

Immunologic postoperative competence after laparoscopy vs laparotomy

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Abstract

Background: Sepsis is a major complication associated with increased morbidity and mortality in patients treated surgically for hepatobiliary and colorectal diseases. Impairment of immune function after surgery may be one of the mechanisms causing increased susceptibility to infection. From November 1999 to October 2001, the perioperative and postoperative immune responses of 20 patients who underwent laparoscopy were compared with those of 20 patients who underwent laparotomy. All the patients were affected by benign pathologies. The current study aimed to elucidate the differences between the immune responses induced by the two different surgical approaches.

Methods: Immunologic function was assessed by a count of lymphocyte subsets (CD3, CD4, CD8, CD3-HLA-DR, CD19, CD16, CD57) and monocytes expressing human leukocyte antigen DR (HLA-DR). Blood samples were obtained 1 day before the surgical therapy, then 2 and 8 days after therapy. For statistical analysis, the continuous variables were compared using Student's *t*-test. Probability values less than 0.05 were considered significant.

Results: With regard to T-lymphocyte function, a fall 2 days after surgery was assessed for both laparoscopy ($p < 0.0005$) and laprotomy ($p < 0.00003$) groups. At 8 days after surgery, these values had returned to the preoperation level on both groups. The activity of B-cells and natural killer cells was not significantly affected, whereas the number of monocytes expressing HLA-DR showed a long-lasting decrease after laparotomy ($p < 0.011$ 2 days after surgery and $p < 0.02$ 8 days after surgery), but not after laparoscopy.

Conclusion: Impairment of cell-mediated immune function after surgery was demonstrated especially in patients treated by laparotomy, as compared with those treated by laparoscopy.

Key words: Immunological competence — Laparoscopy — Laparotomy

The negative influence of surgery and general anesthesia on immunologic function has been investigated by several authors [1, 2]. They agree that intervention itself induces deep, although transient, immunodepression, the extent of which is related to the severity of injury. Sepsis is a major complication associated with increased morbidity and mortality in patients surgically treated for hepatobiliary and colorectal diseases. Impaired immune function after surgery may be one of the factors responsible for increased postoperative susceptibility to infection. As minimally invasive procedures, such as videolaparoscopy, have become progressively more popular, the benefit of access, trauma reduction has been clearly demonstrated [4] and shown to be associated with clinical outcome. Surgical stress generally causes an increase in leukocytes [1, 10], but the lymphocyte count shows a decrease that seems to be mediated by enhanced apoptosis [6, 7]. The ability of peripheral blood monocytes to express the human leukocyte antigen DR (HLA-DR) is critical for the recognition of foreign antigens and the immune response mediated by T-helper lymphocytes [4, 17]. In the current study, we monitored these parameters to clarify the differences in the response of the immune system to the two types of surgical treatment, and to verify our hypothesis that minimally invasive surgery has a less marked immunosuppressive effect than equivalent open procedures.

Methods

The 40 subjects enrolled in this study (authorized by the Ministerial Ordinance 15/7/1997 and the assent of the ethical committee) were admitted to the Department of Surgery, Ospedale Civile of Dolo, Venice, to undergo elective surgery under general anesthesia for benign diseases: cholelithiasis, diverticulitis, and adenomas of the suprarenal gland. They were randomly allocated for laparoscopic or

