J Gastrointest Surg (2011) 15:614–622
DOI 10.1007/s11605-011-1432-y

ORIGINAL ARTICLE

Evaluating Systemic Stress Response in Single Port vs. Multi-Port Laparoscopic Cholecystectomy

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Received: 7 November 2010 / Accepted: 19 January 2011 / Published online: 10 February 2011

Abstract
Background and Aims Acute-phase proteins and inflammatory cytokines mediate measurable responses to surgical trauma, which are proportional to the extent of tissue injury and correlate with post-operative outcome. By comparing systemic stress following multi-port (LC) and single-incision laparoscopic cholecystectomy (SILC), we aim to determine whether reduced incision size induces a reduced stress response.
Methods Thirty-five consecutive patients were included, 11 underwent SILC (mean ± SEM; age 44.8±3.88 year; BMI 27±1.44 kg/m^2) and 24 underwent LC (56.17±2.80 year; 31.72±1.07 kg/m^2, $p<0.05$). Primary endpoint measures included levels of interleukin-6 and C-reactive protein measured pre- and post-operatively. Length-of-stay (LOS) and postoperative morbidity were secondary endpoints.
Results No statistically significant differences were found between SILC and LC for interleukin-6 and C-reactive protein levels, LOS and duration of surgery. There was also no correlation between systemic stress response and operative parameters. There were no intra-operative complications.
Conclusion SILC appears to be a safe, feasible technique with potential advantages of cosmesis, reduced incisional pain, and well-being recommending its use. These data indicate no difference in systemic stress and morbidity between SILC and LC. A larger, multi-centred, randomised prospective trial is warranted to further investigate and confirm this finding.

Keywords Cytokines · Acute phase response · Single incision laparoscopic surgery (SILS) · Cholecystectomy

Presented at London Surgical Symposium, 9th September 2010, London and Association of Laparoscopic Surgeons of Great Britain and Ireland (ALSGBI) Annual Scientific Meeting 2010, 25th–26th November, Nottingham, UK.

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Introduction

Over recent decades, the evolution of laparoscopic techniques has transformed much of traditional surgery. Compared to an open approach, minimally invasive techniques have proven effective in reducing surgical trauma, thereby improving patient recovery and length of hospital stay.[1,2] Benefits of improved postoperative pain and cosmesis are now well established for many operations.[3–6] The benefits displayed by conventional laparoscopic cholecystectomy (LC) have established it as the gold standard for gallbladder removal;[7] however, surgeons have since sought to further reduce the size and number of incisions[5,8,9] with single-incision laparoscopic surgery (SILS) being one of the latest innovations.

SILS is a technique which offers theoretical advantages of reduced pain and complications by limiting the number of incisions to one.

In general surgery, SILS involves the introduction of laparoscopic instruments via the same access point in the abdominal wall; typically the umbilicus, which can result in an almost invisible scar.[10] Issues to overcome with SILS include in-line instrument clashes, and loss of triangulation, with articulating instruments and extra long laparoscopes being developed as a result.[11]

SILS demonstrates feasibility and reproducibility, with surgical safety and outcomes remaining uncompromised in published series of benign disease.[10,12] Although the cosmetic benefit[12,13] and reduced incisional pain are described,[13] the literature has not reported any other significant differences between SILS and conventional laparoscopy. Systemic stress response, postoperative morbidity, and patient satisfaction are yet to be defined.

The extent of surgical trauma has been evaluated by assessing the systemic stress response.[14] Total white cell count (WCC) and acute phase reactant C-reactive protein (CRP) are known indicators of tissue injury.[4,14] In addition, the cytokine response to surgical injury has been well documented.[2,15] An acute-phase response is triggered following surgical injury which can be detected in peripheral blood.[2] The cytokine interleukin-6 (IL-6) is a major mediator of this response. Following acute injury, IL-6 produced by virtually all cells peaks in the circulation 4–6 h post injury.[15] IL-6 levels have been shown to be proportional to the extent of injury;[15] however, this exact mechanism remains unknown. IL-6 can regulate the synthesis of hepatic acute-phase proteins such as CRP. Increases in CRP plasma levels following surgery are positively correlated with the increase seen in IL-6.[16]

