Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(1) 303–310

DOI: 10.1177/0300060518800909
journals.sagepub.com/home/imr
SAGE

Comparison of postoperative lymphocytes and interleukins between laparoscopy-assisted and open radical gastrectomy for early gastric cancer

Xiang Xia, Zizhen Zhang, Jia Xu, Gang Zhao and Fengrong Yu

Abstract

Objective: This study aimed to study the effects of laparoscopic-assisted radical gastrectomy (LAG) and open radical gastrectomy (OG) on immune function and inflammatory factors in patients with early gastric cancer.

Methods: Seventy-five patients with pT1N0M0 gastric cancer in Ren Ji Hospital from August 2017 to January 2018 were studied. Lymphocytes subsets and interleukins were compared preoperatively and on the third postoperative day (POD3) and seventh postoperative day (POD7).

Results: There were no significant differences in age, sex, body mass index, duration of the operation, estimated blood loss, total gastrectomy rate, postoperative first fluid diet, and the levels of preoperative lymphocytes subsets and interleukins between the two groups. The number of CD4+ T cells and the CD4+/CD8+ ratio in the LAG group were significantly higher than those in the OG group on POD3. However, the number of CD8+ T cells, and interleukin-6 and interleukin-8 levels in the LAG group were significantly lower than those in the OG group on POD3.

Conclusions: Laparoscopy can effectively reduce the levels of inflammatory factors and has less effect on the immune system than OG.

Keywords

Early gastric cancer, laparoscopic radical gastrectomy, open radical gastrectomy, immune function, inflammatory factor, lymphocyte, interleukin

Date received: 1 July 2018; accepted: 24 August 2018

Department of Gastrointestinal Surgery, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Corresponding author:
Fengrong Yu, Department of Gastrointestinal Surgery, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, No. 1630 Dongfang Road, Shanghai 200127, China.
Email: yufengrongrj@163.com

Introduction

Laparoscopic-assisted radical gastrectomy (LAG) with D2 lymphadenectomy has been increasingly adopted by gastroenterology surgeons for treating patients with early gastric cancer. LAG has several short-term advantages, such as an earlier recovery, less pain, shorter hospitalization time, and lower complications rate compared with open radical gastrectomy (OG) as shown in clinical trials.[1–3] However, the effects of LAG or OG with D2 lymphadenectomy on immune function and inflammatory factors during hospitalization postoperatively remain largely unknown.

Several investigators have assumed that laparoscopic surgery affects immune function and inflammatory factors.[4–5] Nevertheless, a few studies have shown that laparoscopic surgery contributes to relieving surgical trauma and protecting systemic immunity in clinical practice.[6–7] Therefore, the effects of laparoscopic surgery on immune function and inflammatory factors remain controversial. On the basis of these previous conflicting findings, we designed this retrospective study to examine immune and inflammatory responses in patients with early gastric cancer who underwent LAG or OG without experiencing postoperative complications.

Materials and methods

Ethics statement

The study was approved and supervised by the Research Ethics Committee (2016-135K) of Ren Ji Hospital. This study was also registered in the Chinese Clinical Trial Register (ChiCTR), which is a primary register of the WHO International Clinical Trials Registry Platform (SN. ChiCTR-PIC-17012358). Informed consent was obtained from all individual participants who were included in the study. Research was in compliance with the Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/index.html).

Patients

From August 2017 to January 2018, patients with gastric cancer, which was verified by endoscopy and biopsy, as well as by preoperative enhanced computed tomography scans of the abdominal pelvis indicating cT1-2N0M0 (determined using Japanese Gastric Cancer Association classifications),[8] were enrolled in this study. All patients received preoperative evaluations, including blood tests, chest X-rays, and electrocardiograms. The exclusion criteria included the following: (1) aged younger than 20 or older than 80 years; (2) pregnancy; (3) blood transfusion; (4) postoperative complications occurred; (5) American Society of Anesthesiologists grade III; (6) preoperative chemotherapy or chemoradiotherapy; and (7) not pT1N0M0 stage.

Allocation to groups

Once eligibility was confirmed, patients who were willing to accept laparoscopic surgery were assigned to the LAG group. Concurrently, the other eligible patients were assigned to the OG group.

