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*Original Article*

# High preoperative peripheral blood neutrophil predicts poor outcome in rectal cancer treated with neoadjunctive chemoradiation therapy

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**Abstract:** Distant metastasis impaired the value of neoadjunctive chemoradiation therapy (NCRT) for patients who were not pathological completed response. The objective of this study was to evaluate whether the absolute counts of preoperative neutrophils (pN) could predict survival outcomes of patients treated with NCRT. In this study, 289 locally advanced rectal cancer patients receiving NCRT and radical surgery were recruited between January 2006 and December 2012 at the Fudan University Shanghai Cancer Center. The absolute counts of pN were gathered and analyzed. Survival analysis was used to evaluate the prognostic value of pN. As results, a pN 3.00 was elected as the optimal cutoff points in term of survival by X-tile program. There were 112 patients (38.8%) in high-pN group and 177 patients (61.2%) in low pN group. The 4-year rectal cancer-specific survival (RCSS) and disease free survival (DFS) rate was 48.5% and 80.6%, 50.9% and 76.7% in high and low pN group, respectively. Univariate and multivariate analysis revealed that high-pN predicted poor RCSS and DFS. In conclusion, an elevated pN level was a significantly risk factor for locally advanced rectal cancer patient treated with NCRT, which may serve as a valuable marker to predict the outcomes of those patients.

**Keywords:** Rectal cancer, neoadjunctive chemoradiation therapy, survival analysis, inflammation, neutrophil

## Introduction

Due to the function of decreasing local recurrence rates, neoadjunctive chemoradiation therapy (NCRT) has become gold standard to treat locally advanced rectal cancer [1, 2]. However, distant metastasis reduced the value of NCRT and overall prognosis was not improved [3]. The above treatment failure maybe caused by the combined action of multi-factors, including tumor biology, immunodeficiency [4], inflammation [5] and other factors.

The cognizance that inflammation is a hallmark feature of cancer development and progression is widely accepted [6], and the link between inflammation and the progression and metastasis of colorectal cancer have been established [7]. Moreover, the absolute counts of pre-treatment neutrophils and lymphocyte have been found as an independent predictor for overall survival and recurrence of various malignancies [8-10]. However, the association between the absolute counts of preoperative neutrophils (pN) and prognosis of rectal cancer patients receiving NCRT has not yet been addressed. The purpose of this study is to assess the prognosis role of the pN on those patients.

## Materials and methods

### *Study population*

289 patients who were diagnosed with locally advanced rectal cancer and received NCRT and radical surgery from January 2006 to December 2012 were collected in the Fudan University shanghai cancer center (FUSCC) rectal cancer dataset [11, 12]. The inclusion criteria were rectal cancer as a single primary tumor, located within 10 cm of the anal verge, and completed NCRT. Patients who received local resection, died or recurred within 30 days after surgery,

**Table 1.** Demographic and clinical features of patients with rectal cancer treated with neoadjunctive chemoradiation therapy

| Variable | n | % |
|---|---|---|
| Sex | | |
| Male | 201 | 69.6 |
| Female | 88 | 30.4 |
| Age | | |
| ≥60 | 88 | 30.4 |
| <60 | 201 | 69.6 |
| Histotype | | |
| Adenocarcinoma | 273 | 94.5 |
| Mucinous/signet ring cell | 16 | 5.5 |
| LNs retrieval | | |
| <12 | 179 | 61.9 |
| ≥12 | 110 | 39.1 |
| AJCC Stage | | |
| 0 | 58 | 20.1 |
| I | 66 | 22.8 |
| II | 67 | 23.2 |
| III | 98 | 33.9 |
| TRG score | | |
| 0 | 24 | 8.3 |
| 1 | 45 | 15.6 |
| 2 | 69 | 23.9 |
| 3 | 87 | 39.8 |
| 4 | 65 | 22.5 |
| Lymphovascular invasion | | |
| Negative | 261 | 90.3 |
| Positive | 28 | 9.7 |
| Perineural invasion | | |
| Negative | 247 | 85.5 |
| Positive | 42 | 14.5 |
| Surgical Approach | | |
| Dixon | 100 | 34.6 |
| Miles | 175 | 60.6 |
| Hartman | 14 | 4.8 |
| pCEA (ng/ml) | | |
| ≥5.0 | 48 | 16.6 |
| <5 | 241 | 83.4 |

with chronic inflammatory disease or hematological system diseases or immunodeficiency or hepatic insufficiency were excluded from this study. All patients received neoadjunctive chemoradiation therapy, which consisted of 25 fractions of 5 Gy, a concomitant boost of 45-50 Gy to the pelvis and capecitabine or 5-FU based chemotherapy. Radical surgery was scheduled 6-8 weeks after NCRT.

