The Number of Harvested LNs Is an Independent Prognostic Factor in Lymph Node Metastasis-negative Patients Who Received Curative Esophagectomy

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Abstract. *Background/Aim: The aim of the present study was to evaluate the optimal number of harvested LNs (LNs) in patients who were LN metastasis-negative after curative esophagectomy for esophageal cancer. Patients and Methods: Sixty-one patients who underwent curative surgery for esophageal cancer between 2005 and 2017 and diagnosed as lymph node metastasis-negative were included in this study. Results: The 5-year overall survival rates were 27.8% for 0-20 harvested LNs, 35.7% for 21-30 harvested LNs, 79.4% for 31-40 harvested LNs, and 85.2% for ≥41 harvested LNs. Thirty harvested LNs was regarded as the optimal critical point of classification, considering the 5-year OS rate. The number of harvested LNs was selected as a significant prognostic factor in both univariate and multivariate analyses. The respective 3- and 5-year OS rates were 50.3% and 36.7% for <30 harvested LNs and 82.4% and 82.4% for ≥30 harvested LNs (p=0.003). Conclusion: Thirty or more harvested LNs was a significant prognostic factor in patients with metastasis-negative LNs after curative esophagectomy for esophageal cancer. Therefore, the number of harvested LNs might be useful for predicting the LN metastasis status in esophageal cancer.*

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Esophageal cancer is the seventh-most common cancer in the world and the sixth leading cause of cancer-related mortality (1). Esophagectomy and perioperative adjuvant treatment are essential for curing resectable esophageal cancer (2-4). However, more than half of esophageal cancer patients suffer recurrence, even after curative treatment (5, 6). Therefore, it is necessary to identify prognostic factors to improve the chances of survival of patients with esophageal cancer.

The presence of lymph node (LN) metastases in patients with esophageal cancer was one of the most important prognostic factors (7, 8). Micrometastasis tumor cells are well known to spread *via* the lymphatic system, so esophagectomy with lymphadenectomy are established as critical procedures (9-12). Even patients who have been diagnosed as LN metastasis-negative can develop recurrence with a limited prognosis (13-15). The diagnosis of LN metastasis depends on the number of both harvested LNs and metastasis-positive LNs. Therefore, the accuracy of the LN metastasis-negative diagnosis depends on the number of LNs harvested. If the LN metastasis-negative patients do not receive adequate LN resection or an accurate evaluation of their LNs, the LN metastasis status may be underestimated. However, the TNM staging system does not define the optimal number of LNs to be harvested for accurate nodal staging (16, 17). To improve esophageal cancer patients' chances of surviving, it is necessary to develop a more effective way of assessing the lymph node metastasis status.

We hypothesized that the higher number of negative LNs removed during surgery is associated with more accurate staging and an improved survival in LN metastasis-negative patients. To confirm our hypothesis, we investigated the optimal number of harvested LNs in LN metastasis-negative esophageal cancer patients.

Table I. *Comparison of survival rates stratified by patient characteristics.*

Characteristics	No. of patients (%)	1-year survival rate (%)	3-year survival rate (%)	5-year survival rate (%)	*p*-Value
Age (years)					0.204
<68	28 (45.9)	81.7	61.2	44.1	
≥68	33 (54.1)	84.1	73.9	65.7	
Gender					0.220
Male	49 (80.3)	83.3	63.3	59.1	
Female	12 (19.7)	90.9	90.9	72.7	
Site of tumor					0.439
Upper	23 (37.7)	82.6	64.3	64.3	
Middle or Lower	38 (62.3)	83.4	70.9	61.6	
Number of harvest lymph node					0.013
0-20	10 (16.4)	55.6	27.8	27.8	
21-30	15 (24.6)	85.7	57.1	35.7	
31-40	20 (32.8)	79.4	79.4	79.4	
41-	16 (26.2)	93.8	85.2	85.2	
UICC T status					0.253
T1	34 (55.7)	94.0	72.7	72.7	
T2 to T3	27 (44.3)	73.3	61.7	49.1	
Lymph vascular invasion					0.089
Negative	27 (44.3)	88.1	83.7	83.7	
Positive	34 (55.7)	79.0	57.1	48.2	
Lymph node dissection					0.720
Two-field	40 (65.6)	84.7	73.7	73.7	
Three-field	21 (34.4)	84.5	65.6	56.6	
Neoadjuvant therapy					0.741
Yes	28 (45.9)	88.9	65.3	58.1	
No	33 (54.1)	81.8	69.6	63.2	

UICC: Union for International Cancer Control.

