Does a minimum number of 16 retrieved nodes affect survival in curatively resected gastric cancer?

Title: DOES A MINIMUM NUMBER OF 16 RETRIEVED NODES AFFECT SURVIVAL IN CURATIVELY RESECTED GASTRIC CANCER?

Running head: LYMPH NODE COUNT AFTER CURATIVE GASTRECTOMY

Alberto Biondi MD[1] biondi.alberto@tiscali.it
Domenico D'Ugo MD FACS[1] ddugo@rm.unicatt.it
Ferdinando CM Cananzi MD[2] fcm.cananzi@hotmail.it
Valerio Papa MD[3] valeriopapa@msn.com
Andrea Borasi MD[4] borasi.andrea@gmail.com
Federico Sicoli MD[1] federico.sicoli@yahoo.it
Maurizio Degiuli MD[4] mdegiuli@hotmail.com
Giovanni Doglietto MD[3] gbdoglietto@rm.unicatt.it
Roberto Persiani MD[1] rpersiani@rm.unicatt.it

Affiliations

(1) General Surgery Unit Department of Surgery, "A. Gemelli" University Hospital Catholic University of Rome
(2) Surgical Oncology Unit - Humanitas Clinical and Research Center, via Manzoni 56, 20089 Rozzano (MI) - Italy
(3) Digestive Surgery Unit Department of Surgery, "A. Gemelli" University Hospital Catholic University of Rome
(4) Division of Surgical Oncology, Azienda Ospedaliera Citta della Salute e della Scienza di Torino, Turin, Italy

Corresponding Author
Alberto Biondi, MD
General Surgery Unit, Department of Surgery, "A. Gemelli" University Hospital
Catholic University of Rome
Largo A. Gemelli 8, 00167 Rome, Italy
Tel. 0039.06.30154511 Fax. 0039.06.3051162
e-mail: biondi.alberto@tiscali.it

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Abstract

Background: According to the TNM classification, the analysis of 16 or more lymph nodes is required for the appropriate staging of gastric cancer. The aim of this study was to evaluate whether this number of resected lymph nodes also affects survival.

Methods: This was a multicenter retrospective study based on an analysis of 992 patients with gastric adenocarcinoma who underwent curative resection between January 1980 and December 2009. Patients were classified according to the number of resected lymph nodes (<16 and ≥16 lymph nodes), the anatomical extent of lymph node dissection (D2 vs. D1), and the staging criteria of the seventh edition of the UICC/AJCC TNM staging system. Survival estimates were determined by univariate and multivariate analyses.

Results: Based on the univariate and multivariate analyses, the resection of 16 or more lymph nodes was associated with significantly better survival [p=0.002; hazard ratio (HR) (95% confidence interval [CI]): 0.519 (0.345-0.780)]. Patients with a lymph node count <16 had a significantly worse survival rate than patients with a lymph node count ≥16 in the pN0 (p=0.001), pN1 (p=0.007) and pN2 (p=0.001) stages. In the majority of cases, ≥16 lymph nodes were retrieved when D2 dissection was performed.

Conclusions: in gastric cancer the retrieval of less than 16 lymph nodes may cause inaccurate staging and/or inadequate treatment, thus affecting survival rates. These patients should be considered a high-risk group for stage migration and worse survival compared with those who have a retrieval of more than 16 lymph nodes.

Introduction

Lymph node metastasis is the most important predictor of survival in patients with gastric cancer [1,2]. The classification of lymph node metastasis that is most appropriate for predicting the prognosis of patients with gastric cancer who have undergone curative surgery remains controversial. To date, three main classifications of lymph node metastasis have been applied to predict the prognosis of gastric cancer patients worldwide, namely, classifications that are based on the number of positive nodes, the location of positive nodes, and the ratio between the metastatic and examined nodes. By 2010, the two main lymph node classifications, namely, the 7th edition of the International Union Against Cancer (UICC)/American Joint Committee on Cancer (AJCC) TNM staging system [3] and the 14th Japanese gastric cancer classification [4], were consistent regarding the concept that the absolute number of positive lymph nodes represents the gold standard in the reporting of pathological staging in gastric cancer. Both classifications established the positive node cut-off values for the N categories as follows: N1 = 1–2 nodes; N2 = 3–6 nodes; N3a = 7–15 nodes; and N3b = 16 or more nodes. As a consequence, both classifications recommend the examination of 16 or more regional lymph nodes to determine the correct N status even though this recommendation is not intended to be a stringent requirement.

