Perioperative change in neutrophil count predicts worse survival in esophageal squamous cell carcinoma

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Abstract

Background

Numerous studies reported the prognostic significance of neutrophil count (or preferably NLR) in patients with esophageal squamous cell carcinoma (ESCC), while the relationship between perioperative change in neutrophil count and survival of patients with ESCC after surgery has not been assessed.

Methods

A total of 603 patients with newly diagnosed ESCC after the operation were enrolled in the study. Neutrophil change (Nc) (Nc = Post-Neutrophil−Pre-Neutrophil) was counted according to data within one week before surgery and two week after surgery. The median of Nc was selected to be the cut off value to evaluate the relationship between Nc and Overall survival (OS).

Results

105 (17.4%) patients had pathological stage 1a-1b, 205 (34.0%) patients had pathological stage 2a-2b, and 293 (48.6%) patients had pathological stage 3a-3c.There were 180 (29.9%) patients who had vessel invasive, and 423 (70.1%) patients without vessel invasive. There were 215(35.7%) patients who had nerve infiltration, and 388 (64.3%) without nerve infiltration. In univariate analysis, six parameters including neutrophil change(Nc)(≥ 2.60 vs.<2.60) (P = 0.010, HR = 0.694, 95% CI 0.526–0.915) significantly correlated to worse OS. Multivariate analysis revealed that neutrophil change(Nc)(≥ 2.60 vs. <2.60) was an independent prognostic marker for OS (P = 0.022, HR = 0.720, 95% CI 0.544–0.954). Kaplan-Meier curves shown that Nc < 2.60 was significantly associated with worse OS (P = 0.016).

Conclusions

Perioperative change in neutrophil count predicts worse survival in patients with ESCC after the operation.

Background

Esophageal cancer is one of the most aggressive and common carcinoma worldwide and the fourth most frequent cause of carcinoma-related mortality in China [1]. Esophageal squamous cell carcinoma (ESCC) remains the predominant pathological tissue type in China [2, 3]. The clinical outcome of ESCC patients remains unsatisfactory due to distant metastasis or a high recurrence rate. Although surgical intervention, chemotherapy, radiation and biologically targeted treatments have been improved, the five-year survival rate remains 20%-30% worldwide [4]. Therefore, it is urgent to identify reliable and conventional prognosis markers that may contribute to classify patients with high risk of death and may guide the perioperative management of these patients.

Accumulating evidence indicates neutrophils play a vital role in tumor progression by interacting with immune and cancer cells [5, 6]. Neutrophils are involve in anti- and pro-tumor activities including tumor cell killing, proliferation, aggressiveness, metastasis, angiogenesis, and participating in other immune responses[7–13]. In addition to their effect in tumor site, retrospective researches and functional analyses of neutrophil-to-lymphocyte ratio (NLR) indicate that neutrophils in blood could also play an important value in tumor progression. Oncologists systematically monitored neutrophil counts during cancer management, due to chemotherapy caused neutropenia that makes patients more susceptible to infection [14, 15]. Numerous reviews and meta-analyses reported the prognostic significance of neutrophil count (or preferably NLR) [16–20]. These findings focused on preoperative neutrophil count, while the relationship between perioperative change in neutrophil count and survival of patients with ESCC after the operation has not been assessed.

We hypothesized that the perioperative change in neutrophil count might reflect the efficacy of surgical treatment and predict the clinical outcome of patients with ESCC. Therefore, we investigated whether the perioperative change in neutrophil count might be an independent prognostic factor in patients with ESCC who received surgical intervention.

Methods

Patient selection

A total of 603 patients who were newly diagnosed ESCC and radical esophagectomy were enrolled in the present study at Cancer Hospital of the University of Chinese Academy of Sciences, from 2008 to 2014. All tumor specimens were pathologically confirmed as ESCC after surgery. The blood routine was examined within one week before surgery. If the preoperative neutrophil count was performed on the first day and on the fourth day before surgery, the closest experimental data was selected. The postoperative neutrophil count was checked within two weeks after operation. In order to reduce the risk of stress response after surgery, the neutrophil count farthest within two weeks were collected. The unit of neutrophil count was per L in our clinical laboratory. Patients with incomplete clinical and laboratory data were excluded. Patients with active infection or any simultaneous hematological diseases or other tumors before the operation were excluded. Patients who received neoadjuvant chemotherapy were excluded. Because the preoperative neutrophil counts may be influenced by these diseases or treatments. The present study was approved by the Ethics Committee of Cancer Hospital of the University of Chinese Academy of Sciences (Hangzhou, China). The study was conduct in accordance with the Declaration of Helsinki. In the end, all patients received written informed consent.

Statistical analysis

The preoperative neutrophil counts and the postoperative neutrophil counts were counted as continuous variables, which do not conform to the normal distribution. Continuous data was represented by median and quartile intervals. The clinical characteristics of ESCC patients were analyzed as categorical variables that were presented as numbers and percentage. The chi-square test was used to compare

categorical variables. Overall survival (OS) was calculated from the date of surgery to the date of death and the last follow-up. Overall survival curve was estimated by the log-rank test and the Kaplan-Meier method. The overall survival curve was plotted by GraphPad Prism 7 software. COX regression analyses were used to evaluate independent prognostic markers. All statistical analysis was calculated by SPSS, version 19.0 (SPSS, Chicago, IL, USA). The software CRAN-R (version 3.3.0.) was used to illustrate the beeswarm plot. P less than 0.05 were considered as statistical significance.

