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Clinical Relevance of Sputum Cytology and Chest X-Ray in Patients with Suspected Lung Tumors

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Abstract

Objective To review diagnostic procedures, therapeutic modalities, and follow-up methods in patients with suspected lung tumors.

Methods We retrospectively examined 70 patients who underwent a complete medical checkup because they had been positive for sputum cytology and had presented no chest X-ray findings for the 10-year period between 1994 and 2004. To make a diagnosis, we conducted the first complete medical checkup that included chest X-ray, sputum cytology, chest computed tomography (CT), and bronchoscopy. In the case that no diagnosis could be made, we repeated the chest X-ray and sputum cytology every 3 to 6 months and additionally conducted chest CT and bronchoscopy according to abnormal findings.

Results Among 70 patients, there were 36 and 13 who were diagnosed during the first complete medical checkup and follow-up, respectively, 13 who remained undiagnosed, and eight for whom follow-up was discontinued. Among the 49 diagnosed patients, 40, 8, and 1 patient had lung cancer, upper respiratory tract carcinoma (URTC), and esophageal carcinoma (EC), respectively. Among the 40 patients with lung cancer, 34 had a stage 0 or I tumor and 15 were radically treatable by photodynamic therapy and endobronchial irradiation. Nine among 11 patients whose lung cancer was detected during follow-up had a stage 0 or IA tumor.

Conclusion Not only lung cancer but also URTC and EC were successfully detected in patients who were positive for sputum cytology and presented negative chest X-ray. Radical treatment was possible in 38 (76%) of 50 diagnosed patients, thus indicating the importance of follow-up through these procedures.

Key words: sputum cytology, roentgenographically occult lung cancer, photodynamic therapy, endobronchial irradiation

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Introduction

Lung cancer is a disorder with a poor prognosis because it is rarely detected in a condition where radical treatment can be initiated. Therefore, sorts of efforts are made for its early detection. Among patients whose lung cancer was detected during mass screening only by chest X-ray, two-thirds of them present an advanced or metastatic lesion (1). Hence, mass screening by chest X-ray alone is not considered sufficient for the early detection of lung cancer. Large-scale randomized clinical trials in Europe and the United States (2-6) have not revealed the lung cancer mortality-reducing effect of mass screening for lung cancer that combine chest X-ray to detect peripheral lung cancer with sputum cytology to detect central lung cancer. Therefore, medical institutions in Europe and the United States do not officially recommend the screening. However, case-control studies in Japan (7-10) have demonstrated the lung cancer mortality-decreasing effect of screening, warranting its conduct in Japan.

Screening based on the Elderly Health Law in Japan include chest X-ray for males and females aged 40 years or over, as well as mass screening for lung cancer consisting of sputum cytology for individuals aged 50 years or over who

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Table 1. Patient Characteristics (n=70)

Gender	
Male/female gender, No.	66/4
Age (median), yr	26-81 (69)
Smoking history	
Smoker, No.	55
Ex-smoker, No.	12
Nonsmoker, No.	3
Brinkmann Index (median)	2.25-175 (50) packs/year
≥ 30 packs/year	63
< 30 packs/year	4
Motive of detection	
Mass screening for lung cancer, No.	34
Subjective symptoms, No.	28
Follow-up of another disorder, No.	8
Sputum cytology	
Squamous cell carcinoma, No.	60
Adenocarcinoma, No.	10

Brinkmann index: (Number of cigarette packs per day)×(Number of years the subject has smoked)

have a Brinkmann index of ≥30 packs/year and for symptomatic individuals presenting with bloody sputum (11). Sputum cytology is considered an important screening procedure for lung cancer in heavy smokers. A few study reports are available that have described the follow-up of patients who were positive for sputum cytology and presented no chest X-ray findings (12-14).

