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Development and validation of the optimal circumferential resection margin in pathological T3 esophageal cancer: A multicenter retrospective study

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Running head: Optimal surgical margin in T3 ESCC

Conflict of Interest

The authors declare no conflicts of interest for this article.

Synopsis

This study reported that the circumferential resection margin of 600 μm, set between the Royal College of Pathologists criteria and the College of American Pathologists criteria, is optimal to predict locoregional recurrence for pathological T3 esophageal squamous cell carcinoma.

Abstract

Background: The clinical significance of circumferential resection margin (CRM) in esophageal squamous cell carcinoma (ESCC) remains unclear. Optimal CRM for predicting the recurrence of pathological T3 ESCC was investigated.

Methods: Seventy-three patients were retrospectively investigated in the development cohort. Patients were divided into CRM-negative and CRM-positive groups, and clinicopathological factors and survival outcomes were compared between the groups. The cut-off value was validated in another validation cohort (n = 99).

Results: Receiver operating characteristic analysis in the development cohort showed the cut-off value of CRM was 600 μm. In the validation cohort, patients in the CRM-positive group showed a significantly higher rate of locoregional recurrence ($p = 0.006$) and worse recurrence-free survival (RFS) ($p < 0.001$) than those in the CRM-negative group. Multivariate analysis identified positive CRM as an independent predictive factor for poor RFS (hazard ratio, 2.695; 95% confidence interval, 1.492–4.867; $p = 0.001$). The predictive value of our criteria of positive CRM for RFS was higher than that of the Royal College of Pathologists (RCP) and the College of American Pathologists (CAP) criteria. Stratified analysis in the neoadjuvant chemotherapy groups also revealed that the rate of locoregional recurrence was higher in the CRM-positive group than in the CRM-negative group both in the pathological N0 and N1–3 subgroups.

Conclusions: CRM of 600 μm can be the optimal cut-off value rather than the RCP and CAP criteria for predicting locoregional recurrence after esophagectomy. These results may support the impact of perioperative locoregional control of locally advanced ESCC.

Key words: esophageal cancer, esophagectomy, circumferential resection margin

Introduction

Esophageal cancer is the sixth leading cause of cancer-related deaths worldwide.[1] Transthoracic esophagectomy has been recognized as the standard treatment for esophageal squamous cell carcinoma (ESCC). The complete surgical removal of the invasive tumor is the primary aim of curative surgery for ESCC;[2-4] however, advanced ESCCs often recur after R0 resection, with or without perioperative chemotherapy.

Some factors that can predict prognosis after resection include tumor size, tumor grade, vessel involvement, and lymph node (LN) metastasis.[5,6] Tumor infiltration of the proximal or distal resection margins is associated with poor survival.[7,8]

Regarding the vertical resection margin, the circumferential resection margin (CRM) involvement, which is reportedly a strong predictor of local recurrence in rectal cancer, may be defined by two commonly used criteria in esophageal cancer.[9,10] The Royal College of Pathologists (RCP) criteria define positive CRM as a tumor at or within 1 mm of the cut margin,[11] whereas the College of American Pathologists (CAP) criteria consider only the presence of a tumor at the cut margin as CRM-positive in esophageal cancer.[12] Although some studies have compared the prognostic significance of these two CRM criteria, their usability remain controversial.

In this study, we hypothesized that there is an optimal cut-off value of CRM between 0 and 1 mm to predict locoregional recurrence and prognosis in patients with pathological T3 (pT3) ESCC. We then designed two cohorts at two independent institutions to determine the optimal cut-off value of CRM and validated its prognostic significance in ESCC patients.

Patients and methods

Study population

This study was a retrospective, observational clinical study that included two cohorts from two independent institutions: (i) Hamamatsu University School of Medicine (HUSM, Shizuoka, Japan) and (ii) Shizuoka Cancer Center (ShCC, Shizuoka, Japan). This study included both a development cohort and a validation cohort. Eighty-three patients with pT3 ESCC who underwent esophagectomy at HUSM between July 1, 2009, and December 31, 2020, were recruited in the development cohort. In the validation cohort, 129 patients with pT3 ESCC who underwent esophagectomy at the ShCC between January 1, 2011, and December 31, 2020, were recruited. All enrolled patients were Japanese.

All patients underwent preoperative esophagogastroduodenoscopy (EGD) and computed tomography (CT) from the neck to the pelvis to determine the clinical stage of cancer. Positron emission tomography using 2-[18F] fluoro-2-deoxy-d-glucose (FDG-PET) and endoscopic ultrasound was also performed for some patients. Either current or former smokers were defined as smokers. Patients who regularly drank more than 14 g of alcohol were defined as drinkers, according to the National Institutes of Alcohol Abuse and Alcoholism. The clinical and pathological stage were diagnosed based on the Union for International Cancer Control TNM classification of malignant tumors, 8th edition.[13]

Patients who met the following criteria were enrolled in this study: (1) age > 20 years, (2) Eastern Cooperative Oncology Group performance status of 0 to 1, (3) histological diagnosis of ESCC by endoscopic biopsy, (4) no synchronous cancer, (5) patients who underwent curative esophagectomy, (6) histologically proven invasion to adventitia, (7) no metastasis to distant organs, and (8) no previous irradiation. Patients

were deemed ineligible for enrolment in this study based on the following exclusion criteria: salvage surgery, non-curative surgery, and positive proximal resection margin. Finally, the study population included 73 patients in the development cohort and 99 patients in the validation cohort.

All procedures were conducted in accordance with institutional and national standards for human experimentation, as confirmed by the Ethics Committee of HUSM (approval No: 21-062) and ShCC (approval No: 2965), and with the Declaration of Helsinki of 1964 and its subsequent versions.

Treatment and postoperative complications

The treatment strategies were similar between the two institutions according to the 2017 esophageal cancer practice guidelines in Japan.[2,3] Neoadjuvant chemotherapy (NAC) was performed as a standard treatment for patients with clinical stage II/III ESCC. The treatment regimen was a combination of cisplatin and 5-fluorouracil (CF)[14] or a combination of docetaxel, cisplatin, and 5-fluorouracil.[15] Transthoracic esophagectomy with 2- or 3-field LN dissection followed by gastric conduit reconstruction with cervical anastomosis was performed as a standard surgical procedure.[16,17] Dissection of the supraclavicular LN was performed for patients with upper or middle thoracic ESCC and for those with lower thoracic ESCC, with a clinical diagnosis of T2 or more.[16,18] Although video-assisted thoracoscopic surgery is generally adopted in the thoracic approach, thoracotomy was performed for bulky primary tumors or for those possibly invading the surrounding organs, and for patients who refused thoracoscopy.[16] Postoperative complications were evaluated for pneumonia, anastomotic leakage (AL), and surgical site infection (SSI) according to the Clavien–Dindo (C–D) classification. Pneumonia of C–D

grade 2 or higher and AL and SSI of C–D grade 3 or higher were defined as postoperative complications.[19,20]

Assessment of CRM

All resected ESCC specimens were fixed in formalin and macroscopically examined in detail by a certified pathologist at each institution. The specimens were embedded in paraffin, and thin sections were stained with hematoxylin and eosin for routine microscopic examination. The vertical cut edge of the resected specimens was defined as CRM. The minimum distance of the CRM was measured in micrometers and used to determine the CRM status (Supplementary Fig. 1).

Follow-up

The follow-up schedule was similar between the two institutions. Postoperative follow-up was performed using CT every six months and EGD every year for five years after surgery. When recurrence was suspected, the patients underwent FDG-PET and/or endoscopic examination with biopsy. The initial recurrent sites were classified into the following three patterns: locoregional recurrence including a local recurrence defined as soft tissues newly detected at the circumferential region of the primary tumor and a lymph node recurrence at Group 1 LNs around the primary tumor according to the Japanese Classification of Esophageal Cancer (JCEC) 11th edition;[21] regional LN recurrence, defined as newly detected soft tissues indicative of recurrence at Group 2 LN metastases far from primary tumor in the mediastinal, abdominal, or cervical region, according to the JCEC 11th edition;[21] and distant recurrence, defined as newly detected soft tissues indicative of recurrence at Group 3 or Group 4 LNs, according to the JCEC 11th edition,[21]

or a hematogenous metastasis with organ tumor formation. Recurrence-free survival (RFS) was calculated from the day of surgery to the day of ESCC recurrence or death. Locoregional recurrence-free survival (LRRFS) was calculated from the day of surgery to the day of locoregional recurrence of ESCC or death. Overall survival (OS) was calculated from the day of surgery to the day of death. Patients were followed up until death, until 5 years after surgery, or until the end of the study, i.e., March 31, 2021. Patients who died until 5 years, had interrupted follow-up, or under following up were recognized as censored.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics version 26 for Windows (Chicago, IL, USA). Receiver operating characteristic (ROC) analysis of CRM-based prediction of recurrence until 5 years after esophagectomy was performed to assess the optimal cut-off value of the CRM. Medians and ranges were calculated, and differences were identified using Student's *t*-test. The Mann–Whitney *U test* was used for non-parametric analyses. Differences between each category were identified using the Chi-squared test or Fisher's exact test. Survival curves were generated using the Kaplan–Meier survival method and compared using the log–rank test. Hazard ratios (HRs) and confidence intervals (CIs) were calculated, and univariate and multivariate analyses were performed using Cox proportional hazards regression models. The threshold for significance was set at $p < 0.05$.

