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Review

Post-transplant cholangiopathy: Classification, pathogenesis, and preventive strategies☆,☆☆

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ABSTRACT

Biliary complications are the most frequent cause of morbidity, re-transplantation, and even mortality after liver transplantation. In general, biliary leakage and anastomotic and non-anastomotic biliary strictures (NAS) can be recognized. There is no consensus on the exact definition of NAS and different names and criteria have been used in literature. We propose to use the term post-transplant cholangiopathy for the spectrum of abnormalities of large donor bile ducts, that includes NAS, but also intraductal casts and intrahepatic biloma formation, in the presence of a patent hepatic artery. Combinations of these manifestations of cholangiopathy are not infrequently found in the same liver and ischemia-reperfusion injury is generally considered the common underlying mechanism. Other factors that contribute to post-transplant cholangiopathy are biliary injury due to bile salt toxicity and immune-mediated injury. This review provides an overview of the various types of post-transplant cholangiopathy, the presumed pathogenesis, clinical implications, and preventive strategies.

1. Introduction

Biliary complications are a major cause of morbidity and even mortality after liver transplantation. In general, three types of biliary complications can be distinguished: biliary leakage, anastomotic strictures and non-anastomotic biliary strictures (NAS). NAS are frequently accompanied by the formation of intraductal biliary casts, prestenotic dilatations, and/or intrahepatic biloma formation (Fig. 1). These bile duct abnormalities likely represent different presentations of the same disease. While intrahepatic biloma formation results from full thickness necrosis of the bile duct wall with subsequent leakage of bile into the liver parenchyma this represents the most severe side of the spectrum.

There is no consensus on the exact definition of NAS and different names and criteria have been used in the literature. Alternative names are ischemic-type biliary lesions or ischemic cholangiopathy, based on the radiological similarities with bile duct abnormalities that develop after early hepatic artery thrombosis and subsequent bile duct ischemia [1]. Although there is increasing evidence that ischemia-reperfusion during the transplant procedure plays an important role in de development of bile duct pathology after liver transplantation, the exact mechanisms may remain unidentified in individual patients [2]. Therefore, we propose to use the more general term post-transplant cholangiopathy for the spectrum of multifocal pathologies that affect the macroscopic donor bile ducts in the absence of thrombosis or severe stenosis of the hepatic artery and cannot be explained by recurrent disease (i.e. primary sclerosing cholangitis). Of all possible biliary complications after transplantation, post-transplant cholangiopathy is regarded as the most troublesome complication due to the diffuse and multifocal lesions of the biliary tree and the resistance to therapy, often necessitating re-transplantation of the liver (Table 1).

Incidence rates of post-transplant cholangiopathy, including NAS, vary between recipients of a liver graft from donation after brain death (DBD) and donation after circulatory death (DCD) donors, ranging between 1–10% and 10–30%, respectively [3–6]. The relatively high

Abbreviations: AE2, Anion exchanger 2 (cholangiocyte $Cl^-/HCO3^-$ transporter); ATP, Adenosine triphosphate; BSEP, Bile salt export pump; CFTR, Cystic fibrosis transmembrane conductance regulator; CIT, Cold ischemia time; CMV, Cytomegalovirus; DBD, Donation after brain death; DCD, Donation after circulatory death; DWIT, Donor warm ischemia time; ERCP, Endoscopic retrograde cholangio-pancreaticography; MDR3, Multidrug resistance protein 3; NAS, Non-anastomotic (biliary) strictures; PBG, Peribiliary gland; PVP, Peribiliary vascular plexus

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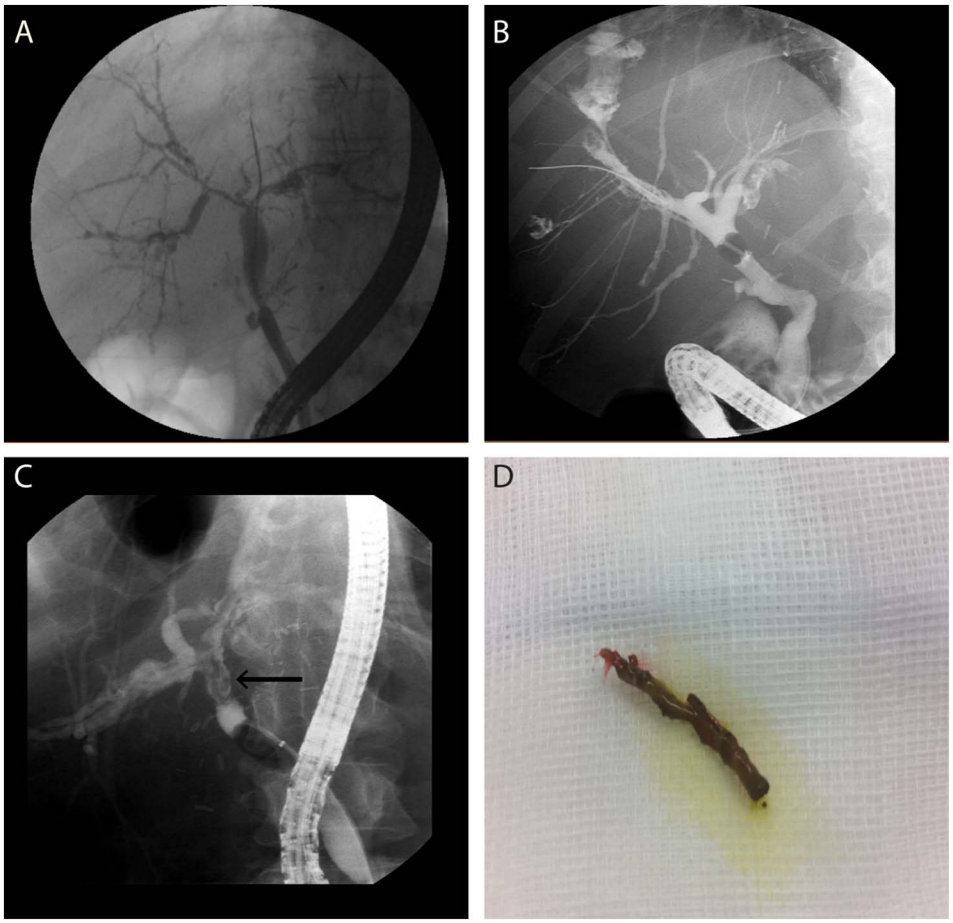

