

Significance of Circumferential Resection Margin Involvement Following Esophagectomy for Esophageal Cancer

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Background : This study was performed to examine the significance of the circumferential resection margin (CRM) involvement by a tumor on the postoperative survival after esophageal cancer surgery. **Methods :** Fifty nine resected cases of esophageal cancers were retrospectively reviewed. The presence of a tumor either at, or within 1 mm of, the CRM was recorded. By an immunohistochemical study for Ki-67, the Ki-67 differential grades (Ki-67 DG) were defined according to the differences between the Ki-67 labeling indices of the central and of peripheral areas of the tumor nearest to the CRM: Ki-67 DG 0 ($\leq 10\%$) and Ki-67 DG 1 ($>10\%$). The CRM involvement was correlated with the clinicopathological factors, Ki-67 DG and survival data. **Results :** CRM involvement was found in 26 (44.1%) of the 59 cases. There were significant differences in the cases, both with and without CRM involvement of tumor cells, in relation to lymph node metastasis, lymphovascular, perineural invasions and tumor stage ($p < 0.05$). Ten (38.3%) of the 26 with, and 3 (9.1%) of 33 cases without, CRM involvement, showed Ki-67 DG 1 ($p = 0.007$). The 3-year survivals of patients with and without CRM involvement were 26.8 and 61.8%, respectively ($p = 0.003$). **Conclusions :** These results show that the CRM involvement status may be used as a predictor of survival after esophageal cancer surgery, and CRM involvement is more an indicator of an advanced disease than of an incomplete resection.

Key Words : Esophageal Cancer–Survival–Prognosis–Circumferential Resection Margin–Ki-67

Over the last 25 years, the incidence of esophageal cancer has risen dramatically,¹ whereas the 5-year survival has remained markedly poor, at less than 10%.² Despite the recent developments in non-surgical treatment, surgery remains the mainstay of potentially curative treatment. The outcome following surgical resection for esophageal cancer is generally poor; with a 5-year survival of approximately 25%.³ The factors affecting the long-term survival following an esophagectomy for esophageal cancer are poorly understood. The presence of a microscopic tumor at the circumferential resection margin (CRM) is one histological factor that has recently been investigated as a possible prognostic marker in esophageal cancer.^{4,5} However, the CRM has less frequently been reported, and the latest study reported that analysis of the survival curves of patients, both with and without CRM involvement, showed no statistically significant difference in the short- or long-term outcomes in esophageal cancer, with CRM involvement being more an indicator of inadequate local surgery than of an advanced disease character.⁶

The proliferating activity of a tumor is a useful parameter in the understanding of tumor behavior. Ki-67 is a proliferation-associated nuclear antigen, expressed in the cells of the whole

cell cycle, with the exception of the resting G₀ phase, and particularly reflects the cells in the S/G₂+M phases.⁷ Ohashi *et al.*⁸ reported that the proliferative activity was increased in the downwardly invading lesions, especially in the peripheral fronts of the invading nests in esophageal squamous cell carcinomas (ESSC), which might be related to the character of tumor cells with an increased proliferative activity.

The purpose of our study was to analyze the prognostic significance of microscopic tumor involvement at the CRM on the postoperative survival following an esophagectomy, and to determine whether or not a prognostic effect may contribute to the relationship of the tumor behavior with an increased proliferative activity at CRM.

MATERIALS AND METHODS

Materials

The materials used in this study were obtained, between 1996 and 2003, from the surgical pathology archival files of the Depart-

