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Changes in T-Lymphocytes' Viability After Laparoscopic Versus Open Cholecystectomy

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Laparoscopic surgery results in decreased immune and metabolic stress response compared to open surgery. Our aim was to evaluate the suspension of host immune defense in terms of apoptosis, necrosis, and survival of peripheral T-lymphocytes in patients undergoing laparoscopic versus open cholecystectomy. Apoptosis, necrosis and viability of peripheral T-lymphocytes were measured preoperatively and postoperatively by means of flow cytometry in 27 patients undergoing laparoscopic cholecystectomy and 25 undergoing open cholecystectomy. White cell count, CRP, and serum glucose levels were also measured. Viable peripheral T-lymphocytes were significantly decreased in open cholecystectomy ($P = 0.02$), while their late apoptotic as well as the overall necrotic rate were significantly increased ($P = 0.01$ and $P < 0.01$, respectively). Open cholecystectomy was also associated with lower levels of surviving circulating T-lymphocytes ($P = 0.01$) and higher percentage of necrotic T lymphocytes ($P = 0.03$) 24 hours postoperatively compared to laparoscopic cholecystectomy. Serum CRP was increased 24 hours after open cholecystectomy ($P = 0.04$). All differences failed to sustain more than 48 hours postoperatively. Increased viability and decreased necrosis of circulating T-lymphocytes were observed in laparoscopic cholecystectomy. Necrosis (and not apoptosis) seems to be the predominant pathway of T-lymphocyte death in open cholecystectomy, in a process reaching its peak at 24 hours and further attenuating 48 hours postoperatively.

Key words: Laparoscopic cholecystectomy – Open cholecystectomy – Immune response – Apoptosis – Necrosis – Flow cytometry – T-lymphocytes

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It has been supported that open surgery is immunosuppressive and causes alterations in multiple immune parameters, including reduction in the number of circulating blood lymphocytes, depression of T-lymphocyte proliferation and diminished neutrophil function.[1,2] On the contrary, many clinical reports have demonstrated that immune response to abdominal surgery is reduced by laparoscopic approach, since reduced surgical wound, injury, and perioperative stress results in reduced systemic endocrine, metabolic and inflammatory response.[3–6] Although these studies have led to a better understanding of immune and metabolic response after open surgery and minimally invasive procedures done under a CO_2 pneumoperitoneum, the underlying mechanisms of these effects have not been fully clarified yet.[7,8]

In order to evaluate the impact of laparotomy and laparoscopy on the suspension of host immune defense, we measured T-lymphocyte apoptosis, necrosis, and viability by flow cytometry in patients undergoing open cholecystectomy (OC) or laparoscopic cholecystectomy (LC). White cell count, serum C-reactive protein (CRP) levels, and serum glucose levels were also measured and compared between the 2 groups as established surrogates of stress response.

Patients and Methods

Apoptosis, necrosis and viability of peripheral T lymphocytes were measured in 27 patients who underwent LC and data were compared with archival measurements from blood samples retrieved from 25 patients who underwent elective OC due to contraindication to a laparoscopic procedure (multiple previous operations, inability to sustain pneumoperitoneum, etc). Both groups were operated on between 2007 and 2012 and were comparable with respect to clinical parameters including age, weight, height, and BMI. Baseline routine blood and flow-cytometry measurements are shown in Table 1. All patients presented with uncomplicated gall stone disease and diagnosis was confirmed by ultrasonography. Serologic tests (SGOT, SGPT, bilirubin, ALP, γGT) were within normal range. Exclusion criteria were cholecystitis; acute pancreatitis; common bile-duct stones; acquired or inherited immunodeficiency or immunosuppression; anemia, autoimmune, metabolic, endocrine, hepatic or renal diseases; malignancy; patients receiving medication interfering with immune function (such as immunosuppressive drugs, steroids, and NSAIDs); and conversion from laparoscopy to laparotomy. All patients presented a low surgical risk (ASA I or II).[9] The study has been approved by a local ethics committee. Signed informed consent was required prior to the participation in the current study and was revalidated in the case of archival blood samples obtained for the purposes of other studies.

Operative procedure

Both surgical procedures were performed by 2 experienced surgical teams (open versus laparoscopic team). Elective LC was carried out following a 4-trocar technique (two 5 mm and two 10 mm) and electrocautery dissection. Carbon dioxide was used for peritoneal insufflation and the intra-abdominal pressure was maintained at 12 to 14 mmHg. OC was performed via a standard right subcostal incision ranging between 6 and 9 cm of length with partial transaction of the ipsilateral rectus abdominis muscle.