Fig. 1. Cholangiographic presentations of the three main types of post-transplant cholangiopathy. Panel A. Severe non-anastomotic strictures, with irregularities of the common bile duct, hepatic bifurcation and all large intrahepatic ducts (i.e. segmental, area, and septal ducts). Panel B Intrahepatic necrotic bile ducts with intraparenchymal leakage of contrast. In addition, intrahepatic biliary irregularities and stenosis are present. The extrahepatic bile ducts show no abnormalities. Panel C. Biliary casts in the common bile duct and hepatic bifurcation extending into the left hepatic duct (arrow). Panel D. A photo of an intraductal biliary cast which was extracted after liver transplantation.

Table 1
Classification of biliary complications after liver transplantation.

A. Biliary leakage
A1. From biliary anastomosis
A2. From hepatic biopsy or parenchymal injury
A3. From gallbladder fossa or cystic duct stump
A4. After removal of biliary drain
B. Anastomotic stenosis of:
B1. Choledocho-choledochostomy
B2. Hepatico-jejunostomy
C. Post-transplant cholangiopathy[a]
C1. Non-anastomotic biliary strictures (of extrahepatic and large intrahepatic ducts)
C2. Intraductal biliary casts
C3. Bile duct necrosis with intrahepatic leakage and biloma formation
D. Biliary abnormalities due to hepatic artery stenosis or thrombosis
E. Biliary strictures due to recurrent disease (i.e. primary sclerosing cholangitis)

[a]) Combinations of the three different subtypes of post-transplant cholangiopathy may occur in one liver graft and overt ischemia due to thrombosis or severe stenosis of the hepatic artery must have been excluded. Examples of the three subtypes of post-transplant cholangiopathy are presented in Fig. 1.

incidence rate of post-transplant cholangiopathy in DCD liver recipients is raising concerns, as DCD livers are increasingly accepted for transplantation. In the Netherlands, the percentage of DCD liver transplants was 34% of the total number of liver transplants performed in 2015 [7]. In the same year in the UK, 22% of all liver transplants were performed with DCD liver grafts [8]. An increased acceptance of DCD livers has also been reported by other countries.

During the last two decades biliary complications after transplantation have been a subject of extensive research and this has led to a better understanding of the pathogenesis, enabling the development of preventive strategies. The aim of this review is to provide an overview of the various types of post-transplant cholangiopathy that can occur after transplantation, and to discuss their presumed pathogenesis and the clinical implications. Moreover, new and emerging strategies to prevent post-transplant cholangiopathy are discussed. Other types of post-transplant biliary complications, such as anastomotic leakage and stricturing, bile leakage (i.e. after liver biopsy, biliary drain removal, cystic duct stump leakage) as well as recurrent PSC, are outside the scope of this review and will not be discussed in detail.

2. Etiology

Although the exact etiology of post-transplant cholangiopathy in an individual patient is often unclear, in general three important groups of mechanisms have been identified in experimental and clinical studies. These three mechanisms include bile duct injury and subsequent fibrosis and narrowing of the macroscopic bile donor bile ducts due to a) ischemia-reperfusion, b) bile salt toxicity, and/or c) immune processes (Table 2). Despite the identification of these three distinct types of pathogenesis, overlap occurs and especially the origin of NAS is often multifactorial (Fig. 2). Intraductal casts and sludge can be detected in combination with NAS, but it can also be found in liver grafts without signs of bile duct strictures [9]. These casts are believed to result from dead biliary epithelium that is sloughed off the bile duct wall and forms a nidus for deposition of bile components (Fig. 1D). Intrahepatic biloma formation represents the most severe form of injury of the biliary tree, resulting in full necrosis of the bile duct wall with subsequent leakage of bile into the liver parenchyma (Fig. 1B).

The high incidence of post-transplant cholangiopathy after DCD liver transplantation and the frequent occurrence of bile duct pathology after early hepatic artery thrombosis provide strong evidence that ischemia plays an important role in the pathogenesis of post-transplant

Table 2
Main causes of bile duct injury in liver transplantation.

A. Ischemia-reperfusion (IR) injury
- Primary IR injury (i.e. during graft preservation and reperfusion)
° Luminal biliary epithelial cell loss, mural necrosis
° Peribiliary glands loss, leading to insufficient epithelial regeneration
- Secondary ischemia
° Damage and thrombosis of the peribiliary vascular plexus after transplantation
B. Immune-mediated injury
- Blood group ABO incompatibility between donor en recipient
- Recurrent disease in recipients transplanted for an immune-mediated liver disease (i.e. auto-immune hepatitis or primary sclerosing cholangitis)
- Cytomegalovirus infection[a]
- Chemokine receptor CCR5 (CRR5- Δ32) polymorphism
- Acute or chronic rejection[b]
C. Bile salt mediated injury of biliary epithelium and duct wall
- High biliary bile salt/phospholipid ratio
- Insufficient protection by the bicarbonate umbrella

[a] Cytomegalovirus infection may lead to endothelialitis and injury of the peribiliary vascular plexus causing secondary ischemia or the inflammatory response may cause direct damage of virus infected biliary epithelial cells.
[b] Acute and chronic rejection mainly affects the small, microscopic bile ductules and not macroscopic bile ducts.

cholangiopathy. Although a liver transplant procedure is currently unavoidably associated with ischemia-reperfusion injury, it is not likely that this can explain the development of biliary complications that occur late (> 1 year) after transplantation. In only 50% of all patients that develop NAS after liver transplantation, NAS is detected within the first year after the procedure. A similar percentage of NAS is presented after more than one year [7]. Clinical observations and associations with risk factors have suggested that late occurring NAS (> 1 year after transplantation) is more likely to be caused by immune-mediated bile duct injury, while ischemia-reperfusion injury usually explains the early manifestation of NAS within the first year after OLT. In addition to the detrimental effects of ischemia-reperfusion, direct bile salt-mediated toxic injury of the bile duct epithelium and bile duct wall contributes to bile duct damage that occurs during and early after the transplant procedure.

