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Original Article

Effect of the number of positive niduses in extranodal soft tissues on the overall survival of gastric cancer patients

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Abstract: Background: This study aimed to elucidate the intensive effect of the number of positive niduses in extranodal soft tissues (PNESTs) on the overall survival of gastric cancer (GC) patients after curative gastrectomy. Methods: The clinicopathological data of 961 GC patients were studied to determine whether increased number of PNESTs was a high-risk factor for the dismal prognosis of GC patients. Results: Survival analyses revealed that the number of PNESTs was negatively associated with the overall survival (OS) (P<0.001) and was an independent prognostic predictor (HR=1.360, P<0.001) for the 961 GC patients. The number of PNESTs, with the smallest BIC value (421.947, P=0.018), was identified as the most intensive independent predictor of prognosis for the 961 GC patients. Correlation analyses showed that the pT stage, pN stage, pTNM classification, tumor size, and type of gastrectomy were significantly related to the number of PNESTs, and that tumor size was the most closely relevant factor in all included GC patients. These findings revealed that increased number of PNESTs was suitable to evaluate the dismal prognosis of GC patients. Conclusions: The number of PNESTs is an important high-risk clinicopathological characteristic for improving the accuracy of prognostic evaluation of GC patients.

Keywords: Stomach, neoplasm, nidus, soft tissue, prognosis

Introduction

Gastric cancer (GC) is the second leading cause of cancer-related deaths worldwide for more than two decades, and the overall survival (OS) of GC patients remains dismal [1, 2]. The number of lymph node metastasis of GC is the one of the most intensive prognostic predictors for the OS of patients after surgery [3, 4]. To date, the prognostic evaluation for GC patients is commonly performed during the N stage, which is based on the positive nodal count, compared with the other categories of lymph node metastasis. However, many researchers stated that the positive niduses in extranodal soft tissues (PNESTs) should be redefined as an important prognostic predictor, owing to the involvement of primary tumor proliferation or the spreading of lymphatic system. According to recent reports, 10%-28% of GC specimens were revealed by the PNESTs through a routine pathological examination [5]. Tanabe et al. [6] reported that esophageal squamous cell carcinoma patients with PNESTs have a higher risk of recurrence, and PNESTs are the most intensive negative factor in evaluating the OS of patients after surgery. Furthermore, PNEST patients with 1-3 metastatic lymph nodes exhibited a lower 5-year survival rate and a significantly higher recurrence rate than the negative NEST (NNEST) patients with 1-3 metastatic lymph nodes. Conversely, Greenberg et al. [7] reported that oral tongue cancer PNEST patients with multiple positive lymph nodes have shorter median OS, lower disease-specific survival rate, and smaller disease-free interval than PNEST patients with a single positive lymph node. Therefore, the PNEST patients with the multiple positive lymph nodes were recommended to participate in the clinical trials to intensify their regional controlling and systemic therapy. A recent systematic review that includes 3250

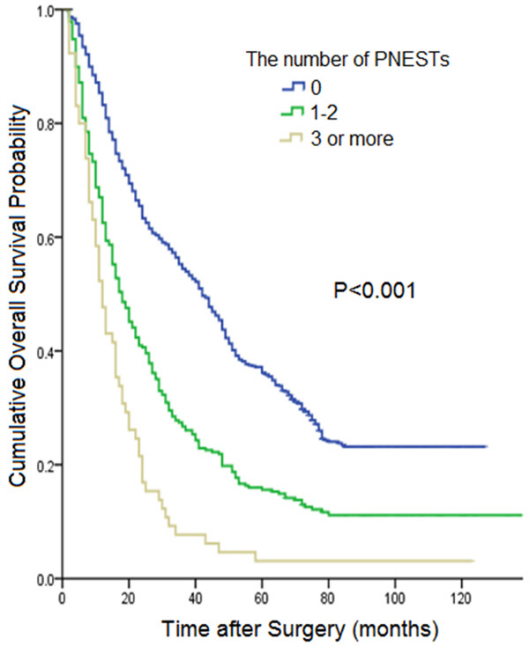

Figure 1. Survival curve of patients according to the number of PNESTs subgroups.

GC patients showed that PNESTs are significantly associated with a higher risk of all-cause mortality, cancer-specific mortality, and disease recurrence compared with other clinicopathological characteristics [8].