Three main factors significantly influence the reporting of pathological lymph node status, including the surgical extent of lymph node dissection, accuracy in the pathological examination, and individual differences in the total number of lymph nodes [5-8]; under-staging is most likely to occur when too few lymph nodes are examined [9].

Together with debates on the staging of cancer, large population studies have demonstrated a strong association between the number of lymph nodes analyzed and improved survival [10,11], which indicates that an adequate number of lymph nodes retrieved through an extended lymph node dissection may have a significant impact on the prognosis of patients with gastric cancer who receive potentially curative treatment.

The extent of lymph node dissection continues to represent an important issue with regard to surgical research in gastric cancer; in addition, whether a higher lymph node count should be considered a requirement for proper staging or as an indicator of adequate surgical treatment is still a matter of controversy.

The aim of this study was to investigate the impact of the lymph node count, with a focus on a cut-off of 16 lymph nodes, on staging and survival in patients with gastric cancer who received curative resection.

Materials and Methods

This multicenter retrospective study was based on an analysis of 1465 patients with gastric adenocarcinoma who underwent surgery with curative intent between January 1980 and December 2009 at 3 surgical units in Italy (1st General Surgery Unit and Digestive Surgery of Catholic University of Rome; Department of Surgery University of Turin, San Giovanni Battista Hospital, Turin). Only patients who underwent curative resection (R0) were included in this study. According to the 7th edition of UICC/AJCC TNM, esophago-gastric junction cancers were excluded from the study. When available, patients with positive peritoneal cytology were excluded from the study as considered R1 resection. The patients with locally advanced gastric cancer (T3/4 N+) who underwent preoperative chemotherapy as a neoadjuvant treatment were excluded from the study. Clinical- and cancer-specific data were collected prospectively and entered into a database. The primary clinico-pathological data, such as age, gender, extent of gastrectomy, extent of lymphadenectomy, tumor diameter, tumor site, Lauren's histotype, tumor stage, grading, total lymph node count (TLC, i.e., total number of harvested lymph nodes), number of metastatic lymph nodes (MtLNs), number of negative lymph nodes (NeLNs), and survival data, were analyzed. The tumor stage was coded according to the TNM system, as described in the UICC/AJCC 7th edition.

For the purpose of the study, the patients were divided into two groups according to the UICC/AJCC recommendation as follows: patients with <16 harvested lymph nodes (TLC- group) and patients with ≥16 harvested lymph nodes (TLC+ group).

The extent of stomach resection was related to the primary tumor site; specifically, total gastrectomy was performed in all proximal tumor locations, and subtotal gastrectomy was performed in distal tumor locations, provided that a 5- to 6-cm safety margin was present. The extent of the lymph node dissection was based on the individual surgeon's judgment and attitude and was defined according to the 14th Japanese Gastric Cancer Association (JGCA) rules. The intent of resection was described as curative when all macroscopic and microscopic disease seemed to have been removed and was considered palliative when there was intraoperative macroscopic evidence of residual or metastatic disease. Immediately after the operation, the specimens were carefully prepared, and the lymph node stations were dissected by a member of the surgical team according to the JGCA rules to achieve an accurate pathological staging.

A close postoperative follow-up was planned for all patients and included clinical history, physical examination, complete blood count and blood serum analysis (every 3 months for the first 2 years and every 6 months thereafter), endoscopy (every 6 months in the first year and once a year thereafter), abdominal ultrasound (every 3 months in the first year and every 6 months thereafter), and chest and abdominopelvic computed tomography (CT) scans (every year). Postoperative adjuvant chemotherapy was administered according to the local hospital’s protocol.

Continuous variables were analyzed using the Mann-Whitney test and t-test (when appropriate), and Spearman's rho was used to estimate the bivariate correlations. The chi-square and Fisher’s exact test were used for the analysis of other parameters. P values of <0.05 were considered statistically significant.

Overall survival was calculated according to the Kaplan-Meier method from the date of operation, while the log-rank test was used to assess any significant differences between the groups.

Multivariate analysis of prognostic factors related to overall survival was performed using Cox's proportional hazards model with the forward likelihood method; variables that were statistically significant at the $p<0.1$ level by Kaplan-Meier analysis were included in the multivariate analysis. The statistical analysis was performed with SPSS (19^{th} edition) software for Windows^{TM}.