Our study was approved by the Ethical Committee and Institutional Review Board of the FUSCC, and this study was in compliance with the Helsinki Declaration. Before included in this study, all patients in FUSCC provided written informed consent.

*Statistical analysis*

We gathered detailed information of 289 patients from FUSCC rectal cancer database, which including age, gender, pN, preoperative lymphocyte (pL), preoperative carcino-embryonic antigen (pCEA), histotype and so on. In consideration of inflammation caused by caducity, the patients were divided into two age groups: <60 years (young) and ≥60 years (old). CEA levels ≥5 ng/ml and was identified as abnormal. In our hospital, the absolute counts of neutrophil ranging from $2.1\times10^9$/L to $7.7\times10^9$/L were regarded as normal. The American Joint Committee on Cancer (AJCC) Tumor-nodes-metastasis staging (TNM) system (7th edition, 2010) was used to determine tumor stages. Tumor regression of the primary tumor was semi quantitatively identified by the amount of viable tumor versus the amount of fibrosis, ranging from no evidence of any treatment effect to a complete response with no viable tumor identified [13, 14]. The patients were divided into two TRG score groups: 0-1 and 2-4 [15].

The optimal pN, and pL cutoff value were achieved by X-tile program in the term of rectal cancer-specific survival (RCSS) [16]. RCSS was the primary endpoint of this study, calculated from the date of diagnosis to the date of cancer specific to death.

Kaplan-Meier Survival Probability Estimates is performed to evaluate 4-year RCSS and 4-year DFS to generate survival curves, differences between the curves were analyzed by log-rank test. Risk factors for survival outcomes and tumor recurrence in rectal cancer patients treated with NCRT were analyzed in Multivariable Cox regression models. Chi-square test and Fisher's exact test was performed for categorical variables. The above analyses were performed using the statistical software package SPSS for Windows, version 22 (IBM Corp, Armonk, NY, USA). Statistical significance was set at two-sided $P<0.05$.

