Gallbladder wall thickness as a predictor of intraoperative events during laparoscopic cholecystectomy: A prospective study of 1089 patients

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A B S T R A C T

Background: Laparoscopic cholecystectomy (LC) has a wide range of technical difficulty. Preoperative risk stratification is essential for adequate planning and patient counseling. We hypothesized that gallbladder wall thickness (GWT) is more objective marker than symptom duration in predicting complexity, as determined by operative time (OT), intraoperative events (IE), and postoperative complications.
Methods: All adult patients who underwent LC during 2010—2018 were included. GWT, measured on imaging and on the histopathologic exam, was divided into three groups: <3 mm (normal), 3—7 mm and >7 mm. Univariate and multivariable analyses were performed to determine the association between GWT and 1) operative time, 2) the incidence of IE and 3) postoperative outcomes.
Results: A total of 1089 patients, subjects to LC, were included in the study. GWT was positively correlated with median OT ($p < 0.001$), the incidence of IE ($p < 0.001$) and median length of hospital stay ($p < 0.001$). GWT independently predicted IE (OR = 2.1 95% CI: 1.3—3.4) and outperformed symptom duration, which was not significantly associated with any of the outcomes ($p = 0.7$).
Conclusions: GWT independently predicted IE and may serve as an objective marker of LC complexity.

Introduction

Laparoscopic cholecystectomy (LC) is one of the most common procedures performed by general surgeons in the United States, accounting for approximately 600,000 cases per year.[1] Although the incidence of major complications and morbidity in LC is less than 3%,[2] there is considerable variability in the range of difficulty of the procedure. The ability to accurately predict the operative complexity or risk of postoperative complications remains unclear. The subjective report of patient's symptoms duration stands as the current standard of estimating operative risk and overall complexity. We hypothesized that the thickness of the gallbladder wall, an objective finding that is a surrogate of inflammation and/or fibrosis, would have a direct association with the complexity of LC. We aimed to evaluate the association of the gallbladder wall thickness (GWT) with operative time and intraoperative events (IE), such as conversion to open cholecystectomy, bile spillage, major bleeding and bile duct injuries. The secondary outcome was the relationship of gallbladder wall thickness with length of hospital stay (LHS), postoperative complications, such as surgical site infections (SSI), organ-space infections, postoperative bleeding and bile leak.

Material and methods

Adult patients who underwent laparoscopic cholecystectomy by the Acute Care Surgery Service of a tertiary center from 2010 to 2018 were prospectively included. Intraoperative details were collected via direct correspondence with the surgeon. In addition, chart review determined patient demographics, preoperative diagnosis, imaging report, histopathology report and comorbidities. Postoperative complications were prospectively recorded 30

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days post discharge, up on patient follow-up. Preoperative measurement of the gallbladder maximal wall thickness was performed by a trained operator who reviewed imaging studies (ultrasound and/or computed tomography) before the procedure. Postoperative information in regard to gallbladder wall thickness was extracted from the histopathology report. In both imaging and pathology, the wall thickness was divided into three subgroups, in terms of severity of thickening. Thickness range below 3 mm was determined as normal.[3] Thickness range from 3 mm to 7 mm and greater than 7 mm were considered as moderate and severe thickening, respectively. Histopathologic specimen report was considered as the most accurate measure of actual wall thickening. Although some degree of discrepancy between pathology and radiology measurements of wall thickness was expected, a positive linear correlation between the two diagnostic disciplines was anticipated. Receiver Operator Characteristics (ROC) or c-statistics were calculated for preoperative ultrasound (US) and computed tomography (CT) to determine their accuracy in detecting actual (histologically confirmed) wall thickening. Finally, univariate analysis and stepwise backwards logistic regression were performed to determine the correlation between thickness of the gallbladder wall and the incidence of intraoperative events, operative time, length of hospital stay and postoperative complications. STATA 14.2, Stata Corp, College Station TX was the software used for the statistical analysis of our data.

