ORIGINAL ARTICLE

# The real incidence of biliary tract complications after adult liver transplantation: the role of the prospective routine use of cholangiography during post-transplant follow-up

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## SUMMARY

Biliary tract complications (BTCs) still burden liver transplantation (LT). The wide reporting variability highlights the absence of systematic screening. From 2000 to 2009, simultaneous liver biopsy and direct biliary visualization were prospectively performed in 242 recipients at 3 and 6 months ($n$ = 212, 87.6%) or earlier when indicated ($n$ = 30, 12.4%). Median follow-up was 148 (107–182) months. Seven patients (2.9%) experienced postprocedural morbidity. BTCs were initially diagnosed in 76 (31.4%) patients; 32 (42.1%) had neither clinical nor biological abnormalities. Acute cellular rejection (ACR) was present in 27 (11.2%) patients and in 6 (22.2%) BTC patients. Nine (3.7%) patients with normal initial cholangiography developed BTCs after 60 (30–135) months post-LT. BTCs directly lead to 7 (2.9%) re-transplantations and 14 (5.8%) deaths resulting in 18 (7.4%) allograft losses. Bile duct proliferation at 12-month biopsy proved an independent risk factor for graft loss ($P$ = 0.005). Systematic biliary tract and allograft evaluation allows the incidence and extent of biliary lesions to be documented more precisely and to avoid erroneous treatment of ACR. The combination 'abnormal biliary tract-canalicular proliferation' is an indicator of worse graft outcome. BTCs are responsible for important delayed allograft and patient losses. These results underline the importance of life-long follow-up and appropriate timing for re-transplantation.

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**Key words**
biliary tract complications, canalicular proliferation, cholangiography, interventional endoscopy, interventional radiology, liver transplantation

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## Introduction

Biliary tract complications (BTCs) are a major source of morbidity and an underrated cause of delayed graft and patient loss after liver transplantation (LT). The reported incidence, in retrospective studies, varies from 10–40% [1–7]. The first report published in 1984 by the Pittsburgh team revealed BTC in 13% of patients [8]. Despite increased experience and refinement of procurement and implantation techniques, this incidence further rose to 30–40% as a consequence of the use of extended-criteria donors and donors after circulatory death (DCD) [9–11]. The lack of routine biliary imaging and of long-term post-transplant follow-up explains the wide variability in BTC reporting. Indeed, the biliary tract is investigated merely when clinically (presence of jaundice, 'biliary fever', itching, nausea, right upper quadrant discomfort) and/or biochemically (presence of abnormal liver tests with or without jaundice) indicated. Yet, recipients can be asymptomatic and have normal liver tests in the presence of a severely damaged biliary tree. Besides, BTCs may induce inflammatory and biliary changes that mimic acute cellular rejection (ACR) on pathology, prompting anti-ACR treatment for initially unrecognized biliary lesions [12]. It is important to diagnose BTC at an early stage to adapt follow-up and treatment in order to avoid the evolution to multidrug-resistant biliary infections, secondary biliary cirrhosis and eventually delayed graft and patient loss. Although important progresses have been made in magnetic resonance imaging (MRI), the direct biliary tree visualization, either by T-tube (TTC), percutaneous transhepatic (PTC) or endoscopic (ERC) cholangiography, remains the superior diagnostic procedure as it documents both nature and extent of BTCs more precisely [13–19].

This prospective single-centre study evaluates the incidence of BTC in a large recipient cohort, using routine invasive cholangiography within the first 6 months after LT, and to correlate the findings of simultaneously performed imaging and pathology in view of their impact on long-term graft and patient outcome.

## Patients and methods

The study was approved by the institutional review board and performed in accordance with the 2000 Declaration of Helsinki and the 2008 Declaration of Istanbul. Informed written consent was obtained from all patients or their next of kin before transplantation. This investigator-driven study was designed, initiated in the year 2000 and managed by the senior author (JL).

During the period January 2000-April 2009, 323 consecutive adult (>16 years) patients underwent LT. The study was limited to this time period because allowing a very long-term follow-up of the liver recipients and so documenting late appearance and consequences of BTCs. After excluding recipients receiving a DCD graft ($n$ = 8), refusing the protocol procedure ($n$ = 49) and dying within 3 months due to nonbiliary complications ($n$ = 24), 242 patients were analysed. The study concerned 236 primary LT and six patients who were immediately re-transplanted (re-LT) because of primary allograft nonfunction; their day of re-LT was considered as baseline. Nine primary sclerosing cholangitis (PSC) and seven primary biliary cholangitis (PBC) patients were included, the aim of the study being to document macroscopic biliary lesions. PSC recurrence, reported to be around 20% five years after LT, is characterized by the delayed (> one post-LT year) appearance of typical macroscopic (e.g. nonanastomotic biliary strictures of the intra-/extrahepatic biliary system with beading and irregularities or microscopic findings (e.g. fibrous cholangitis and fibro-obliterative lesions). PBC recurrence is characterized by very delayed (more than 4 years post-LT) appearance of typical microscopic bile duct lesions only (e.g. florid duct lesions or destructive lymphocytic cholangitis) [20,21].

The LT technique has been reported elsewhere [22]. Biliary reconstruction consisted of a duct-to-duct anastomosis (215 patients; 88.8%); T-tube was inserted 65 times because of bile duct mismatch or small-calibre bile ducts. Roux-en-Y hepatico-jejunostomy (RY-HJ) was performed in 27 (11.2%) patients; nine had PSC and, in 17, the bile duct was unsuitable for duct-to-duct anastomosis. Cold, warm and arterial ischaemia times were defined as the time between cross clamping of the donor aorta and beginning of the cava anastomosis, the time from cava anastomosis to portal reperfusion and the time between portal anastomosis and graft re-arterialization.

All recipients had tacrolimus-based (Prograft®, Astellas Pharma, Tokyo, JP) immunosuppression either as monotherapy (n = 155; 63.7%), or in combination with 2-month low-dose steroids (n = 81) or single-dose antithymocyte globulin (ATG, Grafalon, Neovii Biotech, Gräfelfing, DE, n = 8) [23,24]. Tacrolimus trough levels were kept low (4 to 6 ng/ml). Systematic bile duct visualization was intended also to monitor the safety of this low immunosuppression protocol[23].