Results

Determination of the cut-off values for CRM

ROC analysis of CRM-based prediction of recurrence in the development cohort revealed that the area under the curve was 0.727. The cut-off value of the CRM was set at 600 μm based on the peak point of the Youden index (sensitivity = 0.464, specificity = 0.844, Youden index = 0.308) (Supplementary Fig. 2).

Study flow diagram

Using this cut-off value, patients were divided into the CRM-negative group (CRM > 600 μm, n = 20) and the CRM-positive group (CRM ≤ 600 μm, n = 53) in the development cohort (Supplementary Fig. 3). In the validation cohort, 46 and 53 patients were classified into the CRM-negative and CRM-positive groups, respectively (Supplementary Fig. 4). The median follow-up interval of all patients in the development cohort was 28.6 months, while in the validation cohort, the median follow-up interval of all patients was 23.3 months.

Patient characteristics

While comparing the clinical characteristics, no significant differences were found in the distribution of age, sex, smoking, drinking, tumor location, clinical stage, preoperative therapy, operation time, surgical approach, area of LN dissection, reconstruction organ, or postoperative complications between the CRM-negative and CRM-positive groups in either cohort (Supplementary Table 1). In the validation cohort, the rate of poorly differentiated ESCC in the biopsy specimens was higher in the CRM-positive group than in the CRM-negative group (37.7% vs. 15.2%, p = 0.040)

(Supplementary Table 1).

Clinical impact of the CRM status in the development cohort

Pathological findings were compared between the CRM-negative and CRM-positive groups in the development cohort (Table 1). The tumor diameter, rates of infiltrative growth-c (INFc), lymphatic vessel infiltration (LVI), blood vessel infiltration (BVI), pathological N (pN) status, and pathological stage were comparable between the two groups (Table 1).

The recurrence rate was significantly higher in the CRM-positive group than in the CRM-negative group (71.7 % vs. 35.0 %, $p = 0.007$). Locoregional recurrence was more frequently observed in the CRM-positive group than in the CRM-negative group (41.5 % vs. 2.0 %, $p = 0.002$), whereas the incidence of regional LN recurrence and distant recurrence was comparable between the two groups (Table 1).

RFS was significantly worse in the CRM-positive group than in the CRM-negative group (median survival time [MST]; 10.3 vs. 32.7 months, $p = 0.005$), whereas no difference was seen in OS between the two groups (MST; 21.0 vs. 36.7 months, $p = 0.246$) (Fig. 1). Multivariate analyses identified positive CRM as an independent predictive factor of poor RFS ($p = 0.037$, [HR, 2.482; 95 % CI, 1.056–5.832]) (Table 2).

Validation of the present CRM status

The developed model was then used to predict risks in the validation cohort. Regarding the pathological findings, the rates of INFc, positive LVI, and positive BVI were significantly higher in the CRM-positive group than in the CRM-negative group (INFc, 22.6 % vs. 6.5 %, $p = 0.022$; LVI, 69.8 % vs. 43.5 %, $p = 0.014$; BVI, 86.8 % vs.

65.2 %, p = 0.016, respectively). There was no significant difference in pN status and pathological stage between the two groups (Table 1).

Consistent with the development cohort, the recurrence rate in the validation cohort was significantly higher in the CRM-positive group than in the CRM-negative group (64.2 % vs. 37.0 %, p = 0.009). Regarding the pattern of initial recurrence, locoregional recurrence was more frequently observed in the CRM-positive group than in the CRM-negative group (32.1 % vs. 8.7 %, p = 0.006). Furthermore, the rate of distant recurrence was also higher in the CRM-positive group than in the CRM-negative group (47.2 % vs. 17.4 %, p = 0.003) (Table 1).

Patients in the CRM-positive group had significantly worse RFS and OS than those in the CRM-negative group (MST; RFS, 9.9 vs. 23.3 months, $p < 0.001$ and OS, 18.7 vs. 36.1 months, p = 0.002) (Fig. 2). Consistent with the development cohort, multivariate analyses identified positive CRM as an independent predictive factor for poor RFS (p = 0.001, HR, 2.695; 95 % CI, 1.492–4.867) (Table 2).

Predictive factors of positive CRM

To identify preoperative clinicopathological factors predictive of positive CRM, cox regression analyses for positive CRM were performed in the validation cohort (Supplementary Table 2). In univariate and multivariate analyses, poorly differentiated histology (p = 0.027, HR, 2.647; 95 % CI, 1.119–6.263) and macroscopic Type 1 or Type 3 (p = 0.026, HR, 3.124; 95 % CI, 1.148–8.500) were identified as predictive factors of positive CRM (Supplementary Table 2).

Comparison of the RCP, CAP, and our criteria

The clinical impact of our criteria was compared with the two conventional criteria, i.e., the RCP criteria and CAP criteria, using the data from the validation cohort. The recurrence rate in the CRM-positive group was significantly higher than that in the CRM-negative group according to the RCP criteria (61.7% vs. 35.9%, $p = 0.014$) and CAP criteria (86.7% vs. 45.2%, $p = 0.004$). Locoregional recurrence was more frequently observed in the CRM-positive group than in the CRM-negative group according to the RCP criteria (30.0 % vs. 7.7 %, $p = 0.011$), whereas there was no difference between the two groups according to the CAP criteria (40.0 % vs. 17.9 %, $p = 0.082$) (Supplementary Table 3). The sensitivities of the prediction of locoregional recurrence were 0.857, 0.810 and 0.286 in the RCP criteria, our criteria and the CAP criteria. The specificities of prediction of locoregional recurrence were 0.462, 0.538 and 0.885, respectively. The false positive rate was 0.538, 0.462 and 0.115, and the Youden index was 0.319, 0.348 and 0.171 in the RCP criteria, our criteria and the CAP criteria, respectively.

According to the RCP criteria, patients in the CRM-positive group had significantly worse RFS and OS than those in the CRM-negative group (MST; RFS, 10.4 vs. 28.5 months, $p < 0.001$ and OS, 18.7 vs. 40.2 months, $p = 0.004$). In contrast, according to the CAP criteria, patients in the CRM-positive group had significantly worse RFS than those in the CRM-negative group (MST, 9.5 vs. 19.2 months, $p < 0.001$), whereas there was no significant difference in OS between the two groups (MST, 15.4 vs. 24.2 months, $p = 0.190$).

To evaluate the usefulness of our criteria, patients in the validation cohort were divided into four groups according to CRM status; 0 μm, 0–600 μm, 600–1000 μm, and > 1000 μm (Supplementary Fig. 5). Patients in the 0–600 μm group had significantly worse RFS and OS than those in the 600–1000 μm group (MST; RFS, 10.3 vs. 22.5

months, $p = 0.006$ and OS, 18.5 vs. 32.2 months, $p = 0.013$). Furthermore, there was no difference in OS and RFS between the 0–600 μm and 0 μm groups, and there was also no difference in OS and RFS between 600–1000 μm and > 1000 μm groups (Supplementary Fig. 5).

Univariate analysis demonstrated that the HR of positive CRM for poor RFS was 2.928 (95% CI, 1.453–5.902, $p = 0.003$) in the RCP criteria, 3.167 (95% CI, 1.778–5.644, $p < 0.001$) in our criteria, and 2.236 (95% CI, 1.117–4.475, $p = 0.023$) in the CAP criteria (Table 3).

Survival impact of the CRM adjusted for neoadjuvant chemotherapy and pN status

To evaluate the prognostic value of CRM irrespective of the confounding influence of LN metastasis, stratified analyses adjusted for LN metastasis and NAC were performed. All enrolled patients in the two cohorts were classified into four groups according to the status of NAC and pN: non-NAC and pN0 group (n = 10), non-NAC and pN1–3 group (n = 22), NAC and pN0 group (n = 21), and NAC and pN1–3 groups (n = 119). In the non-NAC group, the incidence of total recurrence was similar between the CRM-positive and CRM-negative groups regardless of the pN status (pN0 groups, $p = 1.000$ and pN1–3 groups, $p = 1.000$). However, the incidence of locoregional recurrence in the NAC group was higher in the CRM-positive group than in the CRM-negative group, both in the pN0 and pN1–3 subgroups (pN0 groups, 37.5% vs. 0.0%, $p = 0.042$ and pN1–3 groups, 39.7% vs. 9.8%, $p = 0.001$, respectively) (Table 4).