Results

A total of 1089 patients, subjects to laparoscopic cholecystectomy, were included in the study. 1051 (96.5%) patients had a complete gallbladder specimen histopathologic report. 924 (84.8%) and 257 (23.6%) had an abdominal ultrasound and a CT scan performed before surgery, respectively. 805 (74.0%) cholecystectomies were performed on a non-elective basis. On the basis of patient's history, physical exam and laboratory and imaging test results, 548 (50.4%) patients were preoperatively diagnosed with acute cholecystitis, 224 (20.6%) had biliary colic, 142 (13.1%) suffered from gallstone pancreatitis, 103 (9.5%) patients were admitted for symptoms of choledocholithiasis, 38 (3.5%) for chronic cholecystitis and 23 (2.1%) patients for development of acute cholangitis. Median operative time was 92 min (IQR 69–120). Briefly, intraoperative events occurred in 688 (64.6%) patients overall. Bile spillage was the most common IE, that was observed in 651 (59.8%) patients. Surgical drain was placed in 114 (10.5%). Unplanned conversion to open procedure occurred in 105 (9.7%) patients. Intraoperative bleeding was observed in 7 (0.7%) patients and bile duct injury in 3 (0.3%). Median length of hospital stay was 2 days (IQR 1–4). Postoperative complications occurred in 141 (12.9%) patients. 73 (6.6%) patients suffered from infectious (surgical site infections, organ-space infections, respiratory and urinary tract infections) and 68 (6.2%) from non-infectious (wound dehiscence, hematoma, postoperative bleeding and bile leak or biloma) complications. Table 1 summarizes patient demographics, comorbidities and perioperative characteristics.

Patients with thickened gallbladder wall were more likely to be men compared to women (p = 0.011), had higher median age [<3 mm: 43 (IQR 31–59) vs 3–7 mm: 49 (IQR 35–65) and >7 mm: 56.5 (IQR 40–71), p < 0.001)] and were more likely to have cholecystitis rather than other presumed diagnosis (p < 0.001). Gallbladder wall thickness was significantly associated with median operative time (<3 mm: 84 min, 3–7: 94 min, >7 mm:110 min, p < 0.001) and there was a positive linear correlation (p = 0.03). Particularly, there was an increase in operative time by 4.2 min for every millimeter of increase in wall thickness (Fig. 1). Intraoperative events - including bile spillage, surgical drain placement,

Table 1
Patients' demographics, comorbidities and perioperative characteristics.

	Data
Age, median (IQR)	47 (33–62)
Male sex, n (%)	413 (37.9)
BMI, median (IQR)	28.7 (25.6–33)
ASA scale, n (%)	
Class I	204 (18.8)
Class II	667 (61.4)
Class III	204 (18.8)
Class IV	10 (0.9)
Class V	1 (0.1)
Diabetes melitus, n (%)	116 (10.7)
Race, n (%)	
White	656 (65.8)
Black	74 (7.4)
Asian	48 (4.8)
Latin American	219 (22.0)
Smoking, n (%)	140 (13.3)
Systemic steroids, n (%)	60 (5.5)
Preoperative diagnosis, n (%)	
Acute cholecystitis	548 (50.4)
Biliary colic	224 (20.6)
Gallstone pancreatitis	142 (13.1)
Choledocholithiasis	103 (9.5)
Chronic cholecystitis	38 (3.5)
Cholangitis	23 (2.1)
Other	10 (0.09)
Histologic thickness range, n (%)	
<3 mm	474 (43.5)
3–7 mm	465 (42.7)
>7 mm	150 (13.8)
Operative time in minutes, median (IQR)	92 (69–120)
Total IE, n (%)	688 (63.2)
Surgical drain, n (%)	114 (10.5%)
Conversion to open, n (%)	105 (9.7)
Bile spillage, n (%)	651 (59.8)
Bleeding, n (%)	7 (0.7)
Bile duct injury, n (%)	3 (0.3)
LHS, median (IQR)	2 (1–4)
Postoperative complications, n (%)	141 (14.8)

IQR: Inter Quartile Range, BMI: Body Mass Index, ASA: American Society of Anesthesiologists, IE: Intraoperative Events, LHS: Length of Hospital Stay.

conversion to open and major bleeding - were significantly associated with GWT (<3 mm: 57.1% vs 3–7 mm: 67.5% and >7 mm: 85.3%, p < 0.001).

