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Application of Fluorescence Image Guided Cholangiography for Assessment of Biliary Anatomy in Patients with Acute Cholecystitis

Summary of the Doctoral Thesis for obtaining the scientific degree "Doctor of Science (*PhD*)"

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Sector – Clinical Medicine

Sub-Sector – Surgery

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Abbreviations used in the work

AC	Acute cholecystitis
BMI	Body mass index
DC	*Ductus cysticus (Lat.) – cystic duct*
DHC	*Ductus hepaticus communis (Lat.) – common hepatic duct*
CRP	C-reactive protein
CVS	Critical View of Safety
EHBD	Extrahepatic bile ducts
EURO-FIGS	European Fluorescence Image-Guided Surgery
FC	Fluorescence cholangiography
FE	Fluorescence effect
IBDI	Iatrogenic bile duct injury
ICG	Indocyanine green
IOC	Intraoperative cholangiography
LC	Laparoscopic cholecystectomy
RAKUS	Riga East University Hospital
TG2018	Tokyo Guidelines 2018
USA	United States of America
WBC	White blood cell
WL	White light

Introduction

Aim of the Thesis

The aim of this study is to evaluate the significance of *the fluorescence effect* in the visualisation of extrahepatic bile duct anatomy in patients with acute cholecystitis.

Tasks of the Thesis

The following tasks have been set to achieve the aim of the Thesis:

1. to include in the study patients with gallstone disease (acute mild / moderate cholecystitis) who have undergone fluorescence-assisted laparoscopic cholecystectomy, dividing the patients into groups according to the severity of acute cholecystitis;
2. to compare fluorescence-assisted intraoperative visualisation of extrahepatic bile ducts between the study groups: before and after following the CVS principle, using an adapted EURO-FIGS Likert scale, *Helpful* scores*, Disturbed* scores (Annex No 3);
3. to compare and evaluate the feasibility of FC-assisted CVS between study groups;
4. to evaluate between study groups: length of hospitalisation, duration of surgery, complications during surgery, as well as postoperative complications and course of treatment.

Hypotheses of the Thesis

1. The use of the fluorescence effect during laparoscopic cholecystectomy in patients with acute cholecystitis improves the visualisation of extrahepatic bile ducts before and after tissue dissection, thereby reducing intraoperative and postoperative complications.
2. The use of fluorescence cholangiography in patients with acute cholecystitis increases patient safety and surgeon confidence by visualising and redistributing critical structures that are analysed using adapted visualisation scores.

Novelty of the Thesis

For the first time internationally, a study has been conducted using fluorescence cholangiography during laparoscopic cholecystectomy in patients with acute cholecystitis to visualise the anatomy of the extrahepatic bile ducts, thereby improving patient safety during surgery.

1 Materials and methods

A prospective study was conducted from October 2021 to April 2024 at the General and Emergency Surgery Clinic of the Riga East University Hospital (SIA "Rīgas Austrumu klīniskā universitātes slimnīca" (Ltd) (RAKUS)), Latvia. RAKUS is one of the largest tertiary healthcare hospitals in the country, offering both planned and emergency healthcare, with nearly 10 000 surgical procedures performed at the clinic each year. The study protocol was developed in accordance with the principles set out in the Declaration of Helsinki 2008 and received a positive opinion from the Medical and Biomedical Research Ethics Committee of the Riga East University Hospital Support Foundation (Annex No 8) (6 August 2020, No 9-A/20). All patients provided written informed consent to participate in the study.

1.1 Patient selection

Inclusion criteria:

1. Age between 18 and 90 years;
2. Diagnosed with mild to moderate acute cholecystitis (Tokyo Guidelines 2018, diagnostic criteria);
3. Patients are candidates for laparoscopic cholecystectomy.

Exclusion criteria:

1. Allergy to indocyanine green or iodine;
2. Actively treated oncological disease or suspected malignant tumor of the gallbladder;
3. Severe coagulopathy or thrombocytopenia;
4. Patients with contraindications to laparoscopic surgery;
5. Patients for whom waiting 12 hours until LC is contraindicated;
6. Patients who refused to provide and did not sign informed consent.

1.2 Surgical procedure

Patients included in the study underwent laparoscopic cholecystectomy with fluorescence cholangiography under general anesthesia. Twelve hours (± 1 h) before the surgical procedure, a fixed dose of 12.5 mg of indocyanine green (ICG*)* dye diluted in 10 cm^3 of physiological solution was administered intravenously. The operations were performed by three certified surgeons specialising in hepato-pancreato-biliary (HPB) surgery with experience and good skills in minimally invasive surgery and FE interpretation. A standard four-trocar approach was used during the operations – a 12 mm trocar *in the epigastrium proprium, a* 10 mm *trocar in the umbo*, and two 5 mm trocars on the right side of the abdomen (see Figure 1.1).

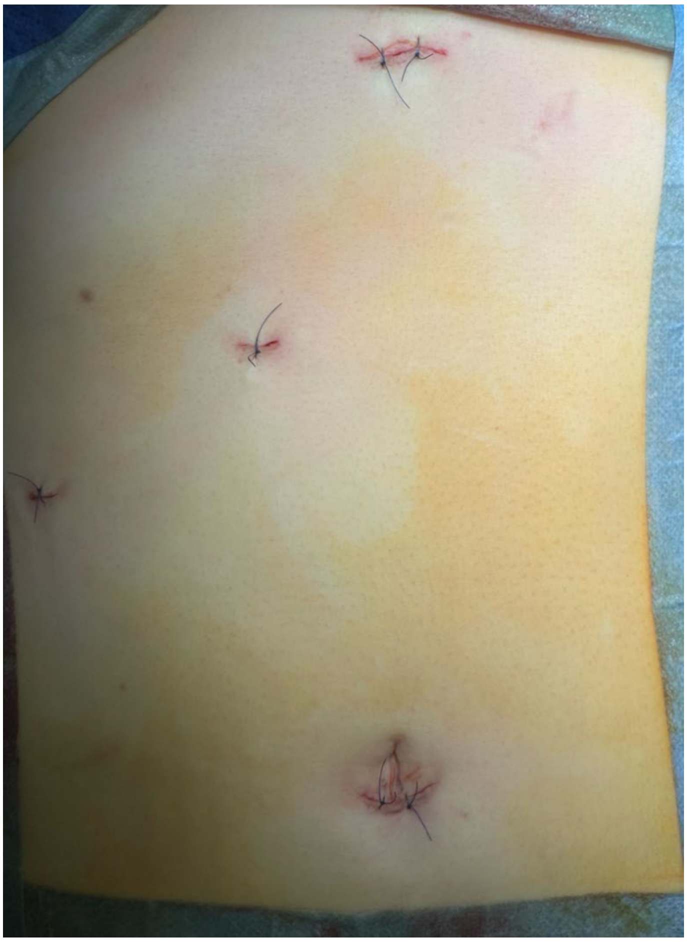

Figure 1.1 **Trocar insertion sites during LC,** author's image

After creating pneumoperitoneum, retraction of the gallbladder was performed cranially. Intraoperatively, the quality of FC visualisation was assessed both before and after dissection using a total of two fluorescence imaging platforms: *System Green* ICG (*Richard Wolf GmbH,* Germany, Figure 1.2) and *Storz 201337 20 SCB D-Light P Cold Light Fluorescence Imaging Xenon Light Source (Karl Storz,* Germany) infrared fluorescence imaging platforms. Tissue dissection was performed according to 8 sequential steps – CVS principles. Hemoclips were used to divide both the *ductus cysticus* and the *arteria cystica*. The aponeurosis was closed with *Novosyn® 3.0* sutures (*BBraun Surgicals,* Spain), while the skin was closed with *Dafilon® 3.0* sutures (B *Braun Surgical,* Spain). Patients who required conversion from laparoscopic to open surgery due to perivesicular infiltrate underwent left subcostal laparotomy, with *Novosyn® 3.0* sutures used to ligate the *ductus cysticus* and *arteria cystica*.

Figure 1.2 ***System Green* ICG infrared fluorescence imaging platform *Richard Wolf GmbH,***
author's image

The surgical procedures were documented on video using an *ENDOCAM Logic 4K Camera Controller* endoscopic 4K camera, *Richard Wolf GmbH*, Germany (see Figure 1.3).

Figure 1.3 ***ENDOCAM Logic 4K Camera Controller* endoscopic 4K camera, *Richard Wolf GmbH*, Germany**, author's image

The documented video recordings after surgery were re-analysed, carefully evaluating and determining the visualisation scores used in the study, and the CVS steps performed and their number were retrospectively analysed. All patients, regardless of the surgical approach used, received standard care in accordance with local and international guidelines, including antibacterial and analgesic therapy. All patients underwent a comprehensive assessment of complications and symptoms prior to discharge from the hospital.

1.3 Assessment of CVS principles

Laparoscopic cholecystectomies were performed according to *the Critical View of Safety (*CVS*)* principles, following an eight-step protocol (Table 1.1). These steps were performed sequentially.

