DOI: 10.15825/1995-1191-2025-2-31-38

INDOCYANINE GREEN FLUORESCENCE IMAGING OF THE COMMON BILE DUCT BLOOD SUPPLY IN THE PREVENTION OF BILIARY COMPLICATIONS IN LIVER TRANSPLANTATION: RATIONALE AND RESULTS

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Objective: to enhance liver transplant (LT) outcomes by developing and implementing intraoperative fluorescence imaging of common bile duct (CBD) blood supply using indocyanine green (ICG). **Material and methods.** The study analyzed treatment outcomes in 203 recipients who received a whole liver from deceased donors. In the first stage, the incidence and potential risk factors of biliary complications were assessed in Group I, comprising 138 patients. The median follow-up period was 35.6 months (IQR: 25–68 months). Group II consisted of 65 cases, with a median follow-up of 7.2 months (IQR: 6.5–13). In this group, intraoperative ICG fluorescence imaging was employed to assess CBD blood supply. Following cholecystectomy, a 5 mL intravenous injection of ICG solution (2.5 mg/mL) was administered. Near-infrared fluorescence imaging was then performed by overlaying near-infrared light onto white light to visualize ICG fluorescence in CBD tissues. In cases where fluorescence imaging indicated hypoperfusion of the distal part of the graft's CBD, the affected segment was excised within the boundaries of well-perfused tissue. In all cases, the resected CBD portions were sent for histological examination. **Results.** In Group I, biliary anastomosis complications were recorded in 13 out of 138 cases (9.4%), all of which were strictures. Analysis of potential risk factors on both the recipient and donor sides did not reveal any statistically significant associations ($p > 0.05$). Comparison of intraoperative fluorescence imaging results with postoperative histological examination demonstrated a sensitivity of 87% and a specificity of 92% for detecting ischemic changes in CBD. In groups with comparable baseline characteristics ($p > 0.05$), the incidence of biliary anastomotic strictures (BAS) was significantly lower in the ICG imaging group: 9.4% in Group I versus 1.5% in Group II ($p = 0.04$). **Conclusion.** The use of fluorescence imaging to assess the blood supply of the CBD in LT is an effective method for preventing biliary complications. This technique enables the formation of biliary anastomosis within a well-perfused tissue, significantly reducing the risk of BAS.

Keywords: common bile duct, biliary complications, liver transplantation, fluorescence.

INTRODUCTION

Biliary complications have long been regarded as the "Achilles' heel" of liver transplantation (LT). These include biliary anastomotic failure, anastomotic strictures (AS), and non-anastomotic strictures (NAS) of the bile ducts. NAS typically encompasses ischemic cholangiopathy resulting from inadequate arterial blood supply to the bile ducts, post-transplant cholangiopathy caused by severe ischemia-reperfusion injury to the graft and its biliary system, and recurrent autoimmune diseases affecting the bile ducts in the transplanted liver [1–2]. In most cases, complications arising from the choledochal anastomosis are primarily attributed to technical peculiarities of biliary reconstruction.

Biliary anastomotic failure typically occurs in the early postoperative period with a 5–10% incidence. The primary contributing factors are conditions that predispose the anastomosis to ischemia, including excessive tension, suture line compromise, and over-reliance on electrocoagulation to control bleeding from the donor and recipient bile duct stumps. Hepatic artery thrombosis is a serious complication that can lead to necrosis of the donor bile duct segment.

An anastomotic stricture (AS) is a localized narrowing at the site of a biliary anastomosis, typically solitary and short, often diagnosed within the first year after LT [3–4]. AS occurs in 7–12% of patients and accounts for up to 86% of all post-transplant biliary strictures. Ischemia and subsequent fibrosis of the bile ducts, often resulting from suboptimal surgical technique or early postoperative complications, are considered primary contributors to AS pathogenesis [5]. Key predisposing factors include small bile duct diameter, prolonged biliary ischemia,

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donor-recipient duct size mismatch, inappropriate suture material, excessive anastomotic tension, redundant donor duct, and overuse of electrocoagulation. Studies have linked prolonged cold ischemia time, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections, and acute and chronic cellular rejection to the development of complications [6].

