The prognostic significance of the positive circumferential resection margin in pathological T3 squamous cell carcinoma of the esophagus with or without neoadjuvant chemotherapy

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A short running head:

Prognostic significance of CRM+ ESCC

Key words: circumferential margin; squamous cell carcinoma; esophageal cancer; esophagectomy; neo-adjuvant chemotherapy.

Abstract

Background: To improve the therapeutic strategy for esophageal squamous cell carcinoma (ESCC), combined neoadjuvant chemotherapy (NAC) followed by surgical resection has been recently applied to patients at clinical stages II/III. Our study aimed to elucidate the impact of the circumferential resection margin (CRM) status of surgically resected specimens on the prognosis of patients undergoing neoadjuvant therapy.

Methods: We enrolled 160 consecutive ESCC patients who underwent esophagectomy. The CRM status of specimens obtained was pathologically examined according to both the College of American Pathologists (CAP) and the Royal College of Pathologists (RCP) criteria. We examined the relationship between CRM status and several clinicopathological factors among ESCC patients with or without NAC.

Results: The local recurrence rate was significantly higher in patients with R1 compared with that of patients with R0 according to CAP criteria (12.5% vs 0.7%; $P = 0.02$; chi-square test). Regarding the prognosis of all patients, the Kaplan-Meier analyses showed that there were significant differences between R0 and R1 groups by CAP or RCP criteria (CAP: $P < 0.001$; RCP: $P = 0.017$). Additionally, the Kaplan-Meier analyses showed that R1 was a significant prognostic factor for poor survival, judged by CAP criteria in both surgery alone ($P < 0.001$) and NAC plus surgery subgroups ($P < 0.001$).

Conclusions: Positive CRM according to CAP criteria after multimodality treatment significantly affects the overall and relapse-free survival of ESCC patients.

Introduction

Although it is widely accepted that exposure of cancer cells at the proximal or distal margin of surgically resected specimens possibly increases the risk of local recurrence,[1 2 3] the influence of positive circumferential resection margin (CRM) status on patient prognosis remains unclear. Previous studies have demonstrated that positive CRM status of surgically resected specimens is a trigger of systematic tumor propagation based on indirect evidence of the dissemination and micrometastases into bone marrow frequently observed in cases of esophageal cancer.[4 5] Surgical resection was the first choice of curative treatment for esophageal cancer until the emergence of radiotherapy and chemotherapy. Therefore, clinicopathological characteristics were examined using surgically resected specimens that were untreated. Some studies have reported that several clinicopathological characteristics, including pathological stage, lymph node metastases, lymphovascular invasion, and CRM status are associated with prognosis.[6 7] Among them, the CRM status, R1, has been reported to be independently associated with poor prognosis for esophageal cancer.[7] Thus far, the CRM status of surgically resected specimens has been examined mainly by the Royal College of Pathologists (RCP) criteria and College of American Pathologists (CAP) criteria. The RCP criteria defines CRM as positive when cancer cells are observed within 1 mm of the resection margin.[8] In contrast, the CAP defines CRM as positive if cancer cells involve the resection margin.[9] Most of the previous reports on CRM status of esophageal cancer have mainly focused on the histological subtype of adenocarcinoma. As far as we know, no previous studies focused specifically on the relationship between CRM status of the histological subtype of esophageal squamous cell carcinoma (ESCC) by CAP and RCP criteria and patient prognosis. Now In Japan, NAC treatment using using cisplatin plus 5-fluorouracil followed by surgery has been a

standard treatment for the patients with ESCC at clinical stage II/III since 2008 according to the results of randomized trial comparing post-operative CT versus preoperative CT for localized advanced ESCC (Japan Clinical Oncology Group (JCOG) 9907) [10]. However, it had not been established the selection criteria for choosing the therapy and almost of all the patients before 2008 had undergone surgery alone. Therefore, this historical background had an influence on selection for therapy. The current selection criteria for neo-adjuvant chemotherapy were as follows: clinical stage II or III excluding T4 disease; resectable disease; Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; sufficient organ function, therefore, the another factors are not considered for deciding the entry in neo-adjuvant chemotherapy followed by surgery.

To establish this combined therapy of NAC followed by surgery as a standard therapy, it is now time to examine the impact of locoregional control of ESCC on prognosis since it is easily speculated that only NAC using cisplatin plus 5-fluorouracil alone might be insufficient for diminishing all ESCC cells completely, that is, it is expected to resect locoregional tumor completely so that R0 resection was accomplished. As a first step, it is necessary to establish how to examine the CRM status of surgically resected specimen for ESCC, which affects the prognosis of the patients with ESCC.

The aim of this study is to examine the impact of the positive status of CRM according to the criteria including CAP or RCP on the prognosis of the patients who underwent NAC followed by surgery. Both groups are studied.

PATIENTS AND METHODS

Patients

A total of 160 consecutive patients with ESCC were enrolled in this study between 1997 and 2011 at the National Cancer Center Hospital East, including 93 patients with pT3M0 who underwent only surgical resection and 67 patients with ypT3M0 who underwent NAC followed by surgical resection. Their cases were retrospectively analyzed according to the approval from the investigational review board at the National Cancer Center (No. 2013-241). The eligibility criteria were as follows: histologically proven squamous cell carcinoma of the esophagus with pathological T3 (Union Internationale Contre le Cancer [UICC] tumor, node metastasis system [TNM] classification).[11]

Follow-up was complete for all patients (100%) enrolled in the present study. All of the patients enrolled in the present study were followed up until the time of death or at least 3 years after initial treatment. Patient information was updated at 6-month intervals in the first and second year after surgery, and annually thereafter. Chest radiograph, thoracoabdominal computed tomography and endoscopy were performed once or twice a year. If recurrence was suspected, patients underwent positron emission tomography/computed tomography, and endoscopic examination with biopsy.

The modes of recurrence were classified into three patterns as follows: local recurrence, defined as a recurrence at the anastomotic site; lymph node recurrence, defined as lymph node metastases in the mediastinal, abdominal, or cervical area; and distant recurrence, defined as hematogenous metastasis with organ tumor formation. In case a patient had multiple recurring lesions, the initial recurring lesion was classified according to the definition as described above. Histologic, cytological, or

unequivocal radiologic proof was required to establish a diagnosis of recurrence, rather than clinical suspicion alone.

Surgical procedure

The operative approach was chosen depending on the patient's physiological condition and tumor characteristics. The open transthoracic or transhiatal approach was performed for 143 and 17 patients, respectively. From 2008 on, minimally invasive thoracoscopic surgery in prone position was performed in only 10 cases. The transthoracic approach was performed in combination with right thoracotomy, laparotomy, or laparoscopy, and a cervical anastomosis. Transhiatal esophagectomy was performed in combination with laparotomy or laparoscopy and cervical anastomosis. All thoracic approaches included a 3-field lymphadenectomy.

Neoadjuvant chemotherapy

Neoadjuvant chemotherapy was performed according to the Japan Clinical Oncology Group clinical practice guidelines, and it comprised two cycles of cisplatin plus 5-fluorouracil, resulting in a total of two courses every 3 weeks. Cisplatin was administered at a dose of 80 mg/m^2 by 2-h intravenous drip infusion on day 1; 5-fluorouracil was administered at a dose of 800 mg/m^2/day by continuous infusion on days 1 through 5. The interval between surgery and chemotherapy tended to be relatively longer than the average 4 to 5 weeks in most patients because the esophagectomy was performed after patients were in good general condition.

Pathological examination

We retrospectively reviewed all pathological records at our institution. The record of each patient was reevaluated and modified by the certified pathologists. All resected ESCC specimens were formalin-fixed and macroscopically examined in

detail. The entire tumors were cut with thickness of 5 mm, including the resected margins of the tumor including proximal, distal and the vertical (circumferential) margins. The specimens were then embedded in paraffin and the thin sections cut with a thickness of 2 to 4 μm from the paraffin-embedded block were stained with hematoxylin and eosin for routine microscopic pathological examination. The proximal and distal margins were defined as the oral and anal edges of the resected specimen, respectively. The minimal distances from the tumor cells at the proximal margin or the distal margin was measured, respectively. The vertical margin was defined as the vertical cut edge of the resected specimen, in a vertical direction to the resected margin. The minimal distance from the tumor cell most closely to the vertical margin was measured. The minimal distances to the proximal or distal margins were measured microscopically in tenths of a millimeter. The minimal distance to the vertical margin was measured in micrometer and to judge the CRM status including R0 or R1 according to the CAP or RCP criteria (Figure 1).

