

# Cellular and humoral inflammatory response after laparoscopic and conventional colorectal surgery.

## Preliminary report



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## Cellular and humoral inflammatory response after laparoscopic and conventional colorectal surgery. Preliminary report

*Our aim is to compare the immune response after colorectal surgery performed laparoscopically and via traditional technique. This response seems to be proportional to the level of the surgical trauma and presumably is directed to improve host defence. This is a prospective reported study based on patients' randomisation. Fourteen patients with colorectal diseases undergoing laparoscopic or open surgery were enrolled. After both laparoscopic and open colorectal surgery, we observed a significant increase of circulating C-Reactive Protein (CRP) levels. The count of lymphocytes subpopulations did not show significant differences after both procedures. IL-6 serum levels increased immediately after laparoscopic approach.*

*IL-6 production was preserved only in the laparoscopic group, while its plasma levels were significantly higher in conventional group. Postoperative cell-mediated immunity was better preserved after laparoscopic than after conventional colorectal resection.*

*Laparoscopy became a popular approach to treat surgically benign and malignant colorectal diseases and several authors reported a better immune response in patients performing laparoscopic surgery after comparing to conventional colorectal surgery. These findings may have important implications in performing a laparoscopic colorectal resection.*

KEY WORDS: C-Reactive Protein IL-6, Laparoscopic Surgery

## Introduction

Surgical trauma evokes a variety of physiologic and immunologic alterations. The body's acute phase response to injury represents a complex interaction between neuroendocrine, metabolic and immune systems. Tissue trauma,

as a result of incisions, tissue dissection, organ manipulation and vascular arrangement stimulates early inflammatory response.

This response seems to be proportional to the degree of the initial insult and presumably is directed to improve host defence<sup>1</sup>. Conventional or laparoscopic surgery is a controlled trauma with a stress response and immunological consequences. In most cases, surgery induces a generalized state of immune-depression.<sup>2</sup>

Postoperative immunosuppression may have important consequences as it has been related to infectious complications and metastasis development<sup>3</sup>.

This is a prospective randomised study, whose aim is to examine the effect of surgical trauma in terms of cellular and humoral inflammatory response, studying laparoscopic versus open surgery.

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## Material and Method

The most considered proteins in literature are cytokines, as IL-6, and C-Reactive Protein (CRP), studied in serum and in peritoneal fluid <sup>5,6</sup>. Cytokines are produced by immune system cells and other tissues and act as mediators of the immune and acute phase response.

In our study, we considered the levels of IL-6, C-Reactive Protein and immune cells' phenotype. Blood samples were taken preoperatively (baseline) and postoperatively, 24 hours and days 7 after surgery.

The level of interleukin-6 (IL-6) is proportional to the severity of surgical trauma <sup>7,8</sup>. Testing blood was drawn *via* peripheral venepuncture preoperatively, 4, 24 hours and days 7 after surgery. Serum samples were obtained by centrifugation for 10 minutes at 3000 rpm at 4°C. All samples were stored in aliquots at -80°C. All IL-6 levels were determined in duplicate using a commercially available solid-phase sandwich enzyme-linked immunosorbent assay kit with monoclonal antibodies specific for IL-6 (Pierce Biothecnology, Inc, USA). The test has a sensitivity <1 pg/ml.

C-Reactive Protein (CRP) is a key marker acute-phase protein and provides an overall marker for acute phase reactants for a lot of trauma and infections. The RCRP method is based on a particle enhanced turbidimetric immune assay (Petia Technique), with a minimum detectable level of 0-3 mg/l).

Major surgical trauma also produces a modification of innate immune system <sup>9</sup>. To study immune cells' phenotype, peripheral venous blood samples were collected in EDTA collection tubes. 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), BD Multitest 6-color TBNK reagent (BD) has been used to characterize lymphocyte subset populations. Briefly, 50 l of well-mixed, anticoagulated whole blood are stained with 10 ml of BD Multitest 6-color TBNK reagent; after

15 minutes in the dark at room temperature, 450 l of lysing solution were added to the tube and after 15 minutes the sample has been analyzed on the flow cytometer FACSCanto II (BD). Results have been reported as percentage of T lymphocytes (CD3+), B lymphocytes (CD19+) and NK lymphocytes (CD16+CD56+).