Materials and Methods

Diagnostic procedures, therapeutic modalities, and follow-up methods were reviewed retrospectively in 70 patients who provided positive results in sputum cytology or screening for lung cancer; due to the lack of chest X-ray findings, they underwent a medical examination in the Department of Respiratory Medicine at Osaka City General Hospital for the last 10 years between 1994 and 2004. Among 70 patients, there were 65 males and 5 females aged 26 to 81 years (median: 69). Regarding smoking history, there were 55 smokers, 12 ex-smokers, and three nonsmokers. The Brinkmann index was 2.25 to 175 packs/year (median: 50) (Table 1).

To make a diagnosis, the first complete medical checkup (X-ray according to the direct method, repeated sputum cytology using pooled sputum, chest CT, and bronchoscopy) was conducted in the above patents. In the case that no diagnosis could be made, chest X-ray and sputum cytology using pooled sputum were repeated every 3 to 6 months. Chest computed tomography (CT) and bronchoscopy were conducted additionally according to abnormal findings. Chest X-ray and sputum cytology were continued wherever possible until making a diagnosis. Furthermore, the clinical stages were determined in accordance with the NCCN guideline (15). A lesion, about which carcinoma *in situ* was considered and whose diagnosis was made not by resected specimen examination but by bronchoscopic biopsy only, could not be diagnosed as a stage 0 tumor unless the biopsy specimen successfully verified the invasion of the basal membrane. Therefore, the lesion was staged 0-IA.

Sputum cytology

Sputum cytology was conducted according not to Saccomanno's method but to the sputum pooling method using sputum that had been pooled for 3 days and the pooling solution that had been improved by the addition of mucosa-dissolving agent and other compounds. The Japan Lung Cancer Society has established the evaluation criteria and guidance for sputum cytology in mass screening for lung cancer according to the pooling method (11). In the present study, cells were assessed in accordance with the criteria, and two professionals (one cytotechnologist and one pathologist) made a diagnosis.

Chest X-ray

Chest X-ray (posteroanterior and lateral) according to the direct method was conducted. Roentgenograms were read by the pneumologist. Chest roentgenograms indicating old lung tuberculosis in fixed foci, pneumoconiosis, lung asbestosis, and pulmonary fibrosis were considered not to show abnormal findings.

Chest CT

CT using the 1-cm slice low-frequency algorithm was conducted to image the region from the upper portion of the clavicle to the level immediately above the diaphragm. One radiologist and one pneumologist read the lung fields and mediastinum. Chest tomograms indicating old lung tuberculosis in fixed foci, pneumoconiosis, lung asbestosis, and pulmonary fibrosis were considered not to show abnormal findings.

Bronchoscopy

After the intramuscular injection of opium alkaloid hydro-

chloride and atropine sulfate, 4% lidocaine hydrochloride was injected to anesthetize the pharynx. Subsequently, the bronchoscopist used a bronchoscope (Olympus BF-200, Tokyo, Japan) to observe the oral cavity, larynx, pharynx, vocal cord, trachea, and at least the third-order bronchi. Abnormal findings were assessed according to the Japanese rule (16). When an abnormality of the bronchial mucosa was found in the region visible to the bronchoscope, bronchoscopic biopsy was conducted actively. When the bronchoscopy was difficult to conduct despite its location in the visible field, the lesion was scraped with a brush. When tests were completed without finding any abnormalities, the sputum collected after the completion of bronchoscopy was subjected to cytology.

Results

The motives for the detection of positive findings in sputum cytology were as follows: mass screening for lung cancer in 34 patients; subjective symptoms in 28 patients; and screening for another disorder in eight patients. Regarding the histological types of the tumor in sputum cytology, there were 60 cases of squamous cell carcinoma (SCC) and 10 cases of adenocarcinoma (AC).

Among 70 patients, there were 36 and 13 who were diagnosed during the first complete medical checkup and follow-up, respectively, 13 who remained undiagnosed, and eight patients for whom follow-up was discontinued, i.e., seven patients at their discretion and one patient who showed deteriorating symptoms of another disease at 3 months (Fig. 1).

