver injury. The primarily of source of infection is intra abdominal (diverticulitis, peritonitis). Jaundice usually manifests within 2-7 days after bacteremia starts. The diagnosis of sepsis-associated cholestasis is base principally on elevated serum concentration of bilirubin, typically with serum bilirubin levels of 5-10mg/dl that can uncommonly, rise to 30-50mg/dl. Cholestasis resolves under appropriate antibiotic therapy.<sup>18</sup>

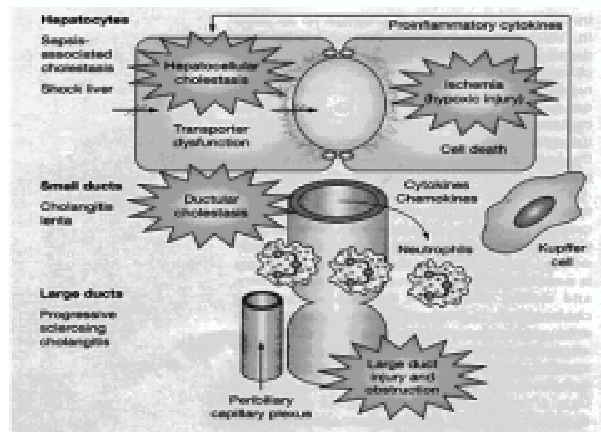


Fig.4. Hepatocellular, ductular and ductal level of cholestasis in sepsis. Proinflammatory cytokines and nitric oxide have crucial roles as Kupffer cell derived mediators of cholestasis. Sepsis associated cholestasis is caused by reduced expression and function of hepatocellular transport systems. Ischemic liver injury is characterized by hepatocyte necrosis. Periductal inflammatory infiltrates, as seen in cholangitis lenta, reflex the ability of the bile duct epithelium (cholangiocyte) to actively secrete cytokines and recruit inflammatory cells, which results impaired ductular bile secretion. At the large duct level, can occur progressive sclerosing cholangitis (rare)

## HYPOXIC LIVER INJURY OR ISCHEMIC HEPATITIS

Hypoxic liver injury also known as shock liver, is a common cause of jaundice during sepsis. The underlying path physiology involves changes in the portal and arterial blood supply as well as in the microcirculation. Ischemic hepatitis should be suspected in patients who have a rapid increase in their ALT levels, to 10-100 times the upper limit of normal in less than 24 h, combined with markedly elevated lactate dehydrogenase levels after IR. Transient episode of hypotension can be sufficient for the development of ischemic hepatitis in patients whose liver is already damaged. A key diagnostic feature of ischemic hepatitis is its rapid reversibility once the underlying cause is corrected. There is low incidence of coagulopathy and encephalopathy among patients with ischemic hepatitis and rarely progress to full-blown liver failure. The diagnosis is usually based on clinical criteria, but if the liver biopsy is performed to rule out other causes of liver injury, centrilobular necrosis is typically observed, as hepatocyte in this location vulnerable to oxygen depletion. Mortality is high in patient with ischemic hepatitis, but is rarely related to their liver disease.<sup>18</sup>

## CHOLANGITIS LENTA

It is unusual manifestation of sepsis-associated cholestasis, that is characterized by the presence of periductal cholangiocytes and inspissated bile within periportal ductules. Patients with cholangitis lenta have a worse prognosis and higher mortality than patients with simple sepsis-associated cholestasis. This finding reflects the ability of the bile duct epithelium to participate actively in the inflammatory process by secreting cytokines and inflammatory cells, resulting in impaired ductular bile secretion.<sup>18</sup>

## PROGRESSIVE SCLEROSING CHOLANGITIS

Occasionally develops in patients after they have suffered septic shock. Typically, these patients have rising levels of alkaline phosphatase and deep jaundice over a period of week or months. Interestingly, bilirubin levels might decrease spontaneously, despite the development of liver cirrhosis. Abdominal ultrasound does not usually show dilated bile ducts, as small bile ducts are more often involved than large bile ducts. A diagnosis of progressive sclerosing cholangitis should be suspected in patients who have rising levels of gamma glutamyl transpeptidase, alkaline phosphatase and bilirubin along with a compatible clinical history of sepsis or septic shock. Confirmation of progressive sclerosing cholangitis is based on endoscopic retrograde cholangiography, which usually reveals severe intrahepatic stones and rarefactions of smaller bile ducts. Liver histology shows ductular proliferation, portal lymphocytic inflammatory infiltrates, and portal and periductular fibrosis. This rare syndrome usually has poor prognosis.<sup>18</sup>

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