Many investigators stated that PNEST should be used as a negative predictor of the OS of patients after gastrectomy; however, the specific contribution of PNESTs to the prognosis of GC remains unknown. Jiang et al. [9] found that the existence of PNESTs, which represents the aggressiveness of the tumor, is a significant independent predictor of reduced disease-free survival and OS in GC patients. Thus, PNESTs should be incorporated into the N stage to improve the accuracy of prognostic evaluation. Chen et al. [10] also reported that PNEST patients have more advanced primary tumor and worse prognosis than NNEST patients, which indicates that PNESTs should be considered as an essential supplementary to the TNM classification for GC.

The correlation between the number of PNESTs and the prognosis of GC patients is not yet explained in any formally published article. In the present study, we analyzed the clinicopathological data and follow-up records of GC patients to elucidate the detailed correlation between the number of PNESTs and the prognosis of GC patients. In addition, we aim to determine whether the number of PNESTs can be used as a novel variable in improving the precious staging of tumor and the accuracy of the prognostic evaluation for patients.

Materials and methods

Patients

The eligible participants in this study include patients aged 20 years or older who underwent surgical resection for GC at the Gastric Cancer Surgery Division, Tianjin Medical University Cancer Hospital from March 2003 to December 2011. Patients were selected according to the eligibility criteria: 1) patients with histologically proven primary cancer of the stomach; 2) patients without a history of gastrectomy or other malignancies; 3) patients without any non-curative surgical factors (such as distant metastasis, positive peritoneal cytology, or peritoneal dissemination); 4) patients with non-esophagogastric junction tumor; 5) patients with pathologically negative resection margins (R0 resection); and 6) patients who remained alive during the initial hospital stay and during the first postoperative month. After applying these criteria, 961 GC patients were included in the study.

Surgical management

All patients underwent standard R0 resection. Primary tumors were resected en bloc using lymphadenectomy (D1, D2, or D2+) according to the guidelines of the Japanese Gastric Cancer Association [11]. Limited lymphadenectomy (D1) entails the removal of the perigastric nodes only, whereas extended lymphadenectomy (D2 or D2+) involves the removal of both perigastric and extragastric nodes. The choice of surgical procedure for gastrectomy (total gastrectomy or subtotal gastrectomy) was according to the attending surgeon's preference and was based on the gastric cancer treatment guidelines in Japan [12].

Follow-up evaluation

After undergoing curative surgery, all patients were required to follow up every 3 or 6 months for 2 years and annually thereafter until death. The median follow-up time for the entire cohort

Table 1. Survival analysis of the 961 gastric cancer patients

Characteristics	Cases	5-YSR (%)	χ^2 value	Univariate *P* value	Multivariate *P* value	Hazard ratio (95% CI)
Age			2.695	0.101		
≤65 yrs	614	28.7				
≥66 yrs	347	27.5				
Gender			0.068	0.769		
Male	688	27.9				
Female	273	27.5				
pT stage			72.410	<0.001	<0.001	1.219 (1.095-1.357)
T1a	9	66.7				
T1b	17	64.7				
T2	99	55.6				
T3	53	37.7				
T4a	742	23.0				
T4b	41	6.0				
pN stage			177.161	<0.001	<0.001	1.294 (1.215-1.377)
N0	306	45.8				
N1	191	28.8				
N2	236	21.2				
N3a	159	11.9				
N3b	69	13.0				
pTNM classification			188.866	<0.001		
Ia	21	71.4				
Ib	62	59.7				
IIa	43	39.5				
IIb	224	42.0				
IIIa	162	24.7				
IIIb	228	18.9				
IIIc	221	8.1				
Tumor size			35.045	<0.001		
≤4	359	37.3				
>4	602	22.1				
Tumor location			22.272	<0.001	0.020	1.080 (1.012-1.151)
Upper third	291	24.7				
Middle third	89	32.6				
Lower third	381	34.9				
>2/3 stomach	200	21.0				
Type of gastrectomy			62.247	<0.001	<0.001	0.796 (0.727-0.871)
Total	255	15.7				
Proximal subtotal	252	25.0				
Distal subtotal	454	36.1				
Extent of lymphadenectomy			17.562	<0.001	<0.001	0.839 (0.779-0.903)
Less than D2	428	22.4				
D2 or D2+	533	32.1				
No. of examined lymph nodes			0.026	0.872		
≤15	525	27.6				
≥16	436	28.0				
Lauren classification			10.678	0.005		
Intestinal	262	34.0				

Diffuse	636	24.2				
Mixed	23	26.1				
PNEST			93.974	<0.001		
Negative	608	36.2				
Positive	353	13.3				
The number of PNESTs			120.904	<0.001	<0.001	1.360 (1.201-1.541)
0	608	36.2				
1-2	288	15.6				
3 or more	65	7.7				

5-YSR 5-year survival rate.