Several authors have examined cytokine profiles following LC, mini-open, and open cholecystectomy (OC).[17] Grande et al.[16] observed that postoperatively, patients undergoing OC had significantly greater increases in serum levels of IL-6 and CRP compared to LC. These findings are also supported by other investigators.[2,4,18,19] Authors have demonstrated a significant difference in the systemic stress profiles following different surgical approaches despite the technique of cholecystectomy remaining the same; supporting that surgical trauma produces a measurable response, the magnitude of which being proportional to the extent of tissue injury.[14,15,19]

With regard to postoperative morbidity, exaggerated elevations in IL-6 have been shown to be linked with the onset of major clinical complications.[20] It has also been reported that postoperative plasma levels of IL-6 are early indicators of postoperative wound infections.[21]

A comparison of inflammatory mediators following SILC and LC provides a model to investigate the extent to which the systemic response is influenced by surgical access. Since the technique of cholecystectomy is the same for both approaches, we hypothesise that any difference in systemic response can be attributed to the difference in the size and number of incisions. We hypothesise the reduction in total incision size seen in SILC will result in a reduced systemic stress response with a potential decrease in postoperative morbidity.

Patients and Methods

Study Design and Subjects

This single centre, non-randomised study received ethical approval by the St. Mary's Hospital Research Ethics Committee (REC ref:08/H0712/146). Data were collected at St Mary's Hospital from February through May 2010. All patients undergoing SILC or LC were considered for inclusion. Patients were excluded if they had co-morbidities resulting in raised inflammatory markers (such as autoimmune disease, malignancy or infection). Informed consent was obtained after verbal and written information were given. Performing the SILC technique was at the discretion of the surgeon.

There is no data in the literature to base a power calculation on; however, based on previous studies comparing cytokine variations in LC and open cholecystectomy,[16,18] we aimed to reach a sample size of at least 11 SILC and 11 LC subjects.

Operative Techniques

All operations were performed by one of five attending-led operative teams. A standardised anaesthetic protocol was followed for all patients.

SILS Cholecystectomy

The technique used at our institution has been previously described in the literature by our institution and the technique used for this study was the same.[22] In short, SILC involved the introduction of laparoscopic instruments and a 5 mm diameter 30° laparoscope into the umbilicus via a 12 mm bladed, but disarmed port and a 5 mm Dexide port (Covidien, Mansfield, MA, USA).[1] With two suspension sutures (0 silk) placed in the right upper quadrant and through the fundus and infundibulum, respectively, traction of the gallbladder was maintained. The principle of cholecystectomy was then carried out in the traditional

[1] Covidien, Mansfield, MA, USA

fashion adhering to principles of safety during dissection, demonstration of the critical view and wide posterior window and clipping of the cystic duct and artery, with gallbladder retrieval into an endo bag through the umbilical incision.

Multi-port Laparoscopic Cholecystectomy

This technique was performed using three 5-mm ports and one 10-mm port. Ports were positioned in the standard fashion at the mid-epigastrium, right lateral, right subcostal and umbilical positions, respectively. Unlike the sutures used in SILC, the assistant surgeon maintained traction of the gallbladder. The principle of cholecystectomy was then carried out as previously described. Conversion to either LC or OC in SILC and LC, respectively, was performed when the surgeon felt it necessary. Reasons for conversion were reported in the operative notes.

We have considered that the larger single incision (12 mm) and multiple fascial incisions used in SILC will be more traumatic than the typical umbilical incision in LC (10 mm); however, we believe it will overall result in a decreased systemic stress response. There are still no robust long-term data on incisional hernia rates following this larger incision however there is a hypothetical increased risk of port-site herniation.

Data Collection

Perioperative data was recorded for all patients as illustrated in Table 1. Primary endpoint measures included plasma levels of IL-6 and CRP. LOS and postoperative morbidity were secondary endpoints.

