

# Laparoscopic Roux-en-Y hepaticojejunostomy reconstruction after iatrogenic bile duct injury: case series report

## Reconstrucción laparoscópica con hepatoyeyunostomía en Y de Roux después de una lesión del conducto biliar iatrogénico: serie de casos

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### Abstract

**Introduction:** Bile duct injury (BDI) is a devastating complication after a cholecystectomy. Laparoscopic management has become a mainstay approach because of the advantages offered to patients; nevertheless, outcomes after repair are influenced by the center, the surgeon's experience, and the type of reconstruction. **Materials and methods:** Patients with common BDI managed at a referral center from January 2013 to June 2018 with a novel modified and simplified laparoscopic technique for a hepatic duct jejunostomy with a Roux-en-Y reconstruction were retrospectively reviewed. **Results:** Twenty patients had a BDI (20/5430-0.3%), 8 (40%) had intraoperative diagnosis, and 12 (60%) patients with diagnosis before 72 h. Type E Strasberg classification, the predominant BDI was Strasberg E1 (65%), the average surgery time was 146.5 min (115-178 min), the average intraoperative bleeding was 15-50 cc. The overall complication rate was 10 % (2/20), 1 (5%) patient required, there were no mortalities, and the mean hospital stay was 5 days. One (5%) patient presented bile leak (Type SE4), and 1 (5%) patient required intensive care unit admission after reintervention. Long-term follow-up showed no bile duct stricture, cholangitis, or recurrent choledocholithiasis up to 5 years after the procedure and with a minimum of 1 year minimum follow-up. **Conclusions:** This simplified laparoscopic approach to bile duct reconstruction with Roux-en-Y anastomoses appears to be an effective and safe alternative to reconstructive open bile duct surgery, offering to patients the benefits of laparoscopic surgery with a low complication rate.

**Key words:** Laparoscopic approach. Bile duct injury. Reconstruction. Bile leakage.

### Resumen

**Antecedentes:** La lesión del conducto biliar es una complicación devastadora tras una colecistectomía. El tratamiento laparoscópico se ha convertido en un enfoque principal debido a las ventajas ofrecidas a los pacientes; sin embargo, los resultados después de la reparación están influenciados por el centro, la experiencia del cirujano y el tipo de reconstrucción. **Método:** Se revisaron retrospectivamente los pacientes con lesión del conducto biliar tratados con reconstrucción biliar en un centro de referencia, desde enero de 2013 hasta junio de 2018, por vía laparoscópica con una técnica modificada y simplificada con hepatoyeyunostomía en Y de Roux. **Resultados:** Veinte pacientes de 5,430 tenían lesión del conducto biliar (0,3%), 8 (40%) tuvieron diagnóstico intraoperatorio y 12 (60%) diagnóstico antes de las 72 horas. Todos con una clasificación de

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Strasberg tipo E, con predominio de E1 (65%). El tiempo quirúrgico promedio fue de 146,5 (rango 115-178) minutos y el sangrado intraoperatorio promedio fue de 15-50 cm<sup>3</sup>. La tasa general de complicaciones fue del 10% (2/20); 1 (5%) paciente requirió reintervención. No hubo mortalidad y la estancia hospitalaria media fue de 5 días. Un paciente (5%) presentó fuga biliar (tipo SE4) y 1 (5%) paciente requirió ingreso en la unidad de cuidados intensivos después de la reintervención. El seguimiento fue de hasta 5 años tras el procedimiento, con un mínimo de 1 año, y no se han documentado estenosis del conducto biliar, colangitis ni coledocolitiasis recurrente. **Conclusión:** Este enfoque laparoscópico simplificado para la reconstrucción del conducto biliar con anastomosis en Y de Roux parece ser una opción efectiva y segura a la cirugía reconstructiva del conducto biliar abierta, ofreciendo a los pacientes los beneficios de la cirugía laparoscópica y con una baja tasa de complicaciones.