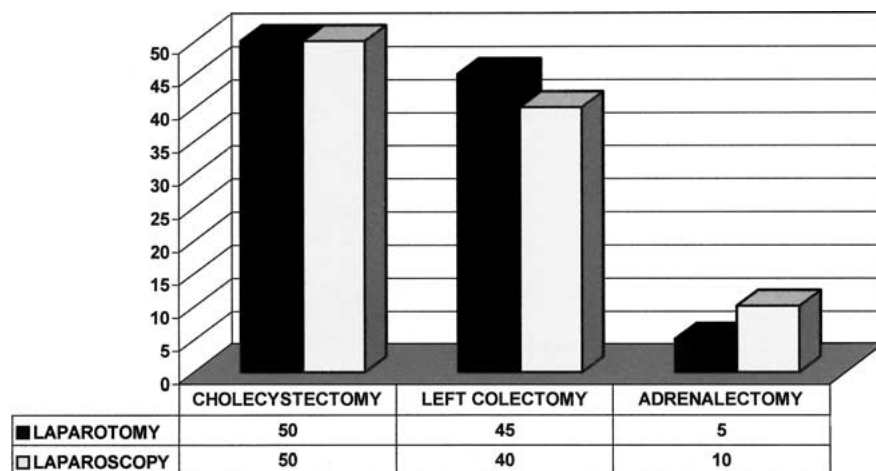


Fig. 1. Distribution percentage of intervention types in the study groups.

Table 1.

	Laparoscopy	Laparotomy	<i>p</i>
Age (years)	59 ± 14	64 ± 13	NS
Sex (m/f)	11/9	14/6	NS
Surgery (min)	160 ± 43	135 ± 45	NS
Hospitalization (days)	4.2 ± 1.2	7.3 ± 1.3	<0.001

NS, not statistically significant

laparotomic surgery after informed consent was obtained (Fig. 1). There were no surgical conversions, and no anesthesia using regional blockade. All patients were subjected to general anesthesia using curari. Demographic data and the average durations of surgery and hospitalization are listed in Table 1.

Peripheral blood samples were drawn 1 day before surgical treatment, then on days 2 and 8 after the intervention, the days on which complications and dismissal are most likely to occur. Analysis of these samples was performed the same morning as the sampling in the clinical pathology laboratory. Total and differential white cell counts were performed on an ADVIA 120 counter (Bayer Corporation) for all the samples. Immunophenotyping of cells to assess immunocompetence was performed on a fluorescence-activated cell sorter scan (FACScan) flow cytometer (Becton Dickinson Immunoscience Systems, MountainView, CA, USA). Specifically, expression of cell surface molecules on circulating lymphocytes and monocytes was analyzed by two-color immunofluorescence staining, in which aliquots of monoclonal antibodies saturated against CD3, CD4, CD8, CD16, CD19, CD57, HLA-DR, fluorescein isothiocyanate (FITC) or PE phycoerythrin-labeled, were incubated with whole blood. As a control, isotype-matched mouse immunoglobulins were included in each experiment. Red blood cells were lysed by adding 10 volumes of FACS lysing solution (Becton Dickinson). Leukocytes were washed twice with phosphate-buffered saline (PBS) and fixed in PBS containing 2% paraformaldehyde. The samples then were analyzed with the FACScan cytofluorimeter equipped with CellQuest Software to obtain the percentage of each subset. Lymphocyte and monocyte gates were set on a forward scatter–side scatter dot plot (Fig. 2). The identity of the monocytes was ascertained by independent determination of reactivity with anti-CD14 Moab (not shown). The lymphocyte subsets CD3, CD4, CD8, CD3coexpressing-HLA-DR, CD19, CD16, and CD57 were measured according to lymphocyte gate, whereas HLA-DR was analyzed according to monocytes region (R1 in Fig. 2).

The lymphocyte and monocyte counts and subset percentages were used to calculate the absolute numbers of each subpopulation. Data are expressed as mean ± standard deviation. Statistical evaluation was performed using Student's *t*-test for paired and unpaired data. The level of significance was set at a *p* value less than 0.05.

Results

The clinical outcome for the patients was satisfactory with both treatments. Only one patient treated by laparotomy experienced an infection of the wound. Figure 3 summarizes the effects recorded in the subjects of the two groups. Both the patients treated by open surgery and those who underwent laparoscopy showed a significant fall in the number of circulating lymphocytes, measured on day 2 after intervention, as compared with the preoperation values ($p < 0.00001$ and 0.0001 , respectively). This decrease was more marked in the patients who underwent open surgery, although the difference between the two groups did not quite meet the requirement for statistical significance ($p = 0.23$). At 8 days after surgery, the mean lymphocyte count had returned to baseline in both groups.