2.1. Ischemia-reperfusion Injury

2.1.1. Primary ischemia

In contrast to blood supply of the liver parenchyma, which is derived from both the portal vein and the hepatic artery, blood supply to the large ducts of the biliary tree is mainly provided by the hepatic artery and arterial branches from the gastroduodenal artery. Together these arteries supply oxygen-rich blood to a fine vascular network encircling the bile ducts, known as the peribiliary vascular plexus (PVP). After transplantation, arterial blood supply to the donor extrahepatic bile duct and the larger intrahepatic branches of the biliary tree is only provided by the hepatic artery. This explains why early hepatic artery thrombosis after transplantation results in overt ischemia of the bile ducts, causing necrosis and subsequent leakage and/or fibrotic stricturing. This type of ischemic cholangiopathy can be diagnosed by direct contrast cholangiography (i.e. via a biliary drain), endoscopic retrograde cholangio-pancreaticography (ERCP) or magnetic resonance

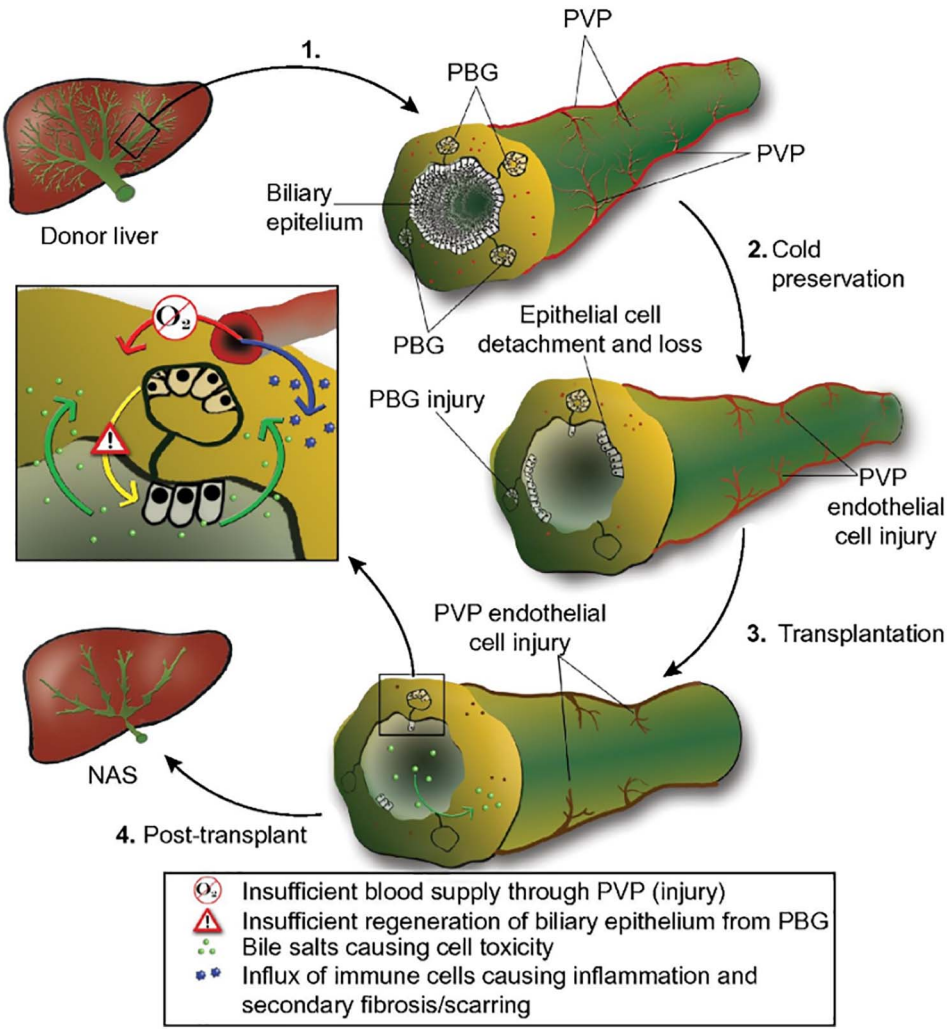

Fig. 2. Multifactorial proposed pathogenesis of post-transplant cholangiopathy. During cold ischemia (cold preservation), warm ischemia and subsequent reperfusion epithelial cell detachment and loss is inevitable. However, epithelial cell detachment or loss alone does not lead to the development of post-transplant cholangiopathy. Critical components of the bile duct wall are the peribiliary glands (PBG) and the peribiliary vascular plexus (PVP). PBG injury and PVP endothelial cell injury are associated with the development of post-transplant cholangiopathy. After transplantation an insufficient blood supply through the PVP, leads to secondary ischemia of the biliary luminal epithelium and the PBG. Moreover, bile salt toxicity and influx of immune cells cause damage to the bile ducts by a combination of inflammatory processes and secondary fibrosis and scarring.
* Figure was obtained from Weeder et al. Machine perfusion in liver transplantation as a tool to prevent non-anastomotic biliary strictures: Rationale, current evidence and future directions, Journal of Hepatology, Volume 63, Issue 1, July 2015, Pages 265–275. Link formal publication: http://dx.doi.org/10.1016/j.jhep.2015.03.008. Link Creative Commons user license: https://creativecommons.org/licenses/by-nc-nd/4.0/