Table 1.1

Overview of *Critical View of Safety (*CVS*)* steps

CVS step	Description
I	Cranial retraction of the *fundus* of the gallbladder.
II	Lateral retraction of the *infundibulum* of the gallbladder.
III	Dissection of the visceral peritoneum with electrocoagulation laterally or medially from the *infundibulum*, ascending to the *fundus* of the gallbladder.
IV	Dissection of the medial fatty tissue of the gallbladder with electrocoagulation, visualisation and release of the *ductus cysticus*, as well as visualisation of its entrance into the gallbladder.
V	Complete dissection of the fatty tissue, formation of a "critical safety triangle" by separating the *ductus cysticus* and *arteria cystica*.
VI	Dissection of the *infundibulum* of the gallbladder from the fatty tissue and mobilisation in the anterior/posterior part, forming Calot's triangle. Visualisation of the edge of the liver.
VII	Clamping of the *ductus cysticus* (distal/proximal) from the gallbladder with a clip and its division. Clamping of the *arteria cystica* with a clip and its division.
VIII	Dissection of the gallbladder from the hepatic bed.

1.4 Visualisation of the extrahepatic bile ducts

Before bile duct extraction or after performing the first step of CVS, the visualisation quality of extrahepatic bile duct (EHBD) structures was assessed using both white light (WL) mode and fluorescence cholangiography (FC) mode (*System Green*, *Richard Wolf GmbH*, Germany). In FC mode, the imaging platform detected fluorescence at a wavelength of 800–850 nm (light source *LEDgreen, Richard Wolf GmbH,* Germany), and a special pedal or button on the laparoscopic camera could be used to switch between WL and FC coverage modes.

After complete dissection of the perivesicular tissue, *ductus cysticus*, and *arteria cystica*, the EHBD structures were re-evaluated in both WL and FC modes. A five-point *Likert scale* was used to assess the quality of structure visualisation, with 1 denoting poor quality and 5 denoting excellent quality (Annex No 2). Intraoperative assessments also included measurements of usefulness or helpfulness (*Helpful* score*)* and quantitative determination of liver fluorescence background interference (*Disturbed Score)*. To assess the extent to which fluorescence imaging was considered useful (*Helpful* score*)* in each case, a four-point score was used, where 0 means that it is not useful and 3 means that it is very useful (Annex No 3).

The Disturbed Score score was used to assess the extent to which liver background fluorescence interferes with the visualisation of the EHBD structure. This was done using a four-point system, where 0 indicates no disturbance and 4 indicates extreme disturbance, making it almost impossible to correctly visualise the EHBD structures (Annex No 4). The accuracy of the intraoperative and postoperative assessments was confirmed by reviewing the video recordings. This was done by the author of this Doctoral Thesis.

1.5 Data collection and statistical analysis

1.5.1 Perioperative patient visit protocols

Perioperative data were collected and compiled during patient visits (patient examination in the ward at the General and Emergency Surgery Clinic) before and after surgery. The first visit for all patients was conducted on the day before surgery, and the second visit was conducted on the day of the patient's discharge from the hospital, following a specifically developed study protocol for perioperative patient visits (Annex No 1). During the first visit, the patient's demographic data, including gender, age, race, body weight, height, and BMI, were obtained and documented. Blood test laboratory parameters: creatinine, glomerular filtration rate, leukocytes, C-reactive protein, total bilirubin, direct bilirubin, as well as alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT). The preoperative visit protocol, or first visit, included the TG2018 diagnostic criteria for acute cholecystitis, as well as the severity of AC, in order to confirm the diagnosis, determine the severity of AC, and include the patient in the study. The protocol of this visit recorded the diagnostic imaging examinations that confirmed the diagnosis of AC, as well as the findings of radiological examinations: thickness of the gallbladder wall, presence of perivesicular infiltrate, total width of the bile duct, and gallbladder empyema. The protocol documented the patient's complaints during the examination and medical history, as well as arterial pressure and pulse rate on *the a.radialis sinistra* or *dextra* (Annex No 1).

The second visit was conducted on the day of the patient's discharge from the hospital, where the patient's complaints, arterial pressure, and peripheral pulse on the aforementioned upper extremity arteries were recorded. The visit included the results of control analysis tests (identical to the first visit). The patient's subjective condition was assessed on a score from 0 to 100. The results related to the operation were also recorded: the duration of the operation, the dose of ICG administered, the CVS steps performed, and the visualisation score indicators.

The postoperative protocol documented visualisation scores: Likert scale, *Helpful* score, and *Disturbed* score. The Likert scale was used to determine the visualisation of specific biliary structures and their junctions and the quality of visualisation before and after tissue dissection, including anatomical structures and their junctions: *ductus cysticus, ductus choledochus, ductus hepaticus communis,* confluence of *ductus cysticus* and *ductus hepaticus communis*, and *ductus cysticus* inflow into the gallbladder. The *Helpful* score determined the extent to which FE is useful in visualising extrahepatic bile ducts: 0 – not useful, 1 – moderately useful (increased reliability of intraoperative visualisation of structures), 2 – useful (facilitates safer tissue dissection), 3 – very useful (can prevent damage to bile ducts). The final visualisation score, which was documented during the second visit, was the *Disturbed* score, determining the extent to which the background fluorescence of the liver interferes with the visualisation of extrahepatic bile ducts: 0 – no disturbance, 1 – slight disturbance (all bile duct structures are visible when using FE), 2 – moderately impaired visualisation (the junction of *the DC* and *DHC* is visible before tissue dissection), 3 – impaired visualisation (the junction of the *DC* and *DHC* is visible only after tissue dissection), 4 – severely impaired visualisation (extrahepatic bile ducts cannot be distinguished). The length of hospitalisation, the fact of drain insertion, the day of drain evacuation after surgery, as well as complications according to the Clavien-Dindo classification (Annex No 5) were also documented.

1.5.2 Statistical analysis

The normality of the distribution of continuous numerical data was tested using the Shapiro-Wilk test and Q-Q plots. Given that the data were distributed asymmetrically due to the different numbers of patients in the study groups, the Gaussian distribution was not used in order to reduce the risk of misinterpretation. Non-parametric tests were used in the study. The Chi-square (χ^2) test or Fisher's exact test was used to test for associations between categorical independent variables. The McNemar test was used for categorical dependent variables in 2 × 2 contingency tables. For R × C cross tables, the McNemar-Bowker symmetry test and the Stuart-Maxwell marginal homogeneity test were used, followed by a *post-hoc* McNemar test with Bonferroni correction for type I error control. A two-tailed p-value of < 0.05 was considered statistically significant. Data analysis was performed using *MS Excel 365* for *Windows 11* and *IBM SPSS* 29.0.0 (*IBM Corp,* USA) for *Windows 11*.

2 Results

The study included a total of 108 patients, of whom 75 (69 %) had mild acute cholecystitis and 33 (31 %) had moderate acute cholecystitis. The average age of patients with mild acute cholecystitis was 54 years, while the average age of patients with moderate acute cholecystitis was 64 years. The mild AC group consisted mainly of women (n = 52) and fewer men (n = 23). The moderate AC group consisted of slightly more women (n = 17) than men (n = 16). At the time of admission, the levels of inflammatory markers such as leukocytes (WBC) and C-reactive protein (CRP) were elevated in both groups, with higher values observed in patients with moderate acute cholecystitis (Table 2.1).

Table 2.1

Patient indicators

Indicators[†]	Mild acute cholecystitis (n = 75)	Moderate acute cholecystitis (n = 33)	p-value
Patient gender			
Male	23 (59 %)	16 (41 %)	$p = 0.076$**
Female	52 (75 %)	17 (25 %)	
Age (years)	54 (24–85)	64 (24–86)	$p = 0.014$**
BMI (kg/m^2)	27.9 (7.1)	28.0 (6.6)	$p = 0.038$*
WBC count at admission (10^9 cells/l)	12.0 (4.0)	14.0 (5.5)	$p < 0.001$*
CRP at admission (mg/l)	16.5 (38.5)	42.0 (67.5)	$p < 0.001$*

[†] Median and interquartile range (IQR) are indicated. Median and range are indicated for age. Number of patients and % are indicated for gender.

* Mann-Whitney U test; ** Chi-square (χ^2) test.

2.1 Intraoperative findings

Patients with mild acute cholecystitis had significantly shorter operating times, partly because perivesicular infiltrates and/or gallbladder empyema were less frequently found during surgery. This also resulted in a significantly higher positive CVS principle step rate in patients with mild acute cholecystitis and, consequently, a lower conversion rate to open surgery (Table 2.2).

Table 2.2

Summary of intraoperative findings

Indicators†	**Mild acute cholecystitis (n = 75)**	**Moderate acute cholecystitis (n = 33)**	**p-value**
Operation time (min)	60.0 (25.0)	85.0 (37.5)	p < 0.001*
Intraoperative perivesical infiltrate			
Yes	15 (20 %)	22 (67 %)	p < 0.001**
No	60 (80 %)	11 (33 %)	
Empyema of the gallbladder			
Yes	10 (13 %)	18 (55 %)	p < 0.001**
No	65 (87 %)	15 (45 %)	
CVS principle implementation (> 4 steps)			
Yes	74 (99 %)	31 (94 %)	p = 0.043***
No	1 (1 %)	2 (6 %)	
Transition to open surgery			
Yes	0 (0 %)	3 (9 %)	p = 0.027***
No	75 (100 %)	30 (91 %)	

† The median and interquartile range (IQR) are indicated. The number of patients and percentage are indicated for all categorical variables.
* Mann-Whitney U test; ** Chi-square (χ^2) test; *** Fisher's exact test, as the assumptions of the Chi-square (χ^2) test were violated.