Thirty-one patients, who were diagnosed during the first complete medical checkup, had a lesion that was located in the region visible to the bronchoscope: 24 with smoking history had lung cancer and seven had upper respiratory tract carcinoma (URTC) (Table 2). The lesion was located in the subsegmental branches inclusive as follows: 0-order bronchi, three patients; first-order bronchi, six patients; second-order bronchi, 10 patients; and third-order bronchi, five patients. Bronchoscopic findings: hypertrophy, 14 patients; nodule, six patients; and polyp, four patients. Regarding histopathology, all but one case of AC were SCC. Simultaneously, there was one patient with double carcinoma.

There were five patients in whom chest CT revealed an abnormality despite the lack of abnormal findings on chest X-ray during the first complete medical checkup. Among them, three patients had lung field mass shadow, one patient had lung field stripes, and one patient had mediastinal lymphadenopathy without lung field lesion. The mass was located at a site that was difficult to discriminate unless conducting chest CT.

Regarding clinical stages and treatment of 24 patients with lung cancer in whom the first complete medical checkup led to a diagnosis under TBB: 13, 9, one, and one had a tumor staged 0-IA, IA, IIIA, and IIIB, respectively. Treatment was photodynamic therapy (PDT) in seven among 13 patients with a stage 0-IA tumor and was endobronchial irradiation in five among them. Another remaining patient

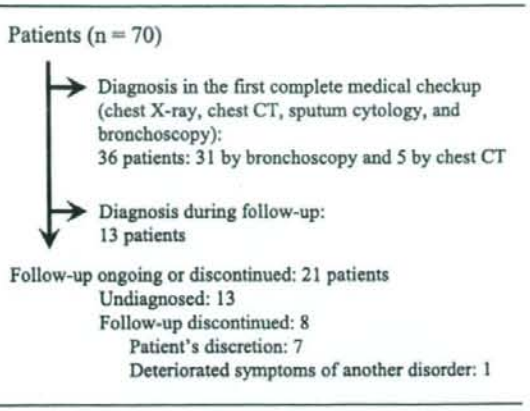

Figure 1. Clinical course until making a definite diagnosis. To make a definite diagnosis in 70 patients who provided positive results in sputum cytology and presented no chest x-ray findings, posteroanterior and lateral x-rays of the chest, sputum cytology using pooled sputum, computed tomography of the chest, and bronchoscopy were conducted as the first complete medical checkup. When no definite diagnosis was obtained, chest x-ray and sputum cytology (pooled sputum method) were repeated every 3 to 6 months.

survived for 5 years and 6 months by external irradiation (EI). We conducted PDT for one SCC and then EI to another SCC in a patient with double cancer, and two sites of them had a complete response. All nine patients with a stage IA tumor underwent surgery, with five assigned to lobectomy, two to segmentectomy, and two to sleeve lobectomy (Table 2).

Five patients with lung cancer in whom chest CT revealed abnormal shadows had tumors categorized to the following clinical stages: IA, one patient; IB, two patients; IIIB, one patient; and IV, one patient. A patient with a stage IA tumor and the patients with a stage IB tumor underwent surgery. Treatment was restricted only to EI toward the mediastinum in the patient with a stage IIIB tumor because of the previous follow-up for chronic disseminated intravascular coagulation (DIC) in Department of Hematology. The patient with a stage IV tumor developed cardiac tamponade due to cancerous pericarditis before receiving anticancer agents and underwent palliative care (Table 3).

EI alone was conducted in five of eight patients with URTC, and all of them had a complete response. In one patient disease recurred after surgery for URTC and was transferred to the hospital where the surgery had been conducted. Another remaining patient underwent tumor resection after preoperative chemotherapy and presented short survival despite subsequent radiation therapy (Tables 2, 4).

Among patients in whom the first