cholangio-pancreaticography (MRCP). Typically, cholangiographic images are characterized by multifocal bile duct wall irregularities (strictures and dilatations), either with or without intraductal cast formation and leakage of contrast into the liver parenchyma (intrahepatic biloma's). Although the cause of ischemic cholangiopathy in patients with early hepatic artery thrombosis after transplantation is obvious, this is often less clear in patients with biliary pathologies in the presence of a proven patent hepatic artery. However, based on the strong similarities between cholangiographic abnormalities found in patients with proven hepatic artery thrombosis, an (post-) ischemic cause is likely to play a role in patients with post-transplant cholangiopathy as well. This is supported by a strong association between post-transplant cholangiopathy and clinical parameters of ischemia and reperfusion that can be identified during transplantation [10].

At multiple time points during liver donation and transplantation ischemia-reperfusion injury may occur. Primary ischemia occurs during preservation of a donor liver. The conventional preservation method of organs for transplantation is based on cooling and subsequent reduction of the metabolic rate and oxygen requirements. This method, during which the liver is flushed out with an ice-cold preservation solution and subsequently stored under melting ice, is called static cold storage. While cooling livers to 0–4 °C significantly reduces the need for oxygen, cellular metabolism never reaches a complete standstill and an oxygen and nutrient deficit is still built up. This results in well-described cellular and mitochondrial perturbations, such as intracellular depletion of adenosine triphosphate (ATP) and cell swelling due to diminished Na/K ATPase activity and subsequent electrolyte shifts. Upon reoxygenation, this is aggravated by the formation of toxic radical oxygen species and other factors, such as danger associated molecular patterns (DAMPs) that activate the immune system, leading to cell death due to apoptosis or necrosis [11,12]. Bile duct epithelial cells or cholangiocytes have been shown to be more susceptible to ischemia-reperfusion injury than hepatocytes. Especially reoxygenation of cholangiocytes in cell culture experiments has been shown to cause more cell death than reoxygenation of hepatocytes. This has been explained by a slower regeneration of intracellular ATP after ischemia, a higher release of radical oxygen species and lower concentration of the antioxidant glutathione in cholangiocytes, compared to hepatocytes [13].

The length of the cold ischemia time (CIT), defined as the time from aortic flush in the donor until graft reperfusion in the recipient, is an established risk factor for the development of NAS. Numerous studies have demonstrated that a prolonged CIT correlates with an increased incidence of NAS. While an a 2% incidence of NAS has been reported in donor livers with a CIT of < 12 h, this increased to 35% after a CIT of > 12 h [1,14,15]. Transplant surgeons are aware of this and generally try to maintain the CIT below 12 h.

Additional to cold ischemia during storage and transportation, liver grafts from DCD donors suffer from warm ischemia in the donor (DWIT) during the time period between withdrawal of life support and initiation of cold flush out with preservation fluid. Several studies have shown that the length DWIT is a significant predictor of the development of NAS [16–19]. One study concluded that each additional minute of DWIT increases the risk of developing NAS by 16% [17]. More specifically, the time from asystole to cold flush is critical in this perspective and significant differences in the incidence of NAS have been found depending of the length of this time period. However, ischemia can also occur between withdrawal of life support and circulatory arrest, since during this time period a drop in blood pressure and oxygen saturation is frequently observed. For example, the duration of a systolic blood pressure below 50 mm Hg from withdrawal of life support until cold flush has been identified as a significant predictor of poor outcome after DCD liver transplantation [20].

Apart from CIT and DWIT, all liver grafts endure a (second) period of warm ischemia during graft implantation, when the liver is taken from ice, placed in the recipient's abdomen and vascular anastomoses are constructed. In most centers portal reperfusion is performed prior to hepatic artery reperfusion, because this allows early decompression of the splanchnic venous outflow. However, portal blood has a relatively low oxygen saturation and does not contribute much to the biliary perfusion. Therefore, portal reperfusion of a donor may result in additional warm ischemia of the biliary tree. Since the bile ducts are mainly dependent on oxygen-rich blood supply from the hepatic artery, artery first or simultaneous reperfusion has been suggested to be superior to sequential reperfusion in preventing additional ischemic injury of the bile ducts. However, evidence for this is not unequivocal and conflicting data have been reported from different clinical studies [21–26]. In one, relatively small, prospective randomized study comparing sequential and simultaneous reperfusion, a significantly lower incidence of NAS was observed in livers after simultaneous reperfusion [22]. However, in another large multicenter analysis comparing different graft reperfusion techniques no differences in graft survival or biliary complications were found between the groups [23]. To this end it should be noted that although the main blood supply to the bile ducts comes from the hepatic artery, portal blood flow contributes to the peribiliary vascularization as well via intrahepatic anastomoses between the portal and arterial circulation. This explains why bleeding from the cut surface of the extrahepatic donor bile duct is frequently seen after portal reperfusion of a liver graft. Interestingly, NAS has also been reported in case reports of portal vein thrombosis or stenosis after transplantation, suggesting that inadequate portal perfusion should be considered a risk factor for the development of NAS as well [27,28].

2.1.2. Secondary ischemia

Apart from primary ischemia that affects the bile ducts during the various stages of the transplant procedure, secondary ischemia may occur after transplantation due to injury of the PVP. When endothelial cells of the small arteries, capillaries and veins of the PVP are damaged due to the ischemia and/or immune-mediated processes coagulation activation and intravascular formation of microthrombi occurs, resulting in secondary ischemia of the biliary epithelium and other bile duct wall components. Therefore, inadequate flush out and preservation of the PVP of donor livers has been proposed as a possible mechanism underlying the development of NAS after liver transplantation. In this regard, the use of preservation fluids with a high viscosity has been associated with a higher risk of NAS, compared to low-viscosity preservation fluids. It is thought that a high-viscosity preservation fluid, such as the University of Wisconsin solution, do not reach and flush out the PVP as well as low-viscosity fluids, such as histidine-tryptophan-ketoglutarate (HTK) and Marshall solution [29,30]. Although some studies have suggested that low-viscosity preservation fluids are indeed associated with a lower incidence of NAS after transplantation, these findings were contradicted by large database studies that showed superior graft survival of livers preserved with UW solution, compared to HTK solution [31–34].

