Prognostic impact of extranodal extension in patients with pN1-N2 lung adenocarcinoma

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Abstract

Purpose: Lymph node involvement is one of the important prognostic factors of patients with lung adenocarcinoma. In the tumor, node, and metastasis classification, lymph node involvement is categorized only according to the anatomical station and not the involvement pattern. The aim of this study was to investigate which morphological pattern of lymph node involvement affects the prognosis of patients with surgically resected lung adenocarcinoma.

Methods: We retrospectively reviewed 168 consecutive patients who underwent surgical resection for primary lung adenocarcinoma with lymph node involvement. The morphological patterns of lymph node involvement (tumor area, number of metastatic lymph nodes, presence of necrosis, and extranodal extension) were histologically examined. The relationships between the patterns of lymph node involvement, clinicopathological features, and survival of patients were analyzed.

Results: Eighty patients had N1 disease, and 88 patients had N2 disease. Univariate analysis revealed that invasive size, history of adjuvant chemotherapy, and presence of extranodal extension were significant prognostic factors in N1 patients, and vascular invasion, pleural invasion, presence of epidermal growth factor receptor mutation, history of adjuvant chemotherapy, and presence of extranodal extension were significant

prognostic factors in N2 patients. In a bivariate analysis including other clinicopathological factors and patterns of lymph node involvement, the presence of extranodal extension was significantly associated with poor 3-year overall and recurrence-free survival of both N1 and N2 patients.

Conclusions: In patients who underwent surgical resection for lung adenocarcinoma with lymph node involvement, the extranodal extension was the most important prognostic factor among morphological lymph node involvement patterns.

Keyword: Lung adenocarcinoma, lymph node involvement, extranodal extension, prognosis

Abbreviations:

EGFR, epidermal growth factor receptor; ENE, extranodal extension; HE, hematoxylin and eosin; LN, lymph node; NSCLC, non-small cell lung cancer; OS, overall survival; RFS, recurrence-free survival.

Declarations

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Ethics approval: This retrospective study was approved by the Institutional Review Board (IRB approval number; 2020-147).

Consent to participate: Informed consent was obtained from all patients.

Consent for publication: Not applicable.

Introduction

Lymph node involvement is one of the most important prognostic factors in lung cancer patients, and the prognosis of patients who underwent surgical resection for non-small cell lung cancer (NSCLC) remains dismal (Asamura H et al. 2015). Nodal staging in the tumor, node, and metastasis (TNM) classification of the International Association for the Study of Lung Cancer (IASLC) is categorized according to the station on lymph node (LN) map of IASLC (Rusch et al. 2009), modifying the Naruke map (Naruke et al. 1978). Although the TNM classification was revised in the eighth edition mainly in terms of the T descriptors, focusing on the size of the invasive component, and the M category was reclassified by the number of extra-thoracic organ metastases, the N classification remained the same based only on the anatomical location of metastatic LN (Goldstraw et al. 2016).

In current nodal staging by TNM classification, nothing has been suggested regarding morphological patterns related to LN involvement, and there has been heterogeneity in the prognosis of lung cancer patients in the same N stage (Caldarella et al. 2006; Nakao et al. 2010). Although many researchers have studied for the rearrangement of the current lymph nodal staging (Giroux et al. 2018), some studies have reported that the number of involved LNs is a prognostic factor in NSCLC patients (Chen

et al. 2019; Chiappetta et al. 2019; Fukui et al. 2006; Katsumata et al. 2019; Wei et al. 2011). Similarly, extranodal extension (ENE) of LN metastasis has been reported to be associated with the prognosis of NSCLC patients (Lee et al. 2007; Luchini et al. 2018). However, it remains unclarified, which morphological pattern of LN involvement is the most important prognostic factor.

Adenocarcinoma and squamous cell carcinoma are the two major histological subtypes of NSCLC (Herbst et al. 2008). Adenocarcinoma is reported to have a higher risk of LN involvement than squamous cell carcinoma (Deng et al. 2019). This study aimed to determine the most important prognostic factor among the morphological patterns of LN involvement in patients with surgically resected lung adenocarcinoma.

Material and Methods

Patients

A total of 1474 consecutive patients with primary lung adenocarcinoma underwent surgery between January 2011 and June 2017 at our hospital and were retrospectively reviewed. We enrolled 1050 patients who underwent complete resection by lobectomy or pneumonectomy with LN dissection, did not receive preoperative therapy and had no synchronous or asynchronous multiple lesions. We excluded 824

patients without metastatic LN and 58 patients whose LN was directly involved in the primary tumor or unevaluable. A total of 168 patients, namely, 80 pathological N1 patients and 88 pathological N2 patients, were enrolled in this study. A flowchart of the patient selection process is shown in Supplemental Fig. 1. This retrospective study was approved by the Institutional Review Board (IRB approval number; 2020-147), and informed consent was obtained from all patients.

Pathological evaluation

Surgical specimens were fixed in 10% formalin, embedded in paraffin, and serially sectioned at 4-μm intervals. The sections were stained with hematoxylin and eosin (HE). We used the Victoria blue-van Gieson staining protocol to evaluate vascular and pleural invasion in all cases. Lymphatic permeation was evaluated with HE-stained slides or using D2-40 staining protocol to visualize lymphatic vessels. Histological typing was based on the 4th edition of the World Health Organization histological classification (William D Travis et al. 2015) and the disease stages were categorized according to the guidelines of the 8th edition of the TNM classification (Goldstraw et al. 2016).

Evaluation of the characteristics of LN involvement

All stained tissue sections were evaluated under a light microscope by two pathologists (K.N. and T.N.). HE-stained slides of all dissected LNs were scanned using the Aperio scan system (Leica Biosystems, Nussloch, Germany). We determined the metastatic tumor and the necrosis by encircling the tumor and necrosis area of each LN and calculated the percentage of the tumor area in LN and necrotic area in tumor area (Fig. 1 a, b, c, d). We used the largest value when multiple LN metastases were present. We defined ENE as cancer cell invasion beyond the capsule of LN (Fig. 1. e, f). We counted and recorded the number of metastatic LNs.

Evaluation of clinicopathological factors

We reviewed the clinical characteristics of patients from the available medical records. The following clinicopathological factors were investigated retrospectively to assess their prognostic effect; age, sex, smoking history, invasive size, pathologic nodal involvement, vascular invasion, lymphatic permeation, pleural invasion, predominant subtype, and adjuvant chemotherapy. The presence of an epidermal growth factor receptor (EGFR) mutation was examined in 157 patients and recorded.

Statistical analysis

Overall survival (OS) and recurrence-free survival (RFS) curves were plotted according to the Kaplan-Meier method and compared using the log-rank test in a univariate analysis. To determine the predictors of OS and RFS, univariate and bivariate analyses were conducted using Cox regression analysis. Two-category comparisons were performed using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. All the *P*-values were two-sided, and the statistical significance level was set at $P<0.05$. All statistical analyses were performed using SPSS version 26.0 software (SPSS Inc., Chicago, IL, USA).