According to current literature, local ischemia plays a key role in the development of biliary anastomotic complications. Due to the bile duct's blood supply anatomy, the distal segment of the donor common bile duct is particularly vulnerable to inadequate perfusion. Numerous studies in abdominal surgery have demonstrated the efficacy of indocyanine green (ICG) fluorescence imaging in assessing tissue perfusion; however, its application in LT remains limited. In this study, we present preliminary results on the use of ICG fluorescence imaging to evaluate blood supply in the donor segment of the ductus hepaticocholedochus (bile duct), with the aim of preventing biliary anastomosis-related complications.

MATERIALS AND METHODS

This retrospective study analyzed the treatment outcomes of 203 recipients who underwent whole LT from deceased donors at Botkin Hospital between 2018 and 2023. Recipients who died or required retransplantation within 1 year post-transplant were excluded from the analysis. In all cases, duct-to-duct anastomosis was performed using a knotted PDS 6-0 suture.

In the first phase of the study, we analyzed the incidence and potential risk factors associated with the development of anastomotic biliary complications in 138 patients operated on between 2018 and 2022 (Group I, control). The cohort included 82 males (59.4%) and 56 females (40.6%). The median recipient age was 48 years (IQR: 36–58), with a median BMI of 26 kg/m^2 (IQR: 22–27) and a median MELD score of 16 (IQR: 12–18). The median donor age was 48 years (IQR: 40–55), with a median BMI of 25.5 kg/m^2 (IQR: 23.0–29.5). Donors met extended criteria in 34 cases (24.6%), while the remaining donors were classified as standard. Median cold ischemia time was 5.6 hours (IQR: 5.2–7.4), and secondary warm ischemia time was 35 minutes (IQR: 35–45). Surgery lasted for 7.4 hours (IQR: 5.5–8.5), with a biliary ischemia time of 40 minutes (IQR: 45–50). Median intraoperative blood loss was 1,400 mL (IQR: 1,000–4,200). The recipients were followed up for a median duration of 35.6 months (IQR: 25–68). Detailed characteristics of Group I are presented in Table 3.

Group II included 65 recipients who underwent LT between 2022 and 2023 with the use of ICG fluorescence imaging. The group comprised 35 males (53.8%) and 30 females (46.2%). The median recipient age was 47 years (IQR: 35–62), with a median BMI of 25 kg/m^2 (IQR: 21–29) and a MELD score of 18 (IQR: 13–25). Donor characteristics included a median age of 52 years (IQR: 32–64) and a median BMI of 27.2 kg/m^2 (IQR: 23.0–32.0). Donors met extended criteria in 21 cases (32.3%), while the remaining observations were standard. Median cold ischemia time was 5.7 hours (IQR: 4.5–6.5), and the median secondary warm ischemia time was 35 minutes (IQR: 30–45). Median operative time was 6.5 hours (IQR: 5.0–7.5), with a biliary ischemia time of 35 minutes (IQR: 35–45). Intraoperative blood loss was 1,250 mL (IQR: 1,000–3,200). The recipients were followed up for a median duration of 7.2 months (IQR: 3.5–11). Detailed characteristics of Group II are presented in Table 3.

Methodology of ICG fluorescence imaging for assessment of ductus hepaticocholedochus perfusion in liver transplantation

Following cholecystectomy and during the preparation of the graft choledochus for biliary reconstruction, 5 mL of ICG solution was administered intravenously by an anesthesiologist at a concentration of 2.5 mg/mL. Fluorescence imaging was then performed using the Karl Storz visualization system, which superimposes near-infrared light on white light to detect ICG fluorescence in the bile duct tissues (Fig. 1).

