# Laparoscopic cholecystectomy in acute cholecystitis: An analysis of the risk factors

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*Background and aim.* Laparoscopic cholecystectomy (LC) is increasingly being used as the initial surgical approach in patients with acute cholecystitis (AC). We describe our experience with LC in the treatment of AC.

*Materials and methods.* In this study 2 412 patients underwent LC, in 315 cases for AC. The diagnosis was based on clinical, laboratory and intra-operative findings. Rates of conversion, complications, length of hospital stay, operating times, and factors associated with conversion or morbidity were analysed.

*Results.* Conversion to open cholecystectomy was necessary in 60 patients (19.04%) with AC. Factors associated with conversion were age >65 years, male gender, presence of empyema, previous abdominal surgery, and fever (temperature >37.5°C). There were no deaths, and the complication rate was 6.4%. The only risk factor for morbidity was a bilirubin level of >20.52 µmol/l. The operating time and hospital stay were significantly longer in AC than in elective cases.

*Conclusions.* LC for AC is technically demanding but safe and effective. With patience, experience, careful dissection and identification of vital structures, the laparoscopic approach is safe in the majority of cases.

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The surgical treatment of symptomatic gallstones has changed radically since the introduction of laparoscopic surgery. Laparoscopic cholecystectomy (LC) has gradually overtaken open cholecystectomy (OC) as the preferred procedure for treating cholelithiasis, offering patients a shorter hospital stay, less postoperative pain, better cosmetic results and earlier recovery.

The laparoscopic approach is, however, more controversial in the setting of acute cholecystitis (AC). Inflammatory tissue reactions make dissection difficult and increase the risk of serious complications. However, increasing experience and confidence in laparoscopic surgery have led increasing numbers of surgeons to attempt to treat the inflamed gallbladder laparoscopically.[1-3]

We describe our experience of the treatment of AC with LC and evaluate pre-operative predictors of adverse outcome in the setting of AC.

## Materials and methods

In this study, 2 412 consecutive patients underwent LC in the Second Department of Surgery at Democritus University of Thrace, Greece. Of these, 315 (13.1%) were treated for AC and had been admitted as emergencies. A standard four-port technique was used. The diagnosis of AC was based on the presence of at least two of the following: primarily, local pain and/or tenderness in the right upper quadrant, temperature higher than 37.5oC, and/or leucocytosis (white cell count (WCC) $\geq 9\times10^9$/l), and ultrasonographic evidence of AC (thickening of the gallbladder wall, distended gallbladder or fluid adjacent to the gallbladder, presence of gallstones) at admission; and secondarily, operative and pathological confirmation of AC. Patients were operated on within 72 hours after the onset of symptoms.

Intra-operative cholangiography (IOC) was initially performed in 18 cases, in association with difficult recognition of the elements, but the results were not considered satisfactory. IOC was subsequently not performed at all as the results did not prevent complications. Patients found to have common bile duct (CBD) stones or missed CBD stones underwent pre-operative or postoperative endoscopic retrograde cholangiopancreatography.

Data sheets for patients, containing demographic, pre-operative, operative and postoperative findings, were prospectively generated. Pre-operative notes contained information on the presence of associated diseases (e.g. cardiovascular disease, hypertension and diabetes), previous abdominal surgery, previous hospitalisation for AC or acute pancreatitis, body mass index (BMI, kg/m$^2$), fever, and laboratory results comprising the WCC and serum bilirubin, aspartate transaminase (SGOT) and alanine transaminase (SGPT) levels. The operative data were macroscopic findings of AC, hydrops and empyema of the gallbladder, reasons for conversion, and duration of surgery. The diagnosis of empyema or hydrops of the gallbladder was based on the presence of pus or mucus in the gallbladder when aspirated laparoscopically. Postoperative notes comprised complications, length of hospital stay, morbidity, and mortality. The overall outcome of LC (conversion rate, complication rate, operative time and hospital stay) for AC was compared with that for elective cholecystectomy. All the above independent factors were tested for their predictive value regarding conversion, complications and operative time in AC.

