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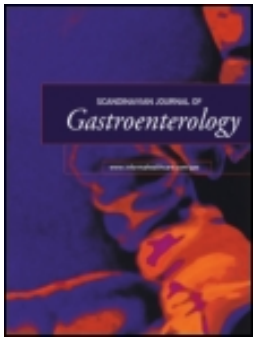
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ORIGINAL ARTICLE

Inflammatory response to surgical trauma in patients with minilaparotomy cholecystectomy versus laparoscopic cholecystectomy: a randomised multicentre study

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ABSTRACT

Objective The aim of the study was to evaluate the inflammatory response to surgical trauma in minilaparotomy cholecystectomy (MC) compared to laparoscopic cholecystectomy (LC). Assessment of inflammatory response to surgical trauma in MC has not been addressed properly. Therefore, we investigated five interleukins (IL) and C-reactive protein (CRP) in MC versus LC group in a prospective randomised trial. **Methods** Initially, 106 patients with non-complicated symptomatic gallstone disease were randomised into MC ($n = 56$) or LC ($n = 50$) groups. Plasma levels of five interleukins (IL-1 β , IL-1ra, IL-6, IL-8, IL-10) and hs-CRP were measured at three time points; before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2). The primary end-point of the study was to compare the plasma levels of five interleukins and CRP in LC versus MC group. **Results** The demographic variables and the surgical data were similar in the study groups. The patients in the MC group had higher elevation of the CRP mean values post-operatively ($p = 0.01$). However, the patients in the MC group had higher elevation of the IL-1ra mean values post-operatively, the mean pre-/post-operative IL-1ra values being 299/614 pg/ml in the MC group versus 379/439 pg/ml in the LC group ($p = 0.003$). There was no statistical significance in IL-6 mean values between the MC and LC groups pre- and post-operatively (POP1). However, the patients in the MC group had higher IL-6 mean values six hours post-operatively (POP2), the mean IL-6 values being 27.6 pg/ml in the MC group versus 14.8 pg/ml in the LC group ($p = 0.037$). In addition, the patients in the MC group had higher elevation of the IL-6 mean values post-operatively, the mean pre-/post-operative IL-6 values being 4.1/27.6 pg/ml in the MC group versus 3.8/14.8 pg/ml in the LC group ($p = 0.04$). There was no statistical significance in IL-8, IL-10, and IL-1 β mean values between the MC and LC groups pre- and post-operatively. **Conclusion** Our results suggest that the inflammatory response in MC versus LC groups was similar based on the IL-8, IL-10, and IL-1 β values. A new finding with possible clinical relevance in the present work is higher relative elevation of the IL-1ra and IL-6 mean values post-operatively in the MC group.

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Introduction

Surgical trauma stimulates the acute inflammatory response and thereby the production of cytokines.[1] The most important cytokines and proteins are interleukin-1 (IL-1), interleukin-6 (IL-6), and C-reactive protein (CRP). The plasma levels of these cytokines are suggested to reflect the extent of trauma, that is, larger the trauma, larger the cytokine response.[2] IL-6 controls cellular metabolic activity and stimulates the production of CRP. The high levels of IL-6 are associated with

increased morbidity and mortality.[2] CPR activates the complement cascade and stimulates phagocytosis by macrophages and neutrophils. The CRP levels will increase from 12 to 72 h post-operatively and return to baseline in two weeks.[2] The inflammatory response to surgical trauma in patients with minilaparotomy cholecystectomy versus laparoscopic cholecystectomy has been assessed in one prospective study. McMahon et al. [3] studied CRP and IL-6 levels in MC ($n = 10$) versus LC ($n = 10$) patients and found no significant difference

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between the study groups. To our knowledge, assessment of the inflammatory response to surgical trauma in MC versus LC with ultrasonic dissection (UsD) in both groups has not been addressed properly. Therefore, we investigated five interleukins (hsIL-1 β , IL-1ra, IL-6, hsIL-8, hsIL-10) and hs-CRP at three time points; before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2). The primary end-point of the study was to compare plasma levels of five interleukins and CRP in LC versus MC group. Because of the benefits of ultrasonic dissection, we used the UsD in both groups. We have previously reported that LC patients reported significantly lower pain score 24 hours postoperatively and a shorter convalescence [4] than the MC patients in a randomised trial. Therefore, the hypothesis of our study was that the outcome difference between MC and LC would also be reflected in the inflammatory response to surgical trauma.