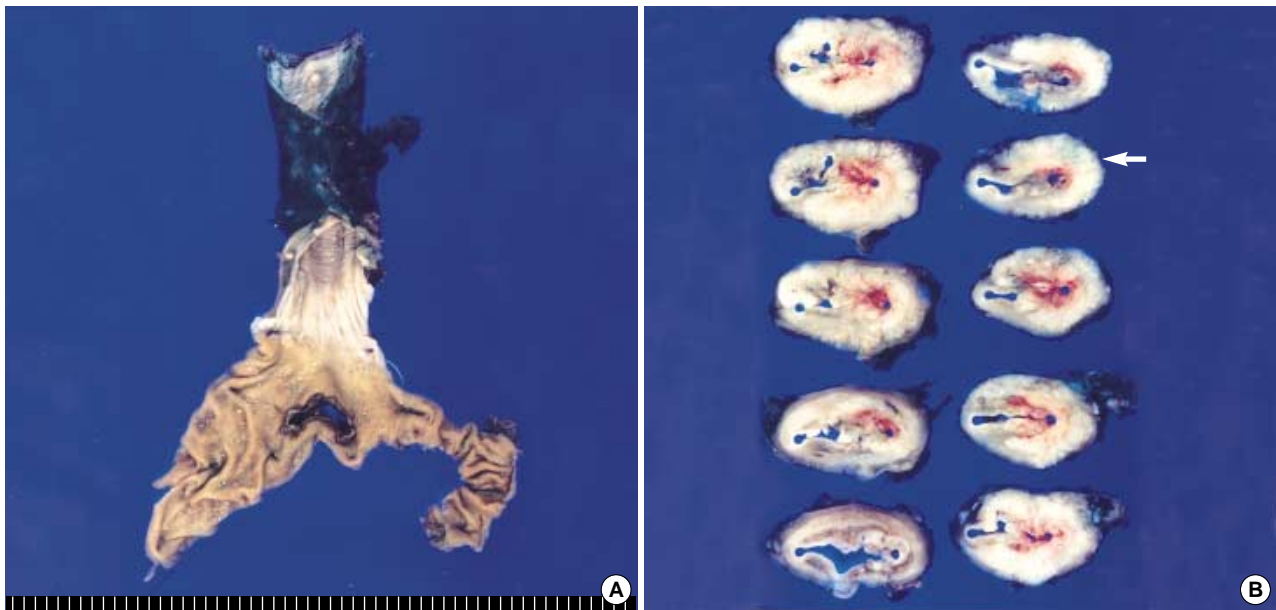


Fig. 1. Macroscopic view of the circumferential resection margin in the esophagectomy specimen. (A) The surgical specimen is opened avoiding the tumor bearing portion of the esophagus following inking of the circumferential resection margin. (B) Serial cross sectional slices of the esophagus show circumferential resection margin involvement by tumor (arrow).

ment of Pathology at Dong-A University Medical Center; and consisted of 76 cases that had undergone an esophagectomy for esophageal cancer. All cases of surgical mortality (defined as death occurring within 30 days of operation), incomplete excision (defined as cases with the presence of microscopic tumor within 1 mm of the proximal and distal margins of excision), tumor with distant metastasis, and other malignancies that occurred before or after the primary esophageal cancer were excluded. Additionally, all those that had undergone neoadjuvant therapy were also excluded. Following these exclusions, the results of the remaining 59 cases were analyzed in detail. All patients had undergone a two- or three-field en-bloc esophagectomy. All resections were regarded as potentially curative at the time of surgery. The clinical records, pathological reports and follow-up information were also obtained where available.

A pathological dissection of each specimen was carried out in the standard fashion, according to a technique based on that originally described by Quirke *et al.*,⁹ but slightly modified in our laboratory. On receipt at the laboratory, the surgical specimens were opened avoiding, where possible, the tumor bearing portion of the esophagus (Fig. 1A). The original operative specimens, stored in formalin, were oriented and pinned, under gentle tension, to a cork board. The CRM was marked with colored ink to allow histological identification and to prevent false-positive involvement of the margin as a result of poor embedding technique. Specimens were then serially sectioned in the transverse

plane and multiple blocks, and then embedded in order to measure the shortest distance (in millimeters) from the outermost part of the tumor to the lateral resection margin (Fig. 1B). The minimum distance between the tumor and CRM was measured on a microscopic stage. Cases where the distance was less than 1mm were deemed to have CRM involvement.