In cases where uniform fluorescence of the entire length of the donor ductus hepaticocholedochus was observed (interpreted as a negative result indicating good perfusion, Fig. 1), the excess length of the graft bile duct was excised, and a standard end-to-end anastomosis was created with the recipient's bile duct. A positive result (Fig. 2) was defined as the presence of hypoperfusion in the distal portion of the donor choledochus. In these cases, the bile duct was transected within the boundaries of adequately perfused tissue.

If the residual length of the donor choledochus was insufficient to create a tension-free anastomosis, the recipient's bile duct was mobilized from surrounding tissues – carefully avoiding skeletonization. Perfusion

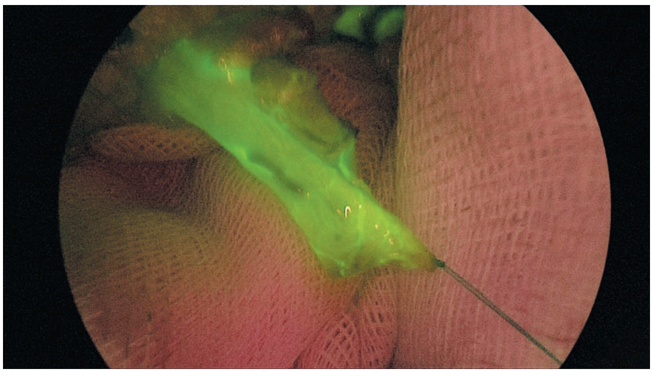

Fig. 1. Intraoperative indocyanine green fluorescence imaging of blood supply of a donor common bile duct (CBD). Satisfactory perfusion of the CBD throughout the whole length

of the recipient's choledochus was also confirmed using ICG fluorescence imaging.

In two cases, additional mobilization of the duodenum was performed using the Kocher maneuver to increase the mobility of the recipient's bile duct and facilitate a tension-free end-to-end anastomosis.

Resected sections of the donor common bile ducts from all cases were submitted for histological examination to assess the severity of ischemic changes in both the proximal and distal segments. To evaluate the diagnostic performance of the fluorescence imaging technique in identifying choledochal ischemia, a comparative analysis was conducted between the intraoperative fluorescence findings and histopathological assessment results.

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 26.0 (IBM Corp., USA). Due to the relatively small sample size, the Mann-Whitney U test was used to compare two groups of quantitative variables, irrespective of distribution. Categorical variables were compared using Pearson's chi-squared test or Fisher's exact test, as appropriate. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Analysis of the frequency and risk factors of anastomotic biliary complications

In the cohort of 138 recipients, biliary anastomotic complications were observed in 13 cases (9.4%), all of which presented as bile duct strictures. The median time from transplantation to the onset of complications was 6.5 months (IQR: 1.0–12.5). Additionally, two cases of non-anastomotic biliary strictures were recorded; one of these was associated with hepatic artery stenosis.

The occurrence of anastomotic biliary strictures (ABS) showed no statistically significant association with early liver graft dysfunction. The incidence among patients with early graft dysfunction was 4 out of 45 (8.9%), compared to 9 out of 93 (9.7%) among recipients with initially satisfactory graft function ($p = 0.415$). Recipient-related variables, including age, sex, BMI, and MELD score, did not demonstrate a statistically significant correlation with ABS ($p > 0.05$). Similarly, donor characteristics and perioperative parameters were not significantly associated with stricture formation ($p > 0.05$). Detailed results are presented in Table 1.

Results of the study on the sensitivity and specificity of ICG fluorescence Imaging for detecting ischemia in the distal donor choledochus

ICG fluorescence imaging was performed intraoperatively during biliary reconstruction in all recipients from the prospective group II ($n = 65$). A positive fluorescence result – indicating hypoperfusion of the distal portion of the donor choledochus – was recorded in 39 cases (60%). In the remaining observations, perfusion was deemed satisfactory along the entire length of the bile duct.