Results

Clinicopathological factors and patterns of lymph node involvement

The clinicopathological characteristics of the 168 patients with metastatic lymph nodes are summarized in Supplemental Table 1. Of the 168 patients, EGFR mutation was examined in 157, and 76 (48%) of them had the mutation. Approximately half of the patients received adjuvant chemotherapy. The pathological characteristics of lymph node involvement are shown in Table 1. The median percentage of the tumor area in the lymph node (tumor area %) was 15% in N1 patients and 27% in N2 patients. The median percentage of the necrotic area in the tumor area of the lymph node (necrosis area %) was

0% in both N1 and N2 patients. ENE was present in 29 patients with N1 and 57 patients with N2, respectively.

Prognostic effect of morphological lymph node involvement patterns on N1 patient survival

We chose 20% as the tumor area % cutoff because the median tumor area % was 22% in all patients. Necrosis was evaluated depending on its presence (positive or negative).

In N1 patients, the median length of follow-up was 4.0 years (range, 0.2-9.2 years). Univariate analysis showed that overall survival was significantly associated with the size of the invasive component of the primary tumor, history of adjuvant chemotherapy, and presence of ENE. Regarding RFS, univariate analysis showed that smoking history and presence of ENE were significantly associated with patient survival (Supplemental Table 2). Because the presence of ENE was significantly associated with both OS and RFS, we assessed the prognostic significance of the presence of ENE using bivariate analysis. Bivariate analysis revealed that the ENE was an independent predictor of poor OS and RFS when adjusted for all other clinicopathological factors (P <0.05) (Table 2). The 3-year OS and RFS rates were significantly lower in patients with ENE

than in patients without ENE (68.6% vs. 89.9%, $P = 0.003$; 30.6% vs. 57.8%, $P = 0.006$: Fig. 2). In patients who did not receive adjuvant chemotherapy, the 3-year OS and RFS rates were significantly lower in those with ENE (84.5% vs. 57.0%, $P = 0.014$; 59.4% vs. 26.3%, $P = 0.030$). In patients who received adjuvant chemotherapy, the 3-year OS and RFS rates tended to be lower in those with ENE (100.0% vs. 90.0%, $P = 0.057$; 66.7% vs. 60.0%, $P = 0.008$; Supplemental Fig. 2).

Prognostic effects of lymph node involvement patterns on N2 patient survival

In N2 patients, the median length of follow-up was 4.0 years (range, 0.3-9.3 years). Univariate analysis showed that OS was significantly associated with the presence of ENE as well as the vascular and pleural invasion of the primary tumor, presence of EGFR mutation, and history of adjuvant chemotherapy. Regarding RFS, univariate analysis showed that the history of adjuvant chemotherapy, multiple metastatic lymph nodes, presence of necrosis in metastatic lymph nodes, and presence of ENE were significantly associated with patient survival (Supplemental Table 3). Because the presence of ENE was significantly associated with both OS and RFS, we assessed the prognostic significance of the presence of ENE using bivariate analysis. Bivariate analysis revealed that the presence of ENE was an independent predictor of poor OS and

RFS when adjusted for all other clinicopathological factors ($P < 0.01$) (Table 3). The 3-year OS and RFS rates were significantly lower in patients with ENE than in those without ENE (73.6% vs. 93.5%, $P = 0.001$; 26.7% vs. 51.1%, $P = 0.001$; Fig. 3). In patients who received adjuvant chemotherapy, the 3-year OS and RFS rates were significantly lower in patients with ENE (96.2% vs. 72.9%, $P = 0.001$; 57.7% vs. 27.5%, $P = 0.001$; Supplemental Fig. 3).

Discussion

The current nodal staging by TNM classification of lung cancer is based on the anatomical station; however, nothing has been suggested regarding morphological LN involvement patterns. In the present study, we evaluated the prognostic impact of multiple morphological patterns of LN involvement, such as the size and number of metastatic LNs, presence of ENE, and presence of necrosis. We compared the prognostic effect of these LN involvement patterns and revealed that ENE had the most powerful prognostic effect among those patterns. To the best of our knowledge, this study is the first to reveal that ENE is the most important factor among morphological LN involvement patterns in patients with lung adenocarcinoma patient with both N1 and N2 disease.

Previous studies have also reported the prognostic significance of ENE in

patients with surgically resected NSCLC (Lee et al. 2007; Li et al. 2020; Liu et al. 2015; Luchini et al. 2018; Olszyna-Serementa et al. 2013). The ENE was detected in 63.3% of patients who underwent the surgical resection for NSCLC with LN involvement, and had a prognostic effect on their OS (Lee et al. 2007). Furthermore, ENE was a significant predictive factor for local recurrence as well as OS and RFS in NSCLC patients (Liu et al. 2015). In our study, bivariate analysis adjusting every other clinicopathological factor revealed that ENE had the strongest effect on OS and RFS in both pN1 and pN2 adenocarcinoma patients. This result indicates that ENE has an important potential for the N classification of lung adenocarcinoma patients.

In our study, as previously reported, ENE was detected more frequently in pN2 patients (Lee et al. 2007; Liu et al. 2015). Furthermore, the presence of ENE was correlated with a high tumor area % and multiple LN metastases (Supplemental Table 4). These results indicate that ENE might be a surrogate marker of the malignant potential of the primary tumor. However, some animal studies showed that metastatic cancer cells in lymph nodes had the potential to invade local blood vessels and disseminate to distant organs (Brown et al. 2018; Pereira et al. 2018). These reports support the hypothesis that ENE itself would invade the local vessels around the LN capsule and result in the poor prognosis of patients. To clarify the mechanism of the association between ENE and

patient prognosis, further studies including more detailed pathological examinations are needed.

Postoperative adjuvant chemotherapy has been reported to improve survival in patients with completely resected stage II to IIIA NSCLC (Douillard et al. 2010; Kris et al. 2017; Pignon et al. 2008). In our study, approximately half of the patients (54%) received adjuvant chemotherapy, and their prognosis tended to be better than that of those without adjuvant chemotherapy. However, the bivariate analysis revealed that ENE was a poor prognostic factor independent of adjuvant chemotherapy. OS and RFS of patients with ENE were poorer in pN1 patients irrespective of adjuvant chemotherapy and pN2 patients receiving adjuvant chemotherapy (Supplemental Fig. 2 and 3). Several studies have shown that postoperative radiotherapy (PORT) improves the prognosis of pN2 patients (Douillard et al. 2008; Shen et al. 2014). However, other study reported that PORT did not improve the prognosis of pN2 patients with ENE (Moretti et al. 2009). These results suggest that patients with ENE might be resistant to adjuvant chemotherapy or PORT. Further prospective investigations are needed to evaluate the efficacy of these therapies in patients with ENE.

There are some limitations to the current study. First, this was a retrospective study in a single institution. Because the number of events in our cohort was small,

overfitting was concerned when we performed multivariate analysis for three or more variables. Therefore, bivariate analysis was substituted to compare the prognostic effect of ENE and each clinicopathological factor. Second, although the effect of adjuvant therapy for patients with N1 and N2 adenocarcinoma had been proved, approximately a half of the patients did not receive adjuvant chemotherapy in our cohorts due to age, comorbidities, endurance, or patient's requests. Third, the evaluation of involved LNs were affected by surgical technique for LN dissection. These problems have been raised by previous researcher (Katsumata et al. 2019). Therefore, we excluded patients with unevaluable metastatic LNs whose structure are disrupted in this study. To predict the prognosis of patients with lung adenocarcinoma more accurately by evaluation of ENE, surgical technique to dissect LNs en block with adjacent adipose tissue as a lump are required. Fourth, we did not evaluate patients with squamous cell carcinoma, which is one of the major histological subtypes of lung cancer as well as adenocarcinoma. Therefore, we should interpret the results of the current study with caution.

