Heterogeneity of Bile Duct Management in the Development of Ischemic Cholangiopathy After Liver Transplantation: Results of a European Liver and Intestine Transplant Association Survey

Nicolas Meurisse^{a,b}, Diethard Monbaliu^b, Gabriela Berlakovich^c, Paolo Muiesan^d, Martin Oliverius^e, René Adam^f, and Jacques Pirenne^{b,*}

^aDepartment of Abdominal Transplant Surgery, University of Liege Academic Hospital, ULg CHU, Liege, Belgium; ^bDepartment of Abdominal Transplant Surgery, University Hospitals Leuven, KU Leuven, Leuven, Belgium; ^cDivision of Transplantation, Department of Surgery, Medical University of Vienna, Vienna, Austria; ^dLiver Unit, University of Birmingham, Birmingham, United Kingdom; ^eDepartment of Surgery of the 3rd Faculty of Medicine Charles University and Kralovske Vinohrady Hospital, Prague, Czech Republic; and ^fAPHP Hospital Paul Brousse, Inserm U985, University Paris Sud, Paris, France

ABSTRACT

Background. Surgical factors and direct cytotoxicity of bile salts on cholangiocytes may play a role in the development of ischemic cholangiopathy (IC) after liver transplantation (LTx). There is no validated consensus on how to protect the bile ducts during procurement, static preservation, and LTx. Meanwhile, IC remains the most troublesome complication after LTx.

Aim. To characterize bile duct management techniques during the LTx process among European transplant centers in cases of donation after brain death (DBD) and circulatory death (DCD).

Method. An European Liver and Intestine Transplant Association–European Liver Transplant Registry web survey designed to conceal respondents' personal information was sent to surgeons procuring and/or transplanting livers in Europe.

Results. Sixty-five percent of responses came from large transplant centers (>50 procurements/y). In 8% of DBDs and 14% of DCDs the bile duct is not rinsed. In 46% of DBDs and 52% of DCDs surgeons prefer to remove the gallbladder after graft reperfusion. Protocols concerning preservation solutions (nature, pressure, volume) are extremely heterogeneous. In 54% of DBDs and 61% of DCDs an arterial back table pressure perfusion is performed. Steroids (20%-10%), heparin (72%-60%), prostacyclin (3%-7%), and fibrinolytics (4%-11%) are used as donor-protective interventions in DBD and DCD cases, respectively. In 2% of DBD and 6% of DCD cases a hepatic artery reperfusion is performed first. In 4% of DBD and 6% of DCD cases, fibrinolytics are administered through the hepatic artery during the bench and/or implantation.

Conclusion. This European web survey shows for the first time the heterogeneity in the management of bile ducts during procurement, preservation, and transplantation in Europe. In the context of sharing more marginal liver grafts, an expert meeting must be organized to formulate guidelines to be applied to protect liver grafts against IC.

ISCHEMIC cholangiopathy (IC), also referred to as non-anastomotic biliary strictures, ischemic-type biliary strictures, intra-hepatic biliary strictures, and post-transplant cholangiopathy, represents one of the most troublesome complications after liver transplantation (LTx) [1]. IC can be

*Address correspondence to Jacques Pirenne, MD, MSc, PhD, Abdominal Transplant Surgery Department, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium. Tel: +32 16 34 87 26; Fax: +32 16 34 87 43. E-mail: jacques.pirenne@uzleuven.be

defined as any irregularity of the intra- or extrahepatic bile ducts of the liver graft at sites other than that of the anastomosis with a patent hepatic artery that is radiologically evident to cause clinical symptoms and requires intervention [2]. Usually resistant to any radiological, endoscopic, or surgical interventions, IC is strongly associated with long hospitalizations, high cost, high rates of graft failure, and high mortality [3]. In addition to ischemic insults and impairment of regeneration, surgical factors and the direct cytotoxicity of bile salts on cholangiocytes may play a role in the development of IC [4]. In the absence of a well-described international consensus, with this anonymous web survey we aimed to characterize the different techniques used in European LTx centers for bile duct management in the course of procurement, preservation, and LTx in cases of donation after brain death (DBD) and donation after circulatory death (DCD) if allowed in the country.

