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Machine perfusion and the prevention of ischemic type biliary lesions following liver transplant. What is the evidence?

Machine perfusion and ITBL prevention

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Abstract

The widespread uptake of different machine perfusion (MP) strategies for liver transplant has been driven by an effort to minimise graft injury. Damage to the cholangiocytes during the liver donation, preservation or early post-transplant period may result in stricturing of the biliary tree and inadequate biliary drainage. This problem continues to trouble clinicians, and may have catastrophic consequences for the graft and patient. Ischaemic injury, as a result of compromised hepatic artery flow, is a well-known cause of biliary strictures, sepsis and graft failure. However, very similar lesions can appear with a patent hepatic artery and these are known as ischaemic type biliary lesions (ITBL) that are attributed to microcirculatory dysfunction rather than main hepatic arterial compromise. Both the warm and cold ischaemic period duration appear to influence the onset of ITBL. All of the commonly used MP techniques deliver oxygen to the graft cells, and therefore may minimise the cholangiocyte injury and subsequently reduce the incidence of ITBL. As clinical experience and published evidence grows for these modalities, the impact they have on ITBL rates is important to consider. In this review, the evidence for the three commonly used machine perfusion strategies (abdominal normothermic regional perfusion [A-NRP], hypothermic oxygenated perfusion [HOPE] and normothermic machine perfusion [NMP]) for ITBL prevention has been critically reviewed. Inconsistencies with ITBL definitions used in trials, coupled with variations in techniques of MP, make interpretation challenging. Overall, the evidence suggests that both HOPE and A-NRP prevent ITBL in DCD grafts when compared to cold storage. The evidence for ITBL prevention in DBD grafts with any MP technique is weak.

Key Words: Liver Transplant; Ischemic Type Biliary Lesions; Hypothermic Oxygenated Machine Perfusion; Normothermic Machine Perfusion; Abdominal Normothermic Regional Perfusion; Donation after Circulatory Death.

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Core Tip: In recent years, the development of different machine perfusion (MP) strategies has generated interest in their use for both the assessment of grafts and optimization during the preservation period. The different mechanisms behind the diverse array of MP strategies may reduce the extent of cholangiocyte and may have the subsequent clinical effect of preventing the development of ischaemic type biliary lesions. This review summarizes the strength and limitations of clinical studies that have been undertaken, their results, and provides a summary of the available literature on machine perfusion and the prevention of ischaemic type biliary lesions.

INTRODUCTION

In recent decades, liver transplantation has made several forward strides. These have been in the area of surgical technique, immunosuppressive drug strategies, treatment and prevention of recurrent viral infections, and the increasing use of alternative preservation techniques. Consequently, recipient and graft survival are greater than 90% at 1 year and long-term survival is considered the norm.[1] A longstanding problem that liver transplantation has faced is the mismatch between the number of donors and the higher number of patients listed for transplant. This has led to long waiting-lists for a graft, and up to 20% of patients don't survive until transplantation.[2, 3]. Furthermore, the recent expansion of transplant indications to include certain oncological scenarios may further aggravate this issue.[4] As a response to this shortage in supply, living donor liver transplantation (LDLT) and using more marginal organs, including those donated after circulatory death (DCD) are strategies that have been used to increase the donor pool[5]. Improving long-term graft survival is vital, as the need for retransplantation creates additional demand on a scarce resource.

Adequate biliary drainage is paramount for the success of a liver transplant, and was previously labelled the 'Achilles heel' of this procedure.[6] Although vascular complications have the largest impact on short-term graft outcomes, biliary complications are the main source of long-term morbidity. These conditions often require costly interventions, cause suffering and adversely affect patients' quality of life.[7] The incidence of these complications is increasing as a result of the growing utilization of extended criteria donor (ECD) organs, mainly from DCD donors [8].

