The neutrophils response after laparoscopic and open cholecystectomy

Ann. Ital. Chir., 2013 84: 153-158
pii: S0003469X13019672

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AIM: *This study want to examine (a) whether neutrophils, the neutrophil-elastase, C-reactive protein and the Interleukin-6 are modified and how, in patients after laparoscopic cholecystectomy open cholecystectomy; (b) whether these findings are indicative of an increased risk to develop infectious complications.*
MATERIALS OF STUDY: *Circulating Interleukin-6 level, C-reactive protein and neutrophil-elastase were measured in 71 patients (35 underwent open cholecystectomy and 36 laparoscopic cholecystectomy). The diagnosis was confirmed by ultrasound examination. During hospitalization the patients were not given antispastic drugs, steroids, or nonsteroidal anti-inflammatory drugs (NSAID).*
RESULTS: *The increase in the serum Interleukin-6 and neurtophil-elastase, during laparoscopic cholecystectomy, was found to be significantly smaller than that during open cholecystectomy and resulted in a smaller extent of postoperative elevations for C-reactive protein. We recorded three cases (8.5%) of postoperative infections in the "open"' group and neutrophil-elastase values normalized later in patient with complications.*
CONCLUSION: *There were significant associations between the response areas of Interleukin-6, C-reactive protein and neutrophil-elastase levels. Neutrophils-elastase level is a more sensible inflammatory marker in comparison to the IL-6 and C-reactive protein. Excessive and prolonged post injury elevations of these mediators are associated with increased morbidity.*

KEY WORDS: Cholecistectomy, Inflammatory markers, Neutrophils response

Introduction

Laparoscopic cholecystectomy (LC) is now considered the treatment of choice for symptomatic gallbladder stone disease. Performed at first in Germany, in 1985, LC rapidly became the elective surgical procedure in the United States and Europe.
When compared to open cholecystectomy (OC), LC presents several advantages, such as reduced postoperative (p.o.) pain, prompt p.o. bowel activity (6-24 hours after operation), reduced hospitalization (1-3 days), earlier return to work, better aesthetic results, and reduced p.o. infections.
It is now clear that the same degree of safety can be achieved with reduced trauma. These facts have lead to the idea that the reduced trauma is associated with a reduction in physical stress for the patient. On the basis of this consideration, the aim of the present study,

Pervenuto in Redazione Aprile 2012. Accettato per la pubblicazione Luglio 2012
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prospective non randomized, is to examine (a) whether the neutrophils, the neutrophils-elastase (PMN-elastase) and the Interleukin-6 (IL-6), produced significantly by neutrophils, are modified and how, in patients after LC compared to patients undergoing OC; (b) whether these findings are indicative of an increased risk to develop infectious complications and whether are they therefore clinically significant.

Materials and Methods

From July 2008 to August 2011 we studied in a prospective non randomized study, 71 patients consecutively (42 women, 29 men; mean age 48.9), all presenting with symptomatic gallbladder stone disease. It was difficult to obtain patients agreement to randomization and so having two sources was the only way to obtain two groups. Including consecutive patients to the study hopefully reduced selection bias.

The diagnosis was confirmed by ultrasound examination. Serologic tests (AST, ALT, bilirubin, serum proteins) were in the normal range and none of the patients had a history of cholecystitis or acute pancreatitis. During hospitalization the patients were not given antispastic drugs, steroids, or nonsteroidal antiinflammatory drugs (NSAID). The patients were classified as grade I or II, according to the American Society of Anesthesiologists (ASA) grading system [1].

Thirty-five patients (22 women, 13 men; mean age 53.1 years) (group 1) (Table I) underwent OC using a right subcostal incision. The remaining 36 patients (20 women, 16 men; mean age 44.8 years) (group 2) (Table I) underwent LC using the standard technique with four trocar incisions. All patients from group 1 were operated using the open technique by a surgical team different from ours, who prefers the OC procedure. The ethical committee of the Department of Surgery at the University of L'Aquila approved the study protocol. All patients gave informed written consent.