Subjects and methods

The study was approved by the Ethics Committee of Helsinki and Uusimaa University District, Helsinki, Finland (DNRO 120/13/02/02/2010, 12 May 2010), it was registered in the ClinicalTrials.gov database (ClinicalTrials.gov Identifier: NCT0172340, Consort diagram, Figure 1), and it was conducted in accordance with the Declaration of Helsinki. Participants gave written consent after receiving verbal and written information. Operations were carried out in two hospitals in Finland; Helsinki University Central Hospital, Helsinki ($n=28$) and Kuopio University Hospital, Kuopio ($n=78$) between March 2013 and May 2015. The flowchart of the study is presented in Figure 1. The study design was a prospective, randomised, multicentre clinical trial with two parallel groups. Altogether 106 patients with uncomplicated symptomatic cholelithiasis confirmed by ultrasound were randomised to undergo cholecystectomy with LC, 50 patients, or with MC, 56 patients. After patient enrolment, randomisation was done with a sealed envelope method either to LC or MC groups. The operations were carried out by three consultant-level surgeons (JH, PJ, ME), and both techniques were familiar to each operator. Only elective patients suitable for day-case surgery with symptomatic gallstones confirmed by ultrasound were included in the study. The exclusion criteria specified American Society of Anaesthesiologists Physical Status class of more than 3, earlier acute cholecystitis, jaundice, suspicion of stones in the common bile duct, previous upper abdominal operation and cirrhosis of the liver or suspicion of cancer. Two patients of the MC group were excluded after the surgery, one with failed anaesthesia protocol

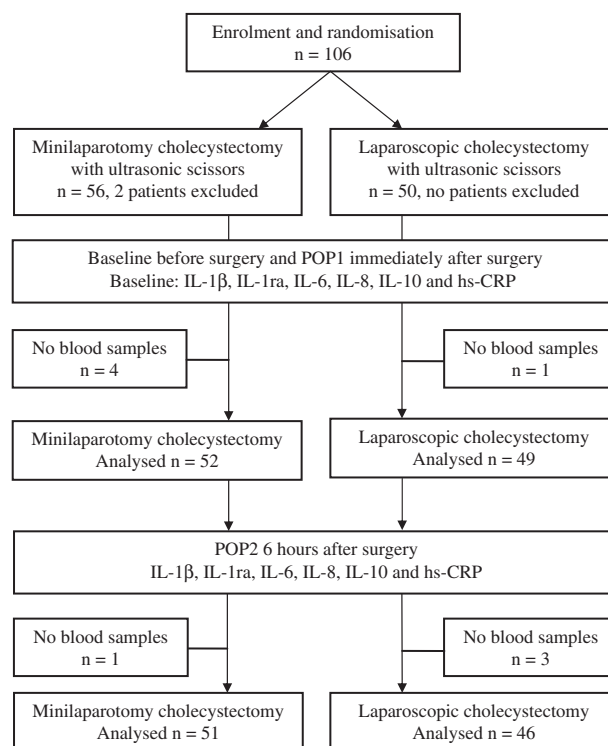


Figure 1. Flowchart of the study design.

and one with a suspicion of a liver tumour and the final number of the study patients was 54 patients in MC group and 50 in LC group (Figure 1).

The surgical techniques used were standardised in both groups.[5–8] The LC procedure was performed using the four-trocar technique (two 10-mm and two 5-mm trocars). An optical trocar was used to penetrate into the abdominal cavity and intra-abdominal pressure was set at 12 mmHg.[3–6] The ultrasonic scissors (Harmonic ACE[®], Ethicon Endo-Surgery, Cincinnati, OH) were used both in the MC and in the LC procedure. The gallbladder was dissected from the liver with ultrasonic scissors. The cystic artery was sealed with ultrasonic scissor and two metal clips were inserted to the cystic duct. The rectus muscle was split, not cut in the MC technique. Cutting the rectus muscle or a skin incision longer than 7 cm in the MC group was considered to be a conversion to conventional open operation.[5–8]. At the end of the operation, the wounds were infiltrated with local anaesthetic (20 ml ropivacaine 7.5 mg/ml) in both groups.

