Bronchial Brushing Cytology of Primary Signet-Ring Adenocarcinoma of the Lung. Another Morphological Feature on the Papanicolaou Smear: A Report of Two Cases

Dear Dr. Bedrossian:

Primary signet-ring adenocarcinoma of the lung (SRA), categorized as a variant of pulmonary adenocarcinoma in the WHO classification,[1] is a special histolopathologic type of mucin-producing lung cancers. In most cases, signet-ring cells (SRCs) are recognized as one component of the tumor (the pure type is very rare) the other being mostly conventional adenocarcinoma, and infrequently, adenosquamous carcinoma.[2] The group in which the SRC component occupies $\geq$50% of the cancer occurs at a younger age and its prognosis is poorer—the 5-year survival rate is 28.4%.[2] Recently, the ALK-rearranged nonsmall cell lung cancers have been implicated in a solid growth pattern with SRCs.[3] Two distinct patterns of growth are recognized in SRA: mainly acinar and occasionally diffuse.[4] The few reports on SRA, from the point of view of cytomorphology on Papanicolaou smears, are probably based on the acinar growth pattern.[5,6] The significant cytological features of SRA are the presence of single SRCs and nuclear pleomorphism, as compared with goblet-cell-type adenocarcinoma.[5] Here, we describe another interesting cytological feature found in bronchial brushing smears of two cases of SRA and confirmed by surgical lung resections.

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Two Japanese men (a 55-year-old nonsmoker with a 7-year indolent growth (patient 1) and a 53-year-old one-pack-a-day smoker (patient 2)), had no symptoms, but computed tomography of the chest revealed a well-demarcated 20 $\times$ 17 mm (patient 1) and 23 $\times$ 20 mm (patient 2) mass in the left inferior lobe of the lung. Cytology specimens obtained by bronchial brushings against the tumors were fixed in 95% alcohol and subjected to Papanicolaou staining. According to the sixth edition of the UICC TNM classification and the results of systemic examinations, the tumors were cT1N1M0, stage IIA (patient 1) and cT1N0M0, stage IA (patient 2); consequently, both patients underwent left lower lobectomy. No further treatment was given to either patient. Three years thereafter, pleuritis carcinomatosa with left pleural effusion was detected in patient 1 during the follow-up. No signs of local recurrence or distant metastasis were observed in patient 2, 2 years after the lobectomy.

Cytologically, both cases showed essentially similar findings. Cytological examination of the smears revealed moderate cellularity and many three-dimensional cell clusters of various sizes in a clear background. The clusters showed strong, mostly well-outlined, and clear overlapping intracellular cohesion and some single cells partly dissociated from the clusters. The uniform round cells in the clusters showed the following signet-ring features: peripheral placed nuclei, mild to moderate anisonucleosis, slightly coarse hyperchromatin, occasional prominent nucleoli, and orangeophilic intracytoplasmic mucin. Mitotic activity was insignificant. These findings have been reported.[5,6] Another noteworthy feature in our cases was the coexistence of clear and amorphous ball-like structures of various sizes

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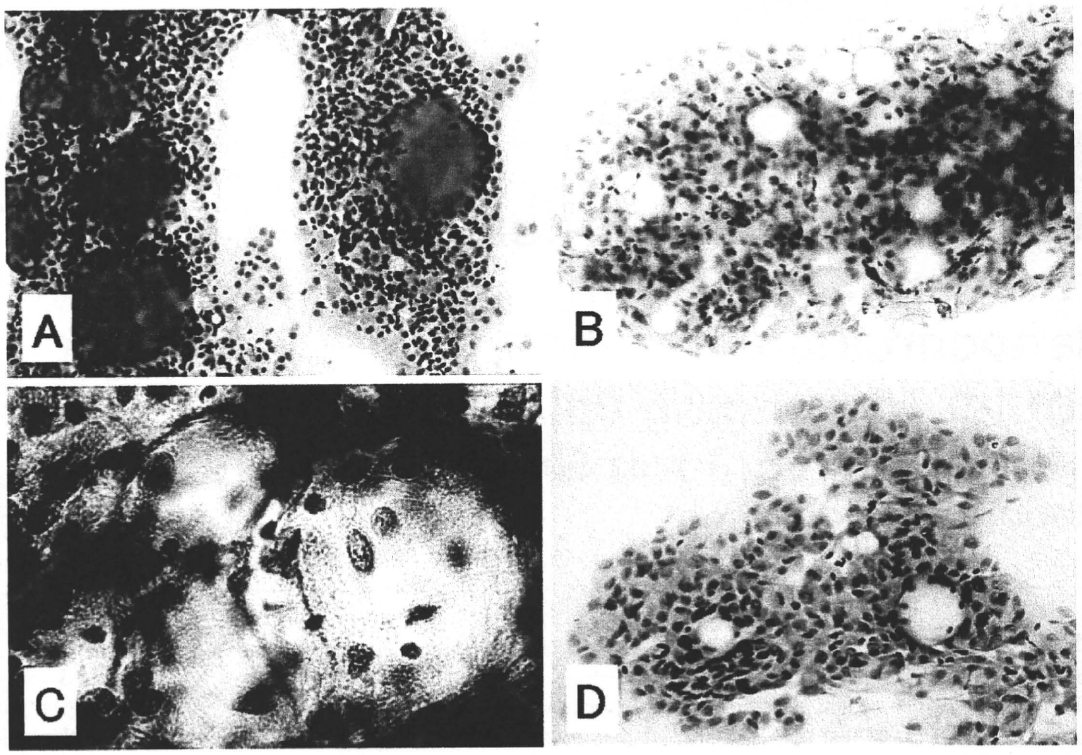

Fig. 1. Bronchial brushing preparations. Note clear and amorphous ball-like structures of various sizes in the clusters of signet-ring cells. (Papanicolaou, A: ×100, B: ×200, C: ×400, D: ×200). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

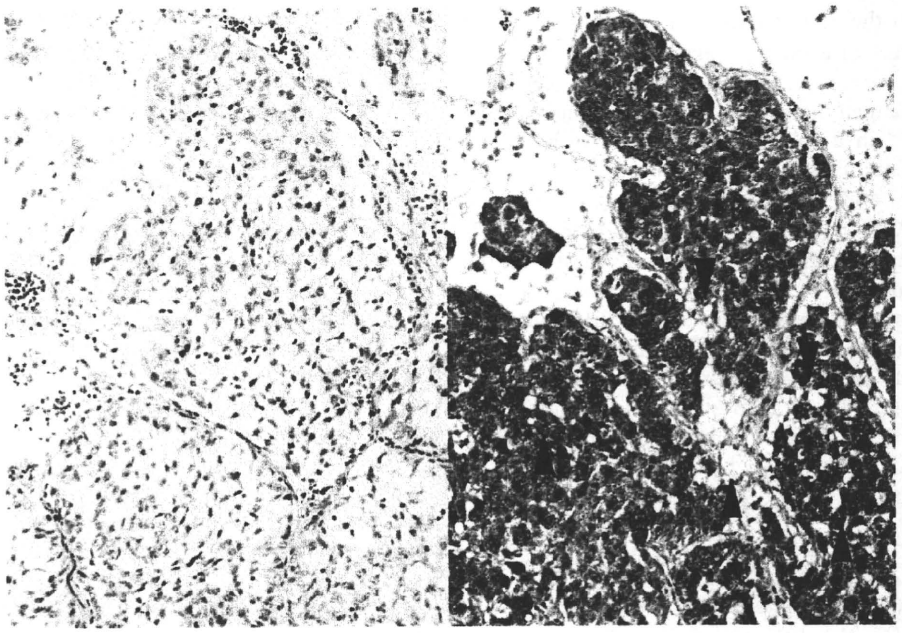

Fig. 2. Preparation of surgically resected lung specimens. Note bubble-like alveolar air spaces (arrowheads) involved in the acini of signet-ring cells (Left: H&E, ×100; Right: Combined Alcian blue-PAS, ×100). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in the clusters (Fig. 1). Focusing on different planes revealed acellularity, but no Papanicolaou staining material in the structure. Cells surrounding the structures showed no polarity, tubuliform or sieve-like patterns. Because of the paucity of cytology preparations by bronchial brushings, we were not able to conduct more special staining.

Histopathological analysis of the surgically resected specimens disclosed pure type SRA (patient 1) and SRA occupied by 90% SRCs (patient 2), with acinar growth patterns. Mucin-rich SRCs with mild to moderate nuclear atypia were packed in alveolar spaces, with no glandular differentiation or sieve-like patterns. Small bubble-like spaces were involved and admixed with the acini (particularly at the periphery of the cancer) that were negative for combined Alcian blue-PAS stain and mucicarmine stain (Fig. 2).