2.2 Number of CVS steps performed and duration of the operation

More than 4 CVS steps were performed in 105 patients (97 %) from the study group. Less than 4 CVS steps were performed in only three patients – one (1 %) patient with mild acute cholecystitis and two (2 %) patients with moderate acute cholecystitis (Table 2.3). The difference in the number of CVS steps performed between patients with mild and moderate acute cholecystitis was found to be statistically significant, with a tendency in favour of patients with mild acute cholecystitis.

Table 2.3

Distribution of patients (n, %) according to the number of CVS steps performed

Number of CVS steps performed	**Mild acute cholecystitis (n = 75)**	**Moderate acute cholecystitis (n = 33)**	**p-value***
0 steps	0 (0 %)	1 (3 %)	0.002
1 step	0 (0 %)	1 (3 %)	
2 steps	1 (1 %)	0	
3 steps	0 (0 %)	0	
4 steps	2 (3 %)	2 (6 %)	
5 steps	5 (7 %)	6 (18 %)	
6 steps	16 (21 %)	9 (27 %)	
7 steps	8 (11 %)	5 (16 %)	
8 steps	43 (57 %)	9 (27 %)	

* p-value obtained using the Mann-Whitney test.

Accordingly, it was observed that performing more than 4 CVS steps significantly reduced the average duration of surgery in patients with moderate acute cholecystitis (Table 2.4). In patients with mild acute cholecystitis, performing more than 4 CVS steps was associated with a 10-minute increase in the average duration of surgery, but the difference was not statistically significant ($p = 0.106$). At the same time, it should be noted that the performance of sequential CVS steps in groups of patients with mild and moderate cholecystitis differed significantly, $p < 0.001$.

Table 2.4

Operation time in minutes based on the number of CVS steps performed and the severity of acute cholecystitis using white light with ICG

Number of CVS steps performed	Operation time (min)				Mann-Whitney p-value
	Mild acute cholecystitis		Moderate acute cholecystitis		
	Median	IQR	Median	IQR	
0–4 steps (n = 7)	45.0	–	105.0	47.5	0.057
5–8 steps (n = 101)	55.0	25.0	75.0	30.0	< 0.001
Mann-Whitney p-value	0.106		0.009		–

(IQR – interquartile range)

2.3 FC Visualisation score (Likert scale)

It was observed that visualisation scores were higher in patients with mild acute cholecystitis compared to patients with moderate acute cholecystitis (see Figure 2.1). Significant differences in *ductus cysticus* visualisation scores were observed between patients with mild and moderate acute cholecystitis both before (82.7 % vs. 51.5 %) and after CVS (88.0 % vs. 69.7 %). In addition, there were significant differences in the proportional distribution of visualisation indicators after CVS between patients with mild and moderate acute cholecystitis for the junction of the *ductus cysticus* and *ductus hepaticus communis* (57.3 % vs. 33.3 %) and for the *ductus hepaticus communis* (70.7 % vs. 45.5 %).

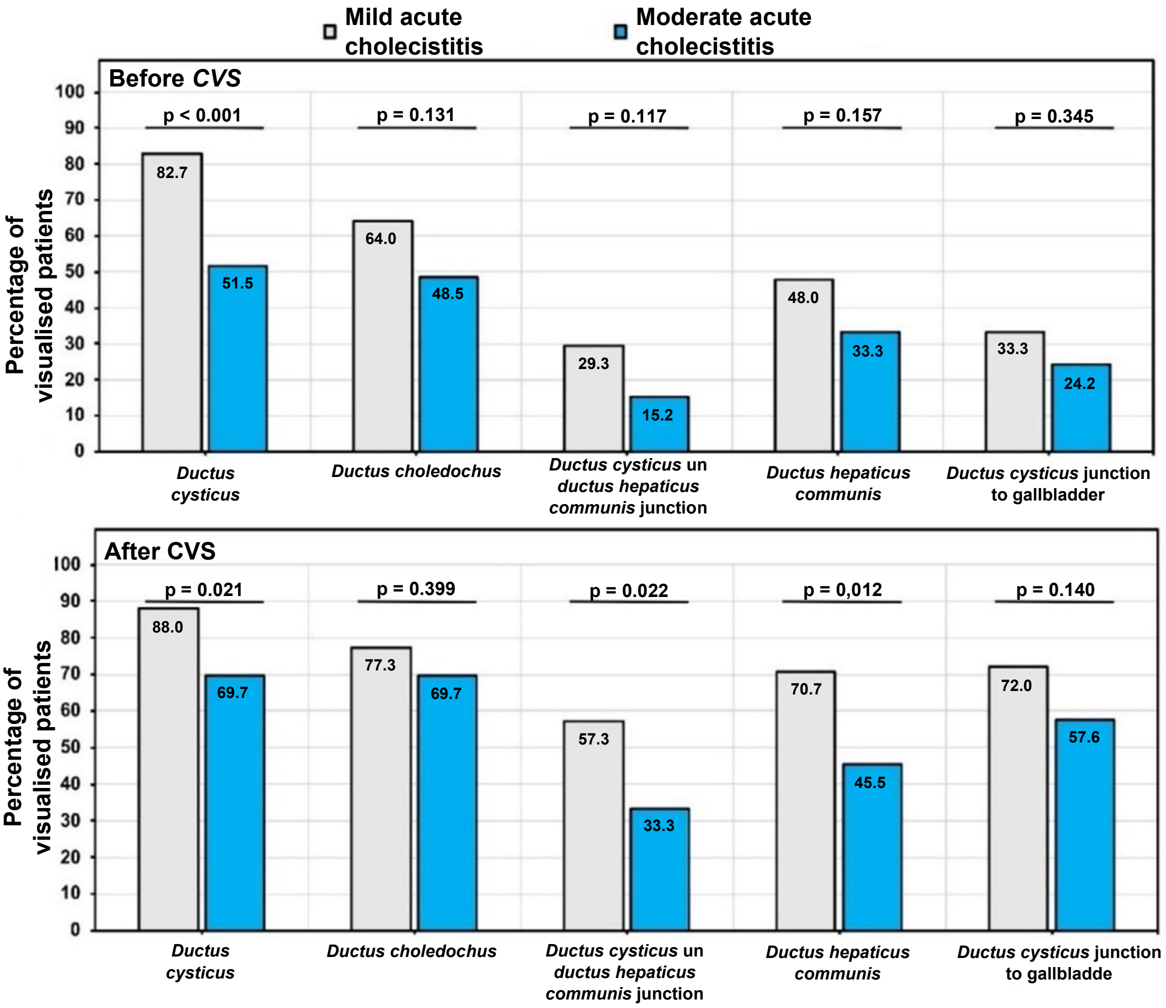

Figure 2.1 **Percentage of visualised bile duct structures before and after CVS, distributed according to the severity of acute cholecystitis**

The percentage was calculated in different groups of acute cholecystitis.
The p-value was obtained from the Chi-square ($\chi 2$) test.

In both patients with mild and moderate acute cholecystitis, a significant improvement in the *ductus cysticus* visualisation index was observed when comparing white light and FC modes before CVS (see Figure 2.2). *The ductus cysticus* visualisation index in both groups approached 100 % after CVS in both white light and FC modes. A significant improvement in the visualisation index of the *ductus choledochus* was observed in both patient groups when comparing white light mode with FC mode before CVS. However, when comparing FC before CVS with FC and after CVS with FC and WL modes, the visualisation of the *ductus choledochus* and *ductus hepaticus communis* did not improve. The above can be explained by the fact that during the operation, the most important thing was to identify critical structures, including *the ductus cysticus.* In cases of severe inflammation, performing all CVS steps to identify all EHBD may lead to biliovascular damage.