Endotracheal anaesthesia and post-operative care were standardised and similar in the two groups. Patients were given 60–120 mg etoricoxib one hour before the surgery and 1 g paracetamol i.v. after the surgery. For rescue analgesia, the patients were given oxycodone 3 mg i.v. every 10 minutes if the pain was at rest 3/10 or higher or during cough or movement 5/10

or higher on an 11-point numeric rating scale (NRS; 0 = no pain; 10 = most pain). After discharge, the patients were prescribed p. o. paracetamol and ibuprofen as analgesics.

Laboratory measurements were given as follows: ethylenediamine tetraacetic acid (EDTA)-plasma samples were taken at the specified time points and centrifuged 1000 *g* for 15 min. Plasma was separated and stored frozen at -70°C until analysed. The plasma interleukins IL-1 β , IL-1ra, IL-6, and IL-10 assays were performed using enzyme-linked immunosorbent assay (ELISA) methods from R&D Systems (Minneapolis, MN). The sensitivity of the assays were as follows: hsIL-10: 0.09 pg/ml; IL-8: 0.13 pg/ml; IL-6: 0.70 pg/ml; IL-1ra: 6.3 pg/ml; IL-1 β : 0.57 pg/ml. Intra-assay CV % at three concentrations ($n = 20$ for each level) were as follows: 4.6–9.3% for hsIL-10, 3.7–7.3% for hsIL-8, 3.3–6.4% for IL-6, 3.7–7.3% for IL-1ra, and 4.3–10.2% for hsIL-1 β plasma high-sensitivity CRP was analysed with a Cobas 6000-analyser (Hitachi, Tokyo, Japan).

The primary outcome measures were the plasma levels of five interleukins (IL-1 β , IL-1ra, IL-6, IL-8, IL-10) and Hs-CRP measured at three time points with high-sensitivity assays: before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2) in the MC versus LC group and the convalescence time (length of sick leave after the operation in days). The secondary outcome measures were the operation time (minutes), length of the skin incision (cm), nausea and vomiting and other complications.

The data were entered and analysed with a statistical software programme (IBM SPSS Statistics 21.0, IBM, Somers, IL). Because of skewing, the marker values were log-transformed for statistical analysis. After transformation, the study variables were normally distributed. The results of the marker values are presented as mean values with standard deviation and median values with interquartile range. The *t*-test and linear mixed model were used for the comparison between the study groups. The linear mixed effect model was used to test overall significance between groups during follow-up period. Group differences in three time points were tested by *t*-test. The Fisher exact test was used to analyse the frequency data. A two-sided *p* value of less than 0.05 was considered statistically significant.

Results

The two study groups were similar in terms of the demographic variables and the perioperative surgical data (Table 1).

There was no statistical significance in hs-CRP mean and median values between the MC and LC

Table 1. Baseline demographic characteristics and surgical data for the two study groups.

Variables	Minilaparotomy <i>n</i> = 56	Laparoscopy <i>n</i> = 50	<i>p</i> value
Age (years)	50.6 (13.5)	52.4 (13.1)	0.477
Sex (male/female)*	11/45	16/34	0.217
Height (cm)	167.4 (7.5)	168.9 (9.6)	0.350
Weight (kg)	77.1 (14.0)	82.2 (17.8)	0.103
BMI (kg/m ²)	27.5 (4.5)	28.8 (5.9)	0.208
Operative time (min)	68.6 (26.9)	68.5 (36)	0.988
Time in the operation theatre (min)	118.2 (27.3)	125.2 (35.8)	0.287
Perioperative bleed (ml)	39.7 (60.7)	29.2 (36.4)	0.287
Conversion rate (n)*	2	3	0.667
Length of the skin incision(s) (mm)	49.3 (12.3)	77.7 (23.5)	<0.001