Differential diagnosis of these cases was challenging because of little cytological data about the SRCs originated in the lung, and both benign and malignant conditions would have had to be considered. Goblet cell metaplasia as a benign condition could cytologically be considered one of the differential diagnoses, and patient 2 might have had smoking-related chronic bronchitis[7]; however, the Papanicolaou smears contained too many SRCs with nuclear atypia, and some single SRCs. The presence of goblet cell metaplasia is usually observed in large airways, although focal goblet cell metaplasia might be identified near adenocarcinoma of the lung.[8] From its frequency of occurrence and simply from the cytomorphological point of view, metastatic SRC carcinoma of the stomach, colon, breast, urinary bladder, and such, necessitates cytological differential diagnosis for determining malignant conditions. Also, cytokeratins and thyroid transcription factor-1 could be examined by immunohistochemistry,[2] but the Papanicolaou smear only, as in our cases, is highly dependable in ruling out the clinical possibility of metastatic carcinoma. Retrospectively, SRA of the lung, with an acinar growth pattern might have shown characteristic clusters with strong cell cohesion; however, only SRC morphology posed great difficulty in differential diagnoses. Regarding other malignant conditions, primary mucin-producing adenocarcinoma, such as mucinous bronchioloalveolar carcinoma, solid adenocarcinoma with mucin, mucinous (colloid) adenocarcinoma, mucinous cystadenocarcinoma, and SRA were also considered in the cytological differential diagnoses, but the salivary gland type of tumor was the most prominent disease, although both cases occurred at the periphery of the lung. Particularly, adenoid cystic carcinoma is known to make gland-like spaces in the clusters (also called globules), cyanophilic hyaline basement membrane material, and ball-like formations, resulting in a sieve-like cell arrangement.[9–14]

In conclusion, we assume that the clear and amorphous ball-like structures (Fig. 1) in the clusters were entrapped alveolar air spaces associated with strong cellular cohesion in an acinar growth pattern of SRA, and that a sufficient amount of bronchial brushing specimens is as good as transbronchial lung biopsy. These findings, we believe, will be of interest to readers of *Diagnostic Cytopathology*, although further study is warranted.

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ORIGINAL REPORT

Phase III Trial Comparing Oral S-1 Plus Carboplatin With Paclitaxel Plus Carboplatin in Chemotherapy-Naïve Patients With Advanced Non–Small-Cell Lung Cancer: Results of a West Japan Oncology Group Study

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This study is registered with University Hospital Medical Information Network Clinical Trial Registry (http://www.umin.ac.jp/ctr/index.htm, identification number UMIN000000503).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

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ABSTRACT

Purpose

The primary goal of this open-label, multicenter, randomized phase III trial was to determine whether treatment with carboplatin plus the oral fluoropyrimidine derivative S-1 was noninferior versus that with carboplatin plus paclitaxel with regard to overall survival (OS) in chemotherapy-naive patients with advanced non–small-cell lung cancer (NSCLC).

Patients and Methods

A total of 564 patients were randomly assigned to receive either carboplatin (area under the curve, 5) on day 1 plus oral S-1 (40 mg/m^2 twice per day) on days 1 to 14 or carboplatin (area under the curve, 6) plus paclitaxel (200 mg/m^2) on day 1 every 21 days.

Results

At the planned interim analysis, with a total of 268 death events available, the study passed the O'Brien-Fleming boundary of 0.0080 for a positive result and noninferiority of carboplatin and S-1 compared with carboplatin and paclitaxel was confirmed for OS (hazard ratio, 0.928; 99.2% CI, 0.671 to 1.283). Median OS was 15.2 months in the carboplatin and S-1 arm and 13.3 months in the carboplatin and paclitaxel arm, with 1-year survival rates of 57.3% and 55.5%, respectively. Rates of leukopenia or neutropenia of grade 3/4, febrile neutropenia, alopecia, and neuropathy were more frequent in the carboplatin and paclitaxel arm, whereas thrombocytopenia, nausea, vomiting, and diarrhea were more common in the carboplatin and S-1 arm. The carboplatin and S-1 arm had significantly more dose delays than the carboplatin and paclitaxel arm.

Conclusion

Oral S-1 with carboplatin was noninferior in terms of OS compared with carboplatin and paclitaxel in patients with advanced NSCLC, and is thus a valid treatment option.

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INTRODUCTION

Lung cancer is the leading cause of death related to cancer worldwide,[1] with non–small-cell lung cancer (NSCLC) accounting for 85% of lung cancer cases. For individuals with advanced or metastatic NSCLC, platinum-based chemotherapy is the mainstay of first-line treatment on the basis of the moderate improvement in survival and quality of life it affords compared with best supportive care alone.[2-5] Thus, there is still a need for new treatment regimens to ameliorate symptoms and prolong survival in patients with advanced NSCLC in a manner that is both convenient and safe.

S-1 (TS-1; Taiho Pharmaceutical Co Ltd, Tokyo, Japan) is an oral fluoropyrimidine agent that consists of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate in a molar ratio of 1:0.4:1.[6,7] A phase II trial of oral S-1 as a single agent for the treatment of advanced NSCLC yielded a response rate of 22% and a median survival time of 10.2 months in 59 patients without prior chemotherapy.[8] We previously performed a phase I/II study of carboplatin/S-1 combination therapy and found that administration of S-1 (40 mg/m^2 twice per day) on days 1 to 14 in combination with carboplatin (area under the curve [AUC], 5) on day 1 of every 3-week cycle yielded efficacy results similar to those of other platinum doublets.[9] The carboplatin and S-1 combination had a more favorable toxicity profile than that typically seen with platinum-based regimens,

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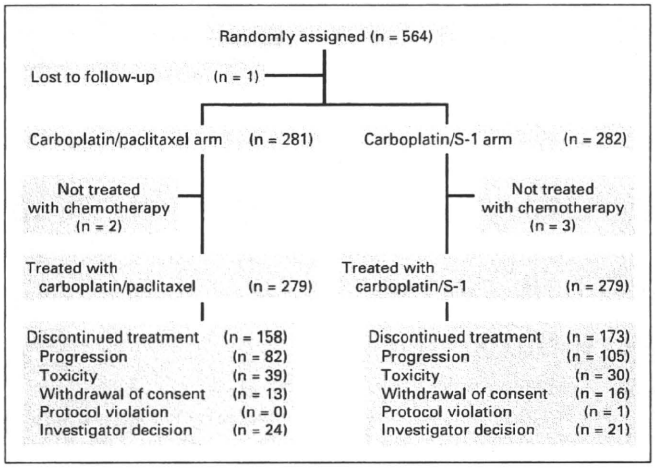

Fig 1. CONSORT diagram for the study.

especially with regard to neutropenia, febrile neutropenia, neuropathy, and alopecia.[9] In addition, replacement of paclitaxel with oral S-1 in combination therapy with carboplatin avoids the need for premedication to ameliorate paclitaxel-induced hypersensitivity and the 3-hour infusions required for paclitaxel administration. We therefore undertook and now report the results of the LETS (Lung Cancer Evaluation of TS-1) study, a multicenter, randomized, phase III, noninferiority trial of carboplatin and S-1 in comparison with carboplatin and paclitaxel combination therapy in chemotherapy-naive patients with advanced NSCLC.

PATIENTS AND METHODS

Patients

The criteria for patient eligibility included a diagnosis of NSCLC confirmed either histologically or cytologically; a clinical stage of IIIB not amenable to curative treatment or of stage IV; a measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST)[10]; no prior chemotherapy; an age of 20 to 74 years; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and a projected life expectancy of at least 3 months. Patients had adequate bone marrow reserve and organ function including a calculated creatinine clearance of $\geq$ 60 mL/min based on the standard Cockcroft and Gault formula. Radiation therapy for metastatic disease was permitted if it was completed at least 2 weeks before random assignment. Main exclusion criteria included active concomitant malignancy, symptomatic brain metastasis, interstitial pneumonia, watery diarrhea, heart failure, uncontrolled diabetes mellitus, active infection, and a past history of drug allergy. These inclusion and exclusion criteria are consistent with those of previous studies involving carboplatin and paclitaxel treatment.[11] Written informed consent was obtained from all patients, and the study protocol was approved by the institutional ethics committee of each of the participating institutions.

Treatment Plan

Eligible patients were randomly assigned to receive either carboplatin (AUC, 6) plus paclitaxel (200 mg/m^2) on day 1[11] or carboplatin (AUC, 5) on day 1 plus oral S-1 (40 mg/m^2 twice per day) on days 1 to 14. Chemotherapy was repeated every 3 weeks for a maximum of six cycles unless there was earlier evidence of disease progression or intolerance of the study treatment.

End Points

The primary objective of this open-label, multicenter, randomized phase III trial was to establish the noninferiority of S-1 plus carboplatin compared with paclitaxel plus carboplatin as first-line therapy in terms of overall survival (OS) in patients with advanced NSCLC. Secondary end points included tumor response, treatment safety, quality of life (QOL), and progression-free survival (PFS).