Results

A total of 1,465 patients underwent surgical treatment for gastric cancer from January 1980 to December 2009, 992 of whom underwent curative resection and were included in this study. The records of 4 patients contained incomplete clinico-pathological data; therefore, those patients were excluded from the statistical analysis. A total of 823 patients (83.3%) had 16 or more lymph nodes harvested (TLC+ group), and 165 (16.7%) had 15 or fewer lymph nodes harvested (TLC- group). The main clinico-pathological data and the differences between the two groups are reported in Table 1. Statistically significant differences were found with respect to age, tumor location, extent of gastrectomy, pN stage, extent of lymphadenectomy, number of metastatic lymph nodes, number of negative lymph nodes, total lymph node count, period of treatment and administration of adjuvant therapy. There were no differences with respect to gender, tumor size, Lauren's histotype, grading, or pTNM stage.

Significant bivariate correlations were identified between TLC and MtLN (Spearman's rho: 0.135; $p<0.001$), TLC and NeLN (Spearman's rho: 0.940; $p<0.001$), and MtLN and NeLN (Spearman's rho: -0.108; $p=0.001$).

The ratio between the number of node-positive patients and the total number of patients (i.e., the positivity rate) was related to TLC (Mann-Whitney test: $p=0.002$; Spearman's rho: 0.886, $p<0.001$; Fig. 1). Additionally, the proportion of patients with a more advanced pN stage was significantly associated with an increased TLC (chi-square test: $p<0.001$; Fig. 2).

Survival analysis

The median follow-up duration from the date of surgery was 44 months (range 1 - 240). Thirty-eight patients were lost at follow up.

The 3- and 5-year overall survival rates were 74.6% and 65.1%, respectively.

The results of the survival data according to the Kaplan-Meier and Cox regression analyses are shown in Table 2. There was a statistically significant difference in the survival rates between the TLC- and TLC+ groups (3-year survival rates, 65.5% vs. 76.2%, respectively; 5-year survival rates, 51.5% vs. 67.6%, respectively; log-rank $p<0.001$ - Fig 3). TLC≥16 remained a significant prognostic factor (p=0,015) also in patients who had D2 lymph node dissection. Conversely, in TLC+ group D2 lymph node dissection did not show any prognostic impact (D1 vs. D2: 3-year survival rates, 82.1% vs. 75.4%, respectively; 5-year survival rates, 77% vs. 66.4%, respectively; log-rank p=0.096).

As regards extent of gastrectomy (subtotal and total gastrectomy) in D2 subgroup (762 patients), TLC≥16 lost his prognostic impact (p=0,056 in subtotal gastrectomy and 0,066 in total gastrectomy group) probably because the number of patients with low TLC (<16) is too small for reliable comparison (40 and 15 patients in subtotal and total gastrectomy group, respectively).

Since three centers were involved in the study, when considering the center effect on lymph node retrieval and survival, no statistically significant differences were found.

When the pN-TNM stage was stratified by the number of harvested lymph nodes, the TLC- group had a significantly worse survival rate than the TLC+ group in the pN0 (p=0.001), pN1 (p=0.007), and pN2 (p=0.001) stages; the group of pN3 TLC- patient was too small to perform any comparison (Table 3). Moreover, similar survival results were observed when the patients were classified by the pTNM classification and stratified by TLC (stage IA: p=0.022, IB: p=0.002, IIA: p=0.048, IIB: p=0.058, IIIA: p=0.032, IIIB: p=0.011, IIIC: p=0.271).

Based on the multivariate survival analysis, age, gender, type of gastrectomy, pT, pN, and TLC were identified as independent prognostic factors (Table 2).

Discussion

The use of the number of metastatic lymph nodes as a classification criterion for nodal staging in gastric cancer was introduced by the AJCC/UICC in 1997 and, more recently, by the JGCA in 2010. Both associations suggest that at least 16 regional lymph nodes should be evaluated to properly assess the nodal stage, although this is currently not mandatory [3,4].

In this large, multicenter, retrospective study, the lymph node count after radical surgery for gastric carcinoma and its relationship with survival were investigated. The principal finding of this study is that patients with curatively resected gastric cancer who had fewer than 16 lymph nodes examined demonstrated a worse prognosis; subgroup analyses also demonstrated that this cut-off point of examined lymph nodes strongly stratifies patient survival regardless of the pTNM and pN staging. In the multivariate analyses, a total of 16 or more examined lymph nodes were an independent prognostic factor that strictly correlated with a better prognosis.