Surgical procedure

LAG and OG were performed by a single gastrointestinal surgical team who had adequate experience in OG and LAG with D2 lymphadenectomy. The resection pattern of distal, proximal, or total gastrectomy and reconstruction methods were dependent on the site of the tumor and the extent of gastrectomy, respectively. In the LAG group, after umbilical puncture, CO_2 pneumoperitoneum was established with maintenance of intra-abdominal pressure at 13 to 15 mmHg. A 12-mm trocar was placed at the umbilicus with a 30°mirror as the

observation port. Punctures were created by the 12-mm trocar below the costal margin of the left anterior axillary line, 5 cm left of the navel, 5 mm below the costal margin of the right anterior axillary line, and 12 mm upper of the right mid-clavicular line. This was performed to position a sheathed needle and laparoscopic camera lens for exploration of the abdomen. According to Japanese gastric cancer treatment guidelines 2014 (ver.4), D2 lymphadenectomy was adopted in the LAG and OG groups.[9] At the end of surgery, the abdominal cavity was cleaned by 2000 mL normal saline and two or three drainage tubes were placed at the incision of right or left anterior axillary line. Antibiotics were routinely provided after surgery.

Measurement of peripheral lymphocyte subsets and interleukins

A total of 10 mL of peripheral venous blood was collected preoperatively and on postoperative day (POD) 3 and POD7. Absolute white blood cell (WBC), neutrophil, and lymphocytes counts were determined with an automated cell counter (Beckman LH750; Beckman Coulter, Brea, CA, USA). Total T lymphocytes, B lymphocytes, CD4+ T cells, CD8+ T cells, and natural killer (NK) cells were measured by flow cytometry (EpicsXL; Beckman Coulter). The monoclonal antibodies used for immunophenotyping were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). Levels of interleukin-6, interleukin-8, and interleukin-10 in plasma were measured using ELISA kits (Dakewei Inc., Beijing, China) according to the manufacturer's instructions. Samples were tested in triplicate.

Statistical analysis

Data are presented as mean ± standard deviation. Data between the groups were analyzed by the Students t test or chi-square test. Differences were considered statistically significant at $P < 0.05$. Statistical analysis was performed using SPSS for Windows, Version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics

Seventy-five patients were enrolled in our study (Table 1). Among the 37 patients who had LAG, 23 were men and 14 were women. The mean age of the LAG group was 60.99 ± 11.2 years. Among the 38 patients who had OG, 25 were men and 13 were women. The mean age of the OG group was 62.76 ± 11.0 years. There were no significant differences in age, the sex ratio, body mass index, duration of the operation, estimated blood loss, gastrectomy patterns, and postoperative first fluid diet between the two groups. However, the LAG group experienced a significantly shorter postoperative hospital stay than did the OG group ($P = 0.001$).

Immune responses in peripheral blood

WBCs, neutrophils, lymphocytes, and the profiles of lymphocyte subsets were tested in our clinical laboratory department. There were no significant within-group differences in immune responses. No significant differences in WBC, neutrophil, lymphocyte, T lymphocyte, B lymphocyte, and NK cell counts were found between the LAG and OG groups at any time point after the operation (Figure 1). However, the number of CD4+ T cells ($P < 0.001$) and the CD4+/CD8+ ratio ($P < 0.01$) were significantly elevated, while the number of CD8+ T cells ($P < 0.05$) was significantly lower on POD3 in the LAG group compared with the OG group (Figure 2). On POD7, the counts of

Table 1. Baseline and operative data.

	LAG (n = 37)	OG (n = 38)	P
Age (years)	60.99 ± 11.2	62.76 ± 11.0	0.087
Sex ratio (M:F)	23:14	25:13	0.744
BMI (kg/m^2)	22.04 ± 2.8	22.44 ± 8.1	0.563
Duration of operation (hours)			0.571
≥3	22 (59.5%)	25 (65.8%)	
<3	15 (40.5%)	13 (34.2%)	
Estimated blood loss (mL)			1.000
≥150	3 (8.1%)	4 (10.5%)	
<150	34 (91.9%)	34 (89.5%)	
Gastrectomy patterns			0.514
Partial	26 (70.3%)	24 (63.2%)	
Total	11 (29.7%)	14 (36.8%)	
Postoperative hospital stay (days)	9.90 ± 2.9	10.48 ± 2.8	0.001
Postoperative first fluid diet (days)	5.50 ± 1.92	5.48 ± 2.06	0.629

Values are mean ± standard deviation or n (%). BMI: body mass index; M: male; F: female; LAG: laparoscopic-assisted radical gastrectomy; OG: open gastrectomy.

