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Bile composition as a diagnostic and prognostic tool in liver transplantation

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Keywords: biliary biomarkers, machine perfusion, outcomes, acute cellular rejection, non-anastomotic biliary strictures, viability assessment, liver transplantation, ischemia reperfusion injury, early allograft dysfunction, bile composition.

Abbreviations

ACR, acute cellular rejection

CA, cholic acid

CDCA, chenodeoxycholic acid

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CDmiR, cholangiocyte-derived micro RNA

CMV, cytomegalovirus

DBD, donation after brain death

DCA, deoxycholic acid

DCD, donation after circulatory death

ECD, extended criteria donor

ERCP, endoscopic retrograde cholangiopancreatography

HDmiR, hepatocyte-derived micro RNA

HMP, hypothermic machine perfusion

^1H NMR, proton nuclear magnetic resonance

IRI, ischemia-reperfusion injury

ITBL, ischemic-type biliary lesions

LDH, lactate dehydrogenase

LT, liver transplantation

miRNA, micro RNA

NAS, non-anastomotic biliary strictures

NMP, normothermic machine perfusion

PC, phosphatidylcholine

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Abstract

Bile secretion and composition reflects the functional status of hepatocytes and cholangiocytes. Bile composition can have a role in the assessment of donor grafts before implantation in the recipient. In addition, changes in bile composition after liver transplantation can serve as a diagnostic and prognostic tool to predict posttransplant complications, such as primary non-function, acute cellular rejection, or non-anastomotic biliary strictures. With the popularization of liver machine perfusion preservation in the clinical setting there is a revisited interest in biliary biomarkers to assess graft viability before implantation. This review discusses current literature on biliary biomarkers that could predict or assess liver graft and bile duct viability. Bile composition offers an exciting and novel perspective in the search for reliable hepatocyte and cholangiocyte viability biomarkers.

Introduction

Bile secretion and composition reflects the functional status of hepatocytes and cholangiocytes and has been used to predict liver function and several diseases. Over the last years, the use of lower quality liver grafts (severely ischemic, steatotic, older) has increased, thereby increasing the risk of post-operative complications. As a consequence, studies have been performed to find predictive markers of graft function and postoperative complications after liver transplantation (LT) (1).

For many years, bile flow and characteristics like bile color have been used as a marker of graft function after LT. More recently, it has become apparent that mere biliary flow rate may not adequately reflect graft function, and, therefore, increasing attention is now given to direct analysis of the composition of bile. There is increasing evidence that bile composition can be used as a tool to monitor and predict liver function in both experimental and clinical studies. At the same time, novel organ preservation techniques such as normothermic machine perfusion (NMP) create a prolonged time window and near-physiological environment in which bile production and biliary quality could be evaluated more effectively. The composition of bile during machine perfusion tends to be a powerful tool to assess liver graft viability and viability of the biliary tree in particular (2).

The present review highlights the importance of bile composition as a biomarker for graft function in LT. We provide a summary on the physiology of bile formation and give an

overview of biliary biomarkers that may be used to assess liver viability and predict postoperative complications. This review may provide a starting point for future studies on bile composition as a biomarker in LT.

How bile is formed

Bile formation is a unique function of the liver and vital to survival of the organism. The fundamental mechanisms of bile composition remained unknown until the mid 20th century, largely because bile is a 'hidden' fluid, which was not as easy to collect as urine, for example (3). Pioneering work in the understanding of bile secretion was performed by researchers Brauer and Sperber, who demonstrated that bile formation is an energy-dependent process (4) and that its production is related to temperature, ceasing around 25°C and reaching its maximum between 38-40°C. Sperber first described that the concentrative transport of solutes in bile created osmotic gradients which stimulates the passive diffusion of water into bile (5).

Bile is a product of hepatic secretory function, and it contains all body components: proteins, lipids, carbohydrates, vitamins, mineral salts, and trace elements. Approximately 95% of bile is formed by water, and 5% consists of inorganic and organic solutes (**Table 1**). The composition of bile is complex, but bile acids, phospholipids, electrolytes, and organic anions are the major components of human bile.

