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Cellular and humoral inflammatory response after laparoscopic and conventional colorectal resections

Results of a prospective randomized trial

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Abstract
Background: Surgical trauma and anesthesia are known to cause transient postoperative suppression of the immune system. In randomized controlled trials, it has been shown that laparoscopic colorectal resections have short-term benefits not observed with conventional colorectal resections. We hypothesized that these benefits were due to the reduction in surgical trauma, leading to a diminished cytokine response and less depression of cell-mediated immunity after laparoscopy.
Methods: In a prospective randomized trial, colorectal cancer patients without evidence of metastatic disease underwent either laparoscopic ($n = 20$) or conventional ($n = 20$) tumor resection. Postoperative immune function was assessed by measuring the white blood cell (WBC) count, the CD4+ and CD8+ lymphocytes, the CD4+/CD8+/ratio, and the HLA-DR expression of CD14+ monocytes. In addition, the production of interleukin-6 (IL = 6) and TNF-α were measured after ex vivo stimulation of mononuclear blood cells with lipopolysaccharide (LPS) and compared to the plasma levels of these cytokines. Postoperative mean levels of the immunologic parameters for the two groups were calculated and compared using the Mann-Whitney U test.
Results: Preoperatively, there were no differences between the two groups in terms of patient characteristics or immunologic parameters. Although the postoperative peak concentrations of white blood cells were significant lower in the laparoscopic group than the conventional group ($p < 0.05$), there were no differences between the two groups in the subpopulation of lymphocytes (CD4+, CD8+). HLA-DR expression of CD14+ monocytes was lower in the conventional group on the 4th postoperative day ($p < 0.05$). The laparoscopic group showed higher values in cytokine production of mononuclear blood cells after LPS stimulation. Postoperative plasma peak concentrations of IL-6 and TNF-α were lower after laparoscopic resection.
Conclusion: Postoperative cell-mediated immunity was better preserved after laparoscopic than after conventional colorectal resection. Cellular cytokine production was preserved only in the laparoscopic group, while cytokine plasma levels were significantly higher in the conventional group. These findings may have important implications for the use of laparoscopic colorectal resection, especially in patients with malignant disease.

Key words: Colorectal resection — Laparoscopic surgery — Inflammatory response — Immune suppression — Surgical trauma — Cancer patients

Laparoscopic colorectal resection is less traumatic than open surgery, has positive effects on postoperative pulmonary function, and produces better cosmetic results [5, 16, 33]. Randomized controlled trials have shown that patients who undergo laparoscopic colorectal resection enjoy clinically relevant advantages not observed with those who have conventional surgery [24, 32]. In a prospective randomized study conducted in our department, we found that laparoscopic patients had a better outcome in terms of pulmonary function as well as less pain and fatigue than those who underwent conventional colorectal resection [28, 29].

It has been hypothesized that the better results after laparoscopic surgery might be related to a modified immunologic response to the laparoscopic approach [11, 26]. Major surgery and its concomitant trauma are known to cause significant modifications of the immune function [9, 20]. Moreover, the immunologic response appears to correlate with the severity of trauma [4]. These postoperative changes in immunologic function seem to be of particular importance in oncologic patients, because postoperative immuno-

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Table 1. Inclusion and exclusion criteria for enrollment in the study

Inclusion criteria
Colorectal tumor
Elective resection by right colectomy, sigmoid resection, rectum resection, or abdominoperineal rectum resection
Exclusion criteria
Rectum carcinoma within 12 cm of the anus, scheduled for sphincter-preserving anterior rectum resection with total mesorectal excision
Tumor of the transverse colon or the flexures scheduled for extended colectomy
Tumor infiltration of adjacent organs
Anesthesia risk >ASA III
Scheduled for abdominoperineal rectum extirpation with dynamic gracilis plasty
Obesity with a body mass index (BMI) >32 kg/m^2
Pronounced peritoneal adhesions from previous interventions
Synchronous extracolonic tumor
Coagulopathy not responding to treatment
Intestinal obstruction
Transverse tumor diameter >8 cm on CT
Immunopathy
Age <18 yr

suppression may be responsible not only for postoperative infections but also for tumor spread and metastases. Therefore, it seems advisable to avoid these changes in patients with malignant diseases, who often present with immunologic depression even before surgical intervention [27]. Therefore, during our earlier prospective randomized study [28], we also investigated cell-mediated immunity as well as the cellular capacity of cytokine production in patients undergoing laparoscopic or conventional colorectal resection.

Patients and methods

Hypothesis, endpoints, and sample-size calculation

The null- hypothesis (H_0) of the data analysis was that there is no difference in cell-mediated immunity between laparoscopic and conventional colorectal resection in the postoperative course. The alternative hypothesis (H_A) was that cell-mediated immunity is different for the two procedures.

The major endpoint of the analysis was HLA-DR expression on CD14+ monocytes. Minor endpoints were the white blood cell (WBC) counts, the CD4+ and CD8+ lymphocytes, the CD4+/CD8 ratio, the cellular capacity of the production of interleukin-6 (IL-6) and TNF-α, and the plasma level of both cytokines.

These parameters were evaluated in a prospective randomized trial comparing laparoscopic and conventional resection of colorectal tumors. There were 20 patients in each group. In prior investigations, we found a mean fluorescence intensity (mfi) of HLA-DR expression on CD14+ positive monocytes of 100 ± 55 on the 1st postoperative day after open resection for colorectal carcinoma. If the laparoscopic approach can lower the decrease in HLA-DR expression by 50%, 20 patients in each arm of the study would be needed in a two-sided test to detect this difference with a power of 80% ($\beta = 0.2$) and a significance level of 0.05 ($\alpha = 0.05$).