Next, we analyzed the survival impact of CRM-positivity according to pN status in patients treated with NAC. Although there was no significant difference, patients in the CRM-positive group tended to show worse RFS (MST; 26.1 vs. 54.7 months, $p = 0.093$)

and OS (MST; 32.4 vs. 54.7 months, $p = 0.087$) in the pN0 subgroups (Fig. 3a, 3c). Furthermore, LRRFS was significantly worse in the CRM-positive group than in the CRM-negative group in the pN0 subgroups (MST; 26.1 vs. 54.7 months, $p = 0.015$) (Fig. 3b). Furthermore, within the pN1–3 subgroup, patients in the CRM-positive group showed significantly worse RFS (MST; RFS, 10.2 vs. 28.5 months, $p < 0.001$), LRRFS (LRRFS, 14.5 vs. 34.9 months, $p < 0.001$), and OS (OS, 20.3 vs. 38.9 months, $p < 0.001$) than those in the CRM-negative group (Fig. 3d, 3e, 3f).

Discussion

This study demonstrated that patients with a CRM ≤ 600 μm (CRM-positive) showed worse RFS and higher rates of locoregional recurrence than those with CRM > 600 μm (CRM-negative), and validated the cut-off value of CRM in another independent cohort. Furthermore, CRM-positivity according to our criteria, had a stronger correlation with locoregional recurrence compared to the RCP and CAP criteria in the validation cohort. These results suggested that the optimal CRM was 600 μm, which was set between the RCP and CAP criteria, to predict locoregional recurrence after esophagectomy for pT3 ESCC.

In the validation cohort, the incidence of locoregional recurrence was higher in the CRM-positive group than in the CRM-negative group according to the RCP criteria, while there was no difference between the two groups according to the CAP criteria. Positive CRM according to the RCP criteria showed higher sensitivity to locoregional recurrence compared to our criteria; however, the false-positive rate was higher. The Youden index, which indicates predictive ability, was the highest in our criteria among the three criteria. In the survival analysis, our criteria showed the highest HR for predicting poor RFS. Moreover, our criteria clearly separated the survival curve in the ambiguous range of CRM between 0 and 1000 μm. These results demonstrate that our criteria are the most useful as factors for predicting recurrence. Some studies have reported the prognostic impact of CRM involvement in esophageal cancer.[22-27] Most studies have compared the CAP criteria with the RCP criteria, and the results remain controversial. Okada et al. reported that positive CRM according to the CAP criteria significantly affected the OS and RFS of pT3 ESCC patients.[22] However, Ghadban et al. reported that there was no correlation, neither according to the CAP nor RCP criteria, with

local recurrence and prognosis in esophageal cancer.[24] If the CRM of tumor specimens is identified between 0 and 1000 μm microscopically after esophagectomy, surgeons often face difficulties in the selection of proper therapeutic options because little is known about the cut-off value for positive CRM. Some single cohort studies proposed a new cut-off value of CRM at 500–600 μm, and CRM-positive patients showed worse OS,[27,28] which is consistent with the results of our study. As per our knowledge, our study is the first to investigate the optimal cut-off value of CRM between the RCP and CAP criteria in pT3 ESCC, and to validate its usability in another independent cohort.

This study demonstrated that tumors in the CRM-positive group were more frequently accompanied by lymphatic and vascular infiltration. Tsutsui et al. pathologically examined the specimens, and reported five types of accessory lesions that existed beyond the main lesion: intraepithelial carcinoma, subepithelial direct extension, intramural metastasis, lymphatic invasion, and vascular invasion.[29] The occurrence of these accessory lesions increased in cases with tumor invasion into the adventitia or deeper. High invasiveness to lymphatic and vascular tissue in pT3, especially close to the surgical margin, led to the discovery of cancer remnants microscopically.[29,30]

According to the 2017 esophageal cancer practice guidelines in Japan, the standard treatment for clinical stage II/III ESCC is NAC followed by radical esophagectomy.[2,3] Stratified analysis in the NAC groups revealed that the rate of locoregional recurrence was higher in the CRM-positive group than in the CRM-negative group, both in the pN0 and pN1–3 subgroups. Furthermore, the CRM-positive group showed worse LRRFS than the CRM-negative group in both the pN0 and pN1–3 subgroups. These results suggest that our criteria could provide the optimal cut-off value of CRM for predicting locoregional recurrence. To date, no studies have evaluated the

survival impact of CRM in patients who underwent standard treatment for ESCC. Our study evaluated the prognostic value of CRM by eliminating the influence of pN metastasis status. These results suggest the importance of adjuvant chemotherapy, radiotherapy or chemoradiotherapy for postoperative local control in CRM-positive cases. A recent phase 3 clinical trial CheckMate 577 showed that postoperative immunotherapy with nivolumab improved disease-free survival in patients with ESCC and esophageal-gastric junction cancer who received neoadjuvant chemoradiotherapy (NACRT) followed by surgery.[31] Adjuvant immunotherapy has become a treatment option of advanced ESCC, although the clinical effect of immunotherapy as adjuvant treatment after surgery following NAC remains controversial.

A previous study reported that pT3 was an independent predictive factor for poor OS and progression-free survival in patients who received NAC followed by surgery for clinical stage II/III ESCC, suggesting the necessity of additional perioperative therapy for pT3 ESCC.[32] In Western countries, where adenocarcinoma accounts for the vast majority of esophageal cancers, chemoradiation is often performed as either neoadjuvant or perioperative treatment, and the association between perioperative chemoradiotherapy and complete resection margin has been reported.[33] In this setting, most tumors after NACRT showed a high pathological regression rate, leading to securing the safe CRM and accomplishing a high complete resection rate.[33] In contrast, our results suggest that NAC consisting of CF might be insufficient to control locoregional recurrence in pT3 ESCC, especially close to surgical margin. In a multivariate analysis, poorly differentiation and macroscopic classification of Type 1 or 3 were identified as predictive factors for positive CRM. These results may contribute to the patient selection to intensive neoadjuvant therapies such as NACRT and more intensive NAC to improve the local

control and to increase the complete resection rate in T3 ESCC.

This study has some limitations. First, this was a retrospective study. However, in terms of CRM, prospective studies are difficult to plan. Second, the treatment strategies of ESCC, including NAC regimen and perioperative management, slightly differed between the institutions in this multicenter study, which could have affected the results. However, both participating institutions were high-volume centers with the ability to deliver high quality care.

A major strength of this study is that it reported the optimal cut-off value of CRM set between the RCP criteria and the CAP criteria in thoracic ESCC, the consistency of which was validated in another independent cohort.

In conclusion, a CRM of 600 μm has the potential to become the optimal cut-off, value rather than the RCP and CAP criteria, to predict locoregional recurrence after esophagectomy for ESCC. These results may support the impact of perioperative locoregional control of locally advanced ESCC.

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Human rights statement and informed consent

All procedures were conducted in accordance with institutional and national standards on human experimentation, as confirmed by the ethics committee of Hamamatsu University School of Medicine (approval number; 21-062) and Shizuoka Cancer Center (approval number; 2965), and with the Declaration of Helsinki of 1964 and its later versions.

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Figure Legends

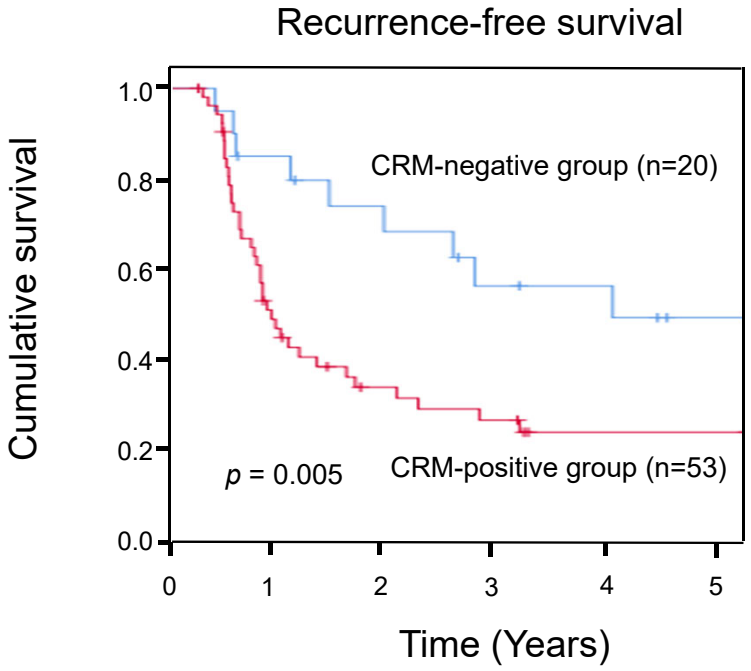
Fig. 1 Kaplan–Meier analysis in the two cohort. **a**. Comparison of recurrence-free survival between the CRM-negative and CRM-positive groups in the development cohort. **b**. Comparison of overall survival between the CRM-negative and CRM-positive groups in the development cohort. **c**. Comparison of recurrence-free survival between the CRM-negative and CRM-positive groups in the validation cohort. **d**. Comparison of overall survival between the CRM-negative and CRM-positive groups in the validation cohort.