METHODS

In 2014, an anonymous web survey (distributed from the University Hospitals of Leuven's Central Computer Department) was designed under the auspices of the European Liver and Intestine Transplant Association (ELITA). Questions were designed to assess management of the bile duct during organ procurement, preservation, and LTx. The Secretariat of the European Society of Organ Transplantation, ELITA, and the European Liver Transplant Registry were asked to provide the email addresses of their members. Surgeons involved in procurement and/or LTx were invited to participate in the survey via a web hyperlink.

In the course of procurement, preservation, and LTx, the following aspects were assessed in cases of DBD and DCD: the site, timing, nature, and volume of the solution used to rinse the bile ducts; the timing of cholecystectomy; the nature, volume, and pressure of the preservation solution for the aorta and timing of the portal vein flushes; the use of ex situ back table hepatic artery pressure perfusion;

donor treatment with steroids, heparin, vasodilators, and/or fibrinolytics; the sequence of hepatic artery and/or portal vein reperfusion; and the use of fibrinolytics in the recipient. Surgeons were asked if their management of bile ducts was based on guidelines. No questions related to dynamic preservation were asked. Relationships between particular practices and the onset of IC are difficult to determine, as each center transplants local or imported livers. Accordingly, no statistical analyses were conducted.

RESULTS

The survey was sent to 475 surgeons from 151 LTx centers in 26 European countries; 210 (44%), 97 (64%), and 23 (88%) responses were obtained, respectively. In decreasing order, surgeons from the following countries completed the web survey (see Fig 1): Italy (16%), Spain (15%), Belgium (10%), Germany (10%), France (9%), United Kingdom (8%), the Netherlands (7%), Poland (3%), Czech Republic (3%), Austria (3%), Portugal (2%), Sweden (2%), Belarus (2%), Hungary (2%), Norway (1%), Bulgaria (1%), Finland (1%), Greece (1%), Croatia (1%), Switzerland (1%), Estonia (1%), and Slovenia (1%).

In total, 11% of the surgeons performed only procurements, 36% were LTx transplant surgeons, and 53% were both procurement and LTx transplant surgeons. Thirty-four percent worked with both DBD and DCD cases and 65% worked in centers performing more than 50 procurements per year.

Eight percent and 14% of surgeons did not rinse bile ducts in DBD and DCD procedures, respectively. When done, only 34% and 27% surgeons, respectively, rinsed bile ducts through the gallbladder via an incision in the common bile duct (CBD); only 38% and 33% did so through both the CBD and the gallbladder in 23% and 29% of cases, respectively; and through the cystic duct only in 5% and 11% in DBD and DCD, respectively (Fig 2).

Fig 1. Geographical distribution and rate of respondents per country (%).

Fig 2. Site(s) chosen by surgeons to rinse common bile ducts (CBD).

Multiple choice questions were provided regarding rinsing bile, which was performed in situ before the start of cold perfusion in 25% and 4% of cases, and/or after cold perfusion in 16% and 33% of cases, and/or ex situ in the donor center before cold storage in 55% and 63% of cases, and/or in the recipient center before implantation in 24% and 25% in DBD and DCD cases, respectively (Table 1).

In situ, sodium chloride (NaCl) in solution form was used to rinse the bile ducts in 25% and 26% of cases; University of Wisconsin (UW) solution in 8% and 11% of cases; histidine tryptophan ketoglutarate (HTK) in 2% and 2% of cases; Celsior in 2% and 4% of cases; and Marshall and Institut George Lopez-1 (IGL-1) in fewer than 1% of DBD and DCD cases, respectively. The abstention rates were 62% and 55% in DBD and DCD cases, respectively. While ex situ, NaCl was used in 8% and 11% of cases, UW in 31% and 46% of cases, HTK in 23% and 17% of cases, Celsior in 12% and 4% of cases, and Marshall and IGL-1 were used in fewer than 1% of DBD and DCD cases, respectively. The abstention rates were 25% and 21% in DBD and DCD cases, respectively (Table 2).

Nineteen percent and 16% of surgeons rinsed bile ducts with less than 100 cc solution without considering the color of the effluent in DBD and DCD cases, respectively, while 19% and 21% did so with more than 100 cc; 62% and 63% of surgeons rinsed bile ducts in DBD and DCD cases, respectively, until the effluent had cleared.

