ORIGINAL ARTICLE

IMPACT OF SPLENECTOMY ON CIRCULATING T-LYMPHOCYTE SUBSETS IN STAGE III GASTRIC CANCER

MIN YOUNG CHO,* MATTHEW D. KROH,† YONG GEUL JOH* AND SUNG OCK SUH*

*Department of Surgery, College of Medicine, Korea University, Seoul, Korea and †Mount Sinai School of Medicine, New York City, New York, United States of America

Background: The role of splenectomy remains unclear in patients with gastric cancer who undergo total gastrectomy. The aim of this study was to prospectively evaluate the impact of splenectomy on circulating T-lymphocyte subsets and survival in advanced gastric cancer.

Methods: Analysis of lymphocyte subsets was performed in 40 patients with American Joint Committee on Cancer (AJCC) stage III gastric adenocarcinoma located on the upper one-third of the stomach, who underwent a curative total gastrectomy with or without splenectomy. Circulating T-lymphocyte subsets were measured on venous blood by using flow cytometry and monoclonal antibodies at preoperative day 1, and postoperative months 1, 3, 6, 12 and 18.

Results: The proportion of lymphocytes and the values of CD3, CD8, CD16 and CD25 subsets were higher in the splenectomy group of patients at postoperative month 3. In the spleen preservation group at the same point of treatment, the proportion of granulocytes and the values of CD4 and CD4 : CD8 ratio were higher. Except for CD16 levels, all T-lymphocyte subsets showed no significant difference between splenectomy and spleen preservation groups after postoperative month 3. Increased CD16 levels in the splenectomy group were not associated with improvement in patients’ 5-year survival rates.

Conclusion: These results suggest that the long-term impact of splenectomy does not play an important role in postoperative quantitative changes of circulating T-lymphocyte subsets of patients with stage III gastric cancer who have undergone total gastrectomy. Furthermore, splenectomy does not give a prognostic benefit, based on tumour recurrence and survival of patients with proximal one-third gastric cancer who undergo total gastrectomy.

Key words: gastric carcinoma, host immunity, lymphocyte subsets, splenectomy, survival.

INTRODUCTION

It is unclear whether patients with advanced gastric cancer who undergo total gastrectomy with D2 lymph node dissection benefit more from splenectomy or spleen preservation. Unresolved issues regarding optimal management include the risk of residual metastases in splenic hilar nodes when the spleen is preserved, the effect of splenectomy on short-term postoperative morbidity and mortality, and the impact of splenectomy on long-term survival.1 Furthermore, the role of the spleen in tumour immunity is still controversial and studies differ on whether it can enhance or suppress the anti-tumour host immunity.2–4

The aim of this study was to prospectively evaluate the impact of splenectomy on postoperative quantitative changes of circulating T-lymphocyte subsets and survival in advanced gastric cancer patients after curative resection. Therefore, the authors investigated T-lymphocyte subsets in patients with AJCC stage III gastric cancer5 who had undergone total gastrectomy with D2 lymph node dissection with curative intent. Patients were divided into splenectomy and non-splenectomy (spleen preservation) groups.

M. Y. Cho MD; M. D. Kroh MA; Y. G. Joh MD; S. O. Suh MD.

Correspondence: Min Young Cho, MD, Department of Surgery, College of Medicine, Korea University, 126–1 5th-Ga, Anam-Dong Sungbuk-Gu, Seoul, 136–705, Korea.

