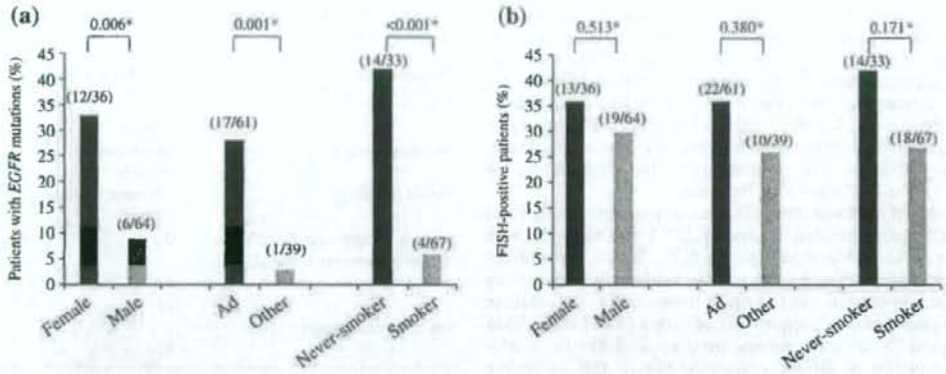

Fig. 1. Sex, tumor histology, and smoking status of patients with advanced non-small cell lung cancer and with either (a) epidermal growth factor receptor (*EGFR*) mutations or (b) a high *EGFR* copy number. Ad, adenocarcinoma. *P-values were determined by Fisher's exact test.

Table 4. Relationship between epidermal growth factor receptor (*EGFR*) mutation and either fluorescence *in situ* hybridization (FISH) status of *EGFR* amplification

Mutation status	FISH status		Gene amplification	
	Positive	Negative	Positive	Negative
Positive ($n = 18$)	8	10	4	14
Negative ($n = 82$)	24	58	2	80
P-value*		0.266		0.009

*Determined by Fisher's exact test.

positivity was not associated with sex, tumor histology, or smoking status (Fig. 1b). Although no relationship was apparent between *EGFR* mutation and FISH positivity (gene amplification or high polysomy), *EGFR* mutation and *EGFR* amplification were significantly associated (Table 4). The clinicopathological and genetic features of patients with *EGFR* mutations are shown in Table 5.

Overall survival. For the total patient population, the median overall survival was 12.3 months, with a 1-year survival rate of 51.7%. Univariate analysis revealed that overall survival was significantly longer in women, never-smokers, patients with a favorable PS, and those with *EGFR* mutations (Table 6; Fig. 2a). In contrast, no difference in overall survival was apparent between FISH-positive and FISH-negative patients (Table 6; Fig. 2b). We also carried out multivariate analysis to identify factors that contribute to overall survival, with covariates including clinicopathological and genetic factors (sex, smoking history, tumor histology, PS, *EGFR* mutation status, FISH status). Female sex and favorable PS were found to be independent prognostic factors (Table 6).

Responsiveness to epidermal growth factor receptor tyrosine kinase inhibitor treatment. Of the 53 patients treated with EGFR TKI, 40 individuals were assessable for objective response. Whereas the rate of response to EGFR TKI treatment for patients with *EGFR* mutations was significantly higher than that for those without such mutations (71.4 vs 11.5%, $P < 0.001$), there was no significant association between FISH status and responsiveness

Table 5. Clinicopathological and genetic features of patients with epidermal growth factor receptor (*EGFR*) mutations

No.	Age (years)	Sex	Smoking status	Histology	Response to EGFR TKI	Type of *EGFR* mutation		*EGFR* copy number
						Sequencing	ARMS	
1	72	F	Never	Ad	PR		L858R	Low trisomy
2	58	F	Never	Ad	PR	L858R	L858R	Gene amplification
3	81	F	Never	Ad	SD	L858R	L858R	High polysomy
4	72	F	Never	Ad	NE		L858R	Gene amplification
5	48	M	Smoker	Ad	SD		L858R	Low trisomy
6	67	F	Never	Ad	SD		L858R	Low trisomy
7	59	F	Never	Ad	PR		L858R	High polysomy
8	78	M	Smoker	Ad			L858R	High trisomy
9	71	F	Never	Ad	PR		L858R	Low polysomy
10	82	F	Never	Ad	PR	L858R	L858R	Low trisomy
11	67	F	Never	Ad		L858R	L858R	High polysomy
12	87	F	Never	Sq	PR	L858R	L858R	Low polysomy
13	78	M	Never	Ad			L858R	Gene amplification
14	56	F	Never	Ad	PR		(E746_A750)del	Low polysomy
15	63	M	Never	Ad	PD	(E746_A750)del	(E746_A750)del	Gene amplification
16	63	M	Smoker	Ad	PR		(E746_A750)del	Low polysomy
17	61	M	Smoker	Ad	PR	(E746_S752)del insV		Low trisomy
18	73	F	Never	Ad	PR	(E746_T751)del insS		High polysomy

Ad, adenocarcinoma; ARMS, amplification-refractory mutation system; NE, not evaluated; PD, progressive disease; PR, partial response; SD, stable disease; Sq, squamous cell carcinoma; TKI, tyrosine kinase inhibitor.

Table 6. Univariate and multivariate analyses of prognostic factors for overall survival

Factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex (female/male)	0.54	0.32–0.91	**0.021**	0.55	0.32–0.93	**0.025**
Smoking history (never-smoker/smoker)	0.50	0.30–0.85	**0.011**			
Histology (adenocarcinoma/other)	0.64	0.39–1.05	0.077	0.68	0.40–1.14	0.141
ECOG PS (0/≥1)	0.44	0.24–0.79	**0.006**	0.48	0.29–0.86	**0.019**
EGFR mutation status (positive/negative)	0.52	0.28–0.97	**0.039**			
FISH status (positive/negative)	1.36	0.82–2.23	0.231	1.49	0.88–2.50	0.130

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridization; HR, hazard ratio; PS, performance status. Multivariate analysis was carried out using the stepwise method (include, <0.05; exclude, >0.2). Significant *P*-values are shown in bold.

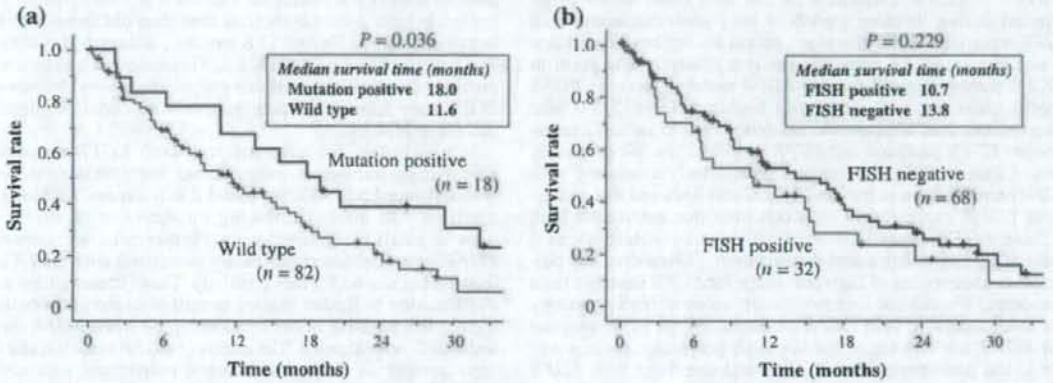

Fig. 2. Kaplan–Meier plots of overall survival in patients with advanced non-small cell lung cancer and either (a) with or without epidermal growth factor receptor (*EGFR*) mutations or (b) with or without a high *EGFR* copy number. FISH, fluorescence *in situ* hybridization.

to EGFR TKI (44.4 vs 29.0% for FISH-positive vs FISH-negative patients, respectively, $P = 0.437$).

Discussion

We have analyzed both *EGFR* mutation and *EGFR* copy number in paired tumor specimens as well as the relationship between these two types of *EGFR* alterations in advanced NSCLC. We used two methods to detect *EGFR* mutations, direct sequencing and Scorpion-ARMS, which identified eight and 16 mutations, respectively. Direct sequencing failed to detect 10 of the 16 mutations identified by Scorpion-ARMS. Of the 10 patients with *EGFR* mutations detected by Scorpion-ARMS alone, seven were assessable for an objective response to EGFR TKI, with five exhibiting a partial response and two having stable disease. Consistent with previous observations,[28–30] our data thus indicate that Scorpion-ARMS is more sensitive than direct sequencing for detection of the two major types of *EGFR* mutation that reflect responsiveness to EGFR TKI. It should be noted, however, that most polymerase chain reaction-based systems for mutation analysis, including Scorpion-ARMS, are able to detect only known *EGFR* mutations targeted by the designed primers. Indeed, two minor variants of deletion mutation in exon 19 were not identified by Scorpion-ARMS in the present study. Given the exclusion of recurrence after surgical resection in our study, most tumor specimens analyzed were obtained either by transbronchial lung biopsy or by percutaneous needle lung biopsy. The amount of tumor tissue obtained by these procedures is limited, but our results suggest that it is sufficient both for histopathological analysis and for the detection of *EGFR* mutations by Scorpion-ARMS in patients with advanced NSCLC.