In recent years, the development of numerous machine perfusion (MP) strategies has generated interest in their use for both the assessment of grafts and optimization during the preservation period.[9] Different techniques of MP have been described and vary in their application, with *in-situ MP* occurring during organ procurement whilst the graft is within the donor and *ex-situ MP* which occurs after the donor hepatectomy is completed. Both hepatocytes and cholangiocytes are vulnerable to ischaemia-reperfusion injury (IRI), and injury to the latter during organ procurement and preservation lies behind the pathogenesis for biliary dysfunction [10]. Consequently, a current trend of research in the MP field is focused on how these different MP perfusion regimens influence post-transplant biliary complications and more specifically, ischaemic type biliary lesions (ITBL). This narrative literature review describes the strength and limitations of clinical studies that have been undertaken, their results, and provides a summary of the available literature on MP and the prevention of ITBL.

Non-Anastomotic Biliary Strictures and Ischemic Type Biliary Lesions

Post-liver transplantation biliary complications can comprise one (or both) of the following entities; strictures (anastomotic and non-anastomotic), and biliary leaks.[11] Anastomotic biliary strictures occur at the site of biliary reconstruction and the surgical technique, and/or local tissue ischemia likely play a role. These strictures are

usually managed with endoscopic or radiological procedures, however surgical revision may be required depending on the timing and type of anastomosis.[12] Non-anastomotic strictures (NAS), as the name implies, occur at a site away from the anastomosis and are most common within the initial 12 mo post-transplant. NAS are one of most feared late complications due to it being associated with high rates of graft loss and mortality, and minimal treatment options except re-transplantation.[8, 13]

NAS is characterised by diffuse fibrotic strictures and dilatation of the biliary tree at any location from the liver periphery to the main extrahepatic ducts (**Figure 1**). In addition, with the disease evolution, the formation of biliary casts and intrahepatic bilomas may occur in its severest form.[14]. The radiological manifestations of NAS are highly variable and range from a peripheral abscess, individual or multiple strictures around the hilum and first order biliary branches, to vanishing ducts seen along the entire biliary tree.[15] NAS can occur as a direct result of ischaemia from an identifiable hepatic artery stenosis (HAS) or thrombosis (HAT). However, very similar lesions can develop in the setting of an entirely patent hepatic artery and this specific situation is termed ITBL, given its similarity to an actual ischaemic cholangiopathy (**Figure 1**).

In a seminal paper by authors from Groningen which excluded patients with HAT, ITBL was classified according to the affected area of the biliary tree.[16] In this study, they demonstrated that the anatomical location of ITBL varied between those that presented early (<1 year) as opposed to late (>1 year). In those presenting late, the peripheral liver (Zone D) was involved more frequently and there was an association with immunological risk factors. In contrast, patients with an early presentation had lesions around the bifurcation and the common bile duct (zone A). This early group had a longer period of both cold and warm ischaemia; therefore, hypoxia is thought to be one of the underlying mechanisms. More recently, a US group from the Mayo Clinic proposed a radiologic classification of ITBL into four distinct patterns that correlate with a distinct natural evolution and prognosis.[17] In this case, those patients with

diffuse necrosis and multifocal progressive patterns experienced more episodes of cholangitis and almost all required stents and eventual re-transplant.

Multiple factors have been associated with ITBL development and are generally divided into three categories: I) ischemia-related injury, II) bile salt mediated injury, and III) immune-mediated injury.[18, 19] The cold (CIT) and donor warm ischaemia time (dWIT), and inadequate microvasculature preservation, predispose cholangiocytes to a subsequent IRI.[10, 18] The increased incidence of ITBL in DCD grafts, which are characterised by a period of dWIT is well established, with ITBL rates of up to 39% following controlled DCD liver transplantation.[13, 20, 21] The toxic effect of bile salts on cholangiocytes at low temperatures during preservation is well known. Certain bile acids have been shown to promote the secretion of inflammatory mediators, and others are directly cytotoxic to cholangiocytes.[22] In addition, new bile after implantation and its altered bile salt/phospholipid ratio have demonstrated a detrimental effect on biliary epithelium and induce NAS.[23-25] An immune-mediated component to the cholangiocyte injury has also been proposed due to several clinical associations, but the mechanism remains under investigation. ITBL has been demonstrated in association with ABO incompatibility, recurrence of immune-mediated liver diseases, cytomegalovirus infection, presence of chemokine receptor CCR5 polymorphism, and acute or chronic rejection.[18]

The pathogenesis of ITBL is multifactorial. Both the cold and warm ischaemic periods induce the formation of reactive oxygen species (ROS). These ROS are generated as a result of Kupffer and polymorphonuclear cells activation, mitochondrial permeability transition (MPT) production, oxidative changes in the structure of the biliary canaliculus and adenosine triphosphate ATP depletion. These all lead to the apotosis and/or necrosis of the cholangiocytes. This mechanism is interrelated with the cytotoxic effect of bile salts, the cholestasis status induced by ischaemia-reperfusion and different immune-mediated mechanisms, which all further propagate the biliary injury.