Values are mean (standard deviation) or *number of cases.

groups pre- and post-operatively. However, the patients in the MC group had a higher elevation of the CRP mean values post-operatively (mean pre-/post-operative CRP values 2.0/3.6 mg/l in the MC group versus 5.1/7.0 mg/l in the LC group, Table 2, $p = 0.01$). Moreover, CRP increased more than 1 mg/l at six hours compared to baseline in 26 out of 50 subjects in the MC group compared to 11 out of 46 in the LC group, respectively ($p = 0.005$). No differences were detected in the IL-1ra values between MC and LC groups pre-operatively and immediately after operation (Figure 2, Table 2). However, IL-1ra level increased in most subjects in the MC group, 46/50, compare to 31 subjects with increased IL-1ra and 15 subjects with decreased IL-1ra at six hours after surgery in the LC group ($p = 0.003$), and the patients in the MC group had higher IL-1ra mean values six hours post-operatively, (mean pre-/post-operative IL-1ra values 299/614 pg/ml in the MC group versus 379/439 pg/ml in the LC group, Figure 2, Table 2, $p = 0.003$). There was no statistical significance in the IL-6 mean values between the MC and LC groups preoperatively and immediately after operation (Figure 3, Table 2). Whereas the patients in the MC group had higher IL-6 mean values six hours post-operatively (POP2), the mean IL-6 values being 27.6 pg/ml in the MC group versus 14.8 pg/ml in the LC group (Figure 3, Table 2, $p = 0.04$). In addition, the patients in the MC group had higher elevation of the IL-6 mean values post-operatively, the mean pre-/post-operative IL-6 values being 4.1/27.6 pg/ml in the MC group versus 3.8/14.8 pg/ml in the LC group. Mimicking changes in CRP, IL-6 increased > 10 pg/l at six hours compared to baseline in 25 out of 50 subjects in the MC group compared to 10 out of 46 in the LC group (Figure 3, Table 2, $p = 0.04$). No statistical significant differences in IL-8, IL-10, and IL-1 β mean values were shown between the MC and LC groups pre- and post-operatively. IL-10 increase in 41 subjects, IL-1 β increase in 14 subjects and decrease in 22 subjects, respectively,

Table 2. Changes in inflammatory markers with the respect to the study group. Values are mean (standard deviation) and median (interquartile range).

Marker	Minilaparotomy	Laparoscopy	p Value
Hs-CRP (mg/l)			0.013*
PRE	2.0 (2.6)	5.1 (10.1)	0.06
POP1	1.3 (0.35–2.85)	1.7 (0.5–3.6)	0.06
POP2	1.5 (0.3–2.85)	1.6 (0.6–3.4)	0.42
POP2	3.6 (3.6)	6.96 (13.1)	0.42
POP2	2.55 (1.1–5.28)	2.55 (0.9–4.2)	
IL-1ra (pg/ml)			0.003*
PRE	298.9 (199.8)	379.2 (339.6)	0.60
POP1	253 (176.6–338.2)	267 (188.1–418.6)	0.79
POP2	382.9 (267.7)	432.0 (403.0)	0.08
POP2	322 (228.1–454)	279 (201.3–491)	
POP2	613.7 (546.3)	438.8 (315.2)	0.08
POP2	403 (289.5–858)	403 (217–563.1)	
IL-6 (pg/ml)			0.04*
PRE	4.13 (4.0)	3.75 (1.6)	0.98
POP1	3.11 (3.11–3.11)	3.11 (3.11–3.11)	0.06
POP2	13.7 (13.7)	8.75 (12.2)	0.06
POP2	7.8 (3.1–19.6)	4.84 (3.11–9.4)	0.04
POP2	27.6 (45.6)	14.8 (19.4)	
POP2	13.3 (8.55–32)	8.36 (6.1–11.31)	
IL-8 (pg/ml)			0.54*
PRE	4.83 (2.1)	5.65 (2.9)	0.12
POP1	4.85 (3.43–5.91)	4.89 (3.93–7.38)	0.55
POP2	5.86 (3.71)	6.11 (3.85)	0.69
POP2	4.59 (3.38–7.84)	5.02 (3.35–7.16)	
POP2	6.85 (6.0)	7.79 (8.8)	
POP2	4.88 (3.98–7.65)	5.06 (3.71–7.12)	
IL-10 (pg/ml)			0.95*
PRE	1.24 (2.9)	1.23 (2.97)	0.91
POP1	0.77 (0.77–0.77)	0.77 (0.77–0.77)	0.67
POP2	3.29 (4.83)	3.49 (6.29)	0.69
POP2	1.33 (0.77–2.87)	0.77 (0.77–2.83)	
POP2	4.32 (8.93)	2.93 (4.65)	
POP2	0.77 (0.77–2.75)	0.77 (0.77–2.47)	
IL-1 β (pg/ml)			0.71*
PRE	0.20 (0.14)	0.24 (0.28)	0.59
POP1	0.12 (0.12–0.21)	0.12 (0.12–0.25)	0.59
POP2	0.22 (0.30)	0.28 (0.42)	0.73
POP2	0.12 (0.12–0.19)	0.12 (0.12–0.17)	
POP2	0.18 (0.13)	0.18 (0.27)	
POP2	0.12 (0.12–0.13)	0.12 (0.12–0.13)	