Many authors have stressed the prognostic impact of the number of lymph nodes assessed, as a greater number of lymph nodes has been correlated with a better prognosis even in patients without lymph node metastases [9,10,12-14].

The underlying mechanism of the strong influence of lymph node counts on survival is a matter of intense debate; the dominant reasons that have accounted for superior survival after the examination of larger numbers of lymph nodes are the therapeutic effect of extended lymph node dissection and stage migration [9,15].

Therefore, if we assume that an extended surgical dissection would yield a larger number of retrieved lymph nodes, we could argue that D2 lymph node dissection, which would provide more retrieved lymph nodes, would result in a survival advantage.

However, the overall therapeutic benefit of extended lymphadenectomy is still a matter of intense debate. Many single-institution studies from Eastern and Western countries [2,16] have shown

excellent survival and low rates of complications and peri-operative deaths after extended lymph node dissection. However, three European randomized controlled trials showed no long-term overall survival benefit [17-21], and two of them demonstrated a higher rate of peri-operative complications and death in the extended lymphadenectomy groups. A recent meta-analysis on this subject demonstrated a trend toward improved survival of T3/T4 patients and patients with preservation of the spleen/pancreas [22]. However, the most recent study from the Dutch Gastric Cancer Group, after 15 years of follow-up, demonstrated lower loco-regional recurrence and tumor-related death rates in the D2 group compared with D1 group [23]. Extended lymph node dissection is not routinely performed in Europe and the USA.

In this study, we were unable to demonstrate any survival benefit in patients who underwent D2 lymph node dissection. Due to the retrospective nature of this study, a selection bias in patients who underwent extended lymph node dissection for locally advanced T stage or overt lymph node metastases is possible. Irrespective of this potential bias, 92% of patients who underwent D2 lymph node dissection and 51% of patients who underwent D1 dissection had more than 16 lymph nodes retrieved. Regardless of the extent of lymph node dissection, the retrieval of more than 16 lymph nodes provided a survival benefit; conversely, in patients with more than 16 retrieved lymph nodes, performing a D2 lymph node dissection provided no survival benefit. A previous autopsy study showed that a mean of 15 nodes can be harvested from a D1 lymphadenectomy, 17–44 nodes (mean 27) can be harvested from a D2 lymphadenectomy, and 25–64 nodes (mean 43) can be harvested from a D3 lymphadenectomy [8]. Assuming that it is impossible to count lymph nodes during surgery, our data indicated that if the retrieval of more than 16 lymph nodes is desired, D2 lymph node dissection is the procedure of choice.

Along with the debate regarding the therapeutic role of D2 dissection, some authors have suggested that extended lymph node dissection combined with rigorous pathological evaluation results in improved staging rather than therapeutic benefit. The lymph node count is certainly influenced by the extension of nodal dissection, as well as by factors beyond the surgeon's control, such as the

accuracy of the pathologist's examination and individual differences in the anatomical distribution of lymph nodes in the abdomen.

The stage migration phenomenon is used to describe the under-staging that occurs due to inadequate lymph node harvesting by the surgeon or inadequate nodal counting by the pathologist, as well as up-staging that occurs from extensive lymph node identification, which may incorrectly suggest a treatment benefit. The issue of stage migration is frequently addressed as the cause of some differences in the outcome of Japanese and Western patients [9, 24]. Japanese surgeons usually dissect and retrieve as many lymph nodes as possible, while extended lymph node dissection is not routinely performed in Western institutions. Pathologists are often reluctant to retrieve more than the minimum number of nodes that is required for correct TNM staging. A retrospective study revealed that after D2 dissections, Japanese pathologists identified an average of 62 lymph nodes, while American pathologists identified only 12 lymph nodes [25].

In this study, in patients who underwent total gastrectomy, an adequate number (≥16) of retrieved lymph nodes was associated with a younger age and with tumors that were located in the upper third of the stomach. Moreover, we observed a positive linear correlation between TLC and metastatic lymph nodes, indicating that patients who had more retrieved lymph nodes were more likely to have an advanced pN stage (Fig. 2). These data, together with the improved survival results for each stage subgroup of patients with more than 16 retrieved lymph nodes, would suggest a stage migration phenomenon even if the stage distribution is not different between patients with an adequate or inadequate number of examined lymph nodes. However, we are unable to distinguish between patients who are under-staged and patients who may benefit from extended lymph node dissection.