Patient demographics, indication for surgery and co-morbidities were recorded. Operative time was measured from the first incision to the closure of the final wound. As operative time can be affected by unforeseen delays such as faulty equipment and/or the experience of the surgeon, the grade of the surgeon and delays were recorded. Operative parameters were documented from the operative notes and personal observation. LOS was measured from the incision time to the patient's discharge time. Any readmissions were added to the patient's original LOS.

Sample Method and Times

Peripheral venous samples were taken preoperatively as baseline. Postoperative samples were subsequently collected at 6±24 h from the incision time. For cytokine analysis, 6 ml blood samples were taken into EDTA blood collection tubes. Within 30 min of collection, samples were centrifuged for 15 min at 1,000×*g*, before the supernatant was separated, aliquoted and samples were stored at −80°C for subsequent analysis.

Table 1 Assessed parameters

Patient demographics	Sex
	Age
	Height, weight and BMI
	Presenting condition
	Co-morbidities—ASA grade
	Past surgical history
Operative parameters	Incision time
	Closure time
	Length of surgery
	Number of incision(s)
	Size of incision(s)
	Total size of incision
	Grade of surgeon
	Bile spillage
	Intra-operative complications/notes
	CO_2 insufflation
	Drain
	Conversion[a]
Outcome	Length of hospital stay (LOS)
	Follow up questionnaires[b]

[a] Conversion is defined as the addition of one or more trocars to the SILS technique and conversation to open in the LC technique

[b] Carried out at 2 weeks and 2 months

Follow Up

Patients were followed up at 2 weeks and 2 months postoperatively. Data collected using a standardised questionnaire included wound healing, postoperative pain, gastrointestinal symptoms and any post-operative medical consultations.

Cytokine Assays

IL-6 levels were measured using a commercially available high-sensitivity enzyme-linked immunosorbent assay (ELISA; Quantikine HS [High Sensitivity] Human IL-6, R&D Systems Europe)[2] according to manufacturer's instructions. All readings taken from the ELISA plates were standardised by assessing positive control values that were assayed in duplicate on each plate. All samples were assayed in duplicate and the standard curve ran from 10 to 0.156 pg/ml.

[2] ELISA; Quantikine HS [High Sensitivity] Human IL-6, R&D Systems Europe

Statistical Analysis

All analyses were carried out using statistical software within GraphPad Prism (Version 5.03, San Diego, CA, USA).[3] Fisher's exact test was used to evaluate significant differences of categorical variables when the sample size was <10. Mann–Whitney *U* test was used in the analysis of non-parametric variables with a larger sample size. Spearman's rank was used for correlations of continuous variables. Statistical significance was set at $p<0.05$.

Results

Patient inclusion and participation is detailed in Fig. 1. The total number of patients available for analysis was 35. The demographics of all 35 patients are shown in Table 2. SILC subjects (2 M:9 F) were typically younger and had a lower BMI compared to LC subjects (7 M:17 F; $p<0.05$). No other significant difference was found in the demographics of patient groups.

Operative Outcomes

Mean duration of surgery was longer for SILC (86.91±8.97 vs. 79.08±4.24 min) however this was not statistically significant. Attendings were more likely to perform SILC (82% vs. 42%; $p=0.04$). No significant difference was seen in the cases of bile spillage and total CO_2 insufflation. Of the LC group, three patients were converted from SILC due to either poor visibility or unclear anatomy. Intra-operatively there were no complications (Table 3) aside from the higher proportion of drains inserted in the LC group (7 vs. 0).

Systemic Stress Response

The systemic stress response of the patient was measured as a change from baseline and compared at 6 and 24 h postoperatively. No significant variations between the baseline values of each group were noted, making them comparable.

IL-6

IL-6 levels significantly increased from baseline to 6 h post operation in the LC group (2.28±0.40–8.65±1.83 pg/ml; $P<0.0001$, Fig. 2a) as well as in the SILC group (1.57±0.28–5.1±1.20; $P=0.0006$), with a greater percentage increase in the LC group, although this was not statistically significant between the groups. At 6 h, IL-6 levels were higher in the LC group compared to the SILC group, but again, this was not statistically significant ($p=0.0673$; Fig. 2a). In the LC group there was a decrease in IL-6 concentration between 6 and 24 h (Table 3). There was no correlation between operative time and systemic stress response ($p=0.94$). There was no significant difference in plasma levels of TNF-α and WCC between the two groups at any time point (data not shown).

