Randomized clinical trial

Fluorescence or X-ray cholangiography in elective laparoscopic cholecystectomy: a randomized clinical trial

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Background: **Safe laparoscopic cholecystectomy may necessitate biliary imaging, and non-invasive fluorescence cholangiography may have advantages over contrast X-ray cholangiography. This trial compared fluorescence and X-ray cholangiography for visualization of the critical junction between the cystic, common hepatic and common bile ducts.**

Methods: **This non-inferiority blinded RCT included patients who had either intraoperative fluorescence cholangiography using 0·05 mg/kg indocyanine green or X-ray cholangiography during elective laparoscopic cholecystectomy.**

Results: **Between March 2015 and August 2018, a total of 120 patients were randomized (60 in each group). There were no drop-outs and 30-day follow-up data were available for all patients. In intention-to-treat analysis, there was no difference between the fluorescence and X-ray cholangiography groups in ability to visualize the critical junction (49 of 60 *versus* 51 of 60 respectively; $P = 0·230$). Fluorescence cholangiography was faster by a few minutes: median 2·0 (range 0·5–5·0) *versus* 4·8 (1·3–17·6) min ($P < 0·001$).**

Conclusion: **Fluorescence cholangiography was confirmed to be non-inferior to X-ray cholangiography in visualizing the critical junction during laparoscopic cholecystectomy. Registration number: NCT02344654 (http://www.clinicaltrials.gov).**

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Introduction

After hernia surgery, laparoscopic cholecystectomy is one of the most common surgical procedures in Europe[1]. Bile duct injury is caused mostly by misinterpretation of the anatomy[2,3]. Since the early era of laparoscopic cholecystectomy more than 30 years ago[4], several techniques have been implemented to avoid bile duct injury. These initiatives include a systematic and careful dissection (critical view of safety[5,6]) guided by intraoperative imaging (either routinely or as deemed necessary) of the anatomical junction between the cystic duct, common hepatic duct and common bile duct (critical junction). X-ray cholangiography is the standard for visualization of the critical junction. A novel non-invasive imaging modality may, however, replace use of X-rays for cholangiography when indicated. Ishizawa and colleagues[7] first described intraoperative fluorescence cholangiography. Several groups[8–12] have found fluorescence cholangiography to be feasible, but it has not been validated against X-ray cholangiography.

Approximately 20 per cent of all laparoscopic cholecystectomies are performed for acute cholecystitis[13]. The perioperative anatomy may be unclear in these patients, and X-ray cholangiography is often not possible owing to an inflamed non-patent cystic duct[14]. X-ray cholangiography does not allow easy switching between X-ray and white light (normal vision). The only RCT[15] so far investigated the efficacy of fluorescence cholangiography compared with white light (normal vision). A drawback of X-ray cholangiography is its time-consuming nature[16]. Therefore, a single-centre non-inferiority RCT was designed to compare fluorescence with X-ray cholangiography. It was hypothesized that fluorescence cholangiography and X-ray cholangiography would have equal ability to visualize the critical junction in patients with complicated gallbladder disease (non-inferiority trial). Detection of choledocholithiasis was not an outcome measure.

Methods

The primary objective was to compare intraoperative fluorescence cholangiography with X-ray cholangiography in terms of ability to visualize the critical junction. Patients were enrolled in the study after providing oral and written informed consent, in accordance with Danish Ethics Committee guidelines and the Helsinki Declaration of 1975. Written consent was obtained at least 1 day before surgery by the study surgeon, and the patients were assigned a sealed randomization envelope. The study was registered with ClinicalTrials.gov (NCT02344654), the Danish National Ethics Committee (H-15000817) and the Data Protection Agency (AHH-2015-005).

Study design

The study design has been described in detail elsewhere[17]. This non-inferiority single-blinded RCT included patients who had either intraoperative fluorescence cholangiography or X-ray cholangiography during elective laparoscopic cholecystectomy for complicated gallstone disease, at a single university hospital centre with unrestricted referral of patients between March 2015 and August 2018. Non-eligible patients were registered in a logbook for drop-out analysis. The two study surgeons were experienced laparoscopic upper gastrointestinal surgeons; each had undertaken more than 400 cholecystectomies, over 100 X-ray cholangiography procedures and more than 20 fluorescence cholangiography procedures. The learning curve for fluorescence cholangiography is steep[18] and probably less than five procedures.

