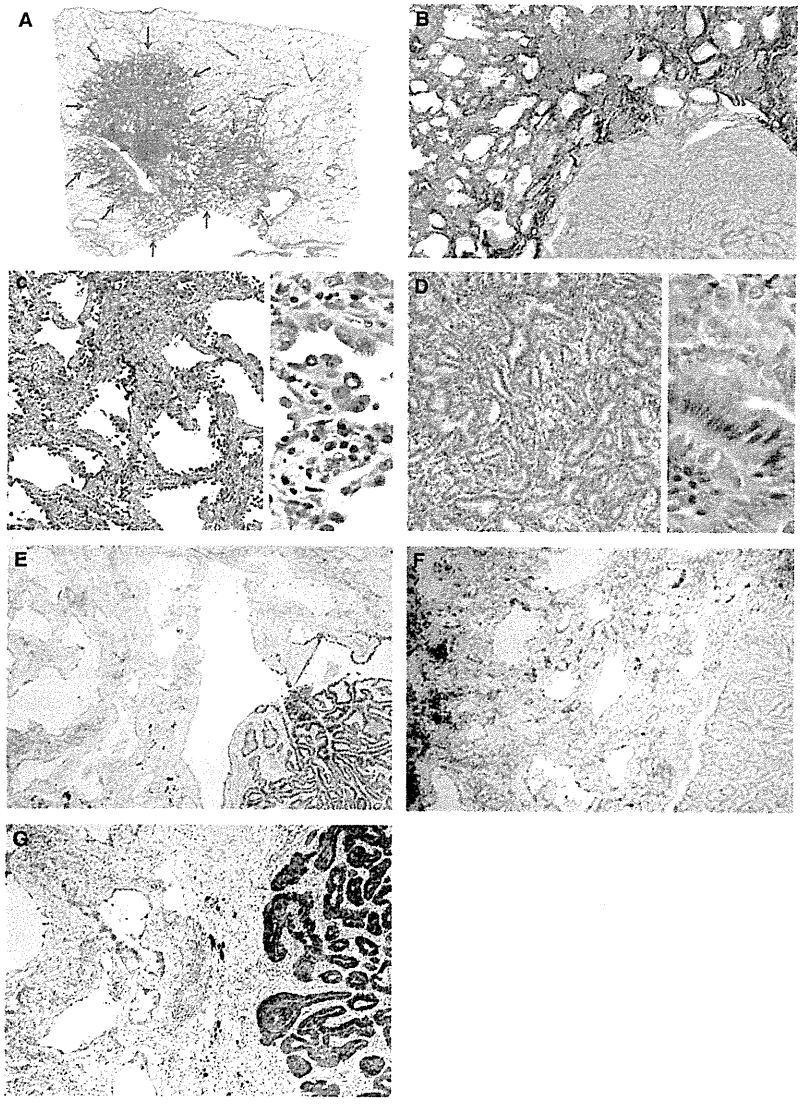

Figure 1. A, A loupe image of the maximum cutting surface shows two component carcinomas: lung adenocarcinoma (surrounded by arrows) and papillary thyroid carcinoma (surrounded by arrowheads). B, Elastic van Gieson staining reveals the complete loss of the pre-existing lung component in the papillary thyroid carcinoma area. C, Lepidic growth with nuclear inclusions and decapitations is observed in primary lung adenocarcinoma. D, Papillary growth with nuclear grooves and nuclear pseudo-inclusions is observed in papillary thyroid carcinoma. E, F, Immunohistochemical results for the two components: the papillary thyroid carcinoma (right part of the tumour) and the primary lung adenocarcinoma (left part of the tumour). E, Staining with the *BRAF* V600E mutation-specific antibody was positive in the papillary thyroid carcinoma area. F, Staining with the *EGFR* L858R mutation-specific antibody was positive in the primary lung adenocarcinoma area. G, Staining with the *EGFR* L858R mutation-specific antibody was visualized with the chromogen diaminobenzene (brown staining) in the primary lung adenocarcinoma area. The *BRAF* V600E mutation-specific antibody was visualized with the Vector VIP system (purple staining) in the papillary thyroid carcinoma area.

was visualized with the Vector VIP system (Vector Laboratories, Burlingame, CA, USA).

Immunohistochemically, both cancer components were positive for TTF-1 and negative for EGFR exon 19 deletion. The papillary thyroid carcinoma component was positive for PAX8 and BRAF V600E, although the lung adenocarcinoma component was negative for both (Figure 1E,G). In contrast, the lung adenocarcinoma component was positive for napsin A and EGFR L858R, whereas the papillary thyroid carcinoma component was negative for both (Figure 1F,G).

Table 1. Previous reports of tumour-to-tumour metastasis to the lung

Author	Donor tumour	Recipient tumour (lung)
Lin *et al.*[3]	Maxillary sinus adenoid cystic carcinoma	Adenocarcinoma
Blanco *et al.*[9]	Maxillary sinus adenoid cystic carcinoma	Adenocarcinoma
Piacentini *et al.*[8]	Breast carcinoma	Squamous cell carcinoma
	Breast carcinoma	Adenocarcinoma
Nonomura *et al.*[10]	Papillary thyroid carcinoma	Squamous cell carcinoma
Roscoe *et al.*[11]	Papillary thyroid carcinoma	Adenocarcinoma
Xue *et al.*[12]	Papillary thyroid carcinoma	Adenocarcinoma, invasive
Kim *et al.*[13]	Papillary thyroid carcinoma	Adenocarcinoma *in situ*
Present case	Papillary thyroid carcinoma	Adenocarcinoma, invasive

Discussion

Here, we report a rare case of tumour-to-tumour metastasis from thyroid carcinoma to lung adenocarcinoma. We used anti-EGFR L858R and anti-BRAF V600E for immunostaining. These genetic mutation-specific monoclonal antibodies contribute as both diagnostic tools and as predictors of molecular-targeted therapy.

In addition to our case, eight cases have been reported in which lung cancer was the recipient of tumour-to-tumour metastasis. In the present case and four of the previous cases, the donor tumour was papillary thyroid carcinoma.[11–13] Of the remaining four cases, breast carcinoma was the donor in two cases and maxillary sinus adenoid cystic carcinoma was the donor in two cases (Table 1).[3,8,9,12,13] The relatively low incidence of lung cancer as a recipient in tumour-to-tumour metastasis has been explained by differences in aggressiveness between the tumours.[13]

In the differential diagnosis of papillary thyroid carcinoma and lung adenocarcinoma with immunohistochemistry, the combined use of PAX8 and napsin A has been considered to be useful. However, by using the mutation-specific antibodies anti-EGFR L858R and anti-BRAF V600E, we were able to simultaneously confirm that this tumour included tumour-to-tumour metastasis and was suitable for molecular-targeted agents, on the basis of only one slide.

Molecular-based analyses, such as polymerase chain reaction-based assays and next-generation sequencing, constitute the gold standard for detecting gene alterations. However, in the current case, these highly sensitive molecular techniques were disadvantageous, because they require detailed microdissection to avoid the contamination of each component. Immunohistochemistry can help to confirm genetic mutations visually and avoid the misleading results that can be provided by molecular-based analyses of both complicated cases (such as our own) and cases with coexisting gene mutations (which have been considered to be mutually exclusive[14]). Moreover, the presence of an adequate number of cancer cells is necessary to detect genetic mutations with molecular diagnosis. Especially in small specimens, such as those acquired through needle aspiration, it is essential to save specimens for subsequent molecular diagnosis. Therefore, we have provided a novel use for mutation-specific antibodies, primarily in order to guide proper methods of molecular diagnosis.

In conclusion, we report a case of tumour-to-tumour metastasis in which papillary thyroid adenocarcinoma metastasized into lung adenocarcinoma. The current antibody selection contributed to both the diagnosis of the case and the efficient assessment of potential driver mutations. A number of molecular-targeted drugs have been developed in recent years, and this trend is likely to continue. Accordingly, each of the multimodal diagnostic tools, including both immunohistochemistry and molecular-based analyses, will help to avoid some pitfalls during diagnosis and help to ensure that patients receive optimal treatments.

Acknowledgements

We would like to thank Sachiko Miura and Chizu Kina for their skilful technical assistance. This work

was supported in part by the National Cancer Centre Research and Development Fund (26-A-13 and 24-A-1), and by a Grant-in-Aid for Scientific Research (C) (Grant Number: 25460446).

Author contributions

Y. Katsuya and K. Tsuta performed the pathological evaluation and wrote the manuscript. S. Watanabe and A. Yoshida contributed to clinical evaluations and productive discussions.

Ethics statement

The patient gave informed consent, and the study was performed according to the Declaration of Helsinki. No ethics committee approval was required, because this was a case report of a single patient.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Surgical Treatment for Synchronous Primary Lung Adenocarcinomas

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Background. Surgical treatment has become the mainstay of treatment for multiple primary lung cancers. In particular, the prevalence of synchronous primary lung adenocarcinomas (SPLA) has recently increased, but few studies have evaluated surgical outcomes of patients with SPLA. We reviewed the clinicopathologic features and surgical outcomes of SPLA to identify factors related to survival.

Methods. Data on 2,041 consecutive patients with primary non-small cell carcinoma who underwent surgical resection in our hospital from 1995 through 2009 were retrospectively analyzed.

Results. The SPLA was pathologically diagnosed in 93 patients, including 26 with bilateral tumors. The rates of overall survival and recurrence-free survival at 5 years were 87.0% and 81.8%, respectively. There was no surgical mortality at 30 days. On univariate analysis, lymph node metastasis ($p = 0.0000$), nonlepidic predominant histologic subtype ($p = 0.0018$), and a solid appearance of the largest tumor on computed tomography ($p = 0.0088$) were significantly related to poor overall survival. On multivariate analysis, bilateral distribution of tumors ($p = 0.031$), lymph node metastasis ($p = 0.004$), and sublobar resection ($p = 0.042$) were independent predictors of poor survival.

Conclusions. Surgery has good outcomes and should be aggressively performed for patients with SPLA. The evaluation of lymph node status has an important role in deciding whether surgery is indicated. Bilateral tumors are a predictor of poor outcomes, requiring that caution be exercised. Lobectomy has a high cure rate and should be performed whenever possible. However, sublobar resection should be considered for patients likely to have poor residual lung function postoperatively.