**Palabras clave:** Abordaje laparoscópico. Lesión del conducto biliar. Reconstrucción. Fuga de bilis.

## Introduction

Bile duct injury (BDI) is still a much-feared complication after gallbladder surgery<sup>1</sup>, and since the era of laparoscopic surgery began the incidence has increased. Even though the laparoscopic approach speeds up patient recovery, BDI secondary to laparoscopic manipulation remains a significant complication with an incidence of 0.08%-1.5%<sup>2-5</sup>. Nevertheless, the laparoscopic management of bile duct pathologies has become a mainstay approach because of the advantages offered to patients<sup>6-8</sup>. When laparoscopic BDI occurs, the consequences can be devastating, such as biliary cirrhosis, liver failure, and the need for complex bile duct reconstruction<sup>9</sup>. The severity of BDI ranges from relatively simple leakage of the cystic duct or liver surface to complete transection or even resection of one or more bile ducts, sometimes accompanied by vascular injuries that mainly involve the right hepatic artery and the right portal vein. Several classification systems exist for BDI, including the Strasberg–Bismuth classification (Table 1)<sup>10,11</sup>.

Various approaches to bile duct reconstruction have been proposed, including endoscopic and open approaches. However, the gold standard is still under debate<sup>9</sup>. The end result and the desired goal are to reestablish bile duct continuity and avoid bile reflux; hence, a hepatic duct jejunostomy along with a Roux-en-Y reconstruction is the surgery of choice<sup>12</sup>. Recent reports have shown satisfactory results using laparoscopy with this technique<sup>13,14</sup>. Nevertheless, elevated incidences of complications such as bile duct stenosis and recurrent cholangitis along with a need for high laparoscopic skill have made this approach unattractive to many surgeons<sup>15</sup>. Successful bile duct reconstruction and anastomosis is determined by the absence of tension, adequate diameter, and proper

**Table 1. Strasberg classification for BDI**

Type	Description
A	Cystic duct leak or leak from small ducts in the liver bed
B	Occlusion of an aberrant right hepatic duct
C	Transection without ligation of an aberrant right hepatic duct
D	Lateral injury to a major bile duct
E	Circumferential injury to a major bile duct
E1	Transection or stricture > 2 cm from the hilum
E2	Transection or stricture < 2 cm from the hilum
E3	Transection at the level of the bifurcation, without loss of contact between the left and right hepatic duct
E4	Transection at the level of the bifurcation with loss of communication between the left and right hepatic duct
E5	Injury of a right segmental duct combined with an E3 or E4 injury

vascularization<sup>16,17</sup>. Although the surgical technique using laparoscopy offers advantages, it has a prolonged surgery time and technical challenges. We introduce a modified approach for bile duct injuries after cholecystectomy with laparoscopic reconstruction perform a hepatic duct jejunostomy with a Roux-en-Y, to reduce the operative time and complications in a case series of 20 patients. This material was presented at the SAGES meeting, Baltimore, 2019 (Abstract ID 93808).

## Materials and methods

### Study population

This study design was a retrospective cohort analysis of 20 patients with BDIs who underwent laparoscopic simplified bile duct reconstruction by iatrogenic

bile duct injuries after laparoscopic cholecystectomy at a referral center in Bogotá, Colombia between January 2013 and June 2018. The study protocol was approved by the ethics committee.

The protocol was implemented in accordance with ethical guidelines of the “World Medical Association (WMA) Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects” adopted by the 18<sup>th</sup> WMA General Assembly, Helsinki, Finland, June 1964, and revised in Tokyo 2004.

As this was an observational retrospective cohort, a descriptive analysis of data was performed prospectively in Microsoft Excel databases and analyzed using SPSS1 version 22.0 (Statistical Package for the Social Sciences). Continuous variables were treated as means (range). Variables were summarized using median, minimum, and maximum values and percentages.

All patients had the surgeon’s email and cell phone number, to ensure strict follow-up and not lose the continuity of clinical surveillance.

