paraesophageal nodes as regional lymph nodes, and supraclavicular nodes as nonregional lymph nodes, irrespective of the site of the primary tumor. There were few reports that had investigated the impact of lymph node metastasis, with a focus on supraclavicular nodes and common hepatic or splenic nodes, on survival.

The purpose of the present study was to assess the ability of the 7th edition of the UICC TNM staging system to predict the overall survival in patients who underwent surgical resection for esophageal carcinoma, and to explore an optimal definition of the regional lymph node.

MATERIAL AND METHODS

Patient Population

Data were collected from the medical records of all patients who underwent surgery at our institution between January 1997–December 2012 for primary esophageal squamous cell carcinoma (ESCC). All 665 consecutive patients underwent esophageal fiberscopy and enhanced computed tomography (CT) from the neck to the abdomen for tumor staging, and in addition there were 585 patients treated after January 2000 who also underwent positron emission tomography.

All 665 patients who met the following inclusion and exclusion criteria were enrolled in this study: (1) subtotal esophagectomy and mediastinal lymph node dissection was performed through the right thoracotomy, (2) curative resection (R0) was achieved, (3) the primary tumor was located in the thoracic esophagus, (4) lack of active malignancy in other organs, and (5) in-hospital mortality due to postoperative complication.

Treatment Protocol

The basic strategy for the treatment of patients with ESCC has been described previously.[9] Patients with clinical Stage (cStage) IA were indicated for surgery without preoperative treatment. Until January 2009, cStage IB and IIA were also indicated for surgery without preoperative treatment. The cStage IB–cStage IIIC, except for cT4, were indicated for neoadjuvant chemotherapy followed by surgery. Patients with clinically metastatic supraclavicular lymph node were indicated for neoadjuvant chemotherapy followed by surgery as with cStage IB–cStage IIIC.

In brief, cT4 were indicated for surgical resection when invasion to the adjacent organ was released by chemoradiotherapy, chemotherapy, or chemotherapy followed by chemoradiotherapy. Surgery was performed 4–8 weeks after preoperative treatment. The chemoradiotherapy and chemotherapy regimen in our hospital was performed as previously described.[10,11] The former consisted of simultaneous radiation with administration of cisplatin and fluorouracil, and the latter consisted of fluorouracil, adriamycin, and cisplatin (FAP), or docetaxel, cisplatin, and fluorouracil (DCF).

Surgery

Surgical resection consisted of a right transthoracic esophagectomy and mediastinal dissection with extensive lymphadenectomy. Abdominal lymphadenectomies were performed to include the paracardial, lesser curvature, left gastric, common hepatic, celiac, and splenic nodes. Patients underwent additional cervical lymph node dissection, based on the location of the primary tumor and the presence or absence of metastases in the lymph node chain along the recurrent laryngeal nerve.[12–14]

Follow-up

All patients were followed up at 3-month intervals for the first 2 years, at 6-month intervals until 5 years, and at 12-month intervals thereafter. All patients underwent physical examination and CT, and were assessed for recurrence or metastasis. The last general follow-up of survivors was done at the end of November 2013. Overall survival was defined as the time between the date of operation and date of death. Surviving patients were censored on the day of the last contact.

STATISTICAL ANALYSIS

Tumors were staged according to the 7th edition of the UICC TNM staging system. Survival was calculated by the Kaplan–Meier method, differences between curves were assessed by the log-rank test. Cox regression was used to evaluate the hazard ratios (HRs), as well as the 95 % confidence intervals. Statistical significance was defined as a P value of <0.05. Statistical significance for each model was set at $P < 0.05$. These analyses were carried out using JMP version 8.0.1 (SAS Institute, Cary, NC) for Windows.

RESULTS

Patient Characteristics

A consecutive series of 665 patients with thoracic ESCC underwent esophagectomy with curative intent. Table 1 lists the demographic parameters of all patients. There were 300 patients who underwent two-field (mediastinal and abdominal) lymph node dissection and 365 who underwent three-field (cervical, mediastinal and abdominal) lymph

TABLE 1 Patients' characteristics

		No. of patients (%)
All patients		665
Age at operation	Median (min–max)	65 (36–85)
Sex	Male	578 (86.9)
	Female	87 (13.1)
Location	Ut	107 (16.1)
	Mt	355 (53.4)
	Lt	203 (30.5)
Histopathological grading	Grade 1	146 (22.0)
	Grade 2	329 (49.5)
	Grade 3	139 (20.9)
	Grade X	51 (7.6)
Preoperative treatment	None	241 (36.2)
	CT	331 (49.8)
	CRT	93 (14.0)
pT	pT0	40 (6.0)
	pT1a	45 (6.8)
	pT1b	157 (23.6)
	pT2	113 (17.0)
	pT3	287 (43.2)
	pT4a	7 (1.0)
	pT4b	16 (2.4)
pN	pN0	261 (39.2)
	pN1	220 (33.1)
	pN2	119 (17.9)
	pN3	65 (9.8)
pM	pM0	567 (85.3)
	pM1	94(14.7)
pStage	0	28 (4.2)
	IA	107 (16.1)
	IB	40 (6.0)
	IIA	67(10.1)
	IIB	109 (16.4)
	IIIA	127 (19.1)
	IIIB	50 (7.5)
	IIIC	43 (6.5)
	IV	94 (14.1)
Lymphadenectomy	Two-field	300 (45.1)
	Three-field	365 (54.9)

CRT chemoradiotherapy, *CT* chemotherapy, *Lt* lower thoracic, *min–max* minumum–maximum, *Mt* mid-thoracic, *pT* pathological T, *pN* pathological N, *pM* pathological M, *pStage* pathological Stage, *Ut* upper thoracic

node dissection. The median number of lymph nodes resected was 55 (12–168), and the mean was 59.2.

There were 404 patients with regional lymph node metastases (60.8 %). Of those 404 patients, 220 (33.1 %) had pN1, 119 (17.9 %) had pN2, and 65 (9.8 %) had pN3. There were 94 (14.1 %) patients with nonregional lymph node metastases (M1).

Survival

The mean follow-up time was 62.9 months (median follow-up time, 59.2 months). The overall 2- and 5-year survival rates were 69.3 and 54.7 %, respectively.