Table 2. Akaike information criterion (AIC) and Bayesian information criterion (BIC) values test of prognosis characteristics for the gastric cancer patients

	AIC value	BIC value	-2 Log likelihood value	P
pT stage	401.396	430.604	389.396	<0.001
pN stage	413.790	442.998	401.790	<0.001
Tumor location	387.245	416.453	375.245	0.743
Type of gastrectomy	398.781	427.989	386.781	0.001
Extent of lymphadenectomy	401.234	430.441	389.234	<0.001
The number of PNESTs	392.739	421.947	380.739	0.018

TNM tumor-node-metastasis.

was 39 months (range of 2-138 months). The follow-up evaluation of all the patients included in this study was completed in December 2016. The OS rate was calculated from the day of surgical resection until the time of death or final follow-up. Ultrasonography, computed tomography scans, chest X-rays, and endoscopy were performed at every visit.

Statistical analysis

To determine the most appropriate cut-off values for continuous data variables, such as tumor size and the number of PNESTs, the cut-point survival analysis [13] was adopted. According to the result of the cut-point survival analysis, the number of PNESTs intervals were as follows: NNEST (PNEST=0), the number of PNESTs between 1 and 2 (1≤PNESTs≤2), and the number of PNESTs of more than 3 (PNESTs≥3). Various clinicopathological factors were analyzed using the method of Kaplan and Meier, and the log-rank test was used to determine the univariate significance. The multivariate analysis includes factors that were potentially important for the univariate analysis (P<0.05). Models of logistic regression or Cox proportional hazards were used for the multivariate analysis. Hazard ratios and 95% confidence intervals were generated. Akaike information criterion (AIC) and Bayesian IC (BIC) values for each category were calculated to measure the discriminatory ability within the Cox proportional hazard regression model. Smaller AIC or BIC value indicates a better model for predicting outcomes [14, 15]. Significance was defined as P<0.05. All statistical analyses were performed using SPSS 22.0 software.

Results

General information

Analysis was performed on the data from 961 gastric cancer patients, including 688 men (71.6%) and 273 women (28.4%). The mean age of the patients was 60.13 ± 11.53 years (range of 20-84 years). The median OS of the patients after curative surgery was 28 months. PNESTs with a mean number of 0.7 ± 1.37 (range 0-13) per patient were dissected for the histopathological examination after surgery. All patients were divided into three categories according to the number of PNESTs: 608 patients had a NNEST (PNEST=0), 288 patients had the number of PNESTs between 1 and 2 (1≤PNESTs≤2), and 65 patients had the num-

Table 3. Correlation analyses between the number of positive niduses in extranodal soft tissues (PNESTs) and other characteristics of the 961 gastric cancer patients

Characteristics	Cases	The number of PNESTs			χ^2 value	*P* value
		0	1-2	3 or more		
Age					0.752	0.686
≤65 yrs	614	383	187	44		
≥66 yrs	347	225	101	21		
Gender					2.552	0.279
Male	688	445	196	47		
Female	273	163	92	18		
pT stage					79.806	<0.001
T1a	9	8	1	0		
T1b	17	0	0	0		
T2	99	94	5	0		
T3	53	41	11	1		
T4a	742	432	252	58		
T4b	41	16	19	6		
pN stage					155.874	<0.001
N0	306	267	34	5		
N1	191	125	55	11		
N2	236	131	87	18		
N3a	159	59	82	18		
N3b	69	26	30	13		
pTNM classification					183.859	<0.001
Ia	21	21	0	0		
Ib	62	59	3	0		
IIa	43	38	5	0		
IIb	224	190	28	6		
IIIa	162	101	53	8		
IIIb	228	121	87	20		
IIIc	221	78	112	31		
Tumor size					33.783	<0.001
≤4	359	268	79	12		
>4	602	340	209	53		
Tumor location					8.835	0.183
Upper third	291	189	85	17		
Middle third	89	56	25	8		
Lower third	381	252	108	21		
>2/3 stomach	200	111	70	19		
Type of gastrectomy					23.324	<0.001
Total	255	134	91	30		
Proximal subtotal	252	166	74	12		
Distal subtotal	454	308	123	23		
Extent of lymphadenectomy					1.778	0.411
Less than D2	428	261	137	30		
D2 or D2+	533	347	151	35		
No. of examined lymph nodes					3.463	0.177
≤15	525	346	146	33		
≥16	436	262	142	32		
Lauren classification					6.760	0.149
Intestinal	262	180	68	14		
Diffuse	636	381	205	50		
Mixed	14	8	1			