The same anesthetic agents were used in both groups. No blood products were administered, and either Ringer's lactate or 5% dextrose water solutions were used for fluid replacement. A standardized drug regimen was used in the perioperative period for both procedures and included the following: premedication (diazepam, 10 mg orally, 1 hour preoperatively) and prophylactic antibiotic treatment (amoxicillin/clavulanic acid, 1.2 g intravenously, 1 hour preoperatively).

Blood sampling and analysis

Peripheral venous blood samples were obtained through an indwelling catheter inserted into a forearm vein, 1 day prior to surgery and on postoperative days 1 and 2. Blood glucose levels were determined by a routine glucose oxidase method (Glukose Analysator 2, Beckman Instruments, Munich, Germany) and CRP was also measured with a high-sensitivity CRP assay.

Apoptosis, necrosis, and viability of peripheral blood T-lymphocytes were measured by flow cytometry. Peripheral blood mononuclear cells (PBMCs) were isolated from 5 to 7 mL of venous blood samples collected in an EDTA Vacutainer tube (Becton Dickinson, Franklin Lakes, New Jersey). Blood was diluted (1:4) with RPMI 1640 (Invitrogen Corporation, Carlsbad, California) and 10 mL were layered over 4 mL Biocoll separating solution (Biochrom GmbH, Berlin, Germany), separated by density centrifugation and washed with 1× PBS. Then, lymphocytes were separated from monocytes by centrifuge and were labeled with Annexin V-FITC and PI using the

Table 1 Patients' demographic characteristics in groups of laparoscopic cholecystectomy (LC) and open cholecystectomy (OC)

	Group A (LC) ($n = 27$)	Group B (OC) ($n = 25$)	P value
Age (years)	59.77	61.62	0.09
Weight (kg)	73.33	77.25	0.08
Height (m)	1.66	1.67	0.74
BMI	26.51	27.71	0.09
Previous operation	18.5%	18.7%	0.98
Duration of procedure (min)	54.00	54.37	0.09
Fluid administration (lt)	1.87	3.25	0.001
Hospital stay (days)	2.04	4.00	0.000

TACSTM Annexin V-FITC apoptosis detection kit (R&D Systems, Minneapolis, Minnesota), according to the manufacturer's instructions. Briefly, 100 μL of cells ($1\times105-1\times106$ cells) were labeled fluorescently for detection of apoptotic and necrotic cells by adding 20 μL of 1× binding buffer, 2.5 μL of Annexin V-FITC, and 5 μL of PI to each sample. Samples were mixed gently and incubated at room temperature in the dark for 15 minutes. Then, samples were washed with 1× PBS and were analyzed by an EPICS XL flow cytometer (Beckman-Coulter International SA, Nyon, Switzerland) within 1 hour. A minimum of 10,000 cells within the gated region were analyzed. Living cells were negative for Annexin V-FITC and PI. Early apoptotic cells were detected after a single staining with Annexin V-FITC, while late apoptosis was determined by concomitant positive staining for Annexin V-FITC and PI. Necrotic cells presented single PI staining.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney U test, the $\chi 2$ test, and the Pearson's correlation coefficient. Statistical significance was defined at a cut-off P value < 0.05. The statistical package used was SPSS 16.0 (SPSS Inc, Chicago, Illinios).

Results

The 2 groups were similar in terms of age, BMI, and other demographics (Table 1). Although operative time was not significantly different between the 2 groups, intraoperative fluid requirements were significantly decreased in patients undergoing LC (1.87 versus 3.25; $P = 0.001$). Postoperative in-hospital stay was also significantly decreased in this group (1.04 days versus 3.0 days; $P < 0.001$).

Preoperative WBC count, CRP values and blood glucose levels were similar between the 2 groups. However, serum glucose levels and CRP values were significantly higher 24 hours postoperatively in OC patients (126 versus 106, $P = 0.01$ and 69.11 versus 44.14, $P = 0.04$, respectively). Moreover glucose levels and total WBC count were significantly higher in OC group compared to LC patients on the second postoperative day (130.1 versus 109.1, $P = 0.04$ and 14.700 versus 8.400, $P = 0.03$, respectively), while CRP values did not differ between the groups (Table 2).