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 11.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test for normality was performed. Normally distributed continuous variables were expressed as means (standard deviation (SD)), while non-normally distributed variables were expressed as the median and range. Categorical variables were expressed as frequencies (and percentages). The chi-square test was used to evaluate any potential association between two categorical variables and the median test was used to assess differences in a continuous variable between two or more groups of patients; *post hoc* analyses were performed using Bonferroni's correction. To assess the independent effect of AC on the outcome of LC, multivariate linear (for operation time and hospital stay) and logistic (for conversion and complications) regression models were constructed. Beta regression coefficients, odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated as the measure of association between AC and the outcome of LC. Variables found to be significantly different between the groups with or without AC were entered as covariates in the analysis. Changes of conversion or complication rates over time were examined with linear regression analysis. All tests were two-tailed and statistical significance was considered for $p$-values less than 0.05.

## Results

### Pre-operative status

The study population of 2 412 patients who underwent LC comprised 1 807 females (74.9%) and 605 (74.9%) males (74.9%), with a mean age of 53.27 (SD 13.93) years (range 15 - 87 years), of whom 2 097 (86.9%) underwent elective LC (1 593 women and 504 men, mean age 52.76 (SD 14.80) years, range 15 - 87 years) and 315 (13.1%) AC (214 women and 101 men, mean age 56.67 (SD 13.96) years, range 19 - 86 years).

The demographic and pre-operative data of patients undergoing elective LC and AC are compared in Table 1. The patients who presented with AC were significantly older than the elective patients ($p<0.001$), and the proportion of men ($p=0.002$) and the incidences of diabetes ($p<0.001$) and previous hospitalisation for AC and pancreatitis ($p<0.001$) were significantly higher. Clinical or laboratory signs of inflammation were seen in 280 (88.9%) of the 315 patients with AC.

**Table 1. Pre-operative status of patients undergoing elective laparoscopic cholecystectomy and patients with acute cholecystitis**

| Pre-operative factor | Elective LC | AC | *p*-value |
|---|---|---|---|
| No. of patients | 2 097 | 315 | |
| Age (mean (SD)) | 52.76 (14.80) | 56.67 (13.96) | <0.001 |
| Gender (*n* (%)) | | | 0.002 |
| Male | 504 (24.0) | 101 (32.1) | |
| Female | 1 593 (77.0) | 214 (67.9) | |
| BMI (*n* (%)) | | | 0.292 |
| ≤24.9 | 501 (23.9) | 67 (21.3) | |
| 25 - 29.9 | 1 052 (50.2) | 154 (48.9) | |
| ≥30 | 544 (25.9) | 94 (29.8) | |
| Cardiovascular disease (*n* (%)) | 71 (3.4) | 17 (5.4) | 0.076 |
| Diabetes (*n* (%)) | 170 (8.1) | 68 (21.6) | <0.001 |
| Hypertension (*n* (%)) | 319 (15.7) | 43 (13.7) | 0.469 |
| Previous abdominal surgery (*n* (%)) | | | 0.965 |
| None | 1 513 (72.2) | 225 (71.4) | |
| Lower | 506 (24.1) | 78 (24.8) | |
| Upper | 78 (3.7) | 12 (3.8) | |
| Previous hospitalisation (*n* (%)) | | | <0.001 |
| None | 1 880 (89.7) | 217 (68.9) | |
| Acute cholecystitis | 161 (7.7) | 85 (27.0) | |
| Acute pancreatitis | 56 (2.6) | 13 (4.1) | |
| Temperature >37.5°C (*n* (%)) | 0 (0) | 166 (52.7) | <0.001 |
| WCC $>9\times10^9$/l (*n* (%)) | 0 (0) | 234 (74.3) | <0.001 |
| Total bilirubin >20.52 µmol/l (*n* (%)) | 0 (0) | 183 (58.1) | <0.001 |
| SGOT >60 U/l (*n* (%)) | 0 (0) | 76 (24.1) | <0.001 |
| SGPT >60 U/l (*n* (%)) | 0 (0) | 92 (29.2) | <0.001 |

Of the patients with AC, 166 (52.7%) had a body temperature >37.5°C, 234 (74.3%) a WCC ≥9ξ10⁹/l, 183 (58.1%) a serum total bilirubin level >20.52 µmol/l, 76 (24.1%) a SGOT >60 IU/l, and 92 (29.2%) a SGPT >60 IU/l. The intra-operative findings in the acute group were acute oedematous cholecystitis in 137 patients (43.5%), hydrops in 66 (21.0%), and empyema in 112 (35.5%).