Email: minyoung@korea.ac.kr

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METHODS

Patients characteristics

Forty-five patients who had undergone curative total gastrectomy with D2 lymph node dissection and splenectomy or spleen preservation by the same surgeon were considered eligible for this study. All patients had histologically proven AJCC stage III gastric adenocarcinoma that was located on the upper one-third of the stomach. Patients were identified preoperatively between 1 January 1995 and 30 June 1996. Patients to undergo splenectomy were determined by whether the tumour was located on the greater curvature of the stomach, especially near the splenic hilum; if preoperative splenic hilar lymph nodes (no. 10) were found on abdominal computed tomography; or if lymph node enlargement was identified in the splenic hilum or along the splenic artery (no. 11) intraoperatively. These patients constituted the splenectomy group. Data regarding patients’ clinical characteristics and lymphocyte subsets were collected and evaluated by a blinded independent surgeon. Patients were excluded from this study if: (i) perioperative or postoperative transfusion was required during follow up; (ii) postoperative major complications occurred, such as single or multiple organ failure, sepsis, pneumonia, anastomosis leakage, intra-abdominal infection or abscess; (iii) re-operation was necessary; (iv) patients failed to maintain regular follow-up; (v) death occurred unrelated to primary cancer. After exclusion criteria, 40 patients were eligible for and enrolled in this study. These patients were followed up regularly at our clinic between 1 January 1995 and 31 February 2000.
2000. Patients were defined as having tumour recurrence or not during follow up based on clinical examination, ultrasound, abdominal computer tomography and tumour markers.

All patients were treated with chemotherapy monthly during the first 6 months after the operation. Continuous infusion of 5-FU was administrated at 500 mg/m² per day, on day 1 to day 5. Bolus cisplatin was given at a dosage of 60 mg/m² per day on day 1. All patients were allocated into one of two groups: patients who underwent splenectomy (splenectomy group, n = 19), and control patients who underwent spleen-preserving D2 lymph node dissection (non-splenectomy group, n = 21). T-lymphocyte subsets were evaluated at preoperative day 1 and postoperative months 1, 3, 6, 12 and 18 from peripheral venous blood draws.

Methods for lymphocyte subsets

Heparinized blood 10 mL was taken from each patient. Aliquots (100 µL) of whole blood were incubated in the dark, with 20 µL of the following monoclonal antibodies: CD3 fluorescein isothiocyanate (FITC)/CD19 R-phycoerythoin (RPE) for mature T-lymphocytes/B-lymphocytes, CD4 FITC/CD8 RPE for helper/suppressor T-cells, CD3 FITC/CD16 plus 56 RPE for T-lymphocyte/natural killer (NK) cells, CD4 FITC/CD25 RPE for activated lymphocytes, immunoglobulin-G FITC plus RPE for control (Beckton Dickinson, San Jose, CA) and CD45 FITC/CD14 RPE for differential percentages of lymphocytes, granulocytes, and monocytes (Immunotech, Paris, France). A total of 2 mL of fluorescence-activated cell sorter-lysing solution (Beckton Dickinson, San Jose, CA) was then added, mixed, and re-suspended in phosphate buffered saline. T-lymphocyte subset distribution was evaluated using the FACScan flow cytometer (Beckton Dickinson, Mountain View, CA, USA). Results were expressed as the total number and percentage of positive cells counted.

Statistical and survival analysis

The data were analysed using the SPSS statistical program (SPSS, Chicago, IL, USA). All values of T-lymphocyte subsets are expressed as mean ± standard deviation of the mean. Mean values were compared using the Mann–Whitney U-test. Continuous data at multiple time points in the same individual were analysed by the repeated measures ANOVA for elucidating within-subject effects and between-subjects effects. The categorical data between the two groups were analysed using the χ² test and Fisher’s exact test. Actual survival and disease-free survival was calculated by the Kaplan–Meier method. Significance between survival curves of populations was evaluated using the log-rank test. Statistical significance was assumed at P < 0.05. Death that was confirmed to be caused by the gastric cancer was treated as an endpoint for survival.