CRP

Plasma CRP levels (Fig. 2b) postoperatively increased in both groups at 6 h; this was not statistically significant. There was a remarkable increase in CRP from 6 to 24 h postoperatively in the LC group ($p<0.05$), demonstrating that CRP may peak at 24 h.

As previously described, there is a learning curve associated with any laparoscopic technique and the complexity of the SILS technique, makes it particularly challenging.[3] Although there was a difference in the grade of surgeon performing the SILC and LC techniques, analysis comparing the grade of surgeon vs. the systemic stress response (Fig. 3a) found no statistically significant difference; incidentally, the attendings generated a slightly higher IL-6 response in both study groups compared to residents but this was not significant. Duration of surgery (Fig. 3b) also showed no significant difference between residents and attendings. Bile spillage between the groups was not shown to be significant.

Postoperative Outcomes

Mean LOS was slightly shorter in the LC group compared to the SILC group (0.97±0.35 vs. 0.86±0.11 days; $p=0.42$). There was one readmission within the SILC group due to erythema and pain at the umbilicus. This readmission was included in the patient's LOS and may have skewed the SILC group's mean LOS.

Follow up revealed that the LC group visited a doctor on more occasions than the SILC group (14% vs. 0%). With regard to the wound(s); within the LC group, 38% stated that at least one wound had not healed compared to 29% of the SILC group. The LC group also had a higher proportion of minor wound site bleeding (9% vs. 0%) and infection (5% vs. 0%; Table 3).

Discussion

The surgical trauma induced inflammatory response is well defined within the literature.[2,15] Primarily, the physiological response to surgical trauma is equal to that of infection or injury i.e. the induction of the acute-phase response;

[3] GraphPad Prism (Version 5.03, San Diego, CA, USA)

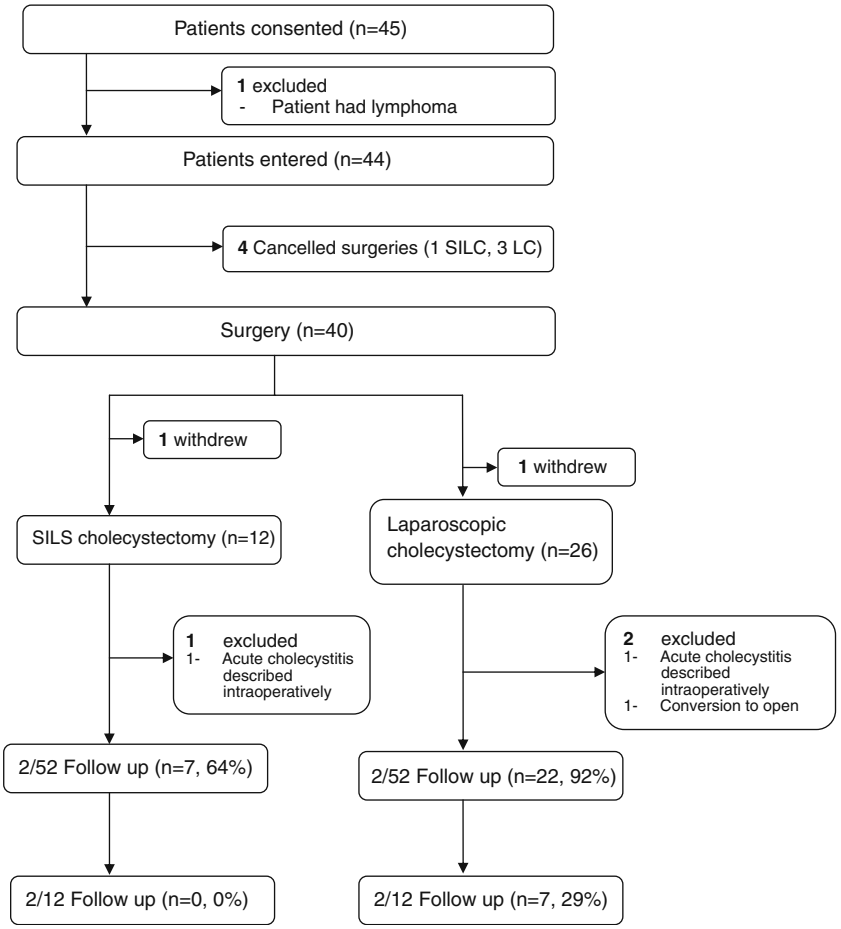

Fig. 1 Flow of subjects (follow up at 11/05/2010)