Scorpion-ARMS identified three E746_A750 deletion mutations in exon 19 and 13 L858R point mutations in exon 21 in the present study. The frequency of the E746_A750 mutation detected by Scorpion-ARMS thus appeared low compared with that of the L858R mutation. Previous studies have shown that the incidence of the E746_A750 deletion is approximately the same as that of the L858R mutation.[10,12] The sensitivity of Scorpion-ARMS for detection of the E746_A750 deletion is equivalent to that for detection of the L858R point mutation. The low frequency of the E746_A750 deletion mutation in the present study is thus likely due to the small number of samples.

Previous studies have revealed a higher prevalence of *EGFR* mutations in East Asians than in Caucasians.[4,10–12,20,22,24,26,27,32–36] The prevalence of *EGFR* mutations in our Japanese cohort was low (18%) compared with values determined previously for East Asian populations. Given that most previous studies examined only individuals treated with EGFR TKI, patient selection based on clinical predictors might have led to an increase in the proportion of subjects with adenocarcinoma histology, a factor known to be associated with *EGFR* mutations. In contrast, our study was carried out with consecutive cases irrespective of EGFR TKI treatment. The relatively low proportion of patients with adenocarcinoma histology (61%) in our cohort is therefore consistent with the low prevalence of *EGFR* mutations. However, the FISH positivity of 32% in our study is similar to that in previous studies that adopted the same criteria, with values ranging from 31 to 48%.[22–24,26,27] Consistent with previous

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results,[1,7–9,12] *EGFR* mutations were significantly more frequent among women, never-smokers, and patients with adenocarcinoma in the present study. In contrast, neither *EGFR* amplification (analysis not shown) nor FISH positivity was associated with any such clinicopathological factor in our study, although the relationship between *EGFR* amplification and never-smoking status approached statistical significance ($P = 0.090$).

The relationship between *EGFR* mutation and FISH positivity (gene amplification or high polysomy) in NSCLC patients has remained unclear.[22–24,26,27] In the present study, we have demonstrated a significant relationship between *EGFR* mutation and *EGFR* amplification, but not between *EGFR* mutation and FISH positivity, in tumor specimens from patients with advanced NSCLC. *EGFR* mutant alleles were previously found to be amplified selectively, resulting in a high *EGFR* copy number, as detected by quantitative real-time polymerase chain reaction analysis.[12] *EGFR* amplification has also been shown to be acquired during invasive growth of lung adenocarcinoma with *EGFR* mutations.[37] Furthermore, recent studies have found that an increase in *EGFR* copy number is a relatively late event in NSCLC pathogenesis[38] and that *EGFR* mutation precedes *EGFR* amplification but not necessarily high polysomy.[37,39] These observations thus support the existence of a close association between *EGFR* mutation and *EGFR* amplification. We previously showed that *EGFR* mutation was significantly associated with *EGFR* amplification in human NSCLC cell lines and that endogenous EGFR expressed in such cell lines that manifested both of these *EGFR* alterations were activated constitutively as a result of ligand-independent dimerization.[25] However, the biological consequences of high polysomy for *EGFR* have not been elucidated. We did not find any cut-off value of high polysomy that was associated with *EGFR* mutation. We therefore propose that *EGFR* amplification, but not high polysomy, plays a key role in the pathogenesis of NSCLC and correlates with *EGFR* mutation.

We sought to determine whether *EGFR* mutation or *EGFR* copy number might affect overall survival of NSCLC patients. Previous studies of EGFR TKI have suggested that *EGFR* mutation is a favorable prognostic indicator for patients with NSCLC.[35,36] We also found that the survival time of patients with *EGFR* mutations was longer than that of those without them (18.0 vs 11.6 months, $P = 0.036$) in the univariate analysis. However, interpretation of this result requires that the effect of EGFR TKI on survival be taken into account, given that 83% (15/18) of patients with *EGFR* mutations were treated with EGFR TKI compared with only 46% (38/82) of those without such mutations. Indeed, analysis of survival after initiation of EGFR TKI treatment as a second-line or subsequent therapy revealed a survival time of 15.6 months for mutation-positive patients vs 6.0 months for mutation-negative patients in our study. It was therefore not possible to determine the prognostic significance of *EGFR* mutation for NSCLC patients. To clarify whether *EGFR* mutation is a predictor of sensitivity to EGFR TKI or a prognostic indicator for NSCLC patients, we are currently carrying out a phase III randomized study comparing platinum-based chemotherapy with gefitinib in chemotherapy-naive NSCLC patients with *EGFR* mutations. Patients with FISH-positive tumors tended to have a shorter survival time than did those with FISH-negative tumors (10.7 vs 13.8 months), although this difference was not statistically significant. This result is consistent with previous observations indicative of an association between high *EGFR* copy number and poor prognosis for certain malignancies, including NSCLC.[1,40]

In conclusion, we have analyzed both *EGFR* mutation and *EGFR* copy number in paired tumor specimens from patients with advanced NSCLC. We found that Scorpion-ARMS is more sensitive than direct sequencing for detection of *EGFR* mutations in small tumor specimens. Furthermore, we showed that *EGFR* mutation was significantly associated with *EGFR* amplification but not with FISH positivity. These observations warrant confirmation in further studies as well as exploration of the biological mechanisms of the relationship between *EGFR* mutation and *EGFR* amplification. The effects of *EGFR* mutation and *EGFR* copy number on clinical outcome in individuals with advanced NSCLC also warrant investigation in a prospective study.

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ORIGINAL ARTICLE

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Brain metastases in patients who receive trastuzumab-containing chemotherapy for HER2-overexpressing metastatic breast cancer

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Abstract

Background. Recently, a high rate of brain metastases has been reported among patients with human epidermal growth factor receptor (HER2)-overexpressing metastatic breast cancer who were treated with trastuzumab. The present study examined risk factors for the development of brain metastasis in patients with HER2-overexpressing breast cancer who were treated with trastuzumab.

Methods. We retrospectively reviewed 204 patients with HER-2-overexpressing breast cancer who were treated with a trastuzumab-containing regimen between 1999 and 2006. Patients with clinical symptoms were diagnosed as having brain metastases when brain magnetic resonance imaging (MRI) or a computed tomography (CT) scan revealed positive findings for brain metastases. The median follow-up time of this cohort was 53.6 months.

Results. Among the patients who received a trastuzumab-containing regimen, 74 patients (36.3%) developed brain metastases. The median survival from the diagnosis of brain metastases was 13.5 months (95% confidence interval [CI], 12.2–14.7 months). The median time interval between the beginning of trastuzumab treatment and the diagnosis of brain metastases was 13.6 months (range, 0.0–45.8 months). Among patients with brain metastases, the median overall survival period was 39 months. A multivariate logistic regression analysis showed that age (≤50 years), recurrent breast cancer, and liver metastases were significant risk factors for the development of brain metastases.

Conclusion. Patients with HER2-overexpressing breast cancer treated with trastuzumab had a high incidence of brain metastases (36.3%). Routine screening for brain metastases 1 year after the start of trastuzumab treatment, may be warranted in younger patients (≤50 years) who had recurrent breast cancer with liver metastases.

Key words HER2-overexpressing breast cancer · Trastuzumab · Brain metastases

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Introduction

In approximately 25% of invasive breast cancers, the human epidermal growth factor receptor (HER) 2 tyrosine kinase receptor is overexpressed.[1,2] HER2 is a member of the epidermal growth factor receptor (EGFR) family, which consists of four different receptors and is associated with cell proliferation, differentiation, and survival. Patients with HER2-overexpressing breast cancer have more aggressive tumors and a poor prognosis.[2,3] Trastuzumab is a recombinant humanized monoclonal antibody targeted against the extracellular domain of HER2[4,5] and is broadly utilized for the treatment of not only advanced but also early-stage HER2-overexpressing breast cancer in an adjuvant setting.[6–8] Initially, trastuzumab in combination with cytotoxic agents as a first-line chemotherapy regimen showed a survival benefit for patients with metastatic breast cancer with HER2 overexpression.[4] Recently, trastuzumab has been found to increase the clinical benefit of treatment in patients with early breast cancer with HER2 overexpression.[6–8]

Recently, a high rate of brain metastases among patients with metastatic HER2-overexpressing breast cancer has been reported.[9–14] Several reasons, including biological factors and treatment-related factors, may be responsible for this trend. A retrospective analysis has identified HER2 as a risk factor for the development of central nervous system (CNS) relapse.[15] The 10-year cumulative incidence of recurrence in the CNS was significantly higher in patients with HER2-overexpressing breast cancer. In addition, the blood-brain barrier causes the brain to act as a sanctuary

site: antitumor agents cannot penetrate the blood-brain barrier in quantities sufficient to achieve an antitumor effect. In particular, trastuzumab has a high molecular weight (145 kDa) and thus cannot enter brain tissue.[9] Because improvements in systemic disease control have enabled patients with metastatic HER2-overexpressing breast cancer to survive for longer periods of time, and because diagnostic magnetic resonance imaging (MRI) and computed tomography (CT) imaging for brain metastases are now routinely used, the incidence of brain metastases is increasing. However, the association between brain metastases and the HER2 status remains unclear.

The aim of the present study was to identify risk factors for the development of brain metastases in patients with HER2-overexpressing breast cancer who were treated with trastuzumab.