Fig. 2 Kaplan–Meier analysis in the validation cohort. a. Comparison of recurrence-free survival between the CRM-negative and CRM-positive groups. b. Comparison of overall survival between the CRM-negative and CRM-positive groups.

Fig. 3 Stratified analysis of survival of patients treated with neoadjuvant chemotherapy according to the pN status. **a**. Comparison of recurrence-free survival between the CRM-negative and CRM-positive groups in pN0 ESCC patients. **b**. Comparison of locoregional recurrence-free survival between the CRM-negative and CRM-positive groups in pN0 ESCC patients. **c**. Comparison of overall survival between the CRM-negative and CRM-positive groups in pN0 ESCC patients. **d**. Comparison of recurrence-free survival between the CRM-negative and CRM-positive groups in pN1–3 ESCC patients. **e**. Comparison of locoregional recurrence-free survival between the CRM-negative and CRM-positive groups in pN1–3 ESCC patients. **f**. Comparison of overall survival between the CRM-negative and CRM-positive groups in pN1–3 ESCC patients.

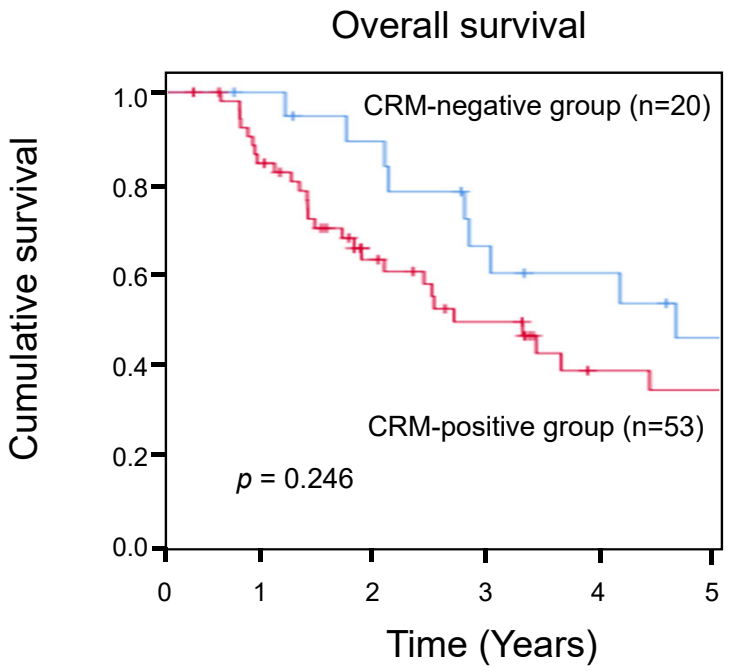
Fig. 1

a



Number at risk						
CRM-negative group	20	16	12	9	5	5
CRM-positive group	53	23	14	11	7	7

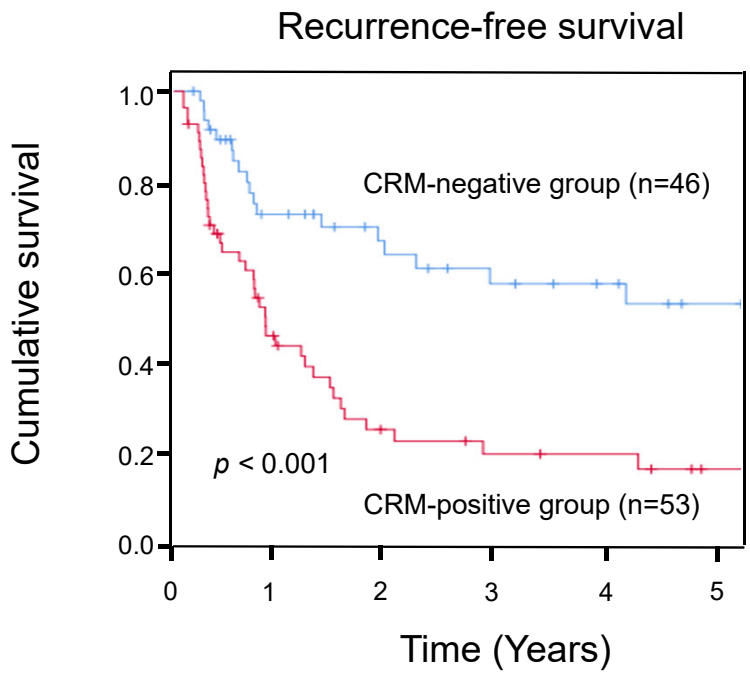
b



Number at risk						
CRM-negative group	20	19	14	10	9	6
CRM-positive group	53	41	23	17	9	8

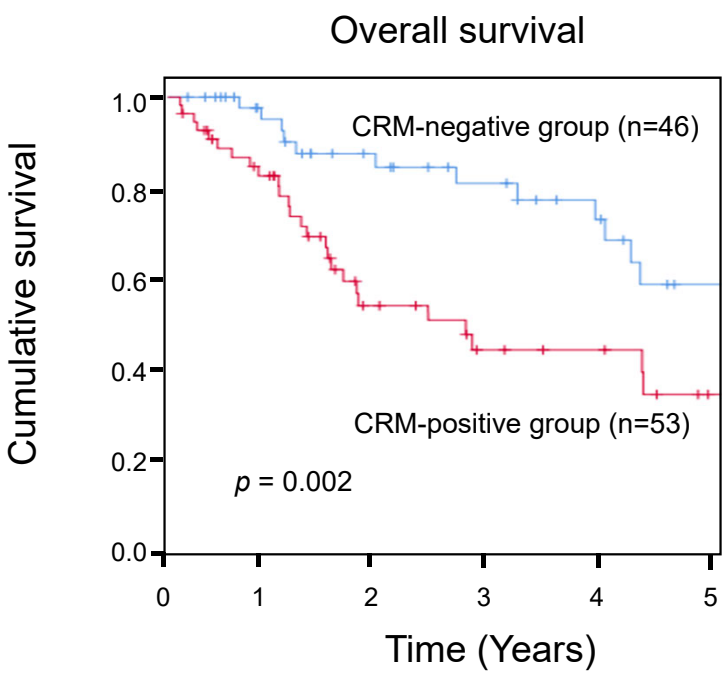
Fig. 2

a



Number at risk						
CRM-negative group	48	30	22	18	15	10
CRM-positive group	55	20	10	7	6	4

b



Number at risk						
CRM-negative group	48	38	28	23	12	10
CRM-positive group	55	38	18	12	10	6