Patients and Methods

Patients. The medical records of consecutive patients diagnosed with primary esophageal adenocarcinoma or squamous cell carcinoma and who underwent complete resection at Yokohama City University from January 2005 to December 2017 were reviewed.

Surgical procedure. Our standard procedures consisted of open subtotal esophagectomy *via* right thoracotomy. A greater curvature tube was used for reconstruction. The patients who had tumors in the middle to lower thoracic esophagus received two-field LN dissection, while those with tumors in the upper thoracic esophagus received three-field LN dissection.

LN harvesting methods and the pathological diagnosis. The LNs were harvested immediately after surgery. First, the surgeons removed the palpable LNs. Second, the surgeons stretched the fat tissues that covered the LNs in order to detect visible LNs. The harvested LNs were then fixed with 10% buffered formalin for 48 h. After standard histological processing, two-step sections were cut from each block and subjected to Hematoxylin-Eosin (H&E) staining. Experienced pathologists screened all slides.

Evaluations and statistical analyses. Fisher's exact test or the χ^2 test was used to assess the correlation between harvested LNs and each parameter. The Kaplan-Meier method and log-rank test were used to calculate and compare the overall survival (OS) and recurrence-free survival (RFS). Univariate and multivariate survival analyses were performed by a Cox proportional hazards model. *p* Values of <0.05 were considered to indicate statistical significance. The SPSS software program (v11.0 J Win; SPSS, Chicago, IL, USA) was used for all the statistical analyses. This study was approved by the Institutional Review Board of the Yokohama City University.

Results

Patients. Sixty-one patients were evaluated in the present study. The median age was 68 years old (range=49-82 years old). Among them, 49 patients were male, and 12 were female. The median follow-up period was 72.5 months (range=15.0-125.2 months). The median operation time was 572 min (range=236-911 min). The median blood loss was 540 ml (range=70-3000 ml). Thirty-six patients received 2-field LN dissection, and 25 received 3-field LN dissection. The median number of harvested LNs was 31 (range=7-118).

Survival analyses. In Table I, the OS stratified by each clinical factor was compared using the log-rank test. There were significant differences with the number of harvested LNs (*p*=0.013) and marginally significant difference with

Table II. *Uni and multivariate Cox proportional hazards analysis of clinicopathological factors for overall survival.*

Factors	No	Univariate analysis			Multivariate analysis		
		OR	95%CI	*p*-Value	OR	95%CI	*p*-Value
Age (years)				0.233			
<68	28	1.000					
≥68	33	1.876	0.668-5.272				
Gender				0.114			
Female	12	1.000					
Male	49	4.035	0.717-22.271				
Number of harvest lymph node				0.006			0.003
<30	25	1.000			1.000		
≥30	36	3.784	1.467-9.763		3.970	1.607-9.806	
Site of tumor				0.238			
Middle or Lower	38	1.000					
Upper	23	1.772	0.686-4.582				
UICC T status				0.146			
T1	34	1.000					
T2 or T3	27	2.563	0.721-9.108				
Lymphovascular invasion				0.161			
Negative	27	1.000					
Positive	34	2.112	0.742-6.012				
Lymph node dissection				0.161			
Two-field	40	1.000					
Three-field	21	1.158	0.396-3.386				
Neoadjuvant therapy				0.534			
Yes	28	1.000					
No	33	1.490	0.423-5.246				

UICC: Union for International Cancer Control.

lymphovasuclar invasion (*p*=0.089). Thirty harvested LNs was regarded as the optimal critical point of classification, considering the 3- and 5-year OS rates. The 3-year OS rates were 27.8% for 0-20 harvested LNs, 57.1% for 21-30 harvested LNs, 79.4% for 31-40 harvested LNs, and 85.2% for ≥41 harvested LNs. The 5-year OS rates were 27.8% for 0-20 harvested LNs, 35.7% for 21-30 harvested LNs, 79.4% for 31-40 harvested LNs, and 85.2% for ≥41 harvested LNs.

Table II shows the results of univariate and multivariate analyses of the prognostic significance for the OS. The number of harvested LNs was found to be a significant prognostic factor in both the univariate and multivariate analyses. The respective 3- and 5-year OS rates were 50.3% and 36.7% for <30 harvested LNs and 82.4% and 82.4% for ≥30 harvested LNs (*p*=0.003). Figure 1 shows the OS curves for <30 harvested LNs and ≥30 harvested LNs.