Bile acids are exclusively synthesized by the liver and represent a major fraction of cholesterol catabolism. The majority of bile acids are conjugated to taurine or glycine, and their secretion generates bile flow. Periportal hepatocytes are mainly responsible for the absorption of bile acids during the enterohepatic circulation. Obstruction of bile flow leads to a more chronic exposure of hepatocytes to bile acids causing various cholestatic diseases (6). The detrimental effect is brought about mostly by hydrophobic bile salts, which may act as detergents that damage cellular membranes and trigger hepatocyte death (7). On the other hand, bile salts appear to promote anti-oxidant defenses and have a stimulatory effect

on liver regeneration up to a certain threshold level. This has been demonstrated in patients undergoing hemi-hepatectomy, whereas patients who had external biliary drainage after the surgery had less liver regeneration compared to patients without external drainage (8).

Phosphatidylcholine (PC) is the major phospholipid in mammalian cells and its main function in bile is to form mixed micelles with bile acids and cholesterol, essential for the emulsification of fats and to encapsulate toxic bile salts to protect cells. PC also has a cytoprotective role in the biliary epithelium and may reduce the cellular toxicity of bile acids (3). The electrolyte content of bile is largely similar to that of plasma. Excretion of sodium, potassium and calcium into bile is closely related to the rate of metabolic processes in the liver and depends on its functional state and on the content of salts in the body. Bile also consists of many proteins, but high concentrations of interfering substances have made proteomics analysis very difficult, which has limited the number of studies to date (9).

The production and secretion of bile requires active transport systems within hepatocytes and cholangiocytes in addition to a structurally and functionally intact biliary tree. Hepatocytes produce bile by secreting conjugated bilirubin, bile acids, cholesterol, phospholipids, proteins, ions, and water into their canaliculi. When conjugated bile acids enter the canaliculus, water follows by osmosis presumably through aquaporin water channels. The gradient allows for passive diffusion of inorganic ions. Canalicular bile flow increases when hydrophobic bile acids (chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA)) are found in higher concentrations than less hydrophobic bile acids (cholic acid (CA)) (10). In humans, up to 90% of bile flow is bile salt-dependent. Bile salt-independent bile flow can be stimulated by active solutes such as glutathione and bicarbonate. Cholangiocytes, or biliary epithelial cells, dilute and alkalize bile through hormone-regulated absorptive and secretory processes, and reabsorb useful solutes like glucose and amino acids. A more alkalotic biliary pH creates a protective barrier against the toxic effects of hydrophobic bile salts, known as the 'bicarbonate umbrella' (11).

Bile as a biomarker in liver transplantation

Biomarkers to predict graft function or viability of the biliary tree before implantation of the graft are increasingly investigated. In particular, predicting post-transplant cholangiopathy has gained considerable interest. **Table 2** gives an overview of literature investigating bile composition as a biomarker in LT. **Table 3** provides a summary of biliary biomarkers that have been identified and their purpose.

Bile composition of the donor graft: obtained during procurement

Already dating back to 1998, Melendez and colleagues performed several studies on donor hepatic bile composition collected during retrieval of the organ (12). Using proton nuclear magnetic resonance (^1H NMR), they were the first to show that bile from steatotic grafts collected during organ retrieval in the brain death donor (DBD) had higher PC content compared to bile from normal grafts (13). A couple of years later, the same group showed that suboptimal donor livers had a different bile acid composition compared to normal grafts: a lower apparent choleretic activity (indicating that bile flow did not increase appropriately with increased bile acid output) and a lower ratio of CA/CDCA (10). Increasing CA/CDCA ratios were observed after recovery from ischemia after LT (14). Instead of graft assessment during machine perfusion, bile collected during procurement may have potential to assess liver quality in DBD donors. However, data on donor bile composition are currently limited, probably due to logistical challenges during procurement.

Bile composition of the donor graft: during machine perfusion preservation

NMP has been used for functional assessment of a donor liver and its biliary tree, shortly before implantation in the recipient (**Figure 1A**) or in reperfusion models in preclinical studies (**Figure 1B**). During machine perfusion, bile is ideally collected under mineral oil to prevent the exchange of CO_2 between the sample and the ambient air (**Figure 1C**). Initially, bile flow was used as a discriminative marker for graft viability during machine perfusion (15,16). Later, it became clear that solely bile flow might be biased due to falsely increased