Outpatient follow-up, done by the same team, consisted of clinical and biochemical evaluation: initially



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weekly, bi-weekly after 3 months, monthly, after 6 months, two-monthly after one year and three-monthly after two years. In the context of the mentioned immunosuppressive minimization strategy and of the availability of an expert interventional transplant radiologist (PG), allografts were deliberately monitored aggressively. The protocol consisted of simultaneous blood sampling, Doppler ultrasound, invasive cholangiography, bile sampling and liver biopsy in one-day hospitalization. Liver biopsies were done on post-LT day seven, after six and 12 months, and, thereafter, every five years and when clinically indicated [23,24]. MRI was performed only in some cases presenting with other hepatic or abdominal problems.

### Cholangiography protocol

All patients received a diagnostic cholangiography after LT, either routinely (routine cholangiography, RCG, i.e. TTC at 3 months or PTC at 6 months) or, earlier, per clinical indication (CICG). Progressively disturbed liver tests, unexplained infection, jaundice, pruritus, fever and nausea, were considered as clinical indications. TTC was performed in 65 patients. In order to avoid later interference with biopsy reading due to possible T-tube related biliary obstruction or infection, the T-tube was removed immediately in all but three patients presenting a small bile leak at the T-tube exit site. PTC and liver biopsy were performed under ultrasound guidance and local anaesthesia using a 22-G Chiba (Becton Dickinson, Franklin Lakes, NJ-US) and 18-G Menghini needles (Hepafix®, Braun, Melsungen, DE). Bile and liver tissue were systematically collected for culture. Single-shot intravenous anti-bacterial prophylaxis consisted of cefuroxime (Zinacef®, GlaxoSmithKline, Verona, IT) and of ceftazidime (Glazidim®, GlaxoSmithKline, Verona, IT); metronidazole (Flagyl®, Baxter, Castlebar, IE) was added in case of high risk for infection (RY-HJ or previous post-LT complications). Patients were discharged eight to twelve hours after the procedure if vital parameters and haematocrit were normal and if symptoms of bleeding, severe pain or organ damage were absent. In case of concordant abnormal imaging and biology findings, biliary lesions were treated.In case of normal liver values and good emptying of the biliary tract, a 'wait-and-see' policy was adopted. Such 'silent' BTC served as an indicator for a tighter post-transplant follow-up. The same authors (JN, DK, JL) retrospectively reviewed all cholangiographies and two experienced liver pathologists read liver biopsies.

### Definitions

BTCs were defined as anatomically identified lesions; these were classified as anastomotic and nonanastomotic according to the anatomic classification proposed by Buis et al.: zone A correlates with lesions in the extrahepatic common bile duct, zone B with lesions between the first and second-order branches, zone C with lesions between the second- and third-order branches and zone D with lesions in the peripheral bile ducts. Liver tests up to 1.5 normal values were considered as normal [25]. Events occurring within 6 months after LT were defined as early.

At histology, particular attention was given to canalicular proliferation, as a sign of BTCs, and to ACR features, possibly elicited by BTCs. Biopsies were scored according Banff criteria [26]. Pathologic moderate and severe ACR correspond to scores of 6–7 and 8–9, respectively. *Clinical* ACR is defined as concordance between liver test abnormalities and Banff scores between 6 and 9, which was approached with an increase in tacrolimus dose and/or 3 to 5 boluses of 200 mg methylprednisolone [23,24].

### Statistical analysis

Binomial variables were reported using numbers and proportions. Numerical variables were reported using medians and interquartile ranges (IQR). Results were compared using Fisher's exact test or Mann–Whitney U test as appropriate. The predictive ability of a number of variables for the risk of BTCs at the initial cholangiography, death and graft loss, was assessed. Variables were selected based on background knowledge to assess the role of biliary variables in the development of adverse outcomes after LT. Cox proportional hazards models were built for time-dependent events. Logistic regressions were run for dichotomous variables. Covariates with $P < 0.157$ were introduced into multivariable models. A backward conditional method was chosen to select significant independent covariates. Hazard ratios and odds ratios, and 95% confidence intervals (CI) were reported for each predictor. The Kaplan–Meier method was used to analyse the rate of death and graft loss. Log-rank tests were run to compare the survival curves. The significance of statistical tests was taken at $P < 0.05$. Analyses were run with SPSS Statistics (version 25, IBM, Armonk, NY-USA).

## Results

Overall, 242 patients received an initial cholangiography, either RCG (210, 86.8%) or CICG (32, 13.2%,

Fig. 1). Median follow-up was 148 (107–182) months. Patients' characteristics are displayed in Table 1.

### Routine cholangiography (RCG)

Overall, 210/242 patients received TTC (58/210, 27.6%), PTC (145/210, 69.0%) or sequential TTC and PTC (7/210, 3.3%). RCGs diagnosed BTCs in 53/210 (25.2%) patients, 32 (60.4%) had neither clinical nor biochemical abnormalities; 12 (22.6%) presented abnormal liver tests, 2 (3.8%) symptom and 7 (13.2%) a combination of both. Liver tests were comparable in BTC and in patients without biliary lesions: total bilirubin 1.2 (0.80–2.25) vs. 0.9 (0.70–1.65) mg/dl, $P = 0.089$), AST 33 (22–67) vs. 33 (22–59) U/l, $P = 0.689$) and γGT 60 (22–208) vs. 47 (26–109) U/l, $P = 0.503$).

### Clinically indicated cholangiography (CICG)

Thirty-two patients received PTC (26/32, 81.2%) or ERC (6/32, 18.8%) earlier than planned RCG, at a median time of 3 (2–4) months after LT. CICG identified BTCs in 23 (71.9%) patients. Indications for cholangiography were abnormal liver tests (12/32, 37.5%), symptoms (3/32, 9.4%), a combination of both (16/32, 50.0%) and suspicion of bile leakage (1/32, 3.1%). Total bilirubin was comparable between BTC and non-BTC cases (1.90 (1.00–4.20) vs. 1.10 (0.65–2.20) mg/dl, $P = 0.112$), whereas BTC recipients exhibited higher levels of AST (52 (42–114) vs. 25 (17–58) U/l, $P = 0.010$) and of γGT (338 (159–619) vs. 71 (55–246), U/l $P = 0.004$).

### Postinterventional adverse events

There was no postinterventional mortality, and morbidity was low (7/242, 2.9%). In the RCG group, four patients experienced complications after PTC: cholangitis needing antibiotics ($n = 2$), haemobilia due to right portal vein puncture, requiring embolization ($n = 1$) and subcapsular hepatic haematoma managed conservatively ($n = 1$); while two complications occurred after TTC: right hepatic haematoma after biopsy, managed conservatively and cholangitis needing antibiotics. In the CICG group, one patient who underwent ERC experienced bleeding after sphincterotomy, which was managed with local haemostasis. Small biliary leaks, occurring three times after T-tube removal, were considered to be associated with the surgical technique and not with the cholangiography per se. All were successfully treated with endoscopic sphincterotomy and short-term stenting.