Statistical analysis

Statistical analyses were performed with JMP® 11 (SAS Institute Inc., Cary, NC, USA). Data were reported as frequencies, means, and median with percentages. The chi-square test was used for comparison of categorical variables. Overall survival (OS) curves were plotted by the Kaplan-Meier method. Log rank tests were applied to identify significant differences in survival or recurrence among groups. A *p* value below 0.05 was defined as significant. Overall survival was defined as the period from the date of treatment initiation until the date of confirmation of survival or death regardless of cause of death. Recurrence free survival (RFS) was defined as period from the date of treatment initiation until the date of recurrence confirmation regardless of recurrence

mode. We used the Cox proportional hazards model for multivariable OS and RFS analyses. Variables potentially related to the risk of OS and RFS with *p* value below 0.10 on univariate analysis were included in the multivariate analysis.

RESULTS

Patient and tumor characteristics

In total, 160 consecutive patients with ESCC were enrolled at our institution between 1997 and 2011, including 93 patients who underwent only surgical resection and 67 patients who underwent NAC followed by surgical resection. The pathological stages of patients who underwent surgery alone and those who received neoadjuvant therapy before surgical resection were pT3M0 and ypT3M0, respectively, according to UICC classification (7th edition).[11] Patients had a median age of 68 (interquartile range [IQR], 36–−90) years; 129 were male (80.6%) and 31, female (19.4%). The patients were divided into two groups: the surgery alone group and NAC plus surgery group. The patients' characteristics are presented in Table 1. There was a significantly greater number of older patients (P = 0.0032; chi-square test) and the tumor size was significantly larger (P < 0.001; chi-square test) in the surgery alone group compared with the NAC plus surgery group. Venous vessel infiltration with carcinoma cells was observed in 138 of 160 patients (86.3%). There was a significant difference between the rate of venous vessel infiltration in the surgery alone group and that of the NAC plus surgery group (95.7% vs 73.1%; P < 0.001; chi-square test). However, there was no significant difference in the rates of lymphatic vessel infiltration and pathological N stage between the groups.

Circumferential resection margin status and pattern of recurrence

Relationships between CRM status and pattern of recurrence in all patients are shown in Table 2. Of the 160 patients, 47 and 113 patients were diagnosed as R0 and R1, respectively, according to the RCP criteria. Conversely, 144 and 16 patients were diagnosed as R0 and R1, respectively, according to the CAP criteria for CRM status. Of 160 patients, 73 (45.6%) presented recurrence, and the median time to recurrence was 22.6 months. The patterns of recurrence according to CRM status, judged by RCP and CAP criteria, are presented in Table 2. According to the CAP criteria, the rate of recurrence of patients with CRM status R1 was higher compared with that of patients with CRM status R0 (68.8% vs 43.1%, $P = 0.09$: chi-square test). When comparing between recurrence modes, the local recurrence rate was significantly higher in patients with R1 compared with that of patients with R0 according to the CAP criteria (12.5% vs 0.7%; $P = 0.02$; chi-square test).

Relationships between CRM status and patterns of recurrence in each subgroup are shown in the below. According to the RCP criteria, 25 (26.9%) and 68 (73.1%) patients in the surgery alone group were diagnosed as R0 and R1, respectively. Conversely, according to the CAP criteria, 80 (86.0%) and 13 (14.0%) patients were diagnosed as R0 and R1, respectively. In the NAC plus surgery group, 22 (32.8%) and 45 (67.2%) patients were diagnosed as R0 and R1, respectively, according to the RCP criteria. Conversely, according to the CAP criteria, 64 (95.5%) and 3 (4.5%) patients were diagnosed as R0 and R1, respectively. There was no significant difference in the population according to CRM status, R0 and R1, between the groups. In the surgery-alone group, 40 of 93 patients (43.0%) developed recurrent disease. Local recurrence, lymph node metastases, and distant organ metastases were recognized in 3 (7.5%), 20 (50.0%), and 17 (42.5%) patients, respectively. In the

NAC plus surgery group, 32 of 67 patients (47.8%) developed recurrent disease. None of the patients developed local recurrence; however, lymph node metastases and distant organ metastases were observed in 12 (37.5%) and 20 (62.5%) patients, respectively. There was no significant difference in recurrence modes between the groups.

Relationship between CRM status and OS and RFS

The median follow-up interval of all patients was 31.2 months. Median OS of patients who were diagnosed as R0 and R1 according to RCP criteria were 39.6 months and 27.1 months, respectively ($P = 0.017$). The median OS of patients, who were diagnosed as R0 and R1 according to the CAP criteria, were 32.7 months and 8.4 months, respectively ($P < 0.001$). The cumulative survival curves plotted by the Kaplan-Meier method are shown in Figure 2. The OS of patients diagnosed as R1 was significantly shorter compared with that of patients diagnosed as R0, according to either RCP or CAP criteria used ($P = 0.017$ and $P<0.001$, respectively, log-rank test). The OS and RFS of patients diagnosed as R1 according to the CAP criteria was significantly shorter compared with that of patients diagnosed as R1 according to the RCP criteria ($P < 0.001$, log-rank test) (Figure.2). Additionally, the multivariate analyses indicated that R1, judged by CAP criteria, was a significantly poor prognostic factor (OS: $P < 0.001$; HR 6.95; 95% CI, 3.47–13.47, RFS: $P < 0.001$; HR 5.73; 95% CI, 2.64–11.65) (Table 3 and 4).

Relationship between CRM status, OS, and RFS

According to the RCP criteria, the 3-year OS rates of patients who were diagnosed as R0 and R1 were 60.0% and 38.2% in the surgery-alone group ($P = 0.036$, log-rank test), respectively, and 68.2% and 62.2% in the NAC plus surgery group, ($P =$

0.32, log-rank test), respectively. According to the CAP criteria, the 3-year OS rates of patients who were diagnosed as R0 and R1 were 50.0% and 7.7% in the surgery alone group ($P < 0.001$, log-rank test), respectively, and 67.2% and 0.0% in the neo-adjuvant group ($P < 0.001$, log-rank test), respectively. The cumulative survival curves plotted by the Kaplan-Meier method are shown in Figure 3. In the surgery alone group, CRM status R1 was associated with significantly shorter OS in patients who were diagnosed according to RCP or CAP ($P = 0.036$ and $P < 0.001$, respectively, log-rank test). In contrast, although R1 according to CAP was associated with significantly shorter RFS, R1 according to RCP was not significantly associated with RFS ($P < 0.001$ and $P = 0.19$, respectively, log-rank test). The multivariate analyses revealed that R1, judged only by CAP criteria, was a significant and independent prognostic factor of poor survival (OS: $P < 0.001$; HR 5.26; 95% CI, 2.37–11.32, RFS: $P < 0.001$; HR 5.53; 95% CI, 2.25–12.83) (Table 3 and 4).

In the NAC plus surgery group, CRM status R1 was associated with significantly shorter OS and RFS in patients diagnosed according to CAP criteria ($P < 0.001$, log-rank test), although there was no significant correlation between the CRM status R1 judged by RCP and shorter OS and RFS (P = 0.32 and $P = 0.094$, respectively, log-rank test) (Figure 4). The multivariate analyses indicated that R1, judged only by CAP criteria, was a significant and independent prognostic factor for poor survival (OS: $P = 0.0066$; HR 10.02; 95% CI, 2.10–37.40, RFS: $P = 0.068$; HR 5.74; 95% CI, 0.85–23.80) (Table 3 and 4). CRM status according to CAP criteria was associated with OS and RFS in both treatment subgroups, whereas CRM status according to RCP criteria was not associated with either OS or RFS.

DISCUSSION

A positive CRM status after radical surgery for multiple cancers has been proposed as an important prognostic factor for survival. The CRM status has been established as a risk factor for survival in rectal cancer.[12] In rectal cancer, RCP criteria are the standard criteria for evaluating CRM status, which is established by clinicopathological examination of rectal cancer specimens. According to the TNM classification, a positive margin (R1) is 0 mm in rectal cancer, meaning that cancer cells are clearly exposed in the resected margin. In contrast, CRM is considered positive within 1 mm of the resection margin, and it is used as a prognostic indicator for local recurrence.[13]

The incision end-line is easily determined in the resection of rectal cancer because there is abundant connective tissue in the rectal area. Additionally, there is an anatomical marker that is useful for determining the incision end-line, which consists of the mesorectal Denonvilliers' fascia in the pelvis. However, the esophagus lacks such an anatomic boundary. Therefore, the CRM status is critical for the prognosis of ESCC, and it should be strictly evaluated. The College of American Pathologists criteria are useful for evaluating CRM in ESCC.