Complicated gallstone disease was defined by the presence of gallbladder stones, verified by ultrasound imaging and/or magnetic resonance cholangiopancreatography (MRCP), and a medical history of at least one of the following conditions: non-operated acute cholecystitis; mild or severe gallstone pancreatitis; cholangitis or common bile duct stones, verified by ultrasonography, MRCP, CT, endoscopic retrograde cholangiopancreatography (ERCP) or serum bilirubin level exceeding 4 g/dl (68 μmol/l). Any common bile duct stones identified before operation were removed by preoperative ERCP to ensure that no included patient had such stones. Patients with acute cholecystitis were not included if the operation was performed during the acute phase (within 5 days of symptom onset). However, they were included if operated at a later time, typically after 3 months.

Blinding and randomization

Patients were randomized to either intraoperative fluorescence cholangiography or conventional X-ray cholangiography in a 1 : 1 allocation ratio. Randomization was performed by the primary investigator using randomisation.com (block size 4) (http://www.randomisation.com). Enrolment and allocation were performed by study surgeons after the patient had been anaesthetized but before the start of the operation. The patient was blinded to the randomization. The primary investigator monitored surgeon adherence to the protocol. The data were anonymized before analysis.

Intraoperative fluorescence cholangiography

Immediately after induction of anaesthesia, 2·5–7·5 mg (0·05 mg/kg) indocyanine green (ICG) (Verdye; Diagnostic Green, Aschheim-Dornach, Germany) was injected intravenously. ICG binds rapidly to plasma proteins, and is excreted exclusively and entirely by the hepatic parenchymal cells into the bile, starting within a few minutes after injection. A laparoscopic imaging system (S343020; Olympus, Tokyo, Japan) for observation of ICG fluorescence with an easy switchable white light–fluorescence mode was used.

The operative field was routinely inspected in the fluorescence imaging mode before dissection of the triangle of Calot. During dissection, the fluorescence imaging mode was used at the discretion of the surgeon to obtain a critical view of safety, as described in detail elsewhere[17]. The duration of the fluorescence cholangiography procedure was defined as the total time in fluorescence mode.

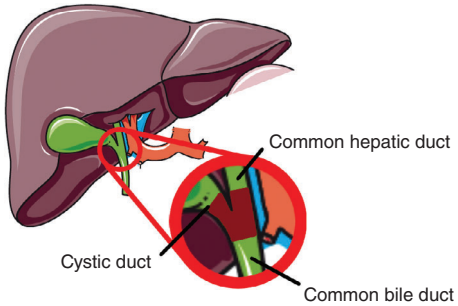
Intraoperative X-ray cholangiography

X-ray cholangiography was performed after dissection of the cystic duct in a standardized manner, by cannulation of the cystic duct with a catheter using either a Kumar or Olsen grasper. Iohexol (Omnipaque™; GE Healthcare, Brøndby, Denmark), diluted 1 : 1 in saline, was injected through a catheter into the cystic duct. A mobile X-ray C-arm system (Ziehm Imaging, Nürnberg, Germany) was used, as described previously[17]. X-ray cholangiography was undertaken by the surgeon and a scrub nurse; no additional staff was needed. For conventional cholangiography, time was measured from application of the Kumar/Olsen grasper until its removal after obtaining a satisfactory cholangiogram.

Outcomes

The primary outcome was ability to visualize the critical junction between the cystic duct, common hepatic duct and common bile duct (at least 1 cm distal from the junction for each bile duct) (*Fig. 1*).

Fig. 1 Critical junction



The critical junction comprises the biliary anatomy at least 1 cm distal from the junction between the cystic duct, common hepatic duct and common bile duct. Modified from https://smart.servier.com/smart_image/liver-and-gallbladder/.

Secondary outcomes were ability to visualize the common hepatic duct (from the junction to the bifurcation), common bile duct (from the junction to the retroduodenal part) and the cystic duct (from the junction to the infundibulum of the gallbladder). The fluorescence or X-ray cholangiography was considered successful when the critical junction was visualized. The duration of cholangiography and surgeon-reported ease of performing the procedure were also registered. The study surgeon registered ease of the procedure immediately after the operation using an arbitrarily chosen numerical rating scale: 1, very easy; 2, easy; 3, acceptable; 4, difficult; and 5, very difficult.

Based on empirical observations, and despite previously published experience[12], a pilot study with 25 patients in each group was undertaken before the present RCT to determine the optimal assessment method for the main study outcome, as recommended by Fairhurst and colleagues[19]. In this pilot study, evaluation of cholangiograms (X-ray images and fluorescence cholangiography videos) was carried out after operation in a blinded manner by a project surgeon and a radiologist. Owing to slight to substantial interobserver and intraobserver variation (Cohen's κ coefficients 0·12–0·69) for fluorescence and X-ray cholangiography, it was decided that the study surgeons would evaluate the cholangiograms during surgery in the RCT.