(Ann Thorac Surg 2014;98:1983–8)

Owing to recent progress in diagnostic imaging techniques and the increased use of computed tomography (CT), patients with a confirmed or suspected diagnosis of multiple lung cancers are occasionally encountered. Recent studies have reported that 2.6% to 7.9% of patients who undergo resection of non-small cell lung cancer (NSCLC) have synchronous lung cancers [1–6], and this trend is increasing [6]. Surgical resection has become the mainstay of treatment for synchronous lung cancers, but the 3-year survival rate broadly ranges from 40% to 92% [1–3, 7–10]. The wide variability in outcomes is attributed not only to differences in treatment timing and demographic characteristics of patients, but also to the lack of standard criteria for differential diagnosis from intrapulmonary metastasis and for the selection of surgical procedures.

The seventh edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system defined the presence of additional tumor nodules in the same lobe as T3 M0 and the presence of additional tumor nodules in other ipsilateral lobes as T4 M0, whereas nodules in the contralateral lung were defined as M1a disease [11]. Such nodules are generally regarded to be intrapulmonary metastases from the primary tumor, but may include separately staged synchronous primary lung cancers that require surgical resection. If multiple lung cancers are of different histologic types, differential diagnosis is relatively straightforward. However, if the histologic type is the same, the differential diagnosis of synchronous primary lung cancers and intrapulmonary metastasis remains challenging.

Recent studies have reported that multiple adenocarcinomas account for 40.3% to 91.3% of synchronous primary lung cancers [3–5, 8–10], making the differential diagnosis of multiple adenocarcinomas particularly important. To date, however, few studies have specifically focused on the diagnosis and surgical outcomes of synchronous primary lung adenocarcinomas (SPLA). Given progress in diagnostic imaging techniques and adenocarcinoma classification systems, we analyzed surgical outcomes in a recent series of patients with SPLA. Our main objective was to define appropriate methods and criteria for diagnosis and selection of surgical procedures on the basis of the outcomes of surgical therapy for patients with SPLA.

Accepted for publication July 7, 2014.

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Published by Elsevier

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2014.07.006

Patients and Methods

This retrospective study was approved by the Ethics Committee of Kanagawa Cancer Center. Among 2,041 consecutive patients with primary lung cancer who underwent surgical resection in our hospital from April 1995 through December 2009, we studied patients with a pathologically confirmed diagnosis of SPLA who underwent complete surgical resection. Patients who had adenocarcinoma admixed with other histologic types were excluded from the study.

Preoperative evaluation included chest radiography, thin-section CT (TSCT) of the chest and upper abdomen, and positron emission tomography-CT. An expert consensus meeting attended by specialists from the fields of respiratory medicine, surgery, and diagnostic radiology was convened to evaluate preoperative findings. Patients with clinical (c) N2 disease were excluded from surgery. If patients had a mediastinal lymph node 1 cm or greater on the short axis on TSCT and a positive finding was identified on positron emission tomography-CT, mediastinoscopy or endobronchial ultrasonography was performed for histologic confirmation. Actually, because all patients in this study were cN0 or N1, no patients were offered endobronchial ultrasonography or mediastinoscopy.

Imaging features of the tumors, the presence or absence of ground-glass opacity (GGO), and tumor disappearance rates (TDR) were evaluated on TSCT. To calculate TDR, the maximum tumor diameter was measured on the lung window image (A) and the mediastinal window image (B), and TDR was calculated by the following formula: $(A - B) / A \times 100$ [12]. In our study, the CT features of tumors were classified into the following three subgroups according to the imaging features of the tumors and the TDR: pure GGO, entire nodules show GGO with a TDR of 100%; mixed GGO, nodules show some consolidation in GGO with a TDR of more than 25%; and solid, nodules consist mainly of consolidation with a TDR of 25% or less.

The same team of surgeons performed all resections. Surgical procedures were selected based on the size, location, and TSCT features of tumors, as well as performance status and pulmonary function. Solid and mixed GGO tumors were usually resected by lobectomy and pure GGO tumors by segmentectomy or wedge resection. For patients with poor pulmonary reserve or performance status, segmentectomy or wedge resection was selected instead of lobectomy.

The pathologic criteria for diagnosis of SPLA in our hospital are based on the Martini-Melamed criteria [13] and incorporate elements of the new international multidisciplinary lung adenocarcinoma classification [14] (Table 1). Patients with adenocarcinoma in situ and those with minimally invasive adenocarcinoma were included in analysis, but those with atypical adenomatous hyperplasia were excluded. For the analysis of survival rates according to the histologic type, adenocarcinoma in situ, minimally invasive adenocarcinoma, and the lepidic predominant subtype of invasive adenocarcinoma were included in the "lepidic predominant" subtype.

The disease stage was reclassified according to the seventh edition of the TNM classification [15]. Each tumor was staged, and the most advanced disease stage of all tumors was used as the disease stage of the patient. Tumor size on pathologic examination and CT features of the largest tumor and second tumor were included in the analysis. Postoperative adjuvant chemotherapy was mainly indicated for patients with pathologic (p) stage II or more advanced disease, and data on these patients were included in analysis.

Table 1. Pathologic Criteria for Diagnosis of Synchronous Primary Lung Adenocarcinomas

1. Major histologic subtypes of tumors are significantly different.
2. Major histologic subtypes are similar, but all tumors have lepidic growth component to a certain proportion, or immunohistologic features or genetic profiles of tumors are different.

Overall survival (OS) and recurrence-free survival were defined, respectively, as the time from initial surgery to the date of death and the date of recurrence or the final follow-up visit. Survival curves were calculated by the Kaplan-Meier method, and log rank tests were used for univariate analysis. Multivariate analysis was performed using a Cox proportional hazards model. Clinicopathologic features were compared according to tumor distribution with the use of Pearson's χ^2 test. All p values of less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed using SPSS 11.0.1 software (SPSS, Chicago, IL).

Results

During the study period, synchronous primary lung cancers were diagnosed in 111 patients. Of these patients, 93 (4.6% of 2,041 patients undergoing resection of NSCLC) with SPLA were included in the study. The other 18 patients had other histologic types of tumors—adenocarcinoma plus squamous cell carcinoma in 8, squamous cell carcinoma plus squamous cell carcinoma in 3, adenocarcinoma plus other histologic types of cancer (large cell neuroendocrine carcinoma, pleomorphic carcinoma, and so forth) in 3, squamous cell carcinoma plus other types of cancer in 3, and a mixture of other histologic types in 1—and were excluded.

Patient Demographics

Demographic characteristics of the patients are shown in Table 2. Median age at the time of initial surgery was 68 years. There were 36 men (39%) and 33 smokers (36%). We confirmed that SPLA is more common among women and nonsmokers than lung cancer in general. The preoperative serum carcinoembryonic antigen level was elevated ($\geq$ 5.0 ng/mL) in 13 patients (14%).

Tumor Characteristics

Tumor characteristics are shown in Table 2. The number of tumors was 2 in 71 patients (76%), 3 in 18 patients (19%), and 4 or 5 in 4 patients (4%). The size of the largest tumor ranged from 10 mm to 57 mm (median 23). The tumor distribution was ipsilateral in 67 patients (same lobe, 31; different lobes of the ipsilateral lung, 36) and bilateral in 26.

Table 2. Patient Characteristics and Clinicopathologic Features

Characteristics	n (%) or Median (range)
Age	68 (49–84)
Sex	
Male	36 (39)
Female	57 (61)
Smoking status	
Current and former	33 (36)
Never	60 (65)
Preoperative CEA elevation, ≥ 5.0 ng/mL	13 (14)
Number of tumors	
2	71 (76)
3	18 (19)
4 or 5	4 (4)
Size of the largest tumor, mm	23 (10–57)
Distribution of tumors	
Ipsilateral same lobe	31 (33)
Ipsilateral different lobe	36 (39)
Bilateral	26 (28)
CT features of tumors, largest + second	
Solid + solid	11 (12)
Solid + mixed GGO	11 (12)
Solid + pure GGO	8 (9)
Mixed GGO + solid	8 (9)
Mixed GGO + mixed GGO	24 (26)
Mixed GGO + pure GGO	26 (28)
Pure GGO + mixed GGO	2 (2)
Pure GGO + pure GGO	3 (3)
Clinical stage	
I	87 (94)
II	5 (5)
IIIA[a]	1 (1)
Pathologic stage	
I	75 (81)
II	9 (10)
IIIA[b]	9 (10)
Histologic subtypes of the largest tumor	
Lepidic predominant[c]	63 (68)
Acinar predominant	10 (11)
Papillary predominant	10 (11)
Micropapillary predominant	3 (3)
Solid predominant	7 (8)

[a] One patient was cT3N1. [b] Eight patients were pT1-2N2 and 1 patient was pT3N1. [c] Adenocarcinoma in situ (n = 24) and minimally invasive adenocarcinoma (n = 11) were included.

CEA = carcinoembryonic antigen; CT = computed tomography; GGO = ground-glass opacity.

The CT features of the largest tumor were mixed GGO in 58 patients, solid pattern in 30, and pure GGO in 5. One or more solid lesions were present in 38 patients (41%).

Pathologic Findings

Pathologic findings are also shown in Table 2. The histologic subtype of the largest tumor was lepidic predominant in 63 patients (68%). The 1 patient with c-stage IIIA disease had T3 N1 cancer. Lymph node metastasis was found in 18 patients (19%); 10 had pN1 disease, and 8 had pN2 disease.

Surgical Procedures

Among the 26 patients with bilateral tumors, one-stage bilateral operations were performed in 6 patients, and two-stage bilateral operations in 20 (Table 3). One patient (1%) underwent pneumonectomy. Sublobar resection (wedge resection and segmentectomy) was included in treatment procedures for 54 patients (58%).