### BTCs and treatment

At *initial* cholangiography, either RCG or CICG, BTCs were diagnosed in 76 (31.4%) patients (Fig. 2).

Concomitant liver histology revealed features of moderate-to-severe ACR in 27 patients (11.2%), six of whom (22.2%) had BTCs. The incidence of rejection in 85 BTC patients was thus 7%.Bile or liver tissue cultures were positive in 18 patients (7.4%): 16 in RCG and two in CICG. Cultures were positive in half of cases with disturbed liver tests but only one patient had documented BTCs.

Concerning BTCs localization, 58/76 (76.3) were anastomotic, 12 (15.8%) nonanastomotic and 6 (7.9%)

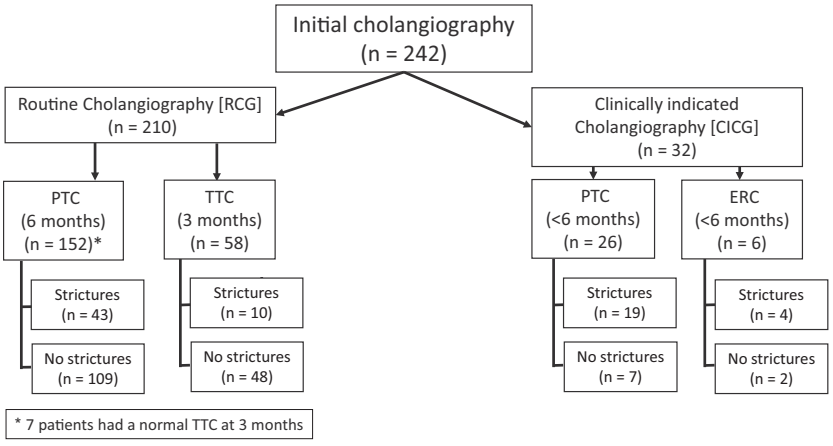



**Figure 1** Flowchart of the cholangiography study protocol. Abbreviations: PTC, percutaneous transhepatic cholangiography; TTC, t-tube cholangiography; ERC, endoscopic retrograde cholangiography.

**Table 1.** Demographics of patients who exhibited biliary tract complications at the initial cholangiography after liver transplantation

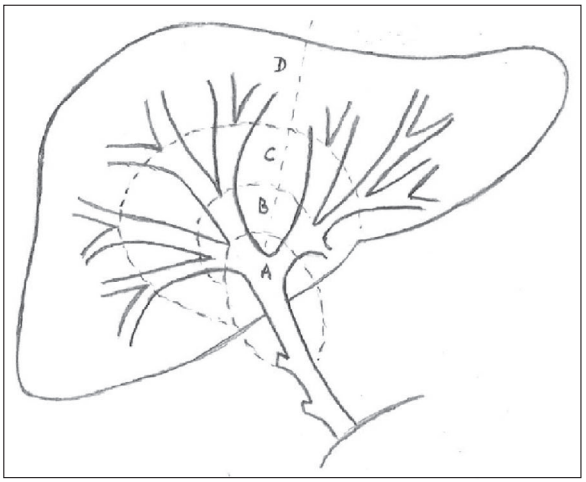
| Variables | Whole population (*N* = 242) Median (IQR) or *n* (%) | No BTC (*N* = 166) | BTC (*N* = 76) | *P* |
|---|---|---|---|---|
| Recipient age (years) | 55 (44–60) | 55 (46–61) | 53 (42–59) | 0.268 |
| Gender (male) | 145 (59.9) | 96 (57.8) | 49 (64.5) | 0.397 |
| Indication for LT | | | | |
| Cancer | 64 (26.4) | 40 (24.1) | 24 (31.6) | 0.272 |
| Viral cirrhosis | 45 (18.6) | 34 (20.5) | 11 (14.5) | 0.291 |
| Alcoholic cirrhosis | 39 (16.1) | 27 (16.3) | 12 (15.8) | 1.000 |
| PBC | 7 (2.9) | 5 (3.0) | 2 (2.6) | 1.000 |
| PSC | 10 (4.1) | 7 (4.2) | 3 (3.9) | 1.000 |
| Secondary biliary cirrhosis | 8 (3.3) | 5 (3.0) | 3 (3.9) | 0.709 |
| ITBL | 8 (3.3) | 7 (4.2) | 1 (1.3) | 0.441 |
| Others | 61 (25.2) | 41 (24.7) | 20 (26.3) | 0.873 |
| Donor age (years) | 48 (38–57) | 48 (34–56) | 49 (39–59) | 0.331 |
| Graft type: | | | | |
| Whole liver | 227 (93.8) | 154 (92.8) | 73 (96.1) | 0.402 |
| Right liver | 14 (5.8) | 11 (6.6) | 3 (3.9) | 0.558 |
| Left liver | 1 (0.4) | 1 (0.6) | 0 (0.0) | 1.000 |
| Cold ischaemia (min) | 639 (531–765) | 640 (543–765) | 629 (472–771) | 0.285 |
| Warm ischaemia (min) | 41 (35–48) | 41 (34–47) | 44 (37–53) | **0.033** |
| Arterial ischaemia (min) | 83 (59–103) | 83 (61–103) | 83 (55–105) | 0.959 |
| Duration of operation (min) | 480 (424–564) | 480 (422–559) | 507 (427–587) | 0.193 |
| Intraoperative transfusions | | | | |
| Patients receiving transfusions | 146 (60.3) | 101 (60.8) | 45 (59.2) | 0.888 |
| Volume of transfusions (ml) | 459 (0–1011) | 460 (0–957) | 466 (0–1207) | 0.567 |
| Biliary reconstruction | | | | |
| Hepatico-jejunostomy | 27 (11.2) | 17 (10.2) | 10 (13.2) | 0.514 |
| Duct-to-duct | 215 (88.8) | 149 (89.8) | (86.8) | |
| 1. Without T-tube | 177 (73.1) | 114 (68.7) | 63 (82.9) | **0.028** |
| 2. With T-tube | 65 (26.9) | 52 (31.3) | 13 (17.1) | |
| Steroid-based immunosuppression | 79 (32.6) | 58 (34.9) | 21 (27.6) | 0.302 |
| Day seven moderate-to-severe ACR | 111/239 (46.4) | 70/163 (42.9) | 41/76 (53.9) | 0.127 |
| Six-month moderate-to-severe ACR | 27 (11.2) | 21 (12.7) | 6 (7.9) | 0.379 |
| Six-month canalicular proliferation | 73 (30.2) | 38 (22.9) | 35 (46.1) | **<0.001** |
| Early clinical ACR | 21 (8.7) | 17 (10.2) | 4 (5.3) | 0.230 |
| Early biliary fistula | 5 (2.1) | 3 (1.8) | 2 (2.6) | 0.651 |
| Total bilirubin at initial cholangiography (mg/dl) | 1.00 (0.80–1.80) | 0.95 (0.70–1.63) | 1.20 (0.90–2.73) | **0.002** |
| AST at initial cholangiography (U/l) | 34 (22–63) | 33 (21–59) | 40 (23–77) | **0.032** |
| γGT at initial cholangiography (U/l) | 60 (28–157) | 50 (27–114) | 109 (29–389) | **0.001** |
| Clinical abnormalities at initial cholangiography* | 88 (36.4) | 44 (26.5) | 44 (57.9) | **<0.001** |
| Follow-up (months) | 148 (107–182) | 149 (108–181) | 147 (90–192) | 0.812 |