In conclusion, we showed that ENE is the most important prognostic factor of morphological LN involvement patterns in lung adenocarcinoma patients with both N1 and N2 disease. Recognizing ENE would provide more accurate prognostic information and would be helpful to devise a treatment strategy for patients with LN involvement, and

eventually to develop the new nodal staging. Further prospective studies are needed to incorporate ENE into N staging using the current TNM classification.

Figure captions

Fig.1 *Microscopic features of lymph node metastasis*

a: Lymph node with a macrometastatic tumor. The black line indicates the area of the metastatic tumor.

b: Lymph node with the micrometastatic tumor. The black line indicates the area of the metastatic tumor.

c: Lower power view of necrosis in a metastatic lymph node.

d: Higher power view of necrosis in a metastatic lymph node. The black line indicates the area of necrosis.

e: Lower power view of a metastatic lymph node with extranodal extension. The solid black line indicates the capsule of the lymph node, and the dotted line indicates capsular invasion by tumor cells.

f: Higher power view of a metastatic lymph node with extranodal extension

Fig. 2 *Kaplan-Meier estimates of the survival of pN1 patients with and without extranodal extension (ENE).*

a: Overall survival (OS) curves of pN1 patients with and without ENE.

b: Recurrence-free survival (RFS) curves of pN1 patients with and without ENE.

Fig. 3 *Kaplan-Meier estimates of the survival of pN2 patients with and without extranodal extension (ENE).*

a: Overall survival (OS) curves of pN2 patients with and without ENE.

b: Recurrence-free survival (RFS) curves of pN2 patients with and without ENE.

Supplemental Figure captions

Supplemental Fig. 1 Flow diagram of subject selection

Supplemental Fig. 2 Kaplan-Meier estimates of the survival of pN1 patients with and without extranodal extension (ENE).

a: Overall survival (OS) curves of pN1 patients with and without ENE who did not receive adjuvant chemotherapy.

b: Recurrence-free survival (RFS) curves of pN1 patients with and without ENE who received adjuvant chemotherapy.

Supplemental Fig. 3 Kaplan-Meier estimates of the survival of pN2 patients with and without extranodal extension (ENE).

a: Overall survival (OS) curves of pN2 patients with and without ENE who did not receive adjuvant chemotherapy.

b: Recurrence-free survival (RFS) curves of pN2 patients with and without ENE who

received adjuvant chemotherapy.