TABLE I - *Open cholecystectomy (group 1) and laparoscopic cholecystectomy (group 2)*

Parameter	Open Cholecystectomy	Laparoscopic Cholecystectomy
N° patients	35	36
Age (years)	36-84 (53.1)	32-83 (44.8)
Sex (F/M)	22/13	20/16
ASA† grade		
1	22	20
2	15	16
Anesthesia (minutes)	36-58 (42.9)	21-64 (48.2)
Operative time (minutes)	30-55 (41.8)	18-51 (39.8)
Postoperative complications‡	3 (8.5%)	0
Postoperative hospitalization (days)§	7-9 (7.3)§	2-3 (2.3)§

*Numbers in parenthesis indicate mean values (except for 8.5%).
†ASA: American Society of Anesthesiologists.
‡bronchopneumonia (2 cases); infection laparotomy wound (1 case).
§P<0.01.

As shown in Table I, age, sex, ASA grades, time of anaesthesia, and operation were comparable in the two groups, but hospitalization was significantly shorter in group 2. There were no indications for intraoperative cholangiography, in either group, nor did they receive blood transfusions. Anaesthesia was obtained in groups using the same procedure. Preanaesthesia was done using atropine (0.01 mg/kg) plus promethazine (0.5 mg/kg), induction using sodium thiopental (5 mg/kg) and atracurium (0.5 mg/kg), and tracheal intubation and assisted ventilation using nitrogen dioxide (NO_2)/oxygen 2:1.

Before the operation (time 0) and 1, 2, 3, 24, 48, 72 hours, 6 and 12 days after the operation (the initial skin incision) the peripheral blood was sampled to measure the serum levels of IL-6, which is thought to play a central role in the development of the metabolic response [2-11], and C-reactive protein (CRP).

All samples were also tested for total white blood cell (WBC) count, WBC population count and PMN-elastase, which is released by activated neutrophils [12], the day before operation and at days 1, 3, 6, and 12 after operation.

In cases where increase in the concentration persisted for more than 12 days, the parameters were measured daily until their values returned to normal range.

The patients postoperative body temperature was monitored every 4 hour and the maximal values on each day were compared between the two groups.

For IL-6 measurement, 2mL of peripheral venous blood was collected. Blood was drawn into ammonium-heparinate-coated syringes (Sarstedt, Numbrecth, Germany). The samples were spun at 2500rpm in a refrigerated 4°C centrifuge (model 3K12; Sigma-Aldrich Corp, St Louis, Mo). The plasma was separated, fractionated and immediately frozen (-70°C) until the time of assay. Circulating IL-6 level was measured using a random access chemiluminescenze-immunoassay system (IMMULITE IL6; DPC Biermann GmbH, Bad Nauheim, Germany). The duration of processing, was approximately 70 minutes and the sensitivity was lpg/mL (range 2-2000pg/mL). The test was standardized according to National Institute for Biological Standards and Control and World Health Organization protocol 89/548.

The plasma concentration of C-Reactive Protein (CRP) was measured using a competitive CRP ELISA kit.

Elastase concentration was determined photometrically, using an immune activation immunoassay (Merck, Damstadt, Germany), as a complex with α_1- proteinase inhibitor, according to the method described by Hafner [13].

We established a reference range for IL-6, CRP and PMN-elastase by measuring the serum concentration in 53 normal control patients without gallbladder cholelithi-

asis or other diseases (31women and 22 men). Reference values were estimated to be 1-11pg/mL for IL-6 and below 1mg/dL for CRP. For PMN-elastase normal values were estimated to be 22±10 μg/l (mean ± SD) and were similar to the values established by other authors [14]. The specificity was 95%, and the higher limit of normal values was 36.7μg/l.

No influence of sex or age on IL-6, CRP and PMN-elastase values could be determined. Statistical analysis was performed using Student's tp<0.05 were considered to be significant. The values were expressed as the mean ± standard deviation (SD).