Regarding the postoperative outcomes, patients with gallbladder wall thickening had longer median hospital stay (<3 mm: 2 days (IQR 1–3) vs 3–7 mm: 3 days (IQR 2–4) and >7 mm: 4 days (IQR 2–6), p < 0.001). Although not statistically significant, postoperative infections were more common in patients with thickened gallbladder wall (<3 mm: 6.3% vs 3–7 mm: 8.0% and >7 mm: 13.0%, p = 0.08). Table 2 compares demographics, comorbidities, intraoperative events and postoperative outcomes among patients with normal gallbladder wall thickness and those who had moderate and severe thickening.

Imaging studies, as preoperative measures of gallbladder wall thickness estimation, were linearly correlated with histopathology. Correlation coefficients of 0.57 and 0.51 were calculated for US and CT, respectively (Figs. 2 and 3). The c-statistics of preoperative abdominal ultrasound and CT scan in detecting severe wall thickening (>7 mm) were 0.75 [95% Confidence Interval (CI): 0.70–0.80)] and 0.72 (95% CI: 0.64–0.82), respectively (Fig. 4) (Fig. 5).

In the multivariate analysis, severe gallbladder wall thickening (greater than 7 mm) was independently associated with the rate of conversion to open and overall intraoperative events (OR = 2.0, p = 0.04, 95% CI: 1.0–6.2 and OR = 2.1, p = 0.003, 95% CI: 1.3–3.4, respectively). Gallbladder wall thickness independently predicted
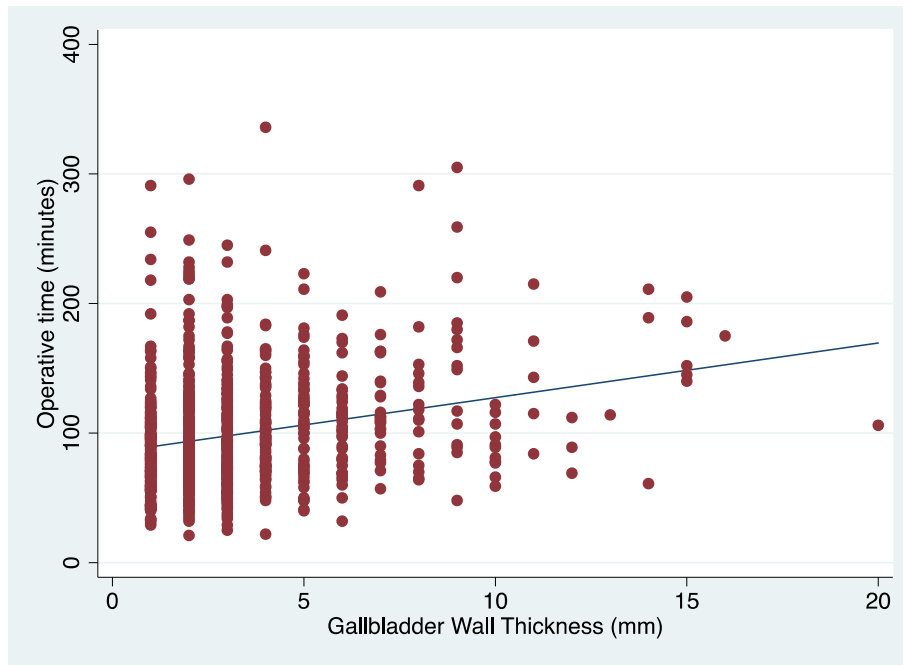
Fig. 1. Linear regression between Gallbladder Wall Thickness and Operative Time (correlation coefficient: 4.2, p = 0.03).

the length of hospital stay. Briefly, the adjusted odds ratio for hospital stay longer than four days was 1.5 (p = 0.024, 95% CI: 1.1–2.1) in patients with moderate thickening and 2.4 (p < 0.001, 95% CI: 1.5–3.8) in patients with severe thickening. Interestingly, duration of symptoms was not significantly associated with any of the outcomes of the study. See Tables 3–5 which demonstrate all the independent predictors of IEs overall, conversion rate and length of hospital stay.