Patients and methods

Patient selection

Two hundred and fifty-two patients with breast cancer received trastuzumab-based chemotherapy between January 1999 and January 2006 at the National Cancer Center Hospital (NCCH), in Japan. Patients meeting the following criteria were retrospectively selected for this study: (1) metastatic or recurrent breast cancer; (2) trastuzumab-containing chemotherapy used for metastatic or recurrent disease; and (3) patients who developed brain metastases after the initiation of trastuzumab therapy. We excluded patients receiving trastuzumab in a neoadjuvant or adjuvant setting and those who had developed symptomatic brain metastases before the initiation of trastuzumab therapy. A total of 204 patients with HER2-positive breast cancer were included in this study. All the tumors were diagnosed as breast cancer by pathologists at the NCCH. The HER2 status, as assessed using Herceptest (Dako, Carpinteria, CA, USA), was considered positive when staining in more than 10% of the cells was graded as 3+ in an immunohistochemical analysis (IHC) or as 2+ using IHC with gene amplification on fluorescence in situ hybridization (FISH). Patients with clinical symptoms suggesting brain metastasis were diagnosed using brain MRI or CT imaging. Data were collected from the patients' medical charts, including the dates of the initial diagnosis of breast cancer and the development of brain metastases, the recurrence of breast cancer, the start of trastuzumab therapy, and death or the last follow-up examination, as well as the sites of disease at the start of trastuzumab therapy and the details of treatment. We also evaluated the clinical response of extracranial metastatic diseases according to the Response Evaluation Criteria in Solid Tumors (RECIST) at the time of the diagnosis of brain metastasis. The patient baseline characteristics and prognostic factors for breast cancer were reviewed, including age, performance status, hormone receptor status, pathological nodal status and tumor size, histological grade, and site of metastases.

Data collection and statistical analyses

The patients' characteristics and prognostic factors, including age, stage, nodal status at initial diagnosis of breast cancer, estrogen receptor (ER) and progesterone receptor (PgR) statuses, histological grade, nuclear grade, site of metastases at the initiation of trastuzumab therapy, and chemotherapy regimens, were compared, using the χ^2 test and the Mann-Whitney U-test, between HER2-overexpressing breast cancer patients with and without brain metastases. In all the analyses, a P value of 0.05 was considered statistically significant. To identify predictive factors for brain metastases, a multivariate logistic regression model was generated. All factors reaching significance at the 0.05 level in a univariate analysis were included in a multivariate model. Disease-free survival (DFS) was measured from the time of the initial diagnosis of breast cancer. Patients with metastatic breast cancer at the time of the initial diagnosis were included in the "within 24 months" DFS group. The time intervals from the start of trastuzumab therapy to the diagnosis of brain metastasis and from the initial diagnosis of breast cancer to the diagnosis of brain metastasis were also calculated. Overall survival (OS) was measured from the diagnosis of breast cancer recurrence or metastatic breast cancer until death or the last follow-up date, using the Kaplan-Meier method, and was compared between breast cancer patients with and those without brain metastases using the log-rank test.

Results

The median follow-up time of this cohort was 53.6 months (range, 0.9–233.2 months). Among the 204 patients with HER2-overexpressing breast cancer who were treated using trastuzumab-containing regimens, 74 patients (36.3%) developed brain metastases. The patients' characteristics are presented in Table 1. The patients with brain metastases were significantly younger than those without brain metastases (P = 0.03). Similarly, significant associations were observed between brain metastases and the number of metastatic sites (P = 0.01), liver metastases (P = 0.004) and bone metastases (P = 0.007). Brain metastases were not associated with the ER or PR statuses, the histological grade, the pathological nodal status, or the tumor size. The use of chemotherapeutic regimens containing anthracycline or taxane before the development of brain metastasis also was not significantly different between the breast cancer patients with and those without brain metastases. No significant association was seen between the disease-free interval and brain metastasis. Table 2 shows the results of a multivariate logistic regression analysis. Patient age at the initial diagnosis (≤50 years; hazard ratio, 1.92; 95% confidence interval [CI], 1.03–3.57; P = 0.04), recurrent breast cancer (hazard ratio, 2.51, 95% CI, 1.16–5.43, P = 0.02) and liver metastases (hazard ratio, 2.10, 95% CI, 1.02–4.34, P = 0.04) were significant predictors of brain metastases. Bone

Table 1. Patient characteristics

	BM+ (%)	BM− (%)	*P* value
Age (years)			
≤50	45 (61)	58 (45)	0.03
>50	29 (39)	72 (55)	
Recurrence or stage IV at diagnosis			
Recurrence	61 (82)	91 (70)	0.05
Stage IV	13 (18)	39 (30)	
Number of metastases			
≧3	28 (38)	28 (22)	0.01
<3	46 (62)	102 (78)	
Sites of metastases at start of trastuzumab therapy			
Liver	32 (43)	31 (24)	0.004
Lung	27 (36)	36 (28)	0.19
Bone	40 (54)	45 (35)	0.007
Lymph	38 (51)	80 (62)	0.16
Pathological LN (n = 147)			
0	17 (28)	19 (22)	0.30
1–3	19 (32)	22 (25)	
4–9	10 (17)	26 (30)	
10≤	14 (23)	20 (23)	
Pathological T (n = 144)			
T0,1, 2	39 (67)	67 (78)	0.15
T3, 4	19 (33)	19 (22)	
Hormone receptor status (n = 204)			
ER+	24 (32)	47 (36)	0.59
PR+	30 (41)	57 (44)	0.70
ER+ or PR+	40 (54)	67 (52)	0.73
Histological grade (n = 178)			
2	13 (21)	22 (19)	0.75
3	49 (79)	94 (81)	
Chemotherapy regimen before development of brain metastases			
Taxane	67 (91)	118 (91)	0.96
Anthracycline	24 (32)	53 (41)	0.24
Disease-free interval (n = 204)*			
≧24 Months	31 (42)	44 (34)	0.25
<24 Months	43 (58)	86 (66)	

*Stage IV breast cancer patients at initial diagnosis were included in the "<24-month group"

Table 2. Multivariate logistic regression analysis of risk factors for brain metastases

	Hazard ratio (95% CI)	*P* value
Age (years)		
≤50	1.92 (1.03–3.57)	0.04
>50		
Disease type		
Recurrence	2.51 (1.16–5.43)	0.02
Stage IV		
Liver metastases		
+	2.10 (1.02–4.34)	0.04
−		
Bone metastases		
+	1.80 (0.94–3.45)	0.07
−		
Number of metastases		
≧3	1.54 (0.69–3.42)	0.29
<3		

metastases tended to be a predictive factor for brain metastasis, but the trend was not significant (P = 0.07).

The median survival period after the diagnosis of brain metastases was 13.5 months (95% CI, 12.2–14.7 months). Among the 74 patients with brain metastases, 68 patients (92%) underwent radiation therapy to treat their brain metastases. Eight patients also underwent metastatic brain tumor resection before radiation therapy. The remaining 6 patients (8%) who did not receive radiation therapy for their brain metastases experienced the aggressive progression of either their brain metastases or other metastases. In 36 (49%) of the 74 patients with brain metastases, extracranial metastases had also progressed at the time when the symptomatic brain metastases were detected. Only fourteen patients (19%) maintained a complete or partial response to systemic therapy. After the appearance of symptomatic brain metastases, 56 (76%) of the 74 patients with brain metastases received further systemic therapy, with a median of one more systemic therapeutic regimen (range, 0–5). In particular, 26 (72%) of the 36 patients with progressive extracranial disease received systemic therapy, and 21 patients (58%) achieved disease control of the extracranial metastases for at least 1 month.

The median time interval between the beginning of trastuzumab therapy and the development of the brain metastases was 13.6 months (range, 0.0–45.8 months). The median time interval between the diagnosis of recurrent or metastatic disease and the development of brain metastases was 19.6 months (range, 0.0–68.5 months). In patients with brain metastases, the median OS was significantly shorter than that in patients without brain metastases (median, 39.0 months; 95% CI, 27.9–50.1 vs 48.1, 95% CI, 38.0–58.1;

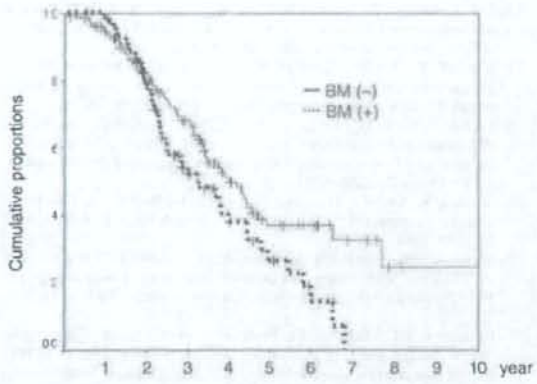

Fig. 1. Overall survival in patients with and without brain metastases (*BM*)

$P = 0.04$). Figure 1 shows the Kaplan-Meier survival curves in patients with and without brain metastases.

Discussion

In our study, patients with HER2-overexpressing breast cancer had a high incidence of brain metastases (36.3%), and this finding was compatible with those of previous reports.[9–15] Clayton et al.[10] reported that 25% of patients treated with trastuzumab developed brain metastases. Although several studies reported that hormone negativity was significantly associated with the development of brain metastases,[10,16,17] our study did not demonstrate any correlation between hormone status and brain metastasis. Age and liver metastases were significantly associated with brain metastases. Younger patients with breast cancer usually have more aggressive tumors. The association between liver and brain metastases suggests that large tumor burdens contribute to the development of hematogenous metastases and supports the result in the present study that breast cancer in half of the patients was refractory to systemic chemotherapy. Lai et al.[11] also revealed that patient age at diagnosis, liver metastases, and a positive lymph node status at presentation were significant predictors of CNS metastases.