Results

Patient characteristics

There were 86 (14.3%) female and 517 (85.7%) male patients with newly diagnosed ESCC. There were 279 (46.3%) young patients whose age at first diagnosis≤60 years and 324 (53.7%) old patients (>60 years). 105 (17.4%) patients had pathological stage 1a-1b, 205 (34.0%) patients had pathological stage 2a-2b, and 293 (48.6%) patients had pathological stage 3a-3c.There were 180 (29.9%) patients who had vessel invasive, and 423 (70.1%) patients without vessel invasive. There were 215(35.7%) patients who had nerve infiltration, and 388 (64.3%) without nerve infiltration. 408 (67.7%) patients with surgery only, 137 (22.7%) patients who received postoperative chemotherapy, and 58 (9.6%) patients who received radiotherapy and chemotherapy after operation. The median value of the preoperative neutrophil count was 3.5, and the quartile interval is 2.8-4.5. The median value of the postoperative neutrophil count was 6.2, and the quartile interval is 5.0-7.6. Details of clinical features were shown in Table 1. Significant differences in the neutrophil count was observed prior to and following surgery (P<0.001) (Figure 1).

Relationship between neutrophil change and clinical features

The clinical data of patients in neutrophil change(Nc) <2.60 group and in neutrophil change(Nc) ≥2.60 group are listed in Table 1. There were no significant difference between the two groups in terms of clinical data including sex, age, depth of tumor, lymph node metastasis, pathological stage, pathology grade, vessel invasive, nerve infiltration, and treatment regimen. The median of Pre-Neutrophil and Post-Neutrophil were both higher in neutrophil change(Nc) ≥2.60 group than in neutrophil change(Nc) <2.60 group (both P <0.001).

Prognostic variables for OS

In univariate analysis, six parameters including neutrophil change(Nc)(≥2.60 vs.<2.60) (P=0.010), depth of tumor (P<0.05), lymph node metastasis (P<0.05), pathological stage (P<0.001), vessel invasive (absence vs. presence) (P<0.001), and nerve infiltration (absence vs. presence) (P<0.001) predict worse OS. Multivariate analysis revealed that neutrophil change(Nc)(≥2.60 vs.<2.60) (P=0.022), lymph node metastasis (P<0.05), and nerve infiltration (absence vs. presence) (P=0.005) could serve as independent prognostic markers of worse OS (Table 2). OS of patients with neutrophil change(Nc)(<2.60) group were notably worse compared to patients with neutrophil change(Nc)(≥2.60) group (P=0.016) (Figure 2).

Subgroup analysis according to other clinical features

To investigate the subgroups of patients with ESCC impacted by neutrophil change(Nc), we classified patients based on gender (male, n=517; female, n=86), age (≤60, n=279; >60, n=324), pathological stage (1a-1b, n=105; 2a-2b, n=205; 3a-3c, n=293), vessel invasive (Yes, n=180; No, n=423), and nerve infiltration(Yes, n=215; No, n=388). OS of male patients, age≤60 patients, patients without vessel invasive and patients without nerve infiltration were dramatically worse for those with neutrophil change(Nc)(<2.60) (P=0.013, P=0.002, P=0.004, and P=0.014), but OS did not differ in female patients, age>60 patients, patients with vessel invasive and patients with nerve infiltration (Figure 3- Figure 7). Patients with pathological stage 3a-3c tend to have worse OS in neutrophil change(Nc)(<2.60) group than in neutrophil change(Nc)(≥2.60), but do not reach statistical difference (P=0.050).

Discussion

Because chemotherapy caused neutropenia, oncologists always evaluated neutrophil counts during the process of chemotherapy treatment [14, 15]. Numerous retrospective studies and meta-analyses reported that neutrophil count (or preferably NLR) could serve as an independent prognostic factor in patients with ESCC [16–20]. However, these reports based on preoperative neutrophil count, the association between perioperative change in neutrophil count and clinical outcome of patients with ESCC undergoing curative surgery has not been investigated.

In the present study, all enrolled patients were divided into two groups including neutrophil change(Nc)(< 2.60) and neutrophil change(Nc)(≥ 2.60) according to the median of neutrophil change(Nc). Our study evaluated perioperative change in neutrophil count and the influence of Nc on survival in patients with ESCC. So far we firstly indicated that perioperative change in neutrophil count could be an independent prognostic factor in patients with ESCC. Based on subgroup analysis we shown that neutrophil change(Nc)(< 2.60) predicted worse OS in male patients, age ≤ 60 patients, patients without vessel invasive and patients without nerve infiltration, but OS did not differ in other subgroup. This finding indicated that neutrophil change(Nc) may serve as prognostic marker specially in these subgroup including male, age ≤ 60, with vessel invasive, and with nerve infiltration. Neutrophil change(Nc)(< 2.60) could assistant clinical doctors to predict and guide prognosis.