The hematoxylin and eosin-stained slides were reviewed in each case, to confirm the original diagnosis, based on the World Health Organization (WHO) criteria.¹⁰ The postoperative pathological staging was determined according to the guidelines of the American Joint Committee on Cancer.¹¹

Immunohistochemistry and assessment of Ki-67 staining

A representative tumor section, showing the shortest distance between the tumor and CRM, was chosen for the immunohistochemical study of Ki-67. The immunohistochemical studies were performed on formalin-fixed, paraffin-embedded, 4- μ m-thick tissue sections, using the avidin-biotin-peroxidase complex method. The primary antibody used in this study was mouse anti-human Ki-67 monoclonal antibody (clone MIB-1, Dako, Copenhagen, Denmark), at a dilution of 1:200. Deparaffinization of all sections was performed through a series of xylene baths, with rehydration through a series of graded alcohol. To enhance the immunoreactivity, microwave antigen retrieval, at 750 W

for 20 min, in citrate buffer (pH6.0), was performed. After blocking the endogenous peroxidase activity, with 5% hydrogen peroxidase for 10 min, the primary antibody incubation was performed, for 30 min, at room temperature. Detection of the immunoreactive staining was carried out by the avidin-biotin-peroxidase complex method, using the Histostain-plus kit (Zymed, CA, USA). The antigen-antibody reaction was visualized using 3-amino-9-ethylcarbazole as the chromogen, with Mayer's hematoxylin counterstaining.

The Ki-67 labeling index (LI) was calculated as the percentage of Ki-67-positive tumor cells divided by the total number of tumor cells examined in each field. The sections were first scanned at low ($\times 40$) and medium power ($\times 200$) for all fields in the central portion of the tumor and the peripheral invading portion of the tumor near to the CRM, respectively, to account for the heterogeneity of the distribution. Fields were selected from the peripheral portion, where invasion was most marked nearest to the CRM and central portion, but showed no massive necrosis, with approximately 1,000 tumor cell nuclei counted in each case. The

Table 1. Relation between circumferential resection margin involvement and clinicopathological characteristics in 59 esophageal cancers

| Clinicopathological characteristics | No. of cases | CRM involvement | | p value |
|-------------------------------------|--------------|----------------------|----------------------|---------|
| | | Positive (%) N=26 | Negative (%) N=33 | |
| Histologic type | | | | 0.08 |
| Well SCC | 25 | 15 (60.0) | 10 (40.0) | |
| Mod SCC | 19 | 6 (31.6) | 13 (68.4) | |
| Poor SCC | 11 | 4 (36.4) | 7 (63.6) | |
| Others | 4 | 1 (25.0) | 3 (75.0) | |
| Lymph node metastasis | | | | 0.03 |
| Negative | 30 | 9 (30.0) | 21 (70.0) | |
| Positive | 29 | 17 (58.6) | 12 (41.4) | |
| Lymphovascular invasion | | | | 0.001 |
| Negative | 37 | 10 (27.0) | 27 (73.0) | |
| Positive | 22 | 16 (72.7) | 6 (27.3) | |
| Perineural invasion | | | | 0.002 |
| Negative | 47 | 16 (34.0) | 31 (66.0) | |
| Positive | 12 | 10 (83.3) | 2 (16.7) | |
| TNM stage | | | | 0.0 |
| I | 12 | 0 (0) | 12 (100) | |
| IIA | 20 | 9 (45.0) | 11 (55.0) | |
| IIB | 7 | 0 (0) | 7 (100) | |
| III | 20 | 17 (85.0) | 3 (15.0) | |
| Recur | | | | 0.07 |
| Negative | 39 | 14 (35.9) | 25 (64.1) | |
| Positive | 20 | 12 (60.0) | 8 (40.0) | |