This study had some limitations. First of all, this is a retrospective study covering a long time period, implying treatment biases resulting from progressively increasing surgical skill and awareness about the most appropriate approaches to gastric cancer surgery, including lymph node

dissection. Second, cause of death report is not always reliable and cancer related survival could not be used as a study end-point.

Regardless of the extent of lymph node dissection, fewer than 16 dissected lymph nodes may lead to inaccurate staging and, possibly, inadequate treatment, thus affecting survival rates. In patients with a TLC<16, if a inadequate examination is suspected then a reassessment of the specimen should be performed. Otherwise, these patients should be considered a high-risk group for stage migration and worse survival who may benefit from adjuvant therapies after surgery.

Irrespective of whether an increased lymph node dissection count affects pathological staging, disease control, or both, the data from this study provide support in favor of D2 lymph node dissection during potentially curative gastrectomy for gastric cancer.

In conclusion, increasing the number of total lymph nodes through a D2 lymph node dissection and through careful pathological examination could improve the accuracy of N staging and may be associated with a better prognosis in curatively resected gastric cancer patients.

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Alberto Biondi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

None declared

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Table 1

		TLC- group: <16 LN ***(n=165)***	**TLC+ group: ≥16 LN** ***(n=823)***	***p***
Age (years)	Mean±SD Median (range)	68.9±12.0 70 (31-95)	63.9±11.9 65 (18-92)	*<0.001*$^{+}$
Sex	Female Male	61 (37.0%) 104 (63.0%)	333 (40.5%) 490 (59.5%)	*ns**
Tumor location	Upper Third Medium Third Lower Third Linitis Plastica Gastric Stump	18 (10.9%) 41 (24.8%) 82 (49.7%) 4 (2.4%) 20 (12.1%)	115 (14.0%) 261 (31.8%) 381 (46.4%) 21 (2.6%) 43 (5.2%)	*0.008**
Gastrectomy	Subtotal Total	130 (78.8%) 35 (21.2%)	554 (67.3%) 269 (32.7%)	*<0.004**
Lymphadenectomy	D1 D2	110 (66.7%) 55 (33.3%)	116 (14.1%) 707 (85.9%)	*<0.001**
Lauren classification	Intestinal type Diffuse type	82 (58.6%) 58 (41.4%)	442 (60.7%) 286 (39.3%)	*ns**
Grading	G1 G2 G3	22 (22.2%) 36 (36.4%) 41 (41.4%)	88 (18.5%) 158 (33.3%) 229 (48.2%)	*ns**
Tumor size	≤40 mm >40 mm	106 (68.8%) 48 (31.2%)	532 (68.2%) 248 (31.8%)	*ns**
pT (TNM – 7^ed.)	1 2 3 4a 4b	65 (39.9%) 29 (17.8%) 33 (20.2%) 30 (18.4%) 6 (3.7%)	294 (35.8%) 174 (21.2%) 194 (23.6%) 140 (17.1%) 19 (2.3)	*ns**
pN (TNM – 7^ed.)	0 1 2 3a 3b	109 (66.5%) 24 (14.6%) 26 (15.9%) 5 (3.0%) -	486 (59.1%) 108 (13.1%) 132 (16.1%) 68 (8.3%) 28 (3.4%)	*0.017**
Stage (TNM – 7^ed.)	IA IB IIA IIB IIIA IIIB IIIC	62 (38.0%) 18 (11.0%) 22 (13.5%) 25 (15.3%) 21 (12.9%) 11 (6.7%) 4 (2.5%)	243 (29.6%) 126 (15.4%) 126 (15.4%) 128 (15.6%) 82 (10.0%) 62 (7.6%) 53 (6.5)	*ns**
Metastatic lymph nodes	Mean±SD Median (range)	1.1±2.0 0 (0-10)	2.5±5.2 0 (0-44)	*<0.001*$^{+}$
Negative lymph nodes	Mean±SD Median (range)	9.1±3.9 10 (0-15)	30.7±14.9 28 (1-124)	*<0.001*$^{+}$
Total harvested lymph nodes	Mean±SD Median (range)	10.2±3.7 (1-15)	33.3±15.0 29 (16-124)	*<0.001*$^{+}$
Period of treatment	1980-89 1990-94 1995-99 2000-04 2005-09	16 (9.7%) 33 (20%) 36 (21.8%) 29 (17.6%) 51 (20.9%)	12 (1.4%) 72 (8.7%) 247 (30%) 287 (34.8%) 205 (24.9%)	*<0.001**
Adjuvant therapy	No Yes	118 (79.7%) 30 (20.3%)	677 (86.2%) 108 (13.8%)	*0.040**