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Fig. 1

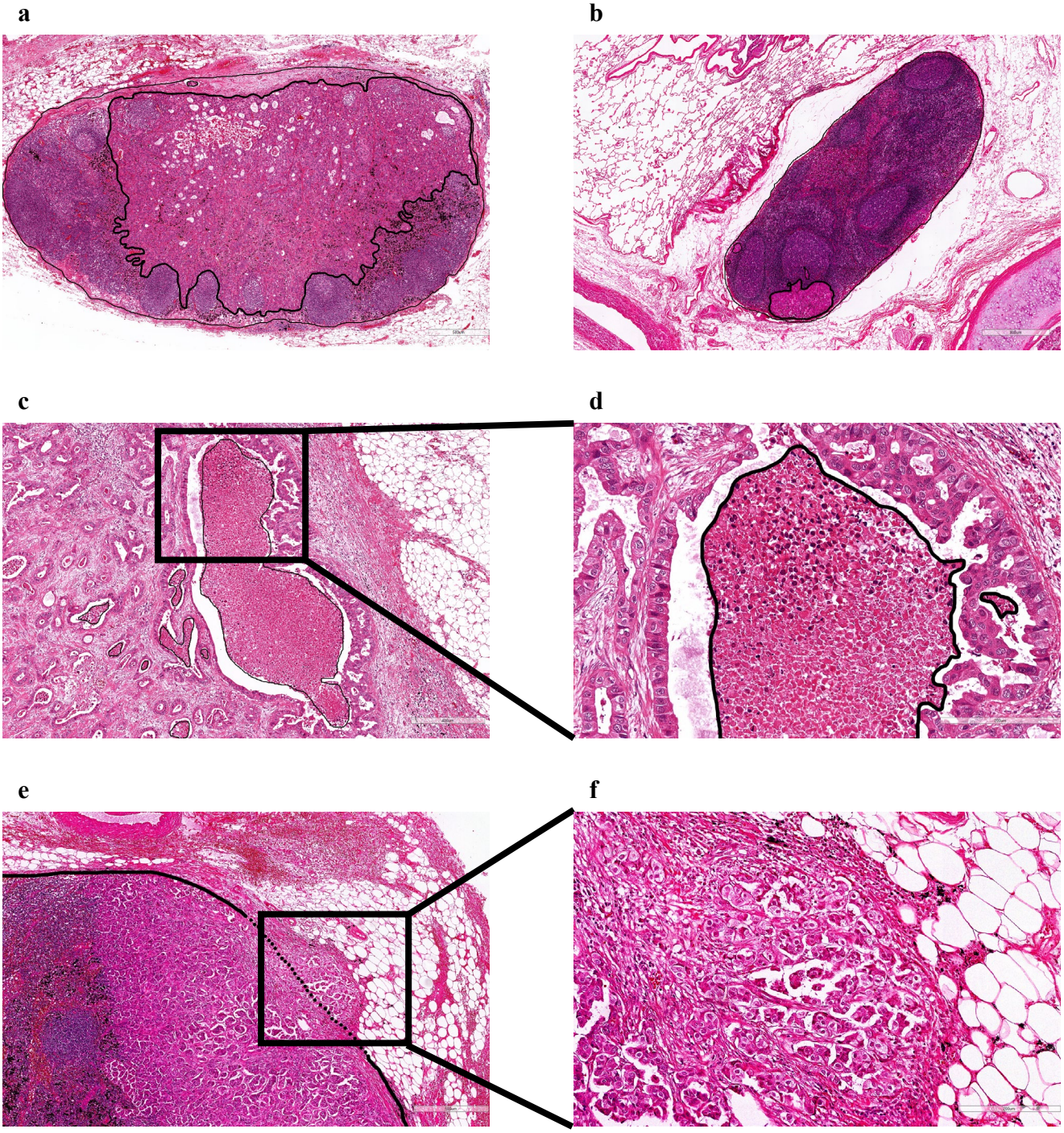
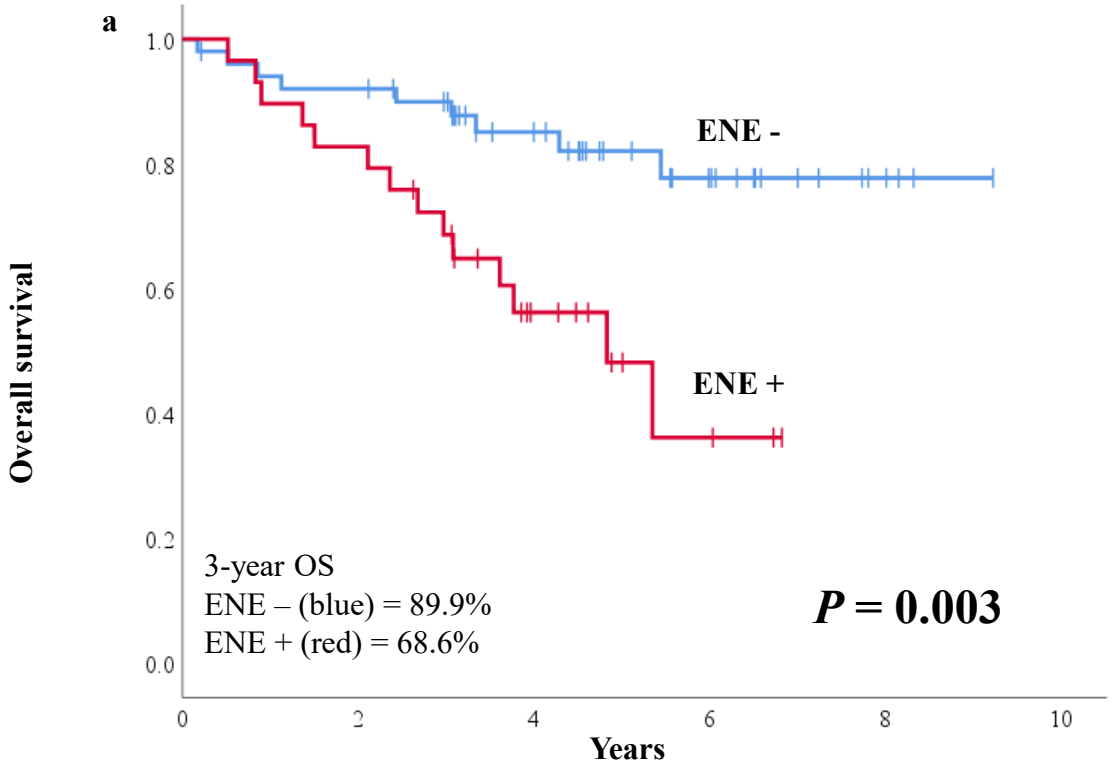
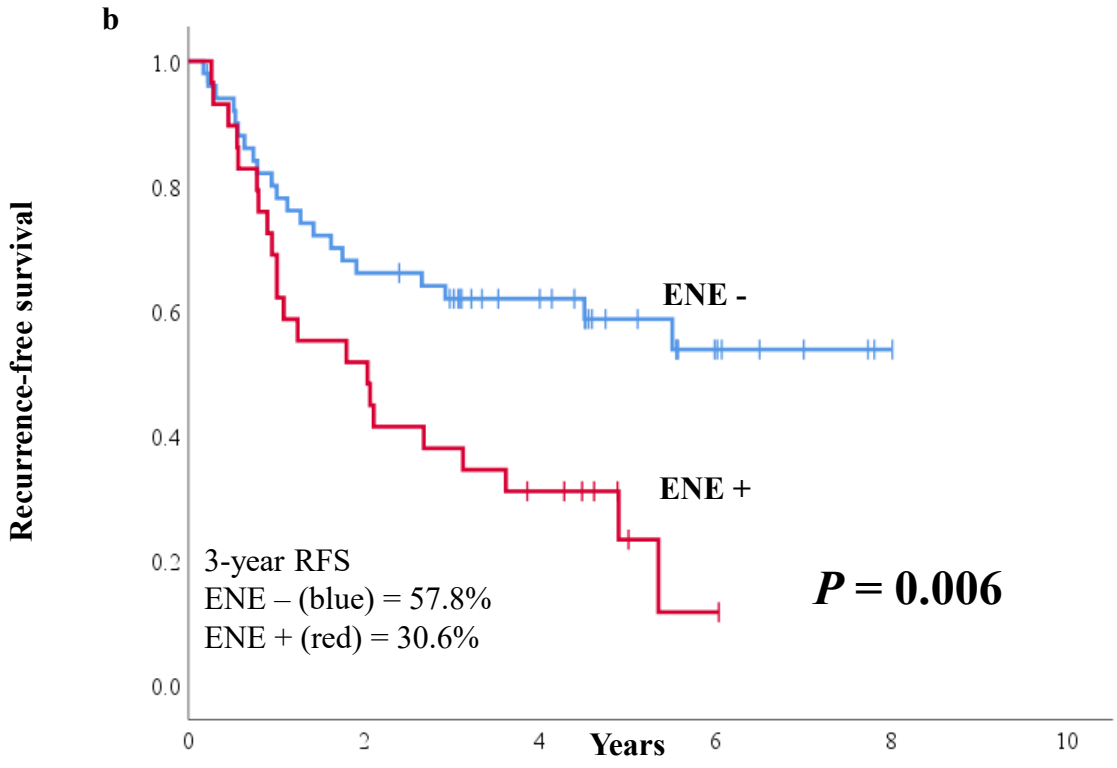