bile volume from the secretion of serum-like fluids from the injured mucosa (17). It has been shown that some livers with poor bile flow had good outcomes while some livers with good bile flow had poor outcomes (18). Moreover, absence of bile flow may be an artifact due to malpositioning of the biliary drain (19). As mentioned earlier, a constant supply of bile acids via the enterohepatic circulation stimulates bile production essential to maintain functional integrity of the liver. Hence, studies have shown deterioration of bile production during long-term perfusions due to bile acid depletion in the circulating perfusate (20). To counter this, several groups now infuse protective, hydrophilic bile salts during ex situ liver perfusions (18,21). For example, the OrganOx *metra* liver perfusion device continuously infuses taurocholate to the perfusion medium (22). The recently published paper by Eshmuminov et al. shows that the majority of livers can maintain bile production during 7-day NMP stimulated by ursodeoxycholic acid infusion (23). It remains investigational whether infusion of bile acids also attenuates ischemia-reperfusion injury (24,25).

In a porcine LT model, it was shown that a lower bile/perfusate glucose ratio (<0.6) and a higher bile/perfusate sodium ratio (≥ 1.2) during NMP was observed in DBD donors or donors with short warm ischemia (up to 70 minutes), compared to donors with long warm ischemia (120 minutes) (26). Bile composition of rat livers assessed during NMP also revealed differences according to the type of ischemic injury. Bile from DBD donors showed increased concentrations of bile acids, glucose and PC, but a decreased concentration of acetate compared to bile before procurement of the graft. In bile from DCD donors these values were further enhanced (27).

Similar observations were made in perfusion studies using human liver grafts. In a preclinical study, Matton et al. determined biomarkers of bile duct injury in 23 discarded human donor livers during 6 hours NMP. During machine perfusion, biliary bicarbonate and pH were significantly higher and biliary glucose was significantly lower in livers with a low histological degree of bile duct injury, compared to livers with high bile duct injury. Low bile duct injury was seen in livers with biliary bicarbonate >18 mmol/L, biliary pH >7.48 , biliary glucose <16 mmol/L, and a bile/perfusate glucose ratio <0.67 after 2.5 hours of NMP (2). Sutton et al.

found that cumulative bile production of >30 g during 6 hours of NMP was associated with better hepatobiliary function. Biochemical analysis of bile samples during NMP revealed a 4-times higher biliary concentration of bilirubin (function of hepatocytes) and a 2-times higher biliary concentration of bicarbonate (function of cholangiocytes) in the high bile output group compared to the low bile output group (28). Recently published results of the DHOPE-COR-NMP trial from Groningen showed that cumulative bile volume did not discriminate viable from non-viable grafts, whereas a biliary pH >7.45 was indicative of biliary epithelial viability (18,29). One recipient of a DHOPE-COR-NMP liver developed cholangiopathy after LT. Even though this liver met all viability criteria, the authors describe that, in retrospect, the difference between biliary and perfusate pH, bicarbonate, and glucose may be more predictive of cholangiocyte viability compared to absolute biliary levels. Similarly, early results analyzing the VITTAL trial demonstrated that whilst bile production during 4 hours of NMP did not correlate with ischemic-type biliary lesions (ITBL), a biliary pH <7.85 differentiates patients that developed ITBL after LT with a sensitivity of 91% (30). In a study from the Cambridge group, 12 initially declined human livers were transplanted after (hepatocellular) viability testing during NMP (31). Three livers developed posttransplant cholangiopathy and the authors found that all these livers were unable to produce a bile pH >7.4 during machine perfusion. This underlines the limitation of current pre-clinical studies assessing bile composition as a marker of cholangiocyte viability. In order to validate viability criteria of bile ducts long-term follow-up is necessary since ischemic cholangiopathy may take several months to develop.

In addition to viability testing, recent studies investigated interventions during NMP aimed to reduce biliary complications after LT. Hence, Boteon et al. supplemented the NMP perfusate with pharmacological drugs that enhance hepatic lipid metabolism. Preliminary results of eight discarded human livers show increased bile production and improved biliary pH (7.70–8.00) after treatment (32). In another NMP study, Raigani et al. aimed to decrease liver macrosteatosis in rats using a defatting cocktail. After 6 hours NMP, bicarbonate in bile was significantly higher in steatotic grafts treated with a defatting cocktail compared to steatotic

livers that were not treated and even compared to lean livers. Cumulative bile production however, remained higher in lean livers (33).