Baseline and Follow-Up Assessments

Baseline evaluations included medical history, physical examination, ECG, tumor status, ECOG performance status, and laboratory analyses. During treatment, blood counts and biochemical tests were performed at least biweekly. A computed tomography scan was performed for tumor assessment within 14 days of initiation of study treatment and was repeated after every 1 to 2 months of planned therapy. All responses were defined according to RECIST. If a patient was documented as having a complete response (CR) or a

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Carboplatin/Paclitaxel (n = 281) No.	Carboplatin/Paclitaxel (n = 281) %	Carboplatin/S-1 (n = 282) No.	Carboplatin/S-1 (n = 282) %	*P*
Age, years					
Median	63		64		.510
Range	36-74		38-74		
Sex					
Male	215	76.5	217	77.0	.902
Female	66	23.5	65	23.0	
ECOG PS					
0	90	32.0	86	30.5	.695
1	191	68.0	196	69.5	
Histology					
Adenocarcinoma	195	69.4	195	69.1	.560
Nonadenocarcinoma	86	30.6	87	30.9	
Clinical stage					
IIIB	68	24.2	68	24.1	.981
IV	213	75.8	214	75.9	
Smoking status					
Smoker	229	81.5	230	81.6	.984
Nonsmoker	52	18.5	52	18.4	

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

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partial response (PR), a confirmatory evaluation was performed after an interval of 4 weeks. Disease control was defined as the best tumor response among CR, PR, or stable disease that was confirmed and sustained for 6 weeks or longer. Patients were evaluated for adverse events during therapy and until 42 days after administration of the last dose of the study treatment. Toxicity was evaluated according to the National Cancer Institute Cancer Common Toxicity Criteria, version 3. QOL was assessed with the lung cancer subscale of the Functional Assessment of Cancer Therapy–Lung (FACT-L)[12] and the neurotoxicity subscale of the FACT/Gynecology Oncology Group-Neurotoxicity (GOG-Ntx) version 4.[13] In addition, alopecia was evaluated on the basis of the single item "I have been bothered by hair loss," which was included in the former version of FACT-L. The maximum attainable scores on the lung cancer subscale, neurotoxicity subscale, and alopecia item were 28, 44, and 4, respectively, with which the patient was considered to be asymptomatic. Patients were asked to complete each instrument at the time of enrollment and at 6 and 9 weeks after initiation of treatment.

Statistical Analysis

Eligible patients were randomly assigned according to a 1:1 ratio to receive either carboplatin and paclitaxel or carboplatin and S-1. After a check of patient eligibility, random assignment was performed centrally at the West Japan Oncology Group data center by minimization with stratification factors including disease stage (IIIB *v* IV), type of histology (adenocarcinoma *v* nonadenocarcinoma), sex (male *v* female), and investigator center. The intent-to-treat (ITT) patient population included all patients who underwent random assignment. The per-protocol (PP) population was defined as the ITT population minus patients considered to have major violations of inclusion or exclusion criteria and those who did not receive any protocol treatment. The safety population was defined as all patients receiving at least one dose of study drugs. The primary end point of the study was OS, which was analyzed in the ITT population by estimation of the hazard ratio (HR) and two-sided 95% CI derived from a Cox regression model with adjustment for the stratification factors with the exception of investigator center. Median OS in both treatment arms was assumed to be 14 months on the basis of data from previous clinical trials.[11] Noninferiority of carboplatin and S-1 was to be concluded if the upper limit of the 95% CI of the HR was lower than 1.33; that is, the null hypothesis that the median OS of the carboplatin and S-1 group would be up to 3.48 months shorter than that of the carboplatin and paclitaxel group was analyzed. Demonstration of noninferiority with a statistical power of 85% at a two-sided significance level of .05 and 2 years of follow-up after 2.5 years of accrual would require 263 patients in each treatment group. Given the possibility of variance inflation due to censoring, the sample size was set at 560 (280 per arm). One interim analysis was planned when all the patients had been enrolled. For analysis of the primary end point, adjustment for multiple comparisons was handled by the method of Lan and DeMets, with the use of the O'Brien-Fleming type α spending function. The significance level was set at .008 for the interim analysis, taking the numbers of observed events (n = 268) and expected events (n = 442) into account. Survival curves (PFS and OS) were analyzed by the Kaplan-Meier method and were compared between groups by the Cox regression model. The 95% CI for median PFS and OS was calculated by the method of Brookmeyer and Crowley. Planned subgroup analyses for OS were performed to examine the interaction effect of treatment arm with each of performance status, sex, disease stage, type of histology, and smoking status. Patient characteristics (ie, sex, ECOG PS, histology, clinical stage, and smoking status) as well as response and toxicity incidence were compared between the two treatment arms by the χ^2 test, and age was compared by the Wilcoxon test. Longitudinal QOL data were analyzed with a linear mixed-effects model. All *P* values were two sided. Statistical analyses were performed with SAS for Windows, release 9.1 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

From August 2006 to May 2008, 564 patients from 30 institutions were enrolled in the study. One patient was excluded from the carboplatin and paclitaxel arm because of loss to follow-up. The ITT population thus consisted of 563 patients: 281 individuals randomly assigned to the carboplatin and paclitaxel group and 282 individuals randomly assigned to the carboplatin and S-1 group (Fig 1). The baseline demographic and disease-related characteristics of the study subjects were well-balanced between the two treatment arms (Table 1). Two patients in the carboplatin and paclitaxel arm and three patients in the carboplatin and S-1 arm did not receive any chemotherapy, with the result that 558 patients were eligible for safety analysis (Fig 1).

Delivered Chemotherapy

The number of treatment courses administered was 1,037 in the carboplatin and paclitaxel arm (median, 4; range, 1 to 6) and 987 in the carboplatin and S-1 arm (median, 4; range, 1 to 6). Dose reductions occurred in 90 (8.7%) of the carboplatin and paclitaxel courses and in 49 (5.0%) of the carboplatin and S-1 courses. Carboplatin and paclitaxel dose reductions were mainly due to neuropathy, whereas those for carboplatin and S-1 were most commonly attributable to thrombocytopenia. Dose delays occurred in 47.9% of carboplatin and paclitaxel courses and 68.5% of carboplatin and S-1 courses. Delays due to

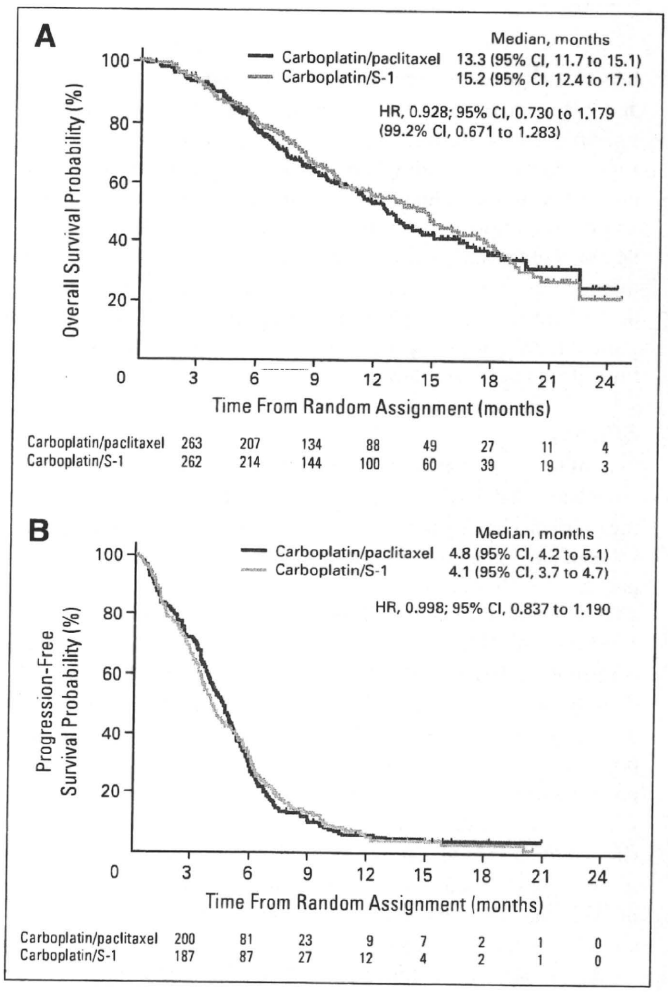

Fig 2. (A) Overall survival and (B) progression-free survival for the intent-to-treat population (n = 563). HR, hazard ratio.