## Conversion and complications

Outcomes in patients undergoing elective LC and in those with AC are set out in Table 2. Among the entire cohort, conversion to OC was necessary in 150 (6.2%) patients, while 43 patients (1.8%) had peri-operative complications; there were no operative deaths. Both conversion (19.4% v. 4.2%, $p<0.001$) and complication (5.7% v. 1.2%, $p<0.001$) rates were significantly higher in patients with AC than in those undergoing elective LC. Among patients with acute oedematous cholecystitis, hydrops and empyema, conversion was necessary in 15.3%, 18.2%, and 25.0% and complications developed in 4.4%, 4.5%, and 8.0% of cases, respectively; there were no significant differences between these three groups ($p=0.152$ and $p=0.419$, respectively). With regard to the effect of surgeons' experience on the outcome of LC, there was a trend towards higher conversion and complication rates during the first year of the study period compared with subsequent years (40.0% v. 17.5%, $p=0.192$, and 40.0% v. 5.8%, $p=0.002$, respectively). Furthermore, during the 13-year study period, simple linear regression analysis showed that an increase of 1 year in surgeons' experience was associated with a 1.6% decrease in conversion rate and an 1.0% decrease in the complication rate. Multivariate logistic regression analyses were performed to evaluate the role of potential confounders on the observed relationship of AC with conversion and complications. The presence of AC remained a strong independent risk factor for developing complications (OR 6.32, 95% CI 2.12 - 18.83; $p=0.001$) and for conversion to OC (OR 2.23, 95% CI 0.95 - 5.31; $p=0.070$).

Extensive inflammation, adhesions, and consequent inadequate recognition of the anatomy at Calot's triangle were the most common reasons for conversion among patients with AC. Other causes for conversion to an open surgical procedure are listed in Table 3. The main difficulties encountered during surgery were adhesions of the gallbladder to surrounding structures and severe inflammation of the hepatic pedicle, which made dissection hazardous. The complications in patients with AC are summarised in Table 4. Among these were 2 transections of the CBD, 1 in each group of patients. The first one to be recognised was in a case of AC; it was confirmed 48 hours later, and Roux-en-Y hepaticojejunostomy was performed. The other one was seen intra-operatively, and end-to-end reconstruction of the CBD over a T-tube was done.

The relationships between conversion to OC and development of complications, and the characteristics of patients with AC, are presented in Table 5. Univariate analysis showed that the risk of conversion was significantly higher in males, in patients older than 65 years and in patients with previous upper abdominal surgery. A tendency towards higher conversion rates was associated with clinical or laboratory signs of inflammation, such as a temperature >37.5oC, a WCC >9×10⁹/l, SGOT >60 IU/l and SGPT >60 IU/l. The combination of previous upper abdominal surgery and a BMI ³30 further increased the risk of conversion (85.7%, 6 of 7 patients). Moreover, only previous lower or upper abdominal surgery was associated with increased complication rates. The presence of concomitant diseases contributed minimally to conversion to OC and the incidence of complications.

**Table 2. Comparison of elective laparoscopic cholecystectomy and acute cholecystitis**

| Pre-operative factor | Elective LC | AC | *p*-value |
|---|---|---|---|
| Conversion rate (%) | 89 (4.2) | 60 (19.04) | <0.001 |
| Complication rate (%) | 25 (1.2) | 18 (5.7) | <0.001 |
| Operative time (min)* | 45 (10 - 170) | 50 (20 - 225) | <0.001 |
| Postoperative stay (d)* | 3 (2 - 9) | 4 (3 - 11) | <0.001 |

*Data are expressed as frequencies and percentages for qualitative variables and as medians and ranges for quantitative variables. Operation time and postoperative stay refer to successful LC only.