Histological analysis confirmed more pronounced ischemic changes in the distal part of the donor choledochus compared to the proximal part in 36 cases (55.3%). An example of histological preparation comparing the proximal and distal segments of the bile duct graft is presented in Fig. 3.

The sensitivity and specificity of the method for detecting ischemia, as confirmed by histological examination, were 87% and 92%, respectively. The positive predictive value was 88.1%, and the negative predictive value was 98.1% (Table 2).

Clinical outcomes of ICG fluorescence imaging in the assessment of ductus hepaticocholedochus blood supply

Comparison of recipient characteristics between the two study groups revealed no statistically significant

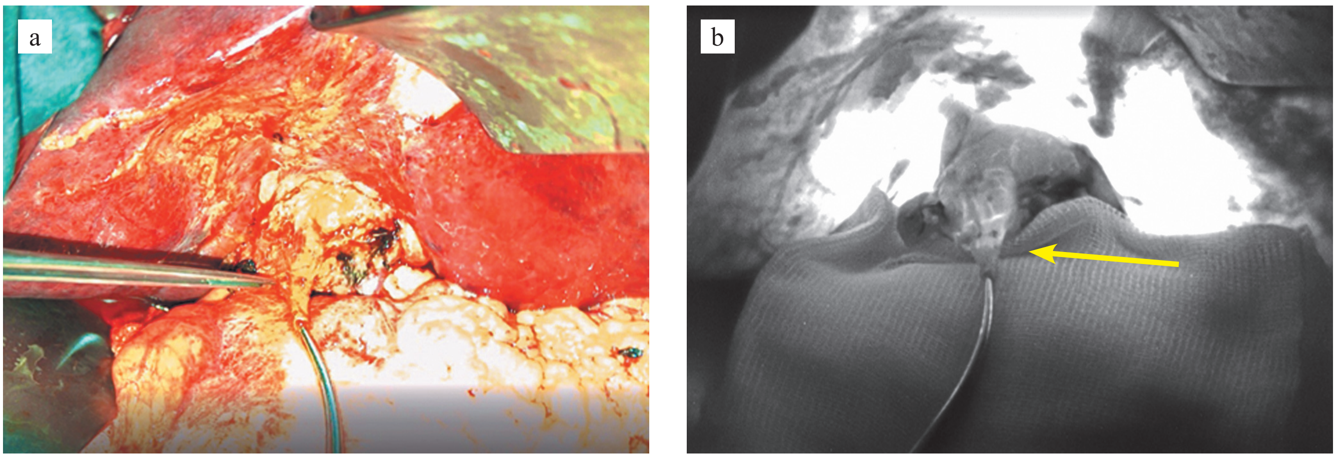

Fig. 2. Intraoperative indocyanine green fluorescence imaging of blood supply of a donor common bile duct (CBD). Poor perfusion of the distal part of the donor CBD (yellow arrow): a, standard mode; b, near infrared light overlay mode

Table 1

Risk factors for biliary anastomotic strictures

Indicator	AS-free, n = 125	AS, n = 13	Significance point (p value)
Recipient risk factors			
Recipient age (years)	43 (IQR: 35–51)	45 (IQR: 32–58)	0.23
Recipient male gender	75 (60%)	7 (53.8%)	0.77
Recipient BMI (kg/m^2)	25 (IQR: 22–27)	25 (IQR: 23–29)	0.52
MELD	16 (IQR: 14–23)	18 (IQR: 15–26)	0.18
Donor risk factors			
Donor age (years)	46 (IQR: 39–56)	44 (IQR: 32–66)	0.4
Donor BMI (kg/m^2)	26 (IQR: 23–29)	27 (IQR: 22–32)	0.48
Macrosteatosis >40%	22 (17.6%)	4 (30.8%)	0.27
Norepinephrine dose >1000 ng/kg/mL or presence of two vasopressors	16 (12.8%)	3 (23.1%)	0.39
Duration of donor's stay in intensive care (hours)	48 (IQR: 41–56)	48 (IQR: 24–72)	0.36
Expanded criteria donor (as defined by Eurotransplant)	27 (21.6%)	4 (30.8%)	0.49
Perioperative risk factors			
Static cold preservation time (hours)	5.5 (IQR: 4.5–6.5)	6.0 (IQR: 4.0–7.0)	0.62
Secondary warm ischemia time (minutes)	40 (IQR: 35–40)	40 (IQR: 35–40)	0.83
Biliary ischemia time (minutes)	40 (IQR: 40–50)	45 (IQR: 40–55)	0.24
Intraoperative blood loss (mL)	1500 (IQR: 800–2500)	1600 (IQR: 1000–2500)	0.6
Cell saver blood reinfusion (mL)	300 (IQR: 250–400)	350 (IQR: 150–550)	0.53
Fresh frozen plasma transfusion (doses)	2 (IQR: 1–3)	2 (IQR: 1–5)	0.1
Erythrocyte suspension transfusion (doses)	1 (IQR: 0–2)	1 (IQR: 0–4)	0.38