Fig. 3

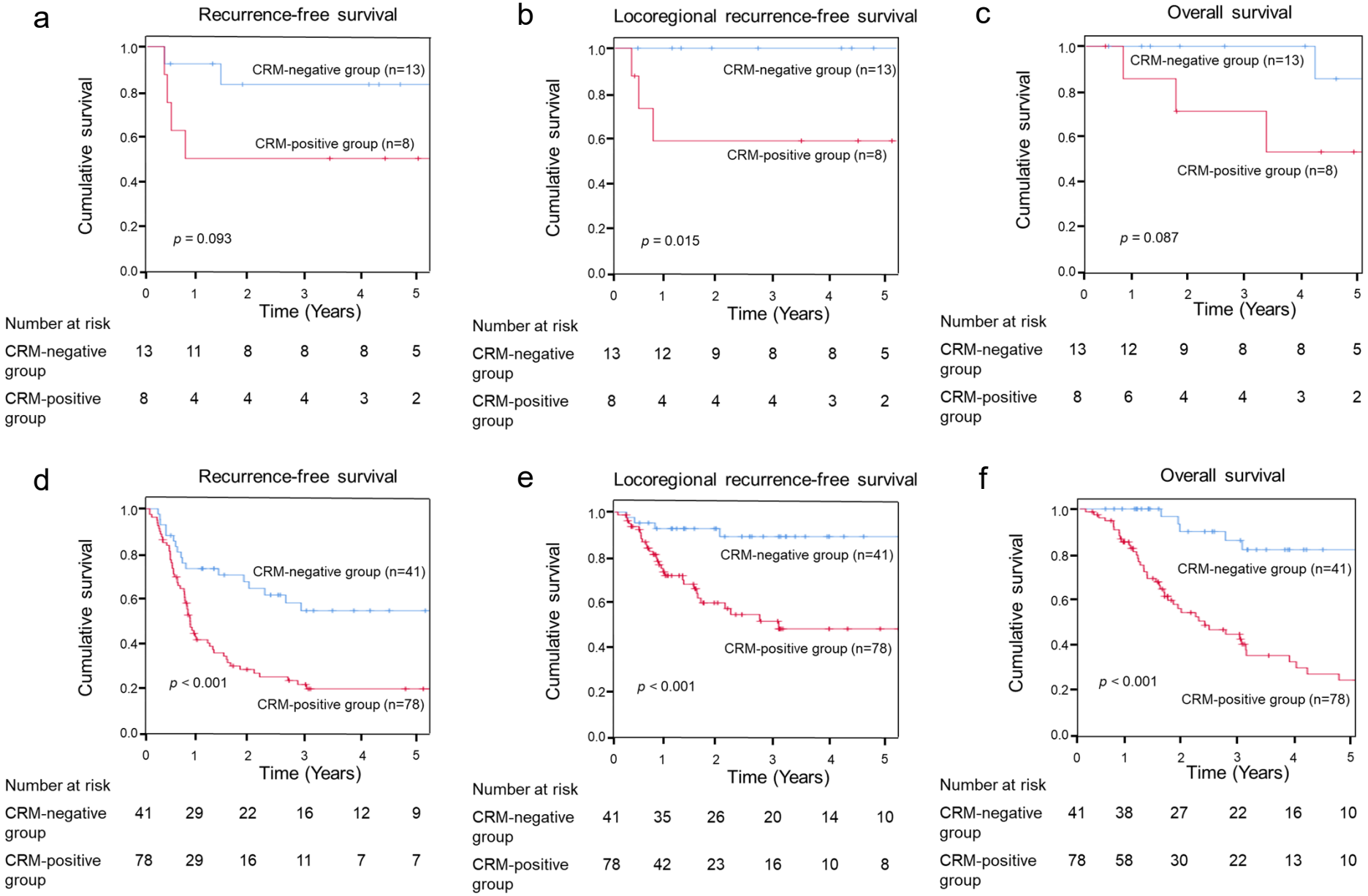

a
Recurrence-free survival
Cumulative survival
CRM-negative group (n=13)
CRM-positive group (n=8)
p = 0.093
Time (Years)
Number at risk
CRM-negative group 13 11 8 8 8 5
CRM-positive group 8 4 4 4 3 2
b
Locoregional recurrence-free survival
Cumulative survival
CRM-negative group (n=13)
CRM-positive group (n=8)
p = 0.015
Time (Years)
Number at risk
CRM-negative group 13 12 9 8 8 5
CRM-positive group 8 4 4 4 3 2
c
Overall survival
Cumulative survival
CRM-negative group (n=13)
CRM-positive group (n=8)
p = 0.087
Time (Years)
Number at risk
CRM-negative group 13 12 9 8 8 5
CRM-positive group 8 6 4 4 3 2
d
Recurrence-free survival
Cumulative survival
CRM-negative group (n=41)
p < 0.001
CRM-positive group (n=78)
Time (Years)
Number at risk
CRM-negative group 41 29 22 16 12 9
CRM-positive group 78 29 16 11 7 7
e
Locoregional recurrence-free survival
Cumulative survival
CRM-negative group (n=41)
CRM-positive group (n=78)
p < 0.001
Time (Years)
Number at risk
CRM-negative group 41 35 26 20 14 10
CRM-positive group 78 42 23 16 10 8
f
Overall survival
Cumulative survival
CRM-negative group (n=41)
p < 0.001
CRM-positive group (n=78)
Time (Years)
Number at risk
CRM-negative group 41 38 27 22 16 10
CRM-positive group 78 58 30 22 13 10

Table 1 Comparison of pathological findings between the CRM-negative and CRM-positive groups in the two cohort

	Development cohort			Validation cohort		
	CRM-negative group n = 20	CRM-positive group n = 53	*p* value	CRM-negative group n = 46	CRM-positive group n = 53	*p* value
Tumor diameter, mm †	37.5 (25.0–70.0)	45 (23.0–150.0)	0.078	48.0 (4.0-95.0)	45.0 (25.0-120.0)	0.679
INF (%)			0.766			0.022
A	0 (0.0%)	2 (3.8%)		2 (4.3%)	0 (0.0%)	
B	20 (100.0%)	48 (90.6%)		41 (89.1%)	41 (77.4%)	
C	0 (0.0%)	3 (5.7%)		3 (6.5%)	12 (22.6%)	
Lymphatic vessel infiltration (%)			0.060			0.014
(-)	6 (30.0%)	5 (9.4%)		26 (56.5%)	16 (30.2%)	
(+)	14 (70.0%)	48 (90.6%)		20 (43.5%)	37 (69.8%)	
Blood vessel infiltration (%)			0.072			0.016
(-)	2 (10.0%)	0 (0.0%)		16 (34.8%)	7 (13.2%)	
(+)	18 (90.0%)	53 (100.0%)		30 (65.2%)	46 (86.8%)	
Pathological N status (%)			0.173			0.105
pN0	5 (25.0%)	5 (9.4%)		13 (28.3%)	7 (13.2%)	
pN1	6 (30.0%)	10 (18.9%)		18 (39.1%)	18 (34.0%)	
pN2	5 (25.0%)	18 (34.0%)		12 (26.1%)	18 (34.0%)	
pN3	4 (20.0%)	20 (37.7%)		3 (6.5%)	10 (18.8%)	
Pathological stage, TNM 8th (%)			0.227			0.186
Stage IIB	5 (25.0%)	5 (9.4%)		13 (28.3%)	7 (13.2%)	

Stage IIIB	10 (50.0%)	25 (47.2%)		29 (63.0%)	36 (67.9%)	
Stage IVA	4 (20.0%)	13 (24.5%)		3 (6.5%)	8 (15.1%)	
Stage IVB	1 (5.0%)	10 (18.9%)		1 (2.2%)	2 (3.8%)	
Initial recurrent site* (%)						
Locoregional recurrence	1 (5.0%)	22 (41.5%)	0.002	4 (8.7%)	17 (32.1%)	0.006
Regional LN recurrence	3 (15.0%)	19 (35.8%)	0.096	9 (19.6%)	7 (13.2%)	0.424
Distant recurrence	6 (30.0%)	22 (41.5%)	0.428	8 (17.4%)	25 (47.2%)	0.003

†Values were presented as median (range).

* Multiple sites of recurrence existed in some patients.

CRM, circumferential resection margin; INF, infiltrative growth; LN, lymph node

Table 2 Prognostic factors for poor recurrence-free survival in the two cohorts

Development cohort

	Univariate analysis			Multivariate analysis		
	HR	*p* value	95% CI	HR	*p* value	95% CI
Age	1.001	0.949	0.968–1.035			
Anastomotic leakage, +	1.134	0.819	0.385–3.338			
Pneumonia, +	1.263	0.621	0.500–3.190			
Lymphatic vessel infiltration, +	2.318	0.109	0.828–6.486	1.250	0.689	0.419–3.733
Blood vessel infiltration, +	0.439	0.441	0.054–3.561			
CRM, positive	3.014	0.008	1.338–6.792	2.482	0.037	1.056–5.832
pStage, III+IV	3.367	0.043	1.039–10.912	2.535	0.130	0.761–8.442

Validation cohort

	Univariate analysis			Multivariate analysis		
	HR	*p* value	95% CI	HR	*p* value	95% CI
Age	0.990	0.548	0.957–1.023			
Anastomotic leakage, +	1.231	0.553	0.619–2.446			
Pneumonia, +	1.265	0.418	0.716–2.233			
INF, c	1.527	0.235	0.759–3.073			
Lymphatic vessel infiltration, +	2.436	0.002	1.374–4.319	1.751	0.065	0.965–3.178
Blood vessel infiltration, +	2.045	0.034	1.056–3.960	1.328	0.427	0.659–2.676
CRM, positive	3.167	<0.001	1.778–5.644	2.695	0.001	1.492–4.867

pStage, III+IV	3.031	0.018	1.209–7.600	2.294	0.083	0.898–5.862

CI, confidence interval; CRM, circumferential resection margin; HR, hazard ratio; INF, infiltrative growth; pStage, pathological stage

Table 3 Comparison of the predictive values of positive CRM for poor recurrence-free survival among three different criteria in the validation cohort

	No. of CRM-positive patients (%)	Univariate analysis		
		HR	*p* value	95% CI
RCP criteria (cut-off, 1000 μm)	60 (60.6%)	2.928	0.003	1.453–5.902
Our criteria (cut-off, 600 μm)	53 (53.5%)	3.167	<0.001	1.778–5.644
CAP criteria (cut-off, 0 μm)	15 (15.2%)	2.236	0.023	1.117–4.475