Patients with brain metastases had a shorter survival period than those without brain metastases and the median survival period from the diagnosis of brain metastases was 13.5 months, which was comparable with data in previous reports.[14,17] In our study, 51% of the patients with brain metastases had maintained stable extracranial metastases at the time of diagnosis of their brain metastases. This dissociation arises from the fact that trastuzumab and cytotoxic agents cannot sufficiently penetrate the blood-brain barrier to control brain metastases. Furthermore, although the remaining 49% of patients with brain metastases exhibited progressive extracranial metastatic disease, three-fourths of them had a general condition that allowed further systemic therapy, from which most of them benefited. Therefore, although many brain metastases seem to appear later in the clinical course in patients with breast cancer, extracranial disease is still more or less chemosensitive, and controlling brain metastases has an important effect on prognosis.

In our study, HER2-overexpressing breast cancer patients seemed to develop symptomatic brain metastases about one and a half years after the diagnosis of recurrent or metastatic breast cancer, leading to the question of whether surveillance for brain metastasis should be performed. If surveillance is to be carried out, the candidate population and timing of the surveillance should be investigated. To date, no evidence suggesting the usefulness of brain metastasis surveillance in patients with breast cancer has been obtained, from the viewpoints of both a survival benefit and cost-effectiveness.[18] Miller et al.[9] reported that survival among patients with occult CNS metastasis was similar to that of patients with symptomatic CNS disease, demonstrating that the earlier detection of brain metastases had no benefit for survival.

Recently, antitumor drugs that appear to be effective for the treatment of brain metastases in breast cancer patients have been developed. For example, lapatinib is an orally active small molecule that inhibits the tyrosine kinases of HER2 and epidermal growth factor receptor (EGFR) type 1. Lin et al.[20] reported that among 38 patients with HER2-overexpressing breast cancer, 2 patients (5%) achieved a partial response and 8 patients had stable disease in the CNS at 16 weeks in the lapatinib arm of a phase II study. Optimal chemotherapy regimens for HER2-overexpressing breast cancer patients with brain metastases must be examined, and whether a survival benefit exists when treatment with drugs effective against brain metastases is initiated prior to the development of symptomatic brain metastases in HER2-overexpressing breast cancer patients should be investigated.

In conclusion, the breast cancer patients with HER2-overexpression in this study had a high incidence of brain metastases and a poor prognosis due to the appearance of symptomatic brain metastases. Risk factors for brain metastases were a younger age, recurrent disease, and liver metastases. We intend to continue examining optimal chemotherapy regimens for patients with brain metastases and the necessity of screening for brain metastases in patients who have the risk factors of younger age (≤50 years), recurrent disease, or liver metastases.

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original article

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CD5 expression is potentially predictive of poor outcome among biomarkers in patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP therapy

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Background: Several biomarkers indicating poor prognosis have been reassessed in patients receiving rituximab combination chemotherapy for diffuse large B-cell lymphoma (DLBCL). However, few studies have investigated outcome in relation to a combination of these biomarkers. In addition, no large-scale studies have reassessed the outcome of patients with CD5-positive DLBCL treated with rituximab.

Patients and methods: We conducted a retrospective study and investigated the predictive value of three biomarkers—BCL2, germinal center (GC) phenotype and CD5—in 121 DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone.

Results: CD5-positive patients showed significantly poorer event-free survival (EFS) and overall survival (OS) than CD5-negative patients (2-year EFS, 18% versus 73%, $P < 0.001$; 2-year OS, 45% versus 91%, $P = 0.001$). However, no significant difference in outcome according to BCL2 or GC phenotype was observed. Multivariate analysis revealed that CD5 expression was a significant prognostic factor for EFS [hazard ratio 14.2, 95% confidence interval (CI) 4.7–43.2] and OS (hazard ratio 20.3, 95% CI 3.6–114.4).

Conclusions: CD5 expression was the only significant prognostic factor among the biomarkers examined in this study. Further studies with larger numbers are warranted to confirm the prognostic significance of CD5 expression for patients with DLBCL receiving rituximab-containing chemotherapy.

Key words: biomarker, CD5, diffuse large B-cell lymphoma, rituximab

original article

introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma (NHL) [1]. It shows an aggressive clinical course and comprises a heterogeneous group of lymphomas in terms of morphology, immunophenotype, molecular abnormality and clinical behavior. Although the cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) regimen has been the mainstay of treatment for aggressive lymphomas for several decades [2], a significantly improved outcome has been obtained in both young and elderly patients by combining the CHOP regimen with rituximab (an anti-CD20 chimeric antibody) [3–5].

In the era when CHOP was used alone, the International Prognostic Index (IPI) was the primary clinical tool employed for prediction of outcome in patients with aggressive NHL [6]. Although the IPI is considered to be the most important prognostic factor for DLBCL, the five risk factors used for assessing it do not provide any information about biologic features. To date, several biomarkers have been shown to predict the outcome and responsiveness of DLBCL to therapy. Overexpression of BCL2 family proteins has also been shown to indicate resistance to chemotherapy both *in vitro* and *in vivo* [7, 8]. BCL6 family proteins are reportedly associated with a better prognosis, and patients with BCL6-positive DLBCL have a relatively favorable outcome when treated with the CHOP regimen [9]. On the other hand, it has been reported that CD5-positive DLBCL has a very poor prognosis and high stage with more extranodal sites in comparison with CD5-negative DLBCL [10, 11]. Moreover, on the basis of the data obtained using complementary DNA (cDNA) microarray, DLBCL has been divided into two distinct subtypes that reflect the different stages of B-cell differentiation, i.e. germinal center

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B-cell-like (GCB) and activated B-cell like (ABC) [12]. The ABC subtype is associated with a poorer prognosis than the GCB subtype. It has been reported that the immunostaining patterns of CD10, BCL6 and MUM1 are an alternative means of identifying germinal center (GC) or non-GC DLBCL including the ABC subtypes and that non-GC DLBCL shows poor responsiveness to anthracycline-based regimens [13].

Recently, it has been recognized that addition of rituximab to anthracycline-based regimens may alter the previously identified prognostic factors, in view of the markedly improved outcome of patients with DLBCL. The study from British Colombia demonstrated that the IPI remained predictive, but reclassified patients into three prognostic groups after reassessing the five prognostic factors [14]. Moreover, several studies have investigated whether these biomarkers predict responsiveness to rituximab combination chemotherapy and outcome. The prognosis of BCL2- or BCL6-overexpressing DLBCL and GC phenotype has been reassessed in patients receiving rituximab combination chemotherapy [15–17]. On the other hand, no large-scale studies of CD5 expression in the rituximab era have been reported.

Although several studies analyzing the prognostic significance of individual biomarkers have been carried out since the introduction of rituximab, none have investigated outcome by considering these biomarkers together. The aims of the present study were to reassess the predictive values of these biomarkers at a single institution and to investigate which factor among BCL2 expression, GC phenotype and CD5 expression has the greatest influence on the outcome of DLBCL patients.

patients and methods

patient characteristics

In the present study, we reviewed the medical records of patients with CD20-positive DLBCL who received CHOP with or without rituximab as a first-line therapy at the Cancer Institute Hospital from April 2004 to May 2007 and were followed until January 2008. The study protocol and sampling were approved by the Institutional Review Board of the Cancer Institute Hospital. Informed consent for retrospective analysis and additional immunophenotypic analysis and gene rearrangement studies was obtained.

Patients were analyzed if they were older than 18 years and had a performance status (PS) of zero to three according to the criteria of the European Cooperative Oncology Group. Patients were excluded if they had clinically relevant cardiac diseases or positivity for antibodies against human immunodeficiency virus-1 or -2. Patients with primary mediastinal large B-cell lymphoma, primary central nervous system lymphoma and primary testicular lymphoma were also not included in this study.

The disease stage was evaluated according to the Ann Arbor staging system. All patients had undergone staging investigations, including physical examinations, blood and serum analysis, bone marrow aspiration and biopsy and computed tomography of the neck, chest, abdomen and pelvis. Magnetic resonance imaging was used for evaluation of involved organs in the head and neck. The following clinical and laboratory data were available at the time of diagnosis: age, sex, serum lactate dehydrogenase level, PS, presence of B symptoms, clinical stage and number of extranodal sites. This information allowed IPI scores to be determined in the included patients. Patients were categorized into either a low-risk group (IPI score, 0–2) or a high-risk group (IPI score, 3–5).

treatment

All patients received rituximab plus CHOP (RCHOP) chemotherapy. For patients with stage IB–IV, rituximab was administered at the standard dose of 375 mg/m^2 once weekly for 8 weeks and CHOP chemotherapy was given concurrently triweekly, as described previously [18]. CHOP chemotherapy was given for a total of six cycles. For patients with stage IA, CHOP chemotherapy was repeated for three cycles and rituximab was continued in the same way as for patients with stages IB–IV, with subsequent radiotherapy.

pathological studies

Biopsy samples collected at the time of diagnosis were fixed in formalin, embedded in paraffin, sliced and stained with hematoxylin and eosin for morphological analysis. Immunohistochemical analysis was carried out using the dextran-polymer method (EnVision+; Dako, Glostup, Denmark) using mAbs against CD10 (56C6, Novocastra, Newcastle-upon-Tyne, UK), BCL6 (PG-B6p, Dako), MUM1 (MUM1p, Dako), BCL2 (124, Dako), CD5 (4C7, Novocastra) and cyclin D1 (P2D11F11, Novocastra) at our institution. For all the antibodies, heat-induced antigen retrieval pretreatment using Target Retrieval Solution, pH. 9 (Dako) was carried out. BCL6, MUM1 and BCL2 were designated as positive when the proportion of stained lymphoma cells was 30% or higher. CD5 and CD10 were considered to be immunohistochemically positive when at least a small population of the neoplastic cells was positive. To classify the samples into immunohistochemically defined GC or non-GC phenotypes, we used an algorithm previously described by Hans et al. [13].