Intraoperative and 30-day follow-up for complications was registered in the regional electronic medical journal database.

Statistical analysis

The sample size was estimated using simulations for a non-inferiority design. Thus, 10 000 data sets were simulated, assuming a success rate of 80 per cent for X-ray cholangiography and 90 per cent for fluorescence cholangiography[17]. A total of 60 patients in each study arm would yield a power of 90 per cent in a one-sided test. The study was discontinued after the successful inclusion of 120 patients.

Student's *t* test or Mann–Whitney *U* test was used to compare continuous variables between the surgical groups, depending on the normality of distribution assessed by the Kolmogorov–Smirnov test. To compare dichotomous variables, χ^2 test or Fisher's test was used, as appropriate. Cohen's κ statistics were used for analysis of inter-rater and intrarater agreement. Data were analysed according to intention-to-treat principles. $P < 0{\cdot}050$ was considered significant. Statistical analysis was done using SPSS® version 25 (IBM, Armonk, New York, USA).

Results

Of 1889 patients assessed for eligibility, 120 were included (*Fig. 2*). Main reasons for exclusion were logistical (non-project surgeon available) and simple gallbladder stone disease (not pancreatitis, history of acute cholecystitis, etc.) (*Table 1*). There were no significant differences between included and excluded patients in terms of sex, BMI and ASA fitness grade. A drop-out analysis showed that included patients were significantly younger than those who were excluded (mean(s.d.) 53·5(13·7) *versus* 57·4(18·2) years; 95 per cent c.i. for difference 0·6 to 7·2 years; $P = 0{\cdot}021$).

Sixty patients were allocated to each surgical group, received the allocated intervention and were included in the analyses. There were no significant differences between groups in biometrics and history of complicated gallstone disease, except for sex ($P = 0{\cdot}013$) (*Table 1*).

All patients in the fluorescence group received the allocated intervention (ICG). In the X-ray group, nine patients did not undergo X-ray cholangiography owing to a non-patent cystic duct (0 *versus* 9 patients; $P = 0{\cdot}003$). Ability to visualize the bile duct anatomy is summarized in *Table 2*. There was no significant intergroup difference regarding visualization of the critical junction ($P = 0{\cdot}231$). The right and left hepatic ducts were visualized significantly less often in the fluorescence group compared with the X-ray group (16 of 60 *versus* 51 of 60; $P < 0{\cdot}001$). There were no differences between groups regarding visualization of the common hepatic duct, cystic duct or the common bile duct.

The median duration of cholangiography was 2·0 (range 0·5–5·0) and 4·8 (1·3–17·6) min for fluorescence and X-ray procedures respectively ($P < 0{\cdot}001$). There was no