Discussion

Our study showed that gallbladder wall thickness was a strong and objective measure of risk and complexity during laparoscopic cholecystectomy, as determined by operative time and intra-operative events. Additionally, GWT was shown to independently predict the length of hospital stay. According to previous studies, in acute cholecystitis, early laparoscopic cholecystectomy (optimally within 72 h and no later than 7 days of symptoms onset) has been shown to be superior to late LC in terms of morbidity, intra-operative complications and hospital length of stay.[4–6] Thus, the subjective report of duration of symptoms remains traditionally the current standard for estimating the level of inflammation and intraoperative difficulty. However, in our cohort, gallbladder wall thickness outperformed symptoms duration, which failed to show significant association with any of the outcomes.

Recent studies have shown a correlation between radiologic

Table 2
Comparison of demographics, comorbidities, intraoperative and postoperative outcomes by gallbladder wall thickness range in histopathologic specimen.

	<3 mm N = 474	3–7 mm N = 465	≥7 mm N = 112	p-value
Demographics				
Age (median, IQR)	43 (31–59)	49 (35–65)	56.5 (40–71)	< **0.001**
Male sex, n (%)	159 (33.5)	180 (38.7)	54 (48.2)	**0.011**
White race, n (%)	289 (66.4)	271 (64.1)	74 (71.2)	0.374
Obesity BMI ≥ 30, n (%)	184 (41.1)	179 (40.8)	48 (45.3)	0.687
ASA>2, n (%)	80 (16.9)	97 (21.0)	33 (29.9)	**0.008**
Diabetes melitus, n (%)	42 (8.9)	52 (11.2)	19 (17.1)	**0.04**
Smoking, n (%)	59 (12.9)	60 (13.5)	16 (14.3)	0.92
Preoperative diagnosis of cholecystitis, n (%)	202 (42.6)	273 (58.7)	90 (81.1)	< **0.001**
Days of symptoms, median (IQR)	2 (1–9)	2 (1–7)	3 (1–7)	0.74
Operative time in minutes, median (IQR)	84 (64–110)	94 (71–122)	113 (89–153)	< **0.001**
Non-elective, n (%)	326 (68.8)	354 (76.1)	96 (86.5)	< **0.001**
Total IE, n (%)	266 (57.1)	305 (67.5)	117 (85.3)	< **0.001**
Surgical drain placement, n (%)	27 (5.7)	51 (11.0)	29 (25.9)	<0.001
Conversion to open, n (%)	26 (5.5)	38 (8.2)	38 (34.2)	< **0.001**
Bile spillage, n (%)	247 (52.1)	289 (62.6)	87 (78.4)	< **0.001**
Bleeding, n (%)	1 (0.2)	2 (0.4)	4 (4.1)	< **0.001**
Bile duct injury, n (%)	1 (0.2)	1 (0.2)	1 (0.1)	0.894
Postoperative complications, n (%)	53 (12.9)	65 (16.2)	19 (19.0)	0.2
Infectious complications, n (%)	26 (6.3)	32 (8.0)	13 (13.0)	0.08
LHS, median (IQR)	2 (1–3)	3 (2–4)	4 (2–6)	< **0.001**