The same trend was observed for subsets of T-lymphocytes, specifically for CD3-, CD4-, CD8-; CD3- and HLA-DR-expressing cells, with the fraction of each subtype remaining approximately constant. After a significant decrease on day 2, they returned to preoperation values in both surgical treatment groups. Figure 4 illustrates this result in the case of CD3-expressing cells. Absolute numbers of B-lymphocytes (CD19), natural killer cells (CD16), and cytotoxic cells (CD57) did not show detectable variations in the determinations performed in this study.

A persistent fall in monocytes expressing antigens of major histocompatibility complex (MHC) class 2 (HLA-DR) was observed on both days 2 ($p < 0.011$) and 8 ($p < 0.02$) after laparotomic surgery. Remarkably, this phenomenon was not recorded in the laparoscopy group (Fig. 5).

Discussion

The three groups of patients were homogeneous for benignity of diseases. The laparoscopic technique now is verified for all the pathologies evaluated in this study. We wanted to compare the two types of surgery using a variety of treated diseases. Open surgery for cholelithi-

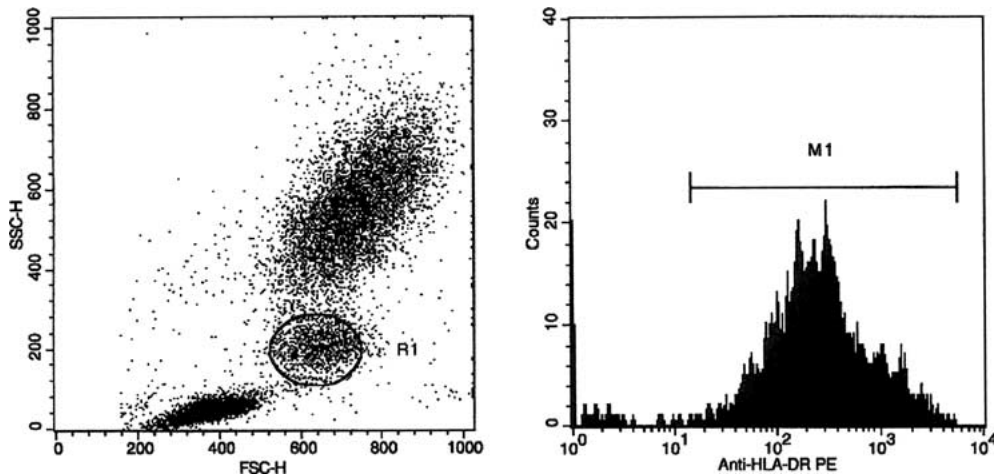


Fig. 2. Fluorescence-activated cell sorter scan (FACScan) identification of monocytes (R1 in dot plot) and monocytes expressing human leukocyte antigen DR (HLA-DR) (M1 in histogram).

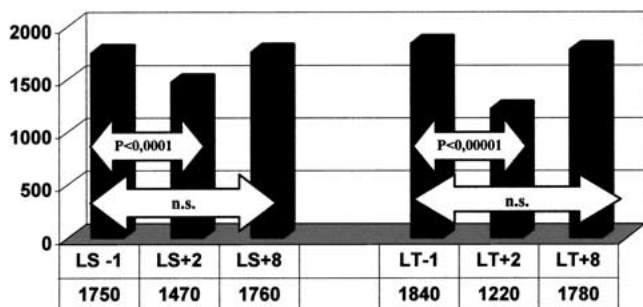


Fig. 3. Comparison of the data for the laparoscopy (LS) and laparotomy (LT) groups 1 day before (-1), then 2 (%+2), and 8 (%+8) days after surgery. Lymphocyte mean count $\times 10^6$ /l.

asis currently is uncommon, and we therefore have relatively limited this surgical treatment.