The importance of the CRM status after esophagectomy has been discussed for decades, but it remains controversial. The first study on CRM in esophageal cancer was published by Sagar et al. in 1993. Sagar et mentioned the possible association of a higher local recurrence rate and CRM involvement.[14] In 2001, Dexter et al. reported the first large-scale study on the impact of CRM involvement on OS.[15] However, these studies have focused mainly on esophageal adenocarcinoma after primary surgery. Further, the CRM status of surgically resected specimens has been examined mainly

by RCP or CAP criteria. Furthermore, many studies reported conflicting results.[16-19] There has been no report on the relationship between the CRM status and the prognosis of patients with ESCC only. In Japan, the CRM criteria are not contemplated in the classification of esophageal cancer. Nevertheless, our study showed that routine pathological assessment of CRM in resected specimens of ESCCs conferred significant information affecting the prognosis of patients with ESCC in addition to other information, except for TNM staging system. The CRM status can only be determined by pathological examination; therefore, the deepest portion of cancer tissue has to be examined pathologically. This study showed the relationship between CRM status according to CAP criteria, OS, and RFS in patients with ESCC. Our study results clearly showed that positive CRM, according to the RCP and CAP criteria, was significantly associated with poor prognosis (Figure 1). Importantly, both univariate and multivariate analysis showed that CAP criteria were significant and independent predictors of poor prognosis regarding OS and RFS in all patients (Table 3 and 4). These results suggest the impact of the positive status of CRM, according to the CAP, on the poor prognosis of patients with ESCC.

In Japan, NAC, comprising cisplatin plus 5-fluorouracil, has been a standard treatment for patients with clinical stages II/III ESCC based on the results of a randomized trial comparing postoperative chemotherapy vs NAC for localized advanced ESCC (JCOG 9907).[10] Thus, it is necessary to examine the influence of CRM status on the survival rate after administering NAC followed by surgical resection as a standard therapy. To the best of our knowledge, this study is the first to describe whether the CAP or RCP criteria are more significant prognostic factors for patients with pure ESCC treated with or without NAC. The CRM status according to CAP

criteria was significantly associated with a poorer prognosis in the NAC plus surgery subgroup. However, there was no significant correlation between the CRM status according to the RCP criteria and OS and RFS. In the previous studies, the RCP criteria were independently predictive of prognosis mainly in patients with esophageal adenocarcinoma.[20-23] Chan DSY et al. reported, in a systematic review and meta-analysis, that a positive CRM, according to the CAP criteria, was an important poor prognostic indicator mainly for patients with esophageal adenocarcinoma who underwent NAC.[24] The relationship between CRM status criteria and prognosis is still controversial, and there is a paucity of reports on the influence of the CRM status on the prognosis of patients with ESCC only treated with or without NAC. Our study results clearly showed that positive CRM, according to the CAP criteria, was significantly associated with poor prognosis in patients with ESCC who underwent NAC followed by surgery (Figure 4).

The CRM status, judged by CAP criteria, was significantly associated with poorer prognosis in both the surgery alone and NAC plus surgery subgroups. In contrast, there was no significant correlation between the CRM status, judged by RCP criteria, and OS and RFS. In conclusion, based on CAP criteria, positive CRM after multimodality treatment affects the OS and RFS of patients with ESCC.

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Figure legends

Figure 1. The formalin-fixed esophagogastric specimen. A. The ulcerative lesion was observed in the thoracic esophagus. B. Longitudinal-sections were made through the whole tumor at a thickness of 3 mm. C. The cut sections of specimen indicate the minimal distance between the tumor and the nearest resected margin macroscopically. D. Microscopic examination defines the circumferential resection margin status according to College of American Pathologists (CAP) or the Royal College of Pathologists (RCP) criteria.

Figure 2. Cumulative overall survival and recurrence free survival curves of all 160 patients with pT3 or ypT3 and were diagnosed as R0 and R1, respectively, according to the College of American Pathologists (CAP) or the Royal College of Pathologists (RCP) criteria

Figure 3. Cumulative overall survival and recurrence free survival curves of the 93 patients with pT3 who underwent surgery alone and were diagnosed as R0 and R1, respectively, according to the College of American Pathologists (CAP) or the Royal College of Pathologists (RCP) criteria.

Figure 4. Cumulative overall survival and recurrence free survival curves in the 67 patients with ypT3 who underwent NAC followed by surgery and diagnosed as R0 and R1, respectively, according to the College of American Pathologists (CAP) or the Royal College of Pathologists (RCP) criteria.

Figure 1

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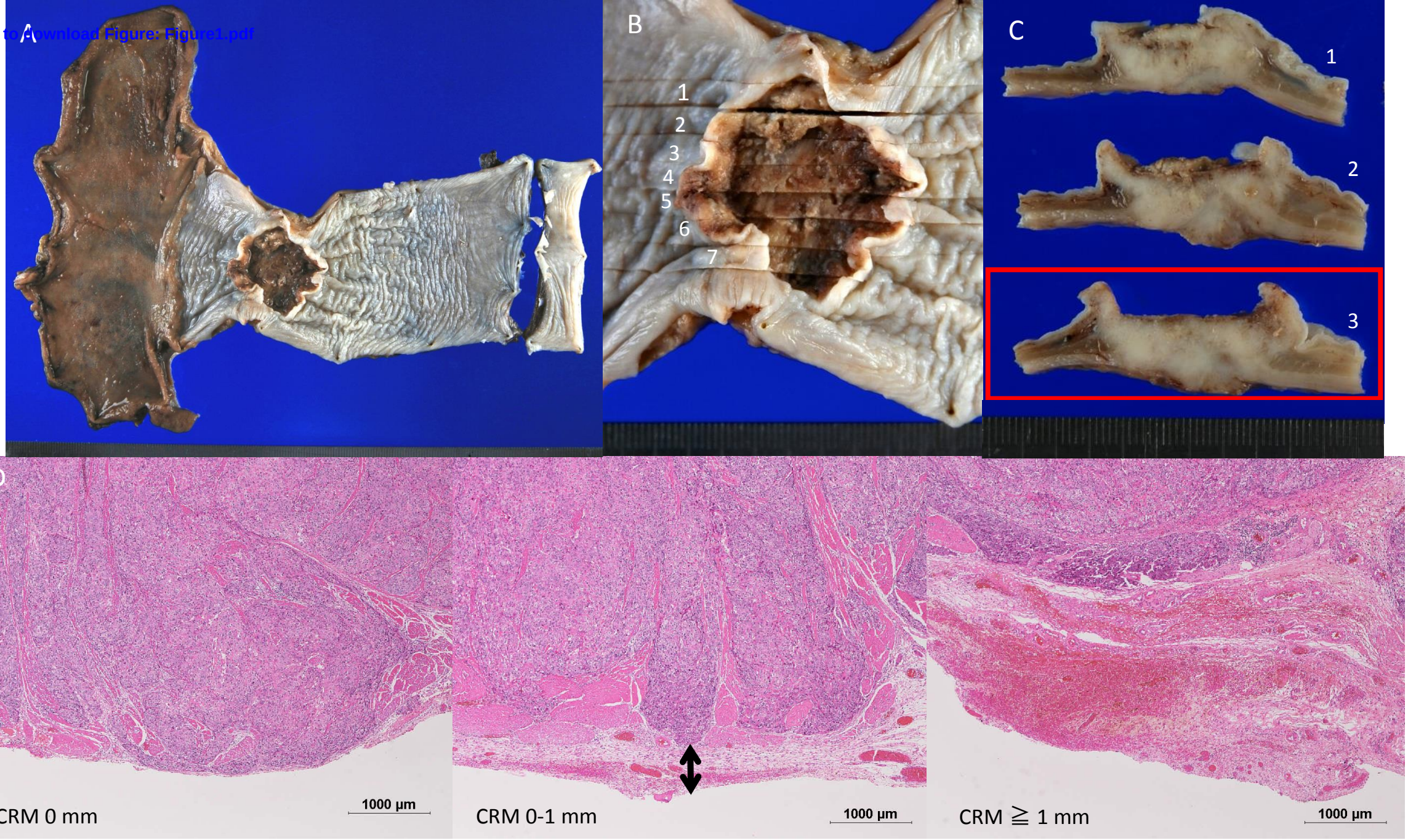