Data between the groups were analyzed by means of *t*-test. Differences between individual groups were considered statistically significant at *P* <0,05. Data are presented as mean±DS.

## Patients and treatment

Fourteen consecutive patients enrolled with colorectal diseases undergoing laparoscopic or open surgery. Inclusion and exclusion criteria are listed in Table I. Written informed consent was obtained from all patients enrolled in this study.

All operations were performed by the same surgeon who has a significant experience with laparoscopic and open technique. For both types of surgery, radical complete tumour excision was performed with ligation as proximal as possible to the pertinent vessels to maximize lymph nodes clearance. Minimally invasive colorectal surgery was performed by laparoscopic procedure with removal of the dissected specimen via a mini-laparotomy (5 cm) like Pfannestil. Laparoscopic surgery was performed using a 3 trocars technique with 1 trocar (10 mm) inserted via a paraumbilical incision (camera port). Two additional (10 mm) trocars were inserted in the up or down right or left abdomen, according to the site of tumour. After removing the dissected specimen and preparation of the stapler anastomosis, we closed the mini-laparotomy and reintroduced pneumoperitoneum to complete the intraabdominal anastomosis.

Conventional colorectal surgery was performed via classical technique.

Table I - Patients' characteristics

	VL <sup>b</sup>	OS <sup>c</sup>	p value
Patients (pt)	7	7	
Age (ys)	67 ± 8,2	74 ±10,4	
Average; Median	66,7; 68	74,1; 74	
Genre: M/F	3/4	4/3	
Primary tumour site Colon/Sigma/Rectum	5/2/0	3/3/1	
Hystotype			
Poorly/Moder./Well differ.	4/1/2	2/3/2	
Timing	261,4 ± 73,4	153,6 ± 56,8	<0.05
Hospitalization (days)	9.7 ± 2.9	9 ± 2.2	
TNM T1/T2/T3/T4	0/0/4/3	0/2/3/2	
Lymph nodes N0/N1/N2	2/2/2	5/2/0	
Metastases M0/Mx/M1	0/7/0	0/7/0	

## Results

Fourteen consecutive patients have been selected to perform this study, which took place in the Unit of General Surgery “V. Bonomo”, University of Bari and it is a prospective reported study based on patients’ randomisation.

Patients’ characteristics can be appreciated in Table I. Seven patients have been selected to perform a laparoscopic surgery and seven patients to perform a conventional colorectal surgery. The average age was  $67 \pm 8,2$  years (mean 66,7; median 68) for laparoscopic patients and the average was  $74 \pm 10,4$  years (mean 74,1; median 74) for open technique. There were no differences between genre, age, drugs or kind of resection performed. Operating time was  $261,4 \pm 73,4$  minutes for laparoscopic approach versus  $153,6 \pm 56,8$  minutes for open surgery ( $p < 0.05$ ). All patients have been discharged in healthy conditions, after 7 days (average 9.7; median 9.7) for what concerns laparoscopic group and after 9 days for what concerns conventional group (average 9.7; median 9.7).

**Interleukin – 6.** Serum pre and post-operative IL-6 levels can be appreciated from Fig. 1 Before the surgery, serum IL-6 concentration is low in both groups ( $18,9 \pm 7,7$  pg/ml *vs*  $15,4 \pm 7,1$  pg/ml,  $p=0,4$ ). After the surgery, post-operative IL-6 levels increased dramatically to a maximum in both groups:  $101,75 \pm 38,5$  pg/ml for laparoscopic patients’ group versus  $204,2 \pm 58,3$  pg/m for conventional patients’ group,  $p=0.002$ . After 24hours, serum IL-6 levels decreased and returned to preoperative

levels ( $61,4 \pm 26,5$  pg/ml *vs*  $73 \pm 56,4$  pg/ml,  $p=0,7$ ). In both groups, IL-6 serum levels returned to normal levels within 6-7 days after surgery,  $18,4 \pm 6,8$  pg/ml *versus*  $19,2 \pm 16,8$  pg/ml,  $p=0,9$  (maximum basically concentration 19,86 pg/ml).