For benign diseases such as diverticulitis, cholelithiasis, and adenomas of the suprarenal gland, laparoscopic surgery ensures a better functional recovery and a shorter stay in hospital than laparotomy. The aim of our study was to understand whether this more favourable postoperative course is accompanied by a better immunologic postoperative competence.

The aforementioned benign pathologies were chosen for this comparative study because cancer itself cause profound alterations in the immune system of the patient, as reported by Ordemann et al. [13]. A study based on cases of both benign diseases and cancer could be expected to yield statistically unreliable results.

Our investigation particularly concerned cell-mediated immune function. In agreement with data reported in other studies [1, 2, 11, 13, 18], we found a decreased response of T-lymphocytes in both groups, suggesting that immune depression after surgery may be related to general mechanisms of apoptosis activation, as demonstrated by Delogu et al. [6, 7] and others [12].

As reported already by Cristaldi et al. [5] and others [8, 9, 14, 18], the comparative statistical analysis between the two groups showed a difference: A greater decrease in lymphocyte and T-cell subset counts occurred soon after intervention in patients who had undergone conventional surgery, although in the current

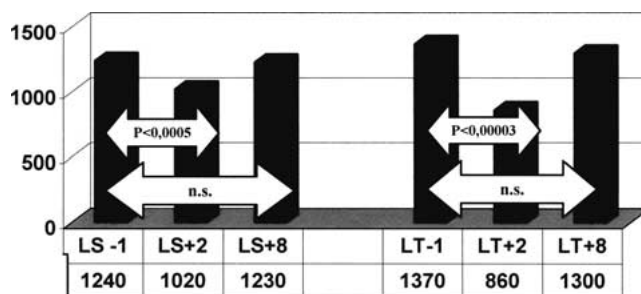


Fig. 4. Comparison of the data for the laparoscopy (LS) and laparotomy (LT) groups 1 day before (-1), then 2 (%+2), and 8 (%+8) days after surgery. CD3 lymphocyte mean count $\times 10^6$ /l.

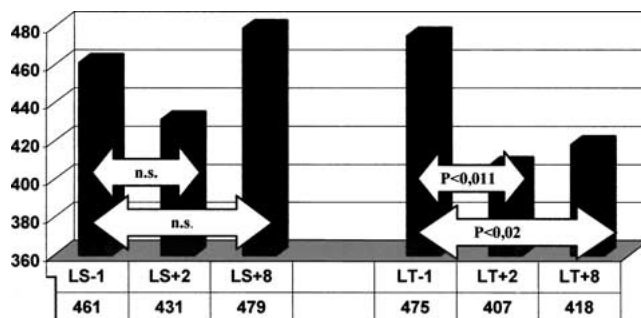


Fig. 5. Comparison of the data for the laparoscopy (LS) and laparotomy (LT) groups 1 day before (-1), then 2 (%+2), and 8 (%+8) days after surgery. HLA-DR + monocyte mean count $\times 10^6$ /l.

study this trend did not reach statistical significance ($p = 0.23$).

Several authors have extensively studied the mechanisms of immune response regarding the antigen presenting-cell, demonstrating that a significant down-regulation of HLA-DR expression in monocytes, recorded in open surgery [2, 3, 11, 15, 17], is related to a less favorable clinical course. In the current study, despite the significant and persistent decrease recorded in the laparotomy group, we observed only one minor postsurgical complication (infection of the wound) in a patient treated by the conventional procedure.

In conclusion, the current study demonstrated impaired cell-mediated immune function after surgery,

especially in patients treated by laparotomy, as compared with those treated by laparoscopy, a surgical technique associated with reduced access trauma. More specifically, the T-lymphocyte count showed a greater fall 2 days after laparotomy (i.e., soon after intervention). Monocytes expressing HLA-DR persistently decreased after laparotomy, whereas after laparoscopy, this function was similar to the original values on both days.

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