CAP	R1	R0	R0
RCP	R1	R1	R0

Figure.2

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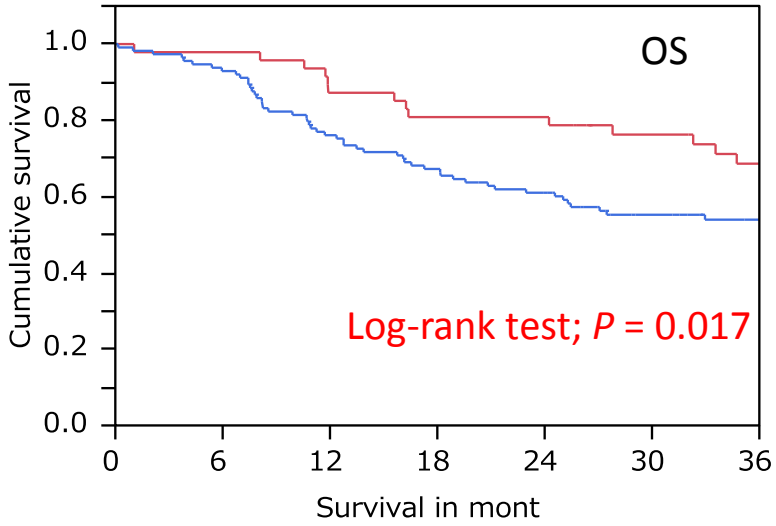
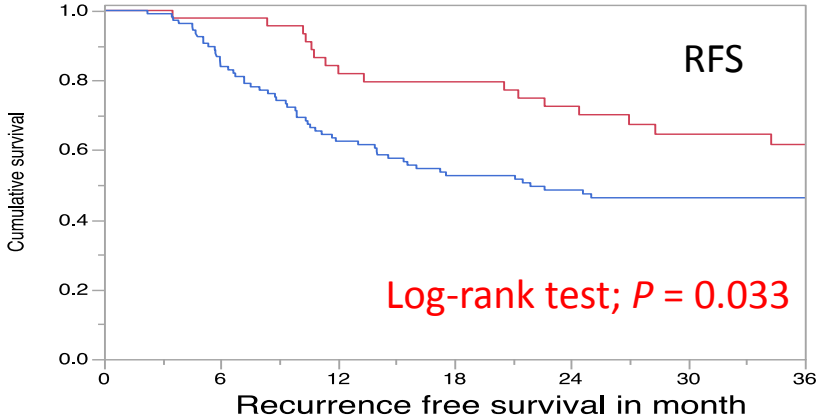
RCP criteria

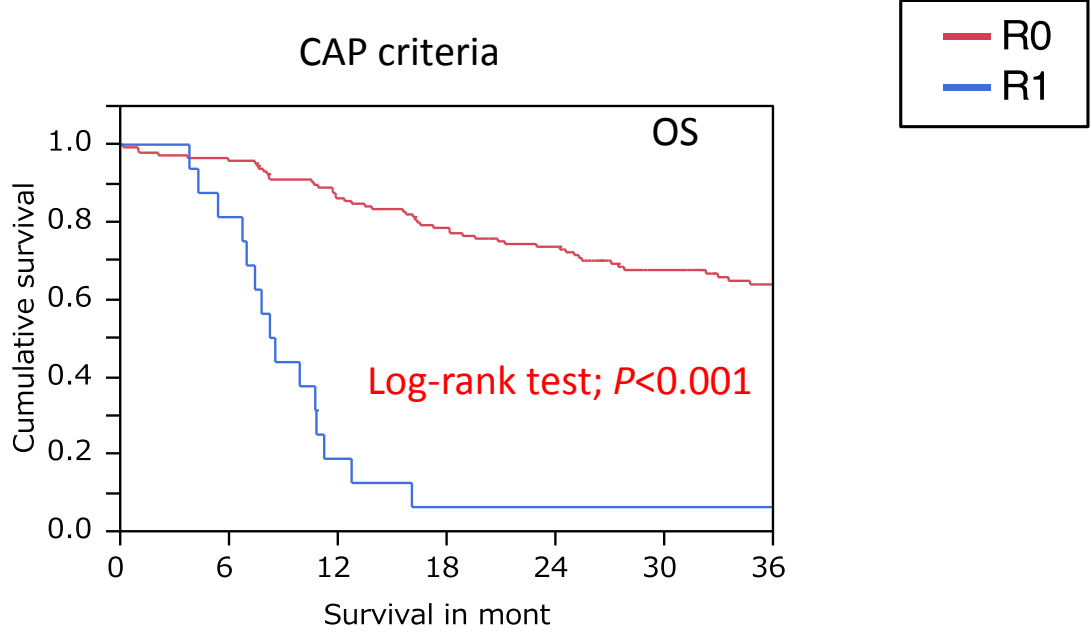


No. at risk	0 months	12 months	24 months	36 months
R0	47	41	38	33
R1	113	73	56	49

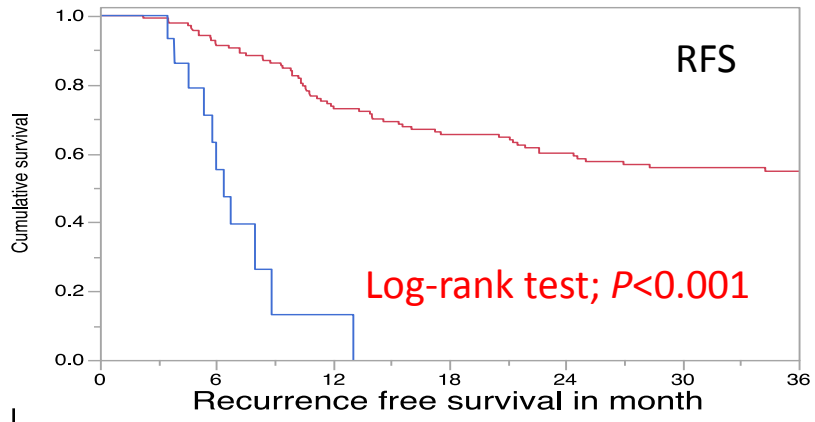


No. at risk	0 months	12 months	24 months	36 months
R0	47	40	35	31
R1	113	74	60	58

CAP criteria



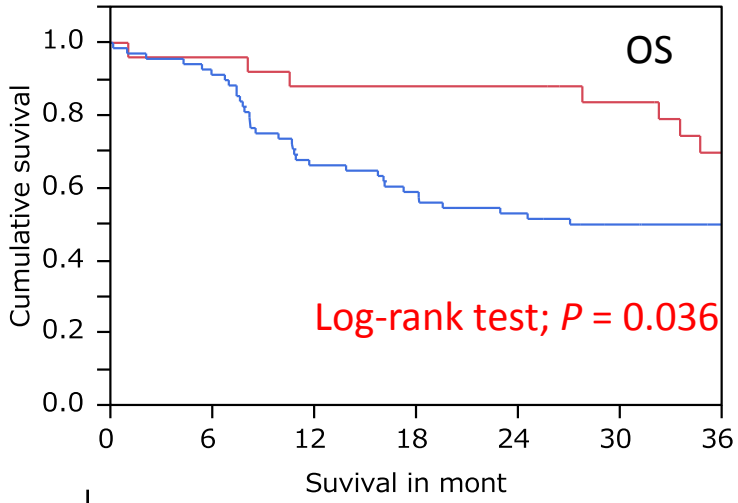
No. at risk	0 months	12 months	24 months	36 months
R0	144	128	110	98
R1	16	3	1	1



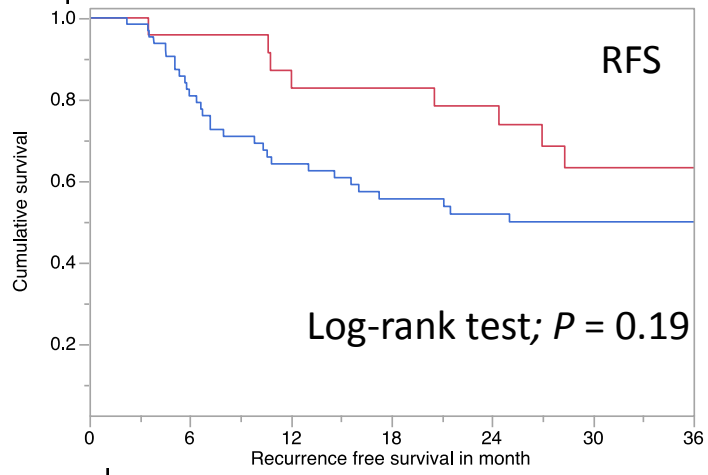
No. at risk	0 months	12 months	24 months	36 months
R0	144	108	90	84
R1	16	6	0	0