For examination of CD5 expression, we reviewed the results of flow cytometry analysis. Cases were defined as CD5 positive if CD5 expression was detected by flow cytometry, irrespective of the result of CD5 immunohistochemistry. Excluded were those positive for cyclin D1 or those with a history of chronic lymphocytic leukemia/small lymphocytic lymphoma. Patients with a small-cell component implying transformation from low-grade/indolent B-cell lymphoma were also excluded. All the histopathology samples were reviewed by an expert hematopathologist (KT), and flow cytometric analyses were reviewed by two of the authors independently (DE and KT).

statistical analysis

The main outcomes of this study were event-free survival (EFS) and overall survival (OS). EFS was calculated from the date of diagnosis to the date of documented disease progression, relapse or death from any cause or to the date on which the study was stopped. OS was calculated from the date of diagnosis until death from any cause or the last follow-up. If the stopping date was not reached, the data were censored at the date of the last follow-up evaluation. Survival curves were estimated by the Kaplan–Meier method, and overall differences were compared by the log-rank test. Cox multivariate analysis was carried out to estimate the prognostic impacts of the biomarkers and IPI risk factors on EFS and OS. Comparisons of basic characteristics between the CD5-positive and -negative groups were tested by Fisher's exact test and Student's *t*-test. Data were analyzed using SPSS software version 11.0 for Windows (SPSS, Chicago, IL).

results

patient characteristics

During the study period, 180 patients were included, and data for all three biomarkers and flow cytometric analysis were available for 121 patients. The characteristics of these patients are listed in Table 1. CD5 was expressed in 11 of 121 patients with DLBCL (9%). None of the CD5-positive patients

Table 1. Patient characteristics

Clinical parameter	Frequency (%)
Sex	
Male	65 (54)
Female	56 (46)
Age	
Median, range	66, 23–88
≤60	37 (31)
>60	84 (69)
Stage	
1–2	85 (70)
3–4	36 (30)
Performance status	
0–1	104 (86)
2–4	17 (14)
Lactate dehydrogenase	
Normal	65 (54)
High	56 (46)
No. of extranodal sites	
0–1	95 (79)
2–4	26 (21)
International Prognostic Index score	
0–2	89 (74)
3–5	32 (26)
BCL2	
Positive	79 (65)
Negative	42 (35)
GC phenotype	
GC type	73 (60)
Non-GC type	48 (40)
CD5	
Positive	11 (9)
Negative	110 (91)

GC, germinal center.

had a history of other lymphoproliferative disorders, and all were found to have *de novo* CD5-positive DLBCL. Of these 11 patients, seven were positive by both flow cytometry and immunohistochemistry and four were positive only by flow cytometry. In all the seven cases defined as CD5 positive by both methods, the lymphoma cells expressed less CD5 than normal T cells in the background. Expression of BCL2 was detected in 79 of 121 cases (65%). CD10 was expressed in 45 cases (37%), BCL6 in 89 (66%) and MUM1 in 55 (45%). Overall, 48 of 121 cases (40%) were categorized into the non-GC group. No significant difference in basic characteristics was found between the 121 and 59 patients for whom all biomarkers were and were not available, respectively. No patients had central nervous system or testicular lesions.

survival analysis

The 2-year OS was 85% and EFS was 79% with a median follow-up of 28 months. We compared the survival curves in accordance with the expression of the three biomarkers. The Kaplan–Meier method revealed that the EFS rates at 2 years were 76% for BCL2-positive patients and 91% for BCL2-negative patients. The corresponding OS rates were 77% and 97%, respectively. Although both survival rates were inferior in BCL2-positive patients, the differences did not reach statistical significance ($P = 0.080$ and $P = 0.060$, respectively, log-rank test). Similarly, the EFS and OS rates at 2 years were 70% and 77%, respectively, for non-GC patients and 90% and 91%, respectively, for GC patients, there being no significant differences in these parameters between the two groups ($P = 0.080$ and $P = 0.120$, respectively). The IPI score at the baseline did not differ significantly according to BCL2 or GC phenotype. On the other hand, the differences in the EFS and OS rates between CD5-positive and CD5-negative patients were significant (EFS, 18% versus 73%, $P < 0.001$; OS, 45% versus 91%, $P = 0.001$) (Figure 1A and B).

For comparison with the biomarkers, we compared the survival curves according to the IPI. The EFS rates at 2 years were 52% for high and high-intermediate IPI and 91% for low and low-intermediate IPI. The OS rates were 64% and 92% for the high and low IPI groups, respectively. The differences in the EFS and OS rates were significant ($P = 0.001$ and $P = 0.010$, respectively) (Figure 1C and D).

The clinical and biological features in relation to CD5 expression are summarized in Table 2. Among the 11 patients who were CD5 positive, the primary site was extranodal in five (bone in two and intestine, thyroid and nasal cavity in one case each). No patient had bone marrow involvement. Significantly more CD5-positive than -negative patients had a poor PS ($P = 0.01$). Although no other significant differences were detected in the distributions of the other patient characteristics, CD5-positive patients were more frequently BCL2 positive ($P = 0.095$).

To further investigate the prognostic impact of CD5 expression, Cox multivariate analysis was carried out adjusted for the IPI categorization. As shown in Table 3, CD5 expression had significant prognostic value for both EFS [hazard ratio 14.2, 95% confidence interval (CI) 4.7–43.2; $P < 0.001$] and OS (hazard ratio 20.3, 95% CI 3.6–114.4; $P = 0.001$). The prognostic significance of CD5 remained even after adjustments by BCL2 expression or GC/non-GC categorization.

discussion

This analysis of biomarkers in 121 DLBCL patients receiving RCHOP highlighted the potentially poor outcome of patients with CD5-positive DLBCL. Multivariate analysis including the IPI revealed that CD5 expression and IPI were independent factors associated with poor prognosis. On the other hand, significant differences in survival were not detected in relation to BCL2 and immunohistochemically defined GC phenotype.

De novo CD5-positive DLBCL, a distinct subgroup that accounts for 5%–10% of all DLBCL, has been reported to be associated with elderly onset, female predominance, frequent involvement of extranodal sites and inferior survival [10, 11]. The largest study of CD5-positive DLBCL demonstrated a 5-year survival rate of 34% for CD5-positive DLBCL treated with an anthracycline-based regimen [11]. The Nordic Lymphoma Study Group also demonstrated that CD5 expression was associated with significantly inferior OS and failure-free survival [19]. In contrast, other authors showed that

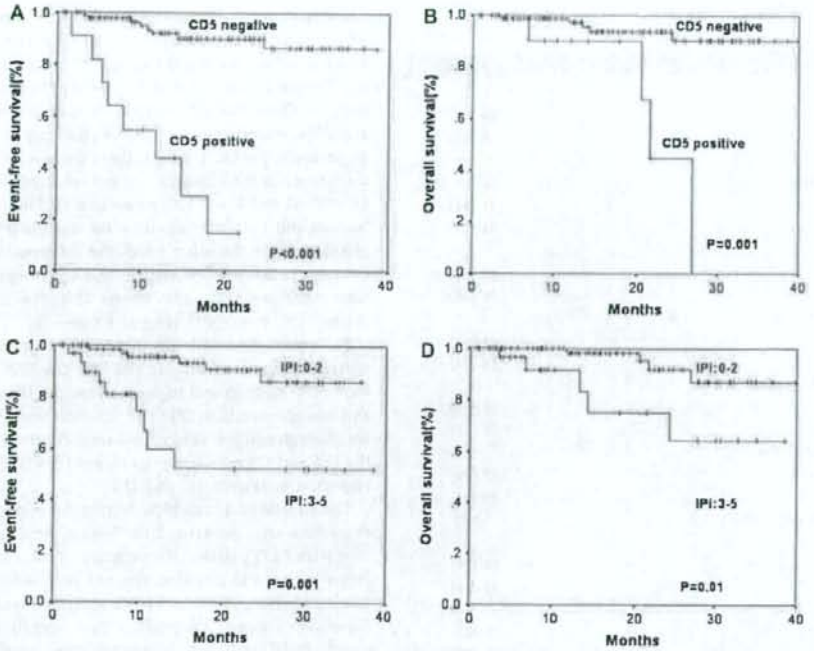

Figure 1. Event-free survival (EFS) and overall survival (OS) curves for diffuse large B-cell lymphoma patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone according to CD5 expression and clinical factors. EFS (A) and OS (B) curves according to positive ($n = 11$) versus negative ($n = 110$) CD5 expression. EFS (C) and OS (D) curves according to the IPI (0–2, $n = 89$ versus 3–5, $n = 32$).