Surgical Outcomes

The follow-up period ranged from 8.1 to 198.1 months (median 56.0). At final follow-up, 12 patients (13%) had died, and 81 (87%) were alive. There was no perioperative death. Twelve patients died in the late phase; the cause of death was lung cancer in 9 patients, postoperative chronic empyema in 1, flare-up of tuberculosis in 1, and unknown in 1. The 3-year and 5-year OS rates were 93.6% and 87.0%, respectively (Fig 1). Recurrence developed in 17 patients (18%). The initial site of recurrence was intrapulmonary metastasis in 7 patients, lymph node metastasis in 5, distant metastasis in 3, pleural dissemination in 1, and recurrence at the resection stump in 1. All patients with pN2 disease had recurrence. The 3-year and 5-year recurrence-free survival rates were 89.2% and 81.8%, respectively (Fig 1).

Table 4 shows the results of univariate analysis of factors related to OS. The presence of lymph node metastasis ($p = 0.0000$), a nonlepidic predominant subtype of the largest tumor ($p = 0.0018$), and solid CT features of the largest tumor ($p = 0.0088$) were significantly related to poor outcomes. Bilateral tumors ($p = 0.0950$) and pathologic T2 to T3 disease ($p = 0.0885$) were slightly, but not significantly, related to poor outcomes. On multivariate analysis including the surgical procedure and tumor distribution in addition to the three significant factors identified on univariate analysis, the presence of lymph node metastasis

Table 3. Operative Details (n = 93)

Distribution and Type of Resection	n
Ipsilateral	
Pneumonectomy	1
Bilobectomy	5
Lobectomy	28
Lobectomy + segmentectomy	3
Lobectomy + wedge	14
Segmentectomy + segmentectomy	1
Segmentectomy + wedge	4
Multiple wedges	11
Bilateral	
Lobectomy + lobectomy	5
Lobectomy + segmentectomy	5
Lobectomy + wedge	5
Segmentectomy + wedge	5
Multiple wedges	6

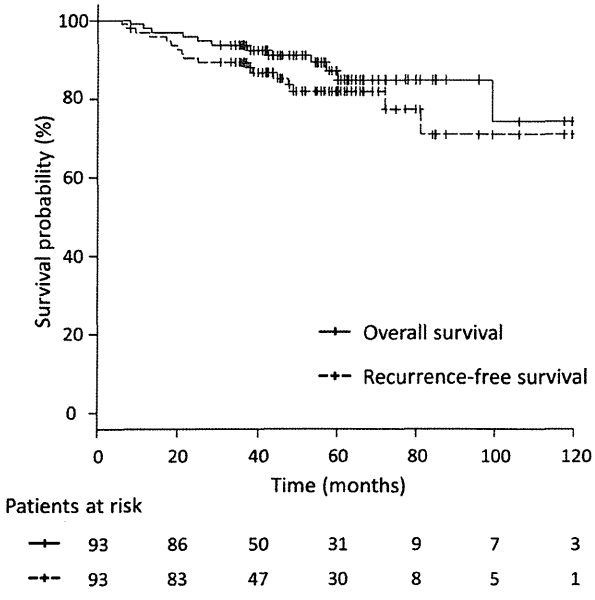

Fig 1. Overall survival (solid line) and recurrence-free survival (dashed line) of 93 patients with synchronous primary lung adenocarcinomas who underwent surgical resection. Five-year rates were 87.0% and 81.8% for overall and recurrence-free survival, respectively.

($p = 0.004$), bilateral distribution of tumors ($p = 0.031$), and the use of sublobar resection ($p = 0.042$) were independent predictors of poor survival (Table 5).

Comment

When we encounter patients with multiple tumors in the lung in clinical practice, it is extremely difficult to distinguish SPLA from intrapulmonary metastasis. The Martini-Melamed criteria [13] have been widely used for differential diagnosis in previous studies. Tumors of the same histologic type that arise in the same segment of the lung are diagnosed as intrapulmonary metastasis. Even if different segments are involved, tumors of the same histologic subtype are diagnosed as intrapulmonary metastasis if metastasis is found at shared lymphatic pathways. However, in the current era of genetic analysis of factors such as epidermal growth factor receptor, SPLA involving multiple lobes of the same lung and accompanied by mediastinal lymph node metastasis have been reported [16]. In addition to the Martini-Melamed criteria, it is therefore necessary to evaluate other factors for diagnosis of this type of cancer. In patients with multiple adenocarcinomas, the histologic subtypes of the tumors must be considered. The recently proposed comprehensive histologic assessment has facilitated the differential diagnosis of multiple primary NSCLC and metastases [17]. However, the problem remains that lepidic predominant primary tumors are likely to be diagnosed as intrapulmonary metastasis if the histologic subtype ratio is similar. Recently, there has been an increasing trend in multiple tumors showing GGO, particularly among nonsmoking women in Asia. Such lesions are likely to be

Table 4. Univariate Analysis of Predictors of Survival

Predictors	n	Overall 5-Year Survival	*p* Value
Age, years			
< 70	53	83.8%	0.9152
≥ 70	40	92.0%	
Sex			
Male	36	77.4%	0.1265
Female	57	93.1%	
Smoker			
Current and former	33	81.8%	0.6533
Never	60	90.0%	
Preoperative serum CEA, ng/mL			
< 5.0	80	90.0%	0.2167
≥ 5.0	13	68.4%	
Size of the largest tumor, mm			
≤ 30	72	84.5%	0.2365
> 30	21	95.2%	
CT features of the largest tumor			
Solid	30	75.6%	0.0088
Mixed and pure GGO	63	92.5%	
Distribution of tumors			
Ipsilateral	67	90.9%	0.0950
Bilateral	26	76.9%	
Number of tumors			
2	71	90.7%	0.3327
≥ 3	22	71.1%	
Highest pT			
T1	66	90.7%	0.0885
T2–3	27	78.6%	
Highest pN			
N0	75	93.4%	0.0000
N1	10	75.0%	
N2	8	41.7%	
Surgical procedures			
Lobectomy	39	92.5%	0.5086
Sublobar included			
Segmentectomy[a]	18	82.1%	
Wedge resection	36	80.4%	
Mediastinal LN management			
Systematic dissection	36	83.3%	0.9118
Sampling	20	80.0%	
Not dissected	37	88.5%	
Histologic subtype of the largest tumor			
Lepidic predominant	63	98.4%	0.0018
Nonlepidic predominant	30	66.9%	
Adjuvant chemotherapy[b]			
Yes	6	83.3%	0.7050
No	12	50.0%	

[a] Nine patients who underwent segmentectomy and wedge resection were included. [b] Eighteen patients with p-stage II or greater disease were included.

CEA = carcinoembryonic antigen; CT = computed tomography; GGO = ground-glass opacity; LN = lymph node.

Table 5. Multivariate Analysis of Predictors of Survival

Variables	n	*p* Value	Hazard Ratio (95% CI)
CT features of the largest tumor			
Solid/mixed and pure GGO	30/63	0.200	0.421 (0.112–1.582)
Distribution of tumors			
Bilateral/ipsilateral	26/67	0.031	4.630 (1.148–18.666)
Lymph node involvement			
Yes/no	18/75	0.004	10.560 (2.142–52.076)
Use of sublobar resection			
Yes/no	54/39	0.042	4.425 (1.054–18.580)
Predominant histology			
Lepidic/nonlepidic	63/30	0.261	2.395 (0.552–10.982)

CI = confidence interval; CT = computed tomography; GGO = ground-glass opacity.

classified as intrapulmonary metastasis. However, tumors with a high GGO ratio are most likely not intrapulmonary metastasis [18]. The diagnostic criteria for multiple lung adenocarcinomas in our hospital have taken this point into account. The good treatment outcomes in our study might have been attributed to the exclusion of patients with intrapulmonary metastasis, which is associated with a poor prognosis.

To our knowledge, this is the second largest, relatively long-term follow-up study of surgical outcomes in patients with SPLA [8]. The 5-year OS rate in our study was 87.0%, which is considered extremely good. Several factors were related to outcomes, and lymph node metastasis had the greatest impact. Previous studies have similarly reported that the presence or absence of lymph node metastasis is a significant prognostic factor [2, 3, 8, 10, 19, 20]. In our study, however, 5-year survival rates were 75.0% for patients with pN1 disease and 41.7% for patients with pN2 disease, better rates than those reported by the International Association for the Study of Lung Cancer lung cancer staging project (38% for pN1 disease, 22% for pN2 disease) [21]. The specialized design of our study in patients with adenocarcinoma might have contributed to better outcomes.

The Martini-Melamed criteria classify cases with N2 nodal involvement as intrapulmonary metastasis, but not multiple cancers. Some studies have excluded patients with N2 disease from the analysis of surgical outcomes [5, 9, 22]. In contrast, because we performed detailed histologic subtyping synchronous primary lung cancers could be diagnosed even in the presence of N2 disease. We, therefore, could obtain a better overall picture of the outcomes of surgical treatment for synchronous primary lung cancers.

Curative chemoradiotherapy is basically indicated for patients with cN2 disease. In our study, 8 cases of pN2 disease (ipsilateral, 4; bilateral, 4) were detected by chance on postoperative pathologic examination. Unexpected pN2 disease was thus detected in approximately 10% of patients, a finding that is generally consistent with the findings of previous studies. Of these patients with bilateral disease, pN2 disease was diagnosed on the second of two-stage bilateral resections in 2 patients and on one-stage bilateral surgery in 1 patient. For patients who underwent two-stage surgery, the side with more advanced lesions or with lesions likely to negatively affect outcomes is usually initially resected. In fact, however, half of all more advanced lesions were not resected at the first operation.

Synchronous surgery for bilateral tumors is considered an effective strategy for preventing disease progression and delays in adjuvant therapy in patients with clinical N0 to pathologic N2 disease. However, synchronous bilateral lobectomy with lymph node dissection is associated with increased risk and therefore should only be performed in carefully selected patients. Given the treatment outcomes in patients with pN2 disease, if N2 disease is detected at the first stage of two-stage resection, treatment options such as chemotherapy and stereotactic body radiotherapy should be also considered instead of performing lobectomy at the second stage.