CRM, circumferential resection margin; Well SCC, well differentiated squamous cell carcinoma; Mod SCC, moderately differentiated squamous cell carcinoma; Poor SCC, poorly differentiated squamous cell carcinoma; Others, 2 small cell carcinoma, 1 adenosquamous cell carcinoma, and 1 adenocarcinoma.

nuclei of the parabasal cells in the normal epithelium were used as positive controls for Ki-67 staining. Stromal cells positive for Ki-67 were carefully excluded from the counting process. Then, two Ki-67 differential grades (Ki-67 DG) were defined, according to the difference between the Ki-67 LI of central and peripheral portions: cases where the difference in the Ki-67 LI was $\leq 10\%$ were defined as Ki-67 DG 0, whereas those where the difference was $>10\%$ were defined as Ki-67 DG 1.

Statistical analysis

The associations between the clinicopathological characteristics, Ki-67 DG and CRM involvement were analyzed using contingency tables. Statistical significance was evaluated using chi-squared tests. Univariate survival analyses for patients with and without CRM were estimated according to the Kaplan-Meier method, with differences in the survival rates assessed using the log-rank tests. The statistical difference was considered to be significant if the p value was less than 0.05. The data were analyzed with the Statistical Package Service Solution software (SPSS for Windows, Standard version 10.1).

RESULTS

Clinicopathological characteristics

The median age of the 59 cases was 60 years, ranging from 29 to 78, with 54 men and 5 women. The median tumor size was 3.5 cm, ranging from 1 to 9 cm. Histologically, the primary tumors were: 55 squamous cell carcinomas (25 well differentiated, 19 moderately differentiated and 11 poorly differentiated), and 4 others (2 small cell carcinomas, 1 adenosquamous cell carcinoma and 1 adenocarcinoma). Thirty patients showed regional lymph node metastasis. There were 22 cases with lymphovascular invasion, and 12 cases with perineural invasion. Twelve, 20, 7 and 20 patients were in stages I, IIA, IIB and III, respectively.

Table 2. Relation between circumferential resection margin involvement and Ki-67 differential grade in 59 esophageal cancers

| Ki-67 DG | No. of cases | CRM involvement | | p value |
|----------|--------------|----------------------|----------------------|---------|
| | | Positive (%) N=26 | Negative (%) N=33 | |
| 0 | 46 | 16 (34.8) | 30 (65.2) | 0.007 |
| 1 | 13 | 10 (76.9) | 3 (23.1) | |

Ki-67 DG, Ki-67 differential grade; CRM, circumferential resection margin.

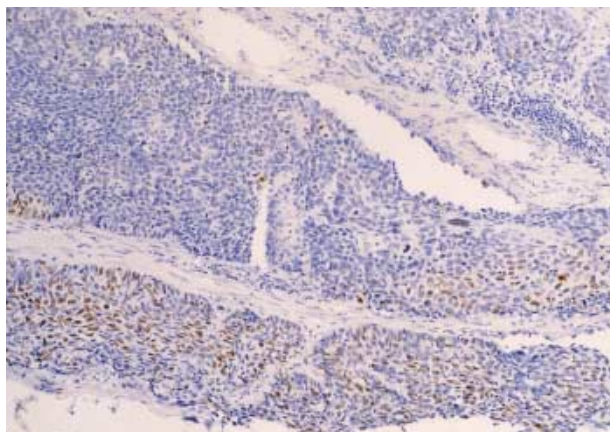


Fig. 2. Ki-67 positive cells are more preferentially distributed at the peripheral, invading fronts than the central tumor area in cases with circumferential resection margin involvement.

Correlation of CRM involvement with clinicopathological factors

There were 26 (44.1%) and 33 (55.9%) cases with and without CRM involvement, respectively. There were significant differences in the percentage of the cases with or without CRM involvement of tumor cells, in relation to lymph node metastasis, lymphovascular invasion, perineural invasion and the tumor stage ($p < 0.05$) (Table 1).