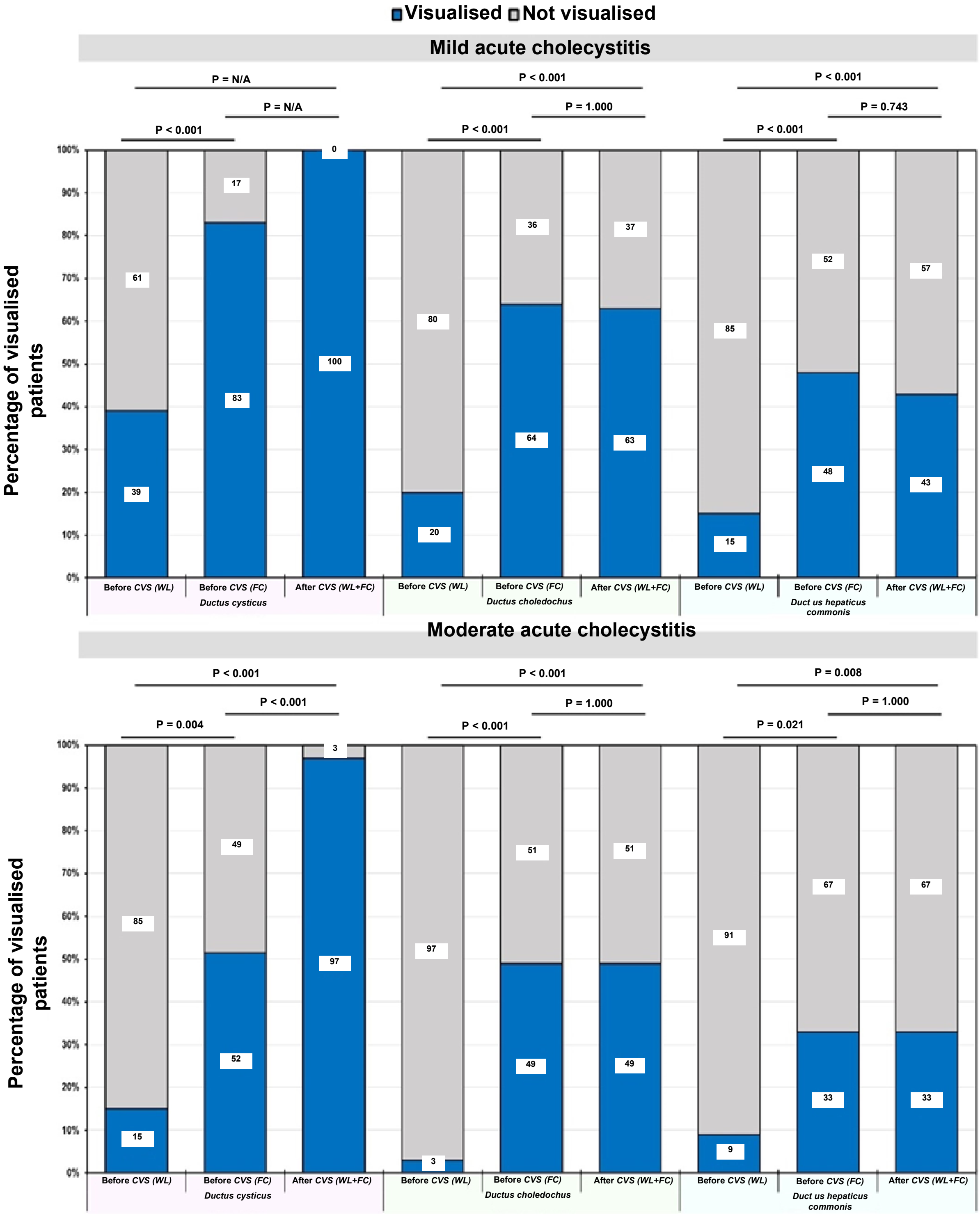

Figure 2.2 **Percentage of visualised EHBD before CVS using white light (WL); before CVS using fluorescence cholangiography (FC) and after CVS using both methods (WL+FC), broken down by severity of acute cholecystitis**

The percentage is calculated for different groups of acute cholecystitis.
P-values were obtained using the McNemar test.

2.4 Assessment of visualisation quality performed with FC *(Helpful* score*)*

Significant changes were observed in the assessment of visualisation quality before CVS (using white light) and after CVS (using white light and FC) (Figure 2.3).

The visualisation quality after CVS mostly improved from good to excellent, especially for the *ductus cysticus* – 35.6 % of patients (Padj < 0.001), the *ductus choledochus* – 28 % of patients (Padj < 0.001), the junction of the *ductus cysticus* and *ductus hepaticus communis* in 37 % of patients (Padj = 0.020), and the *ductus hepaticus communis* in 30 % of patients (Padj = 0.001). In addition, the visualisation quality after CVS significantly improved from almost good quality to good quality in the *ductus cysticus* – 21 % of patients (Padj = 0.002), *ductus choledochus* – 22 % of patients (Padj = 0.001) and *ductus hepaticus communis* – 19 % of patients (Padj = 0.040).

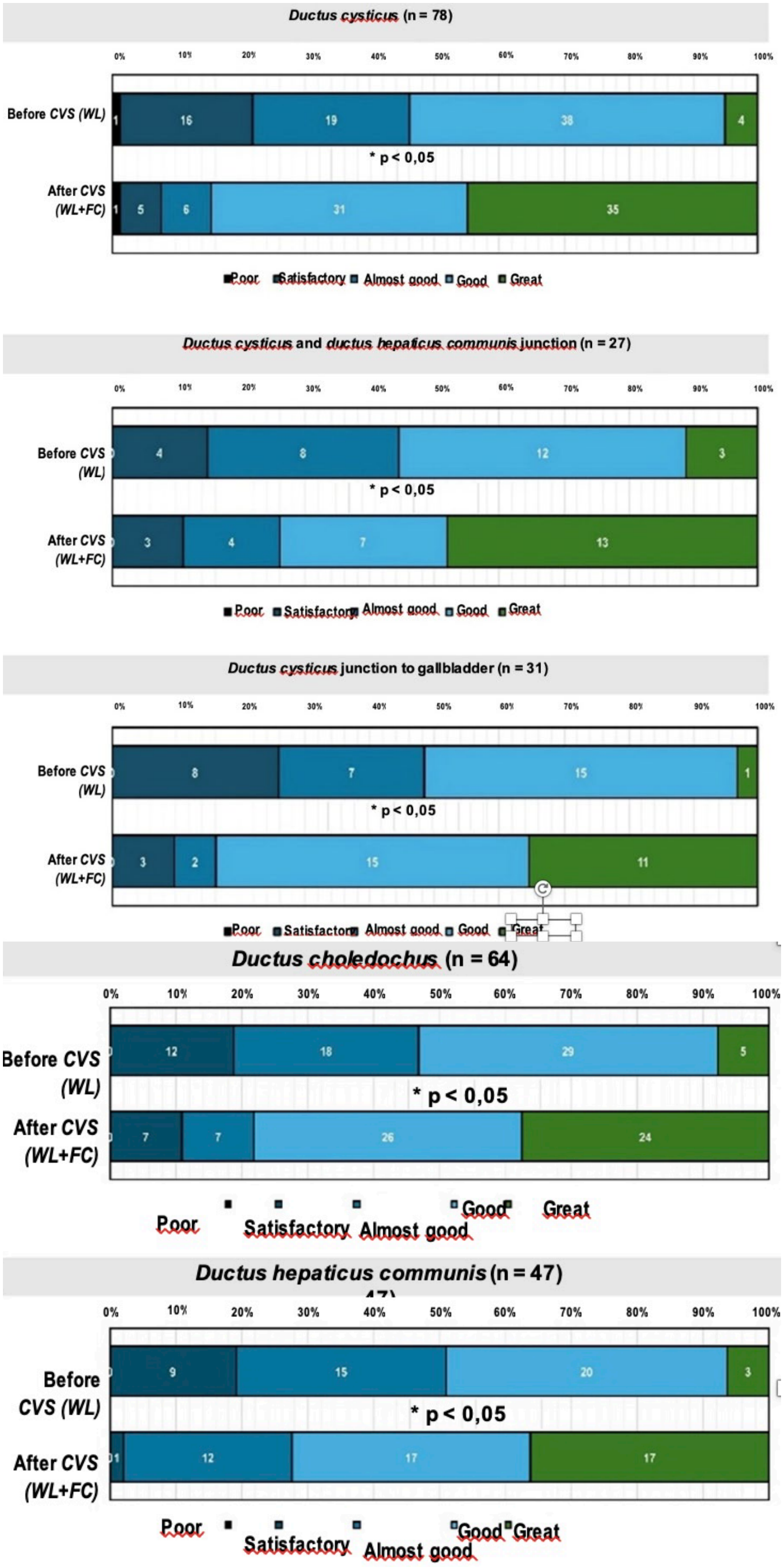

Figure 2.3 **Assessment of the quality of extrahepatic bile duct visualisation before CVS (using white light) and after CVS (using white light and fluorescence cholangiography), expressed as the number of patients (in white font)**

The p-value was obtained using the *McNemar-Bowker* symmetry test and the Stuart-Maxwell marginal homogeneity test (related samples). Significant p-values are marked with asterisks. It should be noted that one patient who had a "*poor"* rating before CVS but a "*good"* rating after CVS was excluded from the analysis in order to maintain equal variables in both columns for statistical interpretation purposes.

2.5 FC usefulness (*Helpful* score) before CVS

A statistically significant correlation was found between the severity of acute cholecystitis and the usefulness of FC before CVS *ductus cysticus* and *ductus hepaticus communis* visualization (Table 2.5). FC was useful for visualisation of the *ductus cysticus* in 83 % of patients with mild acute cholecystitis compared to 49 % of patients with moderate acute cholecystitis ($p < 0.001$). Similarly, FC was useful for visualising the *ductus hepaticus communis* in 49 % of patients with mild disease compared to 27 % of patients with moderate disease ($p = 0.033$).

Table 2.5

Evaluation of the usefulness of FC (shown as n, %)

Structure (n = 108)	**Severity of acute cholecystitis**	**Utility of** ICG **before** CVS		**Chi-square (χ2) test p-value**
		Not useful n (%)	**Useful n (%)**	
Ductus cysticus	Mild (n = 75)	13 (17)	62 (83)	p < 0.001
	Moderate severity (n = 33)	17 (52)	16 (49)	
Ductus choledochus	Mild (n = 75)	27 (36)	48 (64)	p = 0.072
	Moderate severity (n = 33)	18 (55)	15 (45)	
Junction of *ductus cysticus* and *ductus hepaticus communis*	Mild (n = 75)	53 (71)	22 (29)	p = 0.054
	Moderate severity (n = 33)	29 (88)	4 (12)	
Ductus hepaticus communis	Mild (n = 75)	38 (51)	37 (49)	p = 0.033
	Moderate severity (n = 33)	24 (73)	9 (27)	
***Ductus cysticus* junction to gallbladder**	Mild (n = 75)	52 (69)	23 (31)	p = 0.312
	Moderate severity (n = 33)	26 (79)	7 (21)	

2.6 Assessment of liver fluorescence background (*Disturbed Score*)

Patients with mild acute cholecystitis had higher levels of liver fluorescence background disturbance both before (range 9–12 %) and after CVS (range 10–14 %) at all visualisation sites. However, Fisher's exact test did not reveal any significant differences in the proportional association based on the level of liver background fluorescence and the severity of acute cholecystitis (see Figure 2.4). Examples of EHBD visualisation score quality indicators are presented in Figures 2.5, 2.6, 2.7, 2.8, 2.9, and 2.10).