Even though the mechanisms of the association between Neutrophil change(Nc)(< 2.60) and worse OS remains unknown, the interpretation are as follows: (1) Neutrophils account for 50–70% of all circulating white blood cells in healthy adult, and they play an important role in the inflammatory reaction [21]. Neutropenia makes patients more susceptible to infection that belongs to the complication after surgery [22, 23]. In our findings, Neutrophil change(Nc)(< 2.60) group had lower Pre- Neutrophil that may be correlated with a preexisting hypoimmunity. (2) In addition, Neutrophil change(Nc)(< 2.60) group had weak Post-Neutrophil, which may lead to the infection and complication. Therefore Neutrophil change(Nc)(< 2.60) group with both lower Pre- Neutrophil and Post-Neutrophil predicted shorter OS.

An emerging pro-tumor ability of circulating neutrophils has recently been identified. Neutrophils are able to entrap circulating tumor cells (CTCs) by facilitating their extravasation thus promoting metastasis [24–28]. Moreover, neutrophils were described for their ability to infiltrate cancer tissue and are called tumor-associated neutrophils (TANs) [29]. TANs were shown to play a vital role in promoting tumor progression through proliferation, angiogenesis, and interacting with immune cells [30–34]. Although accumulating evidence indicates that TANs are key players in pro-tumor immunity, large researches suggest that contribute to anti-tumor. TANs were able to induce apoptosis through releasing cytotoxic molecules including ROS [35]. In addition, TANs play an important role in recruiting and activating cytotoxic CD8 + and intra-tumor CD4 + T cells [36]. Neutrophils were shown to kill cancer cells by a process termed trogoptosis, which was different from ferroptosis [37]. Therefore Neutrophils contribute to promote cytotoxic T cell reaction by eliminating resistant tumor clones [38].Neutrophil derived extracellular DNA (NETs) were shown to lead to cancer cell death [39]. Because of heterogeneity in neutrophil function, scientists hypothesize that different subgroups of TANs may be involved. The prognostic effect of TANs remains inconsistent because they have relationship with either a worse or better survival [40–45]. Due to the importance of neutrophils in the immunity upon bacterial infection, the neutrophil count in blood routine examination is utilized as a read-out during cancer management related toxicities. Neutrophil count is routine, cheap and reliable in clinical examination. The clinical application of neutrophil count could contribute to predict and guide the prognosis of patients with ESCC specially in these subgroup including male, age ≤ 60, with vessel invasive, and with nerve infiltration.

A beeswarm plot is a two-dimensional visualization technique in which the measured values are drawn in a fixed reference shaft and avoided the overlap of these values. It is good at presenting the experimental data points and the relative distribution of these data point. There were some shortcomings in this study: first, this retrospective design study did not include the validation set neither in our laboratory nor in multicenter hospital. The validation set could improve the suggestion of Neutrophil change(Nc)(< 2.60) may serve as an independent prognostic factor; Second, due to the low patients in the adjuvant treatments such as radiation and chemotherapy, this subgroup were not taken into account. Third, because of retrospective study, the neutrophil count could not be checked on specific days. In order to reduce the risk of stress response after surgery, the post-neutrophil count farthest within two weeks were collected. Despite these limitations, we first investigate the relationship between perioperative change in neutrophil count and clinical outcome in ESCC undergoing curative resection.

Conclusion

In conclusion, this study first indicate that Neutrophil change(Nc)(< 2.60) predict worse OS in patients with ESCC specially in these subgroup including male, age ≤ 60, with vessel invasive, and with nerve infiltration. Neutrophil change(Nc)(< 2.60) could contribute the oncologists to evaluate and predict prognosis based on clinical examination data. In the future, more prospective and multicenter studies are warranted to prove the relationship between them.

Abbreviations

ESCC: Esophageal squamous cell carcinoma; NLR: Neutrophil-to-lymphocyte ratio; Nc: Neutrophil change; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval; CTCs: Circulating tumor cells; TANs: Tumor-associated neutrophils;

Declarations

Ethics approval and consent to participate

All procedures in our study were conducted in keeping with the ethical standards of the World Medical Association Declaration of Helsinki. All the patients provided written informed consent, the protocol was approved by ethics committee at Cancer Hospital of the University of Chinese Academy of Sciences.

Consent to publish

Not applicable.

Availability of data and materials

The data is available by contacting corresponding author.

Competing interests

No conflict of interest.

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Authors' contributions

QS designed the study, and was a major contributor in writing the manuscript.; JW collected material and data and was involved in the statistical interpretation of the data.; SW collected material and data and performed statistical analysis,; SX drafted the manuscript.. All authors read and approved the final manuscript.

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Tables

Table 1. Demographic and clinical data of 603 ESCC patients accroding to neutrophil change(Nc).