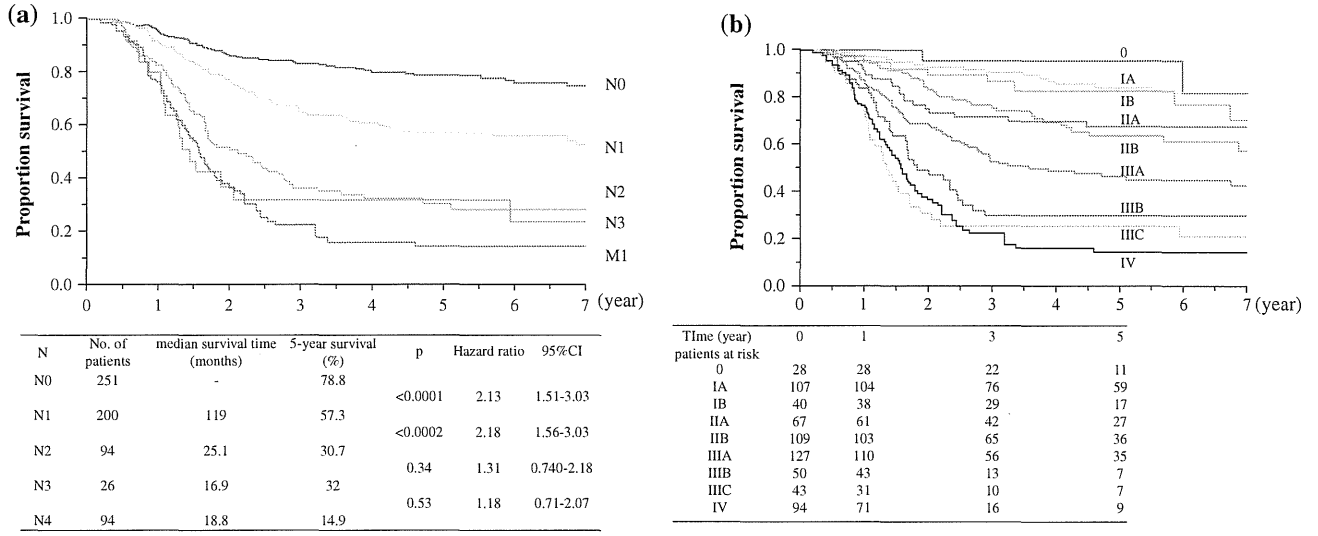
Kaplan–Meier analysis of overall survival, based on lymph node status, N0–N3and M1, and pStage in the 7th edition of TNM classification, showed distinct survival curves to some extent (Fig. 1). However, several subgroups of lymph node status and pStage showed poor discrimination. In particular, the survival curves of the N2, N3, and M1 stages showed considerable overlap and are virtually interchangeable. There were no significant differences in overall survival between N2 and N3 ($P = 0.34$) or N3 and M1 ($P = 0.53$) (Fig. 1a).

Incidences of lymph node metastasis and 5-year survival rates of patients with and without involved nodes were analyzed in each lymph node location (Table 2). The incidence of metastasis in the upper thoracic, mid-thoracic, lower thoracic, and perigastric lymph nodes, which were regarded as regional lymph nodes in both the 6th and 7th editions, was from 12.8–32.8 %. The patients with these lymph node metastases had a relatively good prognosis with 5-year survival rates of 27.7–34.7 %.

The incidence of metastasis in the celiac arterial node and the cervical paraesophageal node, which were regarded as nonregional lymph nodes in the 6th edition, were 2.3 and 10.7 %, respectively. The 5-year survival rates of the patients with coeliac arterial and cervical paraesophageal node involvements were 17.8 and 32.7 %, respectively. The incidences of metastasis in supraclavicular and common hepatic or splenic artery lymph nodes, which were regarded as nonregional lymph nodes in both the 6th and 7th editions, were 10.2 and 3.6 %, respectively. The 5-year survival rates of patients with supraclavicular and common hepatic or splenic artery lymph nodes involvement were 18.6 and 0 %, respectively.

Univariate and Multivariate Analyses of Prognostic Factors

Table 3 presents the results of the univariate analyses for overall survival. Older age (>64 years) ($P = 0.04$), higher histological grade ($P = 0.004$), higher pT stage ($P < 0.0001$), higher pN stage ($P < 0.0001$), preoperative treatment ($P = 0.02$), celiac node involvement ($P = 0.004$), cervical paraesophageal node involvement ($P < 0.0001$), supraclavicular node involvement ($P < 0.0001$), and common hepatic or splenic artery node involvement ($P < 0.0001$) were significantly associated with poorer overall survival.



N	No. of patients	median survival time (months)	5-year survival (%)	p	Hazard ratio	95%CI
N0	251	-	78.8			
				<0.0001	2.13	1.51-3.03
N1	200	119	57.3			
				<0.0002	2.18	1.56-3.03
N2	94	25.1	30.7			
				0.34	1.31	0.740-2.18
N3	26	16.9	32			
				0.53	1.18	0.71-2.07
N4	94	18.8	14.9			

TIme (year) patients at risk	0	1	3	5
0	28	28	22	11
IA	107	104	76	59
IB	40	38	29	17
IIA	67	61	42	27
IIB	109	103	65	36
IIIA	127	110	56	35
IIIB	50	43	13	7
IIIC	43	31	10	7
IV	94	71	16	9

FIG. 1 Kaplan–Meier overall survival curves for 665 patients. The patients were stratified by **a** lymph node status (N), **b** pStage grouping according to the 7th edition of the UICC TNM staging system

TABLE 2 The incidence of lymph node metastasis and 5-year survival rate of the patients with and without involved nodes in the location of lymph node

		No. of patients	5-year survival (%)
Upper thoracic node metastasis	Negative	461	64.4
	Positive	204	33.2
Mid-thoracic node metastasis	Negative	519	60.8
	Positive	146	31.8
Lower thoracic node metastasis	Negative	580	58.2
	Positive	85	27.7
Perigastric node metastasis	Negative	447	64.3
	Positive	218	34.7
Celiac node metastasis	Negative	650	55.6
	Positive	15	17.8
Cervical paraesophageal node metastasis	Negative	594	57.4
	Positive	71	32.7
Supraclavicular node metastasis	Negative	597	58.9
	Positive	68	18.6
Common hepatic and splenic node metastasis	Negative	641	56.4
	Positive	24	0

Variables with a P value of <0.05 in univariate analyses were included in the multivariate analysis. The multivariate analysis demonstrated that pT status ($P = 0.0002$), pN ($P < 0.0001$) status, and common hepatic or splenic artery node metastasis ($P = 0.001$) were independent prognostic factors (Table 3). On the other hand, supraclavicular node metastasis, which was regarded as a nonregional lymph node (M1), was not an independent prognostic factor.

Modified Nodal-Status for UICC Staging System

Based on the results of multivariate analysis, modifications to the current UICC nodal staging system were derived. Patients were stratified into five different nodal status groups: (1) modified (m)-N0, no nodal metastasis; (2) m-N1, one or two regional lymph node metastasis, inclusive of supraclavicular nodes; (3) m-N2, three to six regional lymph node metastases, inclusive of supraclavicular nodes; (4) m-N3, seven or more regional lymph node metastases, inclusive of supraclavicular nodes; and (5) m-M1, non-regional lymph node metastasis, exclusive of supraclavicular nodes. By this modification, 58 of 94 patients with pM1 were shifted from M status to N status; 12, 20, and 26 patients were shifted from M1 to m-N1, m-N2, and m-N3, respectively.

Kaplan–Meier analysis of survival, based on modified lymph node status and pStage according to the proposed staging, showed more ordered and distinct survival curves, especially in N2–M1 and pStage IIIB–IV, than the current classification (Fig. 2). Pair comparison of adjacent subgroups for lymph node status showed improvement in separation of N2–N3 ($P = 0.013$) and N3–M1 ($P = 0.13$), as compared with lymph node status by the current classification.