Taken together, analysis of bile composition during liver machine perfusion offers huge potential to evaluate liver graft and bile duct viability before LT. More experience from clinical studies may improve viability criteria in the future and further prevent the incidence of ischemic cholangiopathy. International registry of machine perfused liver transplants and routine collection and analysis of bile and perfusate samples may be used to apply machine learning/artificial intelligence, which will help the validation of viability criteria (34). Newer techniques such as proteomics or micro RNA (miRNA) analysis of bile offer a new diagnostic window for future machine perfusion studies.

Bile composition after liver transplantation: graft function and cellular rejection

Bile secretion (volume and color) has long been used as an early sign of liver function after LT. The group of sir Roy Calne was already interested in bile composition in the infancy of LT (35). In the late 1980s, a report of one of the first studies on bile composition in an animal model of LT was published. This study in rats showed that bile flow rate in the transplanted liver did not reflect the severity of hepatocellular damage and that during rejection episodes, bile acid and phospholipid output decreased, whereas cholesterol levels were maintained (36). In the decade following, several studies were performed on bile composition after LT, but without very uniform results. A typical postoperative course of bile composition includes low concentrations of bile acids, phospholipids, and cholesterol in the first postoperative days with a steady increase thereafter as the liver recovers from ischemic damage (14). Although some studies found decreased biliary lipid concentrations during periods of graft dysfunction (14,37–40), others did not find impaired lipid composition.(41) In grafts with PNF very low concentrations of biliary lipids are present, indicating total loss of synthesizing and excretory functions of the liver (39).

Several studies investigated post-transplant T-tube drain bile in patients with ACR. The results generally suggest that biliary cytokines (e.g. interleukin 2, 6, and 8, CXC motif chemokine 9 and 10) reflect immunological activity within the liver more closely than serum factors (42–47). A study from 1989 already showed that biliary IL-2 rose 24 hours before the onset of ACR and that biliary levels were more specific and sensitive than serum levels (43). In a study on T-tube bile of 51 LT recipients, biliary IL-6 levels significantly increased with biopsy-proven ACR and 70% of patients with ACR had high biliary IL-6 levels (46). When rejection was resolved, IL-6 levels decreased immediately. Newer techniques such as analysis of miRNAs or proteomics have also been described to identify biomarkers predictive of ACR (45,48,49). For example, Schmuck et al. showed that increased levels of certain miRNAs in bile in the first two postoperative weeks were predictive of ACR within 6 months after LT (44). Verhoeven and colleagues found that levels of hepatocyte- (HDmiRs) and cholangiocyte-derived miRNAs (CDmiRs) in bile strongly correlate with cellular excretory function (48). During episode of rejection, HDmiRs in bile decreased and CDmiRs increased, while this effect opposite in serum. The authors suggest that cholangiocytes cause a release of CDmiRs to bile rather than serum.

It is worth mentioning that routine T-tube placement after LT is no longer common practice in most transplant centers. A systematic review with meta-analysis showed non-superiority of duct-to-duct anastomoses with or without T-tubes in terms of postoperative biliary complications (50). Some studies even suggest that T-tubes after LT are associated with a higher rate of biliary complications. Therefore, post-transplant analysis of T-tube bile might be less relevant for most centers nowadays. In addition, the routine use of T-tube in deceased LT has been mainly abandoned because of the readily availability of endoscopic retrograde cholangiopancreatography (ERCP), which is both diagnostic and therapeutic, and because of post-removal complications such as bile leaks. However, it is still used routinely in living donor/split LT by most centers. In addition, if there was another utility of T-tube besides biliary tree imaging (e.g. bile could be collected from a T-tube post-transplant to provide biomarkers that could reliably predict PNF, ACR, or NAS) the routine use of T-tube could be revisited. The complications related to T-tube removal in the past was likely related

to the large 'classical' T-tubes and choledochotomy for drain insertion. To avoid biliary leaks post-biliary drain removal, we suggest to use straight (not T-shaped) and small-bore tubes placed across the cystic duct stump and surrounded by a small rubber band that will shut the cystic duct stump off after drain removal (51,52) (**Figure 2**).

Bile composition after transplantation: to predict biliary complications

Biliary complications are the most common complication associated with ischemic- and preservation injury. In particular, the high rate of biliary complications after LT of DCD livers remains a major concern. The pathogenesis of ischemic cholangiopathy, or so-called non-anastomotic biliary strictures (NAS) or ITBL, remains largely unclear, and re-LT is often the only treatment. We described earlier that efforts are made in predicting NAS based on bile composition during NMP (2). In addition, bile composition may be used as a diagnostic and prognostic marker for ischemic cholangiopathy after LT.