Note: AS, anastomotic stricture.

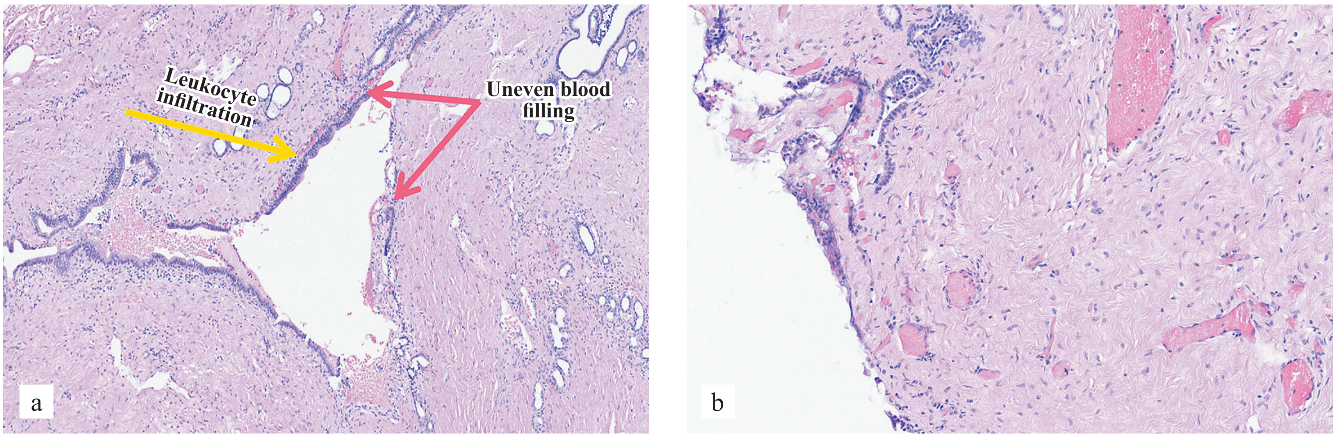

Fig. 3. Morphological examination of the proximal and distal sections of the donor's common bile duct: a, distal section (positive result) – more pronounced inflammation, fibrosis, vascular paresis, irregular blood flow, edema, leukostasis are noted; b, proximal section (negative result): less pronounced inflammation, more pronounced vascular congestion with perivascular hemorrhages

Table 2

Sensitivity and specificity of ICG fluorescence imaging of common bile duct blood supply in relation to ischemia

Histological examination	ICG imaging		Se, %	Sp, %	PPV, %	NPV, %
	Positive	Negative				
Positive	34	2	87	92	88.1	98.1
Negative	5	24				
Total	39	26				

differences in age, sex, BMI, or MELD score ($p = 0.25$, $p = 0.453$, $p = 0.36$, and $p = 0.091$, respectively). Donor characteristics and perioperative parameters were also comparable between the groups ($p > 0.05$). The median follow-up was significantly longer in group I ($p < 0.001$). No significant difference was observed in recipient length of stay in the ICU ($p = 0.921$); however, the total length of stay in the hospital was significantly shorter in group II (fluorescence-guided group) ($p = 0.012$). The incidence