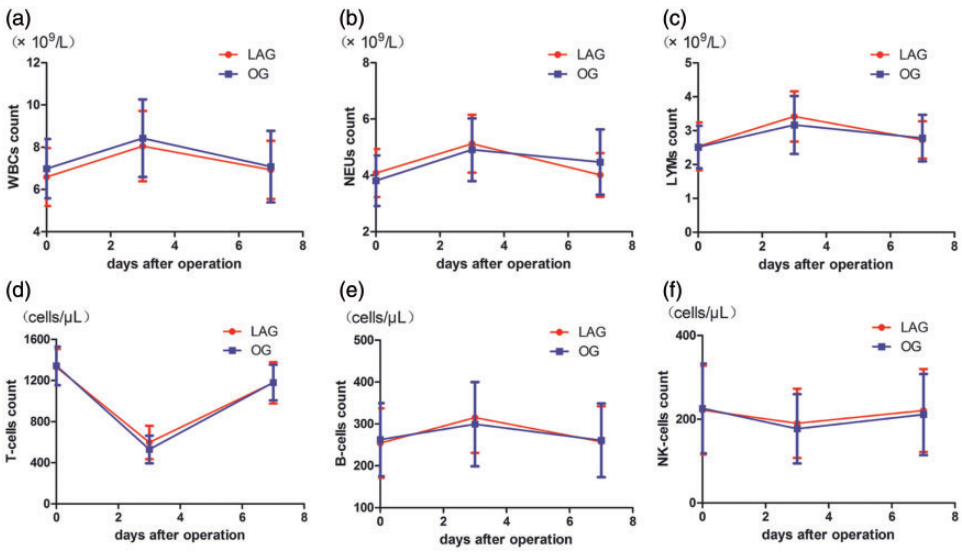

Figure 1. Changes in WBC (a), NEU (b), LMY (c), T cell (d), B cell (e), and NK (f) cell counts in patients who received LAG or OG. Values are presented as mean ± standard deviation.
WBC: white blood cell; NEU: neutrophil; LMY: lymphocyte; NK: natural killer; LAG: laparoscopic-assisted radical gastrectomy; OG: open gastrectomy.

WBCs, neutrophils, lymphocytes, T lymphocytes, B lymphocytes, NK cells, CD4+ T cells, and CD8+ T cells and the CD4+/CD8+ ratio tended to return back to preoperative baseline levels in both groups, with no significant differences were observed between the LAG and OG groups (Figures 1 and 2).

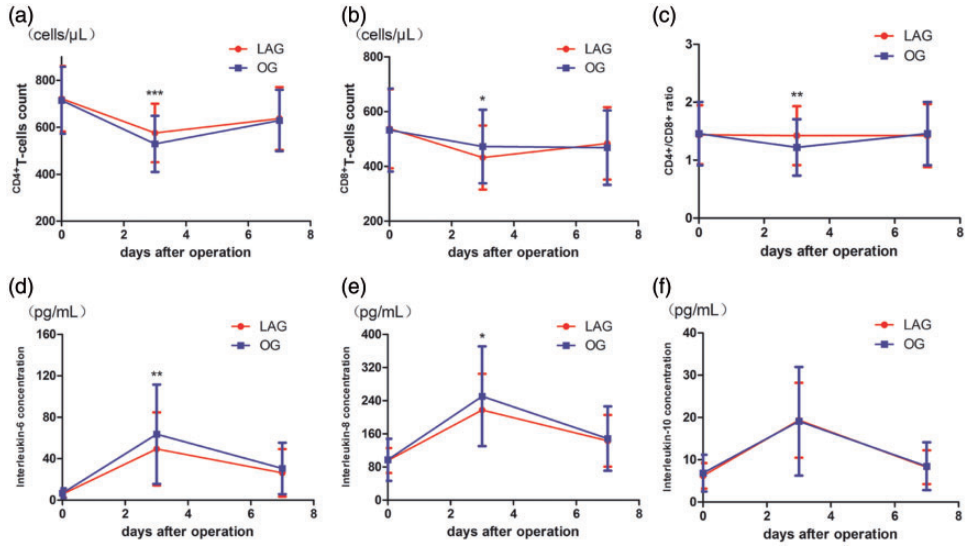

Figure 2. Changes in CD4+ T cells (a), CD8+ T cells (b), the CD4+/CD8+ ratio (c), and interleukin-6 (d), interleukin-8 (e), and interleukin-10 (f) levels in patients who received LAG or OG. Values are presented as mean ± standard deviation. LAG: laparoscopic-assisted radical gastrectomy; OG: open gastrectomy. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared between the LAG and OG groups.

Inflammatory responses in peripheral blood

Inflammatory factors, such as interleukin-6, interleukin-8, and interleukin-10 levels were also analyzed perioperatively. No significant differences in these interleukins were observed between the LAG and OG groups preoperatively. On POD3, interleukin-6, interleukin-8, and interleukin-10 levels were increased in both groups compared with before the operation, but these differences were not significant. However, interleukin-6 ($P < 0.01$) and interleukin-8 ($P < 0.05$) levels in the LAG group were significantly lower than those in the OG group. However, on POD7, these interleukins all returned to preoperative levels and they were significantly different between the LAG and OG groups (Figure 2).