DISCUSSION

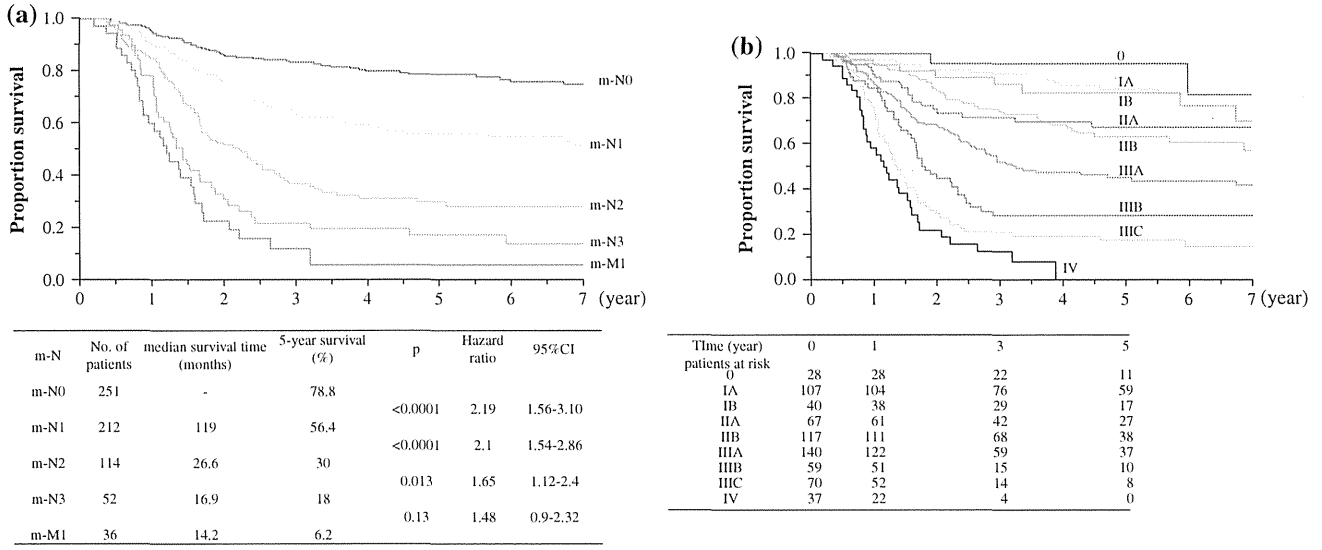
The latest revision of the TNM classification (7th edition) brought substantial changes for esophageal cancer. In particular, the definition of lymph node (N) status was considerably revised in the new TNM classification.

The new classification of N status stratified patients according to the number of positive lymph nodes, not on

TABLE 3 Univariate and multivariate for overall survival

			Univariate			Multivariate		
		Number	HR	95 % CI	*P* value	HR	95 % CI	*P* value
All patients								
Age at operation	≤64/>64	322/343	0.78	0.62–0.99	0.04	0.82	0.64–1.06	0.13
Sex	Female/male	87/578	0.79	0.54–1.1	0.2			
Location	Ut, Mt/Lt	462/203	0.97	0.76–1.26	0.84			
Histopathological grading	1,2/3	475/139	0.67	0.52–0.88	0.004	0.8	0.61–1.05	0.11
pT	T0,1,2,3,4		9.26	5.62–15.5	<0.0001	2.42	1.52–3.89	0.0002
pN	N0,1,2,3		8.8	6.26–12.4	<0.0001	5.13	3.28–8.00	<0.0001
Preoperative treatment	None/CRT, CT	241/424	0.76	0.59–0.96	0.024	0.81	0.62–1.05	0.12
Pathologic node status								
Celiac node	Negative/positive	650/15	0.37	0.22–0.70	0.0037	1.57	0.79–3.37	0.2
Cervical paraesophageal node	Negative/positive	594/71	0.5	0.37–0.70	<0.0001	1.07	0.74–1.56	0.74
Supraclavicular node	Negative/positive	597/68	0.38	0.28–0.52	<0.0001	1.22	0.83–1.76	0.29
Common hepatic or splenic node	Negative/positive	641/24	0.21	0.13–0.34	<0.0001	2.94	1.56–5.23	0.0013

CI confidence interval, *CRT* chemoradiotherapy, *CT* chemotherapy, *HR* hazard ratio, *Ut* upper thoracic, *Mt* mid-thoracic, *Lt* lower thoracic, *pT* pathological T, *pN* pathological N



m-N	No. of patients	median survival time (months)	5-year survival (%)	p	Hazard ratio	95%CI
m-N0	251	-	78.8			
				<0.0001	2.19	1.56-3.10
m-N1	212	119	56.4			
				<0.0001	2.1	1.54-2.86
m-N2	114	26.6	30			
				0.013	1.65	1.12-2.4
m-N3	52	16.9	18			
				0.13	1.48	0.9-2.32
m-M1	36	14.2	6.2			

TIme (year) patients at risk	0	1	3	5
0	28	28	22	11
IA	107	104	76	59
IB	40	38	29	17
IIA	67	61	42	27
IIB	117	111	68	38
IIIA	140	122	59	37
IIIB	59	51	15	10
IIIC	70	52	14	8
IV	37	22	4	0

FIG. 2 Kaplan–Meier overall survival curves for 665 patients. The patients were stratified by **a** lymph node status (m-N), **b** m-pStage grouping according to our modified staging system

the basis of the presence or absence of positive lymph nodes. Some studies, which attempted the validation of this new N status, reported that this nodal classification enabled risk stratification for overall survival after surgery, and was an independent predictor of survival in esophageal cancer.[15,16] Another study reported that the N status in the 7th edition was more useful than that in the 6th edition for risk stratification of patients with lymph node involvement, but it was not satisfactory, due to similar prognoses in patients with N2 and N3 disease.[17,18]

The inconsistent reports on the success of the new N-staging in the 7th edition might result from the number of lymph nodes removed; in the preceding reports, mean or median numbers of lymph nodes removed were 8–23. Peyre et al.[6] proposed that the number of lymph nodes resected was an independent predictor of survival, with a minimum number of 23 regional lymph nodes. The Worldwide Esophageal Cancer Collaboration group has indicated a resection of a minimum of 10 nodes for T1, 20 for T2, and >30 nodes for T3–T4 must be resected to obtain optimal results.[19]

Our data also suggested that the number of lymph nodes dissected, of which the median was 55 (12–168) in this study, was sufficient for adequate nodal staging, and that the nodal classification by the number of involved lymph nodes enables the risk stratification for overall survival after surgery. It is far from adequate nodal staging, and potentially confusing, because the 7th edition of the UICC recommends that the number of lymph nodes resected be at least six for proper nodal classification, but at least seven lymph nodes must be removed to diagnose a patient as N3. The next edition should recommend a greater number of lymph nodes for nodal staging.