A cholecystectomy was performed in the donor center in situ after a cold flush in 13% and 4% of cases, ex situ on the back table prior to cold storage in 25% and 20% of cases, in the recipient center on the bench in 16% and 24% of cases, or after reperfusion and prior to biliary anastomosis in 46%

and 52% of DBD and DCD cases, respectively. In total, 33% and 35% of the surgeons ligated cystic ducts during procurement in situ after a cold flush, independent of the decision to perform a cholecystectomy, in DBD and DCD cases, respectively (Table 3).

UW was used as the preservation solution for aortic flush in 34% and 30% of cases; HTK in 22% and 22% of cases; Celsior in 22% and 20% of cases; and Marshall, Custodiol, Scot-15 and IGL-1 were used in fewer than 5% of DBD and DCD cases, respectively (Table 4). The abstention rates were 17% and 28% for DBD and DCD cases, respectively. If UW was considered, 52% and 28% used less than 5 L, 44% and 66% between 5 and 10 L, and 4% and 6% more than 10 L in DBD and DCD cases, respectively. When HTK was preferred, 9% and 0% used less than 5 L, 80% and 87% between 5 and 10 L, and 11% and 13% more than 10 L in DBD and DCD cases, respectively. When flushing the aorta with Celsior, 30% and 57% used less than 5 L, 70% and 43% between 5 and 10 L, and none used more than 10 L in DBD and DCD cases, respectively. A pneumatic compressive bag

Table 1. Timing of the Rinsing of Bile in the Donor and/or the Recipient Center (Multiple-Choice Questions and Answers)

	DBD	DCD
In situ before the cold flush	25% (52/210)	4% (9/71)
In situ after the cold flush	16% (34/210)	33% (24/71)
Ex situ in the donor center before cold storage	55% (115/210)	63% (45/71)
Before implantation in the recipient center	24% (51/210)	25% (18/71)

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death.

Table 2. Solutions Used to Rinse Bile

	DBD	DCD
In situ		
NaCl	25% (52/210)	26% (19/71)
UW	8% (17/210)	11% (8/71)
HTK	2% (5/210)	2% (2/71)
Celsior	2% (5/210)	4% (3/71)
Marshall, IGL-1	<1%	<1%
No answer	62% (131/210)	55% (39/71)
Ex situ		
NaCl	8% (17/210)	11% (8/71)
UW	31% (65/210)	46% (33/71)
HTK	23% (49/210)	17% (12/71)
Celsior	12% (25/210)	4% (3/71)
Marshall, IGL-1	<1%	<1%
No answer	25% (53/210)	21% (15/71)

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; HTK, histidine tryptophan ketoglutarate; IGL-1, Institut Georges Lopez; NaCl, sodium chloride; UW, University of Wisconsin.

to increase the pressure of the preservation solution was used in 50% and 71% of cases when flushing with UW, in 32% and 43% of cases with HTK, and in 19% and 21% of cases with Celsior in DBD and DCD cases, respectively.

In total, 23% and 18% of surgeons never performed a portal flush in DBD and DCD cases, respectively (based on multiple choice answers). When finished, the portal vein was flushed in situ concomitantly with the aortic flush in 33% and 33% of cases, in the donor center on the back table prior to cold storage in 42% and 45% of cases, and/or in the recipient center on the bench prior to implantation in 19% and 15% of DBD and DCD cases, respectively.

An ex situ hepatic artery back table pressure perfusion was performed by 54% and 61% of surgeons in DBD and DCD cases, respectively. When performed, back table pressure perfusions took place in the donor center prior to cold storage in 52% and 59% of cases or in the recipient center during the bench prior to implantation in 48% and 41% of DBD and DCD cases, respectively.

In total, 20% and 10% of surgeons treated donors with steroids before procurement in DBD and DCD cases, respectively. Seventy-two percent and 60% administered heparin intravenously before donor incision in DBD and DCD cases, respectively. An aortic pre-flush immediately before the start of cold preservation solution was done with vasodilators (prostacyclin) by 3% (DBD) and 7% (DCD) of surgeons, respectively; fibrinolytics (streptokinase, urokinase, recombinant tissue-type plasminogen activator) were

used by 4% (DBD) and 11% (DCD) of surgeons, respectively. Abstinence rates exceeded 20% for each medication.

A sequential liver reperfusion was performed by 70% and 62% of the surgeons using the portal vein first and by 2% and 6% using the hepatic artery first, whereas 9% and 10% preferred a simultaneous reperfusion in DBD and DCD cases, respectively. The abstinence rates for DBD and DCD were 19% and 22%, respectively.