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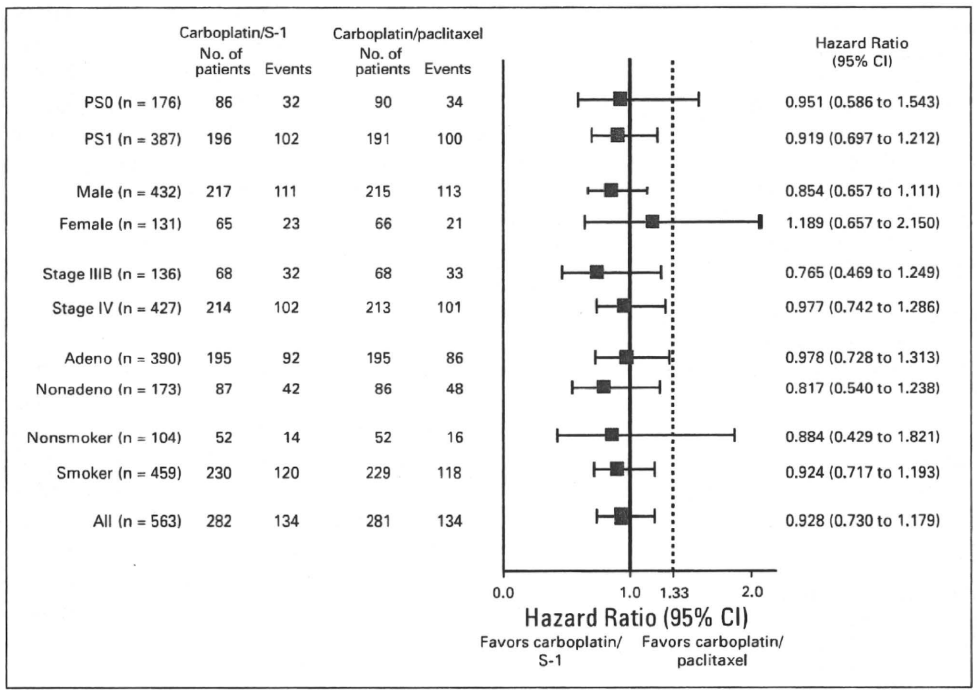

Fig 3. Subgroup analysis of overall survival in the intent-to-treat population (n = 563). PS, performance status; Adeno, adenocarcinoma; Nonadeno, nonadenocarcinoma.

hematologic toxicity occurred in a higher proportion of carboplatin and S-1 courses (51.6%) than carboplatin and paclitaxel courses (9.6%). S-1 was administered for the planned 14 days without interruption in 89.1% of carboplatin and S-1 courses. The median relative dose intensities were high for both carboplatin and paclitaxel (89.6% and 87.6%, respectively) and carboplatin and S-1 arms (83.3% and 94.3%, respectively). The most frequent reason for discontinuation of therapy was disease progression in both arms. Treatment was withdrawn before completion from a similar proportion of patients in each group (13.6% for carboplatin and paclitaxel and 10.7% for carboplatin and S-1) because of adverse events.

Efficacy

At the interim analysis planned for when patient enrollment was completed, 268 death events were available in total. The study passed the O'Brien-Fleming boundary of 0.0080 for a positive result with a *P* value of .002. The HR for OS (carboplatin and S-1 *v* carboplatin and paclitaxel) in the ITT population was 0.928, with a two-sided 99.2% CI after adjustment for multiplicity due to interim analysis of 0.671 to 1.283 (Fig 2A). Noninferiority of carboplatin and S-1 therapy was thus confirmed at the interim analysis by the upper limit of the CI being less than the protocol-specified margin of 1.33. The crude (unadjusted) 95% CI of the HR for OS of 0.928 was 0.730 to 1.179 in the ITT population, and an HR for OS of 0.931 (95% CI, 0.732 to 1.186) was obtained with the PP population. Median OS was 15.2 months (95% CI, 12.4 to 17.1) in the carboplatin and S-1 arm and 13.3 months (95% CI, 11.7 to 15.1) in the carboplatin and paclitaxel arm, with the 1-year survival rates being 57.3% and 55.5%, respectively. Subgroup analysis of OS in the ITT population according to stratification variables and other baseline characteristics were consistent with the primary analysis. A significant interaction effect between treatment arm and subgroups was not observed. The 95% CI for the HR in each subgroup included 1.00 (Fig 3).

The median PFS was 4.1 months in the carboplatin and S-1 arm and 4.8 months in the carboplatin and paclitaxel arm in the ITT population, with a corresponding HR of 0.998 and 95% CI of 0.837 to 1.190 (Fig 2B). In the PP population, the median values of PFS were 4.2 and 4.8 months for the carboplatin and S-1 and carboplatin and paclitaxel arms, respectively, with a corresponding HR of 0.992 and 95% CI of 0.832 to 1.184. Response to treatment was assessed in 279 patients (99.3%) of the carboplatin and paclitaxel group and in 279 patients (98.9%) of the carboplatin and S-1 group. For overall response (CR + PR) rate, carboplatin and paclitaxel was superior to carboplatin and S-1 (29.0% *v* 20.4%; $P = .019$, χ^2 test), whereas the overall disease control (CR + PR + stable disease) rate was similar in both treatment groups (73.5% *v* 71.7%, respectively; $P = .635$).

Safety

The incidence of leukopenia or neutropenia of grade 3 or 4 was significantly lower for patients in the carboplatin and S-1 arm than for those in the carboplatin and paclitaxel arm (leukopenia, 5% *v* 33%; neutropenia, 21% *v* 77%, respectively), as was the incidence of febrile neutropenia (1% *v* 7%; Table 2). Conversely, treatment with carboplatin and S-1 was associated with a higher rate of thrombocytopenia of grade 3 or 4 than was that with carboplatin and paclitaxel (33% *v* 9%, respectively). Platelet transfusion was also necessary for more patients in the carboplatin and S-1 arm than in the carboplatin and paclitaxel arm (8% *v* 2%, respectively; $P = .002$). The overall rates of neuropathy and alopecia were much lower in the carboplatin and S-1 arm (neuropathy, 16% *v* 81%; alopecia, 9% *v* 77%), whereas nausea, vomiting, and diarrhea occurred more frequently in the carboplatin/S-1 arm (Table 2). Death as a result of toxicity occurred in two patients; one death in the carboplatin and S-1 arm was associated with gastrointestinal hemorrhage, and another patient in the carboplatin and paclitaxel arm died of febrile neutropenia and pneumonia.

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Table 2. Incidence of Drug-Related Toxicities in Randomly Assigned and Treated Patients

Toxicity	Regimen by Grade (%)						*P*	
	Carboplatin/Paclitaxel (n = 279)			Carboplatin/S-1 (n = 279)				
	All	3	4	All	3	4	All	3 or 4
Hematologic								
Leukopenia	86.0	29.7	2.9	55.4	5.0	0.4	< .001	< .001
Neutropenia	89.6	31.9	44.8	58.3	18.3	2.9	< .001	< .001
Anemia	82.4	14.3	2.5	86.7	15.5	3.6	.165	.680
Thrombocytopenia	63.1	7.2	2.2	87.4	19.4	13.3	< .001	< .001
Nonhematologic								
Febrile neutropenia	7.2	6.8	0.4	1.1	1.1	0	< .001	< .001
Nausea	49.1	2.2	0	62.4	1.8	0	.002	.475
Vomiting	23.7	1.1	0	34.1	1.8	0	.007	.837
Diarrhea	20.8	1.1	0	32.6	3.2	0	.002	.302
Neuropathy: sensory	81.0	2.9	0	15.8	0.4	0	< .001	.668
Arthralgia	67.4	2.5	0	7.9	0	0	< .001	.357
Alopecia	76.7			9.3			< .001	

NOTE. Differences between the two arms were evaluated by the χ^2 test.

QOL

At random assignment, 99.6% of patients (562 of 564) completed baseline questionnaires, with the questionnaire completion rates being 93.4% at 6 weeks and 90.1% at 9 weeks. Compliance rates were not significantly different between the treatment arms. QOL data were missing in 38 surveys due to death or severe impairment of the patient's general condition, which accounted for 2.3% of the total number of the surveys scheduled. There was no significant difference in the lung cancer subscale of FACT-L between the treatment arms (Fig 4). Scores on the neurotoxicity subscale of FACT/GOG-Ntx had decreased significantly in the carboplatin and paclitaxel arm after two cycles of chemotherapy (Fig 4); the adjusted mean scores at 6 and 9 weeks were 41.2 and 41.0 for the carboplatin and S-1 arm and 38.2 and 37.1 for the carboplatin and paclitaxel arm. The alopecia score was also significantly worse in the carboplatin and paclitaxel arm than in the carboplatin and S-1 arm ($P < .001$, analysis of variance), with the adjusted means at 6 and 9 weeks being 3.8 and 3.7 for carboplatin and S-1 and 1.7 and 1.9 for carboplatin and paclitaxel ($P < .001$ at both 6 and 9 weeks, Tukey-Kramer multiple-comparison test).

Poststudy Treatment

There were no major differences in poststudy treatment between the two arms. Overall, 69.4% of carboplatin and paclitaxel patients and 75.5% of carboplatin and S-1 patients received an additional line of therapy ($P = .103$, χ^2 test). Docetaxel was administered in 43.4% and 52.0% of patients and epidermal growth factor receptor tyrosine kinase inhibitors were administrated in 24.0% and 27.2% of patients in the carboplatin and paclitaxel and carboplatin and S-1 arms, respectively.

DISCUSSION

Our phase III study is the first to evaluate the efficacy of an S-1–containing regimen in comparison with standard platinum-doublet chemotherapy for first-line treatment of patients with advanced NSCLC. The primary objective of the study—determination of the noninferiority of carboplatin and S-1 compared with carboplatin and paclitaxel in terms of OS—was met at the planned interim analysis.