Table 3

Comparative characteristics of the groups and clinical results

Indicator	Group I (Without ICG imaging), n = 138	Group II (ICG imaging), n = 65	Significance point (p-value)
Recipient characteristics			
Recipient age (years)	48 (IQR: 36–58)	47 (IQR: 35–62)	0.25
Recipient male gender	82 (59.4%)	35 (53.8%)	0.453
Recipient BMI (kg/m^2)	26 (IQR: 22–27)	25 (IQR: 21–29)	0.36
MELD	16 (IQR: 12–18)	18 (IQR: 13–25)	0.091
Donor characteristics			
Donor age (years)	48 (IQR: 40–55)	52 (IQR: 32–64)	0.39
Duration of donor’s stay in intensive care (hours)	52 (IQR: 36.0–70.0)	58 (IQR: 36.0–72.0)	0.052
Donor BMI (kg/m^2)	25.5 (IQR: 23.0–29.5)	27.2 (IQR: 23–32.0)	0.08
Norepinephrine dose >1000 ng/kg/mL or two vasopressors (%)	19 (13.7%)	11 (16.9%)	0.534
Na (mmol/L)	143 (IQR: 136–146)	145 (IQR: 134–156)	0.823
AST (U/L)	31.0 (IQR: 22.0–45.0)	37.5 (IQR: 28.0–58.0)	0.061
ALT (U/L)	33.0 (IQR: 26.0–51.5)	28.0 (IQR: 25.0–57.5)	0.236
Macrosteatosis >40%	26 (18.8%)	17 (26.2%)	0.234
Expanded criteria donor (n, %)	34 (24.6%)	21 (32.3%)	0.251
Perioperative risk factors			
Static cold preservation time (hours)	5.6 (IQR: 5.2–7.4)	5.7 (IQR: 4.5–6.5)	0.842
Operation time (minutes)	7.4 (IQR: 5.5–8.5)	6.5 (IQR: 5.0–7.5)	0.063
Secondary warm ischemia time (minutes)	35 (IQR: 35–45)	35 (IQR: 30–45)	0.92
Biliary ischemia time (minutes)	40 (IQR: 45–50)	35 (IQR: 35–45)	0.32
Blood loss (mL)	1400 (IQR: 1000–4200)	1250 (IQR: 1000–3200)	0.12
Reinfusion (mL)	350 (IQR: 50–1000)	200 (IQR: 50–700)	0.461
Fresh frozen plasma transfusion (doses)	2 (IQR: 2–6)	3 (IQR: 2–4)	0.61
Erythrocyte suspension transfusion (doses)	1 (IQR: 0–3)	0 (IQR: 0–2)	0.74
Results			
Length of stay in intensive care unit (days)	3 (IQR: 1–4)	3 (IQR: 1–4)	0.921
Length of stay in the hospital (days)	18 (IQR: 15–34)	13 (IQR: 9–25)	0.012
Early allograft dysfunction (n, %)	45 (32.6%)	16 (24.6%)	0.246
Median follow-up of recipients (months)	35.6 (IQR: 25–68)	7.2 (IQR: 6.5–13)	<0.001
Biliary anastomotic stricture (n, %)	13 (9.4%)	1 (1.5%)	0.04

of early graft dysfunction did not differ significantly between the groups – 45 cases (32.6%) in group I versus 16 cases (24.6%) in group II (p = 0.246).

Importantly, no cases of biliary anastomosis failure were recorded in either group (p = 1). The incidence of biliary anastomotic strictures was significantly lower in the fluorescence imaging group – 1 case (1.5%) versus 13 cases (9.4%) in the control group (p = 0.04).

No adverse events were associated with the administration of ICG in any of the patients. Detailed clinical outcomes are summarized in Table 3.