**Table 3. Reasons for conversion in patients who underwent laparoscopic cholecystectomy**

| | Elective cases | | Acute inflammation | |
|---|---|---|---|---|
| Reasons for conversion | *n* | % | *n* | % |
| Inability to create pneumoperitoneum | 9 | 0.42 | 0 | 0 |
| Intraoperative bleeding | 2 | 0.09 | 2 | 0.63 |
| Suspicion of cancer | 2 | 0.09 | 0 | 0 |
| Injury of the CBD | 1 | 0.046 | 1 | 0.31 |
| Spilled stone | 1 | 0.046 | 0 | 0 |
| Choledochoduodenal fistula | 6 | 0.28 | 0 | 0 |
| Inadequate visualisation of structures | 69 | 3.29 | 57 | 18.09 |
| Total | 90/2 097 | 4.3 | 60/315 | 19.0 |

**Table 4. Complications in patients who underwent LC**

| Complications | Elective cases *n* | Elective cases % | Acute cholecystitis *n* | Acute cholecystitis % |
|---|---|---|---|---|
| Injury of CBD | 1 | 0.046 | 1 | 0.31 |
| Intra-operative bleeding | 2 | 0.09 | 2 | 0.63 |
| Postoperative bleeding | 3 | 0.14 | 1 | 0.31 |
| Subhepatic accumulation | 0 | 0 | 1 | 0.31 |
| Perforation of the colon | 0 | 0 | 1 | 0.31 |
| Bile leakage | 5 | 0.23 | 3 | 0.95 |
| Spilled stone | 0 | 0 | 1 | 0.31 |
| Haematoma abdominal wall | 12 | 0.57 | 2 | 0.63 |
| Pneumonia | 0 | 0 | 6 | 1.90 |
| Spontaneous pneumothorax | 1 | 0.046 | 0 | 0 |
| Subcapsular haematoma liver | 1 | 0.046 | 0 | 0 |
| Mortality | 0 | 0 | 0 | 0 |
| Total | 25/2 097 | 1.2 | 18/315 | 5.7 |

**Table 5. Pre-operative factors associated with conversion and complications in patients with acute cholecystitis (univariate analysis)**

| Pre-operative factor | Conversion rates | *p*-value | Complication rates | *p*-value |
|---|---|---|---|---|
| Gender (M/F) | 26.4/14.0 | 0.012 | 6.2/6.9 | 0.830 |
| Age (</>65 yrs) | 15.1/26.5 | 0.033 | 6.6/5.9 | 0.833 |
| BMI (<24.9/25 – 29.9/>30) | 14.5/15.8/23.4 | 0.256 | 6.5/5.3/8.2 | 0.683 |
| Cardiovascular disease (n/y) | 18.3/0.0 | 0.212 | 6.2/14.3 | 0.391 |
| Diabetes (n/y) | 20.3/9.5 | 0.051 | 6.9/4.8 | 0.540 |
| Hypertension (n/y) | 18.1/15.0 | 0.729 | 6.9/0.0 | 0.224 |
| Previous abdominal surgery (none/lower/upper) | 20.3/5.9/50.0 | 0.001 | 4.0/12.3/10.0 | 0.043 |
| Previous hospitalisation (none/cholecystitis/ pancreatitis) | 19.5/14.7/8.3 | 0.456 | 5.5/7.4/16.7 | 0.290 |
| Severe inflammation (AC/hydrops/empyema) | 13.6/15.3/25 | <0.001 | 4/6.8/9.4 | 0.269 |
| Temperature >37.5°C (n/y) | 8.1/26.9 | <0.001 | 5.9/6.9 | 0.741 |
| WBC count >9×10⁹/l (n/y) | 8.6/21.0 | 0.019 | 1.4/8.1 | 0.049 |
| Total bilirubin >1.2 mg/dl (n/y) | 18.9/17.2 | 0.707 | 1.8/9.5 | 0.011 |
| SGOT >60 U/l (n/y) | 15.1/26.5 | 0.033 | 5.7/8.8 | 0.355 |
| SGPT >60 U/l (n/y) | 11.2/33.7 | <0.001 | 6.6/6.0 | 0.858 |

n/y = no/yes.

After checking for co-linearity, multivariate logistic regression analysis showed that previous upper abdominal surgery was the strongest independent risk factor for conversion (OR 5.9, 95% CI 1.6 - 22.6). Age above 65 years, male gender, temperature >37.5°C, SGOT >60 IU/l and SGPT >60 IU/l also showed significant independent but clearly weaker association with higher conversion rates. Previous lower or upper abdominal surgery were the only independent risk factors for the development of complications (OR 3.39, 95% CI 1.29 - 8.89; $p$=0.013) among patients with AC who underwent LC (Table 6).