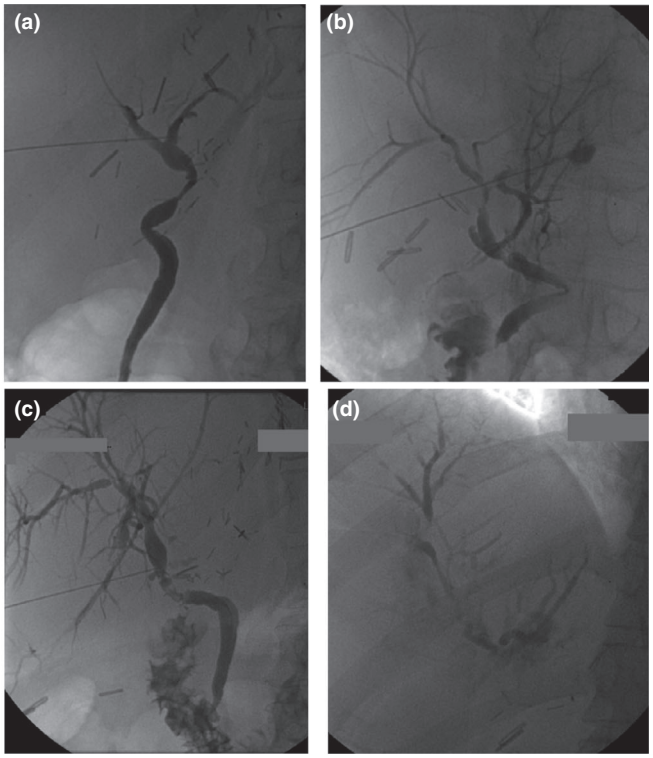
Bold indicates statistically significant values.

ACR, acute cellular rejection; BTCs, biliary tract complications; ITBL, ischaemic-type biliary lesions; LT, liver transplantation; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.

*Either biliary symptoms or liver tests abnormalities.

mixed. Sixty lesions (78.9%) were located in Buis zone A, five (6.6%) in B, four in C (5.3%) and seven in D (9.2%, Fig. 3). Twenty-four (31.6%) patients with BTCs did not require treatment ('wait-and-see' strategy) because showing normal fluoroscopic emptying of the biliary tract, in the absence of bile duct retro-dilation, symptoms or liver tests alteration. Fifty-two (68.4%) recipients needed treatment instead. Zone A lesions required upfront RY-HJ in four cases and interventional radiology and/or endoscopy in 34 recipients, four of these needed secondary RY-HJ. Zone B lesions were treated by RY-HJ (3 patients) and endoscopic stenting




| Type | Early | Late |
|---|---|---|
| D | 7/85 (8.2%) | 2/85 (2.4%) |
| C | 4/85 (4.7%) | 1/85 (1.2%) |
| B | 5/85 (5.9%) | 1/85 (1.2%) |
| A | 60/85 (70.6%) | 5/85 (5.9%) |
| | Detection at initial cholangiography | Detection after a normal initial cholangiography |

**Figure 2** Distribution of biliary strictures according to the anatomic zones as described by Buis et al. [25].

(1 patient). Zone C lesions were treated by interventional radiology, endoscopy or a combination of both. Zone D lesions were treated by interventional radiology or endoscopy (5 patients), and RY-HJ (2 patients).

Among the 52 treated patients, 36 had a good evolution, 13 later developed diffuse biliary lesions (8 pats) leading to refractory infections (7 pats) and pruritus (1 pat); ten developed a secondary biliary cirrhosis and one secondary cirrhosis due to recurrent PSC. Three of four patients were successfully re-transplanted and 11 died as a consequence of BTCs, seven of them while waiting for re-LT. Two of the 24 conservatively managed patients underwent re-LT for recurrent allograft HCV infection and PBC.

A multivariable logistic regression analysis showed that only T-tube insertion is independently negatively associated with the diagnosis of BTCs within 6 months from LT (OR = 0.487, 95% CI = 0.245–0.969, $P = 0.040$, Table 2).

## Late BTCs

Nine (5.4%) of the 166 patients with a normal initial cholangiography developed BTCs after median of 60 (30–135) months from LT. Five developed BTCs in zone A, one each in zone B and C, and two in zone D. All zone A BTCs were treated by interventional radiology, endoscopy or a combination of both; two of them later required RY-HJ. None of the other patients was treated. Two patients were re-transplanted because of recurrent PSC and the other for refractory biliary infections. Three patients died as a consequence of their BTC due to the development of secondary biliary cirrhosis and one due to PSC recurrence.

## Graft loss and death

Eighty-five cases of BTCs were detected overall: 76 thanks to the initial cholangiography protocol and 9 over time. During the whole follow-up, 7 (8.2%) BTC patients needed to be re-transplanted (at 9, 13, 14, 42, 43, 65 and 92 months!) because of refractory biliary infections (3 pats), refractory pruritus (1 pat), secondary biliary cirrhosis (2 pats) and recurrent PSC (1 pat). Thirty-seven BTC patients died, 14 (14 /85 pats, 16.4%) as a direct consequence of their biliary complication (at 9, 11, 15, 19, 33, 44, 46, 71, 113, 128, 164, 202, 220, 238 months!): secondary biliary cirrhosis (7 pats), refractory infections (5 pats), difficult recovery following re-LT for BTCs (2 pats). Ten patients died while waiting for re-LT. On the contrary, 4 (2.5%) of 157 recipients who never developed BTCs got re-LT and