Study population

All patients scheduled for elective resection of a colorectal tumor by right colectomy, sigmoid resection, anterior rectum resection (for tumors >12 cm), or abdominoperineal rectum resection (for tumor infiltrating the sphincter) were included in the study. Inclusion and exclusion criteria are listed in Table 1. The protocol was approved by the local ethics committee, and all patients gave written informed consent.

Study design, randomization, and preoperative treatment

Mechanical bowel preparation and perioperative antibiotics were identical for both groups. Anesthesia was always performed by the same team using a standardized total intravenous technique with atracium, propofol, and sulfentanyl. Patients were ventilated with 50% forced inspiratory oxygenation (FiO_2) throughout the operation. We did not use nitrous oxide, volatile anesthetics, or regional anesthetic techniques.

All patients underwent diagnostic laparoscopy. When the surgeon decided during this procedure that laparoscopic resection of the tumor was feasible, intraoperative randomization was accomplished and a laparoscopic or conventional resection was carried out. If the surgeon decided that laparoscopic resection could not be performed, the patient was excluded from further evaluation and resected conventionally.

All laparoscopic procedures were performed by an experienced laparoscopic team using a standardized five-trocar technique that has been described in detail elsewhere [23]. After laparoscopic dissection of the lymphovascular pedicles and laparoscopic resection had been completed intracorporeally, the specimens were retrieved through mini-laparotomies measuring 3–5 cm in length. In conventional surgery, the tumor-bearing segment was resected through a wide midline incision.

All patients received patient-controlled analgesia (PCA) with morphine sulfate until the morning of the 4th postoperative day. From the 1st postoperative day on, oral feeding was advanced from clear fluids to soup, followed by mashed food and a regular hospital diet according to the status of the individual patients. Patients were not discharged before the 7th postoperative day, so that the immunologic measurements could be completed. All intra- and postoperative complications and deaths were recorded until 30 days after surgery.

Measurement of peripheral mononulear cells

Peripheral heparinized blood samples were collected the day before surgery, after resection of the tumor-bearing bowl segment, 1 and 4 hs postoperatively, and 1, 2, 4, and 7 days after the operation. The samples were processed within 30 min. All samples were tested for total white blood cell count, lymphocyte subpopulations (CD4+ and CD8+ lymphocytes and the CD4+/CD8+ ratio), and HLA-DR+ monocytes.

To determine the total leukocyte count, 10 μl of blood ethylene diamine tetraacetate (EDTA) was dissolved in 20 μl 10% acetic acid for erythrocyte lysis. The leukocyte count was carried out in an air-free environment in a Neugebauer chamber. The detected value was divided by 10, so that the calculated cell count was expressed as giga particle leukocytes per liter of blood (Gpt/L).

The subpopulations of peripheral leukocytes (CD4+ and CD8+ lympocytes, HLA-DR+ monocytes) were analyzed by means of multiparameter FACS (fluorescence-activated cell sorter), (Becton Dickinson, San Jose, CA, USA) using monoclonal antibodies (anti–CD4+ T-cell PE, anti–T-cell FITC8, and anti–HLA-DR PE, (Becton Dickinson). For this test, a round-bottomed Eppendorf tube was used to dilute 400 μl of the preoperatively acquired 3.5 ml EDTA/blood with 400 μl phosphate-buffered saline solution (PBS) (cat. no. 042-04200; Gibco, Paisley, Scotland). It was then divided into five 50-μl aliquots. Afterward, 20 μl of an antibody combination was added. After they were blended to reach a stable fixation of the antibodies and goal cells, the samples were incubated at 4°C for 30 min. After further mixing for 2 min to achieve erythrocyte lysis and fixation, 1.0 ml FACS-lysing solution was added to the samples, which were then incubated for 8 min at room temperature. The cell suspensions were centrifuged twice for 4 min at 922 g, and the supernatants were drawn off and washed in 1.0 ml FACS buffer.

The surface antigen expressions of the leukocyte populations were counted in a FACS apparatus (Becton Dickinson) to determine the quality of the measurement as well as the middle surface antigen expression of the individual cell population (indicator for receptor density of antigens).

Measurement of IL-6 and TNF-α plasma levels and cellular cytokine production of IL-6 and TNF-α

Blood samples for cytokine analysis were drawn into tubes containing 75 μmol/ml ethylene diamine tetraacetic acid and 125 μl/ml aprotinin and immediately centrifuged at 860 g for 5 min at 4°C; supernatants were

Table 2. Age, gender distribution, type of resection, duration of surgery, operative technique

	Laparoscopic group ($n = 20$)	Conventional group ($n = 20$)	p value
Age, yr	62.2 ± 11.2	65.8 ± 12.6	0.7[a]
Duration of surgery, min	210 ± 45	149 ± 49	<0.01[a]
Gender			
Male, *n* (%)	9 (45)	11 (55)	0.8[b]
Female, *n* (%)	11 (55)	9 (45)	
ASA classification			
I, *n* (%)	9 (45)	10 (50)	0.3[b]
II, *n* (%)	9 (45)	9 (45)	
III, *n* (%)	2 (10)	1 (5)	
Type of resection			
Right colectomy, *n* (%)	4 (20)	4 (20)	1.0[b]
Sigmoid resection, *n* (%)	10 (50)	12 (60)	
Rectum resection, *n* (%)	5 (25)	2 (10)	
Abdominoperineal resection, *n* (%)	1 (5)	2 (10)	
Extirpation, *n* (%)			

[a] *t*-test
[b] Fisher's exact test

repeatedly spun at 1730 g rpm (in a micro-centrifuge, Eppendorf GmbH, Hamburg, Germany, model 5410) for 5 min. The plasma was separated and aliquots were stored at −70°C for subsequent analysis of plasma levels of IL-6 and TNF-α. Cytokine levels were analyzed using a commercially available solid-phase sandwich enzyme-linked immunosorbent assay kit with monoclonal antibodies specific for IL-6 and TNF-α (Quantikins; DPC Biermann, Bad Nauheim, Germany).