The boundary between regional (N) and nonregional (M) lymph nodes have also been revised in the 7th edition, which modified celiac axis nodes and cervical paraesophageal nodes as regional lymph nodes, and kept supraclavicular nodes as a nonregional lymph node. Previously, some reports suggested that nonregional lymph node metastasis, such as celiac and cervical paraesophageal nodes in the 6th edition, were resectable and potentially curable, and that they should be classified as N status rather than M1.[20–26] Our analysis also showed that the presence of celiac and cervical paraesophageal nodes did not prove to be an independent, adverse prognostic factor.

The supraclavicular lymph node was still defined as a nonregional lymph node in the 7th edition because there had been conflicting findings regarding the association between the supraclavicular lymph node metastasis and overall survival. A few studies indicated that supraclavicular lymph node dissection should provide a better chance of survival.[25,27,28] On the other hand, Shim et al.[29] suggested that the addition of cervical nodal dissection could guarantee accurate staging, but not lead to a survival benefit. None of them are prospective and randomized studies; thus, this issue remains controversial. Supraclavicular lymph node metastasis did not prove to be an independent adverse prognostic factor in our study, and should be classified as a regional lymph node (N), rather than a nonregional lymph node (M). Our results indicate that the patients with supraclavicular lymph node metastasis should undergo the treatment with curative intent with absence of other noncurative factors, although it remains controversial whether all patients with resectable ESCC should undergo three-field lymphadenectomy or not. Because the focus of this study is on ESCC, it does not enable applying our proposed modification in esophageal adenocarcinoma (EA). In the future it may need the staging system according to histopathological cell types, ESCC and EA.

We understand that there are some limitations to this study that have to be considered in the interpretation of these results. In this study, we regarded the metastatic status of the supraclavicular lymph node as absent and analyzed in 300 patients who did not receive supraclavicular lymph node dissection (i.e., two-field lymphadenectomy), according to our proposal.[12–14] Indeed, only four (1.3 %) of those 300 patients who received two-field lymphadenectomy had a recurrence in the supraclavicular lymph node. Even if we diagnosed the metastatic status of supraclavicular lymph node in these four patients as positive and reanalyzed, supraclavicular lymph node involvement was not an independent prognostic factor (data not shown).

Another limitation of this study is that it is retrospective and uses a single center. This study is on a small scale compared with the worldwide esophageal cancer collaboration database, and consists of a heterogeneous population of which patients undergo not only surgery alone, but preoperative therapy followed by surgery. However, the treatment strategy and follow-up after esophagectomy was highly uniform throughout the entire study period. This study provides information about an optimal definition of the boundary between regional and nonregional lymph nodes. There are few reports, such as this one, that have investigated the celiac axis and cervical paraesophageal nodes, as well as the supraclavicular and common hepatic or splenic artery lymph nodes in detail.

In conclusion, the 7th edition of the UICC classification is useful as an indicator of the survival in patients with ESCC to some extent. The modification of supraclavicular lymph node from nonregional to regional may allow for better stratification of overall survival of the patients.

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Clinicopathological and Prognostic Significance of FOXM1 Expression in Esophageal Squamous Cell Carcinoma

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Clinicopathological and Prognostic Significance of FOXM1 Expression in Esophageal Squamous Cell Carcinoma

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Abstract. *Background: Esophageal squamous cell carcinoma (ESCC) has a poor prognosis because invasion and metastasis are prevalent. To improve diagnosis, it is important to identify and characterize tumor-specific molecular markers in ESCC. FOXM1 is overexpressed and correlates with pathogenesis in a variety of human malignancies. We aimed to investigate the clinical significance of FOXM1 overexpression in ESCC. Patients and Methods: FOXM1 expression was assessed in ESCC specimens from 174 curatively-resected cases. The relationships between FOXM1 expression, clinicopathological parameters, and prognoses were examined. Results: Immunohistochemical analysis showed that 94 (54.0%) tumors were positive for FOXM1 expression. FOXM1 positivity did not correlate with any clinicopathological parameter. However, FOXM1-positive cases had poorer prognoses than FOXM1-negative ones (p=0.0037, log-rank test). In multivariate analysis, the following were independent prognostic factors: pT, pN, neoadjuvant chemotherapy, and FOXM1 expression (hazard ratio=1.69, 95% confidence interval=1.06-2.75, p=0.027). Conclusion: FOXM1 may be a novel prognostic factor in patients with ESCC who undergo curative resection.*

Esophageal cancer is one of the most aggressive diseases of the gastrointestinal tract (1). In Japan and other East Asian countries, the majority of esophageal cancer diagnoses are esophageal squamous cell carcinoma (ESCC). Despite improvements in surgical technique, chemotherapy, and radiation therapy, the mortality rate of ESCC remains high and its prognosis remains poor because of the high prevalence of invasion and metastasis (2). To improve survival, it is important to identify and characterize tumor-specific molecular markers in ESCC that may contribute to its carcinogenesis.

FOXM1 is a member of the Forkhead family of transcription factors (3, 4). FOXM1 acts in the cell cycle by regulating the transition from the G_1 to the S phase, as well as the progression to mitosis (4-6). FOXM1 is predominantly expressed in fetal tissues, but its expression may be maintained in proliferating adult tissues (5, 6). Overexpression of FOXM1 has been observed in cancer of the liver, breast, prostate, brain, cervix, colon, lung, and stomach (7-14). These findings link FOXM1 to the tumorigenesis and progression of several kinds of malignancies. However, the relationship of FOXM1 to ESCC prognosis remains unclear. In the present study, we investigated whether FOXM1 could be used as an independent biomarker to predict prognosis in patients with ESCC.

Patients and Methods

Patients and treatments. The present study included 174 patients with pathologically-confirmed primary ESCC (Table I) who underwent curative surgical resection at Osaka University Hospital between 2001 and 2007. The study population included 19 women and 155 men; the median age was 64 years (range=46 to 81 years). All patients underwent subtotal esophagectomy *via* right thoracotomy with two- or three-field lymphadenectomy. Non-curative resection was excluded, and curative (R0) resection was achieved for all patients. No patients died of postoperative complications. The 63 patients with lymph node metastasis at initial diagnosis received neoadjuvant chemotherapy (NAC), which consisted of two courses of 5-fluorouracil, cisplatin, and adriamycin. After surgery, patients were surveyed every three months by physical examination and serum tumor markers (squamous cell carcinoma antigen, carcinoembryonic antigen), every six months by computed tomographic scanning and abdominal ultrasonography, and every year by endoscopy until tumor recurrence. Patients with tumor recurrence received chemotherapy or chemoradiotherapy as long as they were able to tolerate it. The mean overall survival (OS) was 46.3 months, and the mean recurrence-free survival (RFS) was 42.8 months.

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Key Words: FOXM1, esophageal squamous cell carcinoma, immunohistochemistry.