The relations between surgical procedures and outcomes have been extensively studied. A number of studies have reported no difference in survival according to whether sublobar resection was performed [3, 5, 6, 10, 22]. In our study, sublobar resection was a significant independent predictor of poor outcomes on multivariate analysis. This result is attributed to a negative impact of sublobar resection on curability. In our study, 59% of the patients had tumors with a high GGO ratio, which are associated with relatively good outcomes. The latest American College of Chest Physicians evidence-based clinical practice guidelines recommend that these lesions should be handled separately as multifocal lung cancer and patients should undergo sublobar resection because single tumors with a high GGO ratio have good outcomes [18]. However, clear-cut criteria defining lesions that should be treated by sublobar resection are currently unavailable. Imaging findings of tumors may be useful for determining the range of resection. As shown in our study and previous reports [12, 23, 24], mixed or pure GGO lesions had a high TDR and good outcomes, whereas solid lesions were associated with poor outcomes. Therefore, solid lesions should be treated by radical lobectomy if permitted by lung function.

Interestingly, bilateral tumors were an independent predictor of poor outcomes in our study. Previous studies have reported that OS does not differ significantly according to tumor distribution [3, 8–10]. In contrast to our results, some studies reported that bilateral tumors were associated with significantly better outcomes [2, 20]. To investigate reasons for the poorer outcomes in patients with bilateral tumors, we studied differences in clinicopathologic factors related to tumor distribution. Bilateral tumors were found to be associated with a higher preoperative carcinoembryonic antigen level, a greater number of tumors, a larger size of the second tumor, and a higher proportion of patients who underwent sublobar resection (Table 6). These findings indicate that many of our subjects with bilateral tumors had aggressive disease, and this factor might have led to the difference in outcomes. Moreover, because patients with bilateral tumors had many lesions, it was difficult to perform lobectomy for all lesions. This factor may have also contributed to poorer outcomes.

Table 6. Correlation Between Tumor Distribution and Other Clinicopathologic Features

Variables	Ipsilateral	Bilateral	*p* Value
CT features of the largest tumor			
Solid	22	8	1.000
Mixed and pure GGO	45	18	
Preoperative serum CEA, ng/mL			
< 5.0	61	19	0.032
≥ 5.0	6	7	
Tumor size of the largest tumor, mm			
≤ 30	47	21	0.221
> 30	20	5	
Tumor size of the second tumor, mm			
≤ 20	62	18	0.007
> 20	5	8	
Number of tumors			
2	56	15	0.011
≥ 3	11	11	
Pathologic stage			
Stage I	53	22	0.388
Stage II–III	14	4	
Highest pN			
N0–1	63	22	0.213
N2	4	4	
Surgical procedures			
Lobectomy	34	5	0.009
Sublobar included	33	21	

CEA = carcinoembryonic antigen; CT = computed tomography; GGO = ground-glass opacity.

Finally, our study had several limitations. First, patient selection was biased because this was a single-center, retrospective study. Second, molecular phenotype such as epidermal growth factor receptor mutation was not assessed in all patients at the diagnosis of multiple lung adenocarcinomas. Finally, we did not compare patients with SPLA who underwent surgery with patients who did not undergo surgery or with patients who underwent only incomplete resection. However, because our study was a single-center trial, treatment policy, surgical procedures, postoperative care, and histopathologic evaluations were standardized. We believe that these conditions led to high-quality data.

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Lung Cancer xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Cytokeratin 19 expression in primary thoracic tumors and lymph node metastases

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ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form
18 September 2014
Accepted 22 September 2014

Keywords:
CK19
Lung
OSNA
Immunohistochemistry
TMA
LN

ABSTRACT

Background: The use of one-step nucleic acid amplification (OSNA), which allows for the rapid intraoperative detection of lymph node (LN) metastasis, is becoming more widely accepted in breast cancer. To provide basic data for the development of this method for lung tumors, we conducted a large-scale investigation of cytokeratin (CK) 19 expression in thoracic tumors.
Patients and methods: We examined CK19 expression in specimens from a total of 801 surgically resected samples of primary lung adenocarcinoma (ADC), squamous cell carcinoma (SQC), large-cell carcinoma (LCC), pleomorphic carcinoma (PC), large cell neuroendocrine carcinoma (LCNEC), small cell carcinoma (SCC), and carcinoid tumor (CT) as well as pleural malignant mesothelioma and lung metastatic deposits from breast cancer using tissue microarrays (TMAs) and whole sections. We also compared the CK19 expression status between primary sites and LN metastatic deposits.
Results: The overall rate of CK19 expression as observed on TMAs and whole sections in the 801 analyzed cases was 88.0%. CK19 expression was detected in 94.6% of ADCs, 93.6% of SQCs, 54.5% of LCCs, 54.8% of PCs, 77.4% of LCNECs, 31.8% of SCCs, 34.0% of CTs, and 92.9% of malignant mesotheliomas. Expression of CK19 was also detected in 90.9% of lung metastatic deposits from breast carcinomas. CK19 expression was maintained between CK19-positive primary sites and the corresponding LN metastatic deposits. Of note, a portion of CK19-negative primary tumors showed upregulation of CK19 protein expression in LN metastases.
Conclusions: Most thoracic tumors, except for PCs, CTs, and SCCs, were positive for CK19. We also found that CK19 expression was maintained between CK19-positive primary tumors and the corresponding LN metastatic deposits. These results may be useful in the development of the OSNA method for the intraoperative detection of LN metastasis in non-small cell lung cancer (NSCLC).

1. Introduction

In non-small cell lung cancer (NSCLC), lymph node (LN) metastatic status is one of the most predictable adverse prognostic factors [1–3]. In particular, in deciding on the most appropriate surgical procedure for patients with NSCLC, the presence or absence of intrathoracic metastatic LNs is one of the most important factors. Identification of LN metastasis in decision-making for surgical procedures is also of importance in other tumor types. For example, in breast cancer, sentinel lymph node (SLN) biopsy has recently become a standard surgical procedure in the decision of whether to undertake axillary LN dissection. However, intraoperative diagnosis of SLNs for metastasis using frozen sections has a sensitivity with immunohistochemistry (IHC) of only 50–70% compared with the permanent histologic sections of the same LN [4,5].

Cytokeratins (CKs) are keratin-containing intermediate filament proteins found in the intracytoplasmic cytoskeleton of epithelial tissue. CKs are of two types: acidic type I CKs and basic or neutral type II CKs. CKs are usually found in pairs, comprising a type I CK and a type II CK. Among the 20 epithelial CKs, CK19 is the lowest molecular weight (40 kDa) acidic keratin and is a specific cytoskeletal structure of simple epithelia; its expression is observed in normal and cancerous epithelial cells of organs such as the breast, colon, and lung [6,13].

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http://dx.doi.org/10.1016/j.lungcan.2014.09.018
0169-5002/

The one-step nucleic acid amplification (OSNA) assay is a novel technique that utilizes a loop-mediated isothermal amplification (RT-LAMP) method of gene amplification [7,8]. The assay is characterized by the quantitative measurement of a target mRNA, a brief reaction time, a high specificity for the target mRNA, and an absence of genomic DNA amplification. On the basis of these advantages, the OSNA assay has been developed as an alternative intraoperative method for the detection of tumor metastasis, and has been validated as diagnosis of LN metastases in breast, gastric, and colorectal cancers [9–12]. Also, the potential OSNA utility to non-small cell lung cancer patients has been reported [13]. To provide basic data for the development of the OSNA method for the evaluation of LN metastasis in lung tumors, we used immunohistochemistry to evaluate the CK19 expression rate in several different types of thoracic tumors. In addition, we evaluated the concordance of CK19 expression between primary tumors and their LN deposits.

2. Materials and methods

2.1. Case selection

The Institutional Review Board of our hospital approved the current study (2010-0077). The specimens used in this study were from previously constructed tissue microarray (TMA) blocks, which utilized a core sample measuring 2 mm in diameter, and they included samples from a total of 801 cases seen at the National Cancer Center Hospital (Tokyo, Japan) between 1997 and 2007. The cases consisted of 726 primary lung tumors, including 294 adenocarcinomas (ADCs), 157 squamous cell carcinomas (SQCs), 11 large cell carcinomas (LCCs), 42 pleomorphic carcinomas (PCs), 106 large-cell neuroendocrine carcinomas (LCNECs), 66 small cell carcinomas (SCCs), and 50 carcinoid tumors (CTs), as well as 42 malignant mesotheliomas and 33 metastatic lung deposits from breast carcinomas. Histological diagnoses were based on the latest World Health Organization classification with the aid of immunohistochemical panels [14]. Among all ADC cases, the predominant histological patterns were classified based on the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification [15].

2.2. Immunohistochemistry and evaluation

Heat-induced epitope retrieval with a target retrieval solution 9 (Dako, Carpinteria, CA, USA) was performed. Incubation with primary antibody to CK19 (RCK108, 1:50, Dako) was conducted using an automated stainer (Dako) following the vendor's protocol. ChemMate EnVision™ (Dako) detection kits were used. Immunohistochemical staining was scored independently by two observers (K.M. and K.T.). Discrepancies in judgment were resolved by means of a joint viewing of the slides under a multiheaded microscope. We defined immune-positive cases as those with a mean positive area ≥10%.

First, we performed CK19 staining on TMA sections. Then, the after mentioned 96 CK19-negative cases were subjected to CK19 immunostaining using the whole section from the largest area of the tumor blocks.

2.3. CK19 expression in LN metastasis

To elucidate the differences in CK19 expression between primary sites and their corresponding LN metastases, we selected the CK19-positive cases based on TMA data and the CK19-negative cases based on whole sections at primary tumor sites from patients with LN tumor deposits. CK19-positive cases were defined as those in which more than 5% of the tumor cells in the LN were positive.

2.4. Statistical analyses

Statistical analysis of CK19 expression among the different histological subtypes of ADCs was performed using Fisher's exact test with the SPSS Statistics 22 program (IBM Corporation, Somers, NY, USA).

3. Results

3.1. CK19 expression in TMA sections

CK19 expression was detected in 643 (80.3%) of all 801 analyzed cases. In primary lung and pleural tumors, CK19 expression was detected in 278 (94.6%) of 294 ADCs, 147 (93.6%) of 157 SQCs, 6 (54.5%) of 11 LCCs, 23 (54.8%) of 42 PCs, 82 (77.4%) of 106 LCNECs, 21 (31.8%) of 66 SCCs, 17 (34.0%) of 50 CTs, and 39 (92.9%) of 42 malignant mesotheliomas (Fig. 1). CK19 expression was also detected in 30 (90.9%) of 33 lung metastatic deposits from breast carcinomas (Figs. 1 and 2).