Fig. 2

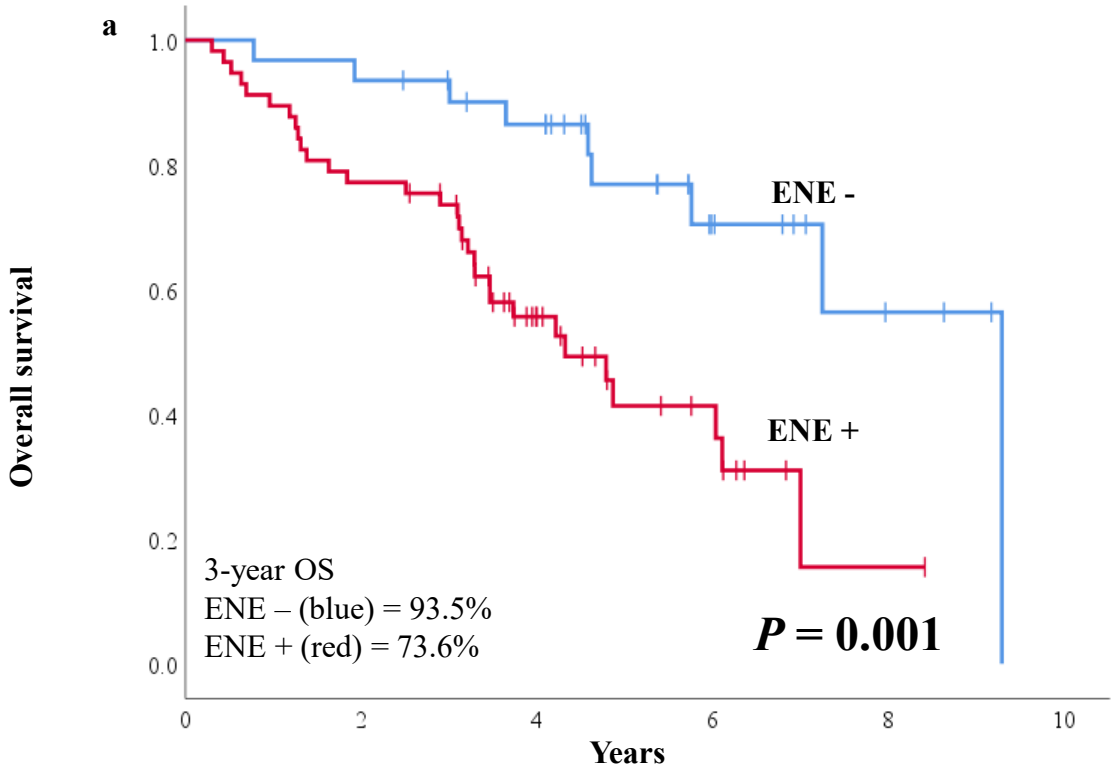


ENE -	51	46	29	14	4
ENE +	29	24	10	3	0

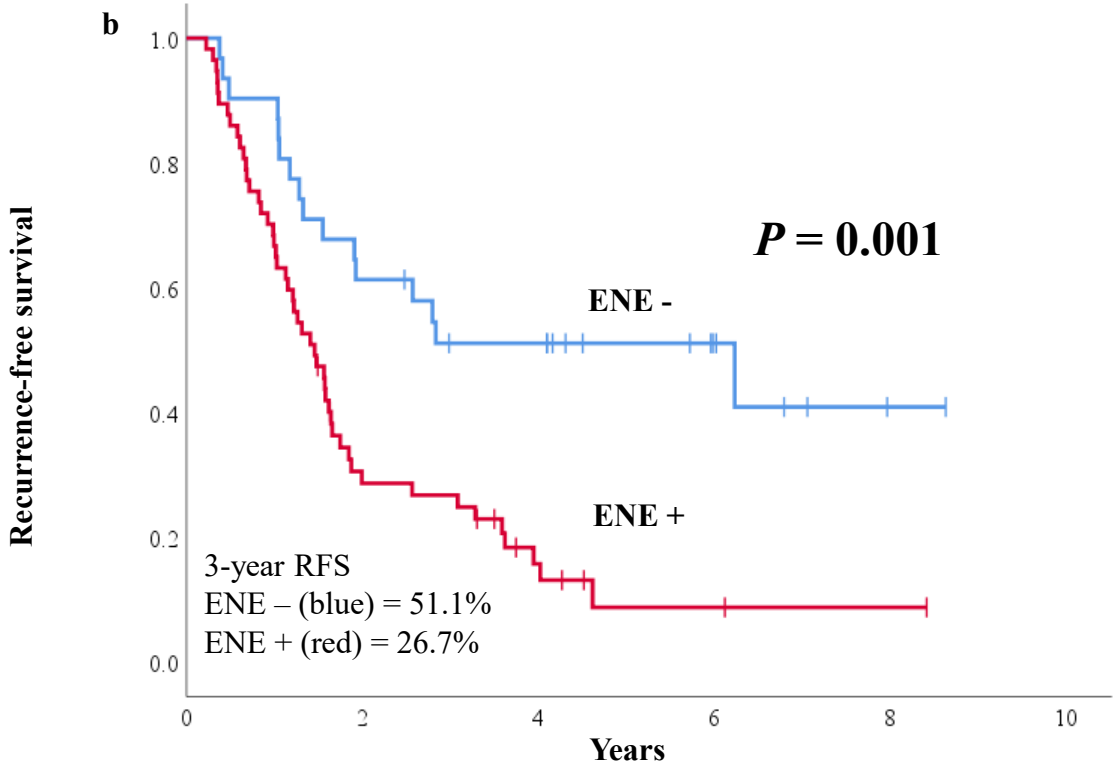


ENE -	51	33	21	7	0
ENE +	29	15	8	1	0

Fig. 3



ENE -	31	29	24	9	3
ENE +	57	44	19	8	1



ENE -	31	19	21	14	1
ENE +	57	15	8	6	1

Table 1. Pathological characteristics of metastatic lymph nodes (N=168)

	N (%)	
	N1 (N=80)	N2 (N=88)
Multiple nodal metastases	36 (45)	77 (88)
Tumor area %[a], median [range]	15 [0-85]	27 [0-90]
Necrosis area %[a], median [range]	0 [0-16]	0 [0-10]
ENE positive	29 (36)	57 (65)

a The largest value was chosen when multiple lymph nodes metastases were present.

ENE, extranodal extension.

Table 2. Bivariate analysis for prognostic significance of extranodal extension in pN1 patients

	Overall survival			Recurrence-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
ENE, positive	3.495	1.489-8.201	0.004	2.295	1.247-4.224	0.008
Age, ≥65	1.334	0.565-3.151	0.511	1.000	0.539-1.856	1.000
ENE, positive	3.337	1.431-7.780	0.005	2.279	1.243-4.178	0.008
Sex, Male	2.662	0.905-7.831	0.075	0.682	0.370-1.258	0.221
ENE, positive	3.457	1.484-8.051	0.004	2.146	1.166-3.951	0.014
Smoking history, ever	1.727	0.637-4.679	0.283	0.410	0.222-0.755	0.004
ENE, positive	3.019	1.285-7.093	0.011	2.212	1.199-4.083	0.011
Invasive size, >3.0cm	2.388	0.974-5.854	0.057	1.248	0.679-2.294	0.475
ENE, positive	3.326	1.418-7.798	0.006	2.300	1.256-4.213	0.007
Vascular invasion, +	0.801	0.336-1.910	0.617	1.119	0.571-2.191	0.743
ENE, positive	3.328	1.411-7.850	0.006	2.214	1.205-4.065	0.010
Lymphatic invasion, +	1.138	0.488-2.657	0.765	1.432	0.773-2.653	0.253
ENE, positive	3.713	1.565-8.807	0.003	2.451	1.331-4.513	0.004
Pleural invasion, +	2.137	0.870-5.249	0.098	1.873	0.995-3.525	0.052
ENE, positive	3.396	1.454-7.931	0.005	2.299	1.255-4.210	0.007
Subtype, non-lepidic	1.230	0.165-9.149	0.840	1.326	0.320-5.486	0.697

ENE, positive	3.258	1.395-7.608	0.006	2.254	1.218-4.172	0.010
EGFR mutation, +	0.691	0.301-1.585	0.382	1.334	0.722-2.463	0.357
ENE, positive	3.592	1.527-8.451	0.003	2.331	1.271-4.274	0.006
AC, positive	0.284	0.096-0.840	0.023	0.760	0.404-1.429	0.394
ENE, positive	3.298	1.411-7.706	0.006	2.287	1.248-4.192	0.007
Metastatic LN, multiple	1.931	0.838-4.445	0.122	1.542	0.844-2.818	0.159
ENE, positive	4.251	1.614-11.199	0.003	2.326	1.177-4.598	0.015
Tumor area %[a], >20%	0.634	0.250-1.613	0.339	0.971	0.494-1.907	0.931
ENE, positive	3.610	1.538-8.469	0.003	2.327	1.267-4.273	0.006
Necrosis, positive	0.270	0.036-2.011	0.201	0.698	0.248-1.961	0.495

a The largest value was chosen when multiple lymph nodes metastases were present.

HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor;

ENE, extranodal extension; AC, adjuvant chemotherapy; LN, lymph node.