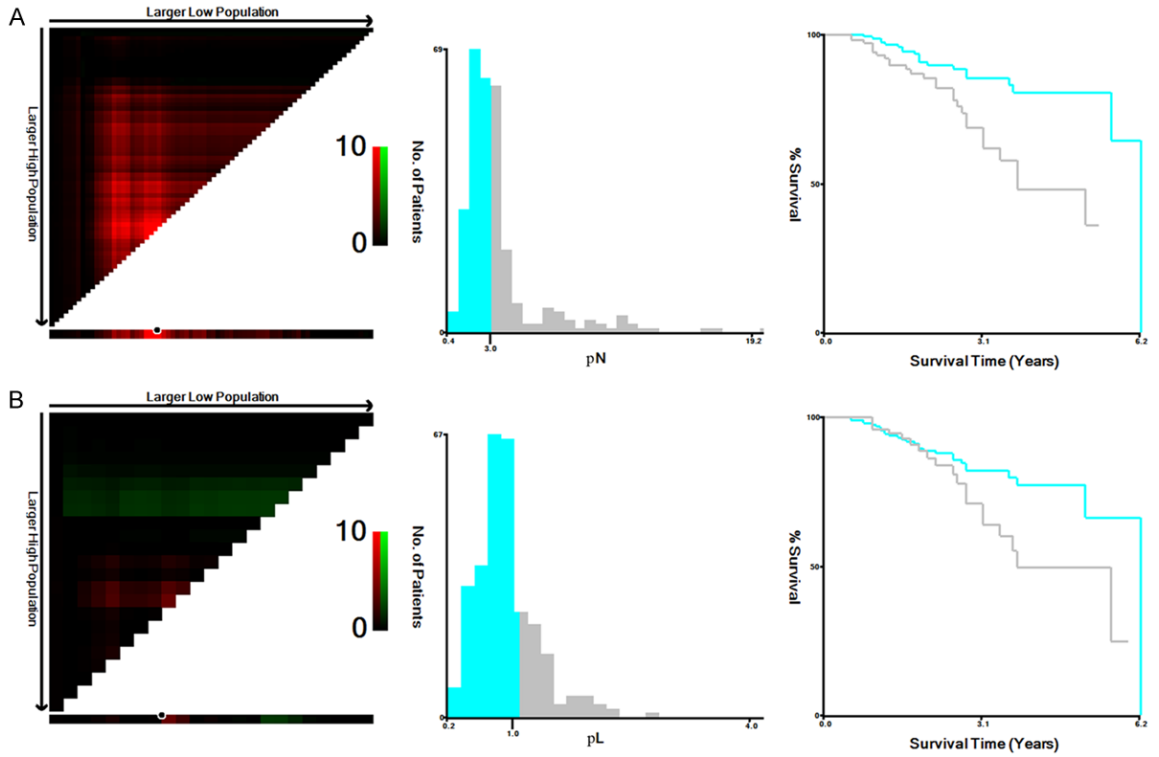


**Figure 1.** X-tile analysis of survival data of patients treated with preoperative chemoradiation therapy. X-tile analysis was performed using patient data, which were equally divided into training and validation sets. X-tile plots of the training sets are shown in the left panels, with plots of matched validation sets shown in the smaller inset. The optimal cut-point highlighted by the black circle in the left panels is shown on a histogram of the entire cohort (middle panels), and a Kaplan-Meier plot (right panels). *P* values were determined using the cutoff point defined in the training set and applying it to the validation set. A: Shows the optimal cutoff point for the pN (3.0, $\chi^2$=12.301, P<0.001). B: Shows the optimal cutoff point for the pL (1.8, $\chi^2$=4.535, P=0.033).

## Results

### *Patient characteristics*

289 eligible patients were recruited from January 2006 to December 2012, with a mean age of 54 years (range, 22-78 years). Patient demographics and pathological features are summarized in **Table 1**. Sixty (20.8%) patients were pathological completed response (pCR). During follow-up, 60/289 patients (20.8%) experienced tumor recurrence after surgical treatment, including 13 (4.5%) who developed local or pelvic recurrence and 47 (16.3%) who developed distant metastasis, and 2 of them were pCR. By the end of follow-up, 47/289 patients (16.3%) had died of rectal cancer.

### *Identification of pN and pL optimal cut-off points*

The mean pN and pL were 3.41 ± 2.58×$10^9$/L and 0.93 ± 0.43×$10^9$/L, respectively. X-tile program was used to determine the optimal cut-off value for the above variables. A pN cutoff 3.00 and a pL cutoff 1.00 were selected as the optimal cutoff point in terms of RCSS (P<0.001 and P=0.033, respectively). Both the optimal cutoff points were used to divide the patients into high and low risk subsets in terms of RCSS by survival analyses (**Figure 1**).

### *Association among, pN, pL and the clinical features of patients*

There were 112 patients (38.8%) in high-pN group and 82 patients (28.4%) in high-pL group. The trend that old patients had a high pN (young VS old, 34.3% VS 48.9%, P=0.020) and pL level (young VS old, 24.4% VS 37.5%, P=0.023) was obvious (**Table 2**). The attribution of other clinical features in high-pN and low-pN group was not different (**Table 2**).

### *Prognostic value of pN and pL on the RCSS*

Upon univariate analysis, the high pN and pL level (P<0.001 and P=0.033, respectively),

**Table 2.** Association among pN and the clinical features in rectal cancer patients treated with neoadjunctive chemoradiation therapy