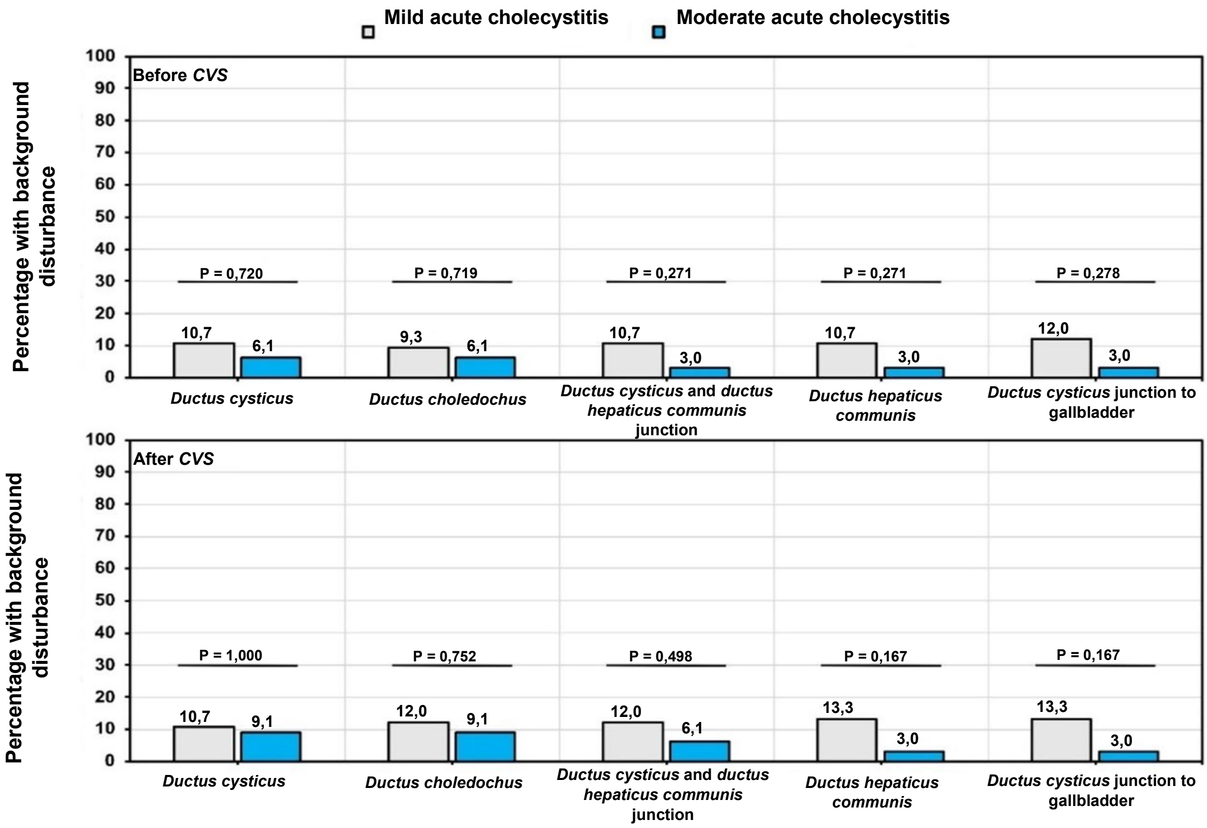

Figure 2.4 **Percentage of patients with background disturbances before and after CVS, categorised by severity of acute cholecystitis**

The percentage was calculated in different groups of acute cholecystitis.
The p-value was obtained using Fisher's exact test.

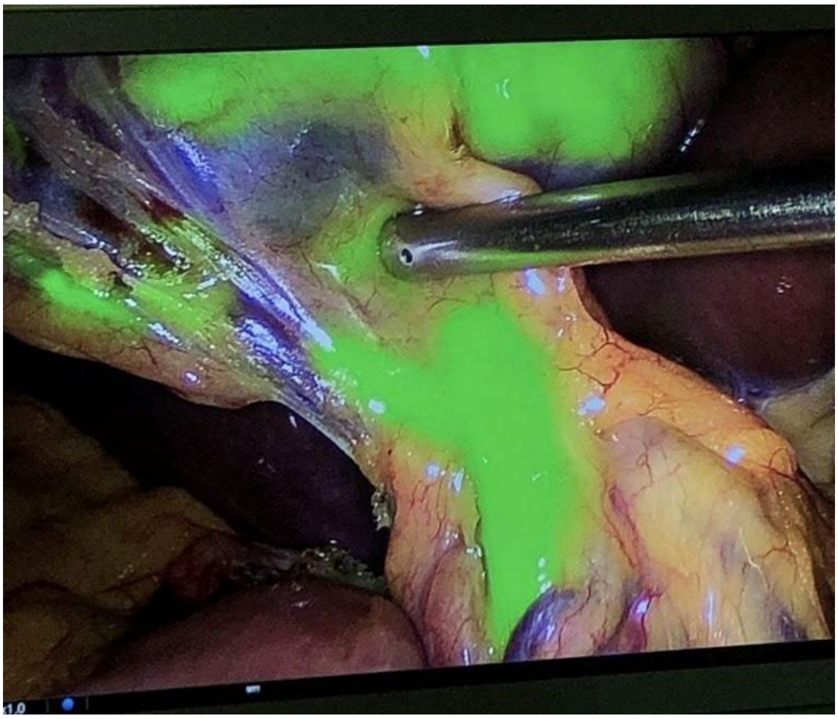

Figure 2.5 **FC assessment before CVS in a patient with mild AC,** author's image

Likert scale *(ductus cysticus)* – excellent (5), *Helpful* score – very useful (3), *Disturbed* score – no disturbances (0).

Figure 2.6 **FC assessment after CVS in a patient with mild AC,** author's image

Likert scale *(ductus cysticus)* – excellent (5), *Helpful* score – very useful (3), *Disturbed* score – no disturbance (0).

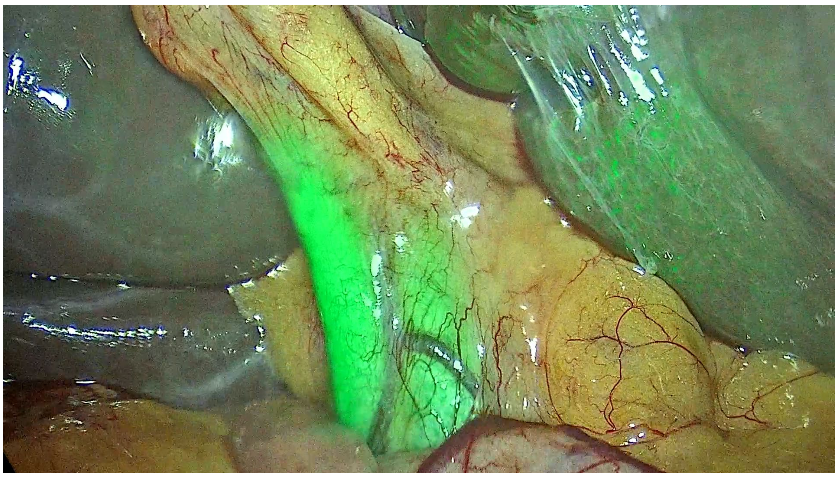

Figure 2.7 **FC assessment before CVS in a patient with moderate AC,** author's image

Likert scale *(ductus cysticus)* – good (4), *Helpful* score – very useful (3), *Disturbed* score – mild disturbance (1).

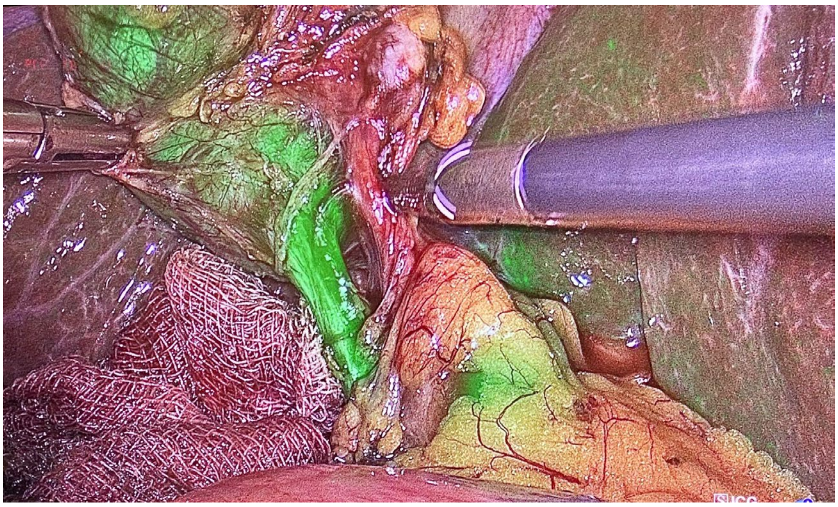

Figure 2.8 **FC assessment after CVS in a patient with moderate AC,** author's image

Likert scale *(ductus cysticus)* – excellent (5), *Helpful* score – very useful (3), *Disturbed* score – mild disturbance (1).

