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Clinical significance of changes in peripheral lymphocyte count after surgery in early cervical cancer

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ABSTRACT

Objective. Immune competence is an important prognostic factor in cancer patients. Surgical management of cancer can cause a variety of immunological disturbances, the clinical consequences of which are still unclear.

Materials and methods. Patients with clinically staged cervical carcinoma (IB to IIA) who were treated at Samsung Medical Center, Seoul, Korea from 1994 to 2007 were retrospectively enrolled. We compared peri-operative peripheral lymphocyte counts, tumor-infiltrating lymphocyte scores, and survival in patients with early cervical cancer treated by abdominal type III radical hysterectomy.

Results. The sample included 756 patients. The median follow-up was 58 months with a range of 3–181 months. There was a positive correlation between pre-operative peripheral lymphocyte counts and tumor infiltrating lymphocyte score. Pre-operative peripheral lymphocyte counts decreased significantly after surgery. In multivariate analyses for recurrence, higher pre-operative peripheral lymphocyte counts and recovery of lymphocyte counts (more than 100/μL from the pre-operative level) on post-operative day 3 were independent positive prognostic factors and LN metastasis was negatively associated with post-operative recovery of peripheral lymphocyte counts.

Conclusion. Peripheral lymphocyte counts after cervical cancer surgery are important prognostic factors, and management aimed at minimizing immune disturbances after surgery should be assessed, especially in patients with LN metastasis.

Introduction

Cervical cancer is the second most common cancer in women worldwide [1] and the leading cause of cancer death in women in developing countries [2].

Immune responses against tumor-specific antigens not only in and around tumors but also in peripheral blood have prognostic significance in many cancers [3], including cervical carcinoma. For example, higher numbers of CD8 + tumor-infiltrating lymphocytes [4] or detection of circulating human papillomavirus-specific T-cells [5] are associated with better prognoses, implying that immune competence is an important prognostic factor in cancer patients [3].

Unfortunately, surgical management can cause a variety of immunological disturbances. Decreases in peripheral lymphocyte numbers and impaired lymphocyte function are often observed after surgery [6]. However, the clinical consequences of immune impairment following surgery are not clear.

The purpose of this study was to investigate the clinical significance of changes in peripheral lymphocyte counts after abdominal type III radical hysterectomy for the treatment of cervical cancer, and to identify clinicopathological factors that are associated with surgery-induced immune disturbances in patients with early cervical carcinoma.

Materials and methods

Patients

Patients with early cervical cancer (FIGO stage IB to IIA) who were treated with abdominal type III radical hysterectomy at Samsung Medical Center, Seoul, Korea, from 1994 to 2007 were retrospectively enrolled in our study. After IRB approval, the data were collected from electronic medical records. Exclusion criteria for this study were as follows: early cervical cancer with microscopic lesions (IA1 and IA2);

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clinically suspected or pathologically proven lymph node (LN) metastasis at the para-aortic area; neoadjuvant chemotherapy cases; histological subtypes except for squamous cell carcinoma, adenocarcinoma, and adenosquamous cell carcinoma; patients who underwent fertility-saving surgery or laparoscopic-assisted type III radical hysterectomy; patients with concurrent hematologic conditions (myeloproliferative disorders, autoimmune diseases, and vasculitis); patients with concurrent infectious diseases including wound infection; immunocompromised patients or patients with human immunodeficiency virus (HIV) infection; patients without data for complete blood cell (CBC) counts with differential cell count within four weeks before starting initial treatment.

To investigate whether there is a difference of surgery related immune disturbances in other operative procedures in benign diseases, we enrolled the patients who underwent surgery for the uterine fibroid in our hospital during the last 6 months from May 1st 2012 and 112 patients were included. The patients' characteristics were shown in Supplementary Table.

Treatment

Standard surgery consisted of abdominal type III radical hysterectomy with bilateral pelvic LN dissection. Bilateral salpingo-oophorectomy and para-aortic LN dissection were not routine procedures. Blood samples were obtained on post-operation days (POD) 1 and 3. Adjuvant therapy after surgery was considered based on pathological risk factors. Patients who had more than one of the three high-risk factors (positive pelvic LN, microscopic parametrial invasion, and positive resection margins with tumor) underwent adjuvant radiotherapy (RT) or platinum-based concurrent chemoradiation (CCRT), which has been considered the preferred treatment since 2000 [7]. Patients with two or more of the three intermediate risk factors (stromal invasion of more than half of the cervix or stromal invasion more than 1 cm, lympho-vascular space invasion (LVSI), and a largest tumor diameter of 4 cm or greater) received adjuvant RT alone. The radiation protocols were as previously described [8].