3.2. CK19 expression rates together with whole section data

Additional whole section analysis increased the CK19 expression from 80.3% to 88.0% of all cases. The expression rate in metastatic deposits from breast carcinoma remained unchanged (90.9%). Among primary lung and thoracic tumors, 62 (40%) of 155 negative cases were found to be changed to positive for CK19-expression using whole section analysis: 14 (87.5%) of 16 ADCs, 4 (40%) of 10 SQCs, 3 (60%) of 5 LCCs, 7 (36.8%) of 19 PCs, 11 (45.8%) of 24 LCNECs, 18 (40%) of 45 SCCs, 4 (12.1%) of 33 CTs, and 1 (33.3%) of 3 malignant mesothelioma (Fig. 3).

Final CK19 expression rates conjunction with TMA and whole sections was 99.3% ADC, 96.2% SQC, 81.8% LCCs, 71.4% PC, 87.7% LCNEC, 59.1% SCC, 42.0% CT, and 95.2% malignant mesotheliomas (Fig. 1).

3.3. Correlation between CK19 expression and IASLC/ATS/ERS classification of lung ADCs

Among the 294 ADCs, the most common histological subtype was papillary-predominant, with 79 cases (26.9%), followed by 66 solid-predominant cases (22.4%), 54 acinar-predominant cases (18.4%), 46 invasive mucinous ADC cases (15.6%), 27 micropapillary-predominant cases (9.2%), and 22 lepidic-predominant cases (7.5%). There were no cases of mucinous adenocarcinoma in situ (AIS) or minimally invasive adenocarcinoma (MIA). When subdividing ADCs based on the IASLC/ATS/ERS classification, there were no statistical differences in CK19 expression ($P=0.31$). Complete positivity was observed in papillary-, acinar-, micropapillary-, and lepidic-predominant ADCs. The other subtypes also demonstrated highly positive CK19 expression, with rates of 98.5% in solid-predominant and 97.8% in invasive mucinous ADCs.

3.4. CK19 expression in LN metastases

We also examined the CK19 expression in LN metastases from 38 lung tumors: 22 cases that were CK19-positive at the primary sites (10 ADCs, 7 SQCs, 1 LCC, 2 LCNECs, 1 SCC, and 1 CT) and 16 cases that were CK19-negative at the primary sites (1 ADC, 2 SQCs, 1 LCC, 3PCs, 4 LCNECs, 2 SCCs, and 3 CTs).

All LN deposits from cases that were CK19-positive at the primary sites were positive for CK19. In addition, 7 (43.8%) of 16 LN

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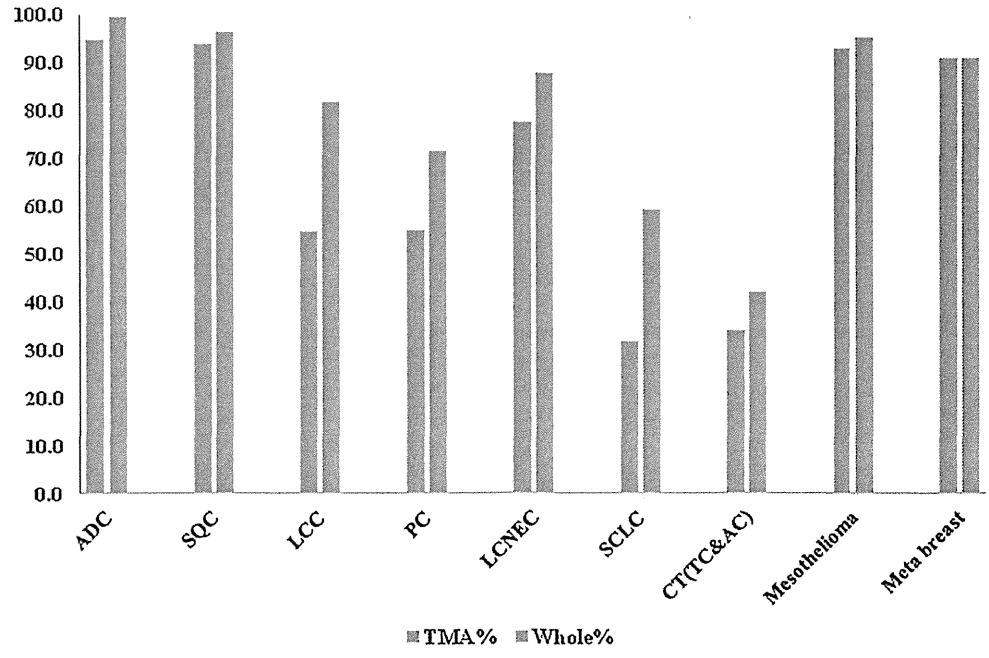

ADC ; adenocarcinoma, SQC ; squamous cell carcinoma, LCC ; large cell carcinoma, PC ; pleomorphic carcinoma, LCNEC ; large-cell neuroendocrine carcinoma, SCC ; small cell carcinoma, CT ; carcinoid tumor, TC ; typical carcinoid, AC ; Atypical carcinoid, and Meta breast ; metastatic lung deposits from breast carcinomas.

Fig. 1. Cytokeratin 19 expression of thoracic tumor and metastatic breast cancer. Purple bar depicting the cytokeratin (CK) 19 expression rate based on tissue microarray and blue bar indicates the final conjunction with tissue-microarray and whole section samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

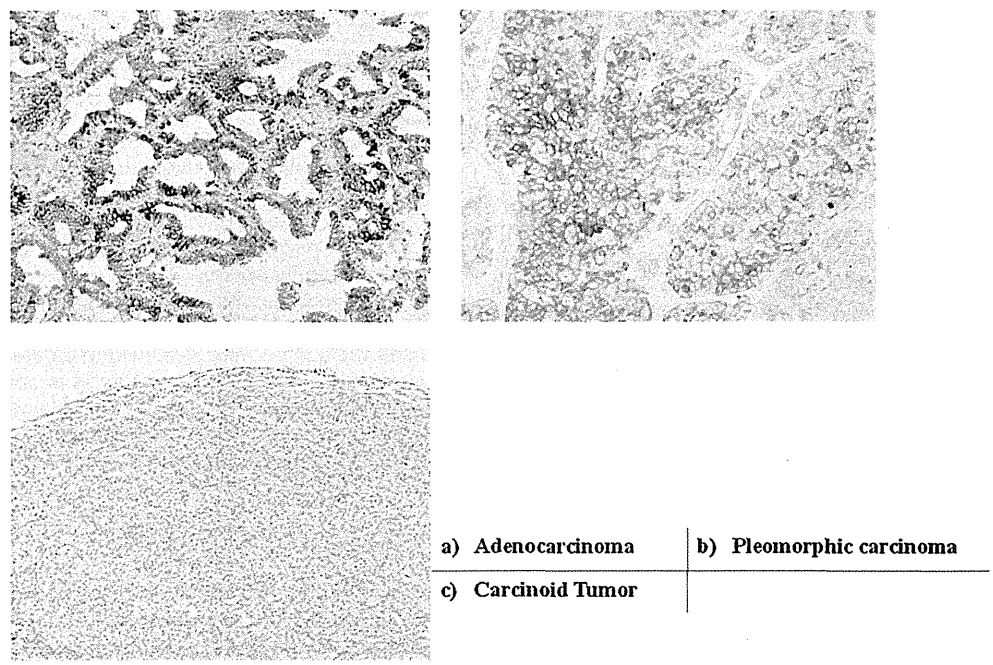

Fig. 2. Representative image of cytokeratin 19 expression. (a) Diffuse and strong staining in lepidic-predominant adenocarcinoma (magnification, 20×). (b) Diffuse and strong staining in pleomorphic carcinoma (magnification, 20×). (c) No staining in carcinoid tumor from tissue-microarray section (magnification, 20×).

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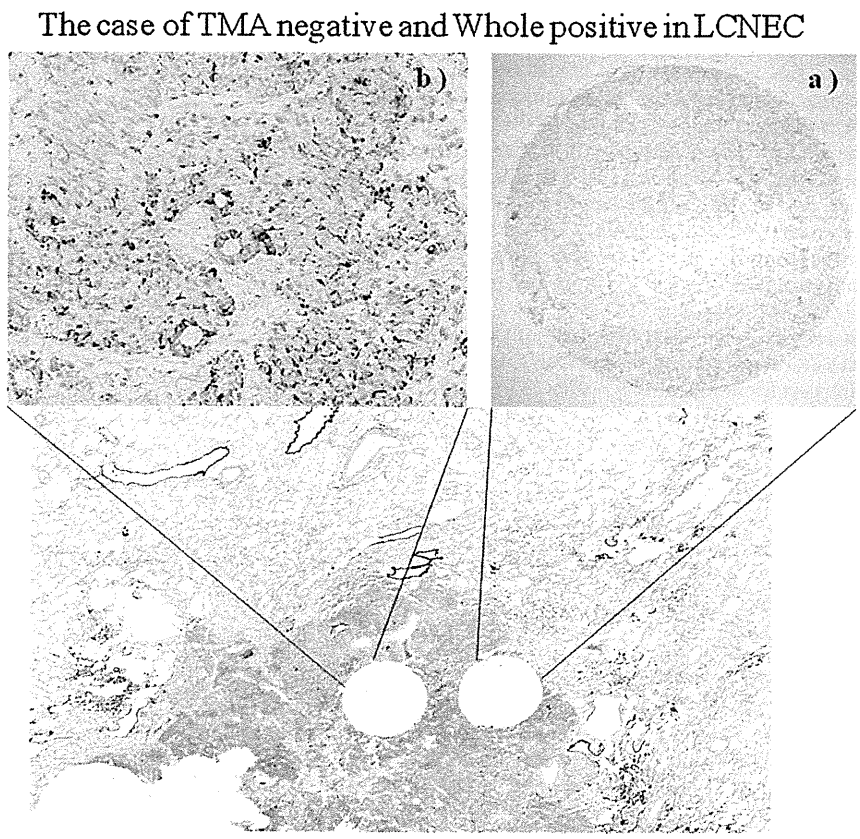

Fig. 3. Differences of cytokeratin 19 expression between tissue-microarray and whole section. (a) No cytokeratin 19 expression in large cell neuroendocrine carcinoma sample from tissue-microarray section. (b) Whole section from the same case showing the cytokeratin 19 negative of central areas including defects of tissue-microarray cores. Whereas, focal moderate staining distributing peripheral area of the tumor.

deposits from cases that were CK19-negative at the primary sites (1 of 1 ADC, 1 of 2 SQCs, 1 of 1 LCC, 3 of 3 PCs, and 1 of 4 LCNECs) demonstrated a change to positive for CK19.