Table I. *Clinical characteristics of 174 patients with esophageal squamous cell carcinoma.*

Parameter	Patients, n (%)
Age, years	64 (46-81)[a]
Gender, male/female	155 (89.0)/19 (11.0)
Histology[b], poor/mod/well	42 (24.1)/93 (53.4)/39 (22.4)
pT[c], 0/1/2/3/4	0 (0)/50 (28.7)/27(15.5)/84 (48.3)/13 (7.5)
pN[c] N0/N1/N2/N3	54 (31.0)/56 (32.2)/37 (21.3)/27 (15.5)
pStage[c] 0/I/II/III/IV	0 (0)/33 (18.9)/41 (23.6)/74 (42.5)/26 (14.9)

[a]Data presented as median (range). [b]Poorly, moderately, and well-differentiated squamous cell carcinoma. [c]pT, pN, pStage (pathological classification) according to the seventh edition of the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification.

Immunohistochemical analysis. FOXM1 expression was evaluated by immunohistochemistry of 4-μm-thick sections of 10% formalin-fixed and paraffin-embedded tissue blocks, as described previously (12). For staining, tissue slides were de-paraffinized in xylene and then rehydrated using graded ethanol. For antigen retrieval, slides were autoclaved in 10 mM citrate buffer (pH 6.0) at 110°C for 20 min. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 20 min. Non-specific binding was blocked with 10% normal serum for 20 min. Subsequently, tissue slides were incubated overnight with FOXM1 antibody (sc502, dilution 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C in a moist chamber. Sites of antibody binding were visualized with the ABC peroxidase detection system (Vector Laboratories, Burlingame, CA, USA). Finally, sections were incubated in 3,3'-diaminobenzidine tetrahydrochloride with 0.05% H_2O_2 for 1 min and counterstained with 0.1% hematoxylin. One representative slide with the deepest tumor invasion was selected from each patient and subjected to immunohistochemistry. The percentage of cancer cells stained with the antibody was then determined. FOXM1 staining for each ESCC sample was defined as positive when more than 10% of the cancer cells in a section were immunoreactive with the FOXM1 antibody; it was defined as negative when 10% or fewer of the cancer cells in a section were positive.

Statistical analysis. Statistical analysis was performed using JMP software (JMP version 9.0.2; SAS Institute, Cary, NC, USA). The relationship between FOXM1 expression and various clinicopathological parameters was assessed using the χ^2 test. RFS and OS were assessed using the Kaplan–Meier method and compared using the log-rank test. All parameters found to be significant in univariate analysis using the Cox proportional hazards model were entered into multivariate survival analysis. *p*-Values <0.05 were considered significant; each *p*-value was derived from a two-tailed test.

Results

FOXM1 expression in ESCC. A total of 174 samples (Table I) that contained both cancerous and non-cancerous lesions were evaluated for FOXM1 expression by immunohistochemistry. Out of these, 94 (54.0%) were positive for FOXM1 expression; staining was mainly cytoplasmic, with faint nuclear staining in tumor cells (Figure 1A). The remaining 80 (46.0%) samples were negative for FOXM1 expression (Figure 1B). In contrast, none of the samples of normal squamous epithelium exhibited substantial FOXM1 staining, although some basal cells exhibited faint nuclear immunostaining (Figure 1C). FOXM1-positive cells were detected in various parts of the tumors, including the surface, central, and deep areas of the esophagus.

Table II. *Correlation between FOXM1 expression and clinicopathological parameters.*

Parameters	FOXM1 expression		
	Positive (%)	Negative (%)	*p*-Value
Age, years			
<65	46 (26.4)	43 (24.7)	0.53
≥65	48 (27.6)	37 (21.3)	
Gender			
Male	84 (48.3)	71 (40.8)	0.90
Female	10 (5.8)	9 (5.2)	
Histology[a]			
Poor, moderate	74 (42.5)	61 (35.1)	0.70
Well	20 (11.5)	19 (10.9)	
Neoadjuvant chemotherapy			
Yes	35 (20.1)	28 (16.1)	0.76
No	59 (33.9)	52 (29.9)	
pT[b]			
T1-2	38 (21.8)	39 (22.4)	0.27
T3-4	56 (32.2)	41 (23.6)	
pN[b]			
N0	24 (13.8)	30 (17.2)	0.089
N1-3	70 (40.2)	50 (28.4)	
pStage[b]			
I, II	36 (20.7)	38 (21.8)	0.22
III, IV	58 (33.3)	42 (24.1)	

[a]Poorly, moderately, and well-differentiated squamous cell carcinoma. [b]pN, pT, pStage (pathological classification) according to the seventh edition of the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification.

Correlation between FOXM1 expression and clinicopathological parameters. Table II lists the correlations between FOXM1 expression and various clinicopathological parameters. No significant correlations were observed between FOXM1 expression and other parameters, including age, sex, histology, use of NAC, or depth of tumor invasion (Table II).

Correlation between FOXM1 expression and survival. The total 5-year OS rate was 52.7%. Patients with FOXM1-positive tumors exhibited poorer OS than those with negative tumors (5-year OS 42.8% *versus* 64.8%, p=0.0037; Figure 2A). Similarly, patients with FOXM1-positive tumors exhibited poorer 5-year RFS than those with FOXM1-