IQR: Inter Quartile Range, BMI: Body Mass Index, ASA: American Society of Anesthesiologists, IE: Intraoperative Events, LHS: Length of Hospital Stay.
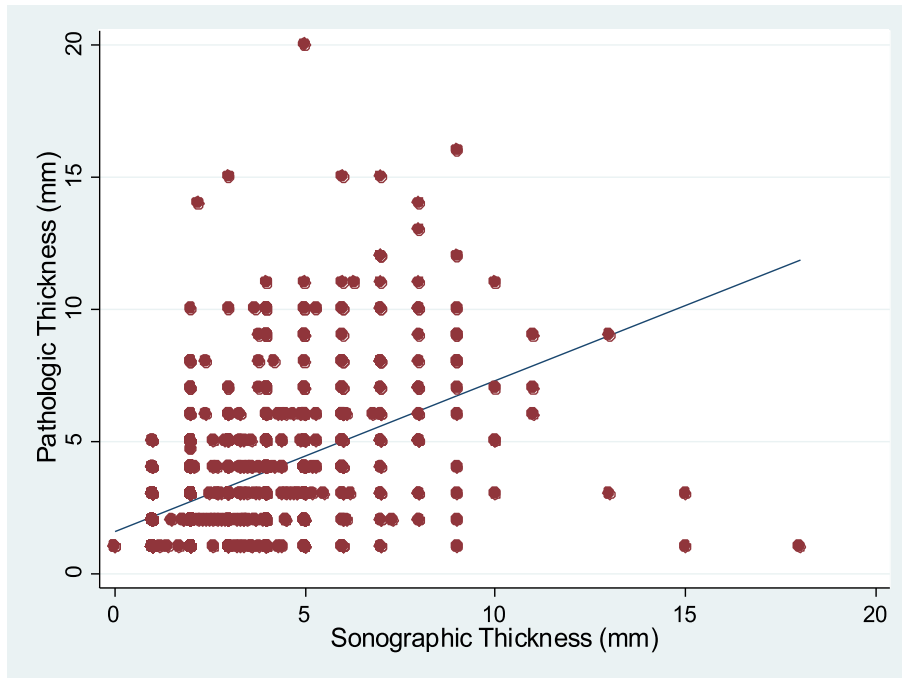
Fig. 2. Linear correlation between pathologic and sonographic gallbladder wall thickness (correlation coefficient: 0.57, p < 0.001).

evidence of gallbladder wall thickness and the rate of conversion to open, LHS and postoperative complications.[7–9] Our study was the first attempt that utilized two different medical disciplines (radiology and histopathology) in order to quantify thickness range of the gallbladder wall and, subsequently, appreciate the relationship of the latter with IE and postoperative outcomes. Engel et al. showed that wall thickness above 3.5 mm accurately predicted the diagnosis of acute cholecystitis.[10] Yang et al. in their metanalysis argued that sonographic findings of gallbladder wall thickness

were directly associated with the probability of conversion to open surgery,[11] evidence that was confirmed by the results of our study, as well. In our cohort, wall thickness - both preoperatively assessed and postoperatively confirmed - not only was correlated with the occurrence of cholecystitis, but it was independently associated with the rate of unplanned conversion to open procedure, regardless of whether the patient was diagnosed with cholecystitis or other diseases of the biliary tree, such as biliary colic, choledocholithiasis, gallstone pancreatitis and acute cholangitis. Terho

Fig. 3. Linear correlation between pathology and preoperative CT scan-based gallbladder wall thickness (correlation coefficient: 0.51, p = 0.017).
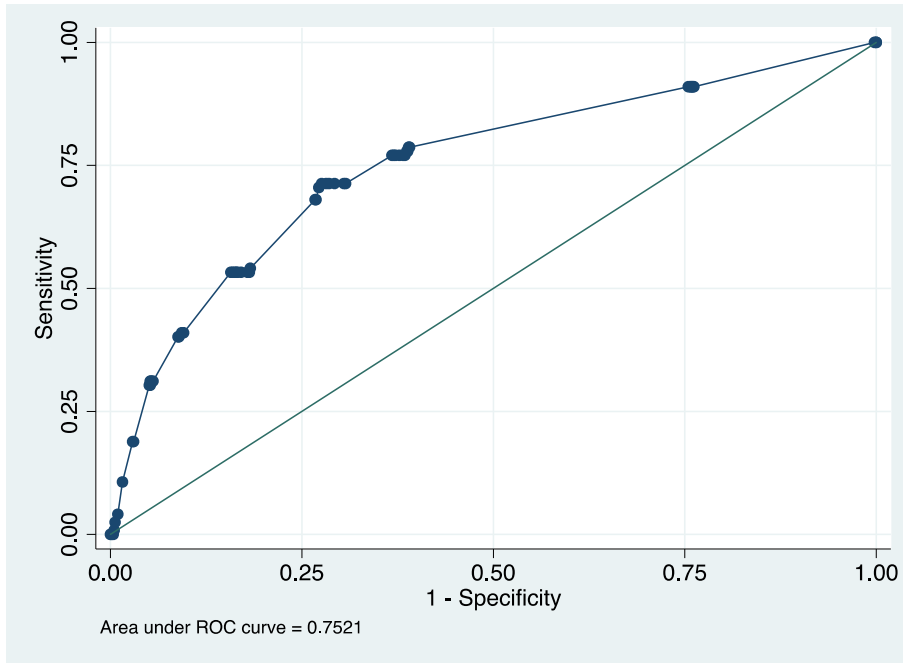
Fig. 4. Area Under ROC for preoperative ultrasonography.