Overall, some inflammatory cells including plasma cells and epithelioid macrophages showed weak CK19 staining in primary sites and LNs.

4. Comment

In this retrospective immunohistochemical study of CK19 expression in lung and pleural tumors, our findings demonstrated that most of the thoracic tumors except for PCs, CTs, and SCCs were positive for CK19. We also revealed that CK19 expression was maintained between CK19-positive primary sites and corresponding LN metastatic deposits. Of note, a portion of CK19-negative primary tumors displayed upregulation of CK19 protein expression in LN metastases.

The high prevalence rates we observed for CK19 expression in most of the lung and pleural tumors were consistent with previous reports, in which the positive rates for expression of CK19 ranged from 70% to 100% [16–19]. The sources of antibodies, dilutions, and the method of antigen retrieval must all be taken into consideration, since all of these methodological variations can significantly affect the results of protein expression using immunohistochemistry. Although there are no previous reports regarding the rate of expression of CK19 in lung metastatic deposits from breast carcinomas, our observed CK19 expression rate in metastatic deposits from breast carcinomas was similar to the range of expression reported in primary sites in previous reports [16,20,21]. Therefore, our method of CK19 detection does not indicate overrepresentation. On the other hand, the low rates of CK19 expression that we observed in PCs, CTs, and SCCs can be taken as genuinely indicative of low or no expression of CK19.

The various reasons for the low expression rates of CK19 in PCs and neuroendocrine carcinomas (NECs) most likely differ. The main reason for the low expression of CK19 in PCs may be epithelial–mesenchymal transition (EMT) [22–25]. During the EMT, epithelial cells lose epithelial phenotypes (downregulation of CK and loss of cell–cell adherence) and gain the mesenchymal phenotypes represented by vimentin expression. EMT is a natural process involved in development and wound healing. Additionally, EMT plays an important role in tumor invasion and metastasis. In PCs, the sarcomatoid component shows EMT [22,23].

On the other hand, the likely reason for the low CK19 expression rates we observed in NECs was that the tumor cells either did not express or expressed very low levels of CK19. Although most neuroendocrine organs are composed of simple epithelium, and thus endocrine tumors usually express the simple epithelial CKs such as CK19, the keratin profiles of these organs are complex due to the fact that some of them are considered to be derived from endoderm (islets of the pancreas, thyroid, parathyroid gland), some from mesoderm (adrenal cortex), and some from ectoderm (pituitary gland, pineal gland, adrenal medulla) [16]. In fact, 25 of 28 (89.3%) cases of pancreatic neuroendocrine tumors (NETs) were CK19 positive [26]. In contrast, low CK19 expression rates were reported in gastrointestinal carcinoid tumors and lung carcinoid tumors [16,27].

Another novel finding of our study was that all tumors that were positive for CK19 at the primary site maintained CK19 expression in LN metastases. Of note, we observed that a portion of

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CK19-negative primary tumors also showed CK19 expression in LN metastases. Similar upregulation of CK19 expression between the primary site and LN metastases was observed in breast carcinomas; 67% of CK19-negative primary breast tumors demonstrated a change to CK19-positive status in the LN metastases[28–31]. One of the hypothesized reasons for this upregulation is reactivation of cytokeratin expression during metastasis [23,31] During the metastatic process, tumor cells at the primary site lose their cell polarity and cell–cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. At the metastatic site, the tumor cells then return to a non-mesenchymal phenotype, via a mesenchymal–epithelial transition [23].

Similar to a previous study [13], we observed some inflammatory cells that reacted with CK19 in primary sites and LNs. Thus, it is necessary to carefully interpret immunostaining results to identify micro-metastases in lymph nodes. Furthermore, a previous report also revealed that CK19 reactivity in epithelioid macrophages induces a false positive result for the OSNA assay (i.e., no lymph node deposit but a positive result for the OSNA assay) [13].

The data from the current study indicate that performing immunohistochemistry for CK19 on biopsy specimens from the primary tumor site prior to OSNA analysis is of less importance. This is because most of the lung carcinomas for which surgery is indicated are positive for CK19 expression. In addition, not only did all CK19-positive tumors maintain their expression at LN metastatic deposits, but a portion of the CK19-negative primary tumors showed expression of CK19 in LN metastases, as well. There are conflicting reports regarding the preoperative evaluation of CK19 expression at the primary tumor site prior to OSNA assay in breast carcinoma [21]. In addition, CTs and SCCs that showed low expression of CK19 less frequently metastasized to LNs and were not indicated for surgical resection, respectively.

In conclusion, we performed a large-scale analysis of the expression of CK19 in thoracic tumor samples. Our data showed that most of the thoracic tumors except for PCs, CTs, and SCCs were positive for CK19. We also revealed that CK19 expression was maintained between CK19-positive primary sites and corresponding LN metastatic deposits. These results may be useful in the development of the OSNA method for the intraoperative detection of LN metastasis in NSCLC. However, since the OSNA assay is based on real-time amplification and quantitation of CK19 mRNA, our protein expression data are not directly applicable to the assay.

Funding

Division of Pathology and Clinical Laboratory, National Cancer Center Hospital (Tokyo, Japan) received research fund during study period from Sysmex Corporation (Kobe, Japan). The sponsor of the study had no role in the conduct of the study, data collection, data interpretation or preparation of the report.

Conflict of interest statement

All authors contributing to this work have no other conflict of interest to declare.

Acknowledgements

We would like to thank Sachiko Miura and Chizu Kina for their skillful technical assistance.

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Prognostic Significance of Tumor Size of Small Lung Adenocarcinomas Evaluated with Mediastinal Window Settings on Computed Tomography

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Abstract

Background: We aimed to clarify that the size of the lung adenocarcinoma evaluated using mediastinal window on computed tomography is an important and useful modality for predicting invasiveness, lymph node metastasis and prognosis in small adenocarcinoma.

Methods: We evaluated 176 patients with small lung adenocarcinomas (diameter, 1–3 cm) who underwent standard surgical resection. Tumours were examined using computed tomography with thin section conditions (1.25 mm thick on high-resolution computed tomography) with tumour dimensions evaluated under two settings: lung window and mediastinal window. We also determined the patient age, gender, preoperative nodal status, tumour size, tumour disappearance ratio, preoperative serum carcinoembryonic antigen levels and pathological status (lymphatic vessel, vascular vessel or pleural invasion). Recurrence-free survival was used for prognosis.

Results: Lung window, mediastinal window, tumour disappearance ratio and preoperative nodal status were significant predictive factors for recurrence-free survival in univariate analyses. Areas under the receiver operator curves for recurrence were 0.76, 0.73 and 0.65 for mediastinal window, tumour disappearance ratio and lung window, respectively. Lung window, mediastinal window, tumour disappearance ratio, preoperative serum carcinoembryonic antigen levels and preoperative nodal status were significant predictive factors for lymph node metastasis in univariate analyses; areas under the receiver operator curves were 0.61, 0.76, 0.72 and 0.66, for lung window, mediastinal window, tumour disappearance ratio and preoperative serum carcinoembryonic antigen levels, respectively. Lung window, mediastinal window, tumour disappearance ratio, preoperative serum carcinoembryonic antigen levels and preoperative nodal status were significant factors for lymphatic vessel, vascular vessel or pleural invasion in univariate analyses; areas under the receiver operator curves were 0.60, 0.81, 0.81 and 0.65 for lung window, mediastinal window, tumour disappearance ratio and preoperative serum carcinoembryonic antigen levels, respectively.

Conclusions: According to the univariate analyses including a logistic regression and ROCs performed for variables with p-values of <0.05 on univariate analyses, our results suggest that measuring tumour size using mediastinal window on high-resolution computed tomography is a simple and useful preoperative prognosis modality in small adenocarcinoma.

Citation: Sakao Y, Kuroda H, Mun M, Uehara H, Motoi N, et al. (2014) Prognostic Significance of Tumor Size of Small Lung Adenocarcinomas Evaluated with Mediastinal Window Settings on Computed Tomography. PLoS ONE 9(11): e110305. doi:10.1371/journal.pone.0110305

Editor: Prasad S. Adusumilli, Memorial Sloan-Kettering Cancer Center, United States of America

Received June 19, 2014; **Accepted** August 22, 2014; **Published** November 3, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

We previously reported that the size of lung adenocarcinoma, evaluated using mediastinal window (MD) settings on computed tomography (CT), is a more important predictive prognosis factor than the total tumour size, evaluated using lung window (LD) settings [1] Various studies have documented the correlation between CT findings and the pathological features of lung adenocarcinoma [2–4]. The ground glass opacity (GGO) component is typically recognized as a bronchioloalveolar carcinoma (BAC) component on microscopic examination, and the BAC is now categorized as an adenocarcinoma in situ that does not affect tumour aggressiveness [5,6]. In contrast, the solid component recognized as invasive lesion being so called scar, which excludes the BAC component in lepidic predominant adenocarcinoma, can be easily defined using MD settings on CT [1,2,5]. Moreover, the solid tumour recognized as a non-lepidic predominant adenocarcinoma, such as acinar, papillary, solid predominant or micropapillary predominant adenocarcinomas, is recognized as invasive

adenocarcinoma and shows much more aggressiveness than that by lepidic predominant adenocarcinoma [2,5,7]. Therefore, we have emphasized the importance of determining the size of the solid tumour component in adenocarcinoma using MD settings when evaluating tumour aggressiveness [1,2,5].