## **Data collection**

During the 5 years study period in this institution, 5430 patients were treated for symptomatic cholelithiasis who underwent laparoscopic cholecystectomy, 20 patients suffered common BDI type E of Strasberg–Bismuth iatrogenic BDI classification (Fig. 1) and underwent to laparoscopic simplified bile duct reconstruction which reduces the surgical complexity and operation time<sup>18</sup>. Were evaluated the following variables: age, sex, type of bile duct pathology, Strasberg–Bismuth iatrogenic BDI classification, surgery time, bleeding, bile leaks, conversion rates, previous endoscopic retrograde cholangiopancreatography (ERCP), oral feeding tolerance, intensive care unit (ICU) stay, hospital stay, reintervention, stenosis, and mortality. Clinical follow-up of the patients was at least 1 year. All patients had a preoperative multidisciplinary team assessment by gastroenterology, internal medicine, infectiology, and general surgery. In addition, all patients had a contrast abdominal computed tomography scan to rule out intra-abdominal collections and vascular involvement. Finally, magnetic resonance cholangiopancreatography was ordered to map out biliary tree anatomy and Strasberg–Bismuth classification (Table 1). Patient consent for laparoscopic bile duct reconstruction and research was obtained before the procedure was started.

## **Inclusion and exclusion criteria**

Over 18 years of age with iatrogenic BDI of Strasberg–Bismuth classification D–E 1-4, diagnosed and treated in first 72 h. The exclusion criteria was patients who had BDI of Strasberg–Bismuth classification E 5; associated vascular injury; acute or chronic malnutrition; uncontrolled intra-abdominal or systemic infections; biliary liver cirrhosis of Child–Pugh B or more; hemodynamic instability; and severe comorbidities that contraindicate laparoscopic management such as severe chronic obstructive pulmonary disease, severe heart failure, among others; active cholangitis; associated vascular injury (Stewart–Way III–IV)<sup>10</sup>; previous Roux en Y surgery; BDI and Caroli disease; malignant biliary obstruction pathology (periampullary tumors) and biliary tract injury; and extrinsic compression of the bile duct from any cause and traumatic biliary duct injury (gunshots and stab wounds).

## **Surgical technique**

### **PATIENT PREPARATION**

All the patients were prepared for simplified laparoscopic bile duct reconstruction just as they would be for an open operation. In the emergency room, we perform correction of electrolyte imbalance, fluid resuscitation, and check the hemodynamic status of the patients. In the case of an intra-abdominal or systemic infection, broad-spectrum antibiotic treatment, and percutaneous drainage (if needed) are indicated. Patients and their families were informed of the surgical risks and the possible need for additional trocars or conversion as well as the risk of mortality.

### **EQUIPMENT AND ROOM SET-UP**

From the case notes, under general anesthesia, all the patients were placed in the lithotomy position with both arms tucked along their sides. Pneumatic stockings were placed on the legs, which were spread. The patient was securely strapped to the surgical bed to facilitate maximum tilting and lateral rotation of the operating table. The surgeon positioned himself between the patient’s legs in the French laparoscopy position. The first surgical assistant stood to the surgeon’s right, and the second assistant stood to the left. The scrub nurse stood to the right of the first surgical assistant.