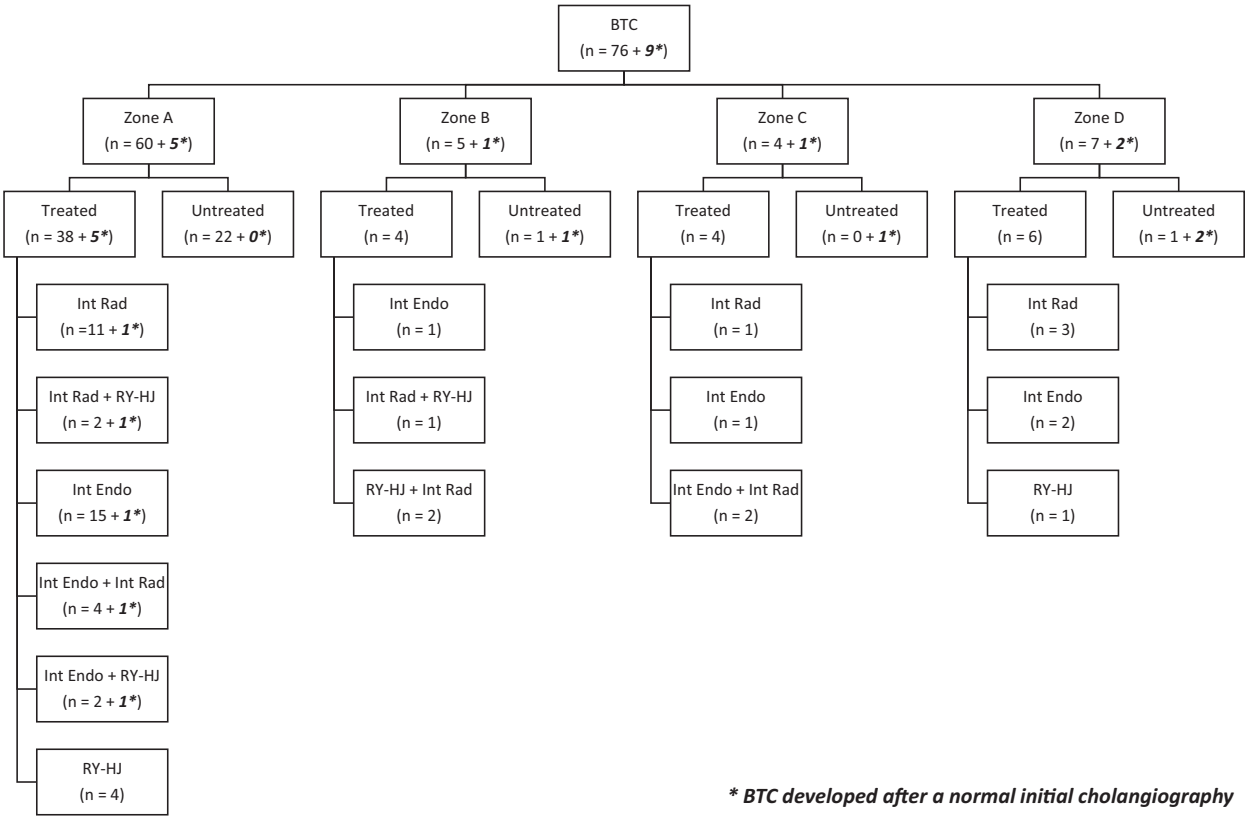



**Figure 3** Therapeutic flow chart of the 83 diagnosed biliary tract complications. Abbreviations: BTC, biliary tract complications; Int Rad, interventional radiology; Int Endo, interventional endoscopy; RY-HJ, Roux-en-Y hepatico-jejunostomy. (*) BTC developed after a normal initial cholangiography.

**Table 2.** Multivariable logistic regression analysis for the diagnosis of biliary tract complications at the initial cholangiography (76/242)

| Variables in the initial model* | OR | 95% CI | *P* |
|---|---|---|---|
| Indication for LT (biliary vs. not) | 0.550 | 0.184–1.643 | 0.284 |
| Donor age (years) | 1.008 | 0.989–1.028 | 0.403 |
| Partial graft (yes vs. no) | 0.727 | 0.178–2.967 | 0.657 |
| Cold ischaemia (min) | 0.999 | 0.997–1.001 | 0.178 |
| Warm ischaemia (min) | 1.021 | 1.001–1.042 | 0.042 |
| Biliary anastomosis (RY-HJ vs. DD) | 1.709 | 0.553–5.283 | 0.352 |
| T-tube (yes vs. no) | 0.552 | 0.271–1.127 | 0.103 |
| Early biliary fistula (yes vs. no) | 1.794 | 0.262–12.269 | 0.551 |
| Variables in the final model† | | | |
| T-tube (yes vs. no) | 0.487 | 0.245–0.969 | 0.040 |

CI, confidence intervals; DD, duct-to-duct biliary reconstruction; LT, liver transplantation; OR, odds ratio; RY-HJ, Roux-en-Y hepatico-jejunostomy.

*Initial model summary (all candidate variables): −2ln likelihood = 287.439; $\chi^2(8) = 13.755$, $P = 0.088$; Nagelkerke $R^2 = 0.078$; Hosmer and Lemeshow $\chi^2 = 7.914$, $P = 0.442$

†Final model summary (after backward conditional elimination): −2ln likelihood = 291.897; $\chi^2(2) = 9.298$, $P = 0.010$; Nagelkerke $R^2 = 0.053$; Hosmer and Lemeshow $\chi^2 = 8.666$, $P = 0.371$.

64 died. BTCs was the fourth reason of late mortality (14/242; 5.8%) after de novo tumour formation (24 pats, 9.9%), cardiovascular events (18 pats,7.4%) and allograft disease recurrence (17 pats, 7%).

Despite the overall burden of BTCs on LT, the occurrence of BTCs at the initial cholangiography was not significantly associated with worse patient or graft survival (log-rank $P = 0.872$ and $P = 0.559$, respectively, Figs 4 and 5). Multivariable Cox regression analyses, performed in the subset of patients who received one-year per-protocol liver biopsy (237/242, 97.9%), revealed that recipient age (HR = 1.036, 95% CI = 1.017–1.056, $P < 0.001$), donor age (HR = 1.017, 95% CI = 1.004–1.031, $P = 0.012$) and one-year canalicular proliferation (HR = 1.883, 95% CI = 1.216–2.914, $P = 0.005$) are independent risk factors for graft loss (Table 3), while only recipient (HR = 1.052, 95% CI = 1.030–1.075, $P < 0.001$) and donor ages (HR = 1.022, 95% CI = 1.008–1.036, $P = 0.002$) are independent risk factors for recipient death (Table S1). Consequently, while patient survival is not influenced by the development of one-year canalicular proliferation ($P = 0.269$, Fig. 6), graft survival is significantly reduced in this set of patients ($P = 0.012$, Fig. 7). The distribution of the most relevant clinical characteristics per the development of one-year canalicular proliferation is detailed in the Table S2.