CAP, College of American Pathologists; CI, confidence interval; CRM, circumferential resection margin; HR, hazard ratio; RCP, Royal College of Pathologists

Table 4 Relationship between our criteria and recurrent site adjusted for neoadjuvant chemotherapy and pathological N status

No neoadjuvant chemotherapy						
	pN0			pN1–3		
	CRM-negative group n = 5	CRM-positive group n = 5	*p* value	CRM-negative group n = 7	CRM-positive group n = 15	*p* value
Initial recurrent site* (%)						
Locoregional recurrence	0 (0.0%)	2 (40.0%)	0.444	1 (14.3%)	3 (20.0%)	1.000
Regional LN recurrence	1 (20.0%)	1 (20.0%)	1.000	3 (42.9%)	5 (33.3%)	1.000
Distant recurrence	0 (0.0%)	0 (0.0%)	1.000	1 (14.3%)	6 (40.0%)	0.350

Neoadjuvant chemotherapy						
	pN0			pN1–3		
	CRM-negative group n = 13	CRM-positive group n = 8	*p* value	CRM-negative group n = 41	CRM-positive group n = 78	*p* value
Initial recurrent site* (%)						
Locoregional recurrence	0 (0.0%)	3 (37.5%)	0.042	4 (9.8%)	31 (39.7%)	0.001
Regional LN recurrence	1 (7.7%)	2 (25.0%)	0.531	7 (17.1%)	18 (23.1%)	0.488
Distant recurrence	1 (7.7%)	2 (25.0%)	0.531	13 (31.7%)	38 (48.7%)	0.083

*Multiple sites of recurrence existed in some patients

CRM, circumferential resection margin; LN, lymph node; pN, pathological N

Supplementary Table 1 Patient characteristics

	Development cohort			Validation cohort		
	CRM-negative group n = 20	CRM-positive group n = 53	*p* value	CRM-negative group n = 46	CRM-positive group n = 53	*p* value
Age (median, years) †	68 (50-76)	68 (40-82)	0.867	68 (36–79)	70 (35–80)	0.771
Sex (%)			1.000			0.798
Male	18 (90.0%)	47 (88.7%)		37 (80.4%)	44 (83.0%)	
Female	2 (10.0%)	6 (11.3%)		9 (19.6%)	9 (17.0%)	
Smoker (%)	17 (85.0%)	48 (90.6%)	0.676	45 (97.8%)	52 (98.1%)	1.000
Drinker (%)	19 (95.0%)	51 (96.2%)	1.000	44 (95.7%)	49 (92.5%)	0.683
Tumor location (%)			0.094			0.481
Ut	1 (5.0%)	4 (7.5%)		4 (8.7%)	5 (9.4%)	
Mt	7 (35.0%)	32 (60.4%)		24 (52.2%)	21 (39.6%)	
Lt	12 (60.0%)	17 (32.1%)		18 (39.1%)	27 (50.9%)	
Macroscopic classification (%)			0.823			0.671
Type1	2 (10.0%)	7 (13.2%)		5 (10.9%)	3 (5.7%)	
Type2	9 (45.0%)	18 (34.0%)		26 (56.5%)	32 (60.4%)	
Type3	9 (45.0%)	27 (50.9%)		15 (32.6%)	18 (34.0%)	
Type4	0 (0.0%)	1 (1.9%)		0 (0.0%)	0 (0.0%)	
Histological differentiation (%)			0.164			0.040
poorly	1 (5.0%)	12 (22.6%)		7 (15.2%)	20 (37.7%)	

moderate	10 (50.0%)	18 (34.0%)		29 (63.0%)	26 (49.1%)	
well	9 (45.0%)	23 (43.4%)		10 (21.7%)	7 (13.2%)	
Clinical Stage, TNM 8th (%)			0.327			0.617
Stage I	1 (5.0%)	0 (0.0%)		10 (21.7%)	8 (15.1%)	
Stage II	8 (40.0%)	17 (32.1%)		29 (63.0%)	40 (75.5%)	
Stage III	10 (50.0%)	34 (64.2%)		6 (13.0%)	4 (7.5%)	
Stage IVA	1 (5.0%)	2 (3.8%)		1 (2.2%)	1 (1.9%)	
Preoperative therapy (%)			0.509			0.426
None	5 (25.0%)	9 (17.0%)		6 (13.3%)	11 (20.8%)	
NAC	15 (75.0%)	44 (83.0%)		39 (86.7%)	42 (79.2%)	
Surgical approach (%)			0.792			0.674
Thoracotomy	13 (65.0%)	32 (60.4%)		29 (63.0%)	36 (67.9%)	
Thoracoscopy	7 (35.0%)	21 (39.6%)		17 (37.0%)	17 (32.1%)	
LN dissection (%)			1.000			0.632
2-field	3 (15.0%)	9 (17.0%)		9 (19.6%)	13 (24.5%)	
3-field	17 (85.0%)	44 (83.0%)		37 (80.4%)	40 (75.5%)	
Reconstruction organ (%)			0.676			0.412
Gastric conduit	17 (85.0%)	48 (90.6%)		42 (91.3%)	51 (96.2%)	
Colon conduit	3 (15.0%)	5 (9.4%)		4 (8.7%)	2 (3.8%)	
Operation time (median, min) †	559 (441–763)	558 (347–927)	0.595	423 (316–671)	434 (258–615)	0.388
Postoperative complications (%)						
AL, C-D grade ≥3	2 (10.0%)	9 (17.0%)	0.716	8 (17.4%)	10 (18.9%)	1.000
Pneumonia, C-D grade ≥2	7 (35.0%)	13 (24.5%)	0.390	12 (26.1%)	16 (30.2%)	0.823

SSI, C-D grade ≥3	0 (0.0%)	1 (1.9%)	1.000	4 (8.7%)	5 (9.4%)	1.000

† Values are presented as median (range)

AL; anastomotic leakage; C-D, Clavien-Dindo classification; CRM, circumferential resection margin; LN, lymph node; Lt, lower thoracic esophagus (thoracic esophagus from inferior half between tracheal bifurcation and esophagogastric junction); Mt, middle thoracic esophagus (superior half between tracheal bifurcation and esophagogastric junction); NAC, neoadjuvant chemotherapy; SSI, surgical site infection; Ut, upper thoracic esophagus (from superior margin of the sternum to tracheal bifurcation)

Supplementary Table 2 Clinicopathological factors to predict positive CRM in the validation cohort

	Univariate analysis			Multivariate analysis		
	OR	*p* value	95% CI	OR	*p* value	95% CI
Age	0.998	0.915	0.954－1.043			
Sex, Male	1.189	0.740	0.428－3.305			
Histological differentiation, poorly	3.377	0.015	1.270－8.976	2.647	0.027	1.119－6.263
Tumor location, Ut	1.094	0.899	0.276－4.341			
Macroscopic classification, Type1 and 3	0.352	0.015	0.152－0.813	3.124	0.026	1.148－8.500
NAC, +	0.587	0.337	0.198－1.740			
Thoracic approach, Thoracoscopy	1.241	0.610	0.541－2.851			

CRM, circumferential resection margin; CI, confidence intervals; NAC, Neoadjuvant chemotherapy; OR, Odds ratio; Ut, upper thoracic esophagus (from superior margin of the sternum to tracheal bifurcation)