PM et al. showed that the risk of postoperative complications is higher after conversion to open cholecystectomy.[12] Based on that evidence, gallbladder wall thickness -as a predictor of conversion- should be highly considered in the context of preoperative risk stratification regarding the risk of unplanned conversion to open procedure and postoperative outcomes. In our cohort, thickness greater than 7 mm, based on the histopathologic specimen, was directly correlated with intraoperative events overall, including surgical drain placement, conversion to open, bile spillage, major bleeding and common bile duct injuries. Furthermore, both moderate and severe wall thickening were associated with longer median hospital stay and, interestingly, independently predicted the odds of patients spending more than four days in the hospital postoperatively. Thus, GWT could be considered by the surgeon as a tool for appropriate patient counseling in regard to operative complexity, the risk of intraoperative events and the estimation of the length of hospital stay.

Surgical drain placement and, particularly, its role in laparoscopic cholecystectomies is still debatable. Literature has shown that it is not beneficial in elective, uncomplicated LC and drains should not be routinely placed in the majority of those cases.[13,14] However, in the context of emergency surgery, drains are
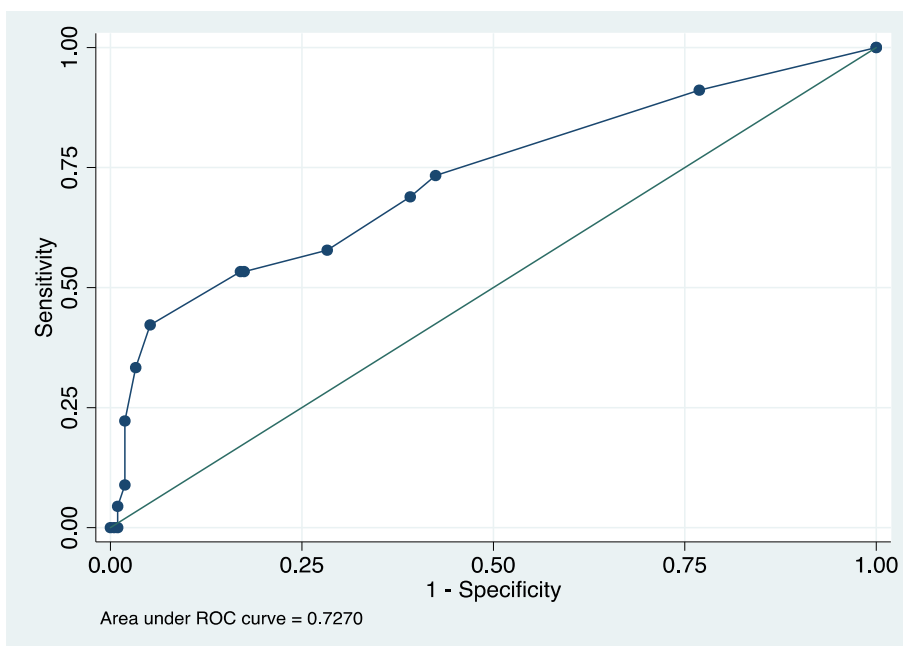

Fig. 5. Area Under ROC for preoperative CT scan.

Table 3
Adjusted odds ratio for intraoperative events (overall).

	Odds Ratio	p-value	95% CI
Histologic thickness			
3—7 mm	1.4	0.08	0.9—1.4
>7 mm	**2.1**	**0.003**	**1.3—3.4**
Radiologic thickness			
3—7 mm	0.8	0.11	0.5—1.1
>7 mm	0.7	0.34	0.4—1.4
Age >60	**1.8**	**0.002**	**1.2—2.5**
Cholecystitis	**2.0**	**< 0.001**	**1.6—2.7**
Male sex	**1.9**	**< 0.001**	**1.4—2.6**
Symptoms >7 days	0.8	0.1	0.5—1.1
ASA > 2	**1.9**	**0.003**	**1.2—2.9**

CI: Confidence Interval; ASA: American Society of Anesthesiologists.