**C- Reactive Protein (CRP).** Serum CRP levels were analyzed before and after surgery and also after 24hours and after 7 days. All results can be appreciated in Fig. 2. There are no differences concerning open surgery results, while there is an increase for what concerns laparoscopic patients. In both groups, an increasing level can be appreciated after 24 hours ( $84,3 \pm 41,1$  mg/L *vs*  $90,7 \pm 51,2$  mg/L) ( $p < 0.05$ ). After 7 days, all levels decreased and return to normal value. There are no differences for what concerns other timing ( $p=0.4$ ).

**Cellular immune response.** To test the cellular immune response, it has been considered to evaluate the levels of White Blood Cells (WBC) and T cell subset (CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, NK cells, CD19<sup>+</sup> e CD4<sup>+</sup>/CD8<sup>+</sup> ratio) (Table II). WBC increased in both groups after surgery and there is an important increase in post-operative time ( $15.9 \pm 5.3$   $10^3/\mu\text{l}$  *vs*  $9.8 \pm 3.4$   $10^3/\mu\text{l}$ ,  $p < 0.03$ ) (Fig. 3). Both groups had a different immune response: laparoscopic patients had the maximum levels after the surgery and those levels progressively decreased in following hours and after 7 days they turned to normal values ( $4-10$   $10^3/\mu\text{l}$ ). At 24 hours, the conventional group has the maximum level and it turned to normal value after the seventh day of surgery. Although the different appearing time of the maximum level (4h *vs* 24 h),

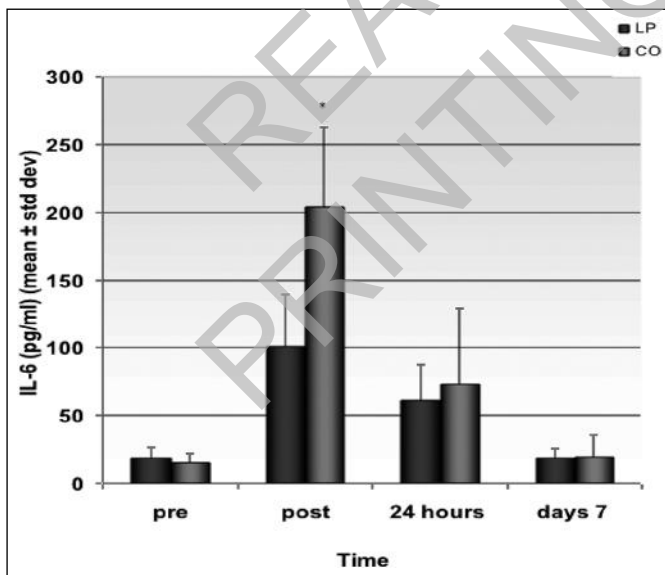


Fig. 1: Interleukin-6 (IL-6) levels preoperative (pre), postoperative (post), twenty-four hours and seven days after surgery in patients undergoing laparoscopic vs conventional colorectal surgery. \*  $P < 0.05$ . VL= videolaparoscopic surgery; CO= conventional surgery.

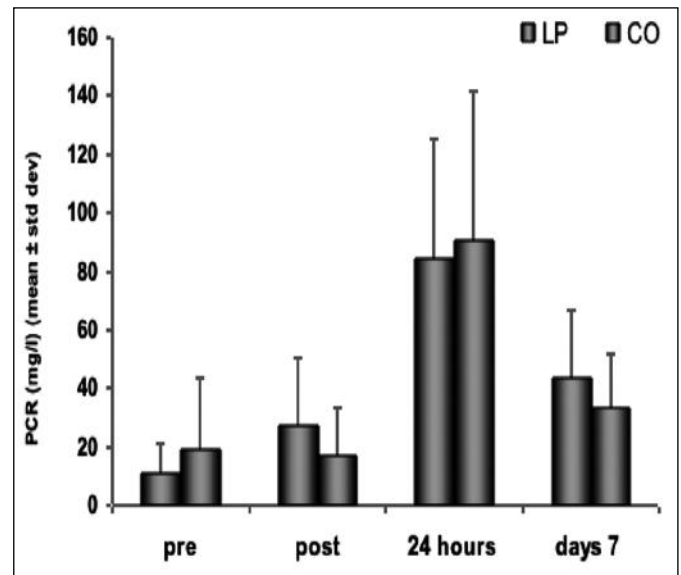


Fig. 2: C-reactive protein (CRP) levels pre (baseline), post (three hours), twenty-four hours and seven days after surgery in patients undergoing laparoscopic vs conventional colorectal surgery. VL= videolaparoscopic surgery; OS = conventional surgery.