For the in vitro stimulation of peripheral mononuclear blood cells, whole blood (800 μl) was mixed 1:1 (V/V) with RPMI 1640 medium (Biochrom KG, Berlin, Germany) and stimulated in microtiter plates with 100 ng/ml LPS (*Escherichia coli*/0127:B8) for 4 h in triplicate. Supernatants were separated from cells by centrifugation and stored at −85°C. ELISA for IL-6 (R&D Systems Minneapolis, MN, USA was performed with aliquots of 10 μl of the supernatant or 50 μl serum. TNF-α ELISA (R&D Systems) was performed with an aliquot of 50 μl of the supernatants.

Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc. Headquarters, 233 S. Wacker Drive, Chicago, IL, USA) was used to analyze the data. Normally distributed parameters are given as means with standard deviations (SD). Parameters not showing normal distribution are given as median and 95% confidence interval (box plots). Data between the groups were analyzed by means of the *t*-test or Mann-Whitney U- test. For all tests, differences between individual groups were considered to be significant at a level of 5% ($p < 0.05$).

Results

Forty patients were randomly assigned to undergo either laparoscopic ($n = 20$) or conventional ($n = 20$) resection of colorectal tumors. The mean age of the patients was 62.2 years (±11.2) for the laparoscopic approach and 65.8 years (±12.6) for the conventional operation. There was no difference between the groups in age, sex, American Society of Anesthesiology (ASA) classification, surgical procedure, or tumor stage (Table 2).

The operative time was 210 min (±45) in the laparoscopic group and 149 min (±49) in the conventional group ($p < 0.01$). On the 1st postoperative day after conventional sigmoidectomy, one patient required relaparotomy because of hemorrhage from the greater omentum. There were two infectious complications (both urinary tract infections) in the laparoscopic group and five in the conventional group (two pneumonia, one late intraabdominal abscess; one central line infection; one secondary perineal wound healing after abdominoperineal resection) ($p > 0.05$). Seventeen days after a conventional right colectomy, an intraabdominal abscess was drained by relaparotomy. Antibiotics were administered to all patients with pneumonia and urinary tract infections.

There was an increase in the total WBC in both groups after the operation; but from postoperative day 1 to day 4, the increase was greater in the conventional group than in the laparoscopic group ($p < 0.05$) (Fig. 1).

There was no postoperative reduction in the number of lymphocyte subpopulations expressing CD4+ and CD8+ after either the laparoscopic or the conventional approach (Figs. 2 and 3). There were no relevant changes in the CD4+/CD8+ ratio in either the laparoscopic or the conventional group (Fig. 4). The two groups did not exhibit different patterns of response following surgery.

Analysis of HLA-DR expression showed that both procedures resulted in a significant postoperative reduction of HLA-DR expression from preoperative values ($p < 0.05$). Although HLA-DR expression was not different on days 1, 2, and 7 in the two groups, on the 4th day after conventional surgery HLA-DR expression was suppressed in comparison to laparoscopic surgery ($p < 0.05$) (Fig. 5).

The postoperative production of IL-6 by peripheral mononuclear blood cells (PMBC) after LPS stimulation was suppressed only in the conventional group ($p < 0.05$); no changes in IL-6 production were detected in the laparoscopic group. The decrease of IL-6 production in the conventional group persisted until 1 day after the operation (Fig. 6). In comparison to IL-6 production, IL-6 plasma levels increased immediately after surgery in both groups. Four hours after conventional colorectal resection, IL-6 levels were higher in comparison to the laparoscopic group. This difference between the two groups was also found on day 1 ($p < 0.01$) (Fig. 7).

TNF-α production of PMBC after LPS stimulation decreased in both groups. The lowest value was noted in both groups 4 hs after surgery. However, the drop in TNF-α production was greater in the conventional group than the laparoscopic group at day 1 after surgery ($p < 0.05$) (Fig. 8). By comparison, the serum concentration of TNF-α was increased over preoperative values in the early postoperative period in both groups. However, from 1 h ($p < 0.05$) until day 2 ($p < 0.05$), plasma concentrations of TNF-α in the conventional group were higher than those in the laparoscopic group (Fig. 9).

Discussion

Because of its short-term benefits in the postoperative period the laparoscopic approach is the preferred technique for a variety of surgical procedures [6, 8, 33]. Moreover, it has been shown that laparoscopic colorectal resection is technically feasible and capable of fulfilling the oncological criteria for cancer surgery [16, 18, 19, 21, 22, 33]. Several controlled randomized trials have demonstrated that laparo-