The importance of adequate preservation of the microvasculature of the bile ducts was recently emphasized in a large clinical study with histological examination of bile duct samples taken at the time of transplantation. The histological grade and severity of injury of the PVP, including necrosis of small arterial branches in the bile duct wall, was strongly associated with the development of NAS after transplantation. Especially DCD liver grafts had significantly more severe PVP injury, compared to DBD grafts [35]. Endothelial injury of the PVP will result in intravascular thrombosis after graft reperfusion, contributing to secondary ischemic damage of the bile ducts (Fig. 2).

Another factor that could predispose to secondary ischemia is steatosis of the liver graft. Due to swelling of lipid loaded hepatocytes, severely steatotic livers have an impaired microcirculation, and steatosis of the liver has been associated with an increased risk for biliary complications [36–39].

2.1.3. Insufficient regeneration of biliary epithelium from the peribiliary glands

Ischemia-reperfusion injury of the epithelial lining of the bile ducts has long been viewed as the main determinant of the development of NAS. However, three independent clinical studies have demonstrated that extensive injury to and loss of the biliary epithelium can be found in over 90% of all livers transplanted [35,40,41]. Despite this extensive injury of the luminal biliary lining, only a minority of these livers develop post-transplant cholangiopathy. This has led to an alternative hypothesis that insufficient regeneration of the biliary epithelium, rather than the initial amount of injury determines whether a donor liver develops post-transplant cholangiopathy (Fig. 2) [35].

Regeneration and repair of biliary epithelium may result from proliferation of mature cholangiocytes aligning the bile duct lumen or from proliferation and migration of epithelial cells from the peribiliary glands (PBG). While proliferation of mature luminal cholangiocytes occurs in case of minimal injury of the epithelium, proliferation of PBG cells can be found after severe injury [42]. PBG are tubulo-alveolar clusters of biliary epithelial cells that are connected with the bile duct lumen via small glandular canals [42,43]. Apart from the secretion of mucus and serous fluids, PBG are thought to play a role in the local mucosal immunity by the production of anti-microbial enzymes. Recently, the PBG of extrahepatic and larger intrahepatic bile ducts have been identified as a local niche of multipotent stem/progenitor cells. These stem/progenitor cells are mainly located in the deeper parts of the peribiliary glands [42]. Interestingly, histological injury of the deep, extramural PBG of the donor bile duct at the time of transplantation has been identified as an important risk factor for the development of NAS after transplantation. Half of the patients who developed NAS had > 50% loss of the epithelial lining of the deep PBG in the donor bile duct, compared to less than 10% of the patients who did not develop NAS (Fig. 3) [35]. These findings support the hypothesis that the regenerative capacity of bile ducts rather than the initial amount of biliary epithelial injury determines whether a livers develops post-transplant cholangiopathy.

2.2. Immune-mediated injury

Immune responses that target the biliary epithelium have been proposed to play a role in the development of post-transplant cholangiopathy. Especially NAS that are discovered relatively late (> 1 year) after transplantation may have an immunological origin, although the exact mechanisms remain to be elucidated. A clinical cohort study has identified an association between immune-related risk factors and NAS occurring > 1 year after transplantation [10]. In contrast to cholangiopathy that occurs early after transplantation and is likely to have an ischemia-reperfusion related origin, in late NAS the smaller bile duct branches in the periphery of the liver are more frequently affected [10]. There are several immunological mechanisms that could predispose to the development of NAS.

Firstly, ABO-incompatibility has been associated with the development of NAS. One of the explanations for this correlation could be that perfusion of the PVP is hampered by the formation of intravascular blood clots, causing secondary ischemic injury of the cholangiocytes. Moreover, biliary epithelial cells express ABO-antigens, which could be targeted by antibodies that are incompatible with the patient's blood type and subsequently cause damage [44–46].

Secondly, patients transplanted for immune-induced hepatobiliary disease, such as auto-immune hepatitis and primary sclerosing cholangitis have an increased risk of developing NAS [2]. It has also been suggested that bacterial reflux into the bile ducts in patients with a Roux-Y bile duct reconstruction have an increased risk of developing NAS due to the inflammatory response induced by recurrent (subclinical) cholangitis [10,14]. However, this correlation between Roux-Y bile duct reconstruction and NAS is biased by the more frequent use of Roux-Y bile duct reconstruction in patients with PSC. In a large series of 486 patients transplanted for PSC, the type of bile duct reconstruction itself was not identified an independent risk factor for NAS [47].

Furthermore, cytomegalovirus (CMV) infection is associated with the formation of anastomotic strictures and bile leakage [47,48]. Although the role of CMV infection in the formation of NAS is less clear, a few clinical studies have provided indirect evidence for possible relationship between CMV and NAS. Post-transplant CMV infection has been identified as a clinical risk factor for NAS [47] and CMV-derived DNA has been detected in the bile of patients with NAS [49]. The underlying pathophysiological mechanism might be either direct or indirect immunological injury of the cholangiocytes. CMV can cause direct injury of biliary epithelial cells. Alternatively, infection of endothelial cells lining the PVP might result in the formation of microthrombi and thus secondary ischemic injury of the bile duct wall and