Table II - Cell Count

	Treatment group	Preoperative	Postoperative		
			4 hours	24 hours	Days 7
WBC	VL	4,7 ± 0,1	13,15 ± 1,8*	12,3 ± 0,8	5,9 ± 0,3
	CO	6,8 ± 1,0	7,8 ± 9,2*	10,4 ± 2,0	5,1 ± 0,9
CD3 <sup>+</sup>	VL	73 ± 9,9	69,5 ± 16,3	62,5 ± 19,1	70,5 ± 9,2
	CO	69 ± 18,1	55,7 ± 30,1	62,7 ± 22,1	64,7 ± 20,1
CD4 <sup>+</sup>	VL	43 ± 2,8	39,5 ± 7,8	26 ± 9,9	42,5 ± 0,7
	CO	47,3 ± 10,5	38 ± 28,2	45,3 ± 20,6	46 ± 14,8
CD8 <sup>+</sup>	VL	16 ± 2,8	17 ± 5,7	22 ± 7,1	18 ± 1,4
	CO	16 ± 3,5	17,3 ± 7,0	16,7 ± 6,1	17,3 ± 6,7
NK cells	VL	16 ± 8,5	19,5 ± 14,8	22 ± 17	17,5 ± 7,8
	CO	23,3 ± 19,2	37,7 ± 31,5	30,7 ± 23,5	27,7 ± 20
CD19 <sup>+</sup>	VL	10 ± 2,8	10,5 ± 2,1	15,5 ± 2,1	12 ± 1,4
	CO	14,0 ± 3,5	14,9 ± 3,9	18,1 ± 3,5	15,8 ± 3,3
CD4 <sup>+</sup> / CD8 <sup>+</sup>	VL	2,1 ± 0,5	2,7 ± 0,7 *	2,1 ± 0,7	2,5 ± 0,7
	CO	2,3 ± 1,2	1,6 ± 1,0	2 ± 1,2	2,1 ± 1

WBC = white blood cells; VL = laparoscopic surgery; CO = conventional surgery; NK = natural killer.

WBC figures are  $10^3/l$ ; CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, NK cells, CD19<sup>+</sup> cells figures are on percentage

\*p< 0,05

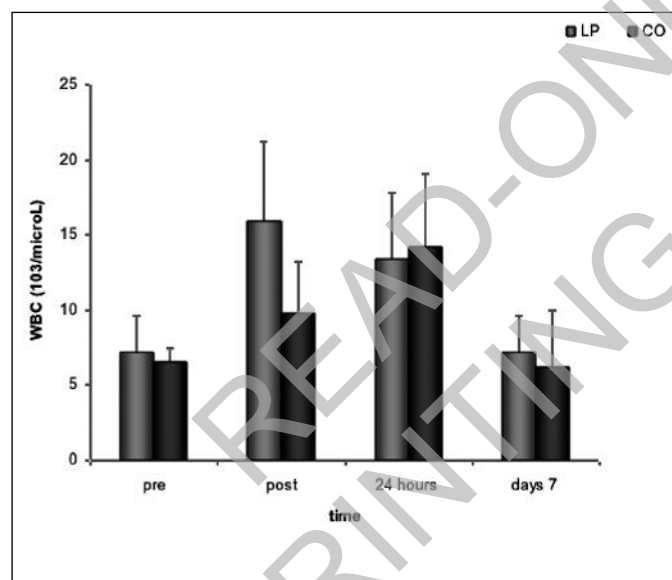


Fig. 3: White blood cells (WBC) count pre (preoperative), post (postoperative), twenty-four hours and seven days after surgery in patients undergoing laparoscopic vs conventional colorectal surgery. VL = laparoscopic surgery; CO = conventional surgery; \* p=0.03.