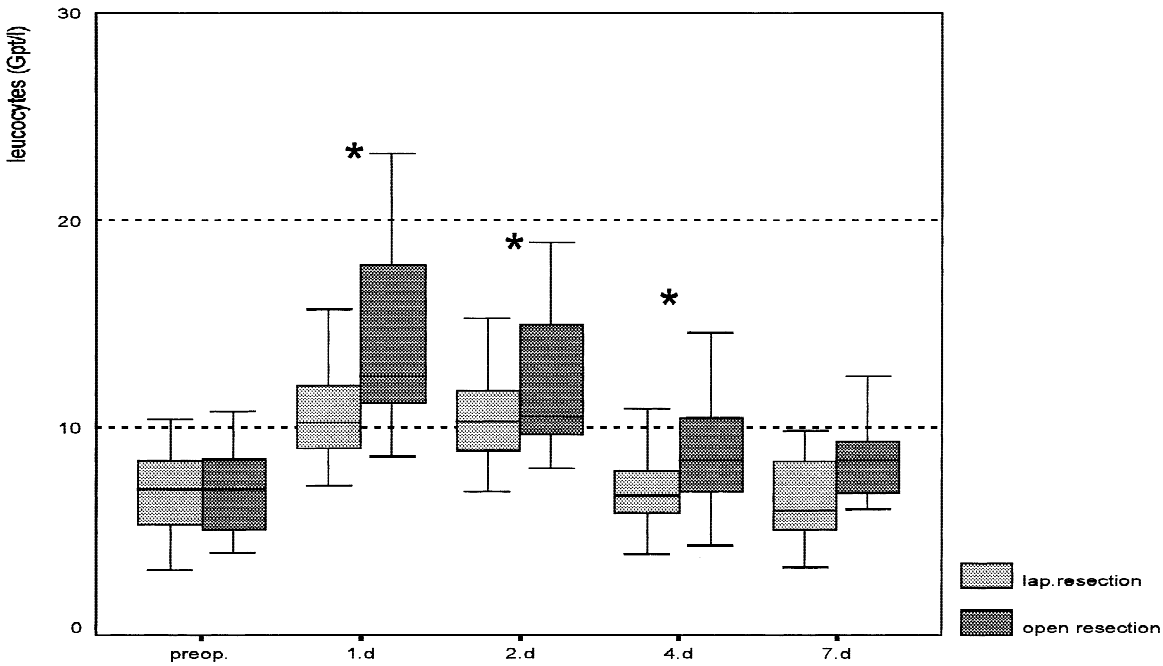

Fig. 1. White blood cell counts in patients with laparoscopic and conventional resection of colorectal tumors *$p < 0.05$ (Mann-Whitney U test).

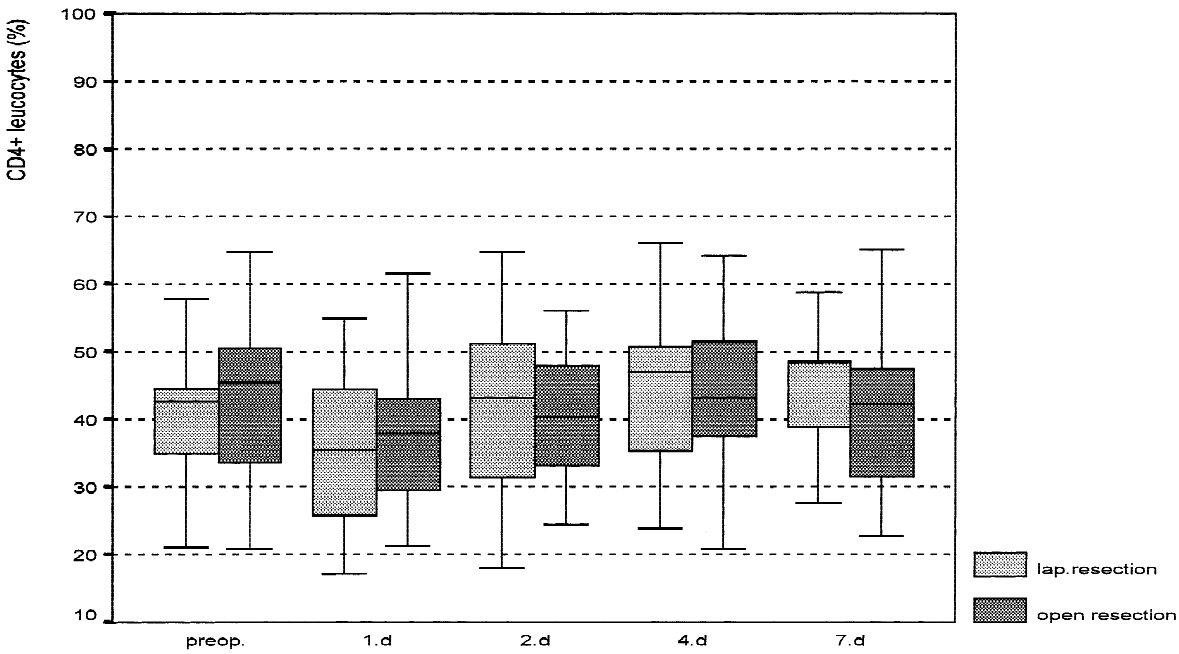

Fig. 2. CD4+ lymphocytes in patients with laparoscopic and conventional resection of colorectal tumors.

scopic surgery has certain clinical advantages—such as reduced postoperative pain, faster postoperative bowel activity, a shorter hospitalization, an earlier return to work, and better cosmetic results—when compared to conventional surgery [19, 23, 25, 28, 32]. Although these clinical benefits are important and are thought to reflect the lesser surgical trauma, long-term survival and the disease-free interval of patients are the most important endpoints for oncological surgery.

The underlying reason for the clinical benefits of minimally invasive surgery may be that the immunological response is different after laparoscopic surgery than it is following conventional surgical procedures. Surgical stress has been shown to be associated with postoperative alterations in host immune functions [9, 20]. Moreover, the immunologic response appears to correlate with the severity of trauma [4]. Evidence has accumulated that laparoscopic surgery results in better preservation of the patient's immunological defenses; there are also clinical studies that have shown a preservation of postoperative immunological function after laparoscopic procedures [1, 17].