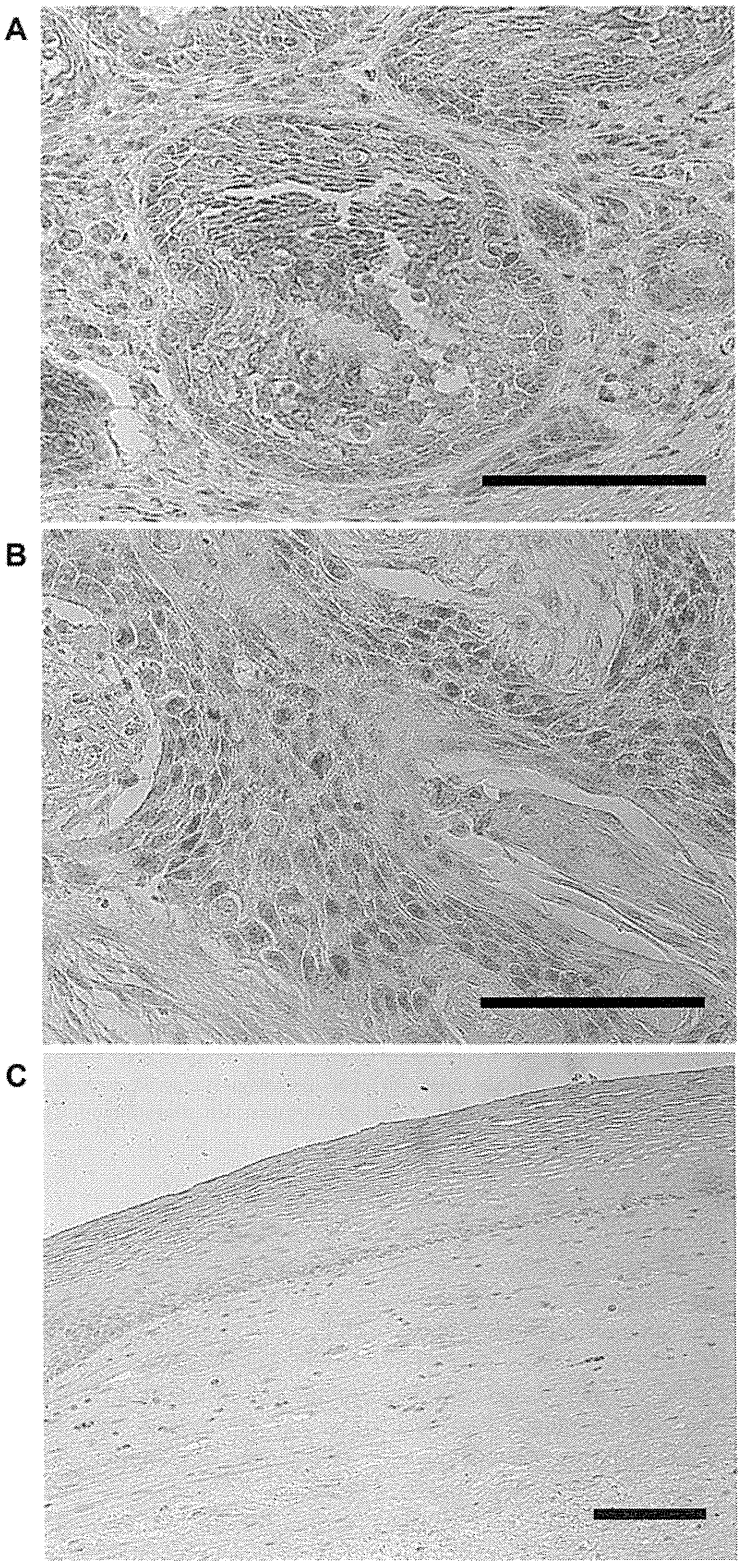

Figure 1. *FOXM1 expression determined by immunohistochemical staining. A: Representative FOXM1-positive esophageal squamous cell carcinoma exhibiting staining mainly in the cytoplasm of tumor cells (magnification ×200). B: Representative FOXM1-negative esophageal squamous cell carcinoma exhibiting almost no staining of tumor cells (magnification ×200). C: Representative normal squamous epithelium that was negative for FOXM1 expression except in a few basal cells (magnification ×100). Scale bars, 100 μm.*

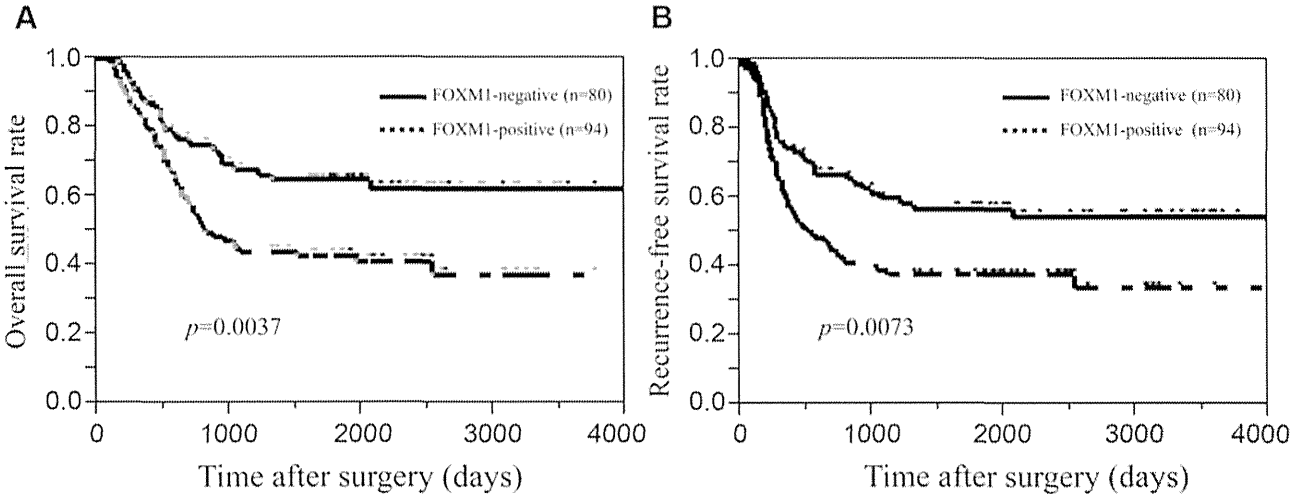

Figure 2. *Survival curves according to FOXM1 expression. A: Overall survival curve according to FOXM1 expression for all patients plotted by the Kaplan–Meier method. B: Recurrence-free survival curves according to FOXM1 expression for all patients. Differences between the two groups were evaluated using the log-rank test.*

Table III. *Univariate and multivariate analysis of overall survival using Cox's proportional hazard model.*

Parameter	Number of cases	Univariate		Multivariate	
		HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age, ≥65 years *vs.* < 65 years	89 *vs.* 85	1.13 (0.73-1.76)	0.57		
Sex, female *vs.* male	19 *vs.* 155	1.02 (0.47-1.93)	0.96		
Histology, poor, moderate *vs.* well[a]	135 *vs.* 39	1.54 (1.87-2.91)	0.14		
pT (T3, 4 *vs.* T1, 2)[b]	97 *vs.* 77	2.48 (1.56-4.05)	<0.0001	1.69 (1.04-2.82)	0.033
pN (N1-3, N0)[b]	120 *vs.* 54	3.56 (2.01-6.93)	<0.0001	2.77 (1.54-5.42)	0.0004
Neoadjuvant chemotherapy, yes *vs.* no	63 *vs.* 111	2.36 (1.52-3.66)	0.0001	1.97 (1.26-3.10)	0.0031
FOXM1 expression, positive *vs.* negative	94 *vs.* 80	1.95 (1.24-3.15)	0.0034	1.69 (1.06-2.75)	0.027

[a]Poorly, moderately, and well differentiated squamous cell carcinoma. [b]pT, pN, (pathological classification) according to the seventh edition of the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification. HR: Hazard ratio; CI: confidence interval.

negative tumors. In univariate analysis, the following were significantly associated with OS: pT [hazard ratio (HR)=2.48, 95% confidence interval (CI)=1.56-4.05, $p<0.0001$], pN (HR=3.56, 95% CI=2.01-6.93, $p<0.0001$), NAC (HR=2.36, 95% CI=1.52-3.66, $p=0.0001$), and FOXM1 expression (HR=1.95, 95% CI=1.24-3.15, $p=0.0034$) (Table III). The four parameters that showed statistical significance ($p<0.05$) in univariate analysis were entered into multivariate analysis. Multivariate analysis revealed that pN was the poorest prognostic factor (HR=2.77, 95% CI=1.54-5.42, $p=0.0004$), followed by NAC (HR=1.97, 95% CI=1.26-3.10, $p=0.0031$), pT (HR=1.69, 95% CI=1.04-2.82, $p=0.033$), and positive FOXM1 expression (HR=1.69, 95% CI=1.06-2.75, $p=0.027$) (Table III).