There was no significant difference in preoperative lymphocyte apoptosis, necrosis, or viability between the 2 groups. Twenty-four hours post-OC viable peripheral T-lymphocyte rates were significantly decreased (85% versus 93.77%; $P = 0.02$), compared to preoperative rates, but similar decline was not recorded in LC (94.86 versus 95.25; $P = 0.25$; Fig. 1A). Early apoptotic rare was increased in both groups, without reaching statistical significance (5.38 versus 3.58, $P = 0.12$ in LC and 8.95 versus

Table 2 Preoperative and postoperative comparison of WBC count, serum glucose, CRP, and percentages of viable, apoptotic, and necrotic T-lymphocytes between Open Cholecystectomy (OC) and Laparoscopic Cholecystectomy (LC)

	WBC	CRP (mg/L)	Glucose (mg/dL)	Lymph number (total)	Lymph alive (%)	Lymph early apoptotic (%)	Lymph late apoptotic (%)	Lymph necrotic (%)
Preoperative								
LC	7.07	6.31	99.6	8.866	95.25	3.58	0.17	0.61
OC	7.21	8.32	107.1	10.501	93.77	5.79	0.26	0.26
p	0.42	0.62	0.28	0.08	0.56	0.40	0.37	0.92
Postoperative (24 hours)								
LC	8.98	44.14	106	7551	94.86	5.38	0.17	0.38
OC	10.6	69.11	126	8664	85.00	8.95	1.44	3.49
p	0.38	0.04	0.01	0.83	0.01	0.40	0.08	0.03
Postoperative (48 hours)								
LC	8.4	63.2	109.1	8590	94.29	4.78	0.20	0.78
OC	14.7	58.5	130.1	8510	89.47	8.65	0.23	0.56
p	0.03	0.30	0.04	0.30	0.10	0.14	0.61	0.43

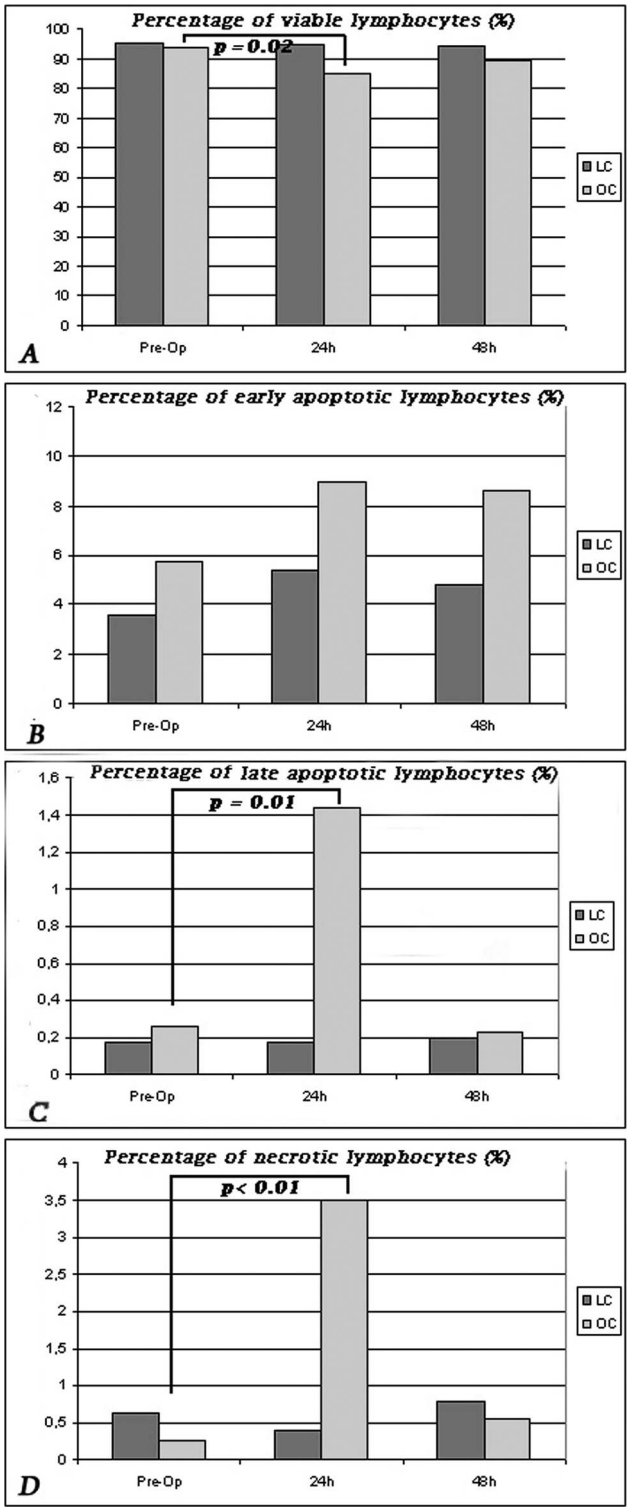

Fig. 1 (A) Percentages of viable lymphocytes. (B) Early apoptotic lymphocytes. (C) Late apoptotic lymphocytes. (D) Necrotic lymphocytes after open and laparoscopic cholecystectomy preoperatively, 24 hours postoperatively and 48 hours postoperatively.