In the present study, both the laparoscopic and the con-

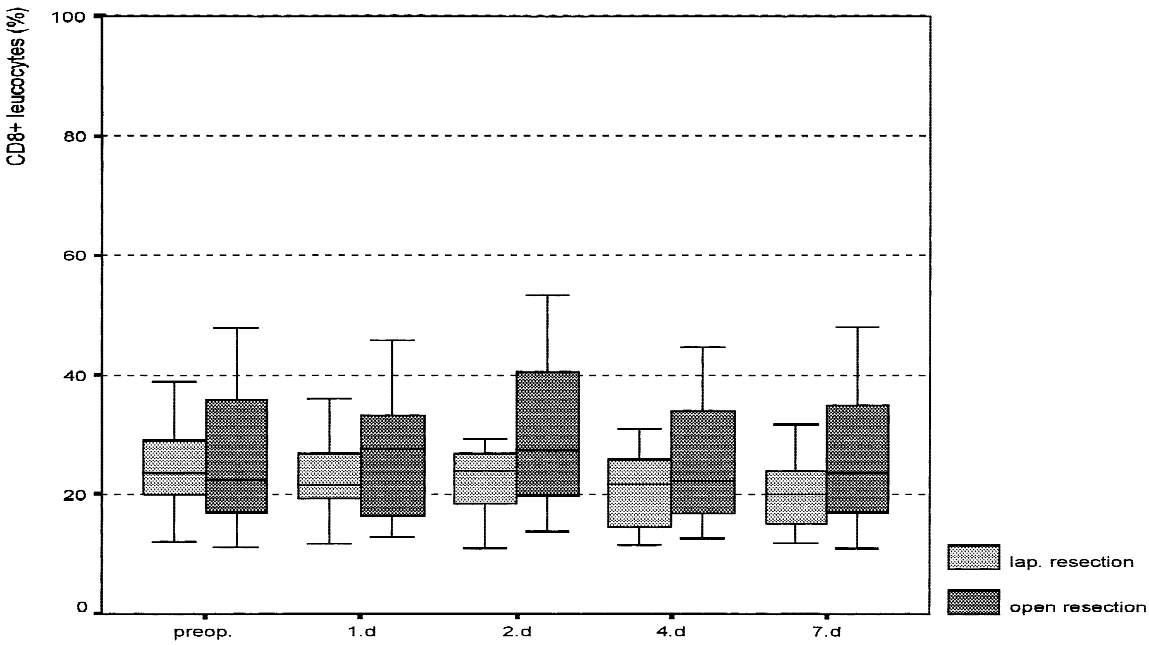

Fig. 3. CD8+ lymphocytes in patients with laparoscopic and conventional resection of colorectal tumors.

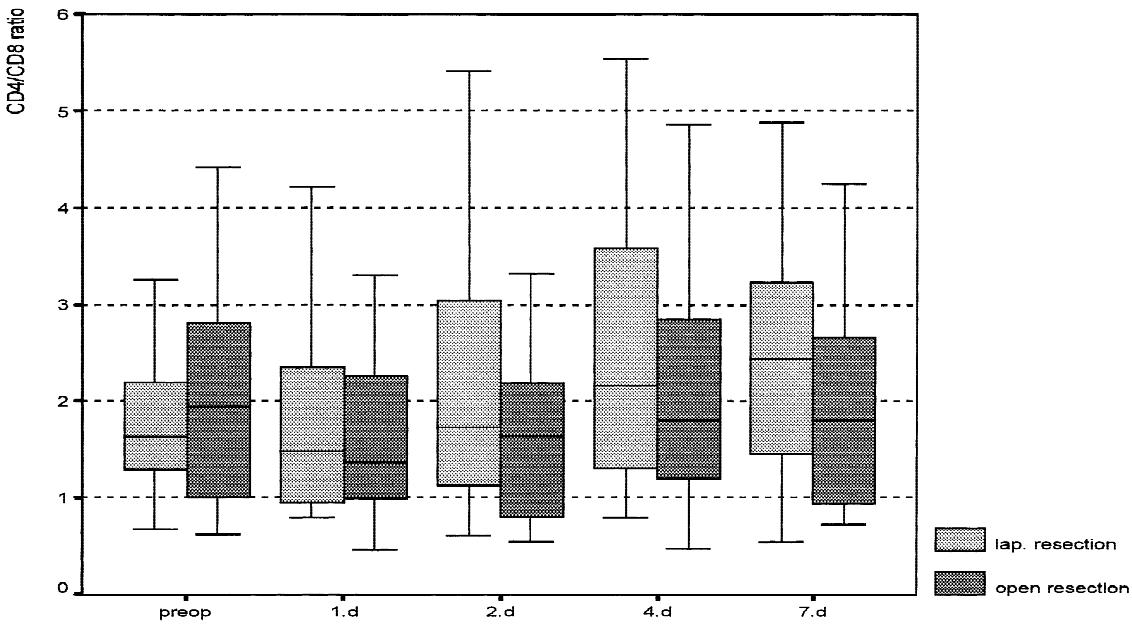

Fig. 4. CD4+/CD8+ ratio in patients with laparoscopic and conventional resection of colorectal tumors.

ventional procedures resulted in an increase in the total number of peripheral blood leukocytes. However, the postoperative leukocyte count was higher in the conventional group than in the laparoscopic group. By contrast, Sietses et al. found no differences in WBC following open and laparoscopic Nissen fundoplication [31]. Similar results were reported by Zieren et al. in their investigation of laparoscopic and open Nissen fundopolication [35]. Nevertheless, Nissen fundoplication can hardly be compared with colon resection, since the extent of intraabdominal trauma is so much greater due to the preparation of the bowel and the resection of the tumor-bearing bowel segment.

The lymphocyte count, an important indication of specific immune response, was lower than preoperative levels from postoperative day 1 to day 4 in both groups, but no differences between the groups were observed in our study. In a randomized trial, comparing the total lymphocyte count after open and laparoscopic cholecystectomy, Cristaldi et al. found a trend toward lower levels after open procedures when compared with the laparoscopic approach during a 7-day period [3].