| Variable | pN level ($10^9$/L) | | P | pL level ($10^9$/L) | | P |
|---|---|---|---|---|---|---|
| | ≤3.00 | >3.00 | | ≤1.00 | >1.00 | |
| Sex | | | 0.282 | | | 0.26 |
| Male | 119 | 82 | | 140 | 61 | |
| Female | 58 | 30 | | 67 | 21 | |
| Age | | | 0.020 | | | 0.023 |
| ≥60 | 45 | 43 | | 55 | 33 | |
| <60 | 132 | 69 | | 152 | 49 | |
| Histotology | | | 0.342 | | | 0.780* |
| Adenocarcinoma | 169 | 104 | | 196 | 77 | |
| Mucinous/signet ring cell | 8 | 8 | | 11 | 5 | |
| pCEA (ug/ml) | | | 0.399 | | | 0.894 |
| <5.0 | 145 | 96 | | 173 | 68 | |
| ≥5.0 | 32 | 16 | | 34 | 14 | |
| LNs retrieval | | | 0.927 | | | 0.631 |
| <12 | 110 | 69 | | 130 | 49 | |
| ≥12 | 67 | 43 | | 77 | 33 | |
| AJCC Stage | | | 0.013 | | | 0.509 |
| 0 | 38 | 20 | | 44 | 14 | |
| I | 41 | 25 | | 43 | 23 | |
| II | 30 | 37 | | 46 | 21 | |
| III | 68 | 30 | | 74 | 24 | |
| TRG score | | | 0.941 | | | 0.295 |
| 0-1 | 42 | 27 | | 46 | 23 | |
| 2-4 | 135 | 85 | | 161 | 59 | |
| Lymphovascular invasion | | | 0.199 | | | 0.677 |
| Negative | 163 | 98 | | 186 | 75 | |
| Positive | 14 | 14 | | 21 | 7 | |
| Perineural invasion | | | 0.924 | | | 0.478 |
| Negative | 151 | 96 | | 175 | 72 | |
| Positive | 26 | 16 | | 32 | 10 | |
| pN | | | | | | 0.096 |
| ≤3.00 | | | | 133 | 44 | |
| >3.00 | | | | 74 | 38 | |

*Fisher's exact test.

AJCC stage (P<0.001), the high pCEA level (>5 ug/ml) (P<0.001), mucinous and signet-ring cancer (P=0.002), poor tumor regression grade (TRG) score (P<0.001), lymphovascular invasion (P<0.001) and perineural invasion positive (P≤0.001) predicted poor RCSS (**Table 3**). Multivariate analysis with Cox regression revealed that the pN level was an independent prognostic factor for RCSS, and a higher pN level exerted a negative effect on RCSS (hazard ratio [HR]=2.987; 95% confidence interval [CI], 1.568-5.691, P=0.001) (**Table 3**). Additionally, high-pCEA cases exhibited a higher likelihood for disease event (HR=2.507; 95% CI, 1.568-5.691, P=0.008) (**Table 3**). However, the pL level was not an independent prognostic factor for RCSS (HR=1.445; 95% CI, 0.765-2.731, P=0.257) (**Table 3**).

*Prognostic value of pN on the DFS*

Univariate analysis revealed that the high pN level (P=0.020), AJCC stage III (P<0.001), the high pCEA level (P<0.001), mucinous and signet-ring cancer (P=0.001), poor TRG score (P<0.001), lymphovascular invasion (P=0.011) and perineural invasion positive (P=0.013) predicted poor DFS (**Table 4**).

Upon multivariate analysis, AJCC stage III, the pN and pCEA level were independent prognostic factors for DFS, and a higher pN level demonstrated a negative effect on DFS (HR=2.010; 95% CI, 1.178-3.430, P=0.010) (**Table 4**).

## Discussion

Although NCRT can decrease the local recurrence rates of locally advanced rectal cancer, the overall survival was not improved, which may indicate the treatment of locally advanced rectal cancer seems to have reached a therapeutic plateau. This disappointed result may be partly induced by changes of the host immune response to cancer cells among the period of NCRT [4, 17].

Accumulating studies has revealed that inflammation exerted variable effects on tumor biology [4, 6, 18, 19]. However, the current researches focus on the cancer-self inflammation, which is not all-inclusive. On account of

**Table 3.** Univariate and multivariate survival analyses evaluating pN influencing RCSS in rectal cancer treated with neoadjunctive chemoradiation therapy