Charateristics		Total (N=603), %	neutrophil change(Nc)		*P* value
			<2.60 (N=296), %	≥2.60 (N=307), %	
Sex	Male	517 (85.7)	251 (84.8)	266 (86.6)	0.517
	Female	86 (14.3)	45 (15.2)	41 (13.4)	
Age (years)	≤60	279 (46.3)	133 (44.9)	146 (47.6)	0.518
	>60	324 (53.7)	163 (55.1)	161 (52.4)	
Pathology grade	Well differentiated	43 (7.1)	24 (8.1)	19 (6.2)	0.107
	middle differentiated	401 (66.5)	208 (70.3)	193 (62.9)	
	Poorly differentiated	145 (24.1)	59 (19.9)	86 (28.1)	
	Undifferentiated	2 (0.3)	1 (0.3)	1 (0.3)	
	Missing	12 (2.0)	4 (1.4)	8 (2.5)	
Depth of tumor	T1a–1b	58 (9.6)	20 (6.8)	38 (12.4)	0.058
	T2	117 (19.4)	57 (19.3)	60 (19.5)	
	T3	428 (71.0)	219 (73.9)	209 (68.1)	
Lymph node metastasis	N0	267 (44.3)	135 (45.6)	132 (43.0)	0.227
	N1	192 (31.8)	84 (28.4)	108 (35.2)	
	N2	102 (16.9)	57 (19.3)	45 (14.7)	
	N3	42 (7.0)	20 (6.7)	22 (7.1)	
Pathological stage	1a–1b	105 (17.4)	50 (16.9)	55 (17.9)	0.700
	2a–2b	205 (34.0)	97 (32.8)	108 (35.2)	
	3a–3c	293 (48.6)	149 (50.3)	144 (46.9)	
Vessel invasive	Yes	180 (29.9)	86 (29.1)	94 (30.6)	0.675
	No	423 (70.1)	210 (70.9)	213 (69.4)	
Nerve infiltration	Yes	215 (35.7)	116 (39.2)	99 (32.2)	0.075
	No	388 (64.3)	180 (60.8)	208 (67.8)	
Treatment regimen	S	408 (67.7)	205 (69.3)	203 (66.1)	0.317
	S plus postoperative C	137 (22.7)	68 (23.0)	69 (22.5)	
	S plus postoperative CRT	58 (9.6)	23 (7.7)	35 (11.4)	
Pre-Neutrophil	Median	3.5 (2.8-4.5)	3.9 (3.0-5.1)	5.0 (4.1-6.0)	**<0.001**
Post-Neutrophil	Median	6.2 (5.0-7.6)	3.2 (2.7-3.9)	7.4 (6.5-8.6)	**<0.001**

Abbreviations: S, surgery; C, chemotherapy; CRT, chemoradiotherapy.

Table 2.Overall survival analyses according to neutrophil change(Nc) in 603 patients with ESCC.

Variables	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P*value
neutrophil change(Nc)(≥2.60 vs.<2.60)	0.694	0.526-0.915	**0.010**	0.720	0.544-0.954	**0.022**
Sex (male vs.female)	1.194	0.791-1.802	0.399			
Age (>60 vs.≤60)	1.122	0.851-1.481	0.414			
Pathology grade						
Well differentiated	0.139	0.017-1.114	0.063			
middle differentiated	0.244	0.034-1.753	0.161			
Poorly differentiated	0.353	0.049-2.561	0.303			
Undifferentiated	1.000					
Depth of tumor						
T1a–1b	0.501	0.264-0.950	**0.034**	0.511	0.219-1.191	0.120
T2	0.611	0.416-0.897	**0.012**	0.533	0.273-1.041	0.066
T3	1.000			1.000		
Lymph node metastasis						
N0	0.179	0.114-0.281	**<0.001**	0.124	0.041-0.377	**<0.001**
N1	0.300	0.194-0.465	**<0.001**	0.295	0.185-0.473	**<0.001**
N2	0.474	0.299-0.753	**0.002**	0.478	0.299-0.762	**0.002**
N3	1.000					
Pathological stage						
1a–1b	0.355	0.219-0.574	**<0.001**	2.701	0.578-12.621	0.207
2a–2b	0.449	0.324-0.622	**<0.001**	1.509	0.604-3.770	0.379
3a–3c	1.000			1.000		
Vessel invasive (absence vs. presence)	1.743	1.313-2.314	**<0.001**	1.195	0.881-1.621	0.253
Nerve infiltration (absence vs. presence)	1.856	1.407-2.449	**<0.001**	1.515	1.131-2.031	**0.005**
Treatment regimen						
S	1.017	0.636-1.626	0.945			
S plus postoperative C	1.098	0.650-1.853	0.727			
S plus postoperative CRT	1.000					