Figure.3
Click here to download Figure: Figure3.ppt

RCP criteria

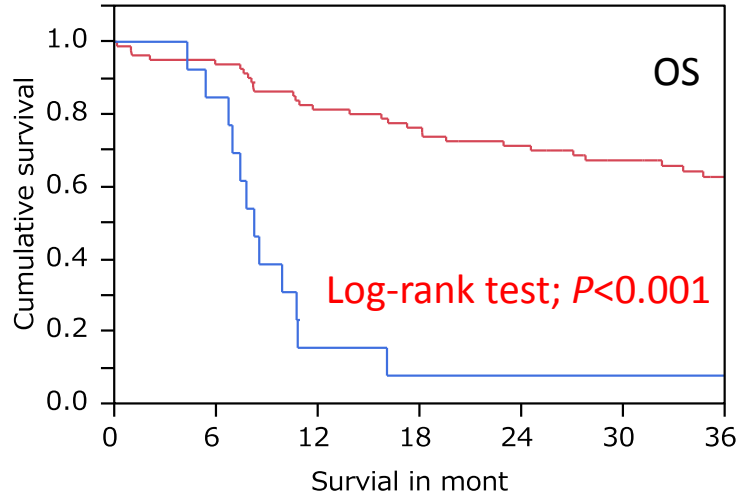


No. at risk	0 months	12 months	24 months	36 months
R0	25	22	22	15
R1	68	45	35	26

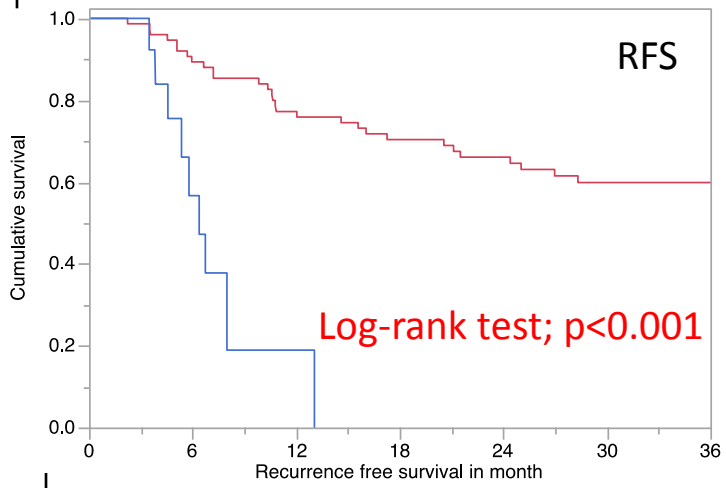


No. at risk	0 months	12 months	24 months	36 months
R0	25	22	20	17
R1	68	46	39	38

CAP criteria



No. at risk	0 months	12 months	24 months	36 months
R0	80	65	56	40
R1	13	2	1	1



No. at risk	0 months	12 months	24 months	36 months
R0	80	63	55	51
R1	13	6	0	0

Figure.4
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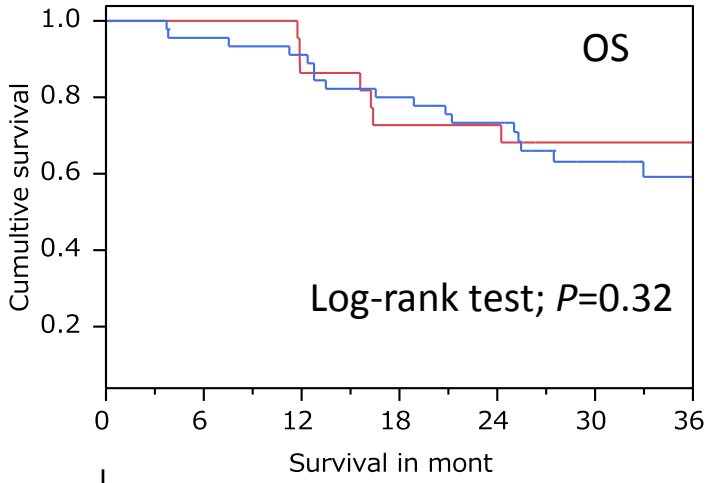
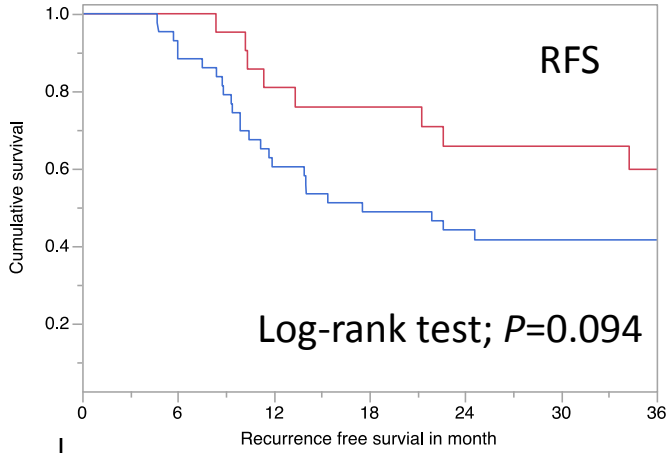
RCP criteria

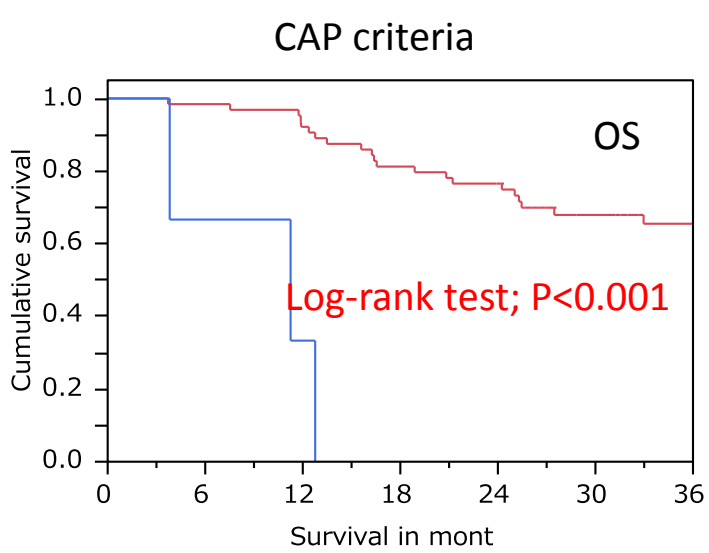


No. at risk	0 months	12 months	24 months	36 months
R0	22	19	16	15
R1	45	41	33	28

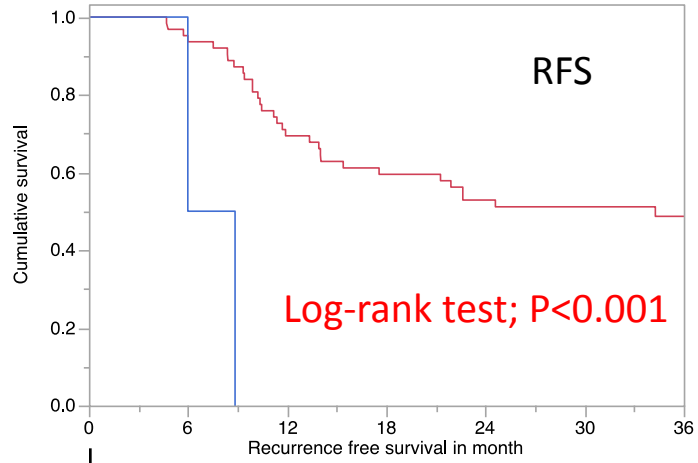


No. at risk	0 months	12 months	24 months	36 months
R0	22	18	15	14
R1	45	28	21	20

CAP criteria



No. at risk	0 months	12 months	24 months	36 months
R0	64	59	49	43
R1	3	1	0	0



No. at risk	0 months	12 months	24 months	36 months
R0	64	45	35	33
R1	3	0	0	0

Table

Table 1. Patients' clinicopathological characteristics

	All (n = 160)	Surgery alone (n = 93)	NAC + Surgery (n = 67)	*P* value
Sex				0.69
Male	129 (80.6)	74 (79.6)	55 (82.1)	
Female	31 (19.4)	19 (20.4)	12 (17.9)	
Age				0.0032
Median (range)	68 (36–90)	70 (42–90)	67 (36–77)	
Localization				
Upper	28 (17.5)	20 (21.5)	8 (11.9)	
Middle	60 (37.5)	26 (28.0)	34 (50.7)	
Lower	72 (45.0)	47 (50.5)	25 (37.3)	
Tumor size				<0.001
Median (range)	50 (14–110)	56 (30-110)	45 (14-90)	
Macroscopic type				0.11
Type1	13 (8.1)	9 (9.7)	4 (5.8)	
Type2	70 (43.8)	45 (48.4)	25 (37.3)	
Type3	65 (40.6)	37 (39.8)	28 (41.8)	
Type4	1 (0.6)	1 (1.1)	0 (0)	
Type5	11 (6.9)	1 (1.1)	10 (14.9)	
Differentiation category				0.68
Well	60 (37.5)	35 (37.6)	25 (37.3)	
Moderately	89 (55.6)	53 (57.0)	36 (53.7)	
Poorly	11 (6.9)	5 (5.4)	6 (9.0)	
Venous vessel infiltration				<0.001
Yes	138 (86.3)	89 (95.7)	49 (73.1)	
No	22 (13.8)	4 (4.3)	18 (22.9)	
Lymphatic vessel infiltration				0.75
Yes	86 (53.7)	49 (52.7)	37 (55.2)	
No	74 (46.3)	44 (47.3)	30 (44.8)	
pN stage				0.27
pN0	58 (36.3)	37 (39.8)	21 (31.3)	

pN1	45 (28.1)	22 (23.7)	23 (34.3)	
pN2	41 (25.6)	26 (28.0)	15 (22.4)	
pN3	16 (10.0)	8 (8.6)	8 (11.9)	
pStage				0.27
pIIA	58 (36.3)	37 (39.8)	21 (31.3)	
pIIIA	45 (28.1)	22 (23.7)	23 (34.3)	
pIIIB	41 (25.6)	26 (28.0)	15 (22.4)	
pIIIC	16 (10.0)	8 (8.6)	8 (11.9)	

Data in the table are presented as n (%), unless otherwise indicated.