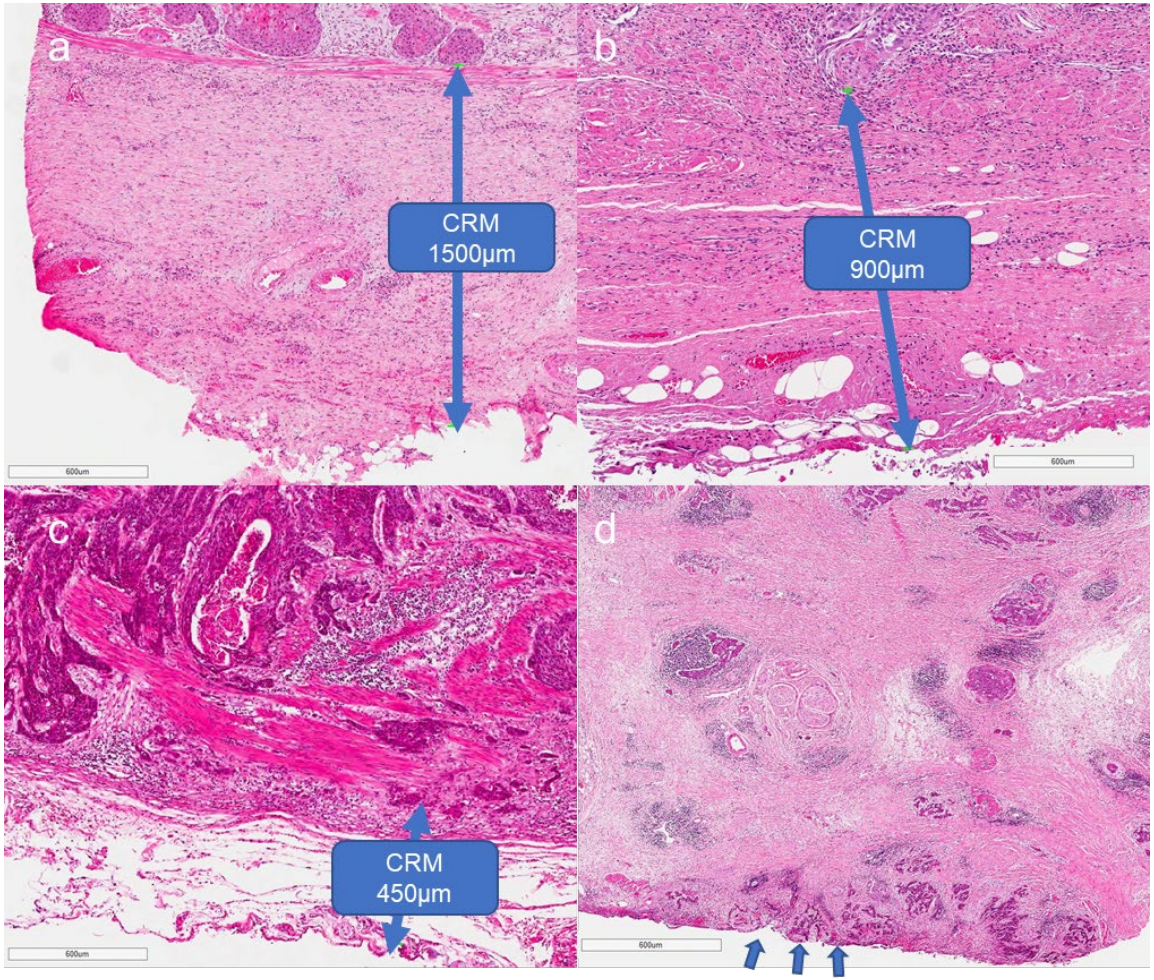
Supplementary Table 3 Comparison between the RCP and CAP criteria in the validation cohort

	The RCP criteria			The CAP criteria		
	CRM-negative group n = 39	CRM-positive group n = 60	*p* value	CRM-negative group n = 84	CRM-positive group n = 15	*p* value
Initial recurrent site* (%)						
Locoregional recurrence	3 (7.7%)	18 (30.0%)	0.011	15 (17.9%)	6 (40.0%)	0.082
Regional LN recurrence	6 (15.4%)	10 (16.7%)	1.000	14 (16.7%)	2 (13.3%)	1.000
Distant recurrence	8 (20.5%)	25 (41.7%)	0.032	23 (27.4%)	10 (66.7%)	0.006

*Multiple sites of recurrence existed in some patients

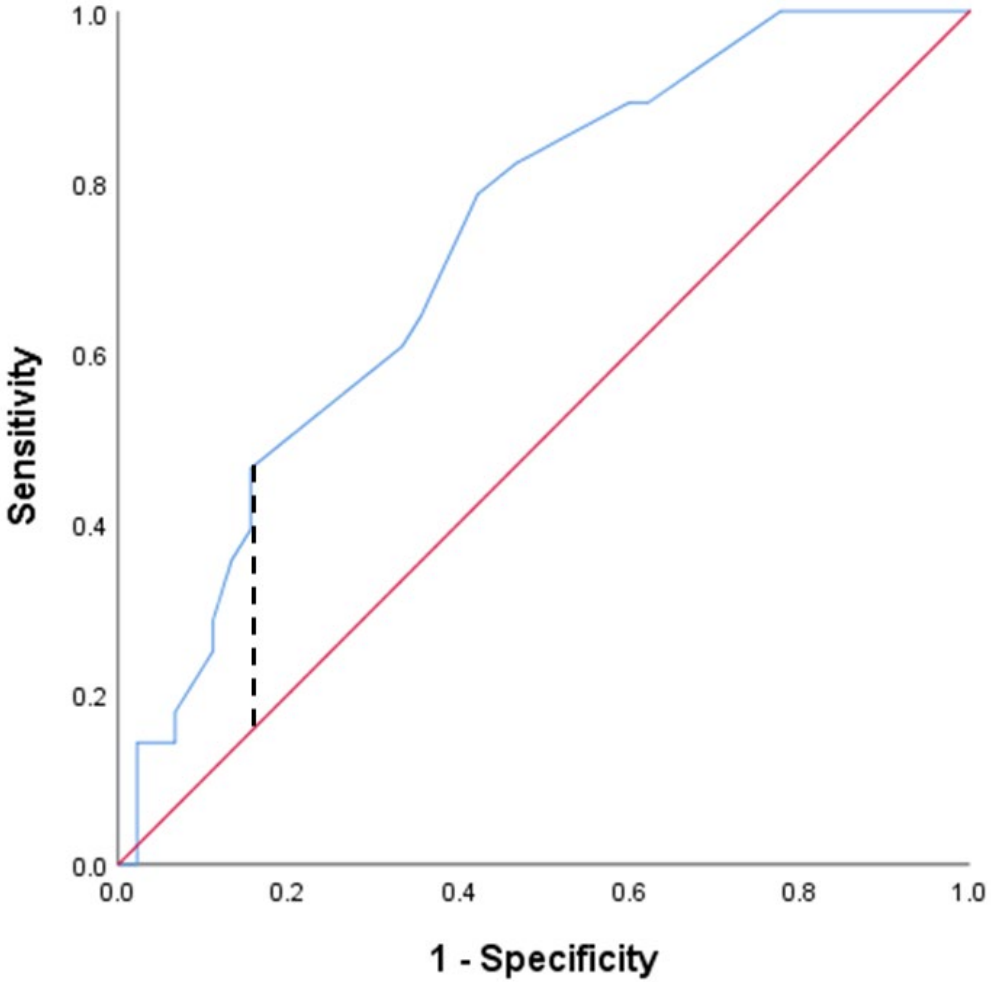
CAP, College of American Pathologists; CRM, circumferential resection margin; LN, lymph node; RCP, Royal College of Pathologists

Supplementary Fig. 1



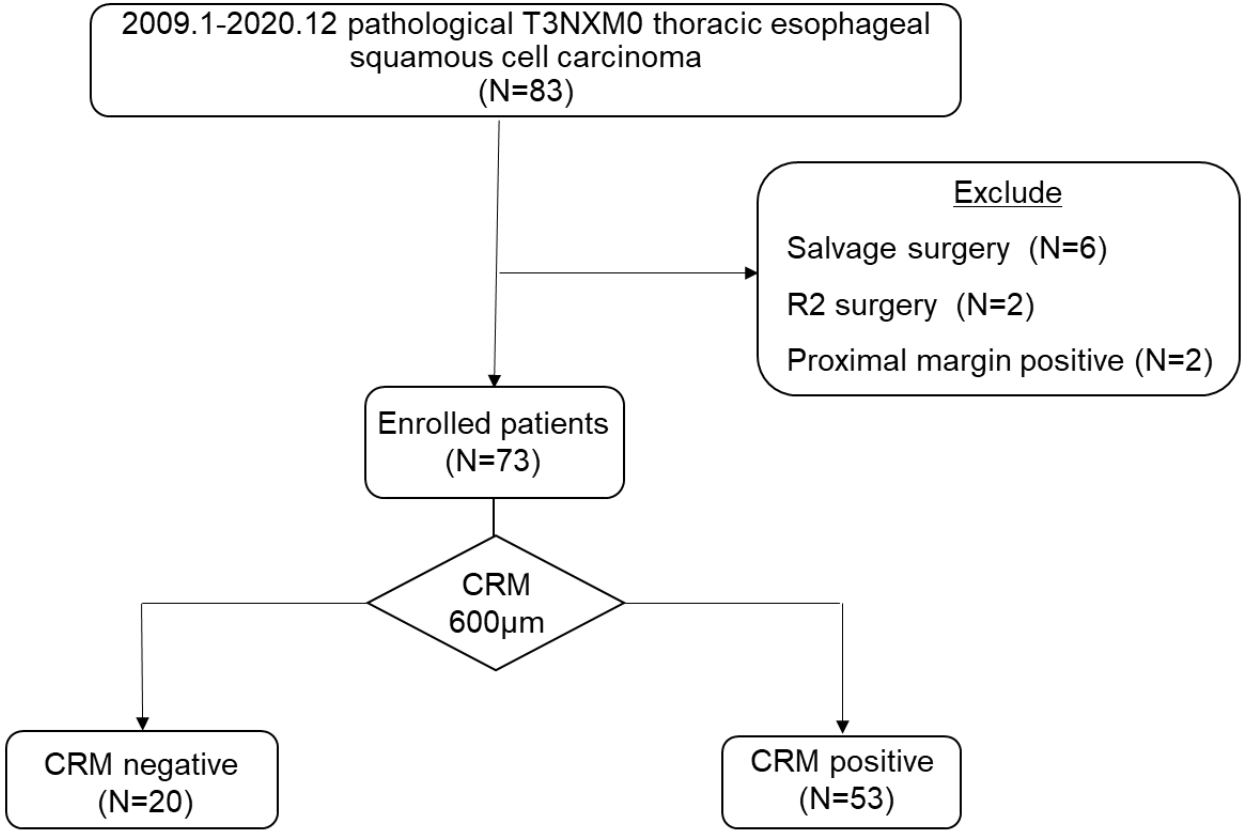
Supplementary Fig. 1 Microscopic measurement of the circumferential resection margin (CRM) of the resected specimens which were stained with hematoxylin and eosin. **a**. CRM > 1000 μm **b**. CRM between 600 and 1000 μm **c**. CRM between 0 and 600 μm **d**. CRM 0 μm.