RESULTS

Clinical characteristics

Table 1. Clinical characteristics of patients following total gastrectomy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-splenectomy (n = 21)</th>
<th>Splenectomy (n = 19)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean ± SD)</td>
<td>53.7 ± 14.5</td>
<td>60.6 ± 8.2</td>
<td>0.155</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>16/5</td>
<td>13/6</td>
<td>0.727</td>
</tr>
<tr>
<td>Recurrence; n (%)</td>
<td>11 (52.4)</td>
<td>10 (52.6)</td>
<td>1.000</td>
</tr>
<tr>
<td>Type of recurrence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematogenous</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Peritoneal</td>
<td>2</td>
<td>2</td>
<td>0.876</td>
</tr>
<tr>
<td>Distant lymph node</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Locoregional</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mean duration of disease free survival (months; mean ± SD)</td>
<td>39.1 ± 16.1</td>
<td>37.2 ± 22.1</td>
<td>0.954</td>
</tr>
<tr>
<td>Mean duration of survival (months; mean ± SD)</td>
<td>43.4 ± 13.7</td>
<td>39.9 ± 20.1</td>
<td>0.840</td>
</tr>
<tr>
<td>AJCC stage; n (%)</td>
<td>3a 14 (66.7)</td>
<td>11 (57.9)</td>
<td>0.745</td>
</tr>
<tr>
<td></td>
<td>3b 7 (33.3)</td>
<td>8 (42.1)</td>
<td></td>
</tr>
<tr>
<td>Depth of invasion; n (%)</td>
<td>T2 2 (9.5)</td>
<td>2 (10.5)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>T3 19 (90.5)</td>
<td>17 (89.5)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis; n (%)</td>
<td>N1 12 (57.1)</td>
<td>9 (47.4)</td>
<td>0.752</td>
</tr>
<tr>
<td></td>
<td>N2 9 (42.9)</td>
<td>10 (52.6)</td>
<td></td>
</tr>
<tr>
<td>Tumor differentiation; n (%)</td>
<td>Well or moderate 11 (52.4)</td>
<td>9 (47.4)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Poor 10 (47.6)</td>
<td>10 (52.6)</td>
<td></td>
</tr>
<tr>
<td>Borrmann classification; n (%)</td>
<td>B II 6 (28.6)</td>
<td>6 (31.6)</td>
<td>0.790</td>
</tr>
<tr>
<td></td>
<td>B III 12 (57.1)</td>
<td>9 (47.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B IV 3 (14.3)</td>
<td>4 (21.0)</td>
<td></td>
</tr>
<tr>
<td>Splenic hilar lymph node metastasis</td>
<td>(no. 10; n (%))</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Clinical characteristics of patients following total gastrectomy
operative adhesion \((n = 1)\). The clinical and histopathological details of all study patients with AJCC stage III gastric cancer studied are shown (Table 1). There were no significant statistical differences between the splenectomy and non-splenectomy groups based on age, sex ratio, recurrence rate, type of recurrence, AJCC stage, depth of tumour invasion, regional lymph node metastasis, tumour differentiation, Borrmann classification and incidence of no. 10 lymph node metastasis. Mean duration of disease-free survival and overall survival was slightly longer in the non-splenectomy group, although not statistically different. The incidence of metastasis in the splenic hilar lymph nodes (no. 10) was 9.5% in the non-splenectomy group and 10.5% in the splenectomy group. The 5-year disease-free survival rate of the splenectomy and non-splenectomy groups was 52.4% and 50.0%, respectively, showing no significant statistical difference between the two groups (Fig. 1a, \(P = 0.651\)). Overall 5-year survival rate of the splenectomy and non-splenectomy groups also showed no significant difference (Fig. 1b, 50.0% and 59.7%, respectively; \(P = 0.915\)).

**Total leucocyte counts and their proportions**

Postoperative total leucocyte counts in the splenectomy and non-splenectomy groups were consistently lower than preoperative values throughout the observation period. Total leucocyte counts showed higher values in the splenectomy group from postoperative month 6, but there was no statistical difference between the two groups (Fig. 2a). All proportions of granulocytes, and lym-
phocytes showed no significant difference during the observation period, except at postoperative month 3 (Fig. 2b). At postoperative month 3, granulocytes constituted a higher percentage in the non-splenectomy group (48.6 ± 12.4% vs 66.0 ± 10.4%; P < 0.05). However, lymphocytes showed a higher percentage in the splenectomy group (37.9 ± 5.3% vs 27.8 ± 5.9%; P < 0.05). Monocytes showed no difference between the two groups and remained relatively stable in both groups (Fig. 2b).