Fig. 2 CONSORT diagram for the trial

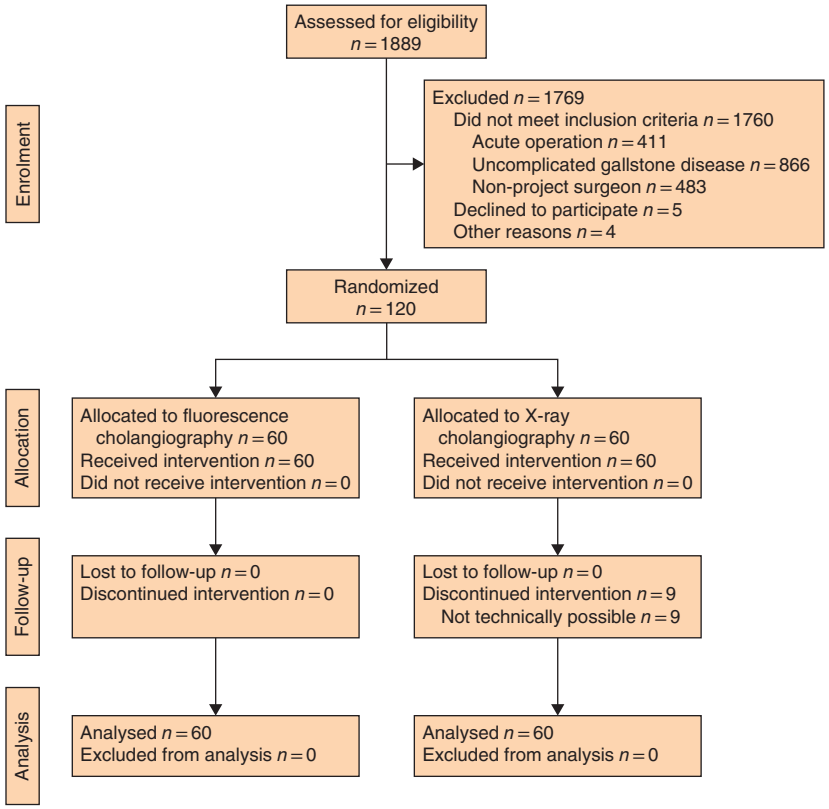


Table 1 Comparison of study group biometrics and history of complicated gallstone disease

	Fluorescence cholangiography (*n* = 60)	X-ray cholangiography (*n* = 60)
Age (years)*	52 (48, 58)	58 (53, 62)
Sex ratio (M : F)	28 : 32	15 : 45
BMI (kg/m^2)†	27·4 (26·2, 28·7)	29 (27·5, 30·5)
ASA fitness grade		
I	16 (27)	15 (25)
II	42 (70)	41 (68)
III	2 (3)	4 (7)
History of gallstone disease		
Gallstone pancreatitis	9 (15)	9 (15)
Acute cholecystitis	37 (62)	26 (43)
Cholangitis	0 (0)	2 (3)
CBD stones	14 (23)	23 (38)

Values in parentheses are percentages unless indicated otherwise; values are *median (95 per cent c.i.) and †mean (95 per cent c.i.). CBD, common bile duct.

Table 2 Bile duct anatomy visualized by fluorescence and X-ray cholangiography

	Fluorescence cholangiography (*n* = 60)	X-ray cholangiography (*n* = 60)	*P*†
No. of failed investigations	0 (0)	9 (15)	0·003
Bile duct anatomy visualized			
Critical junction*	49 (82)	51 (85)	0·231
Right hepatic duct	16 (27)	51 (85)	< 0·001
Left hepatic duct	16 (27)	51 (85)	< 0·001
Common hepatic duct	52 (87)	51 (85)	0·865
Cystic duct	54 (90)	51 (85)	0·925
Common bile duct	56 (93)	51 (85)	0·522

Values in parentheses are percentages. *Anatomical junction between cystic duct, common hepatic duct and common bile duct. †χ^2 or Fisher's exact test.

difference in total median operating time (cholecystectomy and cholangiography) between the two surgical groups: median 45 (25–138) and 54 (33–163) min respectively ($P = 0·092$).

During surgery in one patient in the fluorescence cholangiography group, a lesion in an accessory bile duct was

identified following accidental division of the structure and after the cholangiography procedure had been completed. The patient recovered fully after two ERCP procedures and percutaneous transhepatic cholangiographic drainage. No accessory bile ducts were identified, and there were no intraoperative complications, in the X-ray cholangiography group. The operation was converted to open cholecystectomy in one patient in the latter group, owing to poor anatomical overview after allocation and intervention (X-ray cholangiography was not performed as the cystic duct was non-patent). The postoperative course was uneventful.

One patient in the X-ray cholangiography group developed mild pancreatitis on postoperative day 4 and recovered spontaneously after 2 days. There were no postoperative complications in the fluorescence cholangiography group.

Stones were identified in the common bile duct in two patients in the X-ray cholangiography group. Both underwent postoperative endoscopic ultrasonography and ERCP, which confirmed the presence of a bile duct stone in one of the two patients. The stone was removed during ERCP. No patient in the fluorescence cholangiography group had bile duct stones detected.

There was a significant intergroup difference in surgeon-reported ease of cholangiography performance: mean(s.d.) 1·90(0·89) and 2·36(1·03) for fluorescence and X-ray cholangiography respectively (95 per cent c.i. for difference −0·82 to −0·11; $P = 0·011$).

Discussion

In this study, the ability of fluorescence cholangiography to visualize the critical junction between the cystic duct, common hepatic duct and common bile duct during elective laparoscopic cholecystectomy was non-inferior to that of X-ray cholangiography. Fluorescence cholangiography has a steep learning curve[18,20], but is non-invasive, allows easy switching between white light mode and fluorescence mode, and does not require a patent cystic duct. It is potentially a useful teaching tool for young surgeons[18,21] and might lead to a reduction in the incidence of bile duct injury. In patients with suspected common bile duct stones, X-ray cholangiography will continue to be the standard as fluorescence cholangiography is considered inaccurate.