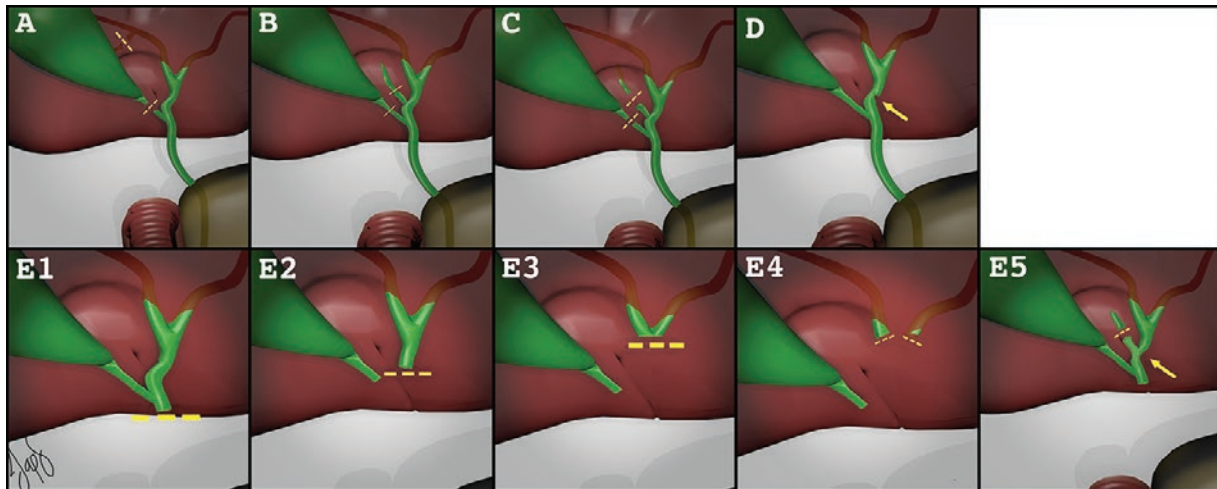


Figure 1. Strasberg–Bismuth classification 3D model.

### **SIMPLIFIED LAPAROSCOPIC BILE DUCT RECONSTRUCTION USING A DOUBLE-OMEGA**

Using an open Hasson umbilical approach, a 12 mm port was introduced for the creation of pneumoperitoneum and maintaining an intra-abdominal pressure of 14 mmHg (Fig. 2). Under direct laparoscopic vision using a 30° lens, additional ports were placed: two 12 mm ports were placed right and left flank and the other in the left para-medial zone, and extra optional 5 mm ports as needed were placed in the right upper quadrant or epigastrium. An ultrasonic laparoscopic (LIGASURE BLUNT TIP) was used for adhesiolysis, and bile collection drainage was performed. As part of the surgical procedure, the porta hepatis and the inferior surface of the liver were exposed, and dissection of the main common hepatic duct and the common bile duct (CBD) was performed (Fig. 3). When the biliary tree anatomy was in doubt, intraoperative cholangiography was performed. Laparoscopic dissection of the hepatic duct was performed until total duct visibility was attained. A 5 mm incision was then made in the anterior border of the common hepatic bile duct to augment its anastomotic diameter prior debridement of devitalized tissue to ensure that the bile duct was adequately perfused (Fig. 4). Debridement of the proximal CBD is performed until vitality is checked, and the closure of the distal portion of CBD is performed as needed, because it is normally clipped or absent.

Longitudinal division of the greater omentum was performed to allow intestinal elevation for anastomosis. The antecolic anterior technique with incision of the

omentum is only performed in obese (body mass index > 30) patients to avoid the tension of the anastomosis, so this approach is optional. The Treitz ligament was identified, and a 60-70 cm omega loop was made and ascended in an anterior colic technique up to the hepatic duct. A lateral–terminal hepaticojejunostomy anastomosis was made using a Hepp–Couinaud approach without tension using simple interrupted sutures with 4-0 polydioxanone (PDS, Ethicon, Inc., Cincinnati, OH) (Fig. 5). Tissue approximation was carried out using a posterior initial suture and continuing anteriorly applying only the necessary number of sutures to prevent leaks, ischemia, and stenosis.