| Variable | 4-year RCSS | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | | Log rank $\chi^2$ test | P* | HR (95% CI) | P* |
| Sex | | 0.012 | 0.912 | | NI |
| Male | 68.0% | | | | |
| Female | 73.4% | | | | |
| Age | | 0.027 | 0.869 | | NI |
| ≥60 | 69.5% | | | | |
| <60 | 68.5% | | | | |
| pCEA (ug/ml) | | 14.849 | <0.001 | | 0.008 |
| <5.0 | 72.9% | | | Reference | |
| ≥5.0 | 41.2% | | | 2.507 (1.74-4.936) | |
| LNs retrieval | | 0.637 | 0.425 | | NI |
| <12 | 65.1% | | | | |
| ≥12 | 75.3% | | | | |
| AJCC Stage | | 24.621 | <0.001 | | 0.147 |
| 0 | 95.5% | | | Reference | |
| I | 82.2% | | | 3.980 (0.476-33.302) | 0.203 |
| II | 67.6% | | | 5.478 (0.675-44.436) | 0.111 |
| III | 46.7% | | | 9.097 (1.098-75.400) | 0.041 |
| TRG score | | 25.915 | <0.001 | | 0.342 |
| 0-1 | 45.6% | | | Reference | |
| 2-4 | 73.2% | | | 0.688 (0.319-1.487) | |
| Lymphovascular invasion | | 12.651 | <0.001 | | 0.223 |
| Negative | 74.2% | | | Reference | |
| Positive | 26.9% | | | 1.602 (0.751-3.416) | |
| Perineural invasion | | 10.332 | 0.001 | | 0.189 |
| Negative | 71.7% | | | Reference | |
| Positive | 53.9% | | | 1.586 (0.797-3.158) | |
| Histotype | | 10.076 | 0.002 | | 0.443 |
| Adenocarcinoma | 69.7% | | | Reference | |
| Mucinous/signet ring cell | 49.2% | | | 1.428 (0.575-3.546) | |
| pN | | 12.301 | <0.001 | | 0.001 |
| ≤3.00 | 80.6% | | | Reference | |
| >3.00 | 48.5% | | | 2.987 (1.568-5.691) | |
| pL | | 4.535 | 0.033 | | 0.257 |
| ≤1.00 | 77.3% | | | Reference | |
| >1.00 | 49.9% | | | 1.445 (0.765-2.731) | |

NI: not included in multivariate survival analysis. *$P$ values refer to the log-rank test of the differences between the two survival curves generated using Kaplan-Meier analysis.

tumor tissue necrosis and inflammation introduced by chemotherapy and radiotherapy, inflammation around primary lesion may be caused by two reasons: cancer-self [5] and therapy [20, 21], and therapy-related inflammation (TRI) exerted variable effects on tumor in the same way [22]. On the one hand, TRI was positive by increasing susceptibility of cancer cells of some patients to immune attack [4], but on the other hand, TRI may be harmful for other patients, in consideration of that TRI may work in the progressive process of cancer [4, 6].

On account of TRI persistently existed until the operative time, pN may be a valuable index of

**Table 4.** Univariate and multivariate survival analyses evaluating pN influencing DFS in rectal cancer treated with neoadjunctive chemoradiation therapy

| Variable | 4-year DFS | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | | Log rank $\chi^2$ test | P* | HR (95% CI) | P |
| Sex | | 0.001 | 0.971 | | NI |
| Male | 66.5% | | | | |
| Female | 70.6% | | | | |
| Age | | 0.003 | 0.959 | | NI |
| ≥60 | 66.2% | | | | |
| <60 | 67.6% | | | | |
| pCEA (ug/ml) | | 12.375 | <0.001 | Reference | 0.015 |
| <5 | 71.4% | | | 2.073 (1.149-3.741) | |
| ≥5 | 44.0% | | | | |
| LNs retrieval | | 1.863 | 0.172 | | NI |
| <12 | 67.1% | | | | |
| ≥12 | 73.8% | | | | |
| AJCC Stage | | 29.865 | <0.001 | | 0.030 |
| 0 | 94.0% | | | Reference | |
| I | 75.9% | | | 4.195 (0.916-19.207) | 0.065 |
| II | 66.0% | | | 4.585 (1.006-21.905) | 0.049 |
| III | 47.2% | | | 8.479 (1.860-38.656) | 0.006 |
| TRG score | | 28.291 | <0.001 | | 0.122 |
| 0-1 | 43.1% | | | Reference | |
| 2-4 | 76.8% | | | 0.613 (0.330-1.139) | |
| Lymphovascular invasion | | 6.496 | 0.011 | | 0.977 |
| Negative | 71.7% | | | Reference | |
| Positive | 34.0% | | | 1.010 (0.495-2.063) | |
| Perineural invasion | | 6.210 | 0.013 | | 0.632 |
| Negative | 70.3% | | | Reference | |
| Positive | 52.1% | | | 1.165 (0.625-2.171) | |
| Histotype | | 11.846 | 0.001 | | 0.365 |
| Adenocarcinoma | 69.9% | | | Reference | |
| Mucinous/signet ring cell | 26.6% | | | 1.446 (0.651-3.213) | |
| pN | | 5.446 | 0.020 | | 0.010 |
| ≤3.00 | 76.7% | | | Reference | |
| >3.00 | 50.9% | | | 2.010 (1.178-3.430) | |
| pL | | 2.265 | 0.132 | | NI |
| ≤1.00 | 71.9% | | | | |
| >1.00 | 55.2% | | | | |

NI: not included in multivariate survival analysis. *P values refer to the log-rank test of the differences between the two survival curves generated using Kaplan-Meier analysis.