Relationship between CRM involvement and Ki-67 DG

Ten (38.3%) of the 26 with CRM involvement, and 3 (9.1%) of the remaining 33 without CRM involvement, showed Ki-67 DG 1 ($p = 0.007$). The tumor cells with CRM involvement showed more increased Ki-67 LI in the downward invading area, especially in the peripheral fronts of the invading nests, than those without CRM involvement (Fig. 2) (Table 2).

Influence of CRM involvement on recurrence and survival

Adequate clinical follow-up information was available for all 59 cases. The mean follow-up of the 59 cases was 36 months, ranging from 1 to 92 months. Thirty-seven (62.7%) were still alive, but 22 (37.3%) died during the follow-up period. Of the latter, 17 died of the documented progressive esophageal cancer, 2 of respiratory dysfunction and 3 of other diseases. Twenty patients (33.9%) had recurrences during the follow-up period: 11 due to distant metastases and 9 to regional recurrences. The mean

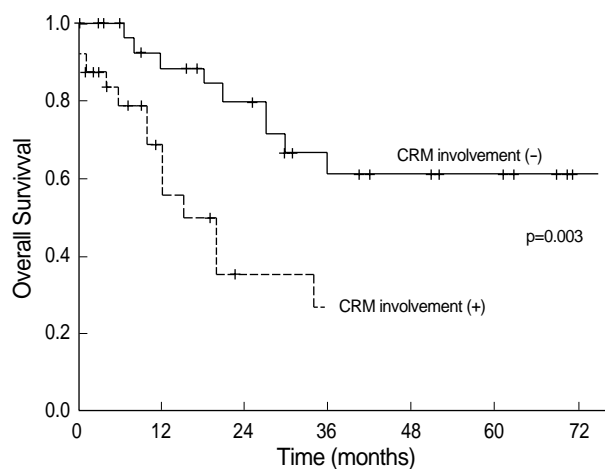


Fig. 3. Survival curves for esophageal cancer patients, with or without circumferential resection margin (CRM) involvement show statistically significant difference ($p = 0.003$).

duration from surgery to recurrence in these 20 patients was 30 months, ranging from 1 to 82 months. Although there was no statistically significant association between CRM involvement and recurrence ($p = 0.07$), the patients with CRM involvement tended to recur more frequently (46.2%) than those without CRM involvement (24.2%).

The overall 3-year survival of the 59 patients in this study was 44.3%, and of the patients with and without CRM involvement were 26.8 and 61.8%, respectively, with statistically significant difference ($p = 0.003$). The Kaplan-Meier survival curves demonstrated that the patients with CRM involvement had significantly shorter survival periods than those without (Fig. 3).

DISCUSSION

In this study, CRM involvement by tumor has been shown to still be one of the important prognostic factors for survival in esophageal cancer. Although the presence of a tumor at the CRM has been suggested as a potential predictor of survival following esophagectomy, it was rather unexpected that only a few studies⁴⁻⁶ have been conducted in esophageal cancer to analyze the significance of CRM involvement. For rectal cancer, the presence of a tumor within 1 mm in the CRM is an important prognostic factor for both local recurrence and survival. Recent studies have shown that CRM involvement in rectal cancer is both a predictor of local recurrence following resection¹² and a marker of the long-term survival.¹³ For esophageal cancer, Sagar *et al.*⁴ reported that CRM involvement was associated with a decreased median survival, and appeared to be a significant cause of local tumor recur-

rence, and Dexter *et al.*⁵ reported that the presence of a tumor in CRM was not only an adverse prognostic factor, but also an independent predictor of survival. However, the results of a recent study showed contradictory findings, in that the analysis of the survival curves, for those cases with and without CRM involvement, showed no statistically significant difference in the short- or long-term outcomes, and the presence of a microscopic tumor at the CRM, following an en-bloc esophagectomy, was not a significant prognostic marker.⁶ In this study, CRM involvement was found to be important in assessing the prognosis after a resection for esophageal cancer, with CRM involvement appearing to be a strong predictor of survival in esophageal cancer.