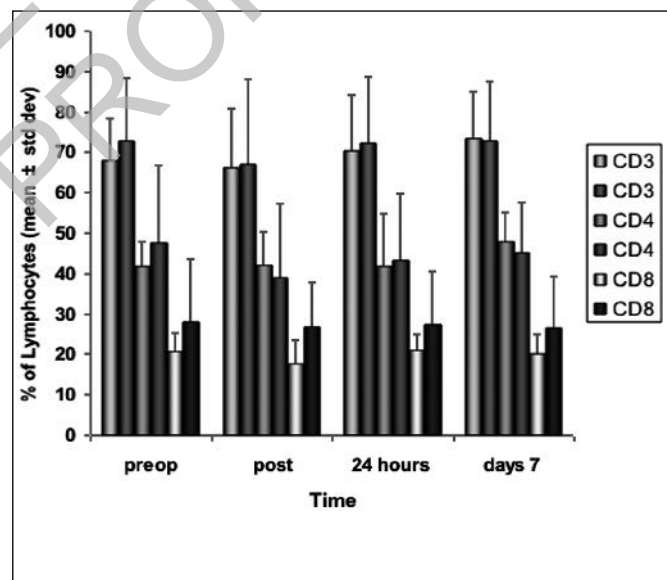


Fig. 4: T cell subsets changes preop (preoperative), post (postoperative), twenty-four hours and seven days after surgery in patients undergoing laparoscopic vs conventional colorectal surgery.

24 hours values appeared higher in laparoscopic patients than in other ones ( $12.3 \pm 0.8 \cdot 10^3/\mu l$  vs  $10.4 \pm 2.0 \cdot 10^3/\mu l$ ). After the surgery, there was a decrease of all the lymphocytes subset (Figs. 3, 4). In both groups there were not relevant changes of T lymphocytes (CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>) and B lymphocytes (CD19<sup>+</sup>). A relevant difference can be appreciated for what con-

cerns post-operative CD4<sup>+</sup>/CD8<sup>+</sup> ratio in both groups ( $p=0.04$ ). After the surgery, CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes count changed relevantly, especially in conventional group ( $2.7 \pm 0.7$  vs  $1.6 \pm 1.0$ ) and then returned to normal levels. NK cells didn't show important changes in both groups (Fig. 4).

## Discussion and Comments

Colorectal cancer is one of the most frequent malignant tumors of digestive system and it presents high morbidity and mortality all over the world. Nowadays, surgery is the first approach to treat colorectal cancer. The development of minimally invasive surgery changed the surgical treatment towards several benign and malignant diseases, because it limits surgical traumas. According to Farinetti and his staff, that compared open versus laparoscopic treatment in colorectal cancer, laparoscopic technique presents a better outcome considering pains, absence of extended scars, discharging time and incisional hernias <sup>10</sup>.

The depression of the immune system has also a significant role in neoplastic cells spreading. Nevertheless surgery has been considered as one of the best method for neoplastic resection, lots of clinical studies demonstrated that surgical trauma reduces the effect of the immune system and this condition should be considered as negative prognosis. In literature there is clear evidence that both techniques seem to be oncologically correct after evaluation of lymphadenectomy and equal for what concerns margins of resection and long-term survival <sup>11</sup>. Also Brisinda evaluated the differences between laparoscopy and open technique and the combined use of intraoperative radiotherapy (IORT) and the findings are good oncologic results in laparoscopy and its application in any kind of colorectal cancer <sup>12</sup>. After the comparison between laparoscopic and open cholecystectomy and acute phase system, the average of studies considers that the laparoscopic approach reduces the acute phase immunological system. Consequently, it's really important to find a way to reduce surgical trauma and also a way to reduce the influence of the immune system. Recent clinical trials evaluated safety and advantages of both laparoscopic and conventional open surgeries and laparoscopic surgery is feasible in cancer treatment <sup>13</sup>. Operative trauma, measured by magnitude and type of intra-operative injury, determines the extent and duration of the postoperative inflammatory and immunological responses. Inflammatory response is mediated by a substances which are produced in wounded and manipulated tissues or at distance and they can act locally and systematically <sup>14</sup>.

IL-6 is considered as the most important marker of the tissue loss and it represents one of the most relevant mediators of acute phase proteins.

According to our work, the highest IL-6 level was found in conventional group in post-operative, 24hours and 7 days after surgery. These data can be also compared to Baigrie's study <sup>15</sup>.

CRP is an important acute phase protein, which increases within 6-24 hours after trauma and then it gradually decreases according to the body assessment. PCR levels were higher in conventional group 24 hours after surgery, therefore PCR is an important prognostic marker.