This investigation aimed to clarify the importance of the tumour size evaluated by MD settings as a preoperative prognostic predictive factor for anatomical pulmonary resection in patients with small adenocarcinomas (1–3 cm). Furthermore, we would clarify that the preoperative evaluation of tumour diameter by CT with MD settings would enable the prediction of prognosis, lymph node metastasis and tumour invasiveness for patients with clinically early-stage tumours.

Materials and Methods

This was a retrospective study conducted between October 2003 and December 2008 in patients with small lung adenocarcinomas (diameters of ≤3 cm) that underwent standard surgical resections (lobectomy with hilar and mediastinal lymph node dissection) at the Cancer Institute Hospital.

Tumour dimension was evaluated under two different CT imaging conditions: LD [level = −500 Hounsfield unit (HU), width = 1500 HU] and MD (level = 60, width = 350 HU). The CT (multi-detector CT, Toshiba, Japan) images were evaluated for the maximum tumour dimension.

Tumour disappearance ratio (TDR) was defined as 1− MD/LD.

"For all patients, preoperative staging was assessed using chest CT, CT or ultrasonography for abdominal metastasis, brain CT or magnetic resonance imaging for the brain metastasis and bone scanning for bone metastasis."

Clinical mediastinal and hilar lymph node status was deemed positive if the chest CT findings revealed a lymph node short axis of >1.0 cm. The status of mediastinal, hilar or interlobar nodes was assessed according to the classification for lung cancer in the TNM Classification of Malignant Tumours, Seventh Edition [8]. The CT findings were reviewed by two independent radiologists

We excluded tumours comprising 100% GGO from this study because most of them were believed, on microscopic examination, to be non-invasive or precancerous lesions. The GGO component was defined as hazy and amorphous with increased lung attenuation, but without obscuration of the underlying vascular markings and bronchial walls. In addition, we excluded a subgroup with BAC or mucinous BAC on microscopic examination that were defined as adenocarcinoma in situ [6]. We also excluded tumours measuring <1 cm because they were few in number and did not undergo standard resections. To select this cohort, the tumour size was used measured by two independent radiologists with CT in LD. Of total 246 patients with small lung adenocarcinomas (diameters of ≤3 cm) who underwent standard surgical resections, 36 were excluded due to lack of thin slice data in CT, 24 were excluded due to adenocarcinoma in situ and 10 were excluded due to size smaller than 1 cm. Therefore, 176 patients were examined in this study.

Patient records were examined for age, gender, preoperative nodal status and tumour size, as evaluated using both MD and LD. Preoperative serum carcinoembryonic antigen (CEA) levels, TDR and pathological status were evaluated using elastic stain, and included lymphatic vessel (ly), vascular vessel (v) and pleural (pl) invasion.

Because individual patients were not identified, our institutional review board (Review Board in Cancer Institute Hospital, Japanese Foundation for Cancer Research) approved this study without the requirement to obtain patient consent. The patient records/information was anonymized and de-identified prior to analysis.

Statistical Analyses

Disease-free survival was assessed. Survival duration was defined as the interval between surgery and either tumour relapse or the most recent follow-up. The Kaplan–Meier method was used to calculate the recurrence-free survival rates. Univariate analyses included a log-rank test, chi-square test and logistic regression. Receiver operating characteristic analyses (ROC) were performed for variables with P-values of <0.05 on univariate analysis using the logistic regression test or the Cox proportional hazards model. All analyses were performed using the JMP 10 software (SAS Institute Incorporated, Cary, North Carolina) and results with P-values of <0.05 were considered statistically significant.

Results

In total, 176 patients were enrolled. This subgroup that excluded BAC and small tumours (<1 cm) comprised 99 females and 77 males, with ages ranging from 34 to 78 (median = 61)

Table 1. Preoperative prognostic factors for disease-free survival with small adenocarcinomas (≤3 cm).

Variables	Odds ratio	95% CI	*P* value
Gender (female)	0.61	0.34–1.07	.08
Age	0.99	0.97–1.03	.74
LD	1.08	1.02–1.14	.007
MD	1.13	1.08–1.18	<.001
TDR	38.6	7.35–	<.001
CEA	0.33	0.99–1.03	.33
High (>5 ng/ml)/normal)	1.89	0.96–3.72	.066
cN (cN0/cN1-2)	0.23	0.11–0.50	<.001

Logistic regression test (Univariate analyses).
LD: diameter using lung window setting, MD: diameter using mediastinal window setting, RFS: recurrence-free survival, TDR: tumour disappearance ratio (TDR = 1− MD/LD), CEA: carcinoembryonic antigen, cN: preoperative nodal status, CI: confidence interval.
doi:10.1371/journal.pone.0110305.t001

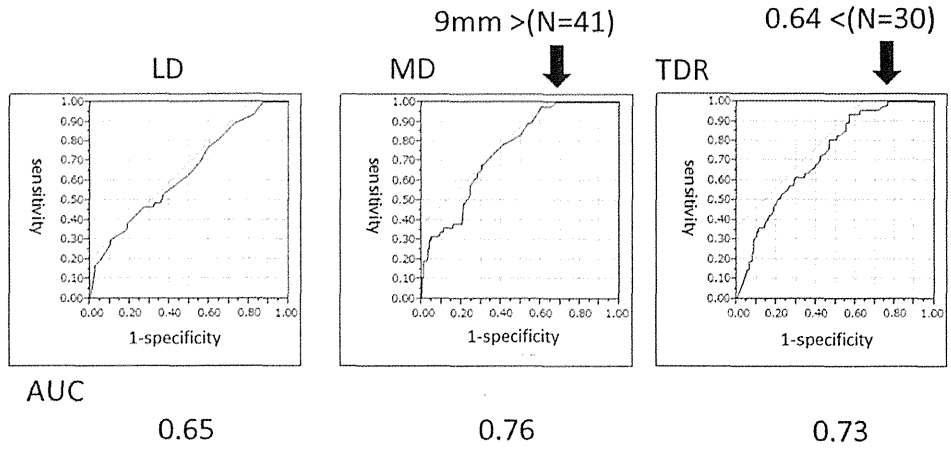

Figure 1. Receiver operating characteristic analyses for recurrence. Tumour dimension was evaluated using lung window (LD) and mediastinal window (MD) settings. TDR: tumour disappearance ratio (TDR = 1− MD/LD). Allow indicated a value at 100% sensitivity.
doi:10.1371/journal.pone.0110305.g001

years. The follow-up periods ranged from 24–84 (median = 49) months.

Preoperative prognostic factors for disease-free survival

As shown in Table 1, LD findings, MD findings, TDR and nodal status (cN) were significant prognostic factors for disease-free survival on univariate analyses. The AUCs for recurrence were 0.76, 0.73 and 0.65, for MD, TDR and LD, respectively (Figure 1).The 5-year disease-free survival rates according to MD were 98.1% for ≤10 mm (N = 52), 71.0% for 11–≤15 mm (N = 52) and 49.0% for>15 mm (N = 72). ($P<0.001$) (Figure 2).

Preoperative Factors Associated with Lymph Node Metastasis

As shown in Table 2, LD findings, MD findings, TDR, CEA levels and cN were significant factors associated with lymph node metastasis on univariate analyses. The area under the curves (AUCs) for lymph node metastases were 0.61, 0.76, 0.72 and 0.66 for LD, MD, TDR and CEA, respectively (Figure 3). The incidence of lymph node metastases according to MD were 0% for <10 mm (N = 52), 34.6% for>10 mm–15 mm(N = 52), 41.2% for>15 mm–20 mm (N = 34) and 50.0% for>20 mm (N = 38) (Figure 4).

Preoperative factors associated with lymphatic vessel, vascular vessel or pleural invasion in small adenocarcinomas

As shown in Table 3, LD findings, MD findings, TDR, CEA levels and cN were significant factors for lymphatic vessel, vascular vessel or pleural (ly/v/pl) invasion on univariate analyses. The AUCs for ly/v/pl invasion were 0.60, 0.81, 0.81 and 0.65 for LD, MD, TDR and CEA, respectively (Figure 5). The incidence of ly/v/pl invasion according to MD were 0% for ≤5 mm (N = 25), 25.9% for>5 mm–10 mm (N = 27), 41.2% for ≥10 mm–15 mm (N = 52) and 79.4% for>15 mm(N = 72) (Figure 6).

Discussion

Tumour diameter is a major prognostic factor for lung cancer. The most common method for determining tumour size before surgery is by CT using lung window settings [8]. Recently, attempts were made to classify small peripheral adenocarcinomas into subgroups according to the patterns of tumour growth, which are considered to be associated with the biological characteristics of tumours derived from clinicopathological examination[4,6,9–11]. These subgroups comprise the following: AIS (adenocarcinoma in situ), minimally invasive adenocarcinoma (3-cm lepidic predominant tumour with an invasion of ≤5 mm), lepidic predominant, acinar predominant, papillary predominant, micropapillary predominant, solid predominant with mucin production and invasive adenocarcinoma variants.

It has been reported that tumour size was not associated with either indicators of proliferation or tumour invasiveness [1,2,5,9,11]. In fact, histological subgrouping based on growth patterns provides a clear indication of the biological characteristics of peripheral small lung adenocarcinoma than simple lesion size [7,9]. That is, papirally, acinar, micropapillery or solid predom-

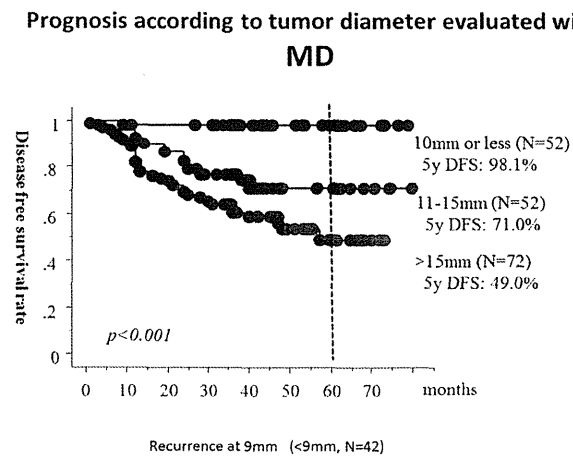

Figure 2. The 5-year disease-free survival curve according to tumour dimension using mediastinal window (MD) settings. Tumour dimension was evaluated using mediastinal window (MD) settings. One case with MD 9 mm showed recurrence and the case is the smallest in MD among all.
doi:10.1371/journal.pone.0110305.g002

Table 2. Preoperative factors associated with lymph node metastasis in small adenocarcinoma (≤3 cm).