ber of PNESTs of more than 3 (PNESTs≥3).

Significant factor characteristics associated with OS of GC after curative surgery

According to the univariate analysis, the following 10 clinicopathological characteristics were significantly associated with OS after the curative surgery for the 961 patients enrolled in the study: pT stage (according to the 7th-edition TNM classification), pN stage (according to the 7th-edition TNM classification), pTNM classification (according to the 7th-edition TNM classification), tumor size, tumor location, type of gastrectomy, extent of lymphadenectomy, Lauren classification, and the number of PNESTs (**Figure 1**; **Table 1**). According to the multivariate analysis (Cox proportional hazards model with Bootstrap procedure), the number of PNESTs was an independent prognostic predictor (HR=1.360, P<0.001) for the 961 GC patients; other qualifying predictors include the pT stage (HR=1.219, P<0.001), pN stage (HR=1.294, P<0.001), tumor location (HR=1.080, P=0.020), type of gastrectomy (HR=0.796 , P<0.001), and extent of lymphadenectomy (HR=0.839, P<0.001) (**Table 1**).

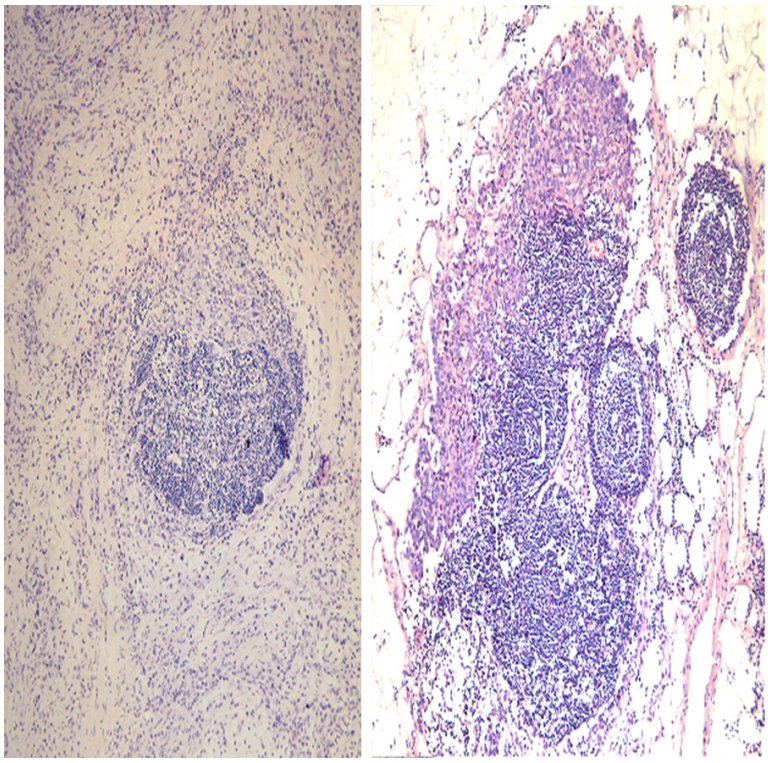

Figure 2. False PNESTs: With the continuous proliferation of cancer cells, some perigastric nodes were destroyed and the normal configuration was disrupted; thus, the positive lymph nodes were mistakenly identified as PNESTs.

Significant factors associated with the number of PNESTs after curative surgery for GC

According to the multinomial logistical regression analysis, the number of PNESTs, with the smallest BIC value (421.947, P=0.018), was the most intensive independent predictor of prognosis for the 961 GC patients (**Table 2**). Furthermore, according to the correlation analyses between the number of PNESTs and other characteristics of the patients, the factors associated with the number of PNESTs include the pT stage, pN stage, pTNM classification, tumor size, and type of gastrectomy (**Table 3**). Tumor size was identified as the most closely relevant factor to the number of PNESTs in all included GC patients.