A recent study by Gaurav et al. collected bile during the first 30 minutes after reperfusion in 100 recipients (53). Twelve recipients developed cholangiopathy and both the difference in glucose concentration between bile and blood, and low biliary sodium levels were found to be predictors. However, no absolute threshold was found. Their results do not show an association between ischemic cholangiopathy and biliary pH or glucose, and the authors suggest that different bile flow rates may play a role: a large volume of bile flowing past cholangiocytes may exceed the cells' capacity to absorb substances while a small volume may not. If certain thresholds for bile biochemistry can be identified in the future, bile collection after reperfusion in the recipient may be a way to collect post-LT bile without use of T-tubes.

It has been suggested that bile cytotoxicity could be involved in the pathogenesis of ischemic cholangiopathy or NAS (54–56). When the recovery of phospholipid secretion is not as rapid as that of bile salt secretion, bile could become more cytotoxic with a relative excess of bile salts. In a study on 111 transplant recipients, the overall biliary secretion of

bile salts, phospholipids, and cholesterol during the first week after LT was significantly lower in patients who later developed NAS, compared to patients who did not develop NAS. The secretion of phospholipids was relatively more affected than bile salt secretion, resulting in a lower biliary phospholipid/bile salt ratio in patients who developed NAS (54). In line with this, another study observed higher biliary bile salt/phospholipid ratios during the first week after LT in livers with histological evidence of severe bile duct injury (57). Also, both extended cold ischemia time as well as prolonged warm ischemia time were related to a more toxic bile composition, due to a high biliary bile salt/phospholipid ratio (56,58). These studies suggest that development of NAS after LT is preceded by early changes in bile composition, several weeks before clinical symptoms of bile duct injury appear.

As mentioned earlier, it is likely that during injury cholangiocytes release specific miRNAs to bile rather than to blood (48). Hence, Lankisch et al. show that biliary concentrations of miR-517a, miR-106a and miR-892a were increased in recipients developing ITBL versus patients with anastomotic strictures or bile ducts stones. However, these miRNAs could not be used to distinguish the severity of ITBL (59). In the corresponding editorial, Verhoeven et al. question whether the miRNAs identified by Lankisch et al. are residing within the cell fractions or are released to the cell-free fraction of bile. They suggest that analysis of distinct fractions of biliary miRNAs are more likely to be informative for pathophysiological mechanisms related to biomarker release, which could increase its sensitivity and specificity as a biomarker (60).

Cytomegalovirus (CMV) is the most common viral infectious pathogen in LT recipients. Before CMV prophylaxis, CMV infection was a risk for biliary complications after LT. A study investigated CMV DNA in 124 biliary samples from living humans. Biliary CMV DNA was detected in a substantial number of LT patients presenting with suspected cholestasis. Moreover, it was not detectable in bile from patients suffering from choledocholithiasis only. Positive findings for biliary CMV were significantly associated with macroscopic or histological NAS. The authors also showed that biliary CMV DNA was detected despite negative blood results for CMV DNA. They hypothesized that chronic CMV latency in biliary

epithelial cells and viral shedding may induce chronic inflammation and, consequently lead to fibrotic reactions in the bile duct system. CMV infection should be considered as a possible contributing etiological factor to NAS complications (61).

Conclusions and future perspectives

Over the last years, suboptimal grafts are increasingly used to overcome the scarcity of donor organs. Although some of these livers turn out to function properly in recipients, their use has also been associated with impaired graft survival due to graft dysfunction or severe biliary complications like NAS. Bile composition seems to be a promising biomarker for bile duct viability or postoperative complications after LT. Dynamic preservation by machine perfusion is entering the clinic and NMP offers a time window in which bile composition can be easily assessed. Besides, bile may be sampled from the graft right after reperfusion, from biliary tubes placed at the time of LT, or by ERCP. As we set out in this review, several biliary biomarkers to assess bile duct viability and to predict postoperative complications have already been identified. Future studies should confirm the clinical and predictive value of these markers in larger cohorts.

In summary, bile composition can be used to diagnose and predict several aspects in the course of LT and can be considered a diagnostic biomarker in experimental and clinical studies. The composition of bile can be most easily assessed during machine perfusion, which offers an effective technique to test viability of the liver and its biliary tree. In addition, detrimental NAS after liver LT can be predicted from bile composition and hopefully prevented in the future.