Appropriate donor selection, the use of preservation fluids and MP are just some of the strategies which are thought to prevent this condition. [26]

The different mechanisms behind the diverse array of MP strategies may reduce the extent of cholangiocyte injury during the transplantation process, in comparison with static cold storage (SCS). This may have the subsequent clinical effect of preventing the development of ITBL. The number of clinical studies on MP strategies continues to increase. With only a few exceptions, the primary outcomes focus mainly on graft and patient survival. In this review, we focus on the available clinical evidence for abdominal normothermic regional perfusion (A-NRP), and ex-situ hypothermic (HOPE) and normothermic perfusion (NMP) in relation to the outcome of ITBL.

2.0 Prevention of Ischaemic Type Biliary Lesions *via* Machine Perfusion

The incidence of ITBL post-transplant varies considerably between institution and nations. These differences likely relate to the type of organ donation permitted, ranging from DBD donors only in some parts of the world, to uncontrolled DCD donors in others. Different national laws regarding the donor age and the 'no-touch' period following asystole in DCD donors likely influences outcome, and these matters also complicate the results and interpretation of clinical trials on MP. In addition, it should be noted that the lack of clear and consistent definitions of ITBL in the currently available literature makes the assessment of the true impact challenging. Many studies either do not differentiate between anastomotic, ischaemic NAS and ITBL, furthermore they may include both symptomatic and non-symptomatic cases.[27] With these limitations in mind, a literature search was performed in October 2022 using PUBMED®, Embase® and Medline®. Publications were restricted to those in English, however no further search filters were applied. The following search terms were used (in different combinations with Boolean operators); Liver, transplant, biliary stricture, ischaemic type biliary lesions, machine perfusion, machine preservation, normothermic,

hypothermic, normothermic regional perfusion. Both prospective and retrospective studies were included if they included outcome data pertaining to biliary strictures. Studies without a control (or comparator) group were excluded. A pooled analysis was not performed due to a significant variation in study methodology, MP application technique, and outcome definitions. Institutional ethical approval was not required.

2.1 Abdominal Normothermic Regional Perfusion and Ischaemic Type Biliary Lesions

A-NRP is an *in-situ* preservation technique using an extracorporeal oxygenated membrane (ECMO), restoring the perfusion of abdominal organs after the donor is declared deceased. This technique may lead to a reduction in the dWIT depending on the timing of cannulation, however it provides a period of resuscitation immediately after the cellular injury incurred from the dWIT. The provision of near physiological conditions during A-NRP provides a period in which cells can recover their energy stores, therefore they tolerate the CIT with minimal additional injury. It can also provide information about graft viability and reduce the effects of the IRI process [28].

In recent years, countries such as Spain, France, UK and Italy have developed different A-NRP protocols as a strategy to improve the outcomes of DCD grafts[29-33]. These protocols differ in the liver viability criteria utilised, "no-touch" periods required, pre-mortem substance administration and vessel cannulation. All countries aforementioned have a mandatory no-touch period of 5 minutes except Italy, which requires 20 minutes. This inevitably lengthens the dWIT. Sedative analgesia administration is not allowed in the UK, in contrast to other countries. In Spain, pre-mortem cannulation is allowed while in Italy and France only the identification of femoral vessels to ease cannulation is permitted. The obvious benefit of premortem cannulation is that it minimises the dWIT and could achieve better outcomes as a result.[34] During A-NRP, certain viability parameters are measured, the most common amongst the different protocols are

transaminase levels. Despite transaminase levels being widely accepted, it only reflects hepatocyte injury and not cholangiocytes. Other viability criteria employed include A-NRP duration [31], lactate clearance [30] and/or the presence of macrosteatosis .[30, 31] However, some of these parameters are considered controversial[28].