**CD3, CD16, CD19 and CD25**

CD3, CD16, CD19 and CD25 levels are shown (Figs 3(a) and 3(b,c), respectively). CD3 levels showed no difference between the splenectomy and non-splenectomy groups, except at postoperative month 3 (1684.0 ± 225.7 cell/mm$^3$ vs 1242.7 ± 248.6 cell/mm$^3$, P < 0.05). CD16 was consistently higher in the splenectomy group after operation. CD16 showed a peak value at postoperative month 3 and a significant difference (826.0 ± 182.4 cell/mm$^3$ vs 483.6 ± 151.9 cell/mm$^3$, P < 0.05). CD16 was also significantly higher in the splenectomy group at postoperative months 3, 6 (668.0 ± 124.1 cell/mm$^3$ vs 445.7 ± 149.6 cell/mm$^3$) and 12 (627.8 ± 136.2 cell/mm$^3$ vs 454.4 ± 121.6 cell/mm$^3$, P < 0.05). There was no difference between the two groups in CD19, and the CD19 level remained relatively stable in both groups during the observation period. CD25 was significantly higher in the splenectomy groups at postoperative month 3, as was CD16 (653.0 ± 179.1 cell/mm$^3$ vs 338.2 ± 162.4 cell/mm$^3$, P < 0.05). However, unlike CD16, CD3 and CD25 levels, showed no difference after postoperative month 3.

**CD4, CD8 and CD4/CD8 ratios**

CD4, CD8, and the CD4/CD8 ratios are illustrated (Fig. 4(a) and 4(b,c), respectively). CD4, CD8 and CD4/CD8 ratios showed no difference between the two groups except at postoperative month 3. CD4 was significantly higher in the non-splenectomy group at postoperative month 3 (982.0 ± 146.4 cell/mm$^3$ vs 715.7 ± 152.7 cell/mm$^3$, P < 0.05). However, CD8 was significantly higher in the splenectomy group at postoperative month 3 (964.0 ± 121.3 cell/mm$^3$ vs 712.1 ± 152.6 cell/mm$^3$, P < 0.05). The CD4 : CD8

![Fig. 3. Changes of: (a) CD3, CD19; (b) CD16; and (c) CD25 in patients with AJCC stage III gastric cancer following total gastrectomy with and without splenectomy. All patients were treated with chemotherapy during the first 6 months after operation. ●, Patients with spleen preservation; ○, patients with splenectomy.](image)

![Fig. 4. Changes of: (a) CD4; (b) CD8; and (c) CD4/CD8 ratios in patients with AJCC stage III gastric cancer following total gastrectomy with and without splenectomy. All patients were treated with chemotherapy during the first 6 months after operation. ●, Patients with spleen preservation; ○, patients with splenectomy.](image)
Table 2. Repeated measures ANOVA of CD16

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sum of squares</th>
<th>d.f.</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-subjects effects (CD16 × splenectomy)</td>
<td>1571240.573</td>
<td>5</td>
<td>7.281</td>
<td>0.001</td>
</tr>
<tr>
<td>Between-subject effects (non-splenectomy × splenectomy)</td>
<td>1103513.890</td>
<td>1</td>
<td>5.680</td>
<td>0.022</td>
</tr>
</tbody>
</table>

ratio was significantly higher in the non-splenectomy group at postoperative month 3 (0.84 ± 0.32 vs 1.38 ± 0.31, \( P < 0.05 \)). Omitting month 3, the remainder of the CD4/CD8 ratios showed similar values in both groups.

Analysis of the repeated measure ANOVA

All lymphocyte subsets except CD16 showed no significant difference between the splenectomy and non-splenectomy groups in the test of within-subjects effects and between-subject effects (Table 2). Only CD16 among T-lymphocyte subsets showed significant differences in these tests (\( P = 0.001, P = 0.022 \), respectively). Therefore, the change of CD16 following operation was significantly different in both groups over the time course of the study and also significantly different between the splenectomy and non-splenectomy groups after correcting for the effects of different points over time in the same individuals, when compared with the preoperative value.