Figure legends

Figure 1: Bile production during normothermic machine perfusion. A) Bile collection during normothermic machine perfusion of human liver grafts. B) Bile collection during normothermic rat liver machine perfusion. C) Bile collection under mineral oil.

Figure 2: Transcystic biliary drainage with a rubber band.

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Table 1. Composition of bile

Components		Bile/plasma ratio
Water 95%		
Electrolytes		
Na	141–165 mEq/L	~1
K	2.7–6.7 mEq/L	~1
Cl	77–117 mEq/L	~1
HCO ₃ ⁻	12–55 mEq/L	~1
Ca	2.5–6.4 mEq/L	~1
Mg	1.5–3 mEq/L	~1
SO ₄	4–5 mEq/L	
PO ₄	1–2 mEq/L	
Organic anions		
Bile salts	3–45 mmol/L	>1
Bilirubin	1–2 mmol/L	>1
Lipids		
Cholesterol	97–310 mg/dL	<1
PC	140–810 mg/dL	<1
Steroid hormones, estrogen		
Proteins	<10 mg/mL	<1
Plasma proteins		
Albumin		<1
Haptoglobin		
IgA		>1
Apo-transferrin		>1
Phermones		
Prolactin		
Insulin		
Hepatocyte proteins		
Alkaline phosphatase		<1
Acid phosphatase		<1
N-acetyl-β-glucosaminidase		<1
β-glucuronidase		
β-galactosidase		<1
5'-nucleotidase		<1
80-kd secretory component of plgA receptor		>1
Peptides and amino acids		
GSH	3–5 mmol/L	>1

GSSG	0–5 mmol/L	>1
Cystinyl glycine		>1
Glutamic acid	0.8–2.5 mmol/L	>
Cysteine		
FGF19		>1
Aspartic acid	0.4–1.1 mmol/L	>1
Glycine	0.6–2.6 mmol/L	>1
Nucleotides		
ATP	0.1–6 µmol/L	
ADP	0.1–5 µmol/L	
AMP	0.06–5 µmol/L	
Heavy metals		
Cu	2.8 mg/L	>1
Mn	0.2 mg/L	>1
Fe	<1 mg/L	>1
Zn	0.2–0.3 mg/L	>1
Vitamins		
25-OH vitamin D		
Cyanocobalamin	15–200 µg/L	
Riboflavin		
Folate	4–50 µg/L	

Values are from measurements in human or rodent bile. Table adapted from Boyer JL. Bile formation and secretion. *Compr Physiol*. 2013;3(3):1035-1078.

Table 2. Overview of studies performed on bile composition as a tool in liver transplantation.

Author, year, reference	Bile biomarker	Analysis	N (specimens /patients)	Clinical application
Gaurav, 2020	Na, bile glucose – blood glucose	Blood gas	–/100	NAS

Cervantes, 2019	Bile/perfusate glucose ratio Bile/perfusate Na ratio	Blood gas	20/–	Bile duct viability
Matton, 2019	Bicarbonate, pH, glucose Bile/perfusate ratio	Blood gas	–/23	Bile duct injury/NAS
Sutton, 2014	Bilirubin, bicarbonate	Blood gas	–/12	Bile duct viability
De Vries, 2019	pH	Blood gas	–/7	Bile duct viability
Boteon, 2019*	pH	Unknown	31/–	ITBL
Habib, 2004	Bile acids, lactate, glucose, PC, acetate	¹ H NMR	8/–	Warm ischemic injury
Boteon, 2019*	pH	Unknown	–/8	Bile duct injury
Raigani, 2019*	Bicarbonate	Unknown	12/–	Graft function after defatting
Melendez, 1998	PC UW	¹ H NMR	–/8 –/4	Donor graft steatosis PGD
Melendez, 2004	Choleretic activity CA/CDC ratio	HPLC	–/35	PGD
Xu, 1993	Bile salts, phospholipids, cholesterol	Spectrophotometry, ELISA	18/–	Graft function
Ko, 1998	Phospholipids	HPLC	–/17	Graft function
Baumgartner, 1995	Bile acids, phospholipids, cholesterol	Not known	–/12	Early graft dysfunction
Sánchez-Bueno, 2000	Bile acids, phospholipids, cholesterol	Spectrophotometry	–/25	ACR/PNF
Chan, 1998	Bile salts Phospholipids	ELISA	16/–	Early graft dysfunction
Verhoeven, 2016	HDmiR CDmiR	RT-qPCR	–/124	ACR
Schmuck, 2017	CD44, CXCL19 miR-122, miR-133a, miR- 148a, miR-194	ELISA, q-PCR	–/45	ACR
Kim, 2012	APN	Proteomics	–/9	ACR
Adams, 1993	ICAM-1	ELISA	–/61	ACR
Adams, 1989	IL2R	Unknown	–/18	ACR
Tono, 1992	IL-6	ELISA	11/–	ACR
Umeshita, 1996	IL-6	ELISA	–/51	ACR
Morimoto, 2014	CXCL10	ELISA	–/41	ACR
Ericzon, 1990	Bile acids CA/CDCA ratio	ELISA, HPLC	–/10	Graft function
Buis, 2009	Bile phospholipid/bile salt ratio	ELISA,	–/111	NAS