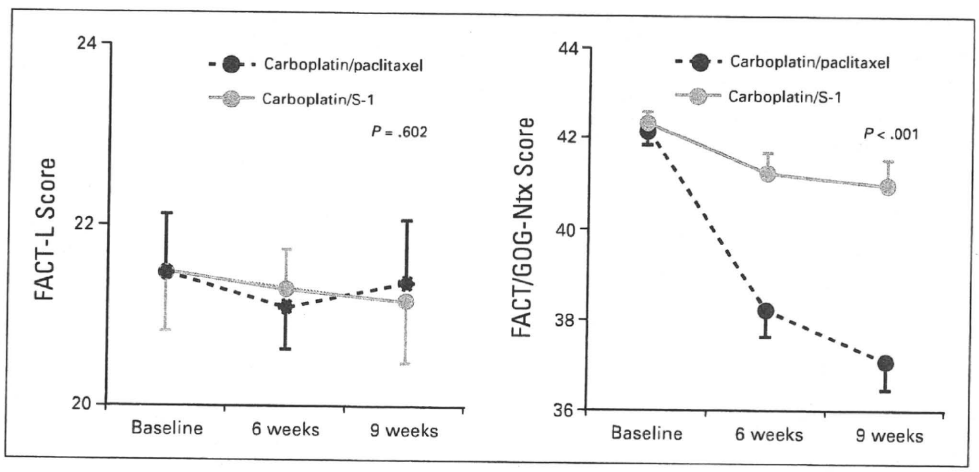

Fig 4. Quality of life assessments with the (left) seven-item Functional Assessment of Cancer Therapy–Lung (FACT-L) and (right) 11-item FACT/Gynecology Oncology Group-Neurotoxicity (GOG-Ntx) scales. Data are least square means ± 95% CI. Higher scores indicate better quality of life. *P* values shown were determined by analysis of variance, with *P* being less than .001 for comparison of FACT/GOG-Ntx scores between the two arms at both 6 and 9 weeks by the Tukey-Kramer multiple-comparison test.

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Analysis of OS in the ITT and PP populations as well as in subgroups of the study subjects demonstrated the noninferiority of carboplatin and S-1. Although there was a significant difference in response rate favoring carboplatin and paclitaxel, disease control rate and PFS were similar for carboplatin and S-1 and carboplatin and paclitaxel. Given that subsequent therapies after discontinuation of the study treatment were well-balanced between the treatment groups, it is unlikely that poststudy therapy confounded survival results. Collectively, our secondary data indicate that the findings of the main analysis are robust. Although the protocol-specified noninferiority margin of 1.33 may be large, the survival curves themselves mostly coincided for the two treatment arms and median OS in the carboplatin and S-1 group was noteworthy at approximately 15 months.

The profile of adverse events associated with carboplatin and S-1 and carboplatin and paclitaxel was as expected, but there were marked differences in the incidence of some of these events. Carboplatin and paclitaxel treatment resulted in a typically high incidence of neutropenia of grade 3 or 4 (76.7%) as well as of febrile neutropenia (7.2%), compared with incidences of only 21.1% and 1.1%, respectively, for carboplatin and S-1. These rates of neutropenia associated with carboplatin and paclitaxel treatment are consistent with those observed in previous studies of Japanese patients.[11,14] Carboplatin and S-1 treatment showed a significantly higher rate of thrombocytopenia, which was the most frequent reason for dose delays in the carboplatin and S-1 group. However, this condition was considered manageable because it was associated with bleeding of grade 3 in only one patient. With regard to nonhematologic toxicities, neuropathy, arthralgia, and alopecia were much less frequent in patients treated with carboplatin and S-1 than in those receiving carboplatin and paclitaxel. Consistent with these results, carboplatin and S-1 treatment showed a clinically relevant improvement in QOL as assessed by the FACT/GOG-Ntx scale and alopecia score. Despite these QOL benefits with carboplatin and S-1, however, there was no significant difference in FACT-L score between carboplatin and S-1 and carboplatin and paclitaxel, possibly because of other more toxic effects of carboplatin and S-1. The incidence of nausea, vomiting, and diarrhea of any grade was higher in patients assigned to the carboplatin and S-1 arm than in those assigned to carboplatin and paclitaxel, although grades 3 or 4 of these toxicities were uncommon ($< 4\%$) in both groups. The relative dose intensity of S-1 was 94.3% in the carboplatin and S-1 arm (median of four cycles administered), and treatment was discontinued in only approximately 10% of patients in this arm because of adverse events. Overall, these data indicate that carboplatin and S-1 was well-tolerated, with continuation of treatment as specified in the protocol not being a problem. According to our previous phase I/II study of carboplatin and S-1,[9] this study excluded elderly (≥ 75 years old) patients. Given its efficacy and favorable toxicity profile, the combination of S-1 and carboplatin warrants further evaluation in elderly patients.

In conclusion, our present study demonstrates the noninferiority of carboplatin and S-1 relative to carboplatin and paclitaxel in terms of OS for patients with advanced NSCLC. Carboplatin and S-1 is therefore a valid therapeutic option for the first-line treatment of patients with advanced NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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RESEARCH ARTICLE Open Access

Possibility of multivariate function composed of plasma amino acid profiles as a novel screening index for non-small cell lung cancer: a case control study

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Abstract

Background: The amino-acid balance in cancer patients often differs from that in healthy individuals, because of metabolic changes. This study investigated the use of plasma amino-acid profiles as a novel marker for screening non-small-cell lung cancer (NSCLC) patients.

Methods: The amino-acid concentrations in venous blood samples from pre-treatment NSCLC patients (n = 141), and age-matched, gender-matched, and smoking status-matched controls (n = 423), were measured using liquid chromatography and mass spectrometry. The resultant study data set was subjected to multiple logistic regression analysis to identify amino acids related with NSCLC and construct the criteria for discriminating NSCLC patients from controls. A test data set derived from 162 patients and 3,917 controls was used to validate the stability of the constructed criteria.

Results: The plasma amino-acid profiles significantly differed between the NSCLC patients and the controls. The obtained model (including alanine, valine, isoleucine, histidine, tryptophan and ornithine concentrations) performed well, with an area under the curve of the receiver-operator characteristic curve (ROC_AUC) of >0.8, and allowed NSCLC patients and controls to be discriminated regardless of disease stage or histological type.

Conclusions: This study shows that plasma amino acid profiling will be a potential screening tool for NSCLC.

Background

Recently, computer-aided systems for data mining, for example by multivariate analysis, are now readily available and have shown promising results when applied to metabolic profiling for diagnostic purposes [1,2]. Currently, several applications of metabolome analysis based on machine learning for human cancer diagnosis using peripheral blood or urine were demonstrated [3-10].

Among metabolites, the amino-acid balance in patients with various diseases often differs from that maintained in healthy individuals, as a result of metabolic changes. Amino acids are considered to be central compounds within metabolic networks. The blood serves as the medium linking the metabolic processes in the different organs of the human body. Human amino-acid metabolism in the blood has been monitored clinically for >30 years. Fischer's ratio, which is defined as the balance between branched-chain amino acids (BCAAs) and aromatic amino acids, has been used as an indicator of both the progression of liver fibrosis and the effectiveness of drug treatment [11]. Specific abnormalities in amino-acid concentrations, as assessed using multivariate analysis, have also been reported in animal models of diabetes, in human liver fibrosis and in other pathologies [12-14].

The metabolism in cancer cells is known to be significantly altered compared with that in normal cells, and these changes are also reflected in the plasma amino-acid profiles of patients with various types of cancer.

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For example, a significant reduction in gluconeogenic amino acids (GAAs) and a significant increase in free tryptophan have been reported in lung cancer patients [15]. Kubota et al. used plasma amino-acid profiles to discriminate between patients with breast cancer, gastrointestinal cancer, and head and neck cancers, and healthy controls [16]. Therefore, detecting metabolic changes from amino-acid profiles could potentially be useful in cancer diagnosis.

Post-genomic technologies also offer possibilities for exploiting amino-acid profiling. Recently, novel methods for analyzing amino acids have been established using high-performance liquid chromatography (HPLC)-electrospray ionization (ESI)-mass spectrometry (MS) [17-19]. This will help to make amino-acid measurements easier and reduce both the time and the cost of analysis.

Therefore, one potentially useful metabolomics tool is the "AminoIndex", which could be a simple and versatile method for monitoring various pathological conditions [12]. Here we investigated the possibility of "AminoIndex" as a novel diagnostic method for the screening of non-small-cell lung cancer (NSCLC).