Figures

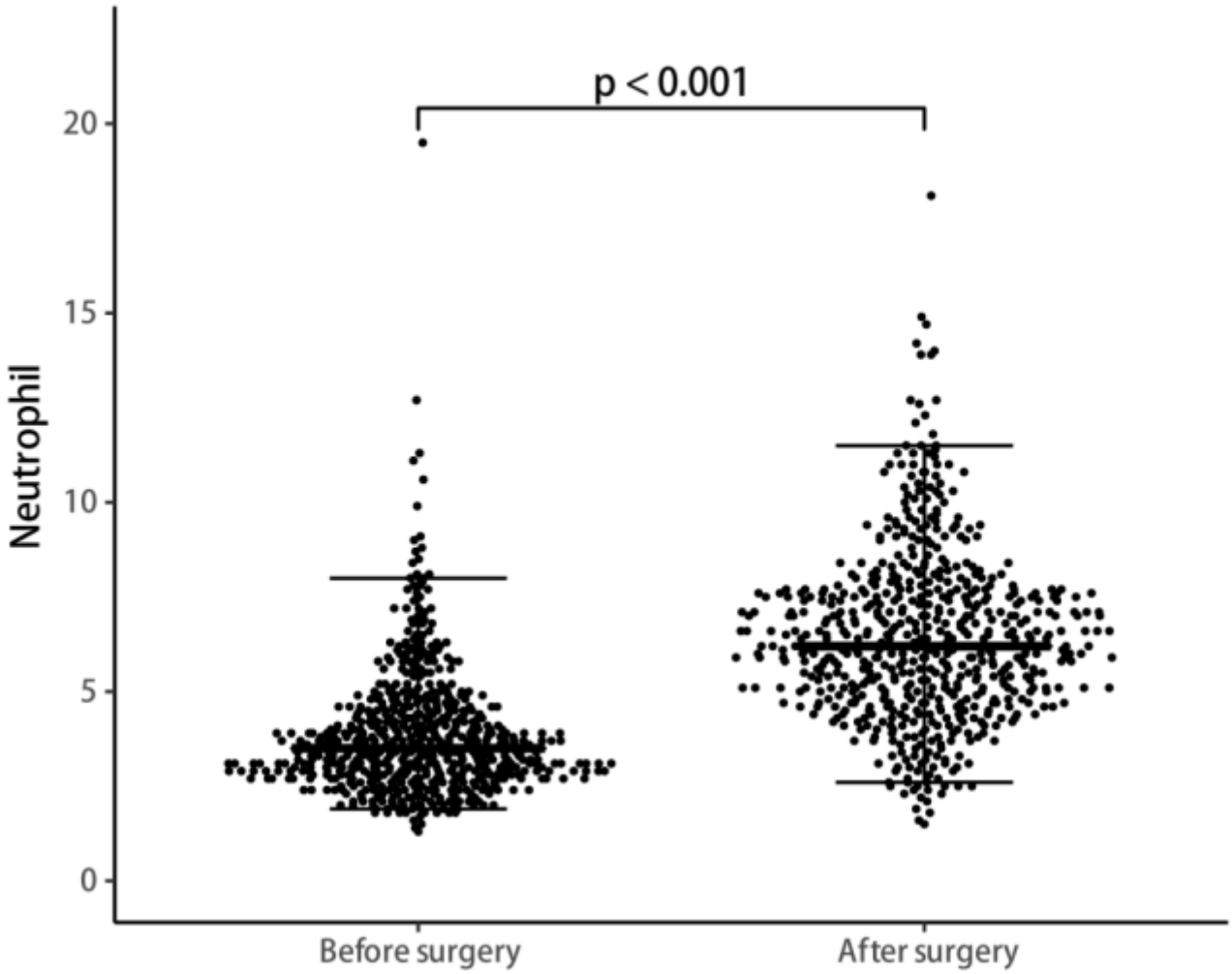

Figure 1

Differences in the neutrophil count prior to and following surgery was illustrated by beeswarm plots. The horizontal line in the middle indicates the median, the horizontal line above represents the 25th-percentile, and the horizontal line below represents the 75th-percentile.