Table 4
Adjusted odds ratio for unplanned conversion to open procedure.

	Odds Ratio	p-value	95% CI
Histologic thickness			
3—7 mm	1.2	0.6	0.6—2.3
>7 mm	**3.5**	**0.002**	**1.7—7.1**
Radiologic thickness			
3—7 mm	1.3	0.34	0.9—4.1
>7 mm	**2.6**	**0.004**	**1.0—6.2**
Symptoms >7 days	0.9	0.7	0.4—1.7
Male sex	**3.5**	**< 0.001**	**1.0—6.7**
ASA >2	**2.0**	**0.04**	**1.0—3.8**

CI: Confidence Interval; ASA: American Society of Anesthesiologists.

commonly placed by acute care surgeons in non-elective, complicated and challenging LCs. Our series showed that wall thickness was significantly associated with the rate of drain placement, thus, supplementarily advocating the hypothesis that GWT may have an implication as a marker of LC complexity.

Although CT scan has been shown to have better sensitivity in detecting wall thickening and cholecystitis than US,[15] in our study it demonstrated lower c-statistics compared to ultrasonography. Additionally, CT scan could not show an independent association with intraoperative events. This finding could be attributed to the fact that the number of patients who underwent CT scan was too low to show statistical significance, compared to those who had an abdominal ultrasound preoperatively in our cohort. However, both ultrasound and CT scan had a positive linear correlation with histopathology as well as a dignified accuracy in detecting severe (>7 mm) gallbladder wall thickening. Thus, they should be featured in preoperative GWT assessment in patients undergoing LC.

Table 5
Adjusted odds ratio for length of hospital stay (>4 days).

	Odds Ratio	p-value	95% CI
Histologic thickness			
3—7 mm	**1.4**	**0.044**	**1.1—2.1**
>7 mm	**2.3**	**< 0.001**	**1.4—3.6**
Radiologic thickness			
3—7 mm	1.2	0.45	0.8—1.7
>7 mm	1.4	0.29	0.8—2.5
Intraoperative events	**1.9**	**0.001**	**1.3—2.7**
Symptoms > 7 days	0.7	0.07	0.5—1.0
Age > 60	**2.4**	**< 0.001**	**1.7—3.4**
Non-elective surgery	**1.6**	**< 0.001**	**1.2—2.5**
Male sex	**1.8**	**< 0.001**	**1.3—2.4**
ASA>2	**5.2**	**< 0.001**	**3.5—7.7**
Cholecystitis	**0.4**	**< 0.001**	**0.3—0.6**
Alcohol use	0.8	0.46	0.4—1.6

CI: Confidence Interval; ASA: American Society of Anesthesiologists.

Despite the fact that postoperative complications were more commonly observed in patients with thickened gallbladder wall (see univariate analysis on Table 2), our cohort did not manage to directly show an independent association between thickness range and postoperative outcomes. It did show, however, a strong association between thickness and bile spillage. The latter was an intraoperative event that, according to a recent study, was shown to be an independent predictor of postoperative surgical site infections.[16] Thus, the statement that gallbladder wall thickness might also indirectly predicted postoperative infectious complications could be a reasonable assumption.

This was a large institutional cohort of more than one thousand patients, who underwent laparoscopic cholecystectomy and were prospectively followed up. Limitations that our study may carry should be addressed, though. It represented a single center experience. Particularly, the vast majority of the patients were operated on a non-elective basis in an acute care surgery setting and the outcomes might not be generalizable. Additionally, variability and discrepancy between imaging and histopathologic measurements could not be diminished entirely for two main reasons. First, some degree of subjectivity of radiology and pathology experts, when examining the organ, should be kept in consideration. Second, studies have shown that histological processing can cause an average of 11% shrinkage between fresh and pathologic specimens due to formalin or other types of fixation.[17,18]

Conclusions

Gallbladder wall thickness was an accurate and independent predictor of intraoperative events and length of hospital stay in patients undergoing laparoscopic cholecystectomy, outperforming symptoms duration, the current standard of assessing overall operative complexity. Thus, it may serve as an objective modality for preoperative risk stratification and appropriate patient counseling.