Methods

All of the patients in the study had been diagnosed histologically with NSCLC at the Osaka Medical Centre for Cancer and Cardiovascular Diseases, Japan, between January 2006 and October 2008. While hospitalized, their informed consent for inclusion was obtained. Data from the first 141 patients enrolled between January 2006 and September 2007 were used as the study data set. A further 4,340 subjects without apparent cancers, who were undergoing comprehensive medical examinations at the Mitsui Memorial Hospital, Japan, in 2008, were recruited as control subjects. Of these, 423 were age-matched, gender-matched, and smoking status-matched with the patients in the study data set group. Data from the remaining patients and control subjects were used as the test data set. Data from an additional 15 SCLC patients, who were hospitalized at the Osaka Medical Centre for Cancer and Cardiovascular Diseases, Japan, between January 2006 and October 2008, were also used. Blood samples were collected from the controls and the NSCLC patients before any medical treatment. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committees of the Osaka Medical Centre for Cancer and Cardiovascular Diseases and Mitsui Memorial Hospital. All subjects gave their informed consent for inclusion before they participated in the study.

Analytical methods

Blood samples (5 ml) were collected from forearm veins, after overnight fasting, in tubes containing ethylenediaminetetraacetic acid (EDTA; Termo, Tokyo, Japan), and were immediately placed on ice. Plasma was prepared by centrifugation at 3,000 rpm and 4°C for 15 min, and then stored at -80°C until analysis. After plasma collection, all samples were stored and processed at the Life Science Institute of Ajinomoto Co., Inc. (Kawasaki, Japan). To reduce any bias introduced prior to analysis, samples were analyzed in random order. The plasma samples were deproteinized using acetonitrile at a final concentration of 80% before measurement. The amino-acid concentrations in the plasma were measured by HPLC-ESI-MS, followed by precolumn derivatization [17-19]. The analytical methods were described in detail previously [17]. The concentrations of amino acids in the plasma were expressed as μM.

Statistical analysis of plasma amino-acid profile

The mean amino-acid concentrations ± standard deviations (SDs) were calculated. Differences between the plasma amino-acid concentrations in NSCLC patients and controls were assessed using the Mann-Whitney U-test and receiver-operator characteristic (ROC) curve. The area under the curve (AUC) for each ROC curve (the ROC_AUC) was calculated for each amino acid.

Principal component analysis (PCA) was also used to assess differences in the plasma amino-acid profile between the controls and the NSCLC patients, with linear combinations of all of the amino acids included as explanatory variables. In PCA analysis the plasma amino-acid concentrations were transformed using the following equation:

$$z_{i,j} = \left(x_{i,j} - \bar{X}_j \right) / \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left(x_{i,j} - \bar{X}_j \right)^2}$$

where $z_{i,j}$ was transformed concentration of of the i-th sample of the j-th amino acid, $x_{i,j}$ was concentration of the i-th sample of the j-th amino acid, n was sample size, and $\bar{X}_j$ was the average concentration of j-th amino acid.

Machine learning and validation

First, an unconditional multiple logistic regression analysis with variable selection was used to construct a criterion for distinguishing NSCLC patients from controls using the study data set with the raw plasma concentrations of 21 amino acids as explanatory variables. The candidate variables of most appropriate logistic regression model, which had the minimum Akaike's information criterion (AIC) value, were selected from among all of the possible combinations in which the number of variables was below seven. A leave-one-out cross-validation (LOOCV) was performed to correct potential

over-optimization for all models in parallel. Briefly, one sample was omitted from the study data set, and the logistic regression model was calculated for the remaining samples, to estimate coefficients for each amino acid. The logistic regression function values for the left-out sample were calculated based on the model. This process was repeated until every sample in the study data set had been left out once, and the function values generated were then used for AIC calculation. Finally, a case-control study was utilized for our study, and so a conditional logistic regression analysis, conditioned on the matching factors (i.e., gender, age, and smoking status), was performed in order to evaluate the association between the combination of amino acids obtained above and NSCLC. The discriminant score, which was defined as a logit of the conditional logistic regression function value, was constructed as a criterion. The degree of discriminancy of this score between NSCLC patients and controls was evaluated through the ROC curve. A distinct test data set, which had not been used in the model generation, was also used to confirm the stability of the obtained model, and to calculate the ROC_AUC values for the discriminant scores.

Subgroup analysis

To assess the effects of cancer stage and histological type, both the study data set and the test data set was stratified according to the analysis parameters. To assess the effects of cancer stage and histological type on the discriminant scores of NSCLC patients, a subgroup analysis was performed using the ROC curve, in each data set. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

Software

All statistical analyses were performed using MATLAB (The Mathworks, Natick, MA), LogXact (Cytel, Cambridge, MA), and GraphPad Prism (GraphPad Software, La Jolla, CA).

Results

Characteristics of patients and control subjects

The study data set comprised 141 patients with NSCLC, and 423 age-matched, gender-matched, and smoking status-matched control subjects, whereas there were 162 patients and 3,917 controls in the test data set; a further 15 SCLC patients were also included (Table 1). Among the patients, 28% and 36% were non-smokers in the study and test data sets, respectively, whereas almost 50% of the control subjects were non-smokers (Table 1). There were no significant differences in body mass index (BMI) between the patients and the control subjects (Table 1). In both the study and test data sets ~50% of the patients were categorized as having stage I disease, ~5% as stage II, ~25% as stage III and ~20% as stage IV (Table 1). The Eastern Cooperative Oncology Group performance status (ECOG) score of most patients was 0 or 1; hence, the majority of the patients were asymptomatic or symptomatic but completely ambulatory (Table 1). The histological type was adenocarcinoma in almost 75% of the patients and squamous cell carcinoma in almost 25%, the other types present included large-cell carcinoma, adenosquamous carcinoma, pleomorphic carcinoma and mucoepidermoid carcinoma (Table 1).

Changes in amino-acid concentrations in NSCLC patients

In the study data set, the plasma concentration of His was significantly lower, and those of Ser, Pro, Gly, Ala, Met, Ile, Leu, Tyr, Phe, Orn, and Lys were significantly higher, in NSCLC patients than in controls (Table 2).

Amino acids in the human body undergo interdependent regulation; comparing single amino-acid concentrations between controls and patients might thus be insufficient to elucidate any changes in plasma amino-acid profiles associated with cancer development. Changes in the balance of the plasma amino acids in the study data set were therefore investigated using principal component analysis (PCA) in the current study. Five PCs with eigenvalues >1 were identified (Table 3). To evaluate their performance, the Mann-Whitney *U*-test was used to compare each PC score between the controls and NSCLC patients. Three of the PCs showed significant p values (< 0.001): PC1, PC3, and PC5 (Table 3). The contributing amino acids for the PCs that had a variance of >0.05 were then extracted; the results identified Ala, Val, Met, Ile, Leu, Tyr, Phe, Trp, and Lys as contributing factors for PC1, Cit, His, Trp, Orn, and Arg as contributing factors for PC3, and Ser, Gly, Cit, His, and Arg as contributing factors for PC5 (Table 3). As a result, fifteen amino acids (Ser, Gly, Ala, Cit, Val, Met, Ile, Leu, Tyr, Phe, His, Trp, Orn, Lys, and Arg) were identified as whose profile in plasma were associated with NSCLC (Table 3).

Classifier for discriminating NSCLC patients

The results described so far suggested that it should be possible to improve the discrimination between cancer patients and normal controls by deriving multivariate functions, using the raw plasma amino-acid concentrations as explanatory variables, which would summarize the changes in metabolic status. Multiple logistic regression analyses by unconditional and conditional likelihood methods were therefore performed with variable selection and LOOCV cross-validation, using the study data set (as described in the Methods). The resulting conditional logistic regression model included six amino acids: Ala (p = 0.007), Val (p < 0.001), Ile (p < 0.001),

Table 1 Characteristics of study participants

		Study data set		Test data set		SCLC
		Controls	Patients	Controls	Patients	patients
Number	Total	423	141	3917	162	15
	(Male, Female, Unknown)	(279,144)	(93,48)	(2363,1554)	(103,55,4)	(15,0)
Age, y	Mean (SD)	61.1(8.7)	62.7(9.2)	52.6(10.8)	65.7(10.4)*	66.8(8.1)*
	Range	32~82	34~83	23~88	34~83	50~76
BMI	Mean (SD)	23.0(3.1)	22.6(2.8)	22.7(3.2)	22.8(3.1)	22.7(3.5)
	Range	16.5~36.4	15.4~29.8	14.0~41.3	15.8~35.1	17.7~30.7
Smoking status	Never	126	42	2020	55	0
	Ex	45	15	1304	25	5
	Current	237	79	554	81	10
	Unknown	15	5	39	1	0
Performance	0		95		129	6
	1		41		31	5
	> 1		2		0	2
	Unknown		3		2	2
Stage**	I		69		93	6
	II		8		16	0
	III		39		30	6
	IV		25		12	3
	Unknown		0		11	0
Histology	Adenocarcinoma		100		123	
	Squamous cell carcinoma		36		33	
	Others		4		5	
	Unknown		1		1	

* Significant at $p < 0.001$ in *t*-test

** In principle, stage indicates pathological stage (p-stage). In some advanced stage (III and IV) patients who had not undergone pathological examinations, clinical stage (c-stage) is indicated instead.

His ($p = 0.035$), Trp ($p = 0.027$) and Orn ($p < 0.001$). The area under the curve (AUC) of the ROC for the discriminant score was 0.817 in the study data set (Figure 1).