Table III shows the results of univariate and multivariate analyses of the prognostic significance for the RFS. The number of harvested LNs, UICC T status, and tumor location were found to be significant prognostic factors in both the univariate and multivariate analyses. The respective 3- and 5-year RFS rates were 42.5% and 29.1% for <30 harvested LNs and 67.2% and 67.2% for ≥30 harvested LNs (*p*=0.003).

Figure 2 shows the RFS curves for <30 harvested LNs and ≥30 harvested LNs.

Table IV compares the sites of first relapse between <30 harvested LNs and ≥30 harvested LNs. When comparing the sites of first relapse, the incidence of bone metastasis was significant for <30 harvested LNs than for ≥30 harvested LNs. In addition, regarding the local site of recurrence, lung metastasis, and liver metastasis were marginally but significantly higher for <30 harvested LNs than for ≥30 harvested LNs.

Discussion

The aim of the present study was to evaluate the optimal number of harvested LNs in patients who were considered LN metastasis-negative after curative esophagectomy for esophageal cancer. The major finding of the present study was that ≥30 harvested LNs was a significant prognostic factor for patients with LN metastasis-negative status after curative esophagectomy for esophageal cancer. Therefore, the number of harvested LNs might be useful for predicting the LN metastasis status in esophageal cancer.

The present study demonstrated that the number of harvested LNs was a significant risk factor for esophageal
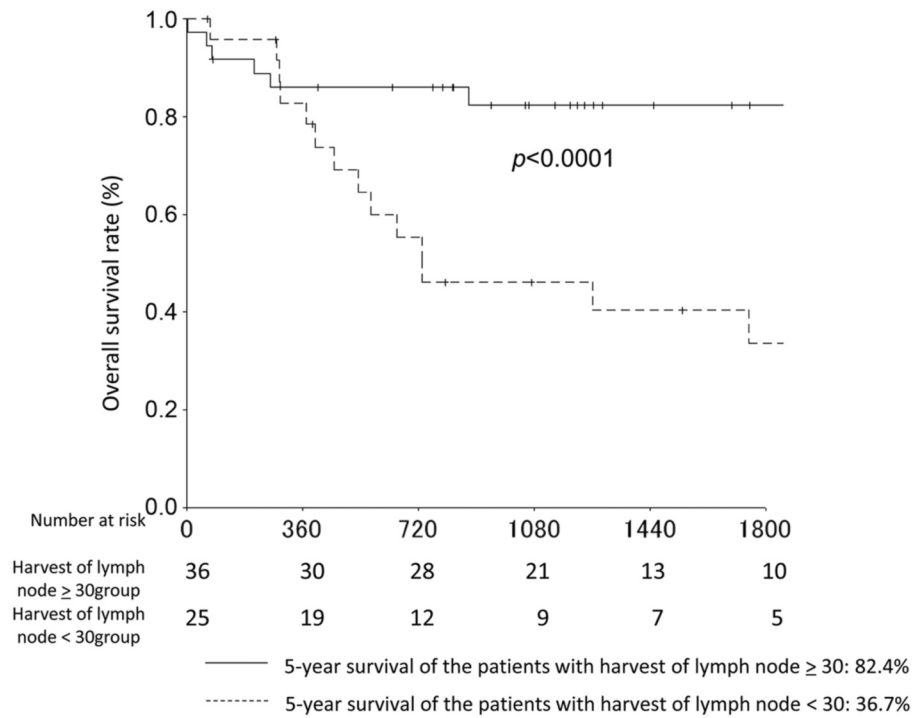
Figure 1. *A comparison of the overall survival for <30 harvested LNs and ≥30 harvested LNs.*

Table III. *Uni and multivariate Cox proportional hazards analysis of clinicopathological factors for recurrence free survival.*

Factors	No	Univariate analysis			Multivariate analysis		
		OR	95%CI	*p*-Value	OR	95%CI	*p*-Value
Age (years)				0.820			
<68	28	1.000					
≥68	33	1.115	0.437-2.845				
Gender				0.174			
Female	12	1.000					
Male	49	2.503	0.666-9.405				
Number of harvest lymph node				0.118			0.026
<30	25	1.000			1.000		
≥30	36	2.015	0.837-4.849		2.540	1.119-5.766	
Site of tumor				0.023			0.046
Middle or Lower	38	1.000			1.000		
Upper	23	2.972	1.159-7.625		2.392	1.014-5.645	
UICC T status				0.017			0.010
T1	34	1.000			1.000		
T2 or T3	27	3.977	1.287-12.292		3.107	1.317-7.329	
Lymphovascular invasion				0.142			
Negative	27	1.000					
Positive	34	2.081	0.783-5.531				
Lymph node dissection				0.384			
Two-field	40	1.000					
Three-field	21	1.624	0.546-4.833				
Neoadjuvant therapy				0.392			
Yes	28	1.000					
No	33	1.618	0.537-4.875				