In our study, the number of lymphocyte subpopulations expressing CD4+ and CD8+ showed no postoperative changes with either the laparoscopic or the conventional approach. There were no differences between the two groups of patients.

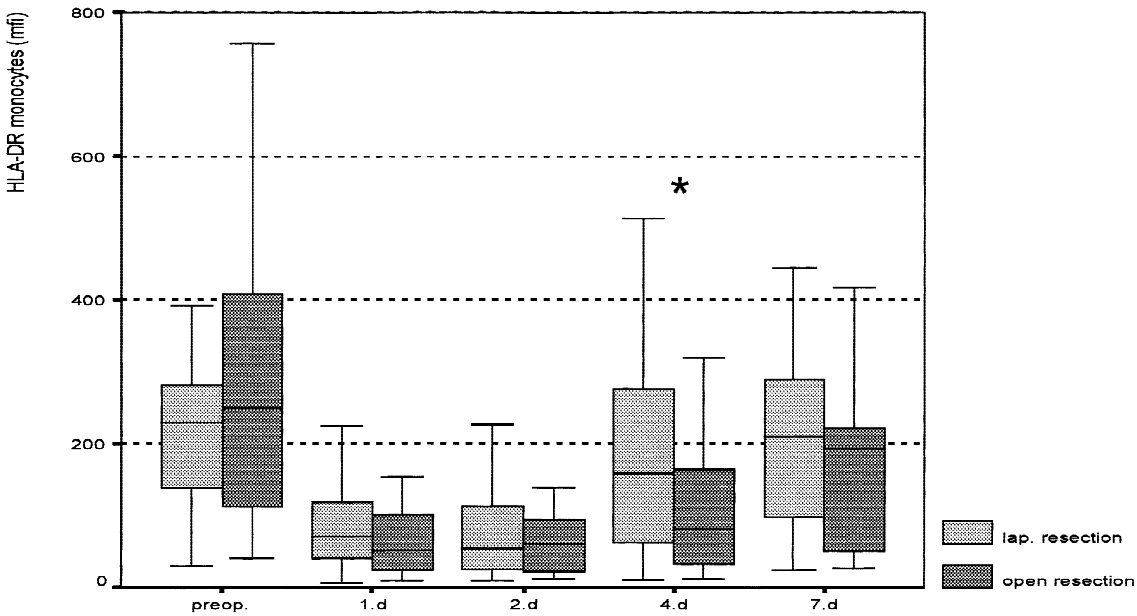

Fig. 5. HLA-DR expression on CD14+ monocytes in patients with laparoscopic and conventional resection of colorectal tumors. *$p < 0.05$ (Mann-Whitney U test).

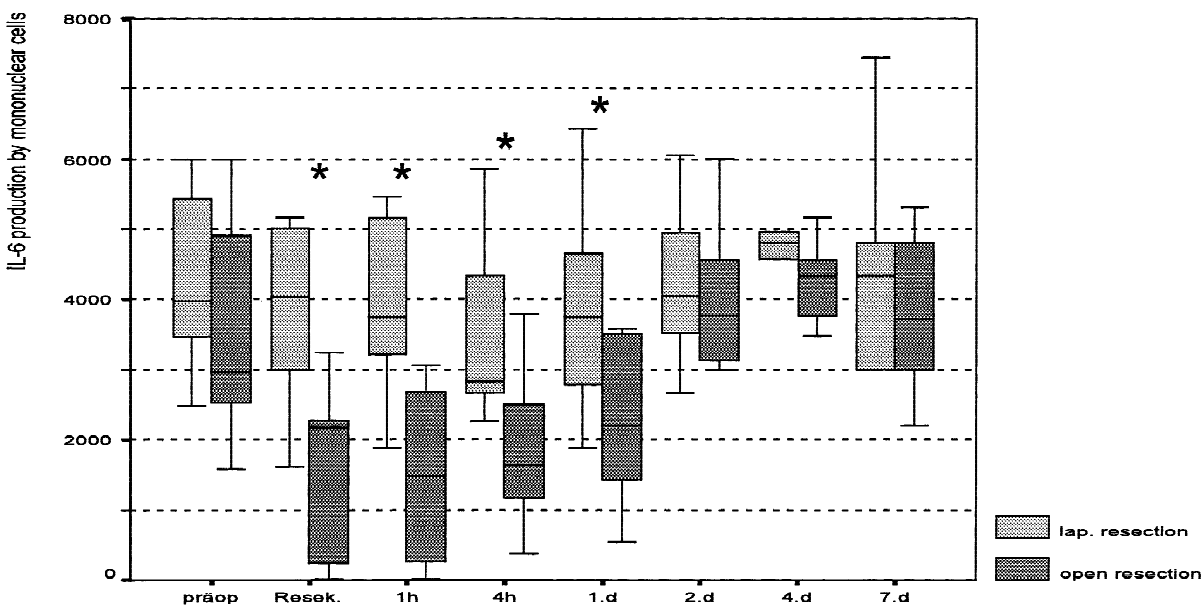

Fig. 6. Perioperative levels of IL-6 stimulated with LPS in patients with laparoscopic and conventional resection of colorectal tumors. *$p < 0.05$ (Mann-Whitney U test).

The effects of mini-laparotomy and laparoscopic cholecystectomy on cell-surface phenotypic profiles (CD4+, CD8+) were also examined by Walker et al. [34]. They found no significant differences between the two groups at any time for CD4+ cells, but there were differences between the two groups for CD8+ cells. In contrast, Dionigi et al. [7] reported a difference between open and laparoscopic cholecystectomy in the percentage of CD3+ cells but no difference in CD4+ and CD8+ cells.