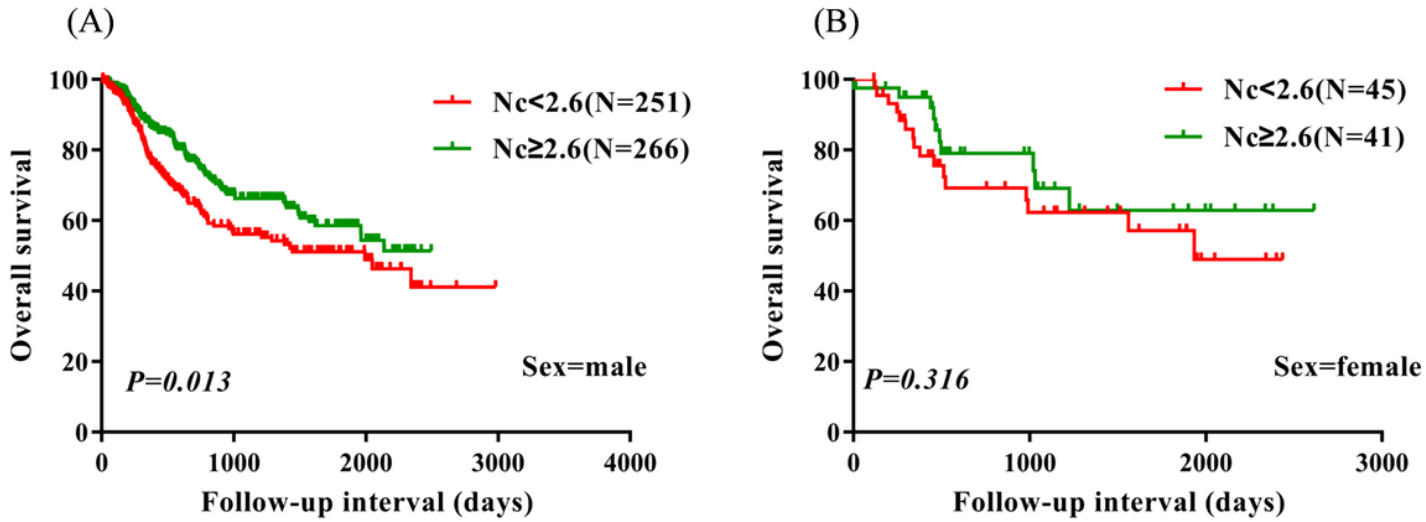

Figure 2

Overall survival analysis in male patients and female patients according to perioperative change in neutrophil count (A, B).

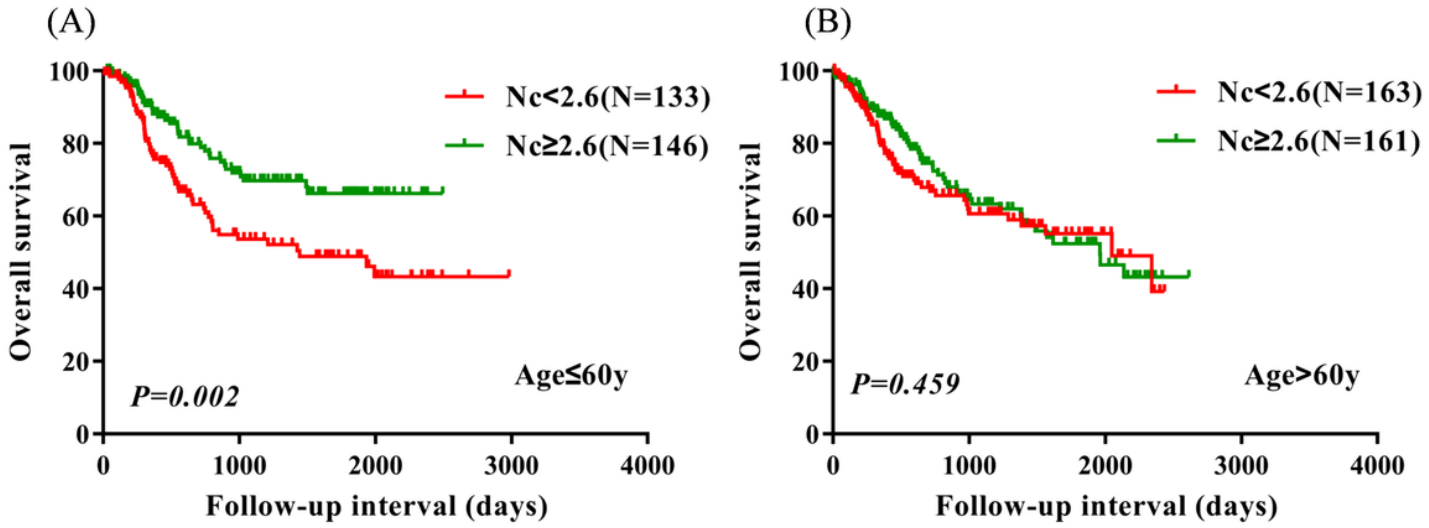

Figure 3

Overall survival analysis in age≤60 patients and age>60 patients according to perioperative change in neutrophil count (A, B).