Discussion

In the present study, we investigated the expression of FOXM1 in ESCC tissues. To our knowledge, this is the largest series of samples analyzed for FOXM1 expression in ESCC to date. Our analysis revealed that FOXM1 expression in ESCC is an independent prognostic indicator for OS. This finding is consistent with previous reports (7, 11, 12, 15, 16). In our series, patients with advanced ESCC received NAC. Thus, NAC became a strong prognostic factor for OS. As far as we are aware, there is just one report on the association between FOXM1 and ESCC in clinical samples (17). In that study, Hui *et al.* reported that FOXM1 overexpression was associated with pathological stage, but not with prognosis of patients with

ESCC. However, it might be premature to conclude that FOXM1 is not associated with the prognosis of patients with ESCC. The report by Hui *et al.*, assessed only 64 patients, and may have been too small to reveal an association between FOXM1 expression and prognosis. Notably, although that study did not find an association between FOXM1 expression and prognosis, it did show a positive association between FOXM1 expression and pathological stage.

FOXM1 is a proliferation-associated transcription factor with important roles in cell proliferation, differentiation, and apoptosis (5, 6, 18). However, the mechanism by which FOXM1 signaling induces tumor growth is not well-understood. Multiple pathways crosstalk with the FOXM1 pathway, including the phosphatidylinositol 3-kinase/protein kinase B (Akt) (19, 20), nuclear factor-κB (21), sonic hedgehog (22), extracellular signal-regulated kinase (23), cyclooxygenase-2 (24), epidermal growth factor receptor (25, 26), vascular endothelial growth factor (27, 28), avian myelocytomatosis virus oncogene cellular homolog (c-MYC) (29, 30), p53 (31, 32), and hypoxia-inducible factor-1 pathways (33). Thus, these reports strongly suggest that FOXM1 is centrally-involved in tumor aggressiveness. In our analysis FOXM1 expression was associated not only with OS but also RFS, this phenomenon was consistent with these mechanisms.

Overexpression of FOXM1 in tumor cell lines is correlated with resistance to apoptosis and to premature senescence induced by oxidative stress, which is strongly implicated in resistance to chemotherapy (34). Recent studies show that FOXM1 is overexpressed in a variety of human cancer types and is crucially-implicated in tumorigenesis (3, 8-10, 35, 36). Furthermore, down-regulation of FOXM1 leads to inhibition of cell growth, migration, and invasion in several cancer types (36-38). These results suggest that FOXM1 may play a crucial role in the development and progression of human cancer. Therefore, although more studies are required, inactivation of FOXM1 may represent a promising strategy for developing novel and selective anticancer therapies.

In conclusion, here we examined the expression of FOXM1 protein in ESCC specimens and investigated correlations between FOXM1 overexpression and clinicopathological characteristics. Patients that were positive for FOXM1 expression had worse prognoses. Thus, evaluation of FOXM1 expression might help identify a subset of patients with ESCC who need more intensive treatment.

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ORIGINAL ARTICLE

Feasibility of laparoscopy-assisted total gastrectomy in patients with clinical stage I gastric cancer

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Abstract
Background Laparoscopy-assisted total gastrectomy (LATG) for gastric cancer is not yet widespread because of the technical difficulty of reconstruction. We have performed LATG on 100 patients with clinical stage I gastric cancer. This study investigated the short-term outcomes of LATG.
Methods Between September 2001 and September 2012, 100 patients with clinical stage I gastric cancer underwent LATG with D1 plus beta or D2 lymphadenectomy. Roux-en-Y esophagojejunostomy was performed intracorporeally using end-to-side anastomosis with a circular stapler (the purse-string suture method). The primary endpoint was the proportion of postoperative complications during hospitalization.
Results Mean operation time was 249 min; mean blood loss was 182 ml. There were no conversions to open surgery. According to the Clavien–Dindo classification, there were 8 grade II (8 %) and 10 grade IIIa/b (10 %) complications. There were no treatment-related deaths or grade IV complications. The most frequent complication was anastomotic or stump leakage (6 %), followed by pancreatic fistula (5 %). Reoperations were required in two patients with leakage.
Conclusions The short-term outcomes of LATG in our study involving 100 patients were outlined. LATG for gastric cancer patients should be attempted preferably in a clinical trial setting by surgeons with sufficient experience in laparoscopic gastrectomy.

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Keywords LATG · LTG · Laparoscopic · Purse-string suture

Introduction

The feasibility of laparoscopy-assisted distal gastrectomy (LADG) has been assessed in many studies [1–3]. A multicenter phase II study has demonstrated that LADG can be performed safely by surgeons with sufficient experience [4]. Large-scale phase III trials of LADG versus open distal gastrectomy for clinical stage I gastric cancer are now ongoing in Japan and Korea. Although long-term outcomes have not yet been evaluated, LADG has recently become a common surgical procedure in both countries.

Nevertheless, laparoscopy-assisted total gastrectomy (LATG) is not yet widespread. The reconstruction required in LATG is technically much more difficult than that in LADG. Only a few Korean studies have evaluated the feasibility of LATG with more than 100 patients [5, 6], so the safety of LATG is still controversial. We have performed LATG with the purse-string suture anastomosis on 100 patients with clinical stage I gastric cancer. This is the first Japanese study involving 100 patients to evaluate short-term outcomes after LATG.

Methods

Patients

Between September 2001 and September 2012, 110 consecutive patients with clinical stage I (T1N0M0, T1N1M0,

and T2N0M0) gastric cancer underwent LATG at the Osaka University Hospital. Cases of stump carcinoma were excluded from this study. Because 10 of 110 cases underwent the OrVil method or the overlap method (side-to-side anastomosis with a linear stapler) anastomosis, we analyzed only 100 cases with the purse-string suture anastomosis in this study. Clinical evaluation of tumor depth (cT) and lymph node metastasis (cN) were determined by preoperative evaluations with both endoscopy and computed tomography. The details of the methods for preoperative T staging have been reported elsewhere [7]. All tumors were histologically diagnosed as adenocarcinoma of the stomach. Clinical stage was classified according to the Japanese Classification of Gastric Carcinoma, second English edition [8]. Informed consent for LATG was obtained from all patients before surgery.

Surgery

Surgeons performed LATG and lymph node dissection according to the Japanese Gastric Cancer Treatment Guidelines in principle [9]. Patients with cT1 carcinoma underwent D1 plus beta dissection, including station nos. 7, 8a, and 9. Patients with cT2 disease underwent D2 or D2 minus splenic hilum node (station no. 10). D2 minus station no. 10 was treated as D1 plus beta in this study.

For reconstruction, Roux-en-Y esophagojejunostomy was performed with the purse-string suture method as previously reported [10]. In brief, the esophageal stump was sewn over with interrupted sutures laparoscopically or by using a device called the Endostich, and the anvil of a circular stapler was inserted into the esophageal stump. The purse-string suture was tied and reinforced with a monofilament pre-tied loop. A circular stapler inserted into the distal side of the jejunum was introduced into the abdominal cavity through the minilaparotomy site, and esophagojejunostomy was performed. Anastomotic leaks were evaluated using air insufflation.