## Discussion

Since the first publications about BTC in LT, little progress has been made to avoid their development despite improved surgical techniques and perioperative care [9,27–35]. In the early Pittsburgh experience the incidence of BTC was 13%. Thirty years later numbers are still similar (20%) in brain death (DBD) donor LT; in DCD LT they reach 30% [7,8,36,37]. BTCs unfortunately remain the Achilles' heel of the procedure, responsible for a significant morbidity and mortality. The magnitude of the problem is underestimated as both systematic and long-term screening of the biliary tract are lacking [36]. The utility of systematic short- and long-term allograft biopsies and, even more so, of invasive examination of the biliary tract have been highly questioned especially when dealing with recipients with good liver function and/or normal liver tests in the absence of clinical symptoms and signs [38]. The varying reported incidence of BTCs has to be interpreted in this context. Three other important elements play an important role in this 'grey zone' of LT, that is, the lack of detailed information about reason for re-LT or of death and death on waiting list. Many patients are not only re-grafted (sometimes very) late because of refractory biliary infections and/or development of liver failure or secondary biliary cirrhosis but this intervention is, additionally, frequently done by other, sometimes very distant, teams without exchanging information; even more importantly, many patients 'disappear' from registries and data-banks because dying or dying on the waiting list without specific information about the underlying cause of death (e.g. liver failure). Dying while waiting should not come as a surprise taking into account the frequently low MELD scores of such patients [25,39].

As the great majority (90% also in this study) of BTCs occur during the first post-transplant year, we implemented in our centre a systematic follow-up of all adult recipients to assess the allograft at histology and by direct visualization of the biliary tract [7,19,23–25]. This unusual and aggressive, protocol was deliberately set up with a triple intention: a) to document the real incidence of BTC; b) to avoid unnecessary reinforcement of immunosuppression (in a context of minimization immunosuppression protocols) due to an erroneous histologic diagnosis of rejection possibly linked to BTCs, and c) to correlate liver pathology and biliary imaging with long-term allograft and patient outcome. This strategy was based on the evidence that recipients presenting with even extensive destruction of the biliary tract can not only be asymptomatic, but also can have normal liver tests, including cholestatic enzymes and that noninvasive examinations can miss or underestimate the extent of BTCs. In the early post-LT period, liver test abnormalities are often explained by preservation injury, vascular problems (e. g. arterial and venous thrombosis or stenosis, splenic steal syndrome), viral de novo (e. g. cytomegalovirus, donor viral disease transmission) or recurrent (e. g. B, C and D virus) hepatitis, drug-induced toxicity, infections and ACR. Timeline, dynamics and degree of modifications of liver tests are of help to make the differential diagnosis of BTC [26,40]. After several months post-LT liver test abnormalities are more likely to be seen in the context of recurrent allograft disease and BTC as ACRs become rarer [12,26,41]. Despite this well-established clinical knowledge, many patients presenting with (undiagnosed) BTCs are treated for the suspicion of ACR. The presence of infiltrates (peri/cholangitis) and distortion of bile duct morphology (especially canalicular proliferation or damage and even *vanishing* bile duct syndrome) explains why BTCs can mimic findings of ACR [26,41]. Consequently such pathology findings in the presence of elevated liver



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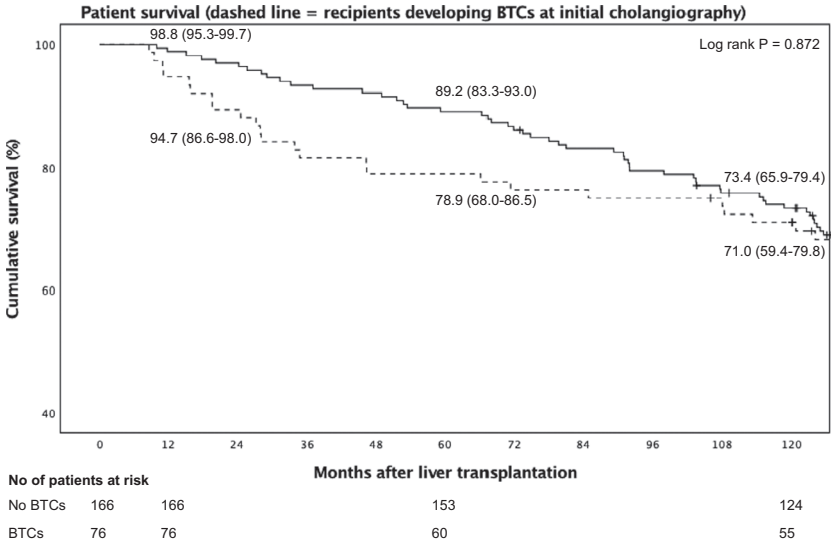


**Figure 4** Patient survival per development of BTCs at the initial cholangiography. Abbreviations: BTCs, biliary tract complications. Survivals expressed as % and 95% confidence intervals at 1, 5 and 10 years after liver transplantation.

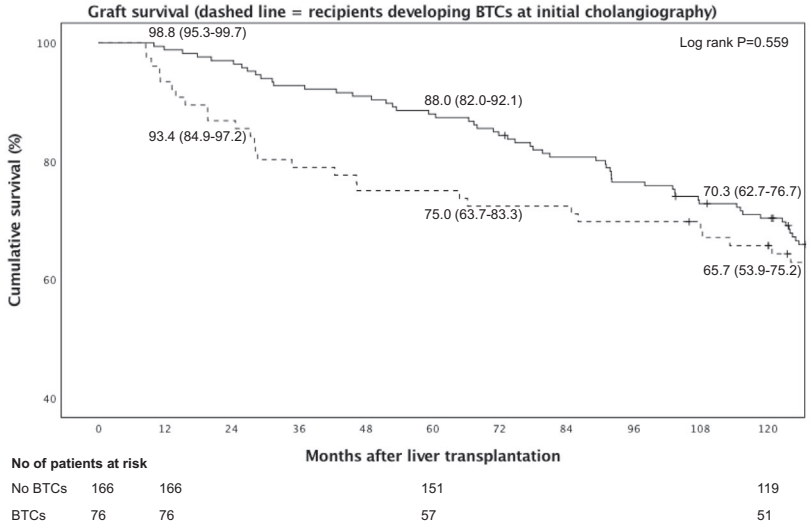


**Figure 5** Graft survival per development of BTCs at the initial cholangiography. Abbreviations: BTCs, biliary tract complications. Survivals expressed as % and 95% confidence intervals at 1, 5 and 10 years after liver transplantation.