Table 2 Patient demographics

Characteristics			Patients with SILC (n=11) (%)	Patients with LC (n=24) (%)	p Value
Sex	Male		2 (18%)	7 (29%)	0.6855
	Female		9 (82%)	17 (71%)	
Mean	Age (years)		44.82 [3.88]	56.17 [2.80]	0.0218
	Weight (kg)		75.21 [6.36]	86.35 [2.33]	0.0190
	Height (m)		1.658 [0.04]	1.657 [0.02]	0.9574
	BMI (kg/m^2)		27 [1.44]	31.72 [1.07]	0.0219
Co morbidities	ASA grades	1	5 (45)	9 (38)	
		2	6 (55)	13 (54)	
		3	0 (0)	1 (4)	
		4	0 (0)	1 (4)	
Indications for surgery	Biliary colic		2 (18)	5 (21)	
	Symptomatic gallstones		6 (55)	16 (76)	
	Abdominal pain		0 (0)	1 (4)	
	Previous gallstone pancreatitis/ cholangitis/cholecystitis		2 (18)	2 (8)	
Past surgical history	Nil		3 (27)	8 (33)	
	Upper GI surgery		0	2 (8)	
	Lower GI surgery		2 (18)	4 (17)	
	Non GI		6 (55)	10 (42)	

[] Standard error of the mean (SEM)

Table 3 Operative, post operative outcomes and systemic stress markers

Characteristics				Patients with SILC (n=11)	Patients with LC (n=24)	p Value
Operative parameters	Operative time (mins)			86.91 [8.97]	79.08 [4.24]	0.3108
	Total incision size (mm)			13.64 [1.26]	33 [1.29]	<0.0001
	Grade of surgeon	Attending		9	10	0.0354
		Resident		2	14	
	Bile spillage	Yes		4	6	0.6889
		No		7	18	
	Conversion			3	0	
	Total CO_2 insufflation (litres)			240.3 [81.12]	118.5 [32.23]	0.1996
	Complications			nil	nil	
Intra-operative findings	Acute cholecystitis			0	0	
	Mucocele			0	3	
	Inflamed gallbladder			3	8	
	Stone impeded in Hartmanns pouch			2	3	
	Gallbladder Adhesions			0	6	
	Gallstone/sludge spillage			0	1	
	Abnormal anatomy			1	2	
	Umbilical hernia			1	1	
	Drain			0	7	
	Liver pathology			1	2	
	Distended gallbladder			2	4	
Inflammatory markers	IL-6 (pg/ml)	Time points	n (SILC, LC)			
		t=0	(11, 22)	1.571 [0.28]	2.278 [0.40]	0.2146
		t=6	(11, 24)	5.100 [1.20]	8.648 [1.83]	0.0673
		t=24	(0, 3)	–	7.422 [0]	–
	CRP (mg/l)	t=0	(11, 22)	3.227 [0.53]	4.682 [0.75]	0.2403
		t=6	(11, 24)	3.609 [0.73]	5.292 [0.79]	0.3251
		t=24	(0, 3)	–	46.000 [25.63]	–
Post operative	LOS (days)			0.97 [0.35]	0.86 [0.11]	0.4238
	Follow up (2/52)			n=7	n=22	
		No complications		4 (57%)	5 (23%)	
		Wound—infection		0 (0%)	1 (5%)	
		Wound—bleeding		0 (0%)	2 (9%)	
		Wound—scarring		1 (14%)	1 (5%)	
		At least one wound not healed		2 (29%)	11 (50%)	
		GI Symptoms		4 (57%)	12 (55%)	
		Non GI Symptoms		3 (43%)	8 (36%)	

[] SEM

LOS Length of stay

The 24 h sample was omitted for SILC cases due to the majority being day cases

reflected in cytokine function and cellular messenger systems.[2] The magnitude of these changes is reflected proportionally to the extent of the surgical trauma.[4]

Many studies support LC as the gold standard over the traditional open approach based on results demonstrated by cytokine response profiles.[4,16,23] To date, there has been no study comparing the systemic stress response of SILC vs. LC. The results of this trial reject the hypothesis that a single incision results in a decreased systemic stress response.