DISCUSSION

LT remains the only definitive and highly effective treatment for end-stage liver disease, offering excellent long-term survival outcomes. However, it is associated with a range of specific complications, particularly involving the biliary anastomosis, which can lead to repeated hospitalizations and increased treatment costs. While advancements in surgery and suture materials have significantly decreased biliary anastomotic failure risk, challenges persist. In our series of 203 liver transplantations, no cases of anastomotic failure were observed. Nonetheless, the incidence of biliary anastomotic strictures (BAS) remained relatively high (9.4%), prompting an in-depth analysis of associated risk factors and the development of strategies for their prevention.

Most authors emphasize that technical challenges during biliary reconstruction are a central cause and risk factor for BAS. These challenges can lead to localized disruption of the bile duct’s blood supply and subsequent scar formation at the anastomotic site. In our study, none of the analyzed recipient, donor, or perioperative parameters showed a statistically significant association with the occurrence of this complication (p > 0.05), which indirectly supports the predominant influence of surgical technique. Accordingly, the most effective strategy for preventing anastomotic strictures is the precision construction of the biliary anastomosis within well-perfused tissue of the ductus hepaticocholedochus. Traditionally, assessment of blood supply to the graft bile duct relies on the surgeon's subjective evaluation of the intensity of bleeding from the duct stump, a method that lacks precision. Fig. 2, a, illustrates an intraoperative view in which visual inspection suggests uniform perfusion of both proximal and distal segments of the choledochus. However, as demonstrated in Fig. 2, b, intraoperative ICG fluorescence imaging reveals a clear hypoperfusion area in the distal segment, which should be excised before forming a biliary anastomosis.

Intraoperative use of ICG fluorescence imaging for assessing tissue perfusion is well-established and used in various abdominal surgerie [7]; however, its application in LT remains limited. To date, we have identified only two published studies specifically addressing the use of this technique for evaluating bile duct perfusion during LT. The first report, by Coubeau Laurent et al. (2017) from a clinical university in Belgium, described a successful case of ICG fluorescence imaging in a liver transplant from a donor after cardiac arrest. Despite the macroscopic appearance suggesting adequate perfusion of the distal bile duct, intraoperative fluorescence imaging revealed hypoperfusion at the distal segment of the donor choledochus. Histological analysis of the resected portion confirmed ischemic injury, including epithelial destruction and separation of epithelial and subepithelial layers. The patient remained free of biliary complications during a 10-month follow-up. The second study, conducted by Panaro et al. (2018) at the University of Montpellier in France, included six clinical cases, although donor type was not specified. In two of these cases, fluorescence imaging prompted the resection of non-perfused segments that were initially deemed viable by visual inspection alone. During a 12-month follow-up, none of the patients developed BAS.

In our larger cohort of 65 recipients, the use of intraoperative fluorescence imaging proved to be an effective method for detecting choledochal ischemia, as confirmed by histological analysis. The technique demonstrated a sensitivity of 87% and a specificity of 92% in identifying ischemic segments. Formation of biliary anastomosis within well-perfused tissue significantly reduced the incidence of anastomotic strictures from 9.3% to 1.5% in groups matched for key clinical characteristics ($p = 0.04$).

Limitations

The main limitations of this study include its retrospective nature and relatively small sample size. It is possible that some contributing factors to biliary complications were not fully accounted for in the analysis. In assition, the median follow-up period in Group II was significantly shorter than that of the control group. However, since the median time to detection of anastomotic stricture identified in the first phase of the study was relatively short, we believe the differences in follow-up duration are not clinically significant.

CONCLUSION

The use of fluorescence imaging to assess bile duct blood supply during LT is a safe and effective method for preventing biliary complications. By ensuring biliary anastomoses occur in well-perfused tissue, this technique significantly reduced anastomotic strictures across key clinical parameters, as demonstrated by statistically significant results in comparable patient groups.

The authors declare no conflict of interest.

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The article was submitted to the journal on 7.10.2024