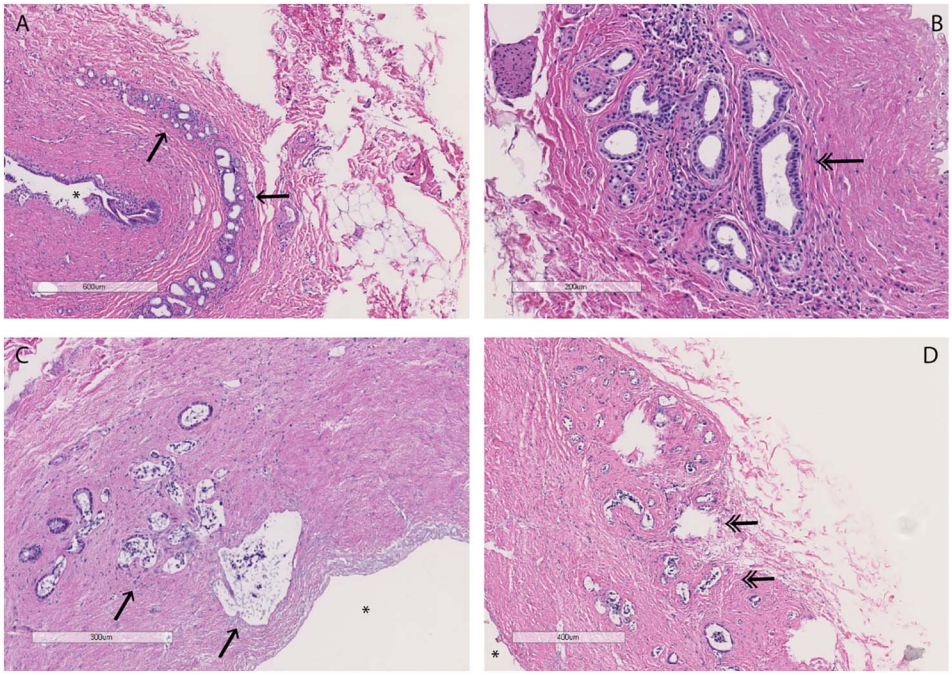

Fig. 3. Histological images of the bile duct illustrating various degrees of ischemic injury. Panel A. Mild injury with minimal injury of the luminal epithelial lining of the bile duct and well preserved peribiliary glands (arrows). Panel B. Higher magnification of intact deep peribiliary glands (double arrowhead). C. Moderate injury with complete disappearance of the luminal epithelial lining, destruction of the subluminal (or intramural) peribiliary glands (arrows) and moderate epithelial cell loss of the deep peribiliary glands. Panel D. Severe injury with complete loss of the luminal epithelium, mural necrosis (loss of nuclei in the fibrous stroma) and severe injury of the deep peribiliary glands (double arrowheads). * indicates the lumen of the bile duct.

epithelium.

Finally, a mutation in chemokine receptor CCR5 (CCR5-Δ32), leading to a reduced expression of CCR5, has been associated with the development of NAS [2,50,51]. Patients carrying the CCR5-Δ32 mutation have a 4 times higher risk of developing NAS, compared to non-carriers. This risk is even higher in patients transplanted for PSC [50]. CCR5 is expressed on immune cells and plays an important role in the attraction of regulatory T cells [52,53]. Biliary epithelial cells express binding sites for CCR5 [54]. It has been hypothesized that reduced expression of CCR5 in carriers of the CCR5-Δ32 mutation leads to impaired attraction of the regulatory T cells to sites of biliary damage associated inflammation. This could subsequently result in an uncontrolled and increased inflammatory response aggravating the bile duct injury.

2.3. Bile salt toxicity

Hydrophobic bile salts have potent detergent effects and can cause cell damage by either destruction of the cellular lipid membranes or by induction of apoptosis after entering the cell [2]. The toxic and detergent effects of bile have long been recognized by transplant surgeons and careful retrograde flushing of the biliary tree has become standard practice during liver procurement. Unfortunately, there are no good studies that have examined the best fluid to flush the bile ducts. While some surgeons use cold saline to flush the bile ducts, others use organ preservation fluids.

In addition to the detrimental effects of intraductal bile that remains present during organ preservation, newly formed bile after transplantation can also contribute to bile salt-induced injury (Fig. 2). The composition of newly formed bile early after liver transplantation is different from “normal” bile. Although early after transplantation biliary concentrations of bile salts and phospholipids are relatively low, the ratio between bile salts and phospholipids can be relatively high. This may increase bile salt toxicity due to insufficient micelle formation.

Bile composition is strongly affected by the expression and functionality of ATP-dependent biliary transporters. Expression of the bile salt excretion pump (BSEP) and multidrug resistance protein 3 (MDR3), responsible for the biliary secretion of bile salts and phospholipids, respectively, is diminished early after transplantation. In addition, function of these transporters is reduced by the low levels of intercellular ATP. The impact of an altered biliary bile salt/phospholipid ratio on the bile ducts has been described in both an experimental animal study and a clinical study. Transplantation of porcine livers from DCD donors with a DWIT of 30 min resulted in a higher bile salt/phospholipid ratio in bile produced immediately after transplantation, compared to DCD livers with a DWIT of 0 or 15 min [55]. In a large clinical study including 111 liver transplant recipients, the biliary bile salt/phospholipid ratio in the first week after transplantation was significantly higher in patients who later developed NAS, compared to recipients who did not develop NAS [56]. These two studies indicate that bile salt toxicity contributes to bile duct damage and the development of NAS in both DBD and DCD liver recipients.

Another factor that has been suggested to cause bile salt toxicity is an insufficient HCO_3^--umbrella. HCO_3^-, secreted by ATP dependent cystic fibrosis transmembrane conductance regulator (CFTR) and cholangiocyte Cl^-/HCO_3^- exchanger (AE2), serves to maintain an alkaline environment near the cholangiocytes [57]. In an alkaline environment hydrophobic bile salts are deprotonated, making them less capable of permeating the lipid cellular membranes of the cholangiocytes. Impaired functionality of ATP dependent CFTR and AE2, caused by ischemia before liver implantation, results in a less alkaline environment near the cholangiocytes [2]. Although this could make cholangiocytes more susceptible to bile salt-induced injury and cell death formal evidence that a diminished HCO_3^--umbrella contributes to formation of NAS after liver transplantation is still lacking.