In total, 4% and 6% of surgeons injected fibrinolytics into the hepatic artery on the bench at the recipient center or during implantation using DBDs and DCDs, respectively. The abstinence rates for DBD and DCD were 20% and 27%, respectively.

Management of the bile duct is left to the discretion of the procuring surgeon in 18% of cases, with no specific guidelines. Twenty-six percent (26%) of surgeons base their technique on international guidelines, for which a written protocol exists in 13% of cases; 17% do so on national consensus, for which a written protocol exists in 14% of cases, and 39% do so on center recommendations, for which a written protocol exists in 33% of cases (Fig 3).

DISCUSSION

This anonymous web survey clearly highlights for the first time the considerable heterogeneity of surgical practices used among European transplant centers for bile duct management in DBD and DCD LTx.

Despite a low rate of answers (44%), relevancy is counterbalanced by a majority of responses coming from experienced centers (65% > 50 procurements/year).

Few surgeons still do not rinse bile ducts at all, despite pre- and clinical data demonstrating a detrimental effect of stagnating bile during cold preservation [5,6] and recommendations [7–9]. The addition of a retrograde rinse through an incision in the CBD should optimize the complete bile flush-out of the graft initiated by an antegrade rinse through the gallbladder (Fig 4). If made in situ, bile ducts can be rinsed before and after the perfusion of preservation solution in case of DBD, while doing so before cross-clamping without increasing the warm ischemia time (defined as the time between the withdrawal of life-sustaining therapy and the start of cold perfusion) in DCD cases is inevitable. Heterogeneity is observed concerning the nature and volume of the solution that should be used to rinse the bile ducts. This result can be explained by the lack of clinical data showing the impact of preservation solutions in decreasing cholangiocyte preservation injuries compared to NaCl during cold storage. Moreover, no guidelines concerning the precise

Table 3. Timing of Cholecystectomy and Ligation of Cystic Duct

	DBD	DCD
In situ after the cold flush	13% (27/210)	4% (3/71)
In the donor center before cold storage	25% (50/210)	20% (13/71)
In the recipient center before implantation	16% (35/210)	24% (17/71)
After implantation	46% (98/210)	52% (37/71)
Ligation of the cystic duct after the cold flush regardless of the decision of a cholecystectomy	33% (69/210)	35% (24/71)

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death.

Table 4. Nature, Volume, and Pressure of the Cold Preservation Solution Perfused Through the Aorta

	UW	HTK	Celsior	Marshall, Custodiol, IGL-1, Scott-15
DBD	34% (72/210)	22% (46/210)	22% (46/210)	<5% (9/210)
Volume				
< 5 L	52% (37/72)	9% (4/46)	30% (14/46)	<5% (4/9)
5–10 L	44% (32/72)	80% (37/46)	70% (33/46)	<5% (4/9)
> 10 L	4% (3/72)	11% (5/46)	0% (0/46)	<5% (1/9)
Pressurized	50% (36/72)	32% (15/46)	19% (9/46)	0% (0/9)
DCD	30% (21/71)	22% (16/71)	20% (14/71)	<5% (2/71)
Volume				
< 5 L	28% (6/21)	0% (0/16)	57% (8/14)	<5% (1/2)
5–10 L	66% (14/21)	87% (14/16)	43% (6/14)	<5% (1/2)
> 10 L	6% (1/21)	13% (2/16)	0% (0/14)	0% (0/2)
Pressurized	71% (15/21)	43% (7/16)	21% (3/14)	0% (0/2)

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; HTK, histidine tryptophan ketoglutarate; IGL-1, Institut Georges Lopez; UW, University of Wisconsin.

volume of solution to obtain a complete rinse of bile ducts exist. Thus, it would be preferable to assess the quality of the rinse through the color of the effluent. Finally, the temperature of the solution should be kept cold, and gentle pressure should be applied for the rinse.

Due to the lack of a surgical consensus, cholecystectomies are performed at either the donor center or the recipient center. The overall goal is to avoid further contamination of stagnating bile in the biliary tree during cold storage. If it is to be removed or if only the cystic duct is to be ligated in the donor center, this procedure should be performed by experienced surgeons. Indeed, one must be aware that dissection made to the hepatoduodenal ligament before implantation can damage the liver hilum, which could compromise the LTx. However, if left in place after a complete anterograde flushing of bile, closing the incision of the gallbladder is advised [8].