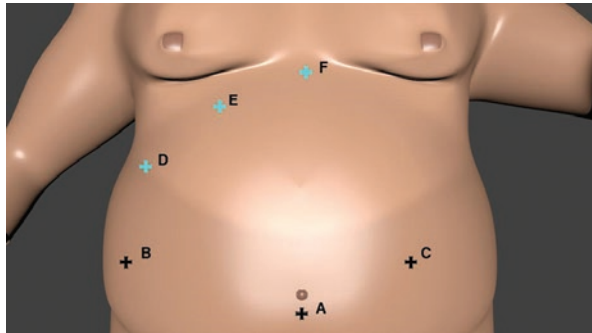
At 100-150 cm distal from the anastomosis, a second omega loop was made with the intestinal tube arising from the duodenum (bile loop), and a lateral–lateral jejunojunction with a EndoGIA, brown reload, 60 mm long (Medtronic, Minneapolis, USA); and the anterior defect with a running suture with 3-0 PDS (Ethicon, Inc., Cincinnati, OH) (Fig. 6).

The posterior wall was sutured using invaginating continuous stitches with 3-0 PDS (Ethicon, Inc., Cincinnati, OH). The mesenteric defect was closed using simple continuous sutures with 3-0 PDS (Ethicon, Inc., Cincinnati, OH). The portion of the small intestine left between the two anastomoses was then separated using a 60 mm endoscopic mechanical linear stapler leaving a Roux-en-Y configuration (Fig. 7). A subhepatic intraperitoneal passive drain was left in place for leak detection.

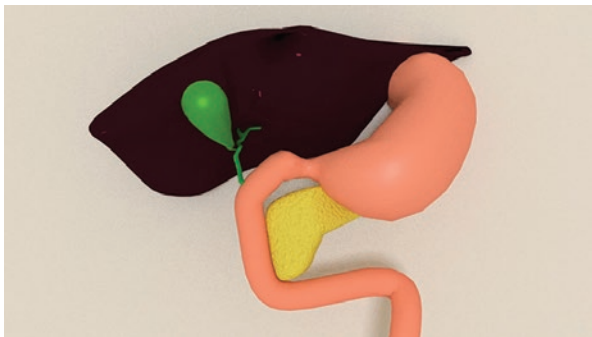
### **Results**

During the period from January 2013 to January 2019, 5430 patients (65% women, and 35% men)





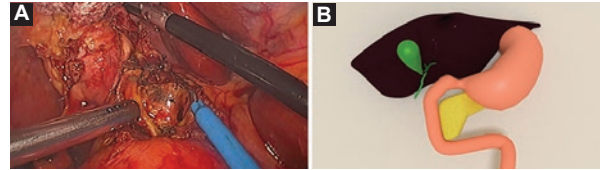
**Figure 2.** Surgical port sites. **A:** to apply pneumoperitoneum using an open Hasson umbilical approach. **B-C:** 12 mm ports; **D-F:** optional ports where a 5 mm port could be used.



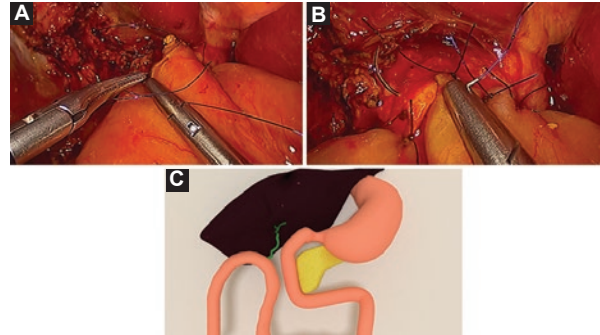
**Figure 3.** Bilioenteric normal anatomy.

median age 45 years (range 34-91) underwent to laparoscopic cholecystectomy, 98% of patients (5339/5430) complete laparoscopic surgery, and 1.6% of patients (91/5430) need convert to open surgery. Twenty patients had a BDI (0.3%), 8 (40%) patient with intraoperative diagnosis during the cholecystectomy, and 12 (60%) patients with diagnosis before 72 h, no diagnoses were presented after this time. In the patient who was diagnosed before 72 h an ERCP was performed and confirmed the injury. All patients presented type E injuries according the Strasberg–Bismuth classification, the predominant BDI was Strasberg E1 (65%) and all underwent simplified laparoscopic bile duct reconstruction at our referral center. Demographic characteristics of the patients are presented in table 2.