Abbreviations: NAC: Neoadjuvant chemotherapy

Table 2. The relationship between CRM status and pattern of the recurrence

	RCP R0 (n = 47)	RCP R1 (n = 113)	P-value	CAP R0 (n = 144)	CAP R1 (n = 16)	P-value
Total recurrence	17 (36.2)	56 (49.6)	0.12	62 (43.1)	11 (68.8)	0.09
Pattern of recurrence						
Local metastasis	0 (0)	3 (5.4)	0.64	1 (0.7	2 (12.5)	0.02
Lymph node metastasis	10 (21.3)	23 (20.4)	0.90	31 (21.5)	2 (12.5)	0.60
Distant metastasis	7 (14.9)	30 (26.5)	0.17	30 (20.8)	7 (43.8)	0.08

Data in the table are presented as n (%). Abbreviations: CRM: Circumferential Resection Margin, RCP: Royal College of Pathologist, CAP: College of American Pathologist

Table 3. The relationship between clinicopathologic parameters and OS by univariate or multivariate analyses

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
All patients (n = 160)				
RCP R1	1.93 (1.14–3.48)	0.014	1.19 (0.67–2.21)	0.57
CAP R1	8.20 (4.31–14.79)	<0.001	6.95 (3.47–13.47)	<0.001
Differentiation category (M/D)	1.52 (0.95–2.48)	0.080	1.17 (0.72–1.94)	0.52
Venous vessel infiltration	2.80 (1.25–7.99)	0.0098	2.11 (0.92–6.12)	0.081
Lymphatic vessel infiltration	2.23 (1.39–3.65)	<0.001	1.31 (0.79–2.22)	0.29
Lymph node metastasis	5.21 (2.80–10.82)	<0.001	4.31 (2.20–9.30)	<0.001
Surgery alone (n = 93)				
RCP R1	2.14 (1.08–4.74)	0.028	1.30 (0.62–2.98)	0.50
CAP R1	6.19 (2.94–12.31)	<0.001	5.26 (2.37–11.32)	<0.001
Differentiation category (M/D)	1.34 (0.75–2.49)	0.33		
Venous vessel infiltration	2.63 (0.57–46.56)	0.26		
Lymphatic vessel infiltration	3.04 (1.66–5.89)	<0.001	1.83 (0.93–3.79)	0.080
Lymph node metastasis	3.86 (1.95–8.55)	<0.001	2.78 (1.30–6.49)	0.0072
NAC + Surgery (n = 67)				
RCP R1	1.55 (0.69–3.93)	0.30		
CAP R1	17.64 (3.72–65.33)	0.0013	10.02 (2.10–37.40)	0.0066
Differentiation category (M/D)	1.81 (0.84–4.21)	0.13		
Venous vessel infiltration	2.94 (1.13–10.05)	0.025	2.56 (0.97–8.81)	0.059
Lymphatic vessel infiltration	1.39 (0.65–3.03)	0.40		
Lymph node metastasis	17.20 (3.66–306.95)	<0.001	15.55 (3.28–278.15)	<0.001

Abbreviations: RCP: Royal College of Pathologist. CAP: College of American Pathologist, M/D: Moderately differentiation category, NAC: Neoadjuvant Chemotherapy, OS: Overall Survival, RFS: Recurrence-free Survival, CI: Confidence Interval

Table 4. The relationship between clinicopathologic parameters and RFS by univariate and multivariate analyses

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
All patients (n = 160)				
RCP R1	1.79 (1.06–3.19)	0.028	1.32 (0.76–2.39)	0.33
CAP R1	8.69 (4.04–17.45)	<0.001	5.73 (2.64–11.65)	<0.001
Differentiation category (M/D)	1.41 (0.88–2.28)	0.16		
Venous vessel infiltration	2.29 (1.08–5.91)	0.030	1.81 (0.84–4.72)	0.14
Lymphatic vessel infiltration	1.81 (1.13–2.93)	0.013	1.11 (0.68–1.84)	0.69
Lymph node metastasis	4.89 (2.67–9.83)	<0.001	4.23 (2.24–8.72)	<0.001
Surgery alone (n=93)				
RCP R1	1.64 (0.81–3.68)	0.17		
CAP R1	7.48 (3.04–17.40)	<0.001	5.53 (2.25–12.83)	<0.001
Differentiation category (M/D)	1.25 (0.66–2.43)	0.50		
Venous vessel infiltration	2.50 (0.54–44.32)	0.29		
Lymphatic vessel infiltration	2.48 (1.30–4.92)	0.0055	1.39 (0.71–2.84)	0.35
Lymph node metastasis	6.95 (2.96–20.33)	<0.001	5.56 (2.27–16.74)	<0.001
NAC + Surgery (n=67)				
RCP R1	1.95 (0.92–4.63)	0.085	1.60 (0.73–3.86)	0.25
CAP R1	10.07 (1.51–40.72)	0.022	5.74 (0.85–23.80)	0.068
Differentiation category (M/D)	1.68 (0.84–3.46)	0.14		
Venous vessel infiltration	2.56 (1.07–7.53)	0.033	2.15 (0.88–6.42)	0.095
Lymphatic vessel infiltration	1.20 (0.60–-2.41)	0.61		
Lymph node metastasis	3.23 (1.42–8.67)	0.004	2.96 (1.29–8.01)	0.009

Abbreviations: RCP: Royal College of Pathologist, CAP: College of American Pathologist, M/D: Moderately differentiation category, NAC: Neoadjuvant Chemotherapy, OS: Overall Survival, RFS: Recurrence-free Survival, CI: Confidence Interval

Supplementary Figure 1

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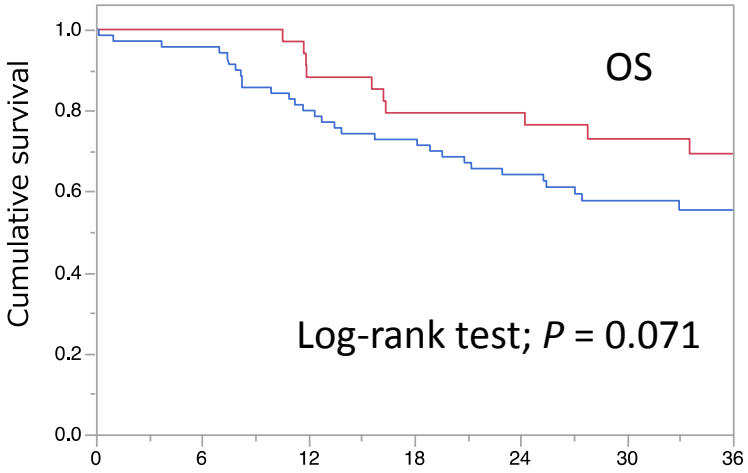
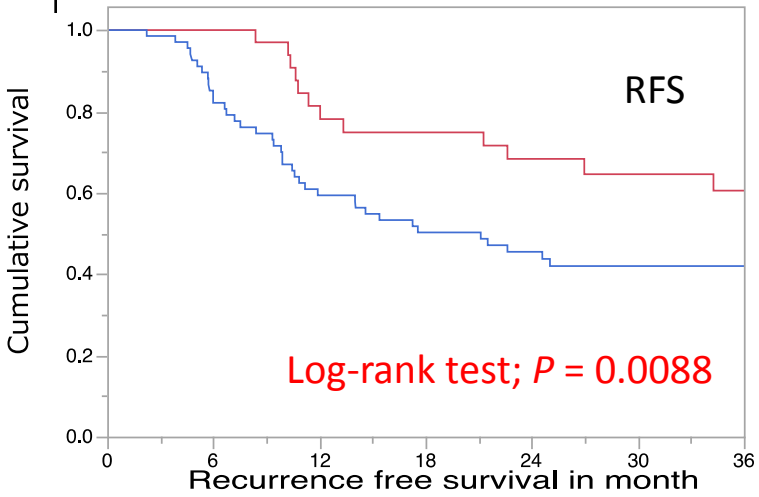
RCP criteria