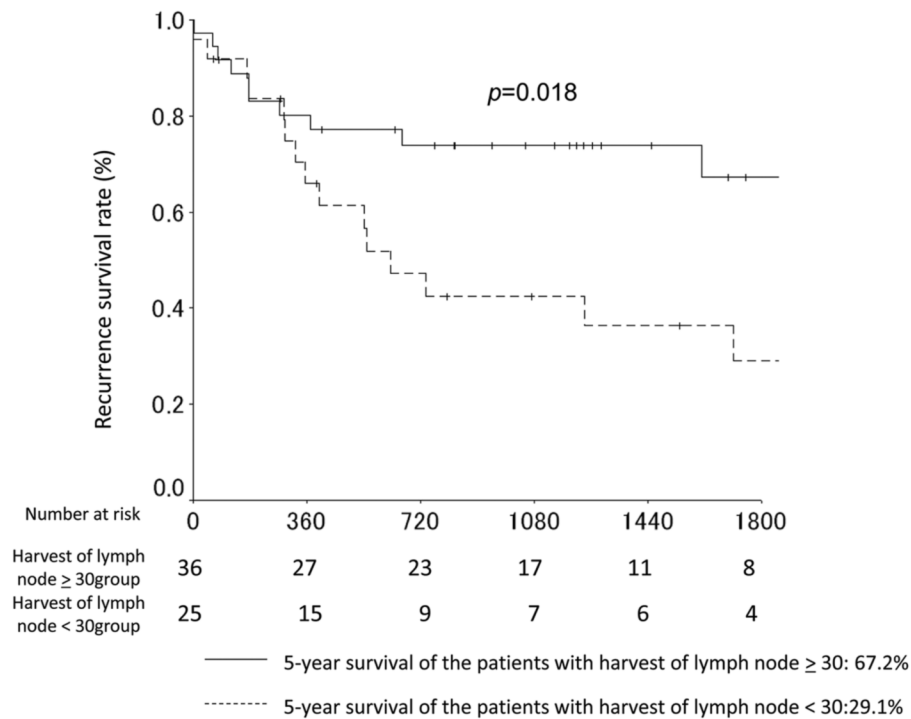UICC: Union for International Cancer Control.

Figure 2. *A comparison of the recurrence-free survival for <30 harvested LNs and ≥30 harvested LNs.*

Table IV. *Patterns of recurrence between the patients with <30 harvested lymph nodes and those with ≥30 harvested lymph nodes.*

Recurrence site	All cases		<30 (n=25)		≥30 (n=36)		*p*-Value
	Number	%	Number	%	Number	%	
Lymph node							
Regional	8	13.1	5	20.0	3	8.3	0.184
Distant	2	3.3	1	4.0	1	2.8	0.792
Local site	5	8.2	4	16.0	1	2.8	0.064
Distant site							
Lung	7	11.5	5	20.0	2	5.6	0.082
Liver	2	3.3	2	8.0	0	0.0	0.084
Bone	3	4.9	3	12.0	0	0.0	0.033
Others	4	6.6	2	8.0	2	5.6	0.704

cancer patients after esophagectomy. Limited studies have shown similar results. Greenstein *et al*. have evaluated the prognostic impact of the total number of harvested LNs in 972 LN metastasis-negative esophageal cancer patients who received curative esophagectomy (18). They divided the 972 patients into 3 groups according to the number of harvested LNs (0-10, 11-17, and ≥18) and found that the total number of harvested LNs was significantly linked to the disease-specific survival rates. The 5-year survival rate was 55% for 0-10 harvested LNs, 66% for 11-17 harvested LNs, and 75%

for ≥18 harvested LNs. They also found that the total number of harvested LNs was an independent prognostic factor for esophageal cancer patients' survival after curative esophagectomy in a multivariate analysis (hazard ratio=3.16, 95% confidence interval=1.34-7.43, *p*=0.02). They suggested that patients undergoing surgical resection for esophageal cancer should have at least 18 LNs removed.