Discussion

Alakus et al. [16] stated that PNEST is an independent negative predictor of prognosis in GC patients. Recently, Jiang et al. [9] also reported that PNEST is closely associated with cancer cell aggressiveness, and the presence of PNEST represents a statistical significance to the OS of GC patients after curative resection. In this study, we further revealed that the count of PNESTs is potentially an independent prognostic factor for GC patients. Thus, PNESTs should be meticulously validated for the subtle discriminations of the survival curves of various subgroups of GC patients. In theory, the quantitative division of PNESTs reflects the biological behavior of the cancer cells, including migration, invasion, proliferation, chemotaxis, and lymphangiogenesis. Therefore, Etoh et al. [17] previously proposed that PNEST should be adopted in the TNM staging system to improve the accuracy of the prognostic prediction of GC.

Although the TNM classification is the optimal indicator in evaluating the prognosis of GC patients, further studies showed that the prognosis of GC is interfered by a variety of clinicopathological characteristics [18, 19]. A large variation of the incidence (between 10% and 28%) of PNEST was reported in several retrospective investigations [5, 17]. However, no consensus was formed about the rational explanation for the mechanisms of the germination of PNEST in GC patients.

Two principal explanations are considered as the potential mechanisms of PNESTs in cancer cases. First, cancer cells depart from the primary lesion and then disseminate in the soft tissues via the lymphatic vessels, blood circulation, or serosal penetration. This explanation of germination of PNESTs is the oretically applicable to patients with systematic spreading or distant metastasis, which indicates that the prognoses of patients are dismal. Furthermore, cancer cells are detected in the perigastric lymph nodes in minor patients with GC. With the continuous proliferation of cancer cells, some perigastric nodes are damaged and the normal configuration is destroyed; thus, the pathologists might mistakenly identify the posi-

tive lymph nodes as PNESTs (**Figure 2**). Positive lymph nodes are false PNESTs but are also significantly associated with the prognosis of GC patients.

In the present study, we showed a significant correlation between PNESTs and pN stage in the GC patients, revealing the necessity to include the prognostic evaluation abilities of various counts of PNESTs in the quantitative analysis. Similar conclusions were proposed by Etoh [17].

In this study, we initially analyzed the contribution and clinical applicability of the count of PNESTs in the accurate evaluation of the OS of GC patients. Among the clinicopathological variables, the count of PNESTs was the most intensive predictor in accurately evaluating the OS of all included GC patients after surgery. The results of multinomial logistical regression analysis also showed that the count of PNESTs effectively improved the accuracy of OS prediction of patients by using the BIC value calculation. As a comprehensive clinicopathological factor in evaluating the OS of GC patients, the number of PNESTs generally helps in the accurate assessment of the patients' prognosis and in the selection of the potential optimal treatment for patients. In the present study, all included patients were strictly selected in accordance with the standard R0 resection plus lymphadenectomy. Therefore, we believe our results provide some positive information for future studies. The limitation of the present study is the relatively small number of patients. A subsequently large scale and multi-center clinical study will be conducted to further confirm the correlation between the number of PNESTs and the prognosis of GC patients.

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Disclosure of conflict of interest

None.

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References

[1] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.

[2] Kanda M, Kodera Y and Sakamoto J. Updated evidence on adjuvant treatments for gastric cancer. Expert Rev Gastroenterol Hepatol 2015; 9: 1549-1560.

[3] Marrelli D, Morgagni P, de Manzoni G, Coniglio A, Marchet A, Saragoni L, Tiberio G, Roviello F; Italian Research Group for Gastric C. Prognostic value of the 7th AJCC/UICC TNM classification of noncardia gastric cancer: analysis of a large series from specialized Western centers. Ann Surg 2012; 255: 486-491.

[4] Deng J, Zhang R, Pan Y, Wang B, Wu L, Jiao X, Bao T, Hao X and Liang H. Comparison of the staging of regional lymph nodes using the sixth and seventh editions of the tumor-node-metastasis (TNM) classification system for the evaluation of overall survival in gastric cancer patients: findings of a case-control analysis involving a single institution in China. Surgery 2014; 156: 64-74.