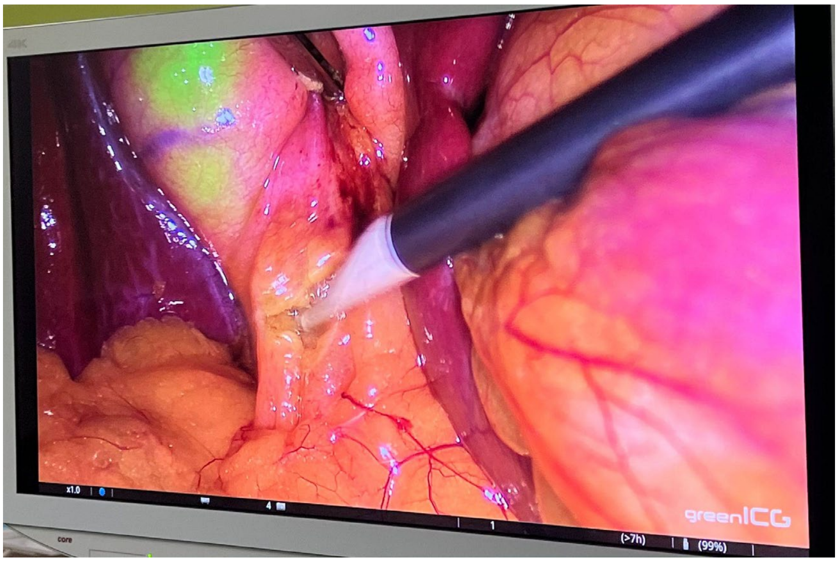

Figure 2.9 **FC assessment prior to CVS in a patient with mild AC**, author's image

Likert scale *(ductus cysticus)* – poor, not identifiable (1),
Helpful score – not useful (0),
Disturbed score – no disturbances (0).

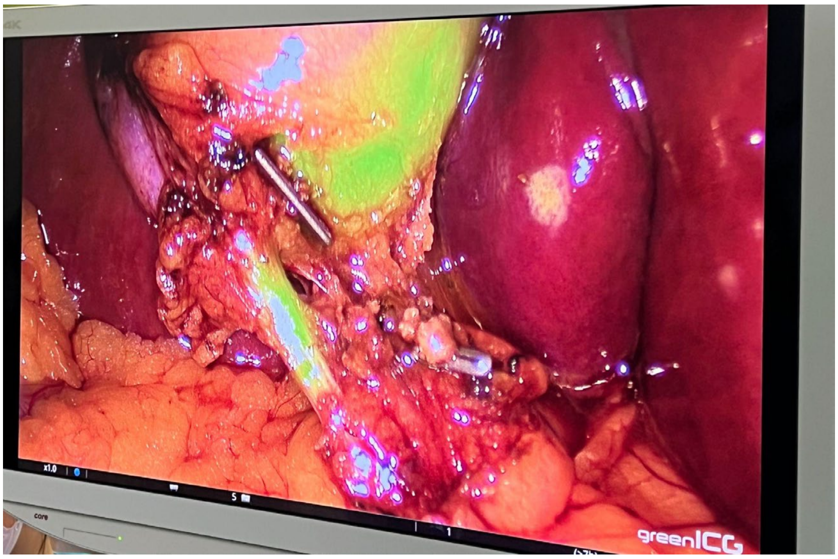

Figure 2.10 **FC assessment after CVS in a patient with mild AC,** author's image

Likert scale *(ductus cysticus)* – almost good (3),
Helpful score – useful (2), *Disturbed* score – no disturbance (0).

2.7 Surgical results

2.7.1 Pathohistological findings

In the mild acute cholecystitis group, the majority of patients had acute phlegmonous cholecystitis (n = 73), while significantly fewer patients had this finding in the moderate acute cholecystitis group (n = 14) (97.3 % vs. 43.8 %). In turn, the morphological finding of acute gangrenous cholecystitis was significantly higher in the group of patients with moderate acute cholecystitis (n = 19) compared to the group of patients with mild AC (n = 2) (56.3 % vs.

2.7 %). The correlation between these results is statistically significant using the *Chi-Square* test, $p < 0.001$ (Table 2.6).

Table 2.6

Results of pathohistological examination in both study groups

AC Histological findings			Histology		Number of patients (n, %)
			Acute phlegmonous	Acute gangrenous	
AC	Mild	Number (n)	73	2	75
		%	97.3	2.7	100.0
	Moderate	Number (n)	14	19	33
		%	43.8	56.3	100
Total		Number (n)	87	21	108
		%	81.1	18.9	100.0

A statistically significant difference was found between the acute phlegmon and acute gangrene pathohistological findings groups in terms of the number of CVS steps achieved ($p < 0.001$; Mann-Whitney U test), with a tendency for a higher number of CVS steps to be achieved in cases of phlegmonous inflammation (Table 2.7).

Table 2.7

Pathohistological findings of cholecystitis and number of CVS steps performed

Number of CVS steps performed	Acute phlegmonous cholecystitis	Acute gangrenous cholecystitis
1	1	1
2	1	0
3	0	0
4	3	1
5	9	2
6	18	7
7	7	4
8	49	0

2.7.2 Intra-abdominal drainage

In the moderate acute cholecystitis group, almost all patients had a drain inserted into the subhepatic space at the end of the operation. In the mild acute cholecystitis group, subhepatic space drainage was performed in 60 % of patients. The association between drain insertion and the severity of AC was statistically significant using the Chi-Square test, $p < 0.001$ (Table 2.8).

The study documented on which postoperative day the drain was evacuated from the abdominal cavity. A total of 77 patients had a drain inserted into the surgical site – the subhepatic space – during surgery. The average number of days until drain evacuation was significantly higher in the moderate AC group (mean days = 2 days, IQR 1–2 days) and shorter in patients with mild AC (mean days = 1 day, IQR 1–2 days).

Table 2.8

Drain insertion during LC, between study groups

			Drainage		**Number of patients (n, %)**
			Yes	**No**	
AC	Mild	Number (n)	45	30	75
		%	60.0	40	100.0
	Moderate	Number (n)	32	1	33
		%	97	3	100.0
Total		Number (n)	77	31	108
		%	71.3	28.7	100.0

2.7.3 Assessment of the patient's subjective condition

During the final visit of the study, the patient's subjective condition was documented by asking the patient to rate their health condition on a score of 0–100. The study data show that patients with mild acute cholecystitis reported significantly better subjective health status compared to patients in the moderate acute cholecystitis group (mild AC median – 90 %, IQR: 80–90 % vs. moderate AC median – 80 %, IQR: 70–80 %, $p < 0.001$).

None of the patients included in the study (n = 108) experienced biliovascular injuries or postoperative complications.

In terms of postoperative complications, all patients were classified as grade I according to the Clavain-Dindo classification. No differences in length of hospital stay were observed between the two patient groups. WBC count and CRP level at discharge were significantly higher in patients with moderate acute cholecystitis (Table 2.9).

Table 2.9

Surgical treatment results

Indicators†	**Mild acute cholecystitis (n = 75)**	**Moderate acute cholecystitis (n = 33)**	**p-value**
Length of hospital stay (days) **Postoperative hospital stay (days)**	6.0 (3.0) 2.0 (2.0)	6.0 (4.5) 3.0 (2.0)	$p = 0.437$* $p < 0.001$*
WBC count at discharge (10^9 cells/L)	7.0 (3.0)	10.0 (4.5)	$p = 0.002$*
CRP at discharge (mg/l)	30.0 (53.0)	60.0 (105.5)	$p = 0.002$

† The median and interquartile range (IQR) are indicated.

* Mann-Whitney U test

3 Discussion

Fluorescence cholangiography during laparoscopic cholecystectomy

3.1 Use of FC in Latvia and worldwide

The first fluorescence-assisted laparoscopic cholecystectomy was performed by surgeon Takeaki Ishizawa in Osaka, Japan, who published the first article related to the use of the fluorescence effect during LC in 2011. The study included a total of seven patients, in whom the visualisation of the EHBD structure was analysed before and after the separation of the Calot triangle (Ishizawa et al., 2011).

In Latvia, fluorescence-assisted LC was first performed and introduced by Professor Haralds Plaudis in 2017. From 2017 to 2019, a total of 12 patients underwent FE-assisted LC at Riga East University Hospital (RAKUS), in most cases for complicated gallstone disease. The above-mentioned patients were included in the international clinical study EURO-FIGS *European Fluorescence Image-guided surgery* (Agnus et al., 2020). Since 2019, the use of fluorescence cholangiography at RAKUS has become a routine additional visualisation method during LC for surgeons, with preoperative assessment indicating that LC is expected to be difficult in more than 150 FC-assisted LC cases.