Patients underwent follow-up examinations approximately every 3 months for the first 2 years, every 6 months for the next 3 years, and once per year thereafter. During routine follow-up, imaging studies including computed tomography (CT) or magnetic resonance imaging (MRI) and chest X-rays were performed each year. When tumor recurrence was suspected based on clinical findings or imaging studies, biopsies of the suspicious lesions were performed on a case-by-case basis. We defined the disease-free survival as the time from the initial treatment to relapse or the last follow-up visit, and overall survival was defined as the time from the initial treatment to death due to cervical carcinoma, or the last follow-up visit.

Quantification of tumor-infiltrating immune cells

To evaluate the relationships of tumor-infiltrating lymphocytes with peripheral lymphocyte counts in patients with cervical cancer, we investigated the tumor-infiltrating lymphocytes of tumor samples in cervical cancer patients who underwent type III radical hysterectomy ($n = 55$). First, each tumor section was evaluated for immune cell infiltration in the tumor stroma. After evaluating the percentage of tumor-infiltrating immune cells in the tumor stroma, we investigated the percentage of lymphocyte count among these tumor-infiltrating immune cells in 5 representative visual fields selected for the most abundant immune cell distribution, under a microscope at 400X magnification. Finally, we calculated the tumor-infiltrating lymphocyte score by multiplying the percentage of tumor-infiltrating immune cells and the percentage of tumor-infiltrating lymphocytes and then divided the scores by 100. Cases were scored blindly with respect to patient history, presentation, and previous scoring by two independent observers.

Statistical analysis

The two-sample *t* test and Wilcoxon rank sum test were used to compare the mean and median values, respectively, after confirming whether the data had non-normal or normal distributions with the Shapiro–Wilks test. The Wilcoxon signed rank test was used to compare the pre-operative and post-operative lymphocyte counts. Frequency distributions between categorical variables among the groups were compared using the χ^2 test. Fisher's exact test was used if the expected frequency was <5. Spearman correlation analysis was used to investigate the relationships between pre-operative peripheral lymphocyte counts and tumor-infiltrating lymphocyte scores. A logistic regression model was used to investigate the relationships between the recovery of the peripheral lymphocyte counts after surgery (recovery was defined as increasing peripheral lymphocyte counts after surgery from the basal level before surgery) and clinicopathological findings. The overall and disease-free survival curves were calculated according to the Kaplan–Meier method with the log-rank test. The Cox proportional-hazards model was used for multivariate analyses. Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL, USA). *P*-values ≤ 0.05 were considered statistically significant and all *P*-values were two-sided.

Results

We enrolled 756 patients with early cervical cancer (IB to IIA). The basal characteristics of patients are shown in Table 1. The median age was 48 years with a range of 23–83 years. The median follow-up was 58 months with a range of 3–181 months and the 5-year survival rate was 93.2%. More than half of the patients were FIGO stage IB1 at the initial visit (IB1: 592/756, 78.3%) and were treated with type III radical surgery alone (405/756, 53.6%). There were 82 cases of cervical cancer recurrence (10.8%) and 50 cases of cancer-specific death (6.6%). Among the study sample, there were no septic complications related to surgery.

The median level of pre-operative peripheral lymphocyte count was $1.864 \times 10^3/\mu L$ and this value decreased to $1.284 \times 10^3/\mu L$ on POD 1 and then slightly increased to $1.364 \times 10^3/\mu L$ on POD 3, which was a statistically significant change, and similar patterns of peripheral lymphocyte count changes was shown in patients with benign uterine fibroid (Fig. 1). This pattern was also found in change of peripheral lymphocyte percentage among total WBC counts. On the

Table 1
Patient characteristics with pre-treatment circulating white blood cell and lymphocyte counts.

	Total
No of patients	756
Median age, y (range)	48 (23–83)
Median white blood cell count, $\times 10^3/\mu L$ (range)	6.110 (2.000–20.900)
Median lymphocyte count, μL (range)	
Pre-operative	1.864 (0.38–5.17)
POD 1	1.284 (0.13–5.49)
POD 3	1.364 (0.18–3.44)
FIGO stage (%)	
IB1	592 (78.3)
IB2	48 (6.3)
IIA	116 (15.3)
Cell type (%)	
SCC	565 (74.7)
AC	142 (18.8)
ASC	49 (6.5)
Treatment (%)	
RH alone	405 (53.6)
RH + adjuvant RT	199 (26.3)
RH + adjuvant CCRT	152 (20.1)

POD = Post-operative day, SCC = Squamous cell carcinoma, AC = Adenocarcinoma, ASC = Adenosquamous cell carcinoma, RH = Type III radical hysterectomy, RT = Radiation therapy, CCRT = Concurrent chemoradiation therapy.