3. Presentation & diagnosis

Symptoms associated with post-transplant cholangiopathy differ greatly among patients and are often non-specific. Symptoms may include jaundice, fever, and abdominal pain. Not infrequently, abnormal liver function test, such as elevated serum gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) are the first signs that indicate bile duct pathology. In more than 50% of the cases patients present with symptoms of NAS within the first year post OLT. As explained above, the time of presentation depends on associated risk factors and the underlying pathophysiological mechanism of NAS [10].

Increased serum bilirubin and/or GGT and/or ALP after liver transplantation in combination with (not necessarily) clinical symptoms will require further diagnostic measures. Firstly, ultrasonography is needed to evaluate patency of the liver vasculature, check for gross parenchymal abnormalities and/or biliary dilatations [58]. However, post-transplant cholangiopathy, especially NAS, is usually not detected by ultrasound. A definite diagnosis of NAS is always based on radiological imaging of the biliary tree. When a biliary drain is used and still *in situ* the preferred imaging technique is direct contrast cholangiography via the biliary drain. Alternatively, cholangiography can be obtained by either ERCP or MRCP. When NAS are suspected, magnetic resonance cholangiopancreatography (MRCP) is preferred as the next step in the diagnostic work-up, since it is non-invasive and is highly accurate with reported sensitivity and specificity to diagnose biliary strictures of 0.94 and 0.95, respectively [59,60]. When an isolated anastomotic stricture is suspected, the preferred diagnostic technique is ERCP, since it gives the opportunity for direct intervention with balloon dilatation and/or stent placement [58].

Different groups have proposed different radiological classifications of NAS [10,61,62]. However, the clinical relevance of these classifications of NAS is not evident as the radiological severity of the strictures does not always correlate well with the severity of the symptoms and disease [61]. In most cases, abnormalities can be found in the entire biliary tree. However, in some cases bile duct abnormalities are limited to one side or segment of the liver or only the most central parts of the biliary tree. These selected lesions of specific parts of the biliary tree may be successfully treated by endoscopy or surgery, as discussed in the next paragraph. Another exception may be the presence of intraductal casts and/or intrahepatic biloma formation. Detection of these expressions of post-transplant cholangiopathy is clinically relevant as it has a negative impact on the prognosis and therefore, influences the choice of treatment. Although in general it is still uncertain how to determine (beforehand) which cases of post-transplant cholangiopathy will progress to severe symptoms, requiring re-transplantation, and which cases will remain stable, the combination of NAS and casts or sludge has been associated with an increased risk to evolve into progressive disease. Moreover, frequently recurring (bacterial) cholangitis in patients with NAS has been associated with progression of NAS towards severe outcome [63]. Finally, intrahepatic biloma formation represents the most severe side of the spectrum of post-transplant cholangiopathy as it results from full thickness necrosis of the bile duct wall with subsequent leakage of bile into the liver parenchyma. This subtype of post-transplant cholangiopathy is associated with a poor prognosis and may require re-transplantation.

4. Treatment

Treatment of post-transplant cholangiopathy is hampered by the often diffuse and multifocal involvement of the biliary tree. When post-transplant cholangiopathy develops, therapy should primarily focus on treatment of infections (i.e. cholangitis) and securing adequate bile flow from the liver into the bowels. Antibiotics, either intravenous or oral maintenance, may relief cholangitis-related symptoms and complaints. Endoscopy with repeated dilatations and/or stenting of the most severe strictures can reduce severity of symptoms in 50–75% of the patients

with NAS. However, even in these successful cases recurrence rates are higher, compared to patients treated for a solitary anastomotic stricture [64]. In case of a Roux-Y bile duct reconstruction it may be difficult to obtain access to the bile ducts and in these cases percutaneous transhepatic cholangiodrainage is an alternative. Surgical resection of the extrahepatic bile ducts and construction of a hepatico-jejunostomy that may involve several bile duct branches can be successful in patients with isolated or predominant involvement of the common hepatic duct and its bifurcation [65].

Unfortunately, in many cases of NAS antibiotics and endoscopic, percutaneous or surgical treatment of biliary strictures does not provide a permanent solution. The only effective treatment for therapy-resistant post-transplant cholangiopathy remains re-transplantation of the liver. Contrary to NAS, re-transplantation for biliary leakage or an anastomotic stricture is rare. In these cases, repeated ERCP with stenting or surgical revision of the biliary anastomosis is usually sufficient [64,66,67].

Reported re-transplantation rates for NAS vary between DBD and DCD liver grafts. While re-transplantation has been reported in 0.6–2.5 % of the patients who developed NAS in a DBD liver graft, the re-transplantation rate may be as high as 11% for patients with NAS after a DCD liver transplantation [16,68].

5. Prevention

Since the treatment of post-transplant cholangiopathy is challenging and often unsuccessful, focus should lie on the prevention of this major complication. All three main etiological factors (ischemia-reperfusion injury, immune-mediated injury, and bile salt toxicity) are potential targets for preventive strategies. Since ischemia has been identified as the most important factor contributing to the pathogenesis of post-transplant cholangiopathy better graft preservation techniques, that result in less ischemia-reperfusion injury, have the greatest potential. To this end, dynamic preservation techniques, such as *in situ* oxygenated regional perfusion methods in DCD donors and *ex situ* machine perfusion of donor livers has attracted increasing research attention [69, 70].