Results

Transient leukocytosis (range 10500-13800/ml) was often (88.4%) present after OC but not after LC, mostly due to an increment of neutrophils (Fig. 1) (day 1, p<0.05). This value returned to the normal range within 48 to 72h. Other WBC types showed no significant variation. A statistically significant change in plasma elastase concentration was recorded p.o., at day 1, 3 and 6 as an increase of this neutral proteinase in patients from group 1 (OC); no changes were noted in LC patients (Fig. 2) (p<0.05). In the OC group plasma elastase concentration returned to normal values within 12 days after operation.

Before the operation, the serum levels of neither IL-6 nor CRP was significantly different between these two groups. Figure 3 shows the chronological change in the serum level of IL-6 both during and after surgery. In the OC group, the serum IL-6 levels began to significantly increase as early as 1 hour from the beginning of the operation revealing a peak at the 6th hour (approximately 4 hours after the operation) and, thereafter, declining to preoperative levels by the 6 days. On the other hand, in the LC group patients, the increase in the serum IL-6 level was delayed and both the intra-operative values and the peak values were significantly lower than those in the OC group (P<0.05).

The mean values of the serum CRP on postoperative days 1 and 3 were also significantly lower in the LC group than those in the OC group (p<0.05) (Fig. 4). In these cases CRP concentrations returned to normal values within 6 days after operation.

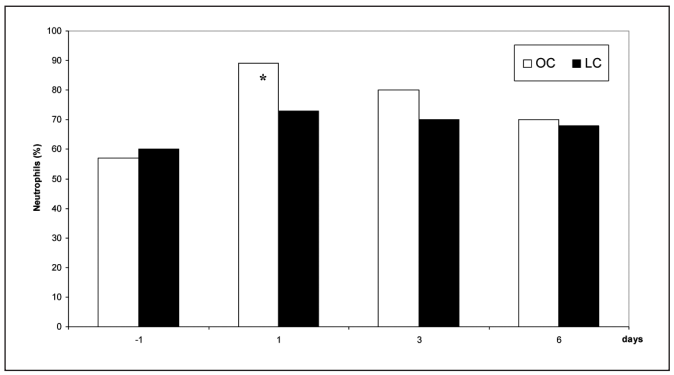

Fig. 1: Neutrophil concentration before and after OC or LC. Transient leukocytosis (range 10500-13800/ml) was often (88.4%) present after open colecystectomy (OC) but not after laparoscopic colecystectomy (LC). *p<0.05 (day 1).

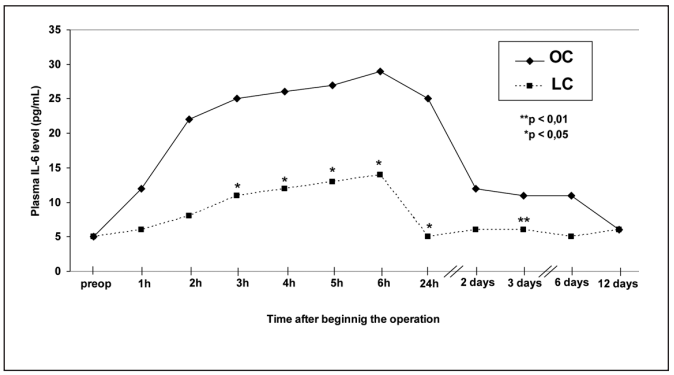

Fig. 3: Plasma concentrations (mean ± SD) of Interleukin-6 (IL-6) before and after laparoscopic cholecystectomy (LC) or open cholecystectomy (OC).

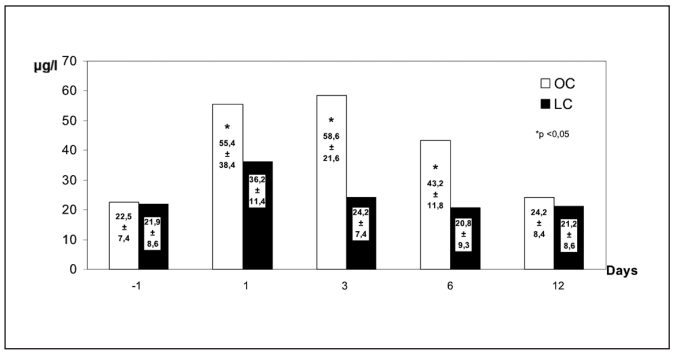

Fig. 2: Plasma Elastase concentration (mean S±D) before and after laparoscopic colecystectomy (LC) or open colecystectomy (OC). *P<0.05 (day 1, 3 and 6).