In recent years, it has been clearly demonstrated that monocytes play a central role in the immune function and that a reduction in HLA-DR expression after surgical intervention is associated with an increase of postoperative infectious complications [2, 13]. Previous studies have shown that HLA-DR expression is related to the severity of the surgical trauma in the pathogenesis of septic processes and their healing [13].

The only randomized study in which HLA-DR expression of monocytes was compared after laparoscopic or conventional colorectal cancer resection was carried out by Hewitt et al. [14]. They found no difference in this parameter between laparoscopic-assisted resection and open surgery. However, since only eight patients were included in each group, the results have to be regarded with a degree of skepticism. In our study, both procedures resulted in a reduction of HLA-DR expression on monocytes from preop-

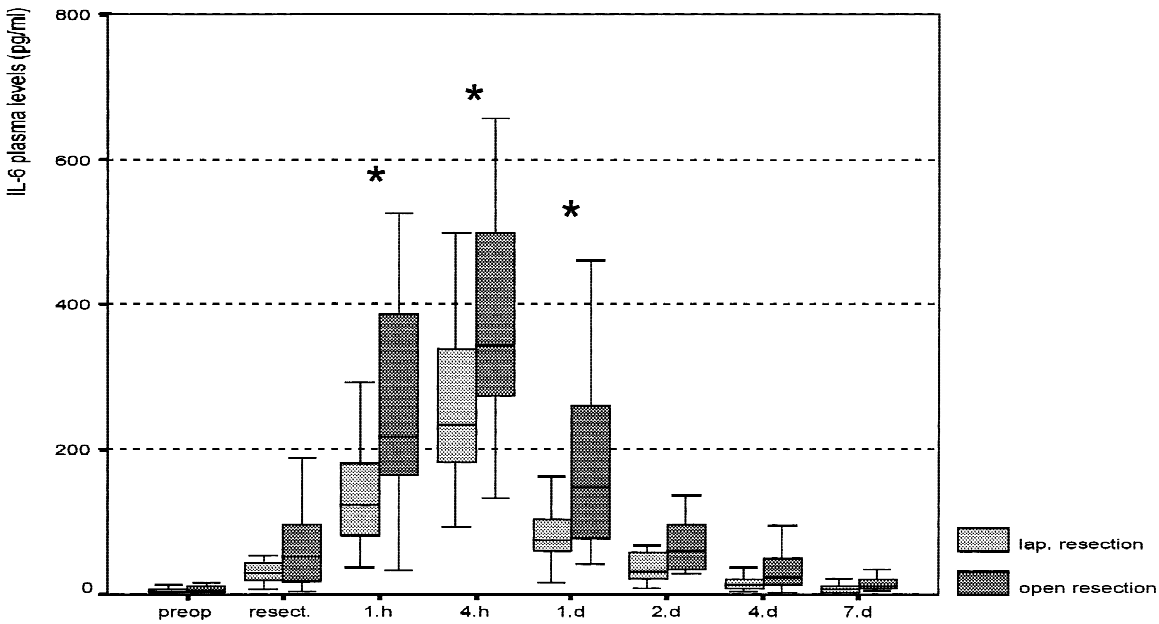

Fig. 7. Perioperative plasma levels of IL-6 in patients with laparoscopic and conventional resection of colorectal tumors. *$p < 0.05$ (Mann-Whitney U test).

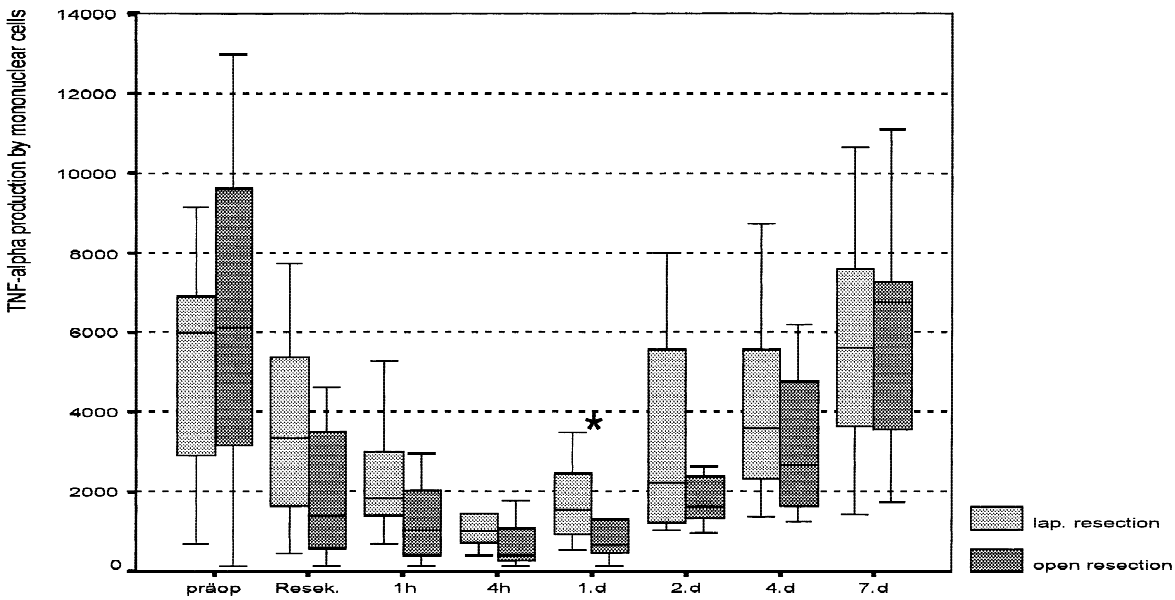

Fig. 8. Perioperative levels of TNF-α stimulated with LPS in patients with laparoscopic and conventional resection of colorectal tumors. *$p < 0.05$ (Mann-Whitney U test).

erative values. Although laparoscopic procedures were found to show a faster recovery in HLADR expression, significant differences between both procedures were only demonstrated on the 4th postoperative day.