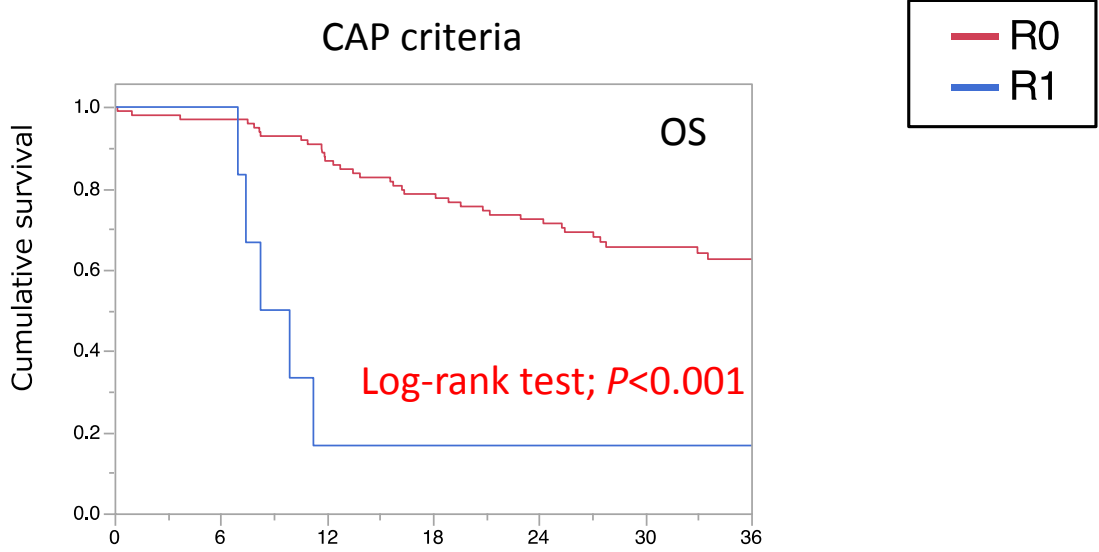


No. at risk	Survival in mont 0 months	12 months	24 months	36 months
R0	34	31	28	27
R1	70	56	45	40

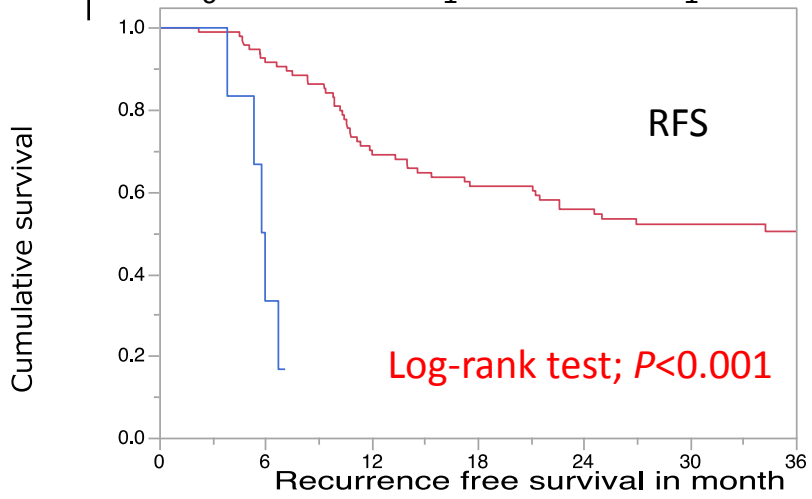


No. at risk	0 months	12 months	24 months	36 months
R0	34	28	25	23
R1	70	43	34	34

CAP criteria



No. at risk	Survival in mont 0 months	12 months	24 months	36 months
R0	98	85	71	63
R1	6	1	1	1



No. at risk	0 months	12 months	24 months	36 months
R0	98	70	57	53
R1	6	1	1	1

Supplementary Figure 2

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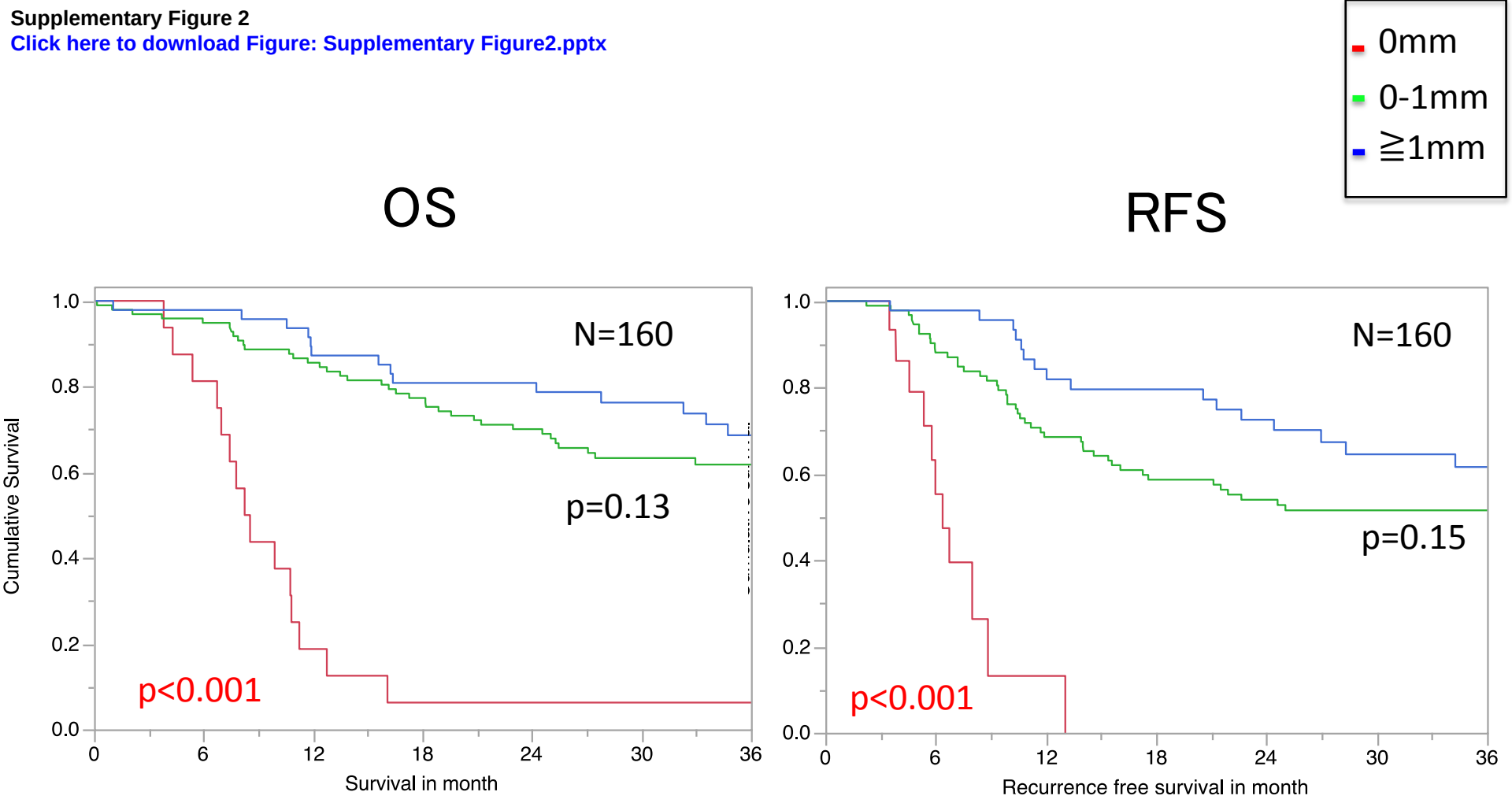

No. at risk	0 months	12 months	24 months	36 months
0mm	16	3	1	1
0-1mm	97	83	68	61
≧1mm	47	41	38	33

No. at risk	0 months	12 months	24 months	36 months
0mm	16	6	5	5
0-1mm	97	68	55	53
≧1mm	47	40	35	31

Supplementary Table 1

Supplementary table1. Patients' clinicopathological characteristics: the overall cohort and PSM cohort