[5] Tanaka T, Kumagai K, Shimizu K, Masuo K and Yamagata K. Peritoneal metastasis in gastric cancer with particular reference to lymphatic advancement; extranodal invasion is a significant risk factor for peritoneal metastasis. J Surg Oncol 2000; 75: 165-171.

[6] Tanabe T, Kanda T, Kosugi S, Ikeda Y, Makino S, Komukai S, Ohashi M, Suzuki T and Hatakeyama K. Extranodal spreading of esophageal squamous cell carcinoma: clinicopathological characteristics and prognostic impact. World J Surg 2007; 31: 2192-2198.

[7] Greenberg JS, Fowler R, Gomez J, Mo V, Roberts D, El Naggar AK and Myers JN. Extent of extracapsular spread: a critical prognosticator in oral tongue cancer. Cancer 2003; 97: 1464-1470.

[8] Veronese N, Fassan M, Wood LD, Stubbs B, Solmi M, Capelli P, Pea A, Nottegar A, Sergi G, Manzato E, Carraro S, Maruzzo M, Cataldo I, Bagante F, Barbareschi M, Cheng L, Bencivenga M, de Manzoni G and Luchini C. Extranodal extension of nodal metastases is a poor prognostic indicator in gastric cancer: a systematic review and meta-analysis. J Gastrointest Surg 2016; 20: 1692-1698.

[9] Jiang N, Deng JY, Ding XW, Ke B, Liu N and Liang H. Node-extranodal soft tissue stage based on extranodal metastasis is associated with poor prognosis of patients with gastric cancer. J Surg Res 2014; 192: 90-97.

[10] Chen XL, Zhao LY, Xue L, Xu YH, Zhang WH, Liu K, Chen XZ, Yang K, Zhang B, Chen ZX, Chen JP, Zhou ZG and Hu JK. Prognostic significance and the role in TNM stage of extranodal metastasis within regional lymph nodes station in gastric carcinoma. Oncotarget 2016; 7: 67047-67060.

[11] Jaehne J, Meyer HJ, Maschek H, Geerlings H, Burns E and Pichlmayr R. Lymphadenectomy in gastric carcinoma. A prospective and prognostic study. Arch Surg 1992; 127: 290-294.

[12] Nakajima T. Gastric cancer treatment guidelines in Japan. Gastric Cancer 2002; 5: 1-5.

[13] Smith DD, Schwarz RR and Schwarz RE. Impact of total lymph node count on staging and survival after gastrectomy for gastric cancer: data from a large US-population database. J Clin Oncol 2005; 23: 7114-7124.

[14] Cho YK, Chung JW, Kim JK, Ahn YS, Kim MY, Park YO, Kim WT and Byun JH. Comparison of 7 staging systems for patients with hepatocellular carcinoma undergoing transarterial chemoembolization. Cancer 2008; 112: 352-361.

[15] Nitsche U, Maak M, Schuster T, Kunzli B, Langer R, Slotta-Huspenina J, Janssen KP, Friess H and Rosenberg R. Prediction of prognosis is not improved by the seventh and latest edition of the TNM classification for colorectal cancer in a single-center collective. Ann Surg 2011; 254: 793-800; discussion 800-791.

[16] Alakus H, Holscher AH, Grass G, Hartmann E, Schulte C, Drebber U, Baldus SE, Bollschweiler E, Metzger R and Monig SP. Extracapsular lymph node spread: a new prognostic factor in gastric cancer. Cancer 2010; 116: 309-315.

[17] Etoh T, Sasako M, Ishikawa K, Katai H, Sano T and Shimoda T. Extranodal metastasis is an indicator of poor prognosis in patients with gastric carcinoma. Br J Surg 2006; 93: 369-373.

[18] Alici S, Kaya S, Izmirli M, Tuncer I, Dogan E, Ozbek H and Sayarlioglu H. Analysis of survival factors in patients with advanced-stage gastric adenocarcinoma. Med Sci Monit 2006; 12: CR221-229.

[19] Orsenigo E, Carlucci M, Braga M, Tomajer V, Di Palo S, Tamburini A, Di Carlo V and Staudacher C. [Prognostic factors of gastric neoplasms: experience with 1,074 cases undergoing surgical treatment at a single center]. Suppl Tumori 2005; 4: S86-87.