DISCUSSION

Total gastrectomy with D2 lymph node dissection is a standard surgical treatment for advanced gastric cancer located on the proximal one-third of the stomach in Korea and Japan.\(^6,7\) However, the role of splenectomy remains unclear in patients with gastric cancer who undergo total gastrectomy.\(^8-12\) Splenectomy has been considered appropriate in order to remove the risk of residual metastases in splenic hilar nodes as a part of extensive surgical management in proximal one-third gastric cancer.\(^13\) Recently, the incidence of metastasis to the splenic hilar lymph nodes has been reported as less than was previously thought, and found to occur at a rate of 10% in advanced gastric cancer.\(^8,12\) The rate of no. 10 lymph node metastasis in our study was similar to the results of other studies. Therefore, the indication for splenectomy in our study was not reliable as a valid predictor of no. 10 lymph node metastasis. Sano et al. reported that preoperative CT, endoscopic ultrasound, and macroscopic diagnosis intraoperatively were unreliable in predicting lymph node metastasis.\(^14\) Mönig et al. also emphasized that a preoperative staging of lymph node metastasis and macroscopic intraoperative evaluation could not be used as a basis for the indication to perform simultaneous splenectomy in gastric cancer resections.\(^8\) Although Maruyama et al. have reported performing splenectomy for complete dissection of the splenic hilar lymph nodes, because it is usually difficult to remove all of the hilar lymph nodes without splenectomy,\(^15\) an accurate and reliable predictor for lymph node metastasis in the splenic hilus is not currently available.\(^1\) Furthermore, the extensive surgery with splenectomy did not appear to be a survival benefit in these patients.\(^9,12\) The present study also demonstrated that patients with total gastrectomy and combined splenectomy did not show a benefit in 5-year disease-free survival and overall survival as compared with spleen preservation surgery. The widespread indication for splenectomy in proximal gastric cancer has become more restrictive and limited, primarily because of higher morbidity and mortality and lower 5-year survival rates.\(^16\)

The spleen plays an important role in host defences against infection and T- and B-cell interaction that is critical for the secondary immune response to previously presented antigens.\(^19\) However, the role of the spleen in tumour immunity is still controversial and evidence does not clearly demonstrate whether it enhances or suppresses the antitumour host immunity.\(^2,3\) To our knowledge, based on the available relevant literature, the status of lymphocyte subsets in patients with gastric cancer who undergo splenectomy has only been reported rarely in either the early postoperative or the late postoperative period. Lersch et al. demonstrated the early postoperative changes of circulating T-lymphocyte subsets of gastric cancer patients after splenectomy and gastrectomy.\(^20\) The absolute number of circulating lymphocytes decreased in these patients during the postoperative 2-week period, whether these patients had undergone splenectomy or not. Absolute numbers of CD3, CD4 and CD8 also decreased postoperatively, similar to the decrease in the number of lymphocytes. A significant decrease of lymphocytes and their subsets (CD3, CD4 and CD8) in the splenectomy patients was demonstrated during postoperative days 1–3 as compared with the gastrectomized only patients. However, the CD4 : CD8 ratios did not differ in either group of patients until 2 weeks postoperatively.\(^20\) Okuno et al. reported that CD3, CD4 and CD8 decreased and CD16 increased in gastric cancer patients with splenectomy more than 2 years following curative total gastrectomy.\(^2\) In our study, the proportion of lymphocytes, and the numbers of CD3, CD8, CD16 and CD25 were higher in the splenectomy group at postoperative month 3, the mid-point of postoperative chemotherapy. On the contrary, the proportion of granulocytes, the number of CD4, and the CD4 : CD8 ratio were higher in the non-splenectomy group at the same point. However, our study did not demonstrate long-term differences of T-lymphocyte subsets, except CD16, after postoperative month 3, whether these patients had undergone splenectomy or not. Only CD16 showed increased levels and higher numbers in the splenectomy group than in the non-splenectomy group during the postoperative period. These increased CD16 levels were not associated with improvement in patients’ 5-year survival rates.

The number of patients who were enrolled in this study was relatively small to fully evaluate the therapeutic effects of splenectomy on postoperative circulating T-lymphocyte subsets and survival. Despite this limitation, these results suggest that the long-term impact of splenectomy does not play an important role on the postoperative quantitative changes of circulating T-lymphocyte subsets of patients with stage III gastric cancer who have undergone total gastrectomy. We also found that splenectomy does not give a prognostic benefit based on tumour recurrence and survival in patients with proximal one-third gastric cancer who undergo total gastrectomy with D2 lymph node dissection.
REFERENCES