To date, there has been no randomized trial comparing A-NRP to the standard retrieval method, known as the super rapid retrieval (SRR) technique. The clinical studies investigating A-NRP that include a comparator group, and provide data on biliary strictures are summarized in **Table 1**. The definition of ITBL used in these studies is relatively homogeneous as most authors have considered ITBL to have occurred when NAS developed in the context of a patent hepatic artery. However, two studies did not include an ITBL definition [35, 36] and two authors [31, 37] considered NAS regardless of the presence of concomitant HAT. Other limitations of these studies include variation in the A-NRP protocols, small sample size, and variability in follow-up periods. These trials can be put into three categories, according the comparator groups included;)1) DCD-A-NRP vs. DBD-SCS 2) DCD-A-NRP vs. DCD-SRR and 3) DCD-A-NRP vs. DCD with other MP.

In the first group of studies [31, 35, 38-40] which compared DCD-NRP-A grafts *vs* DBD-SCS grafts, these authors aimed to demonstrate that DCD grafts after NRP could have similar results of DBD grafts and therefore should not be considered marginal. These studies showed a similar incidence of ITBL between both groups and the results in regard to early allograft dysfunction were promising. However, they had small sample sizes [35, 38, 39], differences in follow-up periods [31, 35, 38, 39] and a high proportion of uncontrolled DCD donors [38]. Other authors have assessed the outcomes of DCD NRP grafts *vs* DCDs grafts recovered by *via* the SRR technique and subsequent SCS.[33, 34, 36, 41] Watson *et al*[33] compared two groups, DCD-A-NRP (n = 43) vs. DCD-SRR (n = 187) and reported both early allograft dysfunction and biliary complication rates. A significantly lower rate of early allograft dysfunction (12% vs. 32%) and ischemic

cholangiopathy (0% vs. 27%) occurred in the DCD-A-NRP group. Similar comparisons have been performed by Spanish transplant groups. Muñoz *et al*[36] did not demonstrate significant differences in their cohort, probably due to the short follow-up period in the A-NRP group and the low number of patients. However, Hessheimer *et al*[41]subsequently found an ITBL incidence of 2% in the NRP group (n = 95) vs. 13% in the SRR group (n = 117). These findings were repeated in a second study [34] with a larger sample size (545 NRP vs. 258 SRR.) In this second study the ITBL incidence was 1% vs. 9% in favor of A-NRP, and this is at present the largest cohort of patients with A-NRP with pre-mortem cannulation in the literature. Recently, Schurink *et al* have reported the safety of NRP to rescue DCD grafts that were declined by the Eurotransplant region for transplantation, with no differences in primary non-function or IC compared to DBD and standard, non-NRP DCD grafts[42].

Finally, other authors [37, 43-45] have compared A-NRP with ex-situ preservation techniques such as NMP or HOPE. However, in many of these trials, accurate data on ITBL are lacking. The only study comparing HOPE vs. NRP was conducted by Muller *et al*[37]. These authors reported the incidence of NAS regardless of hepatic artery status, rather than ITBL specifically. The rate of NAS was reported to be 6.3% in the NRP group and 12.5% in the HOPE group, but was not significantly different. DCD grafts that underwent A-NRP have been compared to DCD grafts undergoing ex-situ NMP in a study by Gaurav *et al* This study showed significantly lower rates of ITBL in the NRP group (6.3% vs. 12.5%) after propensity score matching (PSM)[44]. However, in another study which used PSM analysis [45] (34 NMP vs. 68 NRP), there was no significant differences in both groups ITBL rate (2.9% NRP vs. 8.8% NMP). In this latter study, NMP was applied at source as opposed to the study by Gaurav *et al* which applied it at the recipient hospital in the majority of cases[44].

A-NRP is a widely used in-situ perfusion technique that has improved outcomes and graft utilisation in DCD grafts. Protocol aspects such as the “no-touch” period or pre-

mortem cannulation may impact in these results, due to its association with WIT. Despite the differences between the protocols adopted in different countries, NRP has still achieved superior outcomes in comparison to SRR in regards to ITBL.