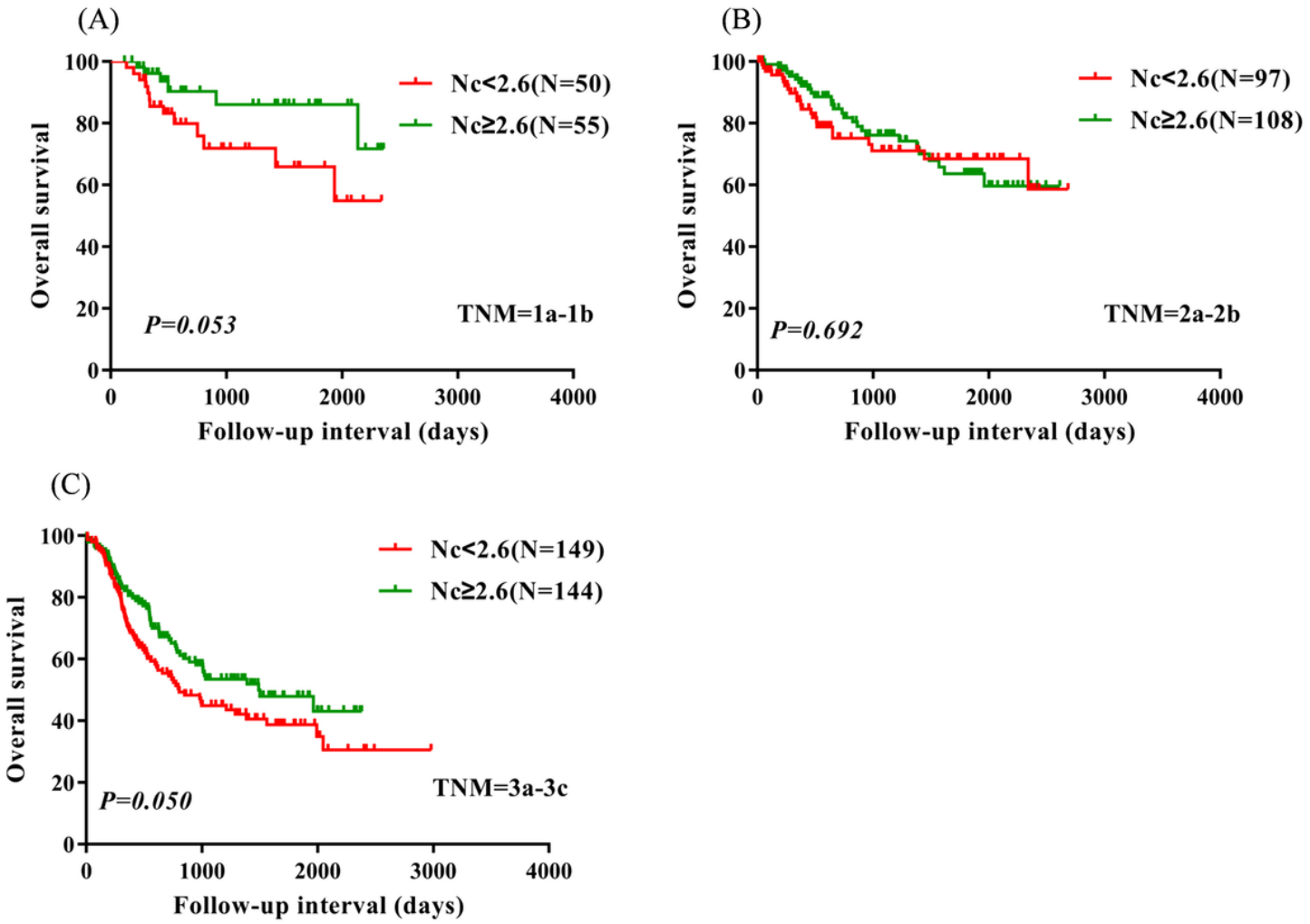

Figure 4

Overall survival analysis in patients with pathological stage 1a-1b, patients with pathological stage 2a-2b, and patients with pathological stage 3a-3c according to perioperative change in neutrophil count (A, B, C).

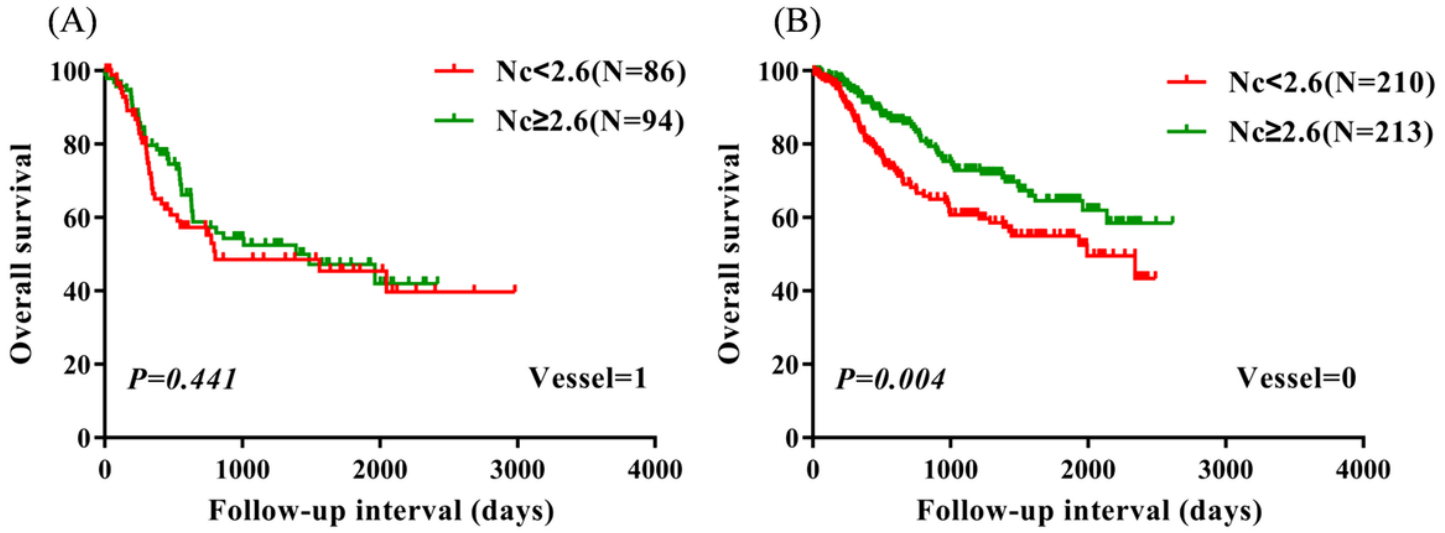

Figure 5

Overall survival analysis in patients with vessel invasive and patients without vessel invasive according to perioperative change in neutrophil count (A, B).