3.2 Demographic data and intraoperative findings

In recent years, FC has gained considerable popularity and is widely used around the world. It has been shown to improve visualisation of the structure of the EHBD while reducing biliovascular injuries during surgery (Broderick et al., 2022). One of the most significant benefits of FC is easier orientation in the anatomy during LC and identification of vital structures that are difficult to distinguish in patients with acute cholecystitis. Factors such as perivesicular infiltrate, adhesions, increased ICP, and anatomical anomalies of the EHBD or blood vessels often make it difficult even for experienced surgeons to accurately visualise critical structures, increasing the risk of iatrogenic biliovascular damage (Chiche et al., 2022). IBDI can cause late complications, such as biliodigestive anastomotic strictures, recurrent cholangitis, and secondary biliary cirrhosis, which require prolonged hospitalisation and repeated surgical interventions (Liu et al., 2023; Pesce et al., 2021). Our study shows that the frequency of visualisation of bile duct structures using FC was higher in patients with mild acute cholecystitis compared to patients with moderate cholecystitis.

The above-mentioned differences may be related to more pronounced inflammation of the gallbladder and surrounding tissues, as patients with moderate acute cholecystitis more often had empyema of the gallbladder, and a significantly larger proportion had gangrenous AC on pathohistological examination. (Pardo et al., 2023; Agnus et al., 2020). Other factors that may affect the quality of the fluorescence effect are increased intra-abdominal fat, especially in *the ligamentum hepatoduodenale (*Liu et al., 2018). Fluorescence cholangiography can be used to visualise EHBD structures if the surrounding tissue does not exceed 10 mm (millimeters), which is related to the ICG's ability to emit light rays at a depth of 5–10 mm (Buchs et al., 2012; Spinoglio et al., 2013). In patients with severe intra-abdominal obesity or perivesical infiltrates, it is not possible to identify structures without tissue dissection, but even in such cases, it is possible to use CVS tissue separation and gradually visualise the structures of the EHBD with FC and perform safe cholecystectomy (Spinoglio et al., 2013). When analysing the results obtained in the Doctoral Thesis regarding increased BMI and EHBD visualisation, no statistically significant correlations were observed. In addition to the above, FC-assisted visualisation of the EHBD can be affected not only by local inflammation or obesity, but also by liver inflammation and cell dysfunction. An animal study in which rats were subjected to acute or chronic liver damage revealed that ICG dye metabolism in hepatocytes was significantly impaired, resulting in incomplete liver fluorescence and a marked decrease in the spectral intensity of green color, or the fluorescence effect. Referring to this study, it can be concluded and hypothesised that a similar process may also occur in humans, where hepatocyte dysfunction resulting from retrograde inflammation of the gallbladder could potentially reduce ICG metabolism and excretion in bile. In the Doctoral Thesis study, cases were observed where it was not possible to identify EHBD structures using the FC method, but the liver fluorescence background remained even after ICG administration. Further studies are needed to evaluate the quality of the FC effect in patients with acute or chronic liver inflammation, or the relationship between ICG metabolism and EHBD visualisation indicators and quality (Onoe et al., 2017).

In February 2023, the European Association for Endoscopic Surgery developed guidelines for fluorescence-assisted surgery, including the use of fluorescence cholangiography during LC. A panel of experts with a high level of evidence determined that fluorescence cholangiography during laparoscopic cholecystectomy improves FCS visualisation before and after tissue dissection compared to standard white light visualisation. The guidelines recommend the use of fluorescence cholangiography whenever available to improve visualisation of the bile ducts. With reference to the above recommendations and the available literature, the diagnosis of chronic and acute cholecystitis is not distinguished, and there are no isolated studies in the literature that examine only acute cholecystitis or patients with varying degrees of AC severity and FC-assisted LC (Cassinotti et al., 2023). Respectively, there are currently no similar studies in the literature regarding the use of FC in groups of patients with acute cholecystitis, and this Doctoral Thesis is the first to conduct a detailed analysis of the use of FC in the identification of EHBD structures in patients with AC, which corresponds to the novelty proposed in the Doctoral Thesis. When comparing chronic inflammation of the gallbladder with acute inflammation, in cases of acute cholecystitis, EHBD structures are more difficult to identify due to local changes caused by acute inflammation, and it is more important to use additional visualisation methods for extrahepatic bile ducts.

3.3 Critical View of Safety: number of steps performed

The RAKUS Surgery Clinic uses an extended CVS protocol, which includes eight consecutive steps, starting with visceral peritoneal dissection and ending with complete removal of the gallbladder from the liver bed. The extended CVS protocol incorporates both the principles of safe cholecystectomy, separating the two critical structures and identifying their inflow into the gallbladder, and the remaining steps to complete the LC. Following these steps not only provides safety for the patient and surgeon, but can also help surgical residents and new surgeons learn LC (Fassari et al., 2023).

In the study, CVS steps were performed with the help of FC, and the results show that the *ductus cysticus* was successfully identified in all cases, which is a very important aspect for performing a safe cholecystectomy. Studies available in the literature describe a less extensive CVS step protocol, focusing on achieving CVS principles and avoiding IBDI. It is important to separate fatty tissue and fibrous tissue from the *trigonum cystohepaticum,* separate the lower part of the gallbladder, and identify the two structures that connect to the gallbladder (*ductus cysticus* and *arteria cystica)* (Onoe et al., 2017). The results of the study prove that FC improves the visualisation of extrahepatic bile ducts, allowing CVS principles to be safely applied even in patients with moderate acute cholecystitis.

In the Doctoral Thesis study, all operations were performed by three certified surgeons specialising in or trained in hepatopancreatobiliary surgery, strictly adhering to the principles of the extended CVS protocol. Referring to the results of the study, it was observed that in the moderate cholecystitis group, the implementation of CVS steps was lower compared to the mild AC group, which can be explained by the surgeon's conscious decision not to always perform all CVS steps, but rather to focus on the visualisation of critically important structures (*d.cysticus, d.choledochus*, and *a.cystica)*, thereby reducing the risk of biliovascular damage. Studies available in the literature also emphasise that in cases of moderate AC, full implementation of CVS principles may be contraindicated as it may cause biliovascular damage during dissection (Dip et al., 2021; Nassar et al., 2022).

3.4 Assessment of the quality of fluorescence effect visualisation, visualisation scores: *Likert, Helpful,* and *Disturbed*

An analysis of the data available in the literature shows a higher rate of extrahepatic bile duct visualisation using FC compared to our study (Bleszynski et al., 2020; Dip et al., 2022; Pesce et al., 2015; Koong et al., 2021). Similar visualisation rates have been published in patients with acute cholecystitis who underwent robotic cholecystectomy with FC, but this study included a significantly smaller number of patients. Using the FC effect during robotic LC, an increase in the identification rates of the *ductus cysticus, ductus choledochus*, and *ductus hepaticus communis* was reported both before and after *trigonum cystohepaticum* dissection (Chen et al., 2021; Sakka et al., 2018; Zhang et al., 2020). In contrast, the FALCON study found a higher visualisation rate for *the ductus choledochus*. However, it should be noted that the patients included in the FALCON study underwent scheduled LC, unlike in our study, where all patients underwent early emergency LC (Dip et al., 2021). It should also be emphasised that in the aforementioned study of robot-assisted LC in patients with acute cholecystitis, these patients were not divided into groups according to the severity of AC, which may explain the observed differences.

In addition, significant differences were found in the ICG administration protocol in the studies. Although a higher dose of ICG (12.5 mg) was used in the protocol of the Doctoral Thesis study than in other studies (Manasseh et al., 2024), in the aforementioned studies, the dye was administered less than an hour before the start of surgery, and repeated doses were administered during surgery as needed. It should be emphasised that the timing and dose of ICG administration can significantly affect the quality of visualisation of structures visualised with FC, as well as the background fluorescence of the liver. Referring to the results of our study, in terms of liver fluorescence background, the liver background did not interfere at all for the majority of patients. When comparing the liver fluorescence background between our study and studies in which ICG was administered one hour before surgery, it can be concluded that administration 12 hours before surgery results in a significantly lower liver fluorescence background. However, it should also be noted that administering ICG 12 hours before LC can be problematic, as it will not always be possible to use this ICG administration protocol for all patients, for example, in cases of indicated early LC, when the patient is admitted urgently and it is not possible to wait 12 hours. As a result, some patients were excluded from the study because they could not wait 12 hours before surgery, which is one of the exclusion criteria (Agnus et al., 2020; Bleszynski et al., 2020; Aranda et al., 2023; Liu et al., 2018; Buchs et al., 2012; Spinoglio et al., 2013; Daskalaki et al., 2014).

3.5 Surgical outcomes of fluorescence cholangiography in patients with AC

3.5.1 Length of hospital stay

Considering that this type of study was conducted for the first time using FC in patients with mild to moderate AC, the analysis and comparison of surgical outcomes is limited. When evaluating the length of hospital stay between the two study groups, the average length of hospital stay is the same, but the length of hospital stay after LC is lower in the mild acute cholecystitis group. Analysing the postoperative duration between the study groups, it can be concluded that in the moderate cholecystitis group, significantly more patients underwent drainage during surgery, increasing the length of hospitalisation, as patients in the moderate AC group were discharged on an outpatient basis only the day after drainage evacuation.

3.5.2 Length of postoperative hospitalisation

After reviewing the available publications, it can be concluded that a shorter postoperative hospital stay is observed in our study. However, it should be noted that the comparative studies involve patients who underwent early LC in cases of acute cholecystitis without the use of FC (Enami et al., 2023; Lucarelli et al., 2015). Compared to the study (FALCON), where FC was used during LC in patients with symptomatic gallstone disease, chronic calculous cholecystitis, or gallbladder polyposis, the average length of postoperative hospitalisation for all patients (n = 294) in the aforementioned study was 1 day (ranging from 0 to 14 days), a lower rate associated with the fact that these patients underwent elective LC (Bos van den et al., 2023).