Discussion

Gastric cancer is one of the most frequent malignant tumors, which have a high morbidity and mortality worldwide, especially in east Asian countries, such as Japan, Korea, and China.[10] To date, surgery remains the primary treatment for gastric cancer, and laparoscopic surgery has gradually spread and prevailed in surgical management of early gastric cancer, especially in east Asia.[2,3,11] In the present study, we explored immune function and inflammatory factors of patients with pT1N0M0 gastric cancer who were treated with LAG or OG with D2 lymphadenectomy without experiencing postoperative complications. After removing the effects of advanced tumors and postoperative complications on the immune and inflammatory system, we found that the number of CD4+ T cells and the CD4+/CD8+ ratio were decreased in the two groups on POD3 compared with before surgery. However, these variables were significantly higher in the LAG group than in the OG group. The number of CD8+ T cells, and interleukin-6 and interleukin-8 levels in the

LAG group were significantly lower than those in the OG group on POD3. However, these indices returned to preoperative levels on POD7.

Fluctuation in immune function and inflammatory responses before and after surgery may reflect the amplitude of trauma and stress of the operation. Theoretically, surgical stress and wounds are directly related to impairment of the immune system and secretion of a variety of cytokines, thus causing negative effects on patients' short-term and long-term outcomes. However, laparoscopic surgery, as a minimally invasive technique, might preserve the innate immune system and attenuate inflammatory responses accompanied by a rapid return to preoperative levels, probably due to decreased surgical trauma and stress.[5,6,12]

The immune reactions caused by tumors are mainly mediated by T lymphocytes. T cell subsets, including CD4+, CD8+, and CD4+/CD8+, are regarded as a reflection of immune function.[13] After being activated, CD4+ is an assistant and inducer T cell that releases a large number of cytokines and plays a major role in antitumor responses. CD8+ is known as a cytotoxic T cell and is capable of cleaning viruses and adhesions.[14,15] The CD4+/CD8+ ratio reflects the steady state of the immune system and if this ratio decreases, this indicates that the immune function is depressed, and consequently, proliferation of cancer cells is activated. Notably, a low CD4+/CD8+ ratio is also generally considered as a predictor for the severity of disease or a poor prognosis.[16,17] Accordingly, our results indicated that less trauma and effects on immune function resulting from laparoscopic surgery might be good for recovery postoperatively and have potential benefits on long-term survival.

Interleukins, which are types of circulating proinflammatory cytokines, can reflect the extent and severity of surgical procedures.[18] Interleukin-6 is a sensitive marker for surgical stress, and generally the greater the surgical trauma is, the greater the change in interleukin-6 levels.[19] Consistent with other studies,[20–22] interleukin-6 levels were significantly lower in the LAG group than in the OG group on POD3, although interleukin-6 levels were increased in both groups. Consequently, LAG treatment might have less surgical stress and reduced inflammatory responses, which would result in a better long-term prognosis. Interleukin-8 is another key mediator that is associated with an inflammatory response, and is secreted by macrophages and lymphocytes in the surgical microenvironment.[6] Interleukin-8 might act as a growth factor in colon cancer cells or a promoter of division. Therefore, lower interleukin-8 levels probably inhibit cancer cell proliferation.

There are a few limitations in our study. First, the sample size of included patients was small. Further large-scale clinical trials are required to confirm if LAG has more benefits in the immune and inflammatory system compared with OG. Second, the long-term relationship among immunity, inflammatory factors, and prognosis caused by LAG or OG needs to be evaluated. Finally, although we minimized bias by excluding patients with advanced pathological stages and experiencing postoperative complications, the retrospective nature of our database may have introduced a few inevitable biases.

In conclusion, comparison between OG and LAG regarding their effects on immune and inflammatory states provides a theoretical foundation for selecting minimally invasive surgery for gastric cancer. The relationships between patients' long-term outcomes and better preserved immune responses, as well as less activated inflammatory factors, remain obscure. Therefore, a longer follow-up of our study is required in the future.

Authors' contributions

XX and ZZZ made substantial contributions to the conception and design of the study, acquisition of data, and analysis and interpretation of data; FRY and JX were involved in drafting the manuscript and revising it critically for important intellectual content; GZ and FRY made substantial contributions to acquisition of data, and analysis and interpretation of data.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This study was supported by the Shanghai Sailing Program (No. 17YF1415700); Shanghai Municipal Commission of Health and Family Planning (No. 2017BR043); and the Hospital Development Center (No. 16CR2022A).