The average surgery time was 146.5 min (115-178 min), the average intraoperative bleeding was 15-50 cc. The overall complication rate was 10% (2/20), one (5%) patient required reintervention because of ischemia and necrosis of the greater omentum segment and converted to open surgery, there were no mortalities, and the mean hospital stay was 5 days. One (5%)



**Figure 4.** A-B: biliary tract injury–Strasberg E1.



**Figure 5.** A-C: laparoscopic hepaticojejunostomy.

patient presented bile leak (Type SE4), and 1 (5%) patient required ICU admission after reintervention, and all patients had oral feeding and adequate tolerance at POP day 1. Long-term follow-up showed no bile duct stricture, cholangitis, or recurrent choledocholithiasis up to 5 years after the procedure and with a minimum of 1 year minimum follow-up (Table 3).

## Discussion

BDI is a significant complication after endoscopic procedures and laparoscopic surgeries of the biliary tree and in most of the cases in gallbladder surgery as we reported; they usually require bile duct reconstruction to re-establish lumen integrity and bile flow. These kinds of injuries are mostly associated with laparoscopic cholecystectomies, with an incidence that increases almost threefold when compared with an open approach. They have also been reported with robot-assisted approaches<sup>19,20</sup>.

The laparoscopic repair of CBD injuries should be attempted only by surgeons with adequate training in bilioenteric reconstruction, as in our study. If a BDI is identified intraoperatively, this allows the possibility of immediate repair. If an injury is suspected, intraoperative cholangiography or magnetic resonance cholangiography can be beneficial in determining the extent of the problem and can help guide definitive repair and control of biomass or bile peritonitis, as in our

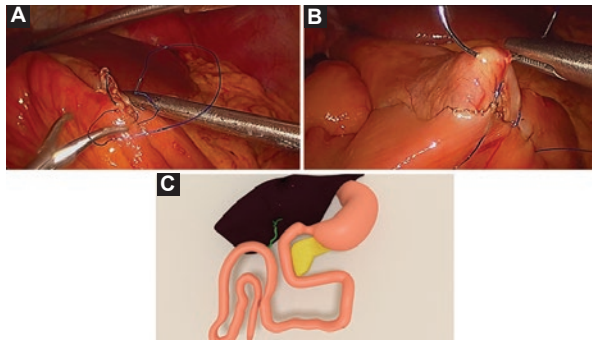


Figure 5. (A-C) Lateral-lateral omega jejunostomy.

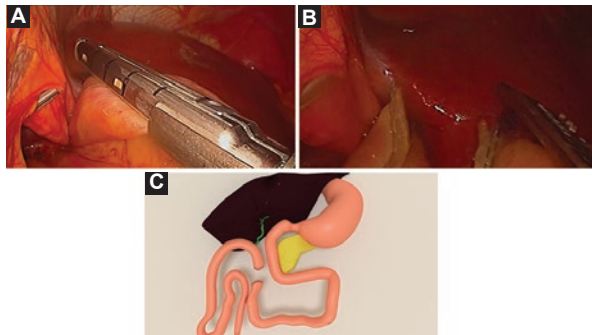


Figure 7. A-C: simplified hepaticojejunostomy in Roux-en-Y.

case series. In cases of limited injury, we advocate leaving a drain in place with a view to treating the injury conservatively with a biliary stent<sup>11,21,22</sup>.

Despite advances in surgical technology, the required technicalities for this type of gastrointestinal reconstructive surgery remain a challenge even to the most expert surgeon. A laparoscopic approach to this type of surgery offers significant advantages over open surgery, among others, better visualization of the biliary tree, less bleeding, and faster recovery time and discharge<sup>9,20</sup>.