5.79, $P = 0.09$ in OC; Fig. 1B). Within the first 24 hours, the lymphocyte late apoptotic rate remained unchanged in the LC group (0.17 versus 0.17, $P = 0.62$) but significantly increased in the OC group (1.44% versus 0.26%; $P = 0.01$; Fig. 1C). Similarly, during the same time the necrotic lymphocytic rate was significantly increased only in OC (3.49% versus 0.26%; $P < 0.01$) but not in LC (0.38 versus 0.61; $P = 0.17$; Fig. 1 D.

When the 2 groups were compared 24 hours postoperatively, the percentage of necrosis of peripheral T-lymphocytes was significantly higher in OC compared to LC (3.49% versus 0.38%; $P = 0.03$), while the percentage of viable peripheral T-lymphocytes was significantly lower (85% versus 94.86%; $P = 0.01$). The postoperative late apoptotic rate of lymphocytes was lower in the LC group; however, this difference did not reach statistical significance (0.17 versus 1.44; $P = 0.08$; Table 2).

Peripheral T-lymphocytes apoptosis, necrosis and viability did not differ significantly when preoperative and 48 hours postoperative measurements were compared (Fig. 1). Furthermore, no difference in T-lymphocyte viability, early apoptosis, late apoptosis, and necrosis was recorded between OC and LC 48 hours postoperatively (Table 2).

Discussion

Surgical trauma is associated with a variety of immunologic disturbances, including suppression of immune function and disruption of host defense mechanism.[1] Laparoscopic techniques are related to smaller surgical trauma, which results in proportionally decreased immune suppression, suggesting a potential advantage for the patient.[10,11] We chose to study the immune response in open and laparoscopic cholecystectomy because uncomplicated gall stone disease represents a nonmalignant noninflammatory disease, eliminating the confounding role of a major factor other than the type of surgery in the viability/response of the peripheral T-lymphocytes.

T-lymphocytes represent a major branch of the acquired immune system being involved in cell-mediated immune functions.[12,13] It has been demonstrated that surgical trauma leads to reduction in the number of circulating lymphocytes and inhibition of T-lymphocyte cellular proliferation, but it has not been clarified if this happens through an apoptotic or a necrotic pathway.[7,14] In the current study, LC patients presented with increased proportion of surviving T-lymphocytes 24 hours post-

operatively, which can be attributed to the significant decreased levels of T-lymphocyte necrosis, compared to OC. Analysis of flow cytometry showed that LC resulted in significantly less postoperative immunosuppression, as reflected by decreased T-lymphocyte necrosis and increased population of viable circulating T-lymphocytes. It also appears that necrosis and not apoptosis suggests the major pathway involved in postoperative peripheral T cell depletion in patients submitted to OC. However, this pathway takes effect mostly during the first 24 hours postoperatively.

Generation of acute-phase proteins is a well-recognized response to injury. Of them, CRP, synthesized by liver after trauma or surgery, is the most extensively studied.[15,16] CRP levels 24 hours after operation were significantly lower after LC, which is in line with previous reports, indicating diminished surgical stress and reduced systemic inflammatory response.[15–18] However, on the second postoperative day, no significant difference of CRP levels between the groups was demonstrated.

WBC count and serum glucose levels, which have been also used as markers of surgical stress and postoperative metabolic response,[8,17,19,20] were significantly decreased 24 hours after LC compared to OC, reflecting diminished metabolic response.

In conclusion, LC was associated with higher levels of surviving circulating T-lymphocytes and lower levels of necrotic T-lymphocytes compared to OC. Necrosis and not apoptosis seems to suggest the major pathway involved in postoperative peripheral T-cell depletion in patients submitted to open surgery. These results further support the hypothesis that host defense is less suppressed in laparoscopic surgery. This effect seems to take place over the first 24 postoperative hours and progressively wears off, suggesting a potential advantage for the patient, in terms of immune response, over the first 24 hours after the operation.

Acknowledgments

The authors declare that they have no conflict of interest.

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