For what concerns WBC, neutrophil granulocytes seem to be increased in both groups and the highest level can be appreciated in laparoscopic group after the surgery ( $15.9 \pm 5.3 \cdot 10^3/\mu\text{l}$  versus  $9.8 \pm 3.4 \cdot 10^3/\mu\text{l}$ ,  $p < 0.03$ ). This growth can pretend that there is a more important inflammatory response in the laparoscopic group, but there less activation of the lymphocytes response B, NK, CD4<sup>+</sup> CD8<sup>+</sup>. This difference needs to be highlighted, because it represents the effective reduction of lymphocytes activity and humoral immune response between laparoscopic and open surgery.

## Conclusions

Inflammatory response seems to be more relevant in traditional group than in laparoscopic one. Considering surgical trauma, in laparoscopy, there is a less activation of the inflammatory response and, consequently, a less negative modulation of the immune system. Nevertheless, in our experience, the times of surgery seem to be longer than in traditional group, all values are anyway better for laparoscopic technique, especially in terms of biohumoral activation. Laparoscopy can give good results in short term and also in long term because of less compromising of the immune system. This point is also verified in literature in terms of better outcome, hospital stay, incisional hernias and respect of incision margins, so laparoscopy has to be considered oncological correct as the open technique <sup>16</sup>.

We are working on this preliminary report, because we want to check other inflammatory parameters as TNF $\alpha$  and we want to continue to follow up.

## Riassunto

La laparoscopia rappresenta un approccio ormai noto per trattare chirurgicamente le patologie benigne e maligne coloretali e numerosi autori hanno riportato una risposta immunologica migliore nei pazienti sottoposti a chirurgia laparoscopica eseguendo un confronto con la chirurgia coloretale tradizionale. Il trauma chirurgico determina una risposta immunitaria locale che può diventare una risposta sistemica in seguito agli stimoli infiammatori e ciò porta ad attivazione del sistema immunitario in difesa dal trauma chirurgico e contemporaneamente una modulazione negativa con immunosoppressione circa l'eventuale difesa dalle infezioni e la diffusione di cellule neoplastiche.

Lo scopo del nostro studio è valutare la differente risposta bioumorale al trauma chirurgico in pazienti sottoposti a resezione colica per via videolaparoscopica ed in pazienti sottoposti ad analogo intervento per via laparotomica convenzionale. Sono stati arruolati quattordici pazienti, con patologia maligna coloretale, di cui 7 sottoposti a chirurgia laparoscopica e 7 a chirurgia tradi-

zionale, selezionati in base all'anamnesi negativa per pregressa patologia neoplastica e/o per patologie particolarmente invalidanti ed in base alla stadiazione del tumore, per cui non vi era evidenza di metastasi a distanza. Tutti i campioni sono stati collezionati prima dell'intervento e dopo di esso, alle 24 ore e a 7 giorni e sottoposti ad analisi di laboratorio: analisi ELISA, determinazione PCR tramite dosaggio immunoturbidimetrico quantitativo, fenotipizzazione delle cellule immunitarie. Abbiamo osservato un incremento significativo dei livelli circolanti di Proteina C-Reattiva (PCR) alle 24h dall'intervento nel gruppo convenzionale. Il valore dell'Interleuchina-6 (IL-6) ha subito un aumento nell'immediato postoperatorio dopo approccio laparoscopico. I livelli delle sottopopolazioni linfocitarie subiscono minor elevazione nel gruppo laparoscopico. In conclusione, la produzione di mediatori dell'infiammazione è maggiormente preservata nel gruppo sottoposto a chirurgia laparoscopica, invece, nel gruppo sottoposto a chirurgia tradizionale, il livello plasmatico di proteine mediatrici dell'infiammazione è incrementato. L'immunità cellulo-mediata nel post-operatorio è preservata maggiormente nel gruppo laparoscopico rispetto a quello convenzionale. Questi risultati possono determinare importanti implicazioni nell'ambito di una resezione per patologia coloretale maligna, in quanto la risposta immunitaria appare più rilevante in chirurgia open ed il trattamento laparoscopico mostra una minor attivazione della risposta infiammatoria e quindi minor modulazione del sistema immunitario.

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