All operations were performed or supervised by surgeons with sufficient experience with laparoscopic gastrectomies and who were certified by the Japan Society for Endoscopic Surgery.

Statistical analysis

The primary endpoint of this study was the incidence of postoperative complications during hospitalization. The grading of complications was based on the Clavien–Dindo classification system [11]. All statistical analyses were performed using SPSS Statistics software, version 20 (Chicago, IL, USA).

Results

The background characteristics of the 100 patients in this study are shown in Table 1. Ninety percent of patients were diagnosed as cT1. Only 6 patients (6 %) had clinically positive lymph nodes.

Surgical results are shown in Table 2. D1 plus beta dissection was performed in 95 cases (95 %) and D2 in 5 cases (5 %). All patients received Roux-en-Y reconstruction. Mean operation time was 249 min and mean blood loss was 182 ml. No patients required conversion to open surgery. Two of 7 patients who received splenectomy did so because of either preoperative comorbidity of thrombocytopenia or intraoperative bleeding from the splenic vein.

Table 3 lists the postoperative complications that occurred during hospitalization. Clavien–Dindo grade II complications occurred in eight patients (8 %) whereas those of grade IIIa/b occurred in ten patients (10 %). There were no treatment-related deaths or grade IV complications. The most frequent complication was anastomotic or stump leakage (6 %), followed by pancreatic fistula (5 %). Among the six leakage cases, four occurred in the esophagojejunal anastomosis, one in the duodenum stump, and one in the duodenum stump and the distal side of the jejunum stump. No patients suffered from anastomotic stricture. Reoperations were required in two patients with leakage.

Table 1 Clinical characteristics

	$n = 100$
Age (years)	
Median	63
Range	29–85
Gender	
Male	75 (75 %)
Female	25 (25 %)
Body mass index (kg/m^2)	
Median	22.5
Range	16.2–28.0
Clinical T	
T1	90 (90 %)
T2	10 (10 %)
Clinical N	
N0	94 (94 %)
N1	6 (6 %)
Clinical stage	
IA	84 (84 %)
IB	16 (16 %)

Clinical TNM stages were classified according to the Japanese Classification of Gastric Carcinoma, second English edition

Table 2 Surgical results

	$n = 100$
Lymph node dissection	
D1 plus beta[a]	95 (95 %)
D2	5 (5 %)
Combined resection	
Spleen	7 (7 %)
Gallbladder	6 (6 %)
Operation time (min)	
Mean ± SD	249 ± 47
Blood loss (ml)	
Mean ± SD	182 ± 183
Number of dissected lymph nodes	
Mean ± SD	38 ± 16

[a] D2 minus station no. 10 was treated as D1 plus beta in this study

Table 3 Postoperative complications

	$n = 100$
Any complications	
Grade II	8 (8 %)
Grade IIIa/b	10 (10 %)
Leakage	
Grade II	0
Grade IIIa/b	6 (6 %)
Pancreatic fistula	
Grade II	1 (1 %)
Grade IIIa/b	5 (5 %)
Bleeding	
Grade II	4 (4 %)
Grade IIIa/b	0
Pneumonia	
Grade II	4 (4 %)
Grade IIIa/b	0
Bowel obstruction	
Grade II	2 (2 %)
Grade IIIa/b	0
Reoperation	2 (2 %)

Grading of complications was based on the Clavien–Dindo classification

Discussion

Laparoscopy-assisted total gastrectomy is still not widespread because of the technical difficulty of the reconstruction. Several reports have been issued on the feasibility of LATG, but only a few Korean studies have evaluated the feasibility of LATG in populations of more than 100 patients [5, 6]. We have performed LATG with the purse-string suture anastomosis in 100 patients with clinical stage I gastric cancer. Compared with the previous Korean studies and a small-scale Japanese study evaluating the safety of LATG [5, 6, 12, 13], we were able to perform LATG with more favorable surgical results in terms of operation time. Regarding the incidence of postoperative complications, our study showed better or similar results compared to previous studies of LATG. The incidence of anastomotic leakage in our study was 6 % (6/100), including 1 case of duodenum stump leakage and 1 case of leakage of the duodenum stump and the distal side of the jejunum stump. Previous randomized controlled studies have reported that incidence rates of anastomotic leakage after open total gastrectomy ranged from 3.8 % to 6.8 % [14–16]. Nomura et al. [17] reported the result of a retrospective large-scale study of open total gastrectomy. Although they reported the esophagojejunal leakage rate after stapled anastomosis as 1.0 % using only the data of the recent 6 years, the overall incidence of esophagojejunal anastomosis leakage was 2.9 % (27/943). Indeed, our result of the incidence of esophagojejunal anastomosis leakage (4.0 %) was slightly higher than their result, so we think we should continue to make efforts for reducing the complication rate.

In this study we used only the purse-string suture anastomosis method. The purse-string suture method is simple and is similar to the anastomosis method in open total gastrectomy. The safety of this method has been already reported by other institutions [18, 19]. It requires fewer devices and costs less, and has the advantage of only rarely causing stenosis. Besides the purse-string suture method, two anastomosis methods (the OrVil method and the overlap method) have been reported as useful anastomosis procedures in LATG [20–23]. Although we have performed LATG with the OrVil method or the overlap method for ten cases outside this study, the incidence of anastomotic leakage of grade IIIa/b was 30 % (3/10). The reason for this high incidence was considered to be the inexperience of the surgeons with these methods. These methods might be safer if they were performed more frequently, thus increasing our overall level of expertise.

The risk of postoperative complications is affected by the skill of each individual surgeon. In our case series, six surgeons performed LATG. There was no clear difference among them in the incidence of postoperative complications. Furthermore, all operations were performed or supervised by surgeons with sufficient experience with laparoscopic gastrectomy and who were certified by the Japan Society for Endoscopic Surgery. Also, all surgeons had abundant experience with open gastrectomy. With regard to the learning curve, the incidence of postoperative complications did not show a clear decrease despite the surgeons' increasing expertise. However, in the recent 2 years (after January 2011), there was only one pancreatic

fistula and no anastomotic leakage. During this period, a fixed team of two surgeons (S.T. and Y.K.) have performed LATG in most cases. Even if the learning curves of individual surgeons do not affect the incidence of complications, a fixed team consisting of the same surgeons could perform LATG more safely.

At this point there are insufficient data concerning long-term outcomes after LATG. Several ongoing randomized control trials are comparing long-term survival between laparoscopic and open distal gastrectomy. Long-term outcomes after LATG should be also evaluated by randomized control trials to establish the possibility of a new standard for the surgical treatment of clinical stage I gastric cancer.

In conclusion, the short-term outcomes of LATG in our study involving 100 patients have been outlined. LATG for gastric cancer patients should be attempted preferably in the clinical trial setting by surgeons with sufficient experience in laparoscopic gastrectomy.

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