Bilioenteric anastomosis, preferably hepaticojejunostomy is the definitive treatment for a severe CBD injury and has shown good long-term results with low complication rates as evidenced in our study, with up to 5 years after the procedure and with a minimum of 1 year minimum follow-up. If the leak is from a very small CBD lesion, it may be amenable to conservative management with a Jackson-Pratt drain to control the leak. Post-operative ERCP with sphincterotomy can be used as an adjunct to ensure low pressure in the biliary system and to promote healing, as reported by Nigro et al. in 2012<sup>21</sup>. In our study, was made in patients had severe CBD injuries of Strasberg E (E1-4)

Table 2. Demographic characteristics of patients referred with bile duct injury

Variable	Sub	n = 20	%
Age (years)		49 (36-62)	
Gender	Female	14	77
	Male	6	23
Indications for cholecystectomy	Symptomatic cholelithiasis	14	70
	Acute cholecystitis	6	30
Initial procedure	OC	1	5
	LC	19	95
ASA classification	ASA 1/2	18	90
	ASA 3/4	2	10
Patients with preoperative ERCP	Yes	12	60
	No	8	40
Moment of diagnosis	During Cholecystectomy	8	40
	Before 72 h	12	60
	After 72 h	0	0
Strasberg classification	E1	13	65
	E2	4	20
	E3	2	10
	E4	1	5
CBD diameter mm(average, mm)		8-12(10)	

ASA: the American society of anesthesiologists physical status classification;  
 CBD: common bile duct; ERCP: endoscopic retrograde cholangiopancreatography;  
 LC: laparoscopic cholecystectomy; mm: millimeter; OC: open cholecystectomy.

class and were not candidates for this conservative management and the injuries with associated vascular injury was ruled out. One patient presented post-operative biliary leak due to the complexity of the injury and because the anastomosis was performed on the biliary plate which resolved spontaneously with expectant management and drainage 3 days after surgery<sup>22</sup>.

In experienced centers, no increased surgery duration with laparoscopy has been reported<sup>23</sup>. This novel technique, which, to our best of knowledge, is the first reported modification of a laparoscopic bile duct reconstruction<sup>24</sup> and it is based on the approach by Ramos-Galvao used in gastric bypass surgery<sup>25,26</sup>.

The criteria for laparoscopic repair that we used in our case series were the same as proposed by Gupta et al.<sup>11</sup>. If the injury is fresh ( $\leq 72$  h), we prefer early repair with laparoscopic simplified bilioenteric anastomosis. Laparoscopic repair is feasible if inflammation in the porta hepatis is not too extensive and there is a healthy duct with good arterial blood supply to perform an end-to-side bilioenteric

**Table 3. Surgical variables and outcomes, 1-5 years follow-up**

Variable	n = 20
Average operation time	146.5 min (115-178 min)
Average intraoperative bleeding	15-50 cc
Mean hospital stay after biliary reconstruction	4.5 days (3-7)
Recurrent choledocholithiasis	0
Post-procedure obstructive biliary syndrome	0
Anastomotic stricture	0
Anastomotic leaks	1
Converted to open surgery	0
Oral feeding and adequate tolerance at PO	1 days
ICU admission	1
Cholangitis PO	0
Mortality	0

PO: post-operative.

anastomosis. The rate of conversion to open surgery is up to 15% in the literature, but in our study no conversions were reported. We prefer an open approach if the CBD is < 3 mm, if the anatomy or injury complexity precludes a straightforward repair as in CBD injury of Strasberg E 4-5, or if port positioning is awkward for laparoscopic repair. We recommend always leaving a surgical drain, and in situations where the operating surgeon cannot perform an adequate repair, he must cannulate the CBD and control sepsis to stabilize the patient for transfer to a tertiary care center for delayed repair. Surgical reconstruction must be performed by specialized hepatobiliary surgeons in a tertiary referral center, as in our study. Stewart and Way<sup>27</sup> reported that only 13% of repairs performed by general surgeons were successful and Perera et al.<sup>28</sup> compared the outcomes of 45 patients treated by non-specialized surgeons to 112 patients treated by hepatobiliary specialists, showing significantly better long-term outcomes and less overall morbidity in patients treated by hepatobiliary specialists. End-to-end bile duct anastomosis is technically simple and has lower rates of post-operative complications compared with conventional hepaticojejunostomy, but de Reuver et al.<sup>29</sup> reported that in a series of 54 patients who underwent end-to-end repair, mostly by general surgeons, 66% of