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Declaration of competing interest

We have no conflicts of interest to report.

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References

1. Pontarelli EM, et al. Regional cost analysis for laparoscopic cholecystectomy. *Surg Endosc.* 2019;33:2339—2344.
2. Lee VS, Chari RS, Cucchiaro G, Meyers WC. Complications of laparoscopic cholecystectomy. *Am J Surg.* 1993;165:527—532.
3. Kaftori J, Pery M, Green J, Gaitini D. Thickness of the gallbladder wall in patients with hypoalbuminemia: a sonographic study of patients on peritoneal dialysis. *Am J Roentgenol.* 1987;148:1117—1118.
4. Cao AM, Eslick GD, Cox MR. Early laparoscopic cholecystectomy is superior to delayed acute cholecystitis: a meta-analysis of case—control studies. *Surg Endosc.* 2016;30:1172—1182.
5. Acar T, et al. Laparoscopic cholecystectomy in the treatment of acute cholecystitis: comparison of results between early and late cholecystectomy. *Pan Afr Med J.* 2017;26:49.
6. Paudel R. Is it safe to perform laparoscopic cholecystectomy for acute calculus

cholecystitis within 7 Days following symptom onset? *J Nepal Med Assoc JNMA.* 2018;56:945–948.

7. Raman SR, et al. The degree of gallbladder wall thickness and its impact on outcomes after laparoscopic cholecystectomy. *Surg Endosc.* 2012;26: 3174–3179.

8. Kania D. Ultrasound measurement of the gallbladder wall thickness in the assessment of the risk of conversion from elective laparoscopic cholecystectomy to open surgery — olkusz county experience. *Pol J Surg.* 2016;88: 334–345.

9. Kama NA, et al. Risk factors resulting in conversion of laparoscopic cholecystectomy to open surgery. *Surg Endosc.* 2001;15:965–968.

10. Engel J, Deitch E, Sikkema W. Gallbladder wall thickness: sonographic accuracy and relation to disease. *Am J Roentgenol.* 1980;134:907–909.

11. Yang TF, Guo L, Wang Q. Evaluation of preoperative risk factor for converting laparoscopic to open cholecystectomy: a meta-analysis. *Hepato-Gastroenterology.* 2014;61:958–965.

12. Terho PM, Leppäniemi AK, Mentula PJ. Laparoscopic cholecystectomy for acute calculous cholecystitis: a retrospective study assessing risk factors for conversion and complications. *World J Emerg Surg.* 2016;11:54.

13. Picchio M, et al. Drain after elective laparoscopic cholecystectomy. A randomized multicentre controlled trial. *Surg Endosc.* 2012. https://doi.org/10.1007/s00464-012-2252-1.

14. Gurusamy KS, Koti R, Davidson BR. Routine abdominal drainage versus no abdominal drainage for uncomplicated laparoscopic cholecystectomy. *Cochrane Database Syst Rev.* 2013. https://doi.org/10.1002/14651858.CD006004.pub4.

15. Fagenholz PJ, et al. Computed tomography is more sensitive than ultrasound for the diagnosis of acute cholecystitis. *Surg Infect.* 2015;16:509–512.

16. Peponis T, et al. Bile spillage as a risk factor for surgical site infection after laparoscopic cholecystectomy: a prospective study of 1,001 patients. *J Am Coll Surg.* 2018;226:1030–1035.

17. El-Fol HA, Noman SA, Beheiri MG, Khalil AM, Kamel MM. Significance of post-resection tissue shrinkage on surgical margins of oral squamous cell carcinoma. *J Cranio-Maxillo-Fac Surg.* 2015;43:475–482.

18. Pereira-Sampaio MA, Marques-Sampaio BPS, Sampaio FJB, Henry RW. Shrinkage of renal tissue after impregnation via the cold Biodur plastination technique. *Anat Rec.* 2011;294:1418–1422.