*: χ2-test $^{+}$: M-W test

Table 2

		Univariate analysis			*Multivariate analysis*	
		3-Yrs Svv (%)	5-Yrs Svv (%)	*p*	HR (95%CI)	*p*
Age	≤70	81.7	73.6	<0.001*	1	<0.001
	>70	61.7	49.1		2.194 (1.589-3.031)	
Sex	F	79.1	69.3	0.009*	1	0.008
	M	71.8	62.5		1.615 (1.133-2.302)	
Tumor Location	Upper	64.7	56.7	0.012		
	Linitis	42.5	42.5			
	Lower	77.3	66.3			
	Medium	77.9	68.1			
	Stump	70.7	68.1			
Lauren classification	Intestinal	76.0	64.8	0.196		
	Diffuse	75.5	68.6			
Grading	1	85.4	80.1	0.001*		
	2	81.1	69.6			
	3	66.0	58.6			
Tumor Size	≤40 mm	81.7	71.0	<0.001*		
	>40 mm	58.1	50.6			
T-stage (TNM-VII)	1	92.4	85.1	<0.001*	1	
	2	73.1	62.1		1.775 (1.069-2.948)	0.027
	3	64.2	54.2		2.854 (1.709-4.768)	<0.001
	4a	57.1	42.8		2.851 (1.806-4.503)	<0.001
	4b	10.3	10.3		7.494 (3.554-15.801)	<0.001
N-stage (TNM-VII)	0	84.4	76.0	<0.001*	1	
	1	69.9	61.5		1.336 (0.838-2.130)	0.224
	2	59.7	48.4		1.966 (1.250-3.090)	0.003
	3a	44.2	21.9		4.081 (2.288-7.279)	<0.001
	3b	8.8	8.8		9.310 (4.657-18.610)	<0.001
Stage (TNM-VII)	IA	93.0	86.0	<0.001		
	IB	84.4	74.8			
	IIA	73.5	67.9			
	IIB	69.9	52.7			
	IIIA	50.3	41.0			
	IIIB	42.2	30.5			
	IIIC	29.1	14.6			
Gastrectomy	Subtotal	78.6	68.1	<0.001*	1	
	Total	65.7	58.5		1.771 (1.277-2.458)	0.001
Lymphadenectomy (site)	D1	74.3	65.3	0.861		
	D2	74.7	65.2			
Lymphadenectomy (number <16; ≥16)	TLC-	65.5	51.5	<0.001*	1	
	TLC+	76.2	67.6		0.519 (0.345-0.780)	0.002
Adjuvant therapy	No	76.7	66.8	0.068*		
	Yes	65.4	59.0			

*: variables considered in the multivariate analysis

Table 3

	TLC -		***TLC +***		
	3-Yrs Svv (%)	5-Yrs Svv (%)	3-Yrs Svv (%)	5-Yrs Svv (%)	*p*
N0	75.4	61.1	86.3	79.2	0,001
N1	48.1	34.3	73.2	65.7	0.007
N2	38.2	19.1	63.6	53.1	0.001
N3	0	0	37.7	19.7	N/A

FIGURE AND TABLE LEGENDS

Figure 1: Relationship between the number of retrieved lymph nodes and the rate of node positive patients.

Figure 2: Relationship between number of retrieved lymph node and pN stage

Figure 3: Survival curve: effect of lymph node count (TLC+ vs TLC-).

Table 1: The clinico-pathological data and differences between between the TLC- and TLC+ groups