### Operation time and hospital stay

Among successfully completed LCs, the median operation time was 45 minutes (range 10 - 225 minutes) and the median postoperative hospital stay 3 days (range 2 - 11 days). Both operation time (mean 50 minutes, range 20 - 225 minutes v. mean 45 minutes, range 10 - 170 minutes; $p<0.001$) and hospital stay (mean 4 days, range 3 - 11 days v. mean 3 days, range 2 - 9 days; $p<0.001$) were significantly longer in patients with AC compared with those undergoing elective LC. The presence of AC remained a strong independent risk factor for longer postoperative hospital

**Table 6. Independent factors associated with conversion and complications in patients with acute cholecystitis (multivariate logistic regression analysis)**

| | Conversion | | Complications | |
|---|---|---|---|---|
| **Independent factors** | **OR (95% CI)** | ***p*-value** | **OR (95% CI)** | ***p*-value** |
| Age >65 yrs | 2.3 (1.2 - 4.5) | 0.016 | | |
| Male gender | 1.9 (1.0 - 3.6) | 0.053 | | |
| Previous upper abdominal surgery | 5.9 (1.6 -22.6) | 0.009 | | |
| Temperature >37.5°C | 2.5 (1.3 - 5.0) | 0.008 | | |
| SGOT >60 U/l | 2.3 (1.2 - 4.6) | 0.017 | | |
| SGPT >60 U/l | 3.8 (2.0 - 7.2) | <0.001 | | |
| Previous abdominal surgery | | | 3.4 (1.3 - 8.9) | 0.013 |

stay (beta coefficient 0.622, 95% CI 1.441 - 2.045; $p<0.001$), but it failed to retain a significant independent relationship with operation time (beta coefficient 3.644, 95% CI -3.200 - 10.487; $p=0.297$) in multivariate linear regression analyses, after all major confounders were included.

The median operative time was significantly shorter in acute oedematous cholecystitis (44 minutes, range 20 - 170 minutes) than in hydrops (50 minutes, range 20 - 115 minutes; $p=0.036$) or empyema (55.5 minutes, range 25 - 180 minutes; $p=0.027$). The median hospital stay was significantly longer in empyema (5 days, range 3 - 11 days) than in acute oedematous cholecystitis (4 days, range 3 - 7 days; $p<0.001$) or hydrops (4 days, range 3 - 9 days; $p<0.001$).

Among patients with AC, both operative time and hospital stay were significantly shorter in successful LC than in cases converted to OC (median operative time 50 minutes, range 20 - 180 minutes v. median 70 minutes, range 45 - 225 minutes; $p<0.001$; median hospital stay 4 days, range 3 - 9 days v. median 9 days, range 7 - 12 days; $p<0.001$). Among patients with AC, excluding the converted cases, multivariate linear regression analysis showed that the duration of surgery was significantly longer in patients with a BMI >25.0 (beta coefficient 9.069, 95% CI 0.212 - 17.927; $p=0.045$), in patients with cardiovascular disease (beta coefficient 18.414, 95% CI 0.594 - 36.234; $p=0.043$) and in patients with previous upper abdominal surgery (beta coefficient 23.604, 95% CI -0.523 - 47.731; $p=0.055$).

## Discussion

LC is now widely accepted as the gold standard for the treatment of symptomatic gallstones, but its application in AC remains questionable. AC was originally considered a contraindication to LC because of the associated technical difficulties, but it has now become an accepted indication for LC thanks to increased experience with the laparoscopic technique.[3-6]

In AC the laparoscopic approach is often technically more demanding than elective LC, as indicated by the median operative times reported above. Although these cases were generally approached as for elective cholecystectomy, with increasing experience it became evident that willingness to complete the procedure laparoscopically was advantageous.[7] Many modifications in the surgical technique have been described, including the use of additional cannulas, more versatile angled and side-viewing laparoscopes, sterile specimen bags to retrieve lost stones or extract infected tissue, decompression of the gallbladder, routine IOC, and liberal use of sutures to control the cystic duct and cystic artery. In addition, when difficult conditions are present within Calot's triangle, subtotal cholecystectomy, either open or laparoscopic, has been reported to be a safe alternative in the case of inflammation or fibrosis. The scope of the procedure is to leave a patch of gallbladder wall along the common hepatic duct, the right side of the hepatic hilum, or the liver bed. In our series, we left the back wall of the gallbladder in place in rare cases, emphasised extensive dissection of Calot's triangle during LC, removed all spilled stones and the gallbladder specimen via a protective endobag, destroyed any residual gallbladder mucosa with a coagulator, and closed the cystic duct with a clip or sutures to avoid postoperative cystic biliary leakage. We do not advocate laparoscopic subtotal cholecystectomy as a routine procedure. Most of these modifications were developed in attempts to facilitate exposure of the biliary anatomy and reduce the incidence of gallstone and bile spillage.