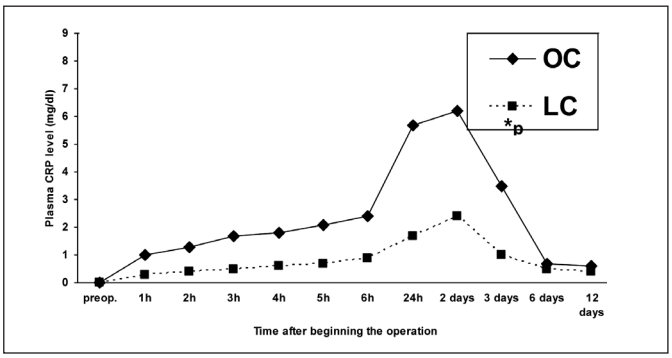

Fig. 4: Plasma concentrations (mean ± SD) of C-reactive protein (CRP) before and after laparoscopic (LC) or open cholecystectomy (OC).

The maximal body temperature postoperatively tended to be lower in the LC group, although the difference was not statistically significant.
There were significant associations between the response areas of IL-6, CRP and PMN-elastase levels ($p<0.00l$). Finally, the ages of the patients did not affect IL-6, CRP and PMN-elastase concentration in either group.
Three patients who had undergone OC (8.5%) developed postoperative infections bronchopneumonia (patient 1 and 2) and laparotomy wound infection of first grade (patient 3) (Table I). These three patients were, respectively, 49, 61 and 65 years old and were all ASA I. There was an increase in the white blood cell count (WBC) after operation. Three days after surgery it reached 16100/ml (82% neutrophils) in patient 1, 13500/ml (78% neutrophils) in patient 2 and 11100/ml (75% neutrophils) in patient 3 (laparotomy wound infection). It then decreased and normalized 8 days after surgery in all patients. Clinical and radiological findings demonstrated basal pneumonia in two patients. IL-6 and CRP concentration which was increased a 1 hour from the beginning of the operation, remained high for 1 and 6 days and returned to normal values 10 (patients 1 and 3) and 12 days (patient 2) after surgery. Plasma elastase concentration was increased a day after surgery, remained high for 3, 6 and 12 days and returned to normal values 13 (patient 3), 14 (patient 1) and 16 days (patient 2) after surgery.
Follow-up after 1 and 6 months is a routine procedure for all patients, who undergo cholecystectomy in our hospital. It showed only a first grade infection of the laparatomy wound in the 62 patients (33 patients that underwent LC, 29 that underwent OC) that accepted follow-up.