Table 2: Survival data according to the Kaplan-Meier and Cox regression analyses

Table 3: Survival rate according to pN-TNM stage

Figure 1

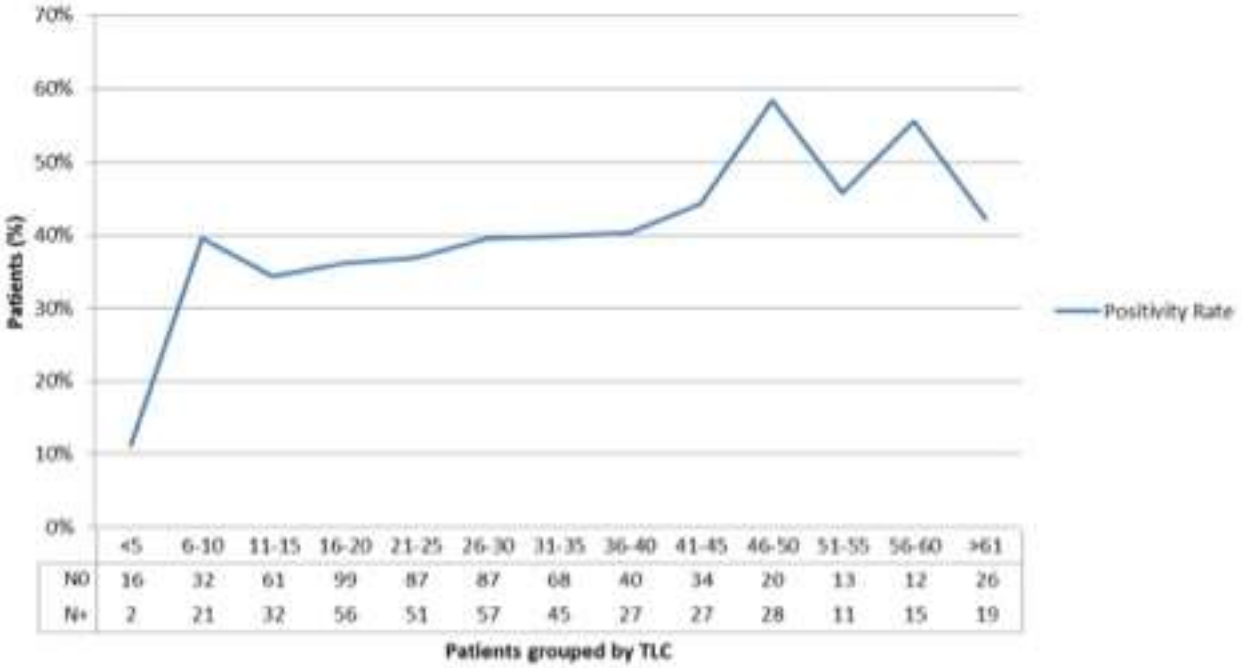

70%
60%
50%
40%
30%
20%
10%
0%
Patients (%)
Positivity Rate
<5 6-10 11-15 16-20 21-25 26-30 31-35 36-40 41-45 46-50 51-55 56-60 >61
N0 16 32 61 99 87 87 68 40 34 20 13 12 26
N+ 2 21 32 56 51 57 45 27 27 28 11 15 19
Patients grouped by TLC