tests can lead to an unnecessary, potentially dangerous, reinforcement of the immunosuppressive load, especially if an underlying biliary infection is present [40]. Such scenario can be explained by the very variable clinical and biochemical presentation(s) of BTC ranging from an asymptomatic to a highly symptomatic (with icteric and anicteric cholestasis and cholangitis) status. Biliary leakage occurs during the early postoperative course and is easily recognized, the more delayed (from several weeks to months and even years) development of biliary strictures explains the difficulty in making a timely diagnosis. When suspected, the usual assessment consists of Doppler ultrasound aiming to evaluate both biliary and vascular status. In the absence of dilated bile ducts, sensitivity is poor. Bile duct dilatation is inconsistent in patients with biliary obstruction, a phenomenon explained by the fact that allograft fibrosis and resistance make the donor biliary system less compliant and less apt to dilate [15]. Although cholangio-MRI has a high sensitivity (94–100%) for the assessment of BTCs, this tool may still fail or underestimate the extent of the situation

**Table 3.** Multivariable Cox regression analysis for the risk of graft loss after liver transplantation in the subset of patients who received one-year per-protocol liver biopsy (237/242, 97.9%, events: 109/237)

| Variables in the initial model* | HR | 95% CI | *P* |
|---|---|---|---|
| Recipient age (years) | 1.038 | 1.018–1.059 | <0.001 |
| Indication for LT (biliary or not) | 1.139 | 0.557–2.327 | 0.721 |
| Donor age (years) | 1.019 | 1.005–1.034 | 0.008 |
| Partial liver (yes vs. no) | 0.844 | 0.302–2.363 | 0.747 |
| Cold ischaemia (min) | 0.999 | 0.998–1.000 | 0.069 |
| Warm ischaemia (min) | 1.003 | 0.989–1.017 | 0.681 |
| Biliary anastomosis (RY-HJ vs. DD) | 1.287 | 0.576–2.874 | 0.538 |
| T-tube (yes vs. no) | 0.685 | 0.412–1.137 | 0.144 |
| Six-month canalicular proliferation (yes vs. no) | 1.258 | 0.824–1.921 | 0.287 |
| One-year canalicular proliferation (yes vs. no) | 1.710 | 1.079–2.712 | 0.022 |
| BTCs at initial cholangiography (yes vs. no) | 0.942 | 0.607–1.462 | 0.790 |
| Variables in the final model† | | | |
| Recipient age (years) | 1.036 | 1.017–1.056 | <0.001 |
| Donor age (years) | 1.017 | 1.004–1.031 | 0.012 |
| One-year canalicular proliferation (yes vs. no) | 1.883 | 1.216–2.914 | 0.005 |

BTCs, biliary tract complications; CI, confidence intervals; DD, duct-to-duct biliary reconstruction; HR, hazard ratio; LT, liver transplantation; RY-HJ, Roux-en-Y hepatico-jejunostomy.

*Initial model summary (all candidate variables): −2ln likelihood = 998.110; $\chi^2(11) = 38.472$, $P < 0.001$.

†Final model summary (after backward conditional elimination): −2ln likelihood = 1000.942; $\chi^2(5) = 36.567$, $P < 0.001$.

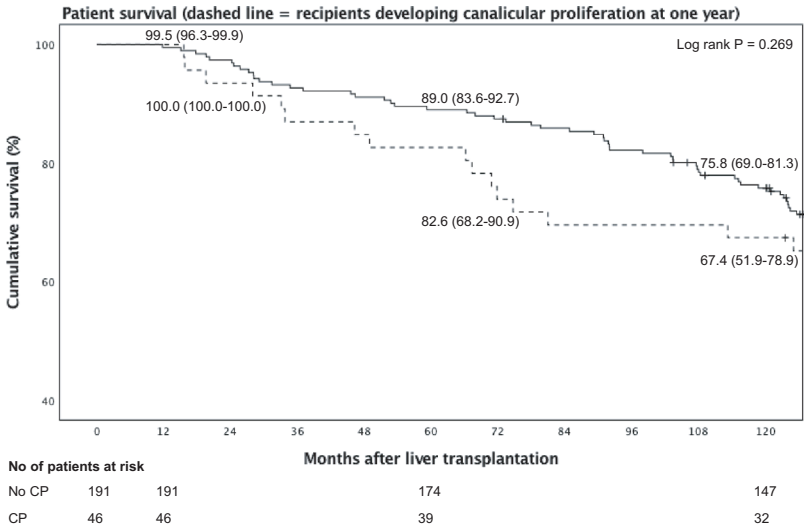


**Figure 6** Patient survival per development of canalicular proliferation one year after liver transplantation. Abbreviations: CP, canalicular proliferation. Survivals expressed as % and 95% confidence intervals at 1, 5, and 10 years after liver transplantation.

especially in case of bilio-digestive anastomosis and of peripheral (zone C and D) lesions [16–18,42]. Despite their invasive nature, PTC and ERC remain the gold standard for BTC diagnosis [19,43–47]. The observations made by Kohli et al. in relation to the diagnostic accuracy of liver tests and noninvasive imaging in the detection of post-LT biliary strictures and those made by Ginat et al. and Rönning et al. in relation to safety of these procedures are in line with our choice of direct visualization of the biliary tree [14,48,49]. The advantages of the ultrasound-guided percutaneous access to the biliary tree around the 3rd to 6th post-LT month are fivefold: (i) the chosen time periods correspond with the highest probability of BTC formation;

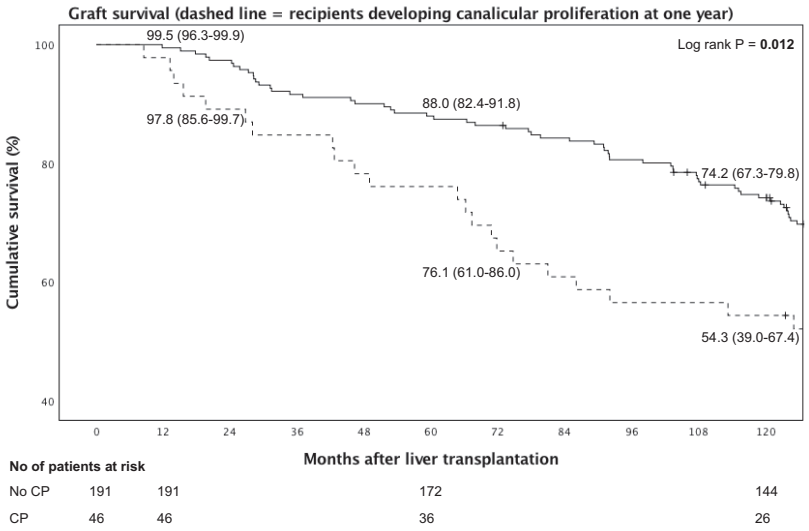


**Figure 7** Graft survival per development of canalicular proliferation one year after liver transplantation. Abbreviations: CP, canalicular proliferation. Survivals expressed as % and 95% confidence intervals at one, five, and ten years after liver transplantation.