Supplementary Fig. 2



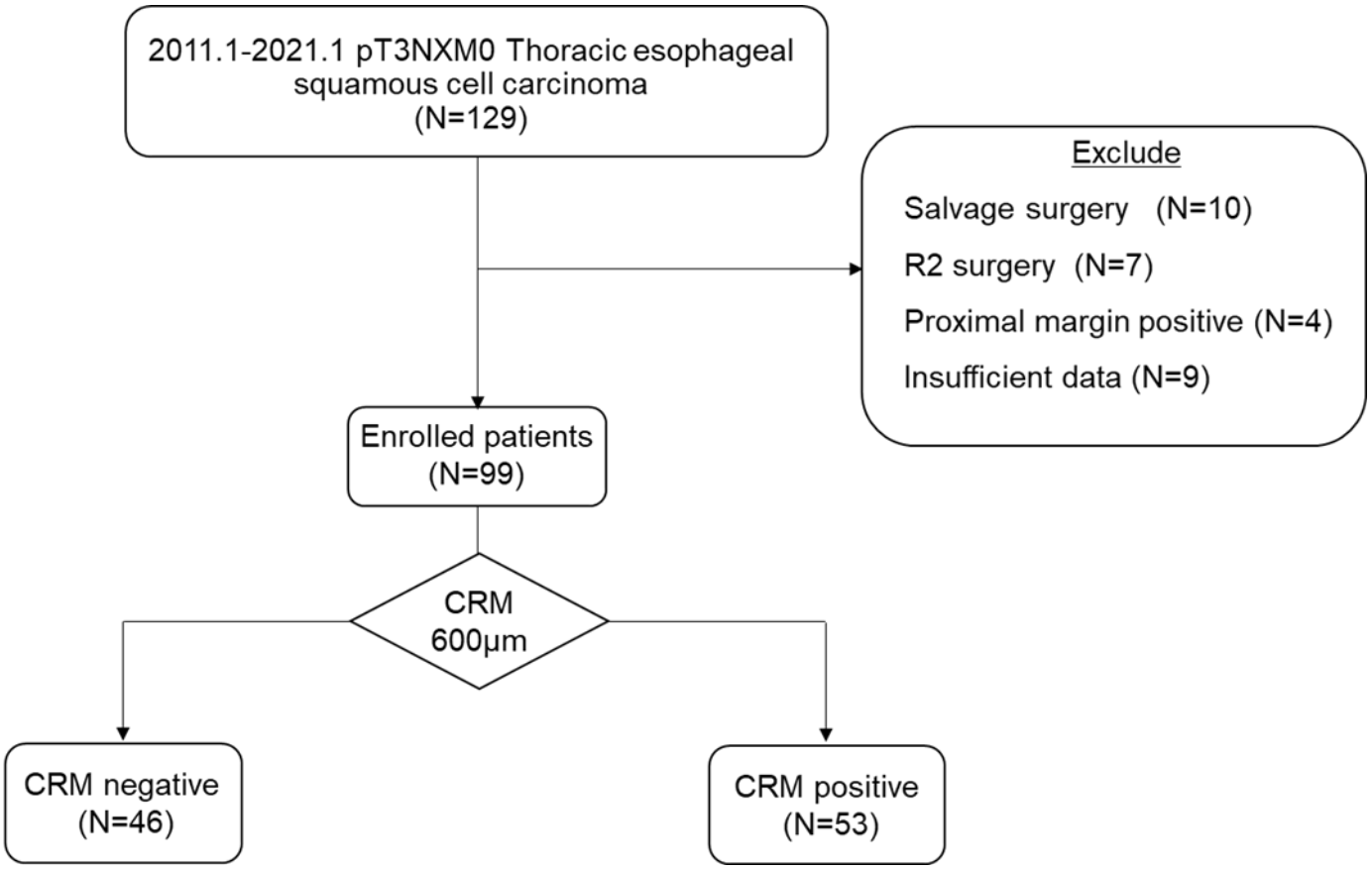
Supplementary Fig. 2 The receiver operating characteristic (ROC) curve of circumferential resection margin for predicting recurrence in the development cohort. The area under the curve was 0.727. The cut-off value was set at 600 μm (sensitivity = 0.464, specificity = 0.844; Youden index = 0.308, dashed line)

Supplementary Fig. 3



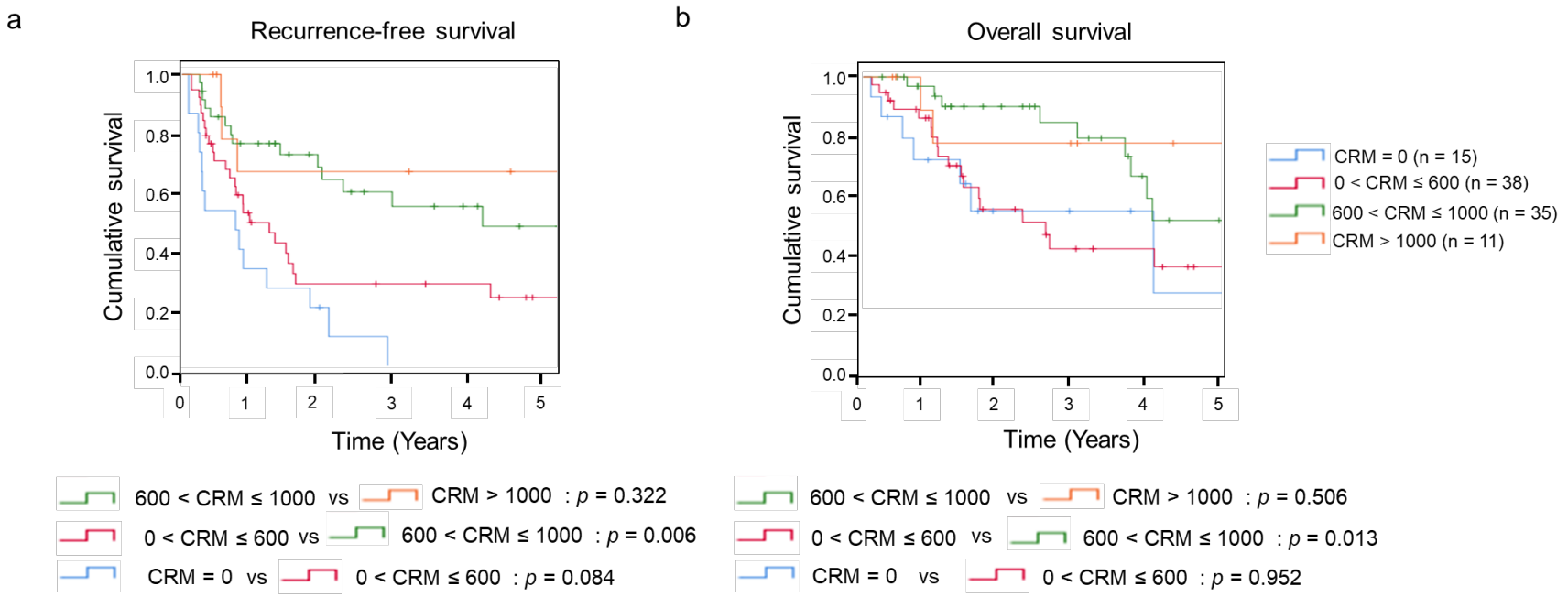
Supplementary Fig. 3 The study flow diagram of the development cohort. CRM, circumferential resection margin.

Supplementary Fig. 4



Supplementary Fig. 4 The study flow diagram of the validation cohort. CRM, circumferential resection margin.

Supplementary Fig. 5



Supplementary Fig. 5 Survival impact according to each cut-off value of CRM in the validation cohort. **a.** Recurrence-free survival. **b.** Overall survival.