Furthermore, to verify the robustness of the resulting model, a ROC curve was generated using the split test data set, which had not been used to construct the model. A ROC_AUC of the ROC for the discriminant score was 0.812 in the test data set (Figure 1), again demonstrating that the obtained model performed well.

Subgroup analysis of the discriminant scores

From the point of view of cancer screening, attention might be paid to whether or not the obtained model also provides sufficient discriminating power to extract effectively patients with early-stage cancer and for all histological types. Thus, to investigate the consistency of the results based on the discriminant scores among different subpopulations defined by cancer stage and histological type, a subgroup analysis was performed using both study data set and the test data set. The discriminant scores of the SCLC patients were also calculated to verify whether the obtained model could discriminate them from the controls.

Interestingly, it was suggested that the model could discriminate lung cancer patients regardless of cancer stage or histological type. Using the discriminant scores, the ROC_AUCs were 0.796 (study data set) and 0.817 (test data set) for stage I patients, 0.906 (study data set) and 0.801 (test data set) for stage II patients, 0.823 (study data set) and 0.843 (test data set) for stage III patients, and 0.836 (study data set) and 0.713 (test data set) for stages IV patients (Figure 2A, B). The model would thus be expected to be effective in detecting early, as well as advanced, cancers. We also demonstrated that the model could detect both adenocarcinomas and other histological types of cancer equally well: the ROC_AUCs were 0.795 (study data set) and 0.796 (test data set) for adenocarcinoma, and 0.860 (study data set) and 0.892 (test data set) for squamous cell carcinoma (Figure 2C, D). Furthermore, the distribution of the discriminant scores for SCLC patients was similar to that for NSCLC patients, with a ROC_AUC of 0.877 (Figure 2D).

Table 2 Plasma amino-acid concentration

Amino acid	Plasma concentration, μM				
	Patients (n = 141)		Controls (n = 423)		*p* value
	Mean	SD	Mean	SD	
Thr	115.6	28.6	115.9	25.4	0.92
Ser	117.1	19.9	111.4	17.9	0.003
Asn	45.1	8.2	44.8	7.2	0.72
Glu	46.7	19.4	45.7	19.5	0.60
Gln	580.5	93.3	587.9	83.5	0.40
Pro	168.0	43.6	150.9	41.4	< 0.001
Gly	263.6	63.2	237.0	57.3	0.000
Ala	422.3	97.4	383.7	88.9	0.000
Cit	34.4	10.6	33.2	8.3	0.20
ABA	24.2	8.4	23.2	6.9	0.20
Val	244.8	47.5	239.8	46.1	0.28
Met	29.4	6.1	28.0	5.2	0.013
Ile	84.3	22.1	69.7	17.5	< 0.001
Leu	131.8	34.3	122.4	27.6	0.003
Tyr	80.7	15.6	75.8	15.5	0.001
Phe	67.9	12.2	63.9	11.2	0.001
His	77.3	15.0	80.8	10.7	0.010
Trp	59.3	12.0	59.8	10.9	0.67
Orn	67.6	19.7	54.4	12.3	< 0.001
Lys	211.5	36.2	200.3	34.1	0.001
Arg	101.3	21.6	98.1	17.8	0.12

Study data set was used.

Table 3 PCA of plasma amino-acid profile of study data set

	PC1	PC2	PC3	PC4	PC5
Thr	-0.204	**0.266**	-0.187	0.096	-0.100
Ser	-0.123	**0.400**	-0.016	**-0.240**	**-0.369**
Asn	-0.211	**0.296**	-0.269	-0.040	-0.031
Glu	-0.146	**-0.374**	0.089	0.072	-0.132
Gln	-0.128	**0.290**	-0.032	0.078	0.169
Pro	-0.211	-0.064	0.153	**0.490**	-0.214
Gly	0.021	**0.384**	0.154	0.073	**-0.435**
Ala	**-0.240**	-0.057	-0.136	**0.295**	-0.220
Cit	-0.138	0.214	**0.379**	0.141	**0.394**
ABA	-0.177	-0.002	-0.033	**-0.396**	-0.212
Val	**-0.287**	-0.190	0.033	-0.235	-0.034
Met	**-0.309**	0.095	-0.101	0.052	0.060
Ile	**-0.294**	**-0.226**	0.205	-0.127	-0.118
Leu	**-0.304**	**-0.228**	0.054	**-0.258**	-0.029
Tyr	**-0.269**	-0.089	-0.005	**0.353**	-0.086
Phe	**-0.240**	-0.117	-0.066	0.005	0.163
His	-0.188	0.088	**-0.484**	-0.010	**0.256**
Trp	**-0.231**	-0.109	**-0.328**	0.090	0.112
Orn	-0.219	0.104	**0.427**	0.041	-0.053
Lys	**-0.231**	0.075	0.153	**-0.367**	0.107
Arg	-0.176	**0.225**	**0.256**	0.020	**0.421**
Eigenvalue	5.897	2.346	1.369	1.214	1.167
p value	< 0.001	0.99	< 0.001	0.23	< 0.001

PCs that had eigenvalues of >1 are indicated. Bold numbers indicate the amino acids of those PCs extracted that had a variance of >0.05. The Mann-Whitney *U*-test *p* values for comparisons of each PC score between the controls and NSCLC patients are shown.

Discussion

Lung cancer has been the leading cause of cancer death since 1998 and >60,000 patients have died since 2005 in Japan. The 5-year survival rate for patients undergoing surgery is only 61%, and an accurate screening method for lung cancer would be an important advance [20]. In Japan, chest X-rays and sputum cytology are used for screening lung cancer. Although chest X-rays are useful for detecting peripheral lung cancer, two-thirds of patients diagnosed in this way have associated metastases, and this method is not sufficient to detect the early stages of the disease [21]. In addition, highly skilled staffs are required to achieve sufficient accuracy. Sputum cytology might be useful for detecting upper respiratory-tract carcinoma, but this method has been reported to be inadequate for detecting peripheral lung cancer and lung cancer in asymptomatic non-smokers [21]. Recently, low-dose helical computed tomography (CT) was reported to be capable of detecting small, early lung cancers in high-risk populations; however, it is not known whether using this method would affect the mortality rate due to lung cancer or whether it would be cost-effective [22].

In comparison to those methods, the "AminoIndex" would be easier to use, as it involves a relatively simple plasma assay, imposes a lower physical burden on patients and does not require advanced technical skills to perform [12]. The current study demonstrated that plasma amino acid profiles were associated with NSCLC. The ROC_AUCs were 0.817 for the study data

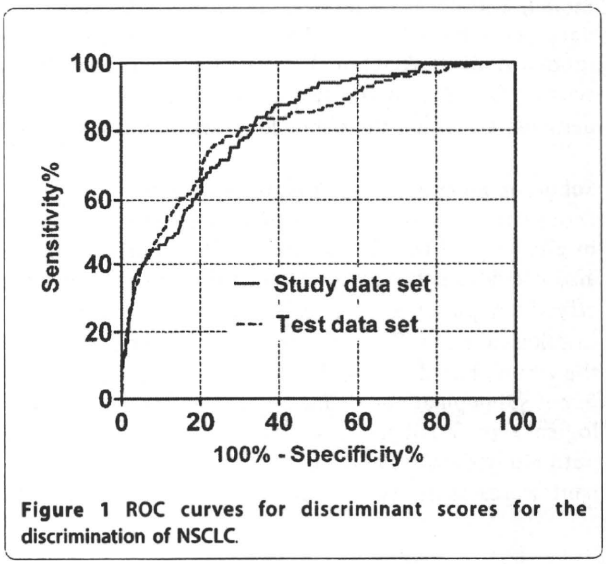

Figure 1 ROC curves for discriminant scores for the discrimination of NSCLC.