These results support earlier findings by Sietses et al. [31] and Brune et al. [1], who also reported a reduction in HLA-DR expression after both laparoscopic and conventional surgery, as well as a faster return to normal values after laparoscopy. Furthermore Kloostermann et al. [17] demonstrated a reduction in HLA-DR expression on day 1 after conventional surgery, whereas in the laparoscopic group there was no reduction at all.

Although the postoperative change in absolute numbers of PMBC is significant, it seems even more important to analyze the function of these cells. One possible way of measuring this parameter is to examine the capacity of cytokine production after stimulation with lipopolysaccharide (LPS). Experimental studies have shown that the determination of the capacity of PMBC to produce cytokines represents a more reliable index of the inflammatory state of surgically treated patients than measuring cytokine levels in the plasma alone [10]. To date, there have been no published studies that have specifically examined the LPS-stimulating capacity of PMBC after laparoscopic and conventional colorectal resection.

In our study, LPS-stimulated TNF-α synthesis was significantly lower in the conventional group than in the laparoscopic group at day 1 after surgery. Interestingly, the

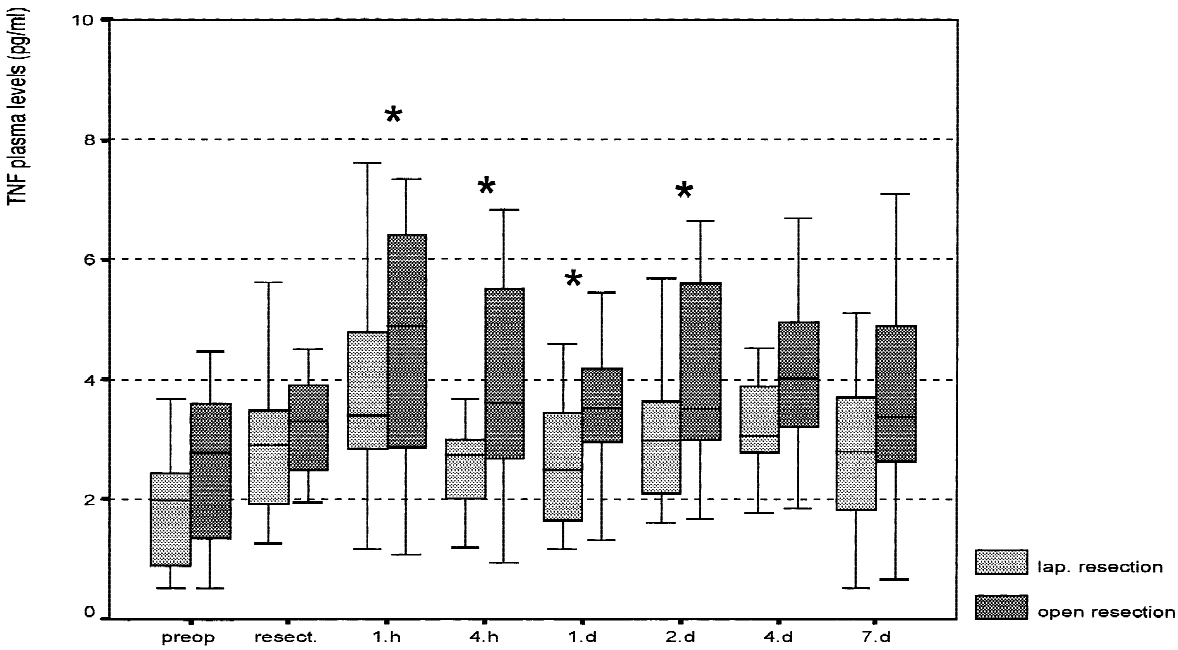

Fig. 9. Perioperative plasma levels of TNF-α in patients with laparoscopic and conventional resection of colorectal tumors. *$p < 0.05$ (Mann-Whitney U test).

TNF-α plasma concentrations were significantly higher in the conventional group than in the laparoscopic group. It may be that the inflammatory response and the acute cell response are higher after conventional surgery than with laparoscopy.

We found similar results when we examined IL-6 production after stimulation with LPS. Although there was a significant decrease in the production of IL-6 in the conventional group, the laparoscopic group did not show any significant changes from the preoperative levels of IL-6 production in the perioperative course.

Furthermore, as reported before by Schwenk et al. [30] and in agreement with other studies [14, 32], the plasma peak levels of IL-6 were higher after conventional surgery than after laparoscopic surgery. Plasma IL-6 levels are known to be proportional to the magnitude of the surgical trauma and a predictor of postoperative complications [12].

Immediately after surgery, an equal increase of IL-6 levels was found in both groups. However, after conventional colorectal resection, the IL-6 level remained elevated in comparison to the laparoscopic group until day 2. Therefore, our results support the hypothesis that laparoscopic surgery causes only minor surgical trauma to the peritoneal tissues.

We conclude that postoperative immune suppression follows conventional and laparoscopic surgery, but the impact of laparoscopic surgery on the postoperative systemic immune response is less than that seen with the conventional approach. Thus, not only does laparoscopic surgery have clinically relevant advantages, it also causes less impairment of the immune system than conventional operations. This effect is mainly represented by a faster recovery of HLA-DR expression, a normal function of cell capacity, and a lower increase in IL-6 plasma levels in laparoscopy patients. The findings of the present study support the hypothesis that immune function is influenced by the size of the abdominal wall incision and the extent of the surgical trauma.

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