The answers regarding the preservation solutions were heterogeneous. No randomized controlled trials (RCT) have shown the superiority of a preservation solution in DBD and DCD LTxs [10]. High-viscosity preservation solutions such as UW have been thought to impair the flush of the peribiliary capillary plexus [5], leading to more biliary complications in retrospective studies compared to those achieved using low-viscosity solutions (such as HTK, IGL-1, and Marshall) [11–13], but the results are conflicting [14]. Finally, large registry analyses have indicated the superiority of UW over HTK in preserving liver grafts in terms of graft survival [15–17].

Systematic reviews demonstrate that there is no clear consensus on the volume of preservation solution [18,19]. It is advised to use a higher volume (4–6 L) of a low-viscosity preservation solution to achieve an adequate flushing rather than the volume required for high-viscosity solutions (3–4 L) [19].

Routine use of a pneumatic compressive bag is not standardized, but its application is more frequent with high-viscosity preservation solutions. In a non-powered RCT, an additional 100 mm Hg of pressure on the UW preservation solution is associated with better early graft function

(international normalized ratio and aspartate transaminase) and superior graft and patient survival at 1 month [20]. In a retrospective study, Pirenne et al hypothesized that pressurized UW could ease the flush of the peribiliary capillary plexus and protect against further IC [21]. Interestingly, Moench et al showed in a pre-clinical model that increasing the pressure of UW and HTK do not improve in situ perfusion flow or pressure in the hepatic artery (HA) due to the venting of the vena cava, despite cross-clamping the thoracic aorta. On the other hand, ex situ perfusion was associated with significantly improved flow and pressure in the HA without any difference between HTK and UW [22]. Consensus for a single aortic flush or a systematic dual aortic and portal perfusion in situ has not been established. A systematic search and meta-analysis showed no differences in terms of the temperature kinetic drop in the liver graft, rate of early allograft dysfunction and primary non-function, or patient and graft survival when comparing a single aortic flush to dual aortic and portal perfusion

Fig 3. Existing protocols of bile duct management according to surgeons.

Fig 4. Anterograde and retrograde rinsing of bile through the gallbladder and the common bile duct, respectively.

[18,19]. There is currently no evidence suggesting the inferiority of the single aortic flush that represents a fast way to flush the liver, especially in DCD donors. Moreover, a single aortic flush avoids portal hypertension and injury to the pancreas, as has been suggested [23]. Overall, an additional ex situ portal/HA flush must be performed at the donor center and/or the recipient center before the implantation can proceed.

Based on the hypothesis that perfusion of the peribiliary capillary plexus is of paramount importance, an arterial back table pressure perfusion (150 mm Hg) of 300 cc UW through the hepatic artery has been identified as an independent protective factor to prevent the development of IC [24]. However, these findings were not confirmed in a recent powered multicenter RCT using ex situ perfusion through HA with 300 cc HTK at a pressure of 100 cm H₂O [25].

A high rate of discrepancy for donor treatment was observed. Steroids (particularly methylprednisolone), which have been shown to reduce inflammatory markers and ischemia reperfusion injury in a powered RCT [26] via modulating brain-death-induced systemic inflammation, are not used systematically despite international recommendations [27]. The routine administration of steroids in DBD continues to be based on conflicting evidence according to a systematic review [28] and recent rodent findings that failed to show a translation of the anti-inflammatory effect on the IRI [29].

Donor heparinization prior to circulatory arrest, which is standard in DBD donors, is performed to prevent thrombi formation and to ensure adequate parenchymal penetration of the preservation solution. In the case of DCDs, it is well known that blood flow rapidly decreases during the agonal phase that ensues after the withdrawal of life sustaining therapy [30], which can lead to thrombi formation [31]. To avoid impairment of organ perfusion, especially the peribiliary capillary plexus, which can potentially lead to biliary duct injuries [5,32], heparin is generally administered immediately prior to stop therapy in Belgium [33] and many US centers [9]. Interestingly, Hessheimer et al recently

demonstrated the anti-inflammatory and cytoprotective effects of heparin given prior to cardiac arrest compared to the administration of fibrinolytics during postmortem regional perfusion in a stringent DCD LTx pig model [34]. However, biological interventions in potential DCD donors before circulatory arrest has occurred are ethically and legally controversial [35]. For this reason, the Human Tissue Act in the UK prohibits giving heparin. Interestingly, the results of the 318 DCD LTx are outstanding in terms of IC (3%) at King's College Hospital London, raising questions about the role of pre-mortem heparin administration [36].