Figure 2

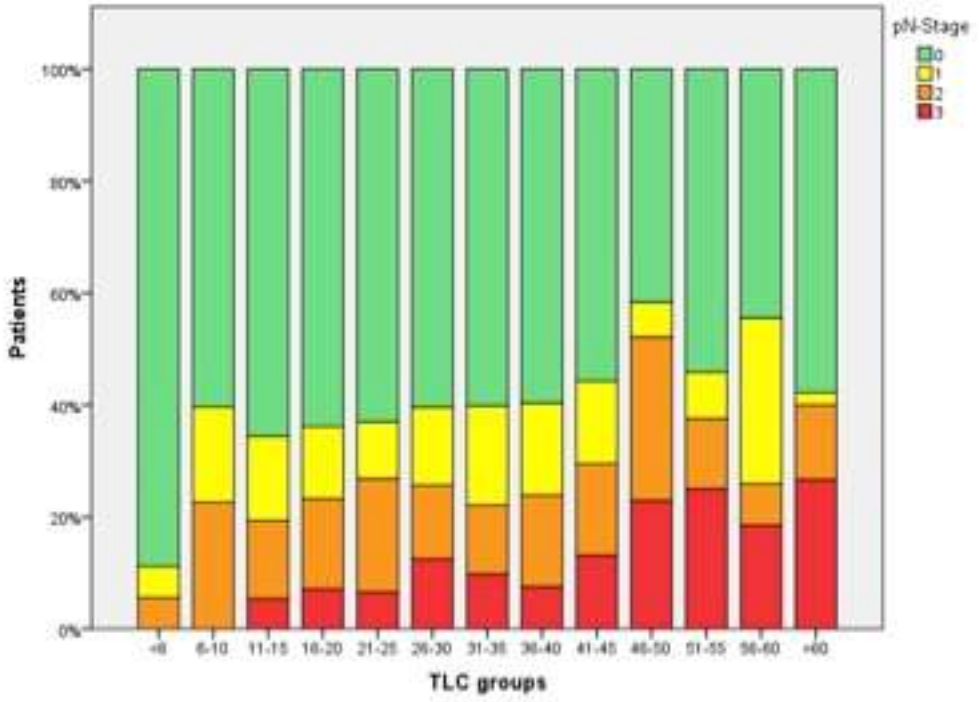
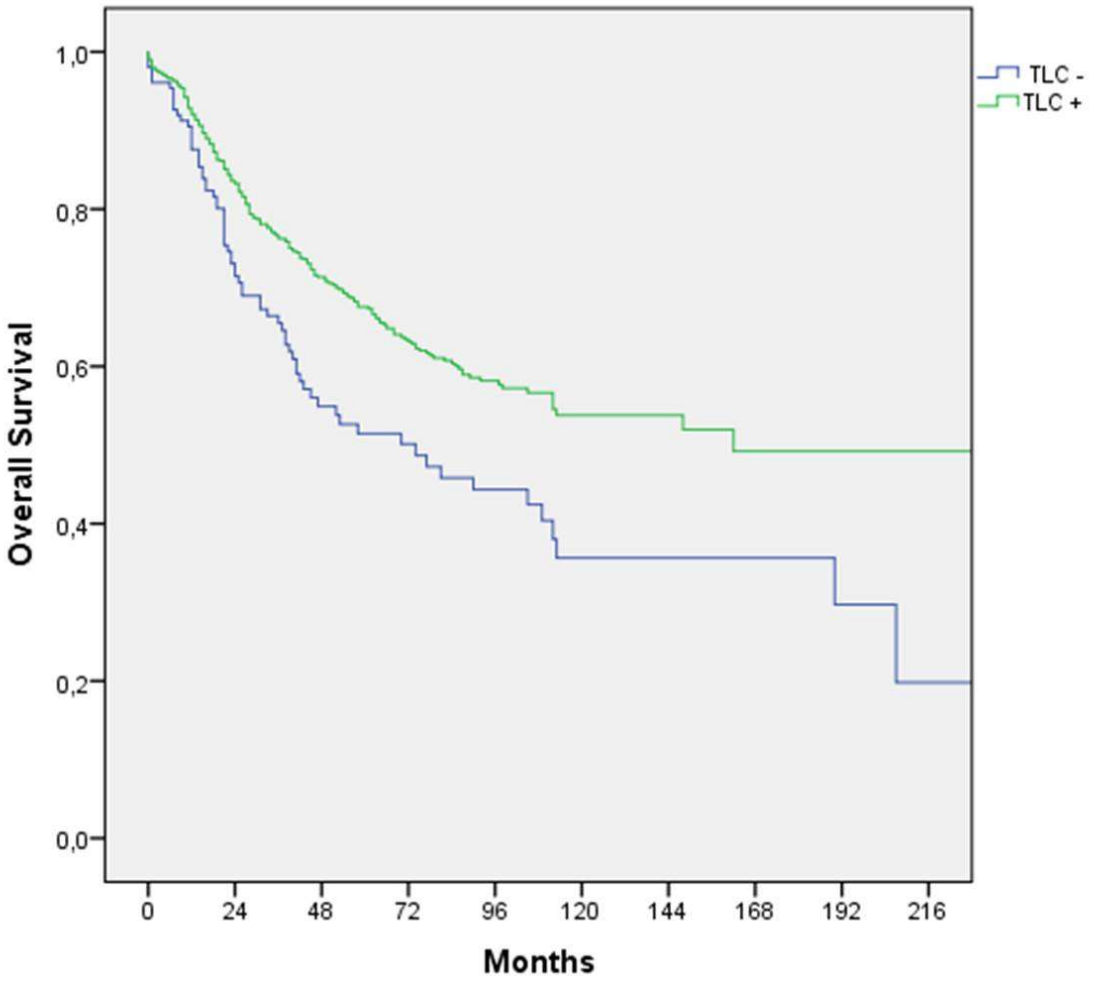

pN-Stage
0
1
2
3
100%
80%
60%
40%
20%
0%
Patients
<6
6-10
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
>60
TLC groups

Figure 3



Patients at risk	**TLC -**	153	91	49	36	27	14	14	10	3	2
	TLC+	797	549	368	218	126	62	30	16	5	3