However, the relationship between survival and CRM involvement is not simple, and assumes different importance according to the extent of lymph node involvement. In our study, CRM involvement significantly correlated with lymph node metastasis and lymphovascular invasion, which are the most significant prognostic factors in patients with esophageal cancer undergoing an esophagectomy.¹⁴ It is likely that CRM involvement represents a proportion of the microscopic residual disease. If the CRM is evaluated on frozen section, during surgery, the surgeon could consider a more radical lymphadenectomy. The CRM should receive close attention from the surgeon at the time of surgery, and subsequently from the pathologist, by careful specimen dissection and histological assessment.

Khan *et al.*⁶ reported that CRM involvement was not a significant prognostic factor, and a partial explanation for the difference in their results with those of others^{4,5} may lie in the more complete surgical resection of the periesophageal tissue in their study group. It is believed that the validity of using the "tumor within 1mm or less" rule, for the definition of CRM involvement, has been confirmed in this study, with this being both a logical and practically useful approach. In our study, even if the carcinoma did not directly encroach on the CRM, there was a strong relationship between poor prognostic factors and the findings of carcinomas at a distance of 1 mm or less from the CRM (data not shown). Therefore, it is considered that CRM involvement is more an indicator of an advanced disease than of an incomplete excision.

An immunohistochemical examination, using monoclonal antibody to Ki-67, has been used to study the proliferative activity of various types of carcinoma, and to estimate its potential as a biomarker for prognosis or tumor progression. ESCC patients with a high Ki-67 LI have lower postoperative survival rates; thus, a high Ki-67 is a prognostic factor of ESCC.^{15,16} Ohashi *et al.*⁸ reported that the proliferative activity, as assessed by Ki-67 LI, was different between intraepithelial spreading and downwardly

invading tumor component, with higher values for the latter component. Ki-67 positive, proliferating cells were preferentially distributed in the peripheral fronts of the invading nests in esophageal cancer. They suggested that the increased proliferative activity in downwardly invading lesions, especially in the peripheral fronts of the invading nests, might be related to the destruction of the basement membrane and budding of the carcinoma nests. In our study, there was no statistical difference between Ki-67 LI, clinicopathological factor and survival. However, the tumor cells with CRM involvement showed greater increases in Ki-67 LI in the downwardly invading lesions, especially in the peripheral fronts of the invading nests, than those without CRM involvement, which might be related to the increased proliferative activity of the tumor cells. It is our assumption that the tumor cells involving the CRM may be types of subclone, with more invasive properties and higher proliferative activities. It also suggests that CRM involvement is more an indicator of an advanced and aggressive disease than of an incomplete excision.

Although the presence of a tumor at the CRM has been suggested as a potential predictor of survival following an esophagectomy, the CRM has only been infrequently reported. A complete pathology report is important in the quality control of surgical treatment, and to predict its outcome. Failure of pathologists to report this feature, therefore, amounts to a disservice to both the clinical team and the patient. CRM involvement for esophageal cancer is likely to remain more important than for rectal cancer, as the esophagus is closely surrounded by vital structures, which cannot be resected en bloc. CRM involvement appears to be a strong predictor of the survival of esophageal cancer. Accurate determination of the CRM in esophageal cancer is important in the determination of local or distant recurrence risks, which might subsequently be prevented by additional therapy. This knowledge can be used in the selection of patients for postoperative adjuvant therapy. Our data, as well as those published by others,^{4,5} suggest that a revision of the TNM staging is necessary, where the distance from the tumor to the CRM should be included. In this way, T3 tumors have to be divided into two categories.

In conclusion, this study has shown that the CRM involvement status may be used as a predictor of survival following a potentially curative esophagectomy, and that it is more an indicator of an advanced disease than of inadequate local surgery. It is suggested that routine assessment of the CRM of esophageal cancer specimens should be an essential part of pathology reporting for esophageal cancer.

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