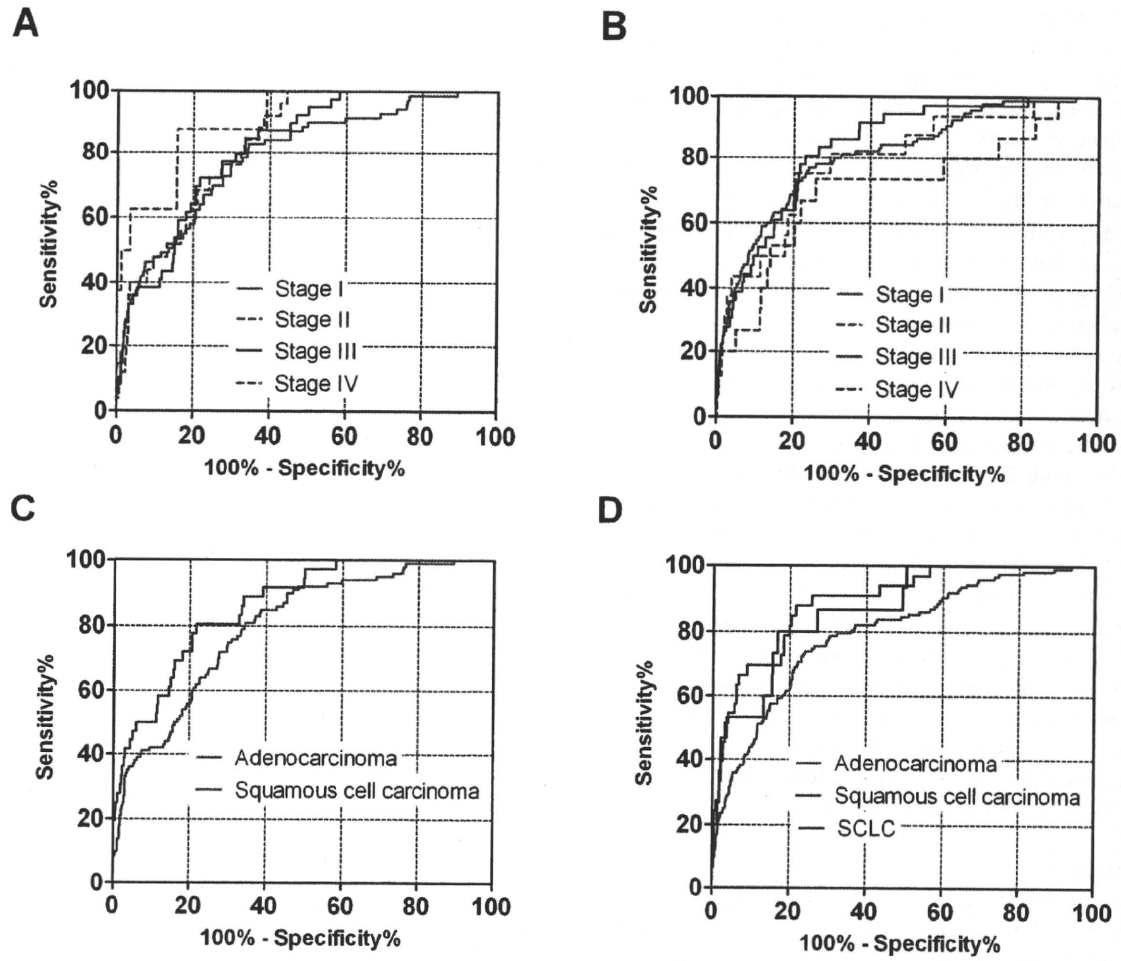

Figure 2 ROC curves for discriminant scores subgrouped by cancer stage and histological type. A. ROC curves for cancer stage of study data set. B. ROC curves for cancer stage of test data set. C. ROC curves for histological type of study data set. D. ROC curves for histological type of test data set (including SCLC patients).

set under the conditional logistic regression analysis conditioned on the matching factors (Figure 1). Okamoto et al. recently reported that plasma amino-acid profiles might be used to screen colorectal and breast cancer [23]. Despite the smaller sample size, they reported ROC_AUCs of 0.860 (with study data) and 0.910 (with test data) for colorectal cancer patients, and 0.906 (with study data) and 0.865 (with test data) for breast cancer patients [23]. Our current study achieved similar discrimination power using data set with a larger sample size under controlling for potential confounders, thereby demonstrating the robustness of the model.

Many reports have shown that the metabolism, including that of amino acids, is notably altered in cancer cells [4,24-26], and that the plasma amino-acid profiles are also changed [15,16,27-30]. Cascino et al. described significant increases in levels of Trp, Glu and Orn in lung cancer patients [15]. Proenza and colleagues also reported an increased level of Orn in patients with lung cancer [29]. Naini et al. reported reduced levels of plasma Arg in lung cancer patients [31].

Changes in the amino-acid balance and an increase in gluconeogenesis have been well documented, especially in cachexic patients with advanced cancer [32,33]. In the current study, the obtained model identified patients at all stages of lung cancer and without cachexia equally well, suggesting that the method did not rely on detecting metabolic abnormalities associated with malnutrition, which might be present in advanced cancer patients (Figure 2A, B). Hirayama et al. reported no

significant correlation between the levels of metabolites, including several amino acids, and the patients' tumour stage [24]. And it was also reported that amino acids were frequently identified compounds among whole metabolites in blood in relation to cancer [3,8]. The metabolism of specific amino acids is known to be associated with specific organs, such as muscle, liver or kidney, changes in the levels of amino acids are affected by their metabolism in, and excretion from, multiple organs of the body. Although it remains unclear how the metabolic changes occurring in tumour cells affect the systemic, plasma amino-acid profile, these results show that the metabolic changes caused by cancer development are at least partially responsible for the changes in plasma amino-acid profile seen even in lung cancer patients with early stage cancer. So, profiling the plasma free amino acids is similar to monitoring metabolic networks in multiple organs and it might better allow us to detect particular conditions in specific organs.

Since this study was designed as a case-control study, the obtained model could not be directly applied to further observation or prediction even though the robustness of the model was preliminarily demonstrated. Therefore model construction and validation using cohort with larger samples will be necessary to clarify its utility. Nonetheless, we believe that this screening technique could be a straightforward diagnostic method for the management of lung cancer.

Conclusions

The current study demonstrated that the plasma amino-acid profile of NSCLC patients differed from that of healthy subjects. And we showed that the multivariate classifier might be effective for discriminating lung cancer patients. Although further prospective validation will be necessary in the future, this method might be an effective and convenient screening tool for lung cancer patients.

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Authors' contributions

AI and HY designed this case control study. JM, MH, TN, MY, FI and KK coordinated the study and collected the background data on the subjects. HY also coordinated the study, and supervised the collection of control data. JM, TD, and AI provided data analysis and wrote the manuscript. JM, MH, AI, TN, HY, TD, MY, FI and KK provided final reviews and approval of the manuscript. All authors read and approved the final paper.

Competing interests

We declare that we are participants in the "AminoIndex" research consortium organized by Ajinomoto, and that we have all seen and approved the final version of this manuscript. Akira Imaizumi and Hiroshi Yamamoto are employees of Ajinomoto. Masahiko Higashiyama, Fumio Imamura and Akira Imaizumi have applied for patents for plasma amino-acid profiling using multivariate analysis as a diagnostic procedure.

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Phase II Study of Sequential Triplet Chemotherapy, Irinotecan and Cisplatin Followed by Amrubicin, in Patients with Extensive-Stage Small Cell Lung Cancer: West Japan Thoracic Oncology Group Study 0301

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Introduction: Combination chemotherapy of irinotecan, a topoisomerase I inhibitor, and cisplatin is a standard treatment in patients with extensive-stage small cell lung cancer (SCLC). Amrubicin, a novel 9-aminoanthracycline, inhibits topoisomerase II. We investigated a sequential triplet chemotherapy consisting of irinotecan and cisplatin followed by amrubicin in patients with extensive-stage SCLC.

Methods: Eligible patients were aged 20 to 70 years and had Eastern Cooperative Oncology Group performance status of 0 or 1, measurable lesions, and adequate organ functions. Chemotherapy consisted of irinotecan 60 mg/m^2 on days 1 and 8 plus cisplatin 60 mg/m^2 on day 1 every 3 weeks for three cycles and then amrubicin 40 mg/m^2 alone on days 1 to 3 every 3 weeks for three cycles.

Results: From September 2004 to September 2006, 45 patients were enrolled, 43 were evaluable for response and survival, and 44 were evaluable for toxicity. Twenty-eight patients (64%) completed the full planned chemotherapy. One patient achieved complete response and 33 had partial response for an overall response rate of 79%. Median progression-free survival was 6.5 months. Median overall survival was 15.4 months. Major toxicity was myelosuppression. Grade 3 or 4 neutropenia, anemia, thrombocytopenia, and febrile neutropenia occurred in 57%, 7%, 0%, and 7% of patients during irinotecan/cisplatin cycles and in 91%, 27%, 9%, and 15% of patients during amrubicin cycles, respectively.

Conclusions: The sequential triplet chemotherapy, irinotecan and cisplatin followed by amrubicin, is an effective and well-tolerated treatment in patients with extensive-stage SCLC. Further investigation of this treatment is warranted.

Key Words: Amrubicin, Small cell lung cancer, Sequential chemotherapy, Triplet chemotherapy.

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Small cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers. Disease extension of SCLC is classified as limited stage or extensive stage. Limited-stage SCLC is defined as tumor confined to the hemithorax of origin, the mediastinum, and the supraclavicular lymph nodes, whereas extensive-stage SCLC as tumor spread outside these limits. For extensive-stage SCLC, chemotherapy is the mainstay of treatment. SCLC is highly sensitive to chemotherapy, with a response rate of 70% to 90% in first-line treatment. However, for most patients with extensive-stage SCLC, the disease recurs within several months, and the 5-year survival rate is less than 1%.[1] It is necessary to develop a new treatment for this serious disease.

Irinotecan, a derivative of camptothecin, inhibits topoisomerase I and shows strong antitumor effect for SCLC. The Japan Clinical Oncology Group conducted a randomized phase III trial (JCOG 9511) comparing irinotecan plus cis-

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