Table 2. Patient characteristics in relation to CD5 expression

Characteristic	CD5 positive ($n = 11$), n (%)	CD5 negative ($n = 110$), n (%)	P
Sex: male	6 (55)	59 (53)	1.0
Age: median, range	68, 33–76	66, 23–88	0.27
IPI score 3–5	4 (36)	28 (25)	0.47
Stages III–IV	4 (36)	32 (30)	0.74
Elevated LDH level	7 (64)	49 (44)	0.54
More than one extranodal site	5 (45)	22 (20)	0.38
PS >1	5 (45)	12 (11)	0.013
BCL2 positive	10 (91)	69 (62)	0.095
Non-GC type	6 (55)	42 (38)	0.54

IPI, International Prognostic Index; LDH, lactate dehydrogenase; PS, performance status; GC, germinal center.

CD5-positive DLBCL did not show distinctive clinical features or inferior survival [20]. In the present study, patients who received immunochemotherapy showed significantly poor OS and EFS, whereas the factors comprising the IPI were similar between the patients who were positive for CD5 and those who were negative. We consider that this poor prognosis of CD5-positive DLBCL in the rituximab era is noteworthy and that a large-scale study is warranted.

CD5 is a 67-kDa transmembrane glycoprotein that is expressed by most normal T cells and less brightly by a subset of B cells known as B1 cells [21]. Reflecting this difference in expression-level neoplastic CD5-positive B cells also usually express less CD5. Therefore, even if successfully stained by immunohistochemistry, these cells are usually stained less strongly than normal background T cells with anti-CD5 antibody. In the authors' experience, until the introduction of antigen retrieval techniques and effective antibodies like mAb 4C7, it was very difficult to detect CD5-positive B cells immunohistochemically on formalin-fixed paraffin-embedded sections [22]. However, even since the introduction of these techniques, CD5 immunohistochemistry using paraffin sections still remains less sensitive than flow cytometry and frozen section immunohistochemistry [23]. In fact, in the present study, only seven cases of DLBCL were positive for CD5 by immunohistochemistry out of 11 cases that were CD5 positive by flow cytometry. In an attempt to overcome this lower sensitivity of CD5 immunohistochemistry for CD5-positive DLBCL, de Jong et al. examined the usefulness of recently developed immunohistochemical enhancement techniques (Powervision; Immunovision Technologies, Duiven, The Netherlands and ChemMate; Dako). However, although they acquired higher sensitivity, there was also a loss of

Table 3. Cox multivariate analysis for EFS and OS

Variable	Unfavorable	HR	95% CI	P
EFS				
CD5	Positive	14.2	4.7–43.2	<0.001
IPI	3–5	7.6	2.5–22.8	<0.001
OS				
CD5	Positive	20.3	3.6–114.4	0.001
IPI	3–5	10.5	1.9–56.8	0.006

HR, hazard ratio; CI, confidence interval; EFS, event-free survival; IPI, International Prognostic Index; OS, overall survival.

reproducibility due to the unacceptable level of background staining [24]. Taken together, CD5 is usually expressed weakly by a subset of normal and neoplastic B cells, and CD5 paraffin immunohistochemistry is less sensitive for these B cells. For these reasons, we consider that flow cytometric analysis or frozen section immunohistochemistry needs to be carried out for detection of CD5 in DLBCL. Differences in the method of CD5 detection might lead to differences among studies in the apparent impact of CD5 on prognosis.

In addition to these differences in clinical aspects, there are several lines of evidence for genetic differences between CD5-positive and -negative DLBCL. Microarray studies have suggested that integrin beta-1 in tumor cells and CD36 in vascular endothelium are expressed more frequently in CD5-positive than in CD5-negative DLBCL [25]. Comparative genomic hybridization studies have revealed that CD5-positive DLBCL has a different pattern of chromosomal gain and loss compared with CD5-negative DLBCL [20, 26]. Loss of 9q21 (*p16 INK4a*), which is strongly associated with lymphoma progression, has been observed more frequently in CD5-positive DLBCL [27].

Previous studies also showed that IPI values remained in patients with DLBCL receiving immunochemotherapy [14–17]. In some studies, IPI category was divided into two risk groups—low or low intermediate and high or high intermediate—and this remained a predictive tool in DLBCL patients receiving immunochemotherapy [15–17]. Other authors have reassessed the IPI risk factors of DLBCL patients treated with RCHOP and divided them into three distinct prognostic groups referred to as R-IPI groups. Significant differences were also demonstrated in the same cohort upon division into two risk groups [14]. In the present study, IPI values were used to delineate two risk groups, and prognostic values were retained, although we did not evaluate each of the IPI risk factors. A consensus will be required for accurate handling of these risk factors in the rituximab era.

BCL2 overexpression was associated with poorer survival in the prerituximab era [7, 8]. In contrast, several studies conducted in the rituximab era demonstrated that addition of rituximab to chemotherapy eliminated the prognostic significance of BCL2 overexpression in DLBCL [15]. However, these studies did not reveal any data on the association between CD5 expression and BCL2 overexpression. Moreover, in previous studies of CD5-positive DLBCL, no association between CD5 expression and BCL2 overexpression in patients with DLBCL was demonstrated [10, 11]. The present study demonstrated that 10 of 11 CD5-positive patients had BCL2 overexpression. The OS and EFS of BCL2-positive, CD5-negative patients were significantly superior to those of patients positive for both BCL2 and CD5 (data not shown), suggesting that the poorer survival trend of patients with BCL2 overexpression in the present series may have been influenced by CD5 expression. A large-scale analysis of BCL2 expression in CD5-positive DLBCL will be needed to clarify the association between expressions of BCL2 and CD5.

There have been several studies of the relationship between CD5 expression and GC/ABC phenotype [27–29]. An analysis of genomic imbalance showed that most cases of CD5-positive DLBCL were included in the ABC type [27], and another study of somatic mutations of the immunoglobulin heavy chain variable region suggested that the cells from which CD5-positive DLBCL arise are predominantly of post-GC origin [28, 29]. These conclusions were based on molecular-based analyses and not by immunohistochemistry. Our study found no association between CD5 expression and GC phenotype. This may be because GC phenotype in the present study was defined by an immunophenotypic algorithm, which reproduced ~80% of the GC phenotype defined by cDNA microarray [13]. A new algorithm using five types of immunostaining—GCET1, MUM1, CD10, BCL6 and FOXP1—has been introduced recently and provided an improved GC/ABC subclassification [30]. Application of this approach to our series might lead to a consistent result.

In conclusion, we have investigated the outcome of DLBCL patients receiving rituximab combination chemotherapy by considering several biomarkers together and demonstrated that CD5 expression is a potentially useful indicator of poor prognosis. To accurately confirm whether CD5 expression influences the outcome of patients receiving RCHOP, further large-scale and prospective studies of CD5-positive patients will be required.

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ORIGINAL ARTICLE

Chronic obstructive pulmonary disease and interstitial lung disease in patients with lung cancer

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ABSTRACT

Background and objective: **Although lung cancer is frequently accompanied by COPD and interstitial lung disease (ILD), the precise coincidence of these diseases with lung cancer is not well understood. The objectives of this study were to determine the prevalence of abnormal CT and spirometric findings suggestive of COPD or ILD in a population of patients with untreated lung cancer, and to estimate the lung cancer risk in this population.**

Methods: **The study population consisted of 256 patients with untreated lung cancer and 947 subjects participating in a CT screening programme for lung cancer. Semi-quantitative analysis of low attenuation area (LAA), fibrosis and ground glass attenuation (GGA) on CT was performed by scoring. Gender- and age-matched subpopulations, with stratification by smoking status, were compared using the Mantel–Haenszel projection method.**

Results: **Inter-observer consistency was excellent for LAA, but not as good for fibrosis or GGA scores. Pooled odds ratios for lung cancer risk using LAA, fibrosis, GGA scores and reduced FEV_1/FVC and %VC were 3.63, 5.10, 2.71, 7.17 and 4.73, respectively ($P < 0.0001$ for all parameters). Multivariate regression analyses confirmed these results.**

Conclusion: **Abnormal CT and spirometric parameters suggestive of COPD and ILD were strong risk factors for lung cancer, even after adjusting for gender, age and smoking status.**

Key words: COPD, interstitial lung disease, lung cancer, radiology, tobacco.

SUMMARY AT A GLANCE

The aim of this study was to determine the frequency of abnormal spirometric and CT findings in lung cancer patients, and whether COPD and interstitial lung disease comorbidity was solely due to smoking. Increased lung cancer risk was associated with airflow limitation and abnormal CT findings after controlling for age, gender and smoking status.

INTRODUCTION

Tobacco is the most significant common risk factor for lung cancer and COPD,[1,2] and possibly also for interstitial lung disease (ILD),[3–6] resulting in a high prevalence of comorbidity for these diseases.[7–15] In lung cancer complicated by COPD and/or ILD, the use of standard lung cancer therapy is sometimes not possible due to reduced pulmonary function and the risk of fatal adverse events with chemotherapeutic agents[11] or thoracic irradiation.[16]

Despite the clinical significance, only a few prospective studies have evaluated the prevalence of COPD among lung cancer patients,[12] although there have been many retrospective evaluations of the frequencies of COPD[7,9,10] and ILD[8,11] among patients with lung cancer. Most studies have been based on a clinical diagnosis of COPD or ILD, with the exception being that of Niho *et al.*,[11] which evaluated ILD on the basis of CT findings. In the present case–control study, the frequencies of COPD and ILD, as assessed by CT findings and spirometry, were determined in a population of consecutive patients with previously untreated primary lung cancer. The results were compared with those from a control population, consisting of consecutive subjects who participated in a CT lung cancer screening programme.