Plasma levels of Hs-CRP and five interleukins (IL-1ra, IL-6, IL-8, IL-10, IL-1 β) were measured at three time points; before operation (PRE), immediately after operation (POP1), and six hours after operation (POP2). For statistical comparison, the marker values were log-transformed to avoid analysing right skewed values.

*The overall p value indicated the relative elevation of the inflammatory marker mean values postoperatively.

and IL-8 increased in 49 subjects and decreased in 47 subjects, with a similar distribution in the two groups.

Discussion

Okholm et al. [2] reviewed 10 studies published from 1999 to 2013 including three randomised trials and seven retrospective studies and concluded that the stress response to surgery depends on the degree of trauma, and the reduction of surgical trauma by laparoscopy-assisted techniques seems to diminish the stress response compared to open surgery. The field of the proinflammatory and anti-inflammatory markers is very

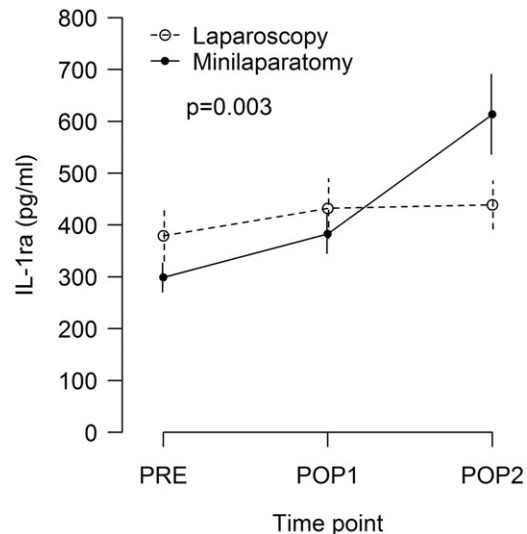


Figure 2. Mean plasma levels of interleukin-1ra measured at three time points; before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2) in LC versus MC group.

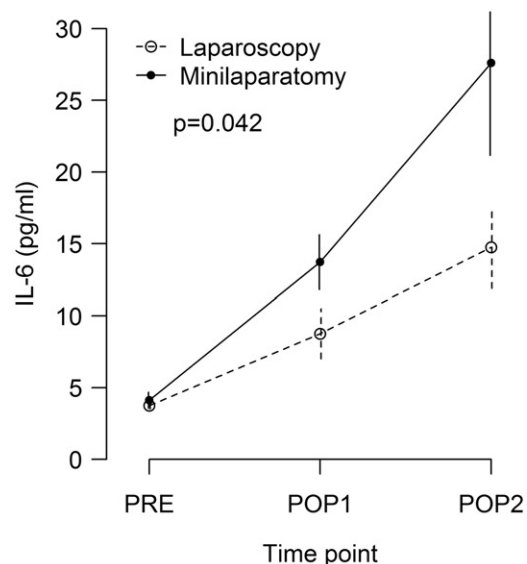


Figure 3. Mean plasma levels of interleukin-6 measured at three time points; before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2) in LC versus MC group.