		spectrophotometry		
Chen, 2009	Bile salt/phospholipid ratio	ELISA	90/–	Bile duct injury/NAS
Geuken, 2004	Bile salt/phospholipid ratio ALP GGT	ELISA	–/28	NAS
Yska, 2008	Bile salt/phospholipid ratio	ELISA, spectrophotometry	30/–	Bile duct injury
Lankisch, 2014	miR-517a, miR-892-a, nmiR-106a, miR-201, miR-337-5p, miR-577, miR-329	RT-qPCR	–/88	NAS
Gotthardt, 2013	CMV	RT-qPCR	–/71	NAS

ACR = acute cellular rejection, APN = alanine aminopeptidase, CDmiR = cholangiocyte-derived micro RNA, CA = cholic acid, CDCA = chenodeoxycholic acid, CXCL10 = CXC motif chemokine 10, CMV = cytomegaly virus, ELISA = enzyme-linked immunosorbent assay, GGT = gamma glutamyl transferase, HDmiR = hepatocyte-derived microRNA, ¹H NMR = proton magnetic resonance imaging, solution, HPLC= high-performance liquid chromatography, ICAM-1 = intracellular adhesion molecule 1, IL-6 = interleukin 6, IL-8 = interleukin 8, IRI = ischemia-reperfusion injury, PC = phosphatidylcholine, PGD = primary graft dysfunction, PNF = primary non-function, miR = micro RNA, NAS = non-anastomotic biliary strictures, RT-qPCR = real-time polymerase chain reaction, TCBA = taurine-conjugated bile acid, UW = University of Wisconsin perfusion. * data obtained from online abstract

Table 3. Biliary biomarkers

Bile composition	Goal
At the time of donor procurement	
↑ PC	Identify steatotic grafts
↓ Choleretic activity	Identify suboptimal grafts
↓ CA/CDCA ratio	Identify suboptimal graft
During NMP	
HCO ₃ ⁻ >18 mmol/L, pH >7.48, glucose <16 mmol/L, bile/perfusate glucose ratio <0.67, and LDH concentration <3689 U/L	Predict low histological degree of bile duct injury
Bile production >10ml and pH >7.45	Predict bile duct viability
pH <7.85	Predict NAS
pH <7.4	Predict NAS
After transplantation	
↓ Na and ↑ bile glucose – blood glucose	Predict NAS
↓↓↓ Lipids	Identify PNF
↑ CDmiR	Predict ACR
↑ miR-133a, miR-148a, miR-194	Predict ACR
↑ IL-2, IL-6, IL-8	Predict ACR
↑ CXCL9/10	Predict ACR
↓ bile salt, phospholipids, cholesterol during the first week after LT	Predict NAS
↑ Bile salt/phospholipid ratio during the first week after LT	Predict histologically severe bile duct injury
↑ miR-517a, miR-106a, miR-892a	Predict NAS

ACR: acute cellular rejection, APN: aminopeptidase N, CA: cholic acid, cICAM-1: circulating intercellular adhesion molecule 1, CXCL: CXC motif chemokine 10, CDCA: chenodeoxycholic acid, IL2R: interleukin-2 receptor, IL-6: interleukin, LDH: lactate dehydrogenase, LT: liver transplantation, Na: sodium, NAS: non-anastomotic

biliary strictures, NMP: normothermic machine perfusion, PC: phosphatidylcholine,
PNF: primary non-function

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