In this trial, postoperative IL-6 levels significantly increased over baseline values in both SILC and LC groups; supporting the described acute-phase response following surgery.[2,15] However, no significant differences

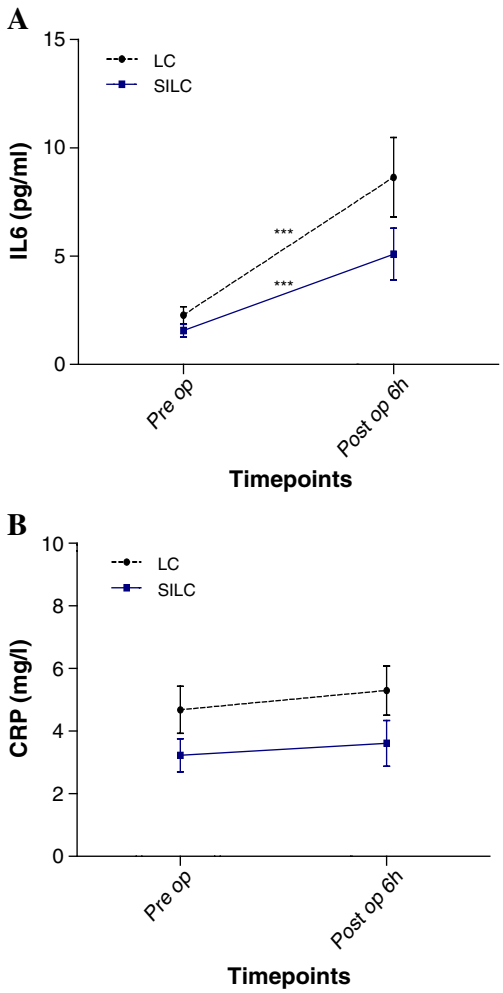

Fig. 2 Plasma concentration of IL-6 and CRP preoperatively (t=0 h) and postoperatively (t=6 h) A: IL-6, a significant difference is seen between t=0 and t=6 in both SILC group (p=0.0006) and LC group (p<0.0001). *Error bars* denote standard error of the mean (SEM). ***p<0.001; **0.001–0.01 (Mann–Whitney U test)