Table 3. Bivariate analysis for prognostic significance of extranodal extension in pN2 patients

	Overall survival			Recurrence-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
ENE, positive	3.541	1.588-7.896	0.002	2.829	1.575-5.082	0.001
Age, ≥65	1.495	0.776-2.880	0.229	1.384	0.837-2.287	0.205
ENE, positive	3.400	1.525-7.581	0.003	2.639	1.477-4.717	0.001
Sex, Male	1.548	0.801-2.992	0.193	1.121	0.683-1.839	0.650
ENE, positive	3.361	1.506-7.498	0.003	2.656	1.487-4.745	0.001
Smoking history, ever	1.333	0.689-2.578	0.394	1.307	0.790-2.162	0.298
ENE, positive	3.345	1.498-7.473	0.003	2.556	1.427-4.579	0.002
Invasive size, >3.0cm	1.243	0.653-2.364	0.508	1.337	0.807-2.217	0.260
ENE, positive	2.991	1.334-6.705	0.008	2.455	1.363-4.420	0.003
Vascular invasion, +	2.714	0.823-8.952	0.101	1.533	0.789-2.978	0.207
ENE, positive	3.512	1.579-7.813	0.002	2.664	1.494-4.751	0.001
Lymphatic invasion, +	1.436	0.759-2.716	0.266	1.094	0.667-1.793	0.723
ENE, positive	3.247	1.444-7.301	0.004	2.568	1.434-4.599	0.002
Pleural invasion, +	1.758	0.928-3.330	0.084	1.471	0.895-2.417	0.128
ENE, positive	3.448	1.551-7.663	0.002	2.816	1.576-5.033	<0.001
Subtype, non-lepidic	0.939	0.285-3.092	0.918	2.394	0.745-7.693	0.143

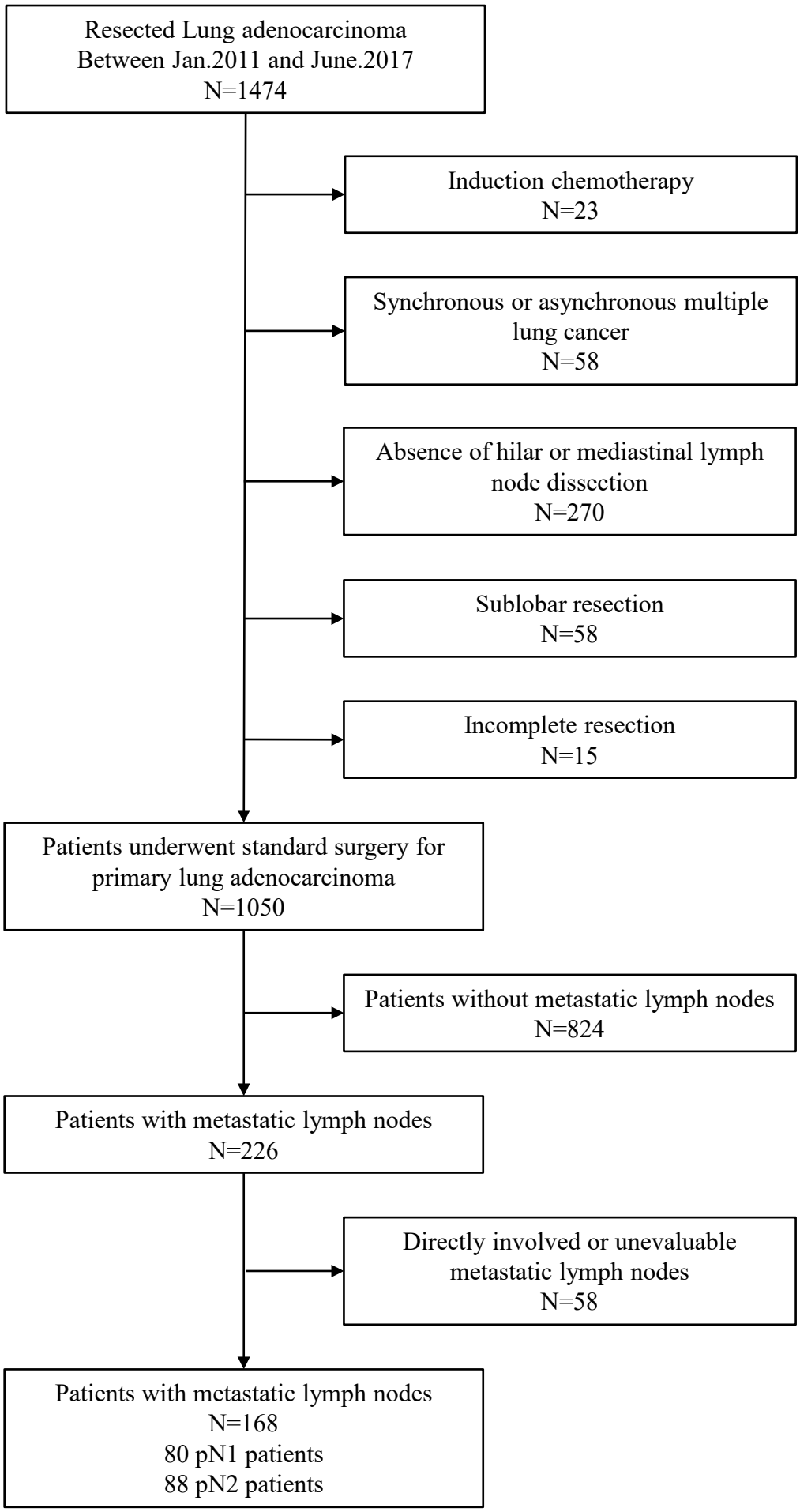
ENE, positive	3.622	1.556-8.427	0.003	2.886	1.592-5.233	<0.001
EGFR mutation, +	0.433	0.221-0.848	0.015	1.438	0.873-2.370	0.154
ENE, positive	3.065	1.359-6.913	0.007	2.423	1.336-4.394	0.004
AC, positive	0.465	0.228-0.948	0.035	0.663	0.385-1.142	0.138
ENE, positive	2.958	1.317-6.646	0.009	2.262	1.242-4.121	0.008
Metastatic LN, multiple	4.166	0.559-31.034	0.164	2.194	0.764-6.303	0.144
ENE, positive	3.372	1.440-7.894	0.005	2.850	1.497-5.425	0.001
Tumor area %[a], >20%	1.061	0.520-2.164	0.872	0.656	0.495-1.557	0.878
ENE, positive	3.413	1.523-7.647	0.003	2.546	1.423-4.557	0.002
Necrosis, positive	1.107	0.426-2.877	0.835	1.806	0.881-3.702	0.106

a The largest value was chosen when multiple lymph nodes metastases were present.

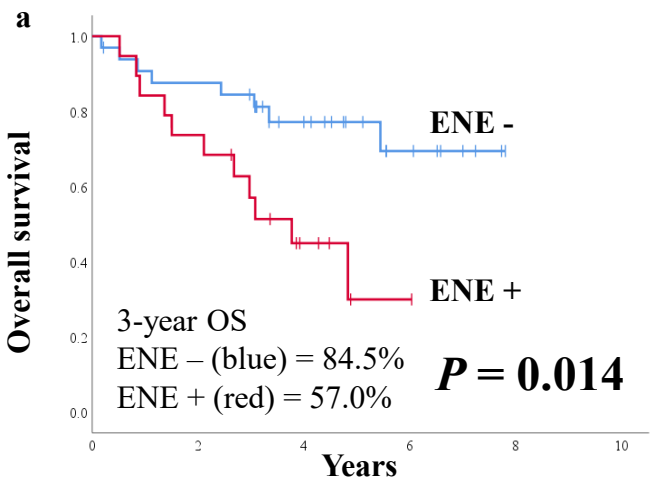
HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor;

ENE, extranodal extension; AC, adjuvant chemotherapy; LN, lymph node.