3.5.3 Provision of intra-abdominal drainage

Routine drainage during LC is not indicated, but it is useful for controlling possible bile leakage or bleeding and reduces the risk of intra-abdominal fluid collection. Placing a drain during LC can reduce postoperative shoulder pain by removing residual carbon dioxide from the intra-abdominal space, but it increases the length of postoperative hospitalisation, which was also observed in our study (Calini et al., 2022). However, another study emphasises that the insertion of a drain increases postoperative pain and the risk of wound infection, recommending that drains should not be inserted even during complicated LC (Yang et al., 2022). Referring to the results of the study conducted in the Doctoral Thesis: in the moderately severe cholecystitis group, 32 (out of 33) patients underwent subhepatic space drainage, with the drain being evacuated on the first postoperative day in most patients and no postoperative complications observed. However, it should be noted that the above-mentioned patients did not undergo follow-up visits after discharge, nor did they undergo follow-up imaging examinations to analyse late postoperative complications.

3.6 Advantages and disadvantages of fluorescence cholangiography

3.6.1 Advantages

Both intraoperative cholangiography (IOC) and fluorescence cholangiography (FC) have been described as safe and practical methods for assessing bile duct anatomy and preventing iatrogenic bile duct injury. Compared to conventional IOC, FC is a minimally invasive technique that does not require cannulation of the *ductus cysticus* and is therefore less traumatic. Another advantage of FC is that images can be obtained before tissue dissection begins. FC images can be obtained both before and during the tissue dissection, but this does not increase the radiation or contrast load on the patient, and this method can be used for both pregnant women and young people, and its repeated use does not pose a risk to the staff in the operating room. IOC allows images to be obtained when the *d. cysticus* is cannulated or a cholangiostoma is created, but in order to reduce the X-ray exposure, the dissection of the structure is usually not performed under X-ray control. Additional medical personnel are required when performing IOC. The advantage of FC is that the camera can be easily switched from infrared light to white light, allowing dissection to be performed under fluorescence imaging control. FC does not prolong the duration of surgery compared to traditional cholangiography and is relatively inexpensive to use, with equipment that can be used many times. FC skills are easy to learn and use (Pavel et al., 2022; Dip et al., 2015; Boni et al., 2015).

3.6.2 Disadvantages

Like all additional visualisation methods, FC has its limitations or disadvantages. One of the disadvantages or prerequisites for performing fluorescence-assisted LC is the need for specialised surgical equipment and dyes. Limited visualisation of the FC effect has been observed in patients with increased body weight and obesity. This aspect is related to the permeability of infrared light rays in tissues. According to available literature, EHBD can be identified before release if the surrounding tissue is no thicker than 10 mm, i. e. the effect of infrared light on ICG achieves a fluorescence effect in the range of 5–10 mm.

As in the case of obesity, in cases of pronounced perivascular inflammation, extrahepatic bile ducts will not always be visible without tissue dissection. Limitations definitely include FC and AC, as it is possible that inflammation retrograde reaches the liver with subsequent liver dysfunction and reduced FC effectiveness, but detailed studies in humans have not yet been conducted, nor are there any similar studies on the use of FC in AC, analysing the severity of AC.

Finally, there are no standard ICG doses and administration times during LC depending on the type of cholecystitis, which may be related to the lack of assessment of the FC effect in patients with acute cholecystitis. However, referring to the results of our study with FC and CVS principles, it was possible to identify the *ductus cysticus* in all patients, following the principles of safe cholecystectomy. The literature provides variable administration times and doses, as well as administration doses and times developed by ISFGS experts; however, when comparing the liver fluorescence background, it cannot be agreed that this dose and administration time are optimal (Pesce et al., 2021; Armstrong et al., 2021; Bos van den et al., 2018; Fassari et al., 2023).

Conclusions

1. The use of fluorescence cholangiography during emergency laparoscopic cholecystectomy is effective in both mild and moderate acute cholecystitis, the results show that, in almost all patients, using the fluorescence effect and the principles of safe cholecystectomy, it was possible to identify the critical structure – the *ductus cysticus*, which allowed the principles of safe cholecystectomy to be followed.
2. The expanded use of the CVS protocol is effective because it helps the surgeon perform a safe, step-by-step operation. Almost all patients underwent at least four consecutive CVS steps, emphasising that the fourth step is the isolation and identification of the critical structure – the *ductus cysticus*. Finally, the study results confirm that FC effectively assists in performing CVS steps, as the additional visualisation method allows for better orientation in both the anatomy of the EHBD and the surgical field.
3. Intravenous administration of 12.5 mg of *indocyanine green* dye 12 hours before surgery results in a low liver fluorescence background, with a tendency towards a slightly lower liver fluorescence background in the moderate cholecystitis study group, as well as good EHBD visualisation indicators; however, it may be technically difficult to perform if the time window between patient admission and surgery is shorter.
4. None of the patients included in the study were diagnosed with biliovascular injuries or significant postoperative complications.

Practical recommendations

1. Fluorescence cholangiography-assisted laparoscopic cholecystectomy is recommended as a routine procedure for all patients with acute cholecystitis, as well as for patients scheduled for complex cholecystectomy – proven atypical anatomy, elevated BMI levels, or a history of acute pancreatitis, or other anticipated technical difficulties during surgery.
2. The additional visualisation method should be used by both residents and young surgeons, regardless of the type and severity of gallbladder inflammation, to avoid biliovascular injuries during surgery.
3. It is recommended that the expanded CVS protocol be implemented as routine practice and applied to all surgeons who plan to perform LC on patients with symptomatic gallstone disease, including a CVS checklist in the patient's medical history so that the CVS steps performed can be noted after surgery, as well as – in the event of biliovascular damage – to assess whether the principles of safe cholecystectomy have been followed.

List of publications, reports, and patents

Publications on the topic of the Doctoral Thesis:

1. **Pavulans, J**., Jain, N., Zeiza, K., Sondore, E., Cerpakovska, K. B., Opincans, J., Atstupens, K., & Plaudis, H. (2025). Fluorescence cholangiography for extrahepatic bile duct visualisation in urgent mild and moderate acute cholecystitis patients undergoing laparoscopic cholecystectomy: A prospective pilot study. *Journal of Clinical Medicine*, 14*(2), 541. https://doi.org/10.3390/jcm14020541

2. **Pāvulāns, J.,** Ivanova, T., Smakiqi, F., Opincāns, J., Sondrore, E., Čerpakovska, B., Zeiza, K., Plaudis, H. (2025) **Surgical Outcomes of Fluorescence Cholangiography–Guided Laparoscopic Cholecystectomy in Moderate Acute Cholecystitis.** *Proceedings of the Latvian Academy of Sciences,* Section B, Vol. 79, No. 5/6 2025, https://doi.org/10.2478/prolas-2025-0019

Reports and theses:

1. Pāvulāns, J. (2021, March 24). *Fluorescence image guided cholangiography in a patient with grade II acute cholecystitis: Case report* [Poster presentation]. Rīga Stradiņš University Research Week 2021, Rīga, Latvia. https://dspace.rsu.lv/jspui/bitstream/123456789/3645/1/KUP_2021_Abstracts-Book.pdf

2. Pāvulāns, J. (2021, March 24). *Application of fluorescence image guided cholangiography for the assessment of biliary anatomy in patients with acute cholecystitis: Review of a case series* [Oral presentation]. Rīga Stradiņš University Research Week 2021, Riga, Latvia. https://dspace.rsu.lv/jspui/bitstream/123456789/3645/1/KUP_2021_Abstracts-Book.pdf

3. Pāvulāns, J. (2021, June 3). *Application of fluorescence image guided cholangiography for the assessment of biliary anatomy in patients with acute cholecystitis* [Oral presentation]. 10th Congress of the Baltic Association of Surgeons, Riga, Latvia. https://site926903.mozfiles.com/files/926903/BAS_abstracts.pdf

4. Pāvulāns, J. (2022, March 29). *Application of fluorescence image guided cholangiography for the assessment of biliary anatomy in patients with acute cholecystitis* [Poster presentation]. 15th IHPBA World Congress, New York, NY, United States. https://www.hpbonline.org/article/S1365-182X(15)30831-5/fulltext#relatedArticles

5. Pāvulāns, J. (2023, June 6). *Fluorescence image guided cholangiography as a method for the safe visualisation of biliary anatomy in patients with acute cholecystitis* [Poster presentation]. 15th Biennial Congress of the European-African Hepato-Pancreato-Biliary Association, Lyon, France.

6. Pāvulāns, J. (2023, April 1). *The use of fluorescence cholangiography in laparoscopic biliary tract surgery* [Oral presentation]. Latvian Surgeons Association Congress, Riga, Latvia.

7. Pāvulāns, J. (2024, April 24). *Application of fluorescence-assisted surgery at the Gaiļezers Hospital* [Oral presentation]. Congress of the Latvian Association of Surgeons, Riga, Latvia.

8. Pāvulāns, J. (2024, November 23). *Application of fluorescence image guided cholangiography for the assessment of biliary anatomy in patients with acute cholecystitis* [Oral presentation]. Latvian Surgeons Association Congress, Jūrmala, Latvia.

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