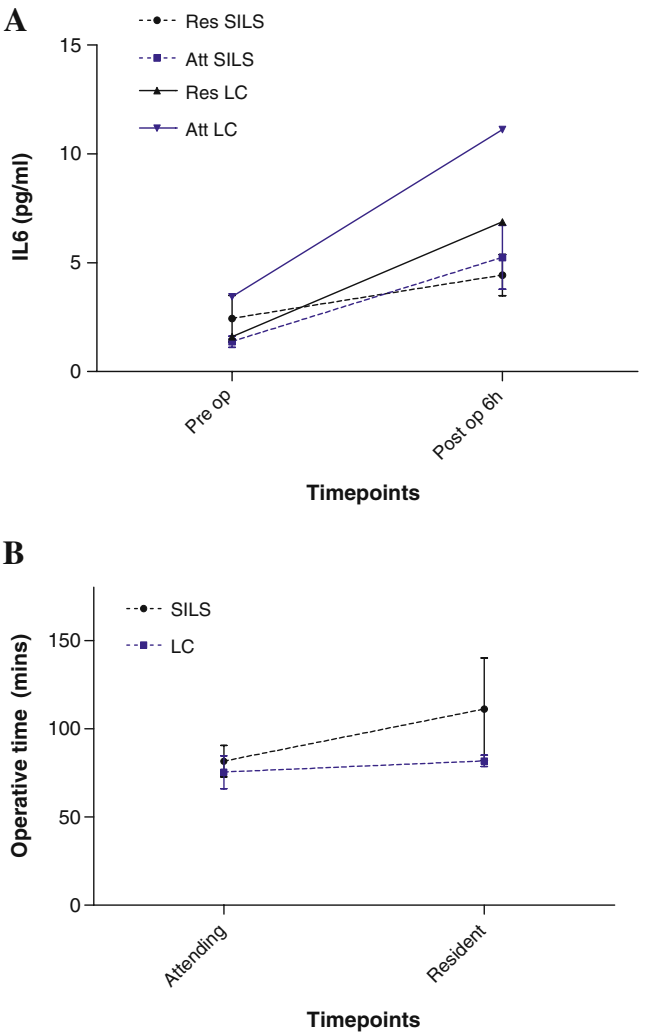

Fig. 3 **a** Grade of surgeon vs. the systemic stress response (IL-6) in SILC and LC. No significant difference found between residents or attendings (Mann–Whitney U Test).

	p value IL-6
Res SILC 6h vs. Att SILC 6h	**0.9091**
Res LC 6h vs. Att LC 6h	**0.2081**

b Grade of surgeon vs. operative time. No significant difference (p= 0.372; Kruskal-Wallis test). *Error bars* denote SEM

were found between the groups although there was a trend for the SILC group to have lower IL-6 plasma levels at 6 h post surgery.

There was no significant difference in the CRP levels postoperatively between SILC and LC. Authors[16] have stated that in the acute-phase response, CRP production is proportional to the increase in IL-6. Our findings conversely showed no significant correlation between IL-6 and CRP (r=−0.23, p=0.29)

The majority of SILC cases were day cases and so the 24 h sample was often omitted. This therefore resulted in a lack of data for SILC at 24 h. As CRP peaks at 24 h,[2] the absence of these time points in the SILC group did not give a representative comparison of CRP in SILC vs. LC. Routine analysis of CRP was performed with staff blind to the two patient groups therefore ruling out potential detection bias.

Total CO_2 insufflation was higher in the SILC group (240.3 L vs. 118.5, p = ns). Recent studies suggest that CO_2 pneumoperitoneum may influence systemic stress.[2] It is thought that the production of cytokines, namely TNF-α and IL-1, in peritoneal macrophages is suppressed due to the acidic environment of CO_2. These findings are not wholly reliable as in SILC, a portion of the total CO_2 "insufflated" is unaccounted for by gas leakage at the port site.[3]

Early studies comparing multi-port LC to OC reported an increased incidence in bile duct injuries.[24,25] However, recent studies have shown that the reported intraoperative complication rate of SILC is comparable to LC;[26–28] in our

study, there were no incidences of complications in either the SILC or LC cohorts. In this trial, we found that there was no difference in the LOS and overall, the SILC group had a better postoperative recovery with fewer cases of consulting a doctor and wound complications. The sample sizes in this study were similar to those of previous studies that demonstrated significance with a comparable methodology.[16,18] Although not suggested by the data, a type 2 error always needs to be considered, especially when dealing with relatively small sample sizes. The ethical issues associated with randomisation meant that we conducted this trial without randomisation. This led to the trial being open to selection bias, as demonstrated in the significant differences in age, weight and BMI. Although this heterogeneity was unavoidable, other characteristics, baseline values and operative parameters were comparable between the groups. The learning curve of the surgeon may have also introduced procedural bias. Nevertheless, our results showed that the grade of surgeon had no effect on systemic stress.

In conclusion, this trial did not demonstrate a significant difference in systemic stress or postoperative morbidity between SILC and LC, identifying SILC to be quite comparable to LC. To overcome the limitations of this study a larger, multi-centred, randomised prospective trial is warranted; to further investigate and confirm our findings. However, based on the results of this trial, we suggest that SILC is a safe and feasible technique, which has at least equivalent peri-operative outcomes to LC with obvious advantages of cosmesis and theoretical advantages of reduced analgesic requirements and well-being in this patient group.

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