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METHODS

Study design and subjects

This was a case–control study to determine the prevalence of COPD and ILD in patients with lung cancer compared with healthy control subjects. The study population consisted of two groups: patients with untreated lung cancer (lung cancer group) and control individuals who had participated in a CT screening programme for lung cancer (control group). For the lung cancer group, consecutive patients with a definitive diagnosis of untreated primary lung cancer, admitted to the Departments of Respirology and Thoracic Surgery, Chiba University, during the period 15 June 2005 to 15 June 2007, were enrolled. For the control group, all participants in the lung cancer screening programme at Makuhari Clinic, Kameda General Hospital during the period 1 October 2002 to 30 September 2003 were enrolled. Subjects proven to have lung cancer through the screening programme were excluded. Subjects with a history of thoracic surgery or known bronchial asthma were excluded from both groups. There was no communication between the two groups. All subjects gave written informed consent and the study was approved by the institutional review boards of the Graduate School of Medicine, Chiba University and Makuhari Clinic, Kameda General Hospital.

CT examination and spirometry

The technical parameters for CT examination without contrast-medium enhancement were 1.375 of beam pitch, 1.25 mm of collimation and reconstruction slice thickness, 120 kV and 110 mA using a 16-row multi-detector spiral CT (Light Speed, GE Healthcare, Milwaukee, WI, USA) for the lung cancer group, and 1.375 of beam pitch, 2.0 mm of collimation and reconstruction thickness, 120 kV and 50 mA using a 4-row multi-detector spiral CT (Aquilion, Toshiba, Tokyo, Japan) for the control group. All CT and spirometry assessments in the lung cancer group were performed prior to any cancer treatment.

Review and evaluation of CT images

Semi-quantitative evaluations of low attenuation area (LAA) for COPD, and fibrosis and ground glass attenuation (GGA) for ILD were performed using the scoring methods proposed by Goddard *et al.*[17] and Kazerooni *et al.*,[18] respectively. Briefly, CT images in three slices, at the upper edge of the aortic arch, the bifurcation of the trachea and 1 cm beyond the upper edge of the right hemi-diaphragm, were reviewed at 1000 and −700 Hounsfield Units (HU) of window width and level, respectively, for scoring LAA. The score consisted of five grades according to the extent of LAA in each slice: no LAA in the slice, grade 0; LAA < 25% of the slice field, grade 1; LAA 25–50%, grade 2; LAA 50–75%, grade 3; and LAA > 75%, grade 4. The total score was calculated by summing all slice scores for the three slices and scoring the right and left sides separately, resulting in total scores ranging from 0 to 24.

The scoring methods for fibrosis and GGA were similar to that for LAA, with some variations. The images were reviewed at 1300 and −500 HU of window width and level, respectively, in the three slices at the same level as for LAA. The scores for fibrosis and GGA consisted of six grades. In the fibrosis score, grade 0 represented no fibrosis in the slice, grade 1 indicated interlobular thickening without honeycombing, grades 2, 3, 4 and 5 indicated areas of honeycombing extending over < 25%, 25–50%, 50–75% and > 75% of the slice, respectively. In the GGA score, grade 0 represented no GGA in the slice, grades 1, 2, 3, 4 and 5 indicated areas of GGA extending over < 5%, 5–25%, 25–50%, 50–75% and > 75% of the slice, respectively. Therefore, the total scores for fibrosis and GGA ranged from 0 to 30.

Although the original method of Kazerooni *et al.*[18] assigned a score for each pulmonary lobe by estimating the score according to the slice level, in the present study, the total score according to slice level (modified method) and the total score according to the lobe, as originally proposed, were documented. All CT images were independently reviewed by four investigators (S.M., Y.T., A.F. and K.M.). The reviewers were not blind to the study groups because of the different CT parameters used. The mean of the total scores, as assessed by the four reviewers, was taken to represent the score for each individual.

Statistical methods

The statistical significance of differences in the frequencies of COPD and ILD between the two groups was determined by the chi-square test. The significance of differences in age and pack-years of smoking were determined by the Mann–Whitney test. To evaluate the inter-observer validity of CT score findings, the intra-class correlation coefficient was used. Spearman's correlation coefficient was used to evaluate the correlation between parameters. For gender- and age-matched comparisons of the lung cancer and control groups, two corresponding subpopulations were randomly extracted, one from each group, at a 1 : 3 ratio, matching gender in each 10-year age category. The resulting pairs of subgroups were stratified into three smoking status categories: current-smoker, ex-smoker and never smoker. Odds ratios for each category were calculated using 2×2 contingency table analyses. Finally, odds ratios for lung cancer risk based on each CT parameter and on spirometric data were calculated using the Mantel–Haenszel projection method that allows data from several groups to be combined while avoiding confounding. Multivariate regression analyses were also performed for variables that were shown to be statistically significant risk factors for lung cancer by univariate analyses on the total population, and gender- and age-matched subpopulations. Differences with a two-tailed P-value < 0.05 were regarded as statistically significant. All

Table 1 Demographic and clinical characteristics, spirometry and CT findings for subjects in the lung cancer and control groups

	Group		
	Control	Lung cancer	*P*-value
Number enrolled	947	256	—
Number evaluated by spirometry	813	245	—
Gender (M : F)	718 : 229	182 : 74	0.124[†]
Median age (range)	56 (22–83)	68 (24–84)	6.2×10^{-48}[‡]
Smoking status			1.4×10^{-33}[†]
Current smoker (%)	320 (33.8)	94 (36.7)	
Ex-smoker (%)	104 (11.0)	106 (41.4)	
Never smoker (%)	522 (55.1)	55 (21.5)	
Unknown (%)	1 (0.1)	1 (0.1)	
Pack-years (median, range)	19.2 (0–36.8)	43.3 (2.5–56.3)	6.8×10^{-25}[‡]
Smoker with ≥ 20 pack-years	360 (39.0)	171 (68.4)	1.7×10^{-17}[†]
Histological type of lung cancer			
Sm : Ad : Sq : La : Oth[*]	—	23 : 158 : 49 : 9 : 17	—
c-Stage (I : II : III : IV)	—	109 : 13 : 60 : 74	—
$FEV_1/FVC < 70\%$	4.2% (34/813)	38.4% (94/245)	6.6×10^{-47}[†]
FEV_1/FVC (mean ± SD)	80.8 ± 6.8	71.1 ± 12.2	8.5×10^{-37}[‡]
VC < 80%	2.2% (18/813)	16.7% (41/245)	3.9×10^{-16}[†]
%VC (mean ± SD)	110.9 ± 16.1	98.2 ± 18.7	1.8×10^{-19}[‡]
LAA score ≥ 1	17.1% (162/947)	58.2% (149/256)	1.7×10^{-40}[†]
LAA score (mean ± SD)	0.8 ± 2.1	4.2 ± 5.4	3.0×10^{-41}[‡]
Fibrosis score ≥ 1	4.2% (40/947)	31.6% (81/256)	2.7×10^{-39}[†]
Fibrosis score (mean ± SD)	0.2 ± 0.5	1.0 ± 1.9	5.2×10^{-27}[‡]
GGA score 1	6.9% (65/947)	28.9% (74/256)	1.2×10^{-22}[†]
GGA score (mean ± SD)	0.2 ± 0.6	0.8 ± 1.4	3.8×10^{-21}[‡]

[*] Sm, small cell carcinoma; Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Oth, others.
[†] chi-square test.
[‡] Mann–Whitney test.
c-Stage, clinical stage of lung cancer; LAA, low attenuation area; GGA, ground glass attenuation.

analyses, including random sampling to generate gender- and age-matched subpopulations, were performed using SPSS version 12.0J (SPSS, Chicago, IL, USA).

RESULTS

Subject characteristics

During the defined periods, 256 patients with lung cancer and 947 control subjects were enrolled in the two study groups. Spirometry was performed in 245 of the 256 patients and in 813 of the 947 control subjects. The characteristics of the subjects are summarized in Table 1.

Inter-observer variability of LAA, GGA and fibrosis scores

Inter-observer variability among the four reviewers for the LAA score, as assessed by the intra-class correlation coefficient, was 0.918 (95% confidence interval (CI): 0.911–0.925). Both the total score according to slice level (modified Kazerooni method) and the original Kazerooni score, in which scores are totalled according to lobe, were recorded. Intra-class correlation coefficients for fibrosis were 0.681 (95% CI: 0.658–0.703) and 0.694 (95% CI: 0.672–0.716) by the original and modified Kazerooni methods, respectively. Intra-class correlation coefficients for GGA were 0.446 (95% CI: 0.416–0.476) and 0.453 (95% CI: 0.424–0.483) by the original and modified Kazerooni methods, respectively. The original and modified Kazerooni methods were strongly correlated, with Spearman's *r*-values of 0.989 ($P < 0.0001$) for fibrosis and 0.973 ($P < 0.0001$) for GGA. Because of this strong correlation, the lower inter-observer variability and the simplicity of the modified Kazerooni method, scoring based on this modified method was used in the further evaluations that were performed in the present study.