complex, but in general, in the inflammatory response, the production of proinflammatory (e.g. IL-1 β , IL-6, IL-8) and anti-inflammatory cytokines (IL-10, IL-1ra) is increased. As the levels of most cytokines are low, we chose those cytokines, which can be measured reliably at specified time points with high-sensitivity assays. Therefore, we investigated five interleukins (IL-1 β , IL-1ra, IL-6, IL-8, IL-10) and hs-CRP at three time points; before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2). The pattern of

production of the inflammation-associated cytokines in MC versus LC with UsD in these groups has not been described previously. The primary end-point of the study was to compare plasma levels of five interleukins and CRP in LC versus MC group.

The most important finding of the present study was that both LC and MC are feasible options of treatment of cholelithiasis, but the MC technique had a higher inflammatory response to surgical trauma than LC. MC has been shown to have a similar perioperative course to LC, and follow-up results on early post-operative recovery indicate that these two techniques share a similar short-term recovery.[9–21] We described earlier the efficacy of monopolar electrosurgical energy (ME) in MC versus LC [5–8] and our results suggest a relatively similar 5-year and 10-year outcome after MC and LC.[22,23]

Considering the positive effects of ultrasonic dissection (UsD) in MC,[24,25] we used UsD for both MC and LC. A new finding with clinical relevance in the present work was a relatively similar short-term outcome in the MC and LC groups when applying the UsD in both groups, although the LC patients reported significantly lower pain scores 24 hours post-operatively and had a shorter convalescence.[4]. The results of our earlier study show that there were no statistically significant differences between the MC and LC groups regarding perioperative outcome.[4] The proportion of conversions was similar, three patients with LC versus two patients with MC. There were no differences in the rescue analgesics consumption, analgesics doses, and nausea/vomiting.[4]

The present study showed that the inflammatory response in MC versus LC groups was similar based on the plasma levels of IL-8, a neutrophil-activating cytokine, and IL-10, an anti-inflammatory cytokine preventing tissue damage caused by inflammation and IL-1 β , a proinflammatory acute-phase protein. A new finding with possible clinical relevance in the present work is a slightly higher relative elevation of the IL-1ra and IL-6 mean values post-operatively in the MC group. Interestingly, although the mean plasma levels of IL-1ra, an anti-inflammatory cytokine, did increase in the LC group, the mean plasma level was doubled in the MC group at six hours after surgery compared to baseline. As the levels of IL-1ra are several folds higher than most cytokines, even small relative changes may be reliably measured. Plasma levels of IL-6, a proinflammatory cytokine, were increased in both groups, but the increase was 6-fold in the MC group compared to 3-fold increase in the LC group at six hours after surgery, respectively. IL-6 is the archetype of cytokines and an inducer of CRP production in the liver in surgical trauma,

and IL-6 serum levels increase two to four hours after incision and peak within six to 12 hours.[26,27] The half-life of cytokines in blood is usually less than one hour [28] as they are rapidly cleared from the circulation. In our study, the six-hour post-operative time point may be optimal for IL-6 and IL-1ra kinetics. The significance of these mean differences should be very carefully interpreted since the plasma levels of Hs-CRP and five interleukins (IL-1ra, IL-6, IL-8, IL-10, IL-1 β) measured at three time points; before operation (PRE), immediately after operation (POP1) and six hours after operation (POP2) were log-transformed to avoid analysing right skewed values (Table 2). The overall *p* value in Table 2 indicated the relative elevation of the inflammatory marker mean values post-operatively. To be careful and comprehensive in the analysis of the results, we will also show the median values of the five interleukins and hs-CRP with interquartile range in Table 2.

In conclusion, our results suggest that the inflammatory response in MC and LC was similar based on the IL-8, IL-10, and IL-1 β values. A new finding with possible clinical relevance in the present work is a slightly higher relative elevation of the IL-1ra and IL-6 mean values post-operatively in the MC group.

Disclosure statement

The authors have no conflicts of interest.

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