Although the two imaging modalities had equal ability to visualize the anatomical structures required for the critical view of safety, visualization of the left and right hepatic ducts was possible in only one-quarter of patients in the fluorescence cholangiography group compared with most patients in the X-ray group. The RCT was not powered to visualize deep bile duct structures, but the limited visualization rate in the fluorescence cholangiography group was probably due to low penetration of the emitted fluorescent light through the overlying liver and fatty tissue[22]. A high BMI may hinder visualization of the bile ducts, although findings have not been uniform[22,23]. The inability of fluorescence cholangiography to visualize the left and right hepatic ducts was not at the expense of its ability to obtain the critical view of safety.

One in six patients in the X-ray cholangiography group had a non-patent cystic duct, so a cholangiogram could not be obtained. This would be even worse in the setting of acute cholecystitis[13]. There were no failures in the fluorescence cholangiography group, but the entire junction of the cystic duct, common hepatic duct and common bile duct was not visualized in some patients. Fluorescence cholangiography failed to visualize either the common bile duct or cystic duct in only two of these. In the X-ray cholangiography group, the critical junction was not identified in 15 per cent owing to complete failure of the method. This study confirmed the finding[12] that fluorescence cholangiography is faster than X-ray cholangiography.

The majority of previous investigations were small, prospective non-controlled and controlled studies[8,10,11,23–31], and these indicated that the ability of fluorescence cholangiography to visualize the critical junction was equal to that of X-ray cholangiography. A recent RCT[15] including 639 patients compared fluorescence cholangiography with white light (normal vision) and not with X-ray cholangiography as the standard. Improved visualization of the bile ducts by fluorescence cholangiography was reported (critical junction: 69 *versus* 45 per cent; $P < 0·001$)[15]. Although the success rate for visualization of the critical junction was lower than that in the present study, the success rate for visualizing the right hepatic duct was similar to the present findings (22 and 27 per cent respectively). However, the study offered no assurance regarding whether the bile ducts detected in white light were the correct ducts. Furthermore, 37 surgeons from eight different centres performed the interventions, which hindered standardization between surgical centres.

The fluorescence technique benefits from the bile-binding abilities of ICG illuminating the bile ducts when inspected in near-infrared light, and offers easy switching between white light and fluorescence mode[12]. Fluorescence cholangiography has the advantages of being non-invasive (requires no dissection or a patent cystic duct), quick to perform and requiring no X-ray radiation. It potentially offers visualization of the bile ducts in patients with acute cholecystitis despite a non-patent cystic

duct, although the capabilities of fluorescence cholangiography during acute cholecystitis have been investigated only scarcely. The disadvantage of fluorescence cholangiography is limited penetration owing to overlying fatty tissue (3·6 mm)[22]. However, once penetrated, the ability to visualize the ICG–bilirubin complex in the bile ducts is principally unlimited.

There are limitations to this RCT. Cholangiography (fluorescence and X-ray) was assessed by only one surgeon. Therefore, the study may be flawed by an inherent risk of observer bias. Overall, drop-out analysis showed no difference between excluded and included patients, except that the included patients were younger. Thus, a positive bias cannot be ruled out[32], but the difference in age was only 4 years. The present study was skewed towards men in the fluorescence cholangiography group, with a risk of negative bias in this group as men have more visceral adipose tissue than women[33]. Nevertheless, as hypothesized, there was no difference between fluorescence and X-ray cholangiography in ability to visualize the critical junction. Recent studies[30,34–36] have indicated that the optimal timing of ICG injection should be at least 3 h before fluorescence imaging to increase the visualization rate. However, data on the optimal preoperative injection time were not available when recruitment to the present trial started, and the study design only allowed injection less than 1 h before fluorescence imaging, which may be a limitation. Fluorescence cholangiography was unsuccessful in some patients; this may be due to visceral adiposity, which was not registered. It cannot be ruled out that prolonging the time from injection would have improved the success rate.

Validation studies of fluorescence cholangiography in elective laparoscopic cholangiography are a prerequisite for its use in patients undergoing laparoscopic cholecystectomy for acute cholecystitis. There is currently insufficient evidence to support the routine use of intraoperative fluorescence cholangiography, but it may be beneficial in selected patients, such as those with acute cholecystitis. Patients with acute cholecystitis rarely have a patent cystic duct, and X-ray cholangiography may be difficult. Future studies should, therefore, assess the ability of fluorescence cholangiography to visualize the bile ducts in patients undergoing laparoscopic cholecystectomy for acute cholecystitis.

Disclosure

The authors declare no conflict of interest.

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