Prevalence of abnormal spirometric and CT findings

Abnormal spirometric data ($FEV_1/FVC < 70\%$, $VC < 80\%$) and CT findings (LAA score ≥ 1, fibrosis score ≥ 1, GGA score ≥ 1) are summarized in Table 1. Age and tobacco consumption were significantly

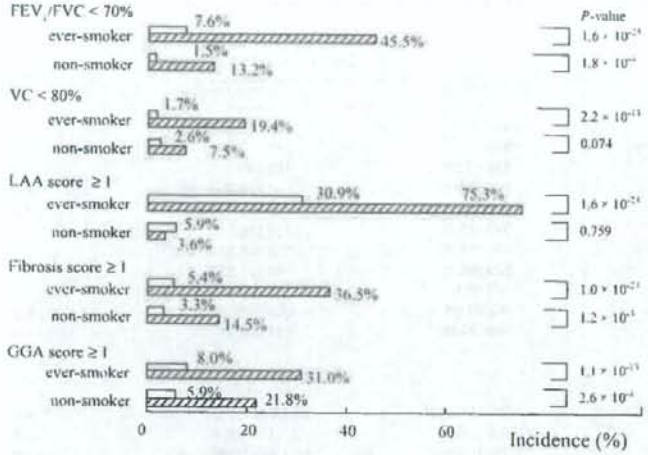

Figure 1 Prevalence of spirometric and CT abnormalities, according to smoking status, in the lung cancer and control groups. Open and shaded bars indicate prevalence in the control and lung cancer groups, respectively. Ever-smokers include current and ex-smokers. For every parameter, the lung cancer group had a significantly higher prevalence than the control group, irrespective of smoking status, with the exception that the prevalence of low attenuation area (LAA) score ≥ 1 in non-smokers was similar between the lung cancer and control groups. GGA, ground glass attenuation.

higher in the lung cancer group than in the control group. The prevalence of abnormal spirometric data and CT findings was significantly higher in the lung cancer group than in the control group (Fig. 1). The prevalence of abnormal spirometric data and CT findings, in both smoker and non-smoker subpopulations, was higher in the lung cancer group than in the control group, except for LAA ≥ 1 in non-smokers, where it was comparable between the two groups.

Risk factors for lung cancer

Gender- and age-matched subpopulations, with stratification by smoking status, were compared using the Mantel–Haenszel projection method, because the lung cancer group contained significantly higher proportions of smokers and older individuals than the control group. The characteristics of the sampled subpopulations are presented in Table 2. There was no intra-stratification difference in smoking index, as assessed by pack-years, between the lung cancer and control groups. The calculated power of the sample sizes was > 0.95 for each parameter in the total, and gender- and age-matched subpopulations. The odds ratios for lung cancer according to smoking status and the pooled odds ratios for each parameter of the spirometric and CT findings are presented in Figure 2. All the five factors analysed were significant risk factors for lung cancer, independent of gender, age and smoking status. Among them, $FEV_1/FVC < 70\%$ had the highest pooled odds ratio for lung cancer (7.17; 95% CI: 4.03–12.74).

A similar comparison was performed between patients with earlier stage (clinical stages I and II) and later stage (clinical stages III and IV) lung cancer. Again, gender- and age-matched subpopulations ($n = 93$ for each subpopulation) were compared using the Mantel–Haenszel test, with stratification for smoking status. VC < 80% was identified as a significant risk factor for advanced stage lung cancer, with a pooled odds ratio of 2.60 (95% CI: 1.11–6.08). None of the other factors was identified as a risk factor for advanced stage disease, with pooled odds ratios of 1.22 (95% CI: 0.66–2.55) for $FEV_1/FVC < 70\%$, 1.35 (0.64–2.83) for LAA score ≥ 1, 1.12 (0.60–2.10) for fibrosis score ≥ 1 and 1.18 (0.63–2.22) for GGA score ≥ 1.

Multivariate regression analyses for the total population produced similar results, and identified the independent risk factors for lung cancer as older age (odds ratio 1.10; 95% CI: 1.08–1.13; $P = 5.4 \times 10^{-18}$), smoking history (3.50; 2.31–5.29; $P = 2.8 \times 10^{-9}$), $FEV_1/FVC < 70\%$ (5.02; 3.01–8.38; $P = 6.8 \times 10^{-10}$), VC < 80% (5.70; 2.74–11.81; $P = 3.0 \times 10^{-6}$), fibrosis score ≥ 1 (3.52; 1.94–6.37; $P = 3.4 \times 10^{-5}$) and GGA score ≥ 1 (1.83; 1.04–3.25; $P = 0.038$). Multivariate analyses for the gender- and age-matched subpopulations revealed that the independent risk factors for lung cancer were smoking history (odds ratio 2.98; 95% CI: 1.76–5.07; $P = 5.2 \times 10^{-5}$), $FEV_1/FVC < 70\%$ (4.93; 2.70–8.98; $P = 1.9 \times 10^{-7}$), VC < 80% (3.88; 1.66–9.08; $P = 0.0018$), LAA score ≥ 1 (1.73; 1.02–2.94; $P = 0.042$) and fibrosis score ≥ 1 (3.75; 1.99–7.05; $P = 4.1 \times 10^{-5}$).

DISCUSSION

In the present study, the CT findings in terms of LAA, fibrosis and GGA, and spirometric data were evaluated in 256 (245 for spirometry) patients with previously untreated lung cancer and in 947 (813 for spirometry) control subjects who participated in a CT screening programme for lung cancer. The presence of LAA and/or air-flow limitation is not directly related to a definitive diagnosis of COPD, although the

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Table 2 Characteristics of subjects in the gender- and age-matched subpopulations

	Subgroup		
	Control	Lung cancer	*P*-value
Number	423	141	
Number evaluated by spirometry	423	141	
Gender (M : F)	321 : 102	107 : 34	
Median age (range)	62 (22–79)	63 (24–78)	
Smoking status			
Overall median pack-years (range)	0.0 (0.0–40.0)	42.0 (5.0–53.1)	7.6×10^{-141}
Current smoker			
Number (%)	122 (28.8)	57 (40.4)	
Median pack-years (range)	45.5 (7–132)	49.5 (10–118)	0.264[†]
Ex-smoker			
Number (%)	46 (10.9)	55 (39.0)	
Median pack-years (range)	42.5 (8–192)	43.0 (0.2–230)	0.360[†]
Never smoker			
Number (%)	255 (60.3)	29 (20.6)	
Histological type of lung cancer			
Sm : Ad : Sq : La : Oth[‡]	—	15 : 91 : 21 : 5 : 9	
c-Stage (I : II : III : IV)	—	41 : 11 : 36 : 43	
$FEV_1/FVC < 70\%$	5.7%	39.7%	1.1×10^{-23}
FEV_1/FVC (mean ± SD)	79.7 ± 7.2	72.0 ± 11.5	7.3×10^{-15}
VC < 80%	3.3%	14.2%	2.6×10^{-6}
%VC (mean ± SD)	108.0 ± 16.5	100.2 ± 17.6	2.3×10^{-6}
LAA score ≥ 1	18.4%	55.3%	2.3×10^{-17}
LAA score (mean ± SD)	0.8 ± 2.3	4.1 ± 5.2	1.4×10^{-16}
Fibrosis score ≥ 1	5.9%	29.1%	1.2×10^{-13}
Fibrosis score (mean ± SD)	0.2 ± 0.6	0.9 ± 1.5	1.3×10^{-11}
GGA score ≥ 1	9.0%	22.7%	1.9×10^{-5}
GGA score (mean ± SD)	0.3 ± 0.6	0.6 ± 1.1	4.7×10^{-7}

[‡] Sm, small cell carcinoma; Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Oth, others.
[†] Mann–Whitney test.
[§] chi-square test.
c-Stage, clinical stage of lung cancer; LAA, low attenuation area; GGA, ground glass attenuation.

presence of LAA is strongly suggestive of destruction of alveolar structure and pulmonary emphysema. In addition, the presence of GGA and/or fibrosis most likely indicates common forms of ILD such as IPF or non-specific interstitial pneumonia, because these features are observed in patients with otherwise non-specific clinical manifestations, namely the absence of acute symptoms, lymphadenopathy, concomitant collagen vascular diseases, known occupational exposure to hazardous dust or other special conditions.

The lung cancer group had a higher prevalence of abnormal CT findings and abnormal spirometric data than the control group, both among smokers and non-smokers, with the exception of the LAA score in non-smokers. As the lung cancer group had a higher proportion of smokers and consisted of older individuals than the control group, these observations are very likely to be biased. To minimize potential bias, gender- and age-matched subpopulations of the two groups were compared by the Mantel–Haenszel projection method with stratification for smoking status. In fact, the number of pack-years smoked by the lung cancer and control groups according to smoking status, that is, current and ex-smokers, was very similar, suggesting efficient exclusion of the intra-stratification imbalance with smoking status. The results showed that all five factors, LAA score ≥ 1, fibrosis score ≥ 1, GGA score ≥ 1, $FEV_1/FVC < 70\%$ and VC < 80%, were risk factors for lung cancer. Multivariate analyses also produced similar results with slight variations.

The potential shortcomings of this study should be noted. To minimize radiation exposure in healthy individuals who participated in the CT screening programme, the technical parameters for CT in the control group were different from those in the lung cancer group. Low-dose CT may lead to underestimation of CT findings, especially LAA. The concordance of the CT findings with the spirometric data, however, seems to support the validity of the CT findings. Second, abnormal spirometric data might be the consequence of impaired pulmonary function due to lung cancer. This possibility is supported by the observation that the prevalence of VC < 80% was significantly higher in the subpopulation with advanced lung cancer (stages III and IV) than in that with earlier stage lung cancer (stages I and II). As the prevalence of $FEV_1/FVC < 70\%$ was, however, similar in the two