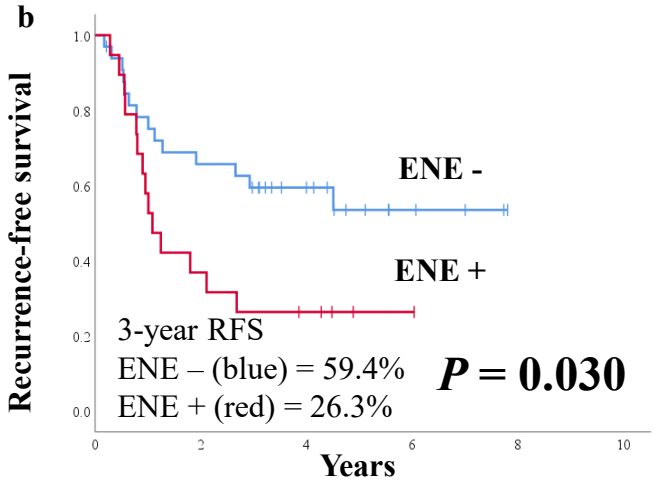
Supplemental Fig. 1



Supplemental Fig. 2



ENE -	33	28	16	7	0
ENE +	19	14	5	1	0



ENE -	33	21	12	4	0
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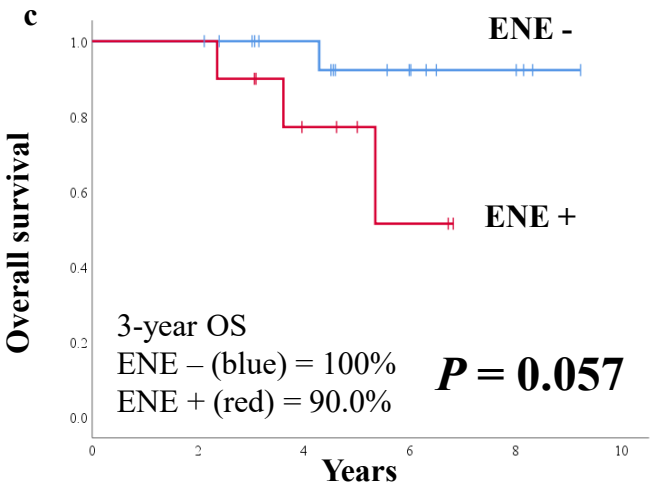
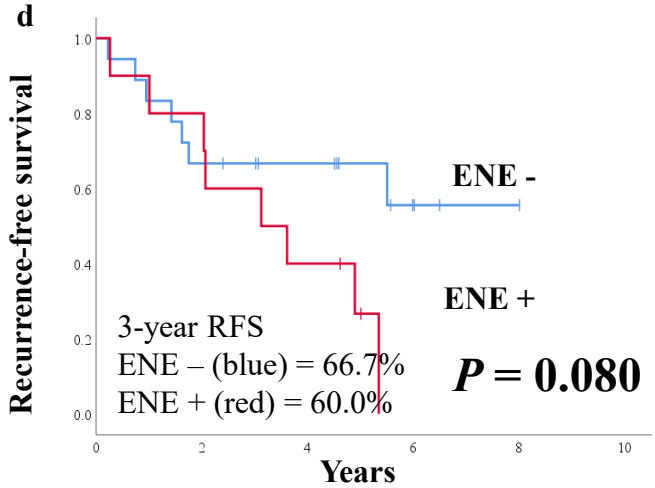
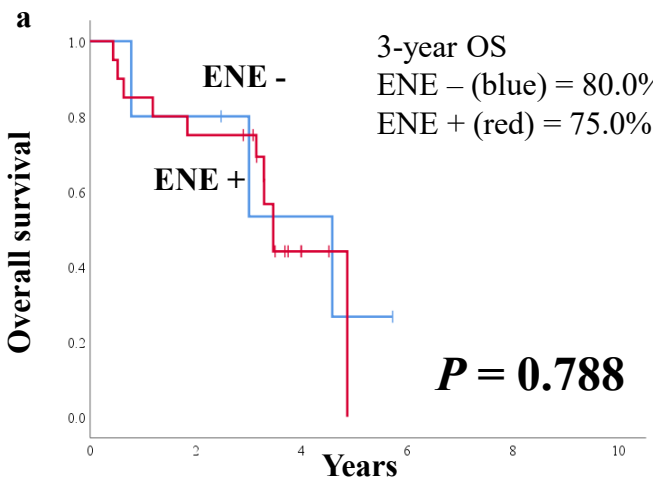