	Overall cohort (n=160)			PSM cohort (n=104)		
	Surgery alone (n = 93)	NAC + Surgery (n = 67)	*P* value	Surgery alone (n = 52)	NAC + Surgery (n = 52)	*P* value
Sex			0.69			0.45
Male	74 (79.6)	55 (82.1)		44 (84.6)	41 (78.9)	
Female	19 (20.4)	12 (17.9)		8 (15.4)	11 (21.1)	
Age			0.0032			0.87
Median (range)	70 (42–90)	67 (36–77)		65 (54–78)	65 (54–77)	
Localization			0.11			0.047
Upper	20 (21.5)	8 (11.9)		10 (19.2)	5 (9.6)	
Middle	26 (28.0)	34 (50.7)		17 (32.7)	27 (51.9)	
Lower	47 (50.5)	25 (37.3)		25 (48.1)	20 (38.5)	
Tumor size			<0.001			<0.001
Median (range)	56 (30-110)	45 (14-90)		54 (30-110)	45 (14-90)	
Macroscopic type			0.11			0.81
Type1	9 (9.7)	4 (5.8)		8 (15.4)	4 (7.7)	
Type2	45 (48.4)	25 (37.3)		24 (46.2)	20 (38.5)	
Type3	37 (39.8)	28 (41.8)		19 (36.6)	22 (42.3)	
Type4	1 (1.1)	0 (0)		0 (0)	0 (0)	
Type5	1 (1.1)	10 (14.9)		1 (19.2)	6 (11.5)	
Differentiation category			0.68			0.69
Well	35 (37.6)	25 (37.3)		22 (42.3)	20 (38.5)	
Moderately	53 (57.0)	36 (53.7)		26 (50.0)	28 (53.8)	
Poorly	5 (5.4)	6 (9.0)		4 (7.7)	4 (7.7)	
Venous vessel infiltration			<0.001			0.013
Yes	89 (95.7)	49 (73.1)		3 (5.8)	13 (25.0)	

No	4 (4.3)	18 (22.9)		49 (94.2)	39 (75.0)	
Lymphatic vessel infiltration			0.75			0.69
Yes	49 (52.7)	37 (55.2)		26 (50.0)	24 (46.2)	
No	44 (47.3)	30 (44.8)		26 (50.0)	28 (53.9)	
pN stage			0.27			0.40
pN0	37 (39.8)	21 (31.3)		18 (34.6)	14 (26.9)	
pN1	22 (23.7)	23 (34.3)		14 (26.9)	20 (38.5)	
pN2	26 (28.0)	15 (22.4)		15 (28.8)	12 (23.1)	
pN3	8 (8.6)	8 (11.9)		5 (9.6)	6 (11.5)	
pStage			0.27			0.40
pIIA	37 (39.8)	21 (31.3)		18 (34.6)	14 (26.9)	
pIIIA	22 (23.7)	23 (34.3)		14 (26.9)	20 (38.5)	
pIIIB	26 (28.0)	15 (22.4)		15 (28.8)	12 (23.1)	
pIIIC	8 (8.6)	8 (11.9)		5 (9.6)	6 (11.5)	

Data in the table are presented as n (%), unless otherwise indicated.

Abbreviations: NAC: Neoadjuvant chemotherapy, PSM: propensity score matching

Supplementary table2. The relationship between CRM status and pattern of the recurrence in each treatment subgroup: overall cohort and PSM cohort

	Overall cohort (n=160)			PSM cohort (n=104)		
	Surgery alone (n = 93)	NAC + Surgery (n = 67)	*P*-value	Surgery alone (n = 52)	NAC + Surgery (n = 52)	*P*-value
CRM status RCP			0.41			0.21
Negative-R0	25 (26.9)	22 (32.8)		14 (26.9)	20 (38.5)	
Positive-R1	68 (73.1)	45 (67.2)		38 (73.1)	32 (61.5)	
CRM status CAP			0.09			0.09
Negative-R0	80 (86.0)	64 (95.5)		47 (90.4)	51 (98.1)	
Positive-R1	13 (14.0)	3 (4.5)		5 (9.6)	1 (1.9)	
Total recurrence	40 (43.0)	32 (47.8)	0.55	23 (44.2)	28 (53.4)	0.33
Pattern of recurrence						
Local	3 (7.5)	0 (0)	0.25	2 (8.7)	0 (0)	0.11
Lymph node metastasis	20 (50.0)	12 (37.5)	0.29	11 (47.8)	11 (39.3)	0.74
Distant metastasis	17 (42.5)	20 (62.5)	0.09	10 (43.5)	17 (60.7)	0.22

Data in the table are presented as n (%).

Abbreviations: CRM: Circumferential Resection Margin, RCP: Royal College of Pathologist, CAP: College of American Pathologist, NAC: Neoadjuvant Chemotherapy, OS: Overall Survival, RFS: Recurrence-free Survival, PSM: propensity score matching.

Supplementary table3. The relationship between clinicopathologic parameters and OS by univariate or multivariate analyses: PSM cohort

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
PSM patients (n = 104)				
RCP R1	1.89 (0.99–3.91)	0.053	1.38 (0.70–2.90)	0.36
CAP R1	5.57 (1.89–13.19)	0.0038	8.98 (2.73–25.8)	<0.001
Differentiation category (M/D)	1.90 (1.05–3.57)	0.034	1.21 (0.65–2.33)	0.55
Venous vessel infiltration	1.78 (0.77–5.17)	0.19		
Lymphatic vessel infiltration	1.84 (1.03–3.40)	0.041	0.90 (0.48–1.71)	0.74
Lymph node metastasis	8.76 (3.19–36.18)	<0.001	10.17 (3.30–45.19)	<0.001
NAC or Surgery alone	1.11 (0.62–2.01)	0.71		

Abbreviations: RCP: Royal College of Pathologist. CAP: College of American Pathologist, M/D: Moderately differentiation category, NAC: Neoadjuvant Chemotherapy, OS: Overall Survival, RFS: Recurrence-free Survival, CI: Confidence Interval, PSM: propensity score matching.

Supplementary table4. The relationship between clinicopathologic parameters and RFS by univariate and multivariate analyses: PSM cohort

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
PSM patients (n = 104)				
RCP R1	2.06 (1.11–4.13)	0.021	1.58 (0.83–3.21)	0.17
CAP R1	12.38 (3.77–36.29)	<0.001	9.25 (2.77–27.72)	<0.001
Differentiation category (M/D)	2.10 (1.19–3.82)	0.01	1.53 (0.85–2.84)	0.16
Venous vessel infiltration	2.0 (0.88–5.79)	0.11		
Lymphatic vessel infiltration	1.52 (0.87–2.69)	0.14		
Lymph node metastasis	4.24 (2.02–10.35)	<0.001	3.65 (1.71–9.01)	<0.001
NAC or Surgery alone	1.27 (0.73–2.25)	0.39		

Abbreviations: RCP: Royal College of Pathologist, CAP: College of American Pathologist, M/D: Moderately differentiation category, NAC: Neoadjuvant Chemotherapy, OS: Overall Survival, RFS: Recurrence-free Survival, CI: Confidence Interval

Supplementary table5. The relationship between CRM status and responses to NAC treatment

	RCP R0	RCP R1	*P*-value	CAP R0	CAP R1	*P*-value
NAC plus Surgery (n=67)	22 (32.8)	45 (67.2)		64 (95.5)	3 (0.5)	
Responses to NAC			0.99			0.15
PR (n=29)	9 (31.0)	20 (69.0)		26 (89.7)	3 (10.3)	
SD (n=38)	13 (34.2)	25 (65.8)		38 (100.0)	0 (0)	

Abbreviations: RCP: Royal College of Pathologist, CAP: College of American Pathologist, M/D: Moderately differentiation category, NAC: Neoadjuvant Chemotherapy, PR: Partial Response, SD: Stable Disease, PD: Progressive Disease

Supplementary table6. The relationship between CRM status and pattern of the recurrence in each treatment subgroup

	Surgery alone (n = 93)	NAC + Surgery (n = 67)	*P*-value
CRM status RCP			0.41
Negative-R0	25 (26.9)	22 (32.8)	
Positive-R1	68 (73.1)	45 (67.2)	
CRM status CAP			0.09
Negative-R0	80 (86.0)	64 (95.5)	
Positive-R1	13 (14.0)	3 (4.5)	
Total recurrence	40 (43.0)	32 (47.8)	0.55
Pattern of recurrence			
Local	3 (7.5)	0 (0)	0.25
Lymph node metastasis	20 (50.0)	12 (37.5)	0.29
Distant metastasis	17 (42.5)	20 (62.5)	0.09

Data in the table are presented as n (%).

Abbreviations: CRM: Circumferential Resection Margin, RCP: Royal College of Pathologist, CAP: College of American Pathologist, NAC: Neoadjuvant Chemotherapy, OS: Overall Survival, RFS: Recurrence-free Survival.