5.1. Machine perfusion

In DCD donors, a period of *in situ* oxygenated regional perfusion of the abdominal compartment after declaration of circulatory death and before organ procurement may enable resuscitation of the organs and restore intracellular energy sources. Normothermic regional perfusion of the abdominal compartment of DCD donors has been associated with a reduced incidence of NAS after transplantation. It remains to be established whether regional perfusion should be performed at body temperature, or can be successfully performed under hypothermic conditions as well [71].

Ex situ machine perfusion of isolated donor livers has great potential to become the new standard of organ preservation for transplantation. Although machine perfusion devices were already developed in the pioneering years of organ transplantation in the 20th century, the renewed interest in this technology has sparked an extensive output in experimental and clinical research. An important advantage of machine perfusion, compared to conventional static cold storage, is that it provides better perfusion and flush out of the liver, provides oxygen and nutrients and, when performed at body temperature, enables functional testing of a donor liver prior to transplantation. Currently, different methods of machine perfusion of donor livers are being explored, including perfusions at different temperatures. Of these, devices that provide hypothermic (4–12 °C) or normothermic (35–37 °C) machine perfusion of donor livers have entered into clinical practice and research [70,72]. Many aspects of machine perfusion, however, remain to be determined. For example, it remains unknown whether machine perfusion should be performed during the entire period between organ procurement and implantation, or can be performed for a shorter period (i.e. short before transplantation). The first clinical studies indicate that a short period of hypothermic oxygenated machine perfusion after conventional static cold storage (so called end-ischemic machine perfusion) is safe and results in significant reduction of ischemia-reperfusion injury. In addition, it remains to be established whether optimal bile duct preservation can be obtained by single portal perfusion, or requires also perfusion via the hepatic artery. Since the bile ducts are largely dependent on arterial blood supply, it has been argued that combined portal and arterial perfusion provides better bile duct and PVP preservation, compared to single portal perfusion [73,74].

End-ischemic hypothermic oxygenated machine perfusion is a safe first step that may provide better preservation of the donor bile ducts. Hypothermic oxygenated machine perfusion has been shown to resuscitate mitochondrial function and restore intrahepatic concentrations of ATP [75–77]. The restoration of ATP and resuscitation of mitochondria before transplantation is important as it results in decreased formation of radical oxygen species, less Kupffer cell and endothelial cell activation, and reduced activation of the innate immune system after graft reperfusion. Since cholangiocytes are susceptible to ATP depletion and most hepatobiliary transporters are ATP dependent, it is conceivable that machine perfusion offers better preservation and protection of the bile ducts [13].

Histological assessment of the bile ducts in experimental studies support the possible protective effects of machine perfusion on the bile ducts. In a porcine model of DCD livers, hypothermic oxygenated machine perfusion resulted in significantly less arteriolonecrosis of the PVP, compared to static cold storage [78]. Other experimental and preclinical studies have shown a reduction in microscopic bile duct injury and increased bile production after various types of machine perfusion [79–82].

In the first clinical series of hypothermic machine perfusion, Guarrera et al. have shown improved hepatobiliary function and less biliary complications after transplantation of machine-preserved high risk DBD livers, compared to conventional static cold storage [83]. In a matched case analysis Dutkowski et al. have found a significant difference in overall and cholangiopathy-free graft survival between DCD livers that were subjected to hypothermic oxygenated machine perfusion and non-perfused livers [84]. Clinical studies on normothermic machine perfusion have not yet been able to show improvement in biliary preservation and a reduction of post-transplant cholangiopathy [85,86]. However, when normothermic machine perfusion replaces static cold storage and is performed throughout the entire period of storage and transportation of a donor liver, this may significantly reduce ischemic bile duct injury. Currently, several clinical trials on machine perfusion are in progress. In one European multicenter randomized controlled trial the effect of hypothermic oxygenated machine perfusion on the incidence of NAS after DCD liver transplantation is being investigated (NCT02584283).

Apart from better protection of the bile ducts against ischemia-reperfusion injury, machine perfusion creates the possibility to stimulate regeneration and repair of the biliary tree of donor livers prior to transplantation. Extrahepatic and large intrahepatic bile ducts have been shown to contain niches of stem/progenitor cells in the PBG [42,87]. This makes PBG an interesting target for machine perfusion. Addition of agents that stimulate proliferation and maturation of PBG progenitor cells to the perfusion fluid, especially under normothermic conditions, could initiate timely repair of injured biliary epithelium, preventing the development of NAS.

5.2. Prevention of bile salt-mediated injury

To minimize bile salt induced injury of the biliary epithelium during donor liver preservation, it is current practice to flush the biliary tree during organ procurement. Most surgeons flush the bile ducts with either a standard organ preservation solution or saline. Most of these

solutions, however, are pH neutral or slightly acidotic, while bile is usually alkalotic and biliary epithelial cells protect themselves against bile salt toxicity by providing an alkalotic layer on their luminal membrane (so called bicarbonate umbrella). Therefore, it can be questioned whether current organ preservation fluids have the optimal composition and pH to protect and flush out the bile ducts. Ideally, a specific preservation fluid to flush the bile ducts which is not too viscous or acidotic should be developed.

6. Conclusion and future perspectives

Post-transplant cholangiopathy remains to be the most challenging complication after liver transplantation with high morbidity and mortality rates. Post-transplant cholangiopathy includes a spectrum of abnormalities of the macroscopic donor bile ducts, ranging from biliary stricture and cast formation to full thickness bile duct necrosis with leakage of bile into the liver parenchyma. By definition, post-transplant cholangiopathy occurs in the presence of a patent hepatic artery. Nevertheless, ischemia-reperfusion injury has been identified as one of the most important underlying causes of this type of biliary complication. Other factors involved in the pathogenesis are bile salt toxicity and immune-mediated injury. Since treatment options for post-transplant cholangiopathy are limited and often not successful, emphasis should lie on prevention. Experimental and first clinical research data have provided good hope that machine perfusion technology for organ preservation enables better protection of donor bile ducts and will reduce the incidence of biliary complications after transplantation.

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