Nevertheless, despite its feasibility as shown by previous studies and the present study, LC in patients with AC is associated with significantly higher rates of conversion to OC, longer operating times, longer hospital stays, and an increased complication rate compared with elective LC.

A conversion rate of 17.9% for LC in AC versus 3.1% ($p<0.001$) for elective LC is consistent with the finding that AC is a risk factor for conversion.[8] Conversion rates in the literature range from 6% to 35%.[9-13] The most common reason for conversion – inflammatory adhesions obscuring the anatomy – is compatible with other studies on LC for AC. Several studies have already investigated conversion in elective cases and AC.[14-18] In our study we found that conversion of LC to open surgery for AC was associated with severity of inflammation, age, gender, diabetes, previous hospitalisation for AC or pancreatitis, temperature >37.5°C, WCC $>9\times10^9$/l, total bilirubin >20.52 μmol/l, SGOT >60 U/l and SGPT >60 U/l. Various degrees of inflammation of the gallbladder culminate as AC, but obviously mild inflammation at the early stages of AC does not impair LC, whereas advanced necrotising tissue reactions severely hamper the safe identification and dissection of the anatomical structures. In our study, we categorised the severity of inflammation into three types according to the intra-operative findings in the gallbladder. The conversion rate increased with the severity of the inflammation, and was significantly higher when empyema was identified. The association between a history of previous hospitalisation for AC or pancreatitis and a high rate of conversion to OC is explained by local changes induced by repeated and chronic inflammation. The decision on when to convert to an open procedure is largely a subjective one, and it is difficult (if not impossible) to lay down absolute rules.

LC in an acutely inflamed gallbladder remains controversial due to increased complication rates, mainly bile duct injuries. OC has been the accepted treatment for AC, with a mortality rate of 0.2% and a morbidity rate of 10 - 25%.[19] There were no deaths in our series, and the complication rate was 6.4%. Compared with patients who had elective surgery, a higher proportion of patients who underwent LC for AC developed complications. The difference was essentially accounted for by general complications, with rates for biliary complications not differing significantly, but the complication rate compares favourably with rates reported for OC for AC. We had 2 cases of bile duct injury, 1 in each group of patients, giving a rate of bile duct injury of 0.062% in elective cases and 0.36% in patients with AC. This rate is comparable to those reported for patients with AC in other series, in which the bile duct injury rate ranges from 0% to 0.9%,[19-23] and is acceptable in comparison with rates in open surgery for AC.[24,25] The only factor found to be independently associated with complications when LC was attempted for AC was a pre-operative total bilirubin level exceeding 20.52 μmol/l. A large randomised study comparing LC with OC for AC would be the only way to confirm our conclusions, but it would probably be difficult to recruit patients for such a study, because of the popularity enjoyed by laparoscopy.

Average postoperative stay was somewhat longer for AC than for elective LC, but was shorter than that for patients who required conversion to OC. This was not due to prolongation of hospitalisation because of complications, but rather to intravenous antibiotics usually being continued for 48 - 72 hours postoperatively. Furthermore, all our patients underwent drainage of the hepatorenal space, and the drain was usually not removed until the 2nd or 3rd postoperative day.

## Conclusion

It can be concluded that LC for AC is technically demanding but safe and effective. It does not increase mortality, and the rate of complications appears to be lower than in OC for AC. Furthermore, it is associated with a shorter hospital stay and a more rapid return to normal activity for patients. With patience, experience, careful dissection and identification of vital structures, LC can be completed safely in the majority of cases of AC. However, a high rate of conversion to OC must be accepted, as must longer operative time.

**Conflicts of interest.** The authors declare that there are no conflicts of interest.

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