In addition, Yu *et al*. evaluated the prognostic influence of the total number of harvested LNs in 576 esophageal cancer patients who received curative esophagectomy (19). They divided the

576 patients into 4 groups according to the number of harvested LNs (0-9, 10-14, 15-19, and ≥20) and found that the total number of harvested LNs was significantly linked to the survival rates. The 3-year survival rate was 21.7% for 0-9 harvested LNs, 40.0% for 10-14 harvested LNs, 61.2% for NLNs of 15-19 harvested LNs, and 77.5% for ≥20 harvested LNs. They also found that the total number of harvested LNs was an independent prognostic factor for esophageal cancer patients' survival after curative esophagectomy in a multivariate analysis. Taken together, our results and those of other investigators suggest that the number of harvested LNs may influence survival of esophageal cancer patients after curative surgery.

In the present study, we set the cut-off value of harvested LNs at 30, based on the 3- and 5-year OS rates. The cut-off value for harvested LNs differed somewhat between the present and previous studies. An important limitation to consider concerning the available data on harvested LNs, including those from the current study, is the lack of a consensus regarding the most appropriate cut-off point for the evaluation of harvested LNs. Mo *et al*. set the cut-off value at 21 in their study of 768 esophageal cancer patients, Greenstein *et al*. set the cut-off value at 18 in their study of 972 esophageal cancer patients, and Xia *et al*. set the cut-off value at 14 and 15 in their study of 7356 esophageal cancer patients (18, 20, 23). Of course, there are some differences between the present and previous studies that should be mentioned. First, the sample size was differed among studies. Second, the perioperative adjuvant treatment approach was also differed among studies. Previous reports analyzed only patients who were treated with surgery alone, while the present study analyzed only those who had been treated with surgery as well as perioperative adjuvant treatment. The outcomes of patients with esophageal cancer have gradually improved due to effective adjuvant treatment. Theoretically, effective adjuvant treatment could improve the patient survival by inhibiting micrometastases. Indeed, a previous study has shown that perioperative adjuvant treatment was associated with a reduced LN ratio in other gastrointestinal cancers (21, 22). Third, the median number of harvested LNs was differed among the studies. In the present study, the median number of harvested LNs was 31, and the cut-off value of the LNs was 30. In contrast, Mo *et al*. have reported that the mean number of harvested LNs was 23, and the cut-off value of harvested LNs was set at 21 (23). Furthermore, Xia *et al*. have reported that the median number of harvested LNs was 12, and the cut-off value of harvested LNs was set at 14 and 15 (20). These differences might have affected the cut-off values of the harvested LNs.

Special attention is required when interpreting the present findings, as this study is associated with several potential limitations. First, our study was a retrospective analysis performed in a single institution. We cannot deny the possibility that our findings were observed by chance. Second, there was a selection bias in the patients in this series. Surgeons often avoid performing esophagectomy in some patients because the procedure is associated with high rates of morbidity and mortality (40%-60% and 1-5%, respectively). Third, there was a time bias in the present study, as the surgical procedure, perioperative care, and adjuvant treatment changed throughout the study period. Given these limitations, the results must be confirmed in another cohort or in a prospective, multicenter study.

In conclusion, the OS and RFS of LN metastasis-negative esophageal cancer patients who underwent curative surgery differed significantly based on the number of harvested LNs. It is necessary to develop an effective means of using the LN metastasis status in these patients.

Conflicts of Interest

The Authors declare no conflicts of interest in association with the present study.

Authors' Contributions

Toru Aoyama and Yosuke Atsumi made substantial contributions to conception and design. Norio Yukawa, Toru Aoyama, Hiroshi Tamagawa, Ayako Tamagawa, Yosuke Atsumi, Shinnosuke Kawahara, Yukio Maezawa, Kazuki Kano, Masaaki Murakawa, Keisuke Kazama, Masakatsu Numata, Takashi Oshima, Munetaka Masuda,and Yasushi Rino made substantial contributions to acquisition of data, or analysis and interpretation of data. Norio Yukawa, Toru Aoyama, Hiroshi Tamagawa, Ayako Tamagawa, Yosuke Atsumi, and Shinnosuke Kawahara have been involved in drafting the manuscript or revising it critically for important intellectual content. Norio Yukawa, Toru Aoyama, Hiroshi Tamagawa, Ayako Tamagawa, Yosuke Atsumi, and Shinnosuke Kawahara have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All Authors read and approved the final manuscript.

Acknowledgements

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