ENE -	18	18	13	7	4
ENE +	10	10	5	2	0

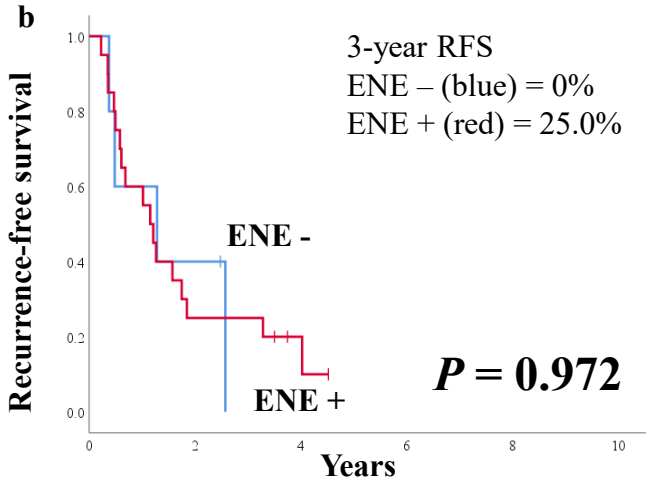


ENE -	18	12	9	3	1
ENE +	10	8	4	0	0

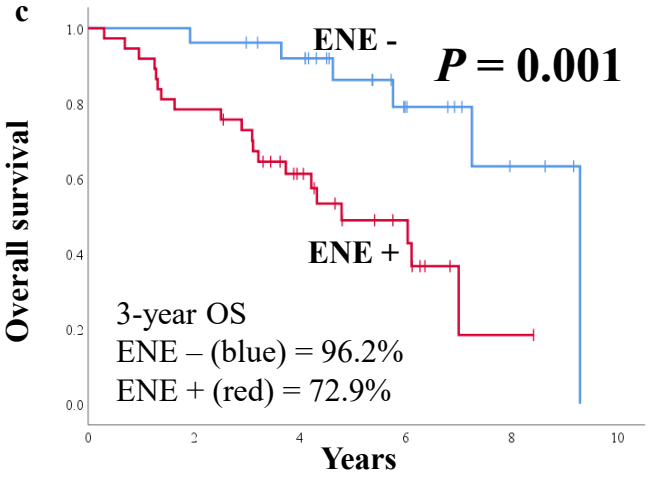
Supplemental Fig. 3



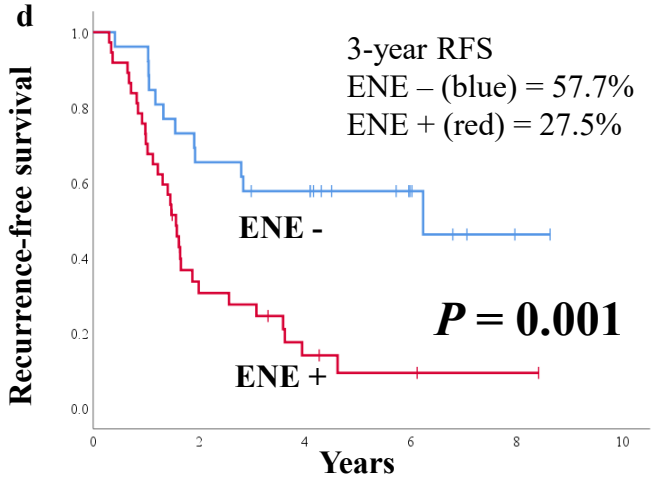
ENE -	5	4	2	0	0
ENE +	20	15	2	0	0



ENE -	5	2	0	0	0
ENE +	20	5	2	0	0



ENE -	26	25	22	9	3
ENE +	37	29	17	8	0



ENE -	26	17	14	6	1
ENE +	37	10	4	2	0

Supplemental Table 1. Patient's clinicopathological characteristics (N=168)

	N (%)
Age, y, median [range]	66 [31-86]
Sex, male	101 (60)
Smoking history, ever smoker	105 (63)
Tumor size (>5.0cm)	13 (8)
Invasive size (>3.0cm)	70 (42)
Pathological N stage	
N1	80 (48)
N2	88 (52)
Vascular invasion, positive	125 (74)
Lymphatic invasion, positive	72 (43)
Pleural invasion, positive	83 (49)
Predominant subtype	
lepidic	10 (6)
papillary / micropapillary	57 (34)
acinar	38 (23)
solid	61 (36)

other	2 (1)
EGFR mutation, positive (n=157)	76 (48)
Adjuvant chemotherapy, positive	91 (54)

EGFR, epidermal growth factor receptor.

Supplemental Table 2. Univariate analysis for prognostic significance in pN1 patients

	Overall survival			Recurrence-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
Age						
≥65	1.334	0.565-3.151	0.511	0.907	0.492-1.674	0.755
Sex						
Male	2.721	0.925-8.003	0.069	0.670	0.364-1.236	0.200
Smoking history						
ever smoker	1.616	0.600-4.358	0.343	0.385	0.120-0.706	0.002
Invasive size						
>3.0cm	2.760	1.134-6.717	0.025	1.409	0.773-2.567	0.263
Vascular invasion						
positive	0.710	0.298-1.688	0.438	1.091	0.559-2.133	0.798
Lymphatic invasion						
positive	1.367	0.592-3.160	0.464	1.553	0.842-2.867	0.159
Pleural invasion						
positive	1.855	0.763-4.512	0.173	1.713	0.914-3.213	0.093
Predominant subtype						
non-lepidic	1.197	0.161-8.887	0.861	1.287	0.311-5.325	0.728
EGFR mutation						
positive	0.658	0.285-1.521	0.328	1.384	0.749-2.556	0.299
Adjuvant chemotherapy						
positive	0.302	0.103-0.892	0.030	0.798	0.425-1.496	0.481
Metastatic lymph node						
multiple	2.028	0.883-4.660	0.096	1.552	0.850-2.832	0.152

Tumor area %[a]						
>20%	1.240	0.547-2.811	0.606	1.401	0.769-2.553	0.270
Necrosis						
positive	0.326	0.044-2.421	0.273	0.750	0.268-2.101	0.584
ENE						
positive	3.394	1.453-7.930	0.005	2.295	1.253-4.203	0.007

a The largest value was chosen when multiple lymph nodes metastases were present.

HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; ENE, extranodal extension.

Supplemental Table 3. Univariate analysis for prognostic significance in pN2 patients

	Overall survival			Recurrence-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
Age						
≥65	1.414	0.740-2.705	0.295	1.187	0.723-1.949	0.498
Sex						
Male	1.626	0.843-3.135	0.147	1.229	0.750-2.012	0.413
Smoking history						
ever smoker	1.487	0.772-2.865	0.235	1.345	0.813-2.226	0.249
Invasive size						
>3.0cm	1.451	0.769-2.739	0.251	1.524	0.924-2.512	0.099
Vascular invasion						
positive	3.570	1.097-11.617	0.035	1.904	0.992-3.653	0.053
Lymphatic invasion						
positive	1.379	0.731-2.604	0.321	1.128	0.689-1.846	0.632
Pleural invasion						
positive	1.994	1.057-3.760	0.033	1.613	0.986-2.639	0.057
Predominant subtype						
non-lepidic	0.804	0.245-2.630	0.718	1.923	0.602-6.148	0.270
EGFR mutation						
positive	0.407	0.208-0.794	0.008	1.316	0.803-2.157	0.275
Adjuvant chemotherapy						
positive	0.375	0.186-0.756	0.006	0.521	0.307-0.885	0.016
Metastatic lymph node						
multiple	6.264	0.858-45.732	0.070	3.227	1.169-8.904	0.024
Tumor area %[a]						

>20%	1.644	0.843-3.204	0.145	1.413	0.847-2.355	0.185
Necrosis						
positive	1.535	0.597-3.945	0.374	2.161	1.062-4.397	0.034
ENE						
positive	3.456	1.557-7.670	0.002	2.761	1.499-4.761	0.001

a The largest value was chosen when multiple lymph nodes metastases were present.

HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; ENE, extranodal extension.

Supplemental Table 4. Correlations between extranodal extension and clinicopathological characteristics

	Nodal involvement pattern		
	ENE -	ENE +	
	82	86	*P*
Age			
<65	31	37	
≥65	51	49	0.532
Sex			
Female	32	35	
Male	50	51	0.875
Smoking history			
never smoker	30	33	
ever smoker	52	53	0.874
Invasive size			
≤3.0cm	50	48	
>3.0cm	32	38	0.534
Pathological N stage			
N1	51	29	
N2	31	57	<0.001
Vascular invasion			
negative	24	19	
positive	58	67	0.296
Lymphatic invasion			

negative	51	45	
positive	31	41	0.215
Pleural invasion			
negative	42	43	
positive	40	43	0.879
Predominant subtype			
lepidic	3	7	
non-lepidic	79	79	0.330

ENE, extranodal extension.

Supplemental Table 4. Continued

	Nodal involvement pattern		
	ENE-	ENE +	
	82	86	*P*
EGFR mutation			
negative	41	43	
positive	37	42	0.876
Adjuvant chemotherapy			
negative	38	39	
positive	44	47	1.000
Metastatic lymph node			
single	38	17	
multiple	44	69	<0.001
Tumor area%[a]			
≤20%	58	22	
>20%	24	64	<0.001
Necrosis			
negative	76	73	
positive	6	13	0.145

a The largest value was chosen when multiple lymph nodes metastases were present.

ENE, extranodal extension; EGFR, epidermal growth factor.