Discussion

While laparoscopy is "minimally invasive", systemic immune responses are still invariably activated [15-16]. Overall, responses to surgery in general are reflected in terms of cytokine function and cellular messenger systems. While cytokine levels do not directly reflect immune status, they give us a framework to understand systemic immunity in terms of underlying immune activation. Because alterations are proportional to the extent of injury, the physiologic response to minimally invasive surgery may, intuitively, be different than those for traditional open surgery. The acute phase protein response appears to be one example. The cytokines Interleukin-1 (IL-1), tumour necrosis factor (TNF) and Interleukin-6 are known to be major mediators of the acute phase response. IL-6 plays a central role in the acute-phase of inflammation seen after surgery [4,12,17]. IL-6 induces the production of acute-phase protein, such as C-reactive protein in hepatocytes and also causes fever [14]. It has been reported that surgical stress causes the serum IL-6 level to increase and that the extent of such an increase is closely correlated to the subsequent increase in the serum CRP levels [12]. The C-reactive proteins rise approximately 4 to 12 hours after surgery and peak at 24 to 72 hours. Subsequently, C-reactive proteins remain elevated for approximately 2 weeks [12].
The acute-phase response after laparoscopic surgery has been studied in several clinical trials, measuring IL-6 levels after laparoscopic cholecystectomy [6,11,17-25]. IL-6 levels have been noted to be reduced in patients undergoing laparoscopic procedures compared to traditional laparotomy [6,19,20-24,26]. Additionally, a linear correlation between peak concentrations of IL- 6 and C reactive proteins has been noted [6-19]. Other studies, however, have shown contrary finding. Roumen et al. [18] reported that IL-6 levels only were detected in patients after the age of 60 undergoing laparotomy. Furthermore, they found no relative correlation between plasma concentrations of IL-6 and C-reactive proteins. McMahon [2] showed no significant difference between laparoscopic cholecystectorny and mini-laparotomy cholecystectomy groups. That study found that IL- 6 levels in both laparoscopic and mini-laparotomy cholecystectomy groups were similar to historical reports of standard cholecystectomy levels. The IL-6 levels correlated with those for C-reactive proteins and so were thought to suitably reflect the acute phase response [2]. PMN-elastase is a neutral proteinase (30kd) consisting of 218 aminoacids, present mainly in the azurophilic granules of segmented granulocytes. Its function is to contribute to tissue repair after trauma, inflammation or necrosis and can also cause, via non specific proteolysis, tissue injuries and breakdown of regulatory proteins, thus sustaining the inflammatory process. During the surgical procedures there is a massive release of elastase from the neutrophils , along with other proteinases.
In this connexion it is necessary to state that although monocytes, endothelial cells and fibroblasts are thought to be the major in vivo source of IL-6, some recent evidence suggests that circulating neutrophils contribute significantly to IL-6 production [27].
The strong correlation between changes in IL-6 and PMN-elastase, which are released by activated neutrophils [13] and the immediate rise in PMN-elastase, found in the present study, are consistent with this hypothesis. Similarly, Suzuki [15] studied patients undergoing LC (group 1) and major abdominal operation (group 2). The authors found a significant difference in granulocytic PMN-elastase levels in the two groups. No correlation was found between elastase and IL-6 levels.
McMahon [18], in his study consisting of two groups of patients treated with LC and OC, considered the elastase levels and found a significant correlation with alterations in IL-6 and CRP. However no significant difference between mini-laparortomy cholecystectomy and laparoscopic group was shown.
Our study demonstrates that the serum level of IL-6 and

C-reactive protein is correlated with that of the PMN-elastase. It should be noted however that IL-6 and C-reactive protein values normalize about 6 days postoperatively in OC patients and 10-12 days postoperatively in patient with complications, while PMN-elastase values normalized later on. Therefore we consider that PMN-elastase level is a more sensible inflammatory marker in comparison to the IL-6 and C-reactive protein.

Although PMN-elastase, IL-6 and CRP are considered to be a mediator of the physiologic-short term phase reaction to the injury, excessive and prolonged post injury elevations are associated, according to our opinion, with increased morbidity. Indeed in our 3 patients who developed postoperative infections after OC the IL-6 and CRP concentration remained high for 1, 3 and 6 days and normalized 10-12 days after surgery. Instead the PMN-elastase concentration normalized after 13, 14 and 16 days.

The complete documentation of postoperative complications is a laborious undertaking. In many studies only the in-hospital period is analyzed. Because this interval is significantly shorter after laparoscopic operations it is difficult to interpret the results of these studies. A considerable number of complications occurs only after patients has been discharged. Jakeways and Carli [28] suggested that the follow-up of patients in randomized trial be continued for several weeks into the postoperative period. Therefore we have attempted to see all patients again 1 and 6 month after surgery, reporting only one infection of laparotomy wound.

In conclusion, although some studies have shown contrary finding, an increase in the serum PMN-elastase and in the IL-6 levels during LC is lower in comparison to OC and results in lower postoperative elevations in CRP. Laparoscopic surgery, associated with a small skin incision and the avoidance of open laparotomy, can thus minimize surgical stress, and provide more favourable postoperative conditions for patients. Indeed excessive and prolonged post-injury elevations of PMN-elastase, IL-6 and CRP are associated with increased morbidity. Moreover, the PMN-elastase is a more sensible marker of inflammation in comparison to the IL-6 and CRP.

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