A small percentage of surgeons treated donors with profibrinolytic agents. These agents have been proposed to adequately remove preformed microthrombi, as heparin has no effect on pre-existing clots in the microcirculation [37]. These maneuvers can be performed as a donor pre-flush [2], on the bench, and/or during implantation in DBD and DCD LTxs. Observational studies indicate that the administration of fibrinolytics both to the preservation solution and directly into the hepatic artery on the back table at the donor center [38], into the donor hepatic artery immediately before implantation [39], or through the hepatic artery during portal anastomosis or after portal reperfusion right before the arterial anastomosis is associated with less IC without increasing the risk for bleeding in controlled DCD LTx [40]. A thrombolytic protocol is now described [40,41], and an RCT has been designed to determine the efficacy and safety of this strategy (NCT01197573). However, thrombi formation during warm ischemia time in DCD donors is not supported by current evidence [36,42]. Hyperfibrinolysis has even been reported at the declaration of death or after resuscitation on a molecular basis and according to thromboelastometry [42], suggesting that fibrinolytic therapy could be obviated.

A low percentage of surgeons treat the donor with prostacyclin analogs, which are well known for their strong vasodilation and platelet aggregation inhibition activity. In a pre-clinical pig DCD LTx model, the infusion of OP-2507 (prostacyclin analog) before cardiac arrest was associated with the improvement of survival [43]. In a stringent DCD LTx model, Monbaliu et al [44] developed a strategy combining epoprostenol (a prostacyclin analog) with a series of agents targeting specific pathways of IRI (streptokinase, glycine, glutathione, alfa-1-glycoprotein, alfa-tocopherol, and mitogen-activated protein kinase inhibitor) administered immediately before the cold flush that resulted in the elimination of primary non-function, improved liver function, reduced biliary salt toxicity, and improved survival. Moreover, this combined approach was found to suppress inflammation-regulating genes [45]. In a clinical setting, Klein et al demonstrated in a non-powered RCT that the administration of epoprostenol (100 µg) before cold perfusion significantly reduced the IRI via a clear reduction in the postoperative peak of transaminase [46]. Finally, donor treatment with epoprostenol was identified in a retrospective study as a protective factor against the development of IC by Pirenne et al [21].

In this survey, the most commonly used procedure for revascularization of the liver graft was initial reperfusion via the portal vein, followed by subsequent reconstruction of the hepatic artery. This is based on the experience that portal blood flow alone is sufficient for adequate hepatocellular function [47]. However, the timing of reperfusion (sequential vs simultaneous portal and arterial reperfusion) is still a matter of debate in the literature. A recent meta-analysis has shown that IC can be prevented by performing a simultaneous arterial and portal reperfusion, thus reducing warm ischemic damage to the bile ducts, which depends solely on arterial blood supply [48]. However, prolongation of anastomosis time and re-warming the graft associated with simultaneous reperfusion may have an overall negative impact on postoperative graft function [49]. Special attention must be given to prepare the recipient hepatic artery right after the recipient liver explantation to expedite arterial anastomosis, regardless of the reperfusion sequence.

Finally, excluding surgeons who did not follow any recommendations, the answers related to guidelines to which surgeons refer were extremely heterogeneous in terms of their origin (international, national, or local), emphasizing the lack of scientific evidence describing the best way to handle bile ducts in LTx.

CONCLUSION

This European web survey shows for the first time the significant heterogeneity in surgical techniques used to manage bile ducts among respondents between countries, centers, and even surgeons from the same institution. The absence of evidence-based data and a well-described international consensus defining the best practice for managing bile ducts in the course of procurement, preservation, and LTx is obviously emphasized. Due to exchange programs for more high-risk organs, an expert consensus meeting must be organized to clearly state guidelines that should be applied to protect liver grafts against IC. Finally, European educational initiatives (European Union of Medical Specialists, European Society of Organ Transplantation, ELITA) should be encouraged for surgeons dedicated to organ procurement and/or LTx to avoid obvious mistakes, such as the omission of bile rinse-out before cold storage.

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