patients underwent subsequent endoscopic stenting and 32% underwent a hepaticojejunostomy. However, our case series using the simplified laparoscopic hepaticojejunostomy presented minimal post-operative complications<sup>22,27-29</sup>.

Cancer and blood diseases institute (CBDI) repaired with a minimal delay has a much more favorable outcome. Moreover, performing timely repair should be prioritized over delay for laparoscopic repair, as shown by Gazzaniga et al. in 2001<sup>22</sup>, because this may prevent the clinical deterioration of the patient and also leads to a shorter hospitalization period and lower costs. The rationale of delayed surgical repair is that it allows adequate sepsis control, restoration of vascular damage, and optimization of the clinical condition of the patient, and may allow bile duct ischemia to reach its final state, ensuring that the anastomosis is made on an adequate level to serve as definitive repair. In our study, the early and delayed reconstructions have the same outcomes as in the publications by Barauskas et al.<sup>30</sup>, Booi et al.<sup>31</sup>, Russell Kirks et al.<sup>32</sup>, and Felekouras et al.<sup>33</sup>, which all showed similar short- and long-term results for early and delayed repair. Our group believes that an individualized approach, taking into account the type of injury and the patient's clinical condition, is the best option<sup>28,29</sup>. Similar findings were presented by Russell et al. in 2016 when they published a study of 61 patients who required biliary reconstruction because of CBDI; the study showed that early repair when compared with delayed repair had no clinically relevant differences, because the timing did not change the length of hospital stay, readmission rate, or mortality risk<sup>32</sup>.

This approach offers a safe and effective alternative to laparoscopic bile duct reconstruction, with fewer complications and surgical complexity. Our results differ from those reported in the literature using the traditional hepaticojejunostomy approach, which was associated with a bile leak incidence of 17% and a surgery time of 240 min<sup>9,14</sup>. Bile leaks are the most common and significant complications after CBD reconstruction. None of our patients had leaks or stenosis after the surgery<sup>18,34,35</sup>.

Several factors have been reported to be associated with worse outcomes. The risk factors for anastomotic stricture are vascular injury, injury level, sepsis or peritonitis, and post-operative bile leakage. This can lead to secondary biliary cirrhosis, portal hypertension, end-stage liver disease, and death. Patients who have complex vasculobiliary injuries or

high intrahepatic BDI may require a partial liver resection and cannot be candidates for simplified laparoscopic hepaticojejunostomy. In our study, none of the patients developed post-operative anastomotic stricture, because all our patients had distal CBD injuries (E1-3) with the absence of vascular injury and were taken to reconstruction without sepsis. No incisional hernia was reported with the simplified laparoscopic hepaticojejunostomy in our case series, and in most of the studies in the literature, the rate of incisional hernias is not reported. No mortality was seen in our study, but the long-term mortality after BDI is considerable; BDI-related mortality varied between 1.8% and 4.6%<sup>11,21,29</sup>. The main limitation of this study is that it is a retrospective observational study without randomization and with no control group. Therefore, it is subject to selection bias. In addition, as the frequency of complications is low, the N size and the follow-up of our study may be too small and too short to find the incidence of complications for this technique.

## Conclusions

This simplified laparoscopic approach to bile duct reconstruction with Roux-en-Y anastomosis appears to be an effective and safe alternative to reconstructive open bile duct surgery, offering patients a faster surgery time with few complications. Although our series shows low complication rates with this modified technique, additional research is warranted to assess long-term results in a larger series.

## Conflicts of interest

The authors declare have no they conflicts of interest.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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