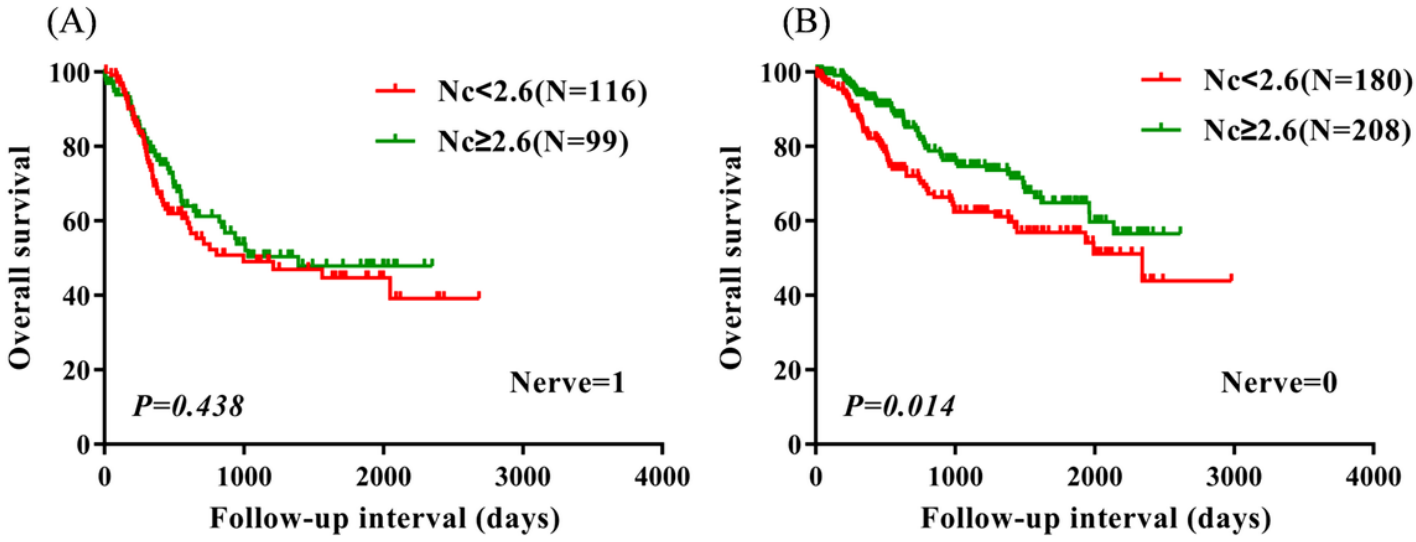

Figure 6

Overall survival analysis in patients with nerve infiltration and patients without nerve infiltration according to perioperative change in neutrophil count (A, B).

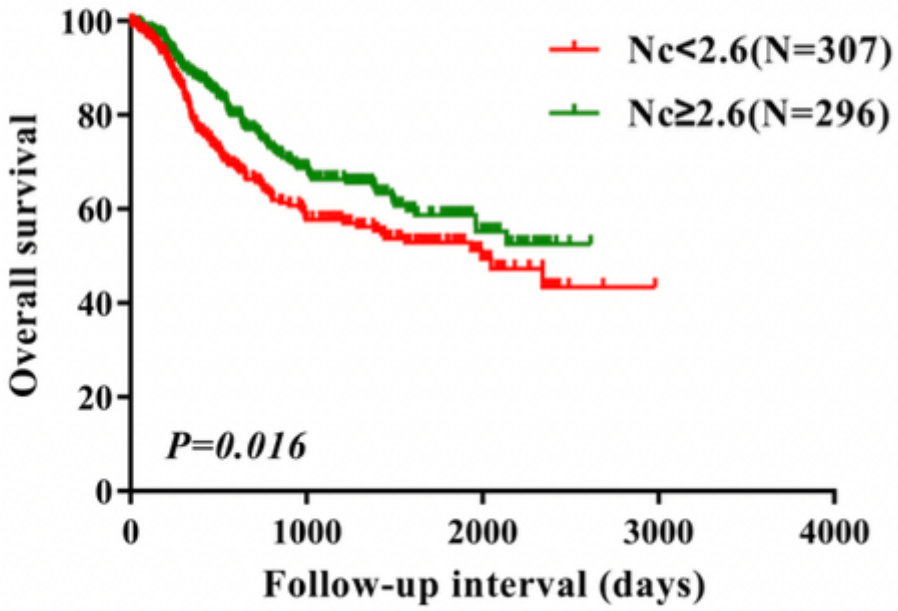

Figure 7

Overall survival analysis in all 603 patients with ESCC according to perioperative change in neutrophil count.