TRI. Moreover, prior studies have found that neutrophils present in the tumor bed is suspected to stimulate cancer growth [18, 23]. So, we hypothesized that the prognosis value of pN on survival may indirectly inflect the impact of TRI on tumor.

This study evaluated the prognosis role of pN, pL and other clinical factors on RCSS and DFS. We found that high-pN was an independent risk factor on RCSS. More importantly, High-pN was able to predict poor DFS. For patients who had an abnormal pN level, system inflammation caused by other reasons was drastic during NCRT, and exerted a predominant effect on tumor biology, which may induce poor prognosis. However, high-pN was still an independent risk factor on DFS (Supplementary Table 1),

when patients with abnormal pN (n=19) were omitted.

Certainly, there were some limitations in this study, including a single-center retrospective study, insufficient number of patients, short follow-up time, and TRI need to be clarified by further preclinical and clinical researches.

In summary, an elevated preoperative pN level was a significantly risk factor for locally advanced rectal cancer patient treated with NCRT, which may serve as a valuable marker to predict outcomes of those patients.

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## Disclosure of conflict of interest

None.

## Authors' contribution

Conceived and designed the study: Xianke Meng, Qingguo Li, Guoxiang Cai. Acquisition of data and providing data: Hongtu Zheng, Ye Xu, Debing Shi, Sanjun Cai. Drafted the manuscript: Xianke Meng, Qingguo Li, Guoxiang Cai. All authors read and approved the final manuscript.

## Abbreviations

NCRT, neoadjunctive chemoradiation therapy; pN, preoperative neutrophils; RCSS, rectal cancer-specific survival; DFS, disease free survival; pCEA, preoperative carcino-embryonic antigen; pCR, pathological completed response; pL, preoperative lymphocyte; TRI, therapy-related inflammation.

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**Supplementary Table 1.** Univariate and multivariate survival analyses evaluating pN influencing DFS in rectal cancer treated with neoadjunctive chemoradiation therapy (patients with abnormal pN (n=19) were omitted)

| Variable | 4-year DFS | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | | Log rank $\chi^2$ test | P* | HR (95% CI) | P |
| Sex | | 0.002 | 0.967 | | NI |
| Male | 66.8% | | | | |
| Female | 70.3% | | | | |
| Age | | 0.003 | 0.956 | | NI |
| ≥60 | 65.6% | | | | |
| <60 | 68.0% | | | | |
| CEA (ug/ml) | | 11.916 | 0.001 | | 0.010 |
| <5 | 71.3% | | | Reference | |
| ≥5 | 46.5% | | | 2.222 (1.209-4.084) | |
| LNs retrieval | | 1.923 | 0.166 | | NI |
| <12 | 63.8% | | | | |
| ≥12 | 74.0% | | | | |
| AJCC Stage | | 28.069 | <0.001 | | 0.024 |
| 0 | 94.0% | | | Reference | |
| I | 73.4% | | | 4.828 (1.056-22.067) | 0.042 |
| II | 68.2% | | | 3.918 (0.843-18.218) | 0.082 |
| III | 48.2% | | | 8.439 (1.862-38.257) | 0.006 |
| TRG score | | 27.962 | <0.001 | | 0.155 |
| 0-1 | 42.9% | | | Reference | |
| 2-4 | 77.4% | | | 0.617 (0.317-1.199) | |
| Lymphovascular invasion | | 3.591 | 0.058 | | NI |
| Negative | 71.1% | | | | |
| Positive | 37.9% | | | | |
| Perineural invasion | | 3.811 | 0.051 | | NI |
| Negative | 69.7% | | | | |
| Positive | 55.6% | | | | |
| Histotype | | 12.094 | 0.001 | | 0.333 |
| Adenocarcinoma | 70.2% | | | Reference | |
| Mucinous/signet ring cell | 26.6% | | | 1.484 (0.667-3.303) | |
| pN | | 5.557 | 0.018 | | 0.012 |
| ≤3.00 | 76.7% | | | Reference | |
| >3.00 | 49.6% | | | 2.031 (1.171-3.523) | |

NI: not included in multivariate survival analysis. *$P$ values refer to the log-rank test of the differences between the two survival curves generated using Kaplan-Meier analysis.