Variables	Odds ratio	95% CI	*P* value
Gender (female)	0.66	0.34–1.28	.22
Age	1.03	0.99–1.03	.86
LD	1.07	1.01–1.15	.03
MD	1.15	1.09–1.21	<.001
TDR	66.7	10.0–	<.001
CEA	1.11	1.01–1.12	.01
High (>5 ng/ml)/normal)	3.0	1.31–6.87	.009
cN (cN0/cN1-2)	30.3	3.76–250	<.001

Logistic regression test (Univariate analyses).
LD: diameter by lung window setting, MD: diameter by mediastinal window setting, TDR: tumour disappearance ratio (TDR =1− MD/LD), CEA: carcinoembryonic antigen, cN: preoperative nodal status, CI: confidence interval, LN: lymph node.
doi:10.1371/journal.pone.0110305.t002

inant adenocarcinoma have more aggressive character than lepidic predominant adenocarcinoma when tumoue size is limited to 3cm or smaller [7–11].

Furthermore, it has been reported that the larger or more desmoplastic fibrous scars is asosiated with more aggressive tumour invasion and poorer prognoses [7,9–11]. In addition, we previously reported that tumour size, excluding the BAC component (lepidic growth), was an important indicator of tumour invasiveness and that tumour diameter evaluated using MD was associated with the tumour growth pattern (i.e. non-lepidic growth component) and scars [1,2,5]. The solid lesion evaluated with MD in CT is the tumor lesion without lepidic growth adenocarcinoma. As an easy and simple extracting method of the solid area representing an invasive lesion (scar) of the adenocarcinoma or non-lepidic predominant adenocarcinoma (invasive adenocarcinoma), we examined the usefulness of tumour size as evaluated using MD. In other words, using LD to evaluate the total tumour diameter cannot directly detect the solid lesion associated with tumour aggressiveness, but can detect the associated BAC (lepidic growth) component, which is not associated with tumour aggressiveness [1,2,5,14].

In the present study, we confirmed that tumour dimensions determined using MD settings provided additional useful prognostic data that could not be evaluated using LD settings [1,2,5]. Furthermore, tumour size was a significantly better prognostic factor when evaluated using MD instead of LD. The MD were an important predictive factor for prognosis as well as for lymph node involvement and tumour invasiveness in small lung adenocarcinoma (1–3 cm). A new concept −minimally invasive adenocarcinoma (MIA)− has been proposed. This is a small, solitary adenocarcinoma (≤3 cm) with a predominantly lepidic pattern and invasion of ≤5 mm in the greatest dimension in any one focus. Patients with an MIA have a nearly 100% disease-specific survival if it is completely resected. The invasive component to be measured in an MIA is defined as follows: (1) histological subtypes other than a lepidic pattern (i.e., acinar, papillary, micropapillary, and/or solid) or (2) tumor cells infiltrating into myofibroblastic stroma. MIA is excluded if the tumor (1) invades lymphatics, blood vessels, or pleura or (2) has tumor necrosis [6]. Accordin to the present study, when the MD findings were equal or smaller than 5 mm, no patient showed vessel invasion, plural invasion, or tumor relapse. Therefore, MD may be a promising criteria to be

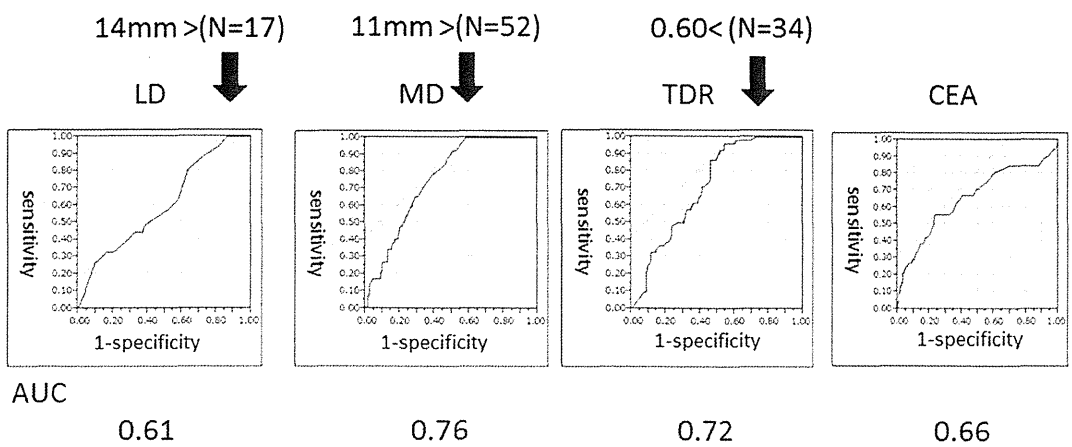

Figure 3. Receiver operating characteristic analyses for lymph node metastasis. Tumour dimension was evaluated using lung window (LD) and mediastinal window (MD) settings. TDR: tumour disappearance ratio (TDR =1− MD/LD), CEA: carcinoembryonic antigen. Allow indicated a value at 100% sensitivity.
doi:10.1371/journal.pone.0110305.g003

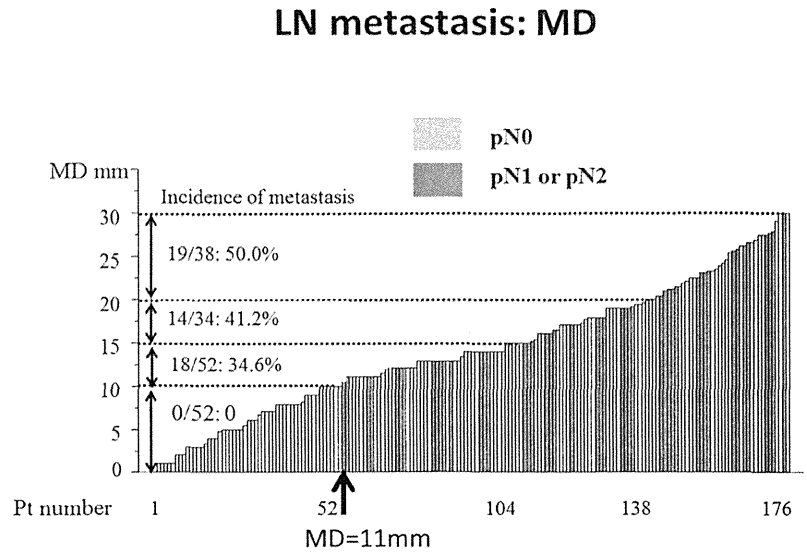

Figure 4. Incidence of lymph node metastasis according to tumour dimension using mediastinal window (MD) settings. Tumour dimension was evaluated using mediastinal window (MD) settings. A black bar showed a patient with lymph node metastasis and a gray bar showed a patient without lymph node metastasis.
doi:10.1371/journal.pone.0110305.g004

used for CT classification as the newly proposed minimally invasive adenocarcinoma.

Tumour markers are used clinically to assist in the diagnosis of patients with non-small-cell lung carcinoma and to monitor progression, recurrence and/or efficiency of treatment. Among these markers, CEA is considered to be the most prevalent marker for the diagnosis and monitoring of lung adenocarcinoma. Serum CEA levels are an important prognostic factor for early-stage adenocarcinoma, such as clinical stage IA tumours [12–14]. In the present study, univariate analysis revealed that CEA levels were correlated with lymph node metastasis and v or pl invasion, with a tendency for predicting postoperative prognosis. However, CEA levels were not a significant prognostic factor for lymph node metastasis following multivariate analyses with MD findings. This may be explained by the high correlation between CEA levels and MD findings. When CEA levels were compared among the tumour size groups, they gradually and significantly increased with tumour stage progression ($P<0.01$). When the mediastinal size was>20 mm, one-fourth of the patients in this cohort showed serum CEA levels beyond cut off value at 5 ng/ml.

TDR is an established and important prognostic factor. Suzuki et al. reported that radiological non-invasive (neither ly nor v) peripheral lung adenocarcinoma could be defined as an adenocarcinoma of ≤2.0 cm with ≤0.25 consolidation [15]. The results of our study were similar to those of a previous study on TDR [16.17].

The solid type adenocarcinoma on CT was highly associated with non-lepidic predominant adenocarcinoma, such as acinar predominant, papillary predominant and solid predominant with mucin production [2,3,5]. In invasive adenocarcinoma, lepidic predominant adenocarcinomas have a much better prognosis than non-lepidic predominant adenocarcinomas [1,2,5,9–11]. It was therefore suggested that the size of a non-lepidic tumour component evaluated using MD findings on CT was a useful indicator of solid adenocarcinoma aggressiveness.

Table 3. Preoperative factors associated with pleural, lymphatic and vascular invasion in small adenocarcinomas (≤3 cm).

Variables	Odds ratio	95% CI	*P* value
Gender (female)	0.66	0.36–1.20	.17
Age	1.00	0.97–1.03	.87
LD	1.06	1.00–1.12	.03
MD	1.21	1.14–1.30	<.001
TDR	6.76	3.50–13.2	<.001
CEA	1.23	1.07–1.40	.002
High (>5 ng/ml)/normal)	5.9	2.12–16.3	<.001
cN (cN0/cN1-2)	11.1	1.40–90.9	.03

Logistic regression test (Univariate analyses).
LD: diameter by lung window setting, MD: diameter by mediastinal window setting, TDR: tumour disappearance ratio (TDR = 1− MD/LD), CEA: carcinoembryonic antigen, cN: preoperative nodal status, CI: confidence interval, ly: lymphatic vessels, v: vascular vessels, pl: pleura.
doi:10.1371/journal.pone.0110305.t003