References

1. Yoshimura F, Inaba K, Kawamura Y, et al. Clinical outcome and clinicopathological characteristics of recurrence after laparoscopic gastrectomy for advanced gastric cancer. *Digestion* 2011; 83: 184–190.
2. Kim W, Kim HH, Han SU, et al. Decreased morbidity of laparoscopic distal gastrectomy compared with open distal gastrectomy for stage i gastric cancer: short-term outcomes from a multicenter randomized controlled trial (KLASS-01). *Ann Surg* 2016; 263: 28–35.
3. Quan Y, Huang A, Ye M, et al. Comparison of laparoscopic versus open gastrectomy for advanced gastric cancer: an updated meta-analysis. *Gastric Cancer* 2016; 19: 939–950.
4. Ishikawa M, Nishioka M, Hanaki N, et al. Perioperative immune responses in cancer patients undergoing digestive surgeries. *World J Surg Oncol* 2009; 7: 7.
5. Okholm C, Goetze JP, Svendsen LB, et al. Inflammatory response in laparoscopic vs. open surgery for gastric cancer. *Scand J Gastroenterol* 2014; 49: 1027–1034.
6. Karanika S, Karantanos T and Theodoropoulos GE. Immune response after laparoscopic colectomy for cancer: a review. *Gastroenterol Rep (Oxf)* 2013; 1: 85–94.
7. Wu MH, Wang MY, Yang CY, et al. Inflammatory response attenuation in patients undergoing gasless laparoscopic gastrectomy. *Hepatogastroenterology* 2013; 60: 439–42.
8. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; 14: 101–112.
9. Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2014 (ver. 4). *Gastric Cancer* 2017; 20: 1–19.
10. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017; 67: 7–30.
11. Huscher CG, Mingoli A, Sgarzini G, et al. Laparoscopic versus open subtotal gastrectomy for distal gastric cancer: five-year results of a randomized prospective trial. *Ann Surg* 2005; 241: 232–237.
12. Cui M, Gong C, Jiang B, et al. Evaluation of immune responses of gastric cancer patients treated by laparoscopic and open gastrectomy. *Med Oncol* 2015; 32: 253.
13. Ma Z, Bao X and Gu J. Effects of laparoscopic radical gastrectomy and the influence on immune function and inflammatory factors. *Exp Ther Med* 2016; 12: 983–986.
14. Larsen SK. Cellular immune responses towards regulatory cells. *Dan Med J* 2016; 63: B5188.
15. Brentville VA, Metheringham RL, Gunn B, et al. Citrullinated Vimentin presented on MHC-II in tumor cells is a target for CD4+ T-cell-mediated antitumor immunity. *Cancer Res* 2016; 76: 548–560.
16. Jackute J, Zemaitis M, Pranys D, et al. Distribution of CD4(+) and CD8(+) T cells in tumor islets and stroma from patients with non-small cell lung cancer in association with COPD and smoking. *Medicina (Kaunas)* 2015; 51: 263–271.
17. Chen M, Mohtize M, Mattei MF, et al. Reduced levels of both circulating CD4+ CD25+ CD127(low/neg) and CD4+ CD8 (neg) invariant natural killer regulatory T cells in stable heart transplant recipients. *Clin Exp Immunol* 2011; 163: 104–112.

18. Bertram P, Junge K, Schachtrupp A, et al. Peritoneal release of TNF alpha and IL-6 after elective colorectal surgery and anastomotic leakage. *J Invest Surg* 2003; 16: 65–69.
19. Jess P, Schultz K, Bendtzen K, et al. Systemic inflammatory responses during laparoscopic and open inguinal hernia repair: a randomised prospective study. *Eur J Surg* 2000; 166: 540–544.
20. Sammour T, Kahokehr A, Zargar-Shoshtari K, et al. A prospective case-control study of the local and systemic cytokine response after laparoscopic versus open colonic surgery. *J Surg Res* 2012; 173: 278–285.
21. Wu FP, Sietses C, von Blomberg BM, et al. Systemic and peritoneal inflammatory response after laparoscopic or conventional colon resection in cancer patients: a prospective, randomized trial. *Dis Colon Rectum* 2003; 46: 147–155.
22. Shu ZB, Cao HP, Li YC, et al. Influences of laparoscopic-assisted gastrectomy and open gastrectomy on serum interleukin-6 levels in patients with gastric cancer among Asian populations: a systematic review. *BMC Gastroenterol* 2015; 15: 52.