(ii) both cholangiography and liver biopsy can be realized in one single session; (iii) the visualization of the peripheral bile ducts is better and can be done using less contrast medium compared to ERC; (iv) the 'bilio-dynamic' consequence of a lesion permits immediate evaluation of the evacuation of the contrast medium thereby allowing withholding of any treatment despite the presence of BTCs ('wait-and-see' strategy and finally (v) if treatment is judged to be necessary a first step (e.g. drainage) can immediately be taken [14,19,21]. Expert interventional radiology allows this combined procedure to be done safely even when the intrahepatic biliary tree is not dilated. The 'wait-and-see' policy, based on the dynamic imaging, avoided pre-emptive insertion of quality-of-life-compromising biliary stents or drainages in about one third of anatomically documented BTC. The lesser amount of used contrast medium may represent another benefit in case of the frequently compromised renal function.

The results from this invasive biliary tract follow-up protocol convey several important messages for the liver transplant community. Firstly, the real incidence of BTC after LT has to be set around one third of recipients. Secondly, the evidence that T-tube confers a putative protection against the development of BTCs might be biased because the surgeons chose this option in selected, merely unfavourable, cases. Nonetheless, the absence of a clear prognostic factor for early BTCs confirms the usefulness of a direct visualization protocol in order to correctly diagnose BTCs. Thirdly, BTCs in the absence of symptoms and liver test abnormalities are frequent (61% in this study). Consequently, no firm statements can be made in relation to the 'positive impact' on the biliary tree of different types of preservation solutions, storage methods and machine perfusion as long as systematic (direct) visualization of the biliary tree is not part of the study protocol. In such studies BTCs are underreported because investigated only in case of abnormal liver tests and/or symptoms [25,50,51]. Fourthly, BTCs may lead to pathologic features mimicking phenomena seen in acute and chronic rejection (7% in this study). In order to avoid unnecessary reinforcement of immunosuppression, BTCs ought to be excluded beforehand [12,23,24,38,52]. Reversal of all identified rejection features (even of the description of *vanishing* bile duct syndrome), were confirmed by per-protocol 5- and 10-year routine liver biopsies and the paucity of graft loss in the absence of BTCs. Fifthly, while our wide-ranging multivariable analyses might be biased because of our reduced sample size, one-year canalicular proliferation intriguingly appeared to jeopardize long-term graft outcomes. Anomalies on six-month biopsies proved less critical because histological evidences of biliary lesions possibly need a longer time to establish. Secondary biliary cirrhosis is indeed known to be a protracted process. Such histological features should incite the transplant team to follow-up such patients more closely. Sixthly, BTCs burden LT with graft and patient loss, biliary infections and decompensated secondary biliary cirrhosis, suggesting that the decision to list the patient for re-LT is frequently untimely or overdue. As MELD score in these patients is

frequently low in the process of the development of BTCs, access to bonus points should be advocated in order to reduce this cause of delayed mortality after transplantation, similarly to what happens for infected PSC patients. Seventhly, the long gap between LT and re-LT (up to 92 months in these series) or death (up to 238 months) because of BTC underlines the importance of a rigorous long-term follow-up. Many of these late graft (7.4% in this study) or patient (5.8% in this study) losses are usually not documented, leading to an underestimation of the impact of BTCs on outcomes after transplantation. Almost one fifth of patients (18/85 pats) presenting BTC will lose their graft during follow-up. For this reason, we focussed on a cohort of patients who received transplantation up to 10 years ago.

This study has some limitations such as the use of two different imaging techniques (TTC and PTC), performed at two, although approximated, different time points (three and six months). However, both imaging techniques allowed a direct biliary tract visualization and most BTCs are known to occur within the first six months after LT [19,25,36,37]. Additionally, we did not systematically report donor-specific antibodies (DSA) because the rarity of data did not allow an appropriate analysis to discern their role in the development of BTCs. Nonetheless, it is improbable that DSA might have distorted our conclusions because 1.6% (4/242) patients from this cohort lost their graft due to chronic rejection due to noncompliance (2 pats), severe tacrolimus related neurotoxicty (1 pat) and HBV vaccination (1 pat)).

Sensibly, the implementation of this invasive biliary follow-up protocol should be left to the discretion of the LT teams. The context of our centre's minimization immunosuppression study offered a unique opportunity to define more precisely the underestimated incidence of BTCs as well as their short- and long-term consequences. Our results might incite the transplantation community to use more deliberately cholangio-MRI to document possible BTC, especially when recipients show unexpected modifications of liver tests. In case of an unsatisfactory evaluation by these noninvasive technique, cholangiography should be advocated. Based on the results of this report, our centre has launched a prospective study to compare direct biliary visualization and cholangio-MRI.

In conclusion, systematic simultaneous evaluation of liver histology and biliary tree precisely documents the incidence and consequences of BTCs after LT. BTCs in the absence of symptoms and liver test abnormalities are frequent. The combination of abnormal biliary tract at imaging and canalicular proliferation at pathology is an early indicator of significantly reduced graft survival and, therefore, represents a useful tool to intensify follow-up and to help decide when to list patients for re-LT. Firm conclusions about the impact of different preservation solutions and modalities should be made with caution if direct biliary tree visualization is not included in the study protocol.

## Authorship

JL: conceived and designed the work; JN, DK, KA, MN, KBM, PB and JL: collected data; SI and JL: analysed data; JL, PG, EBR, OC, LC and TM: contributed to clinical management and interpretation of data; JN, SI and JL: drafted the article; all authors revised the article and finally approved it.

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## Conflict of Interest

The authors have no conflict of interest to declare in relation to the content of the submitted paper. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1** Multivariable Cox regression analysis for the risk of death after liver transplantation in the subset of patients who received one-year per-protocol liver biopsy (237/242, 97.9%, events: 101/237).

**Table S2** Characteristics of recipients who showed canalicular proliferation or not, at histological examination one year after liver transplantation.

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