2.2 Hypothermic Oxygenated Machine Perfusion and Ischaemic Type Biliary Lesions

Hypothermic machine perfusion (HMP) was first introduced in clinical practice in 2010 by Guarrera *et al* demonstrating in their pilot case-controlled series that was a feasible and safe preservation method.[46]Subsequently in preclinical studies that investigated the active oxygenation of the hypothermic perfusate over a short period, it demonstrated restored mitochondrial integrity. This implied a reduction of oxygen free radicals and damage-associated molecular patterns after transplantation.[47-49] Clinical studies also reported promising results in preventing biliary complication and graft function compared to standard SCS.[50, 51]

HOPE is employed as an end-ischaemic treatment after SRR or standard procurement in the case of DCD and DBD livers respectively, followed by a variable period of SCS. The devices available are not portable and HOPE needs to be applied at the recipient hospital. Its use limits the CIT and extends graft preservation time, avoiding the damage associated with extended periods of preservation. Two main perfusion strategies are currently employed which combine hypothermia with a highly oxygenated perfusate: 1) single HOPE or 'HOPE', which consists of single perfusion through the portal vein; 2) dual or 'D-HOPE' consisting of dual perfusion through the portal vein and the hepatic artery.[15, 52] Currently, there are no clinical studies comparing the two strategies, although preclinical studies performed on pigs did not find differences regarding the preservation of hepatobiliary or endothelial function when comparing both strategies.[53]Advocates of D-HOPE emphasise that dominant vasculature of the bile ducts comes from arterial supply and single portal perfusion may not provide optimal preservation of the biliary tree. On the other hand, many

argue the potential risk of mechanical damage to the hepatic artery intima that may occur during cannulation could cause a higher incidence of acute HAT following liver transplant.[51] Researchers from Zurich have proposed methods for liver graft assessment during HOPE using real-time quantification of flavin mononucleotide (FMN) in the perfusate.[54] FMN is a molecule part of complex I of the mitochondrial respiratory chain. [37] Its concentration is determined by fluorescence spectroscopy and levels in perfusate correlate with graft function, complications, and graft survival in DCD livers.[54]

The available studies on the use of HOPE as a preservation method, with a control group and reporting data on biliary strictures are summarized in Table 2. At present, these include four randomised controlled trials (RCT) and five retrospective cohort studies with an appropriate control group of SCS grafts, and a single study comparing HOPE against NRP. The influence of HOPE on ITBL prevention has been studied only to a limited extent because in the majority, the primary endpoint was not related to biliary complications.

The relationship between ITBL and DCD livers is well established.[20] However, only 4/10 published cohort studies and one RCT have studied the influence of HOPE on DCD graft outcomes.[51, 52, 55] Thus, most published studies included only DBD livers so there is little or no ITBL incidence, and are not powered for this endpoint.[56-60] The most relevant study on ITBL prevention by *ex-situ* machine perfusion was the multicentre clinical trial led by the Groningen group.[52] This European trial had symptomatic NAS within 6 mo after transplantation as the primary outcome. The study included a clear definition of NAS, which included the presence of a patent hepatic artery and therefore the NAS in this study were all ITBL. The authors demonstrated that two hours of end-ischemic D-HOPE led to a lower risk of symptomatic NAS after liver transplantation of grafts from DCD donors. NAS occurred in 5/78 (6%) of the patients in the HOPE group compared to 14/78 (18%) of SCS grafts with two patients from the SCS group requiring

a re-transplant because of severe NAS. Furthermore, the D-HOPE group showed a reduction by a factor of almost four in treatment interventions required for NAS.

Trials published by Italian and German groups have reported the efficacy of HOPE for the prevention of complications and a shorter intensive care unit stay.[56, 57] There was no mention of ITBL in both trials, and grafts studied were from DBD donors so one could assume that most of the biliary complications were from an anastomotic origin. While the primary endpoint in the German [57] study was the peak ALT within 1 wk after transplantation and demonstrated a 47% reduction in the serum peak of this enzyme in the HOPE group, the authors did not find any difference in biliary complication. rate (4/23, 17% *vs* 26% 6/23). A trial [56]demonstrated benefits of HOPE, with a reduction in EAD and better graft survival rates. This study did provide a clear definition of biliary complications, although reported a rate of biliary complication different from bile leak of 5/55 (9%) in the HOPE group compared to 6/55 (11%). Two patients in each group (4%) had biliary strictures, but it is unclear if these were ITBL. A recent multicentre trial on DBD livers from the Zurich group[61] reported the effects of HOPE on preventing postoperative complications. Although the primary endpoint was not reached, and the proportion of patients with at least one Clavien [3] III complication did not differ , findings suggested that HOPE may decrease the risk of severe liver graft related events. The study did not include a clear definition of NAS and state if hepatic artery patency was required. In this study the NAS incidence was 1/85 (1.2%) in the HOPE group compared to 3/85 (3.5%) in the SCS group.

The only study comparing A-NRP and HOPE for liver grafts from DCD donors included an A-NRP cohort from 6 high-volume French centres and the HOPE cohort from the Zurich group[37]. In the A-NRP cohort, femoral artery cannulas were introduced post-mortem after a ''no-touch'' period of 5 minutes. NAS was defined as strictures with or without the presence of concomitant HAT or arterial complications and both groups showed similar rates of HAT, PNF and NAS (6/132 in the A-NRP

group *vs* 8/93 in the HOPE group). D-HOPE has an encouraging role in preventing symptomatic ITBL in liver transplants from DCD grafts, due to mitochondrial injury prevention and ATP recovery under the hypothermic aerobic conditions.

2.3 Normothermic Machine Perfusion and Ischaemic Type Biliary Lesions

NMP provides oxygenated blood at a physiological temperature *via* both the hepatic artery and portal vein, whilst the liver graft is *ex-situ*[62]. Several different NMP devices are commercially available, and the majority are transportable even with graft connected. The only major differences between the devices are the nature of the arterial flow (pulsatile or non-pulsatile) and the caval outflow (open or closed). Since the phase 1 first-in-man study that demonstrated safety and feasibility of NMP, there has been a significant uptake in this preservation modality around the world and two large RCTs[63-65]. In contrast to *ex-situ* HMP, the more physiological nature of NMP allows cellular function to continue and this can be assessed *via* surrogate parameters[66, 67]. Numerous biochemical parameters within the NMP perfusate or bile have been used to predict hepatocellular and cholangiocyte function respectively, in an effort to identify the grafts destined to demonstrate severe EAD or biliary complications[68]. Therefore, an accurate discussion of the impact NMP has on ITBL post-transplant needs to consist of two components; 1) the incidence of ITBL in NMP preserved grafts in comparison to other modalities 2) the accuracy of NMP parameters to identify livers destined to develop clinically significant ITBL.

Published outcomes for NMP are confounded by the fact that this preservation modality is more frequently applied to organs of marginal quality to facilitate transplantation[69], and it may be applied for the entirety of the preservation period from the donor hospital ("at source") or following a period of cold storage during transport to the recipient centre ("back to base"). The early clinical trials of NMP included both DBD and DCD grafts, with a focus on its feasibility, safety and impact on the ischaemia-reperfusion injury as indicated by markers of early graft function[63, 64].

These multicentre trials included standard risk donors that were accepted for transplant regardless of the preservation modality (NMP or SCS). Subsequently, clinical practice and research shifted to investigate the ability of NMP to resuscitate poor quality grafts that were otherwise deemed untransplantable with SCS[70-72]. Complicating matters further, supposedly untransplantable livers are a heterogenous group with a varying overall risk of ITBL[71, 73]. As an example, a DBD graft with severe macrosteatosis has a different risk of ITBL than a DCD graft with 60 minutes of dWIT, but both will fall into the declined group due to differing clinical concerns. The increased utilisation of NMP for livers otherwise deemed to high risk for transplant following SCS has pragmatic consequences for trial design. This loss in equipoise over the safety of randomising high-risk livers to SCS results in a greater reliance on cohort studies.

The trails that have investigated NMP in the clinical setting, *via* either an RCT or cohort study with a representative control group, are listed in Table 3. Many of these trials unfortunately lack clear definitions of what was considered ITBL, and whether complete artery patency was required for the diagnosis. To date, three RCTs of NMP have been completed [64, 65, 74] (Table 3). The first multi-centre RCT published by Nasralla *et al* in 2018 included standard risk grafts from both DBD and DCD donors and applied NMP *at source*. This study included a protocol MRCP at 6 mo to assess for biliary complications of which only a small proportion of subjects completed. The difference in stricture incidence was greatest in the DCD subgroup, with a rate of 26.3% in the DCD-SCS group as opposed to 11.1% in the DCD-NMP group. Whether these ischaemic strictures were ITBL or the result of a vascular lesion is unclear, as HA patency was not possible to determine from the data provided.[64] The smaller randomised trial conducted by Ghinolfi *et al* comprised a sample of elderly DBD donors (≥70 years) and applied NMP in a back-to-base approach[74]. However, the small sample size and lower overall risk of ITBL in DBD grafts makes the findings of this study less informative. In this study, only one patient developed a biliary stricture, and they were in the NMP group. A recent large RCT from the U.S randomised nearly 300 Livers (both DBD and

DCD) to either at-source NMP or SCS[65]. These authors did provide a definition of what they considered an ischaemic biliary complication (Table 3) and this occurred in 4 and 14 of the NMP and SCS livers respectively. Once again, the granularity of the data presented does not allow determination of the proportion of patients with ischaemic biliary complications (IBC) that were truly ITBL. It should be noted that none of these randomised trials performed any formal viability testing of the grafts prior to transplant and were not powered appropriately to assess for biliary complications.

Both prospective and retrospective cohort studies have investigated biliary complications following NMP (Table 3). In the VITTAL trial, Mergental *et al* reported the outcomes of a prospective cohort of 22 Livers (12 DBD, 10 DCD) destined for discard that were transplanted following back-to-base NMP. After 6 mo follow up, 3/10 of the DCD livers had developed symptomatic non-anastomotic biliary strictures. requiring retransplantation. Fodor *et al* subsequently reported a retrospective cohort study of predominantly DBD grafts (49 DBD and 9 DCD) preserved *via* back-to-base NMP and reported both a lower incidence and severity of ITBL in the NMP group in comparison to SCS[75]. This study had a clear definition of ITBL and applied viability testing which included bile output and pH, however specific parameters for these were not reported. Recently, Gaurav *et al* published a single centre retrospective cohort study of DCD grafts preserved by NMP (back-to-base and at-source), NRP and CS with the primary outcome being NAS[44]. These authors also provided a clear definition of NAS and this required hepatic artery patency. In the NMP group, 12/69 developed NAS and in 7/12 it was clinically significant. This was comparable to the SCS group (22/97, 12/22 clinically significant) but higher than the NRP group (4/69, 0/4 clinically significant). It must be noted that this study applied biliary viability testing to NMP preserved livers and 77% of these NMP preserved DCD livers proceeded to transplant.

Utilising NMP perfusate and bile parameters to predict (and avoid) certain outcomes remains controversial. The risks associated with liberal use of NMP preserved livers is

associated morbidity and mortality. The risks associated with being too stringent on the NMP viability criteria, when predictive accuracy is less than perfect, is the discard of a liver that would have resulted in acceptable outcomes. NMP indicators of biliary injury and/or function have been studied by several groups in human livers[76, 77]. The early experience of associating bile pH, bicarbonate and glucose with cholangiopathies was reported by Watson *et al* in 2018, from 16 transplanted livers. In this group of livers, a biliary pH <7.4 occurred in 3/16 and all three of these developed a cholangiopathy. A lower biliary bicarbonate and higher biliary glucose concentration was also associated with subsequent cholangiopathy. Recently, the same group have reported outcomes in a much larger cohort of 144 transplanted livers[78]. Interestingly, 15 of these transplanted livers did not meet their previously reported cholangiocyte viability criteria and in a further three livers no bile was produced which precluded this assessment. Clinically significant non-anastomotic strictures developed in 9/144 recipients and all of these had a bile pH >7.5. Matton *et al* investigated biliary NMP parameters in both the laboratory and a small clinical trial (n = 6)[76]. These authors did not have any cases of ITBL in the recipients within the trial, but demonstrated an inverse correlation between bile pH and bicarbonate concentration with histological evidence of biliary injury in a group of non-transplanted livers. A pH and bicarbonate concentration of <7.48 and <18mmol/L had a positive predictive value of 75% and 91% for significant biliary injury. In summary, the prediction (and avoidance) of ITBL using NMP requires further research. This will undoubtedly be of assistance to the transplant community, however at present the accuracy remains sub-optimal and it must be improved to avoid the unnecessary discard of grafts.

2.4 Machine combination approaches

In theory, the different rationale behind the various MP strategies could work synergistically to prevent ITBL. A-NRP abbreviates the dWIT induced damage and provides in-situ resuscitation prior to the cold ischaemic period, whereas the ex-situ

techniques may dampen IRI. Conversely, the application of sequential machine perfusion strategies may follow the rule of diminishing returns and the resources may not be justified. Despite the report that individual grafts had the combination of A-NRP and NMP, clarity in the ITBL rate with this combination is lacking as they were excluded from the analysis of this study[44, 79]. The Groningen group have also reported their experience with sequential ex-situ end-ischemic D-HOPE, controlled oxygenated rewarming and NMP for high-risk livers (mostly DCD grafts).[80] The application of this protocol is proposed to protects these livers against IRI (D-HOPE phase) and enables viability assessment (NMP phase) prior to transplantation, resulting in promising outcomes.

In Italy, as aforementioned circulatory death during DCD procurement is declared after a stand-off period of 20 min. This prolonged dWIT time has been a general reluctance to use such DCD grafts for transplantation due to the probable high risk of graft failure. The combination of A-NRP and D-HOPE has contributed to increase the donor pool in this high-risk donor context. In a retrospective cohort study, this combination has reported satisfactory outcomes in terms of ITBL when compared with relatively low-risk donor control group (A-NRP + D-HOPE 3% vs. SCS 8%)[43]. Recently, a new procedure called ischaemia-free liver transplantation has been proposed, during which liver grafts are procured, preserved and implanted under continuous NMP. Its applicability and clinical impact are yet to be determined[81]. Further studies are required to determine if additional benefits are achieved by combining different techniques.

3.0 Evidence summary

The volume of data on MP and its impact on the development of ITBL following liver transplant is growing. However, with only a few exceptions, ITBL was not the primary outcome under investigation in these studies and therefore were not designed and

reported with this entity in mind. It is difficult in many published studies to tease out what biliary complications represent the development of ITBL, as opposed to strictures of another cause. With this considered, the evidence for prevention of ITBL with MP is distinctly different for DCD as opposed to DBD grafts.

The highest quality evidence for ITBL prevention with MP in DCD grafts is D-HOPE, in comparison to SRR and SCS. This is based on finding from a randomised controlled trial[52]. However, a larger quantity of lower quality evidence supports A-NRP in comparison to both SRR with SCS, and NMP *via* a 'back to base' approach. At present, there is no evidence that D-HOPE is superior to A-NRP or that the combination of these two MP techniques results in a further reduction of ITBL than each one in isolation. Based on the DCD subgroup from one randomised trial [64], there is a suggestion that NMP applied 'at source' reduces ITBL in comparison to SRR and SCS[64]. There is no good quality evidence that NMP applied in a 'back to base' approach for DCD grafts prevents ITBL. In DBD donors, the incidence of ITBL is lower and therefore studies with a large sample size will be required to demonstrate a noticeable effect. The available evidence to suggest that either NMP 'at source' or 'back to base' for DBD grafts is weak, and consists of only one cohort study [75]. There is no trial evidence to support a reduction in ITBL for DBD grafts with HMP strategies.

CONCLUSION

ITBL remains an ongoing issue and the notion that biliary complications are the 'achilles heel' of liver transplantation remains true. However, with the introduction of MP technology, gains are being made in the prevention of this highly morbid condition. The greatest area of improvement is for DCD grafts with RCT evidence for D-HOPE, and large cohort studies supporting A-NRP. Given the demonstrated benefits these modalities have over SCS for DCD grafts, a loss of equipoise within the transplant community is diminishing the opportunity for further RCTs that include a SCS group. As an organ donor often generously gives numerous organs, the impact A-NRP has on

other abdominal viscera may influence the decision regarding the most appropriate MP strategies. Multiple factors likely interact to cause ITBL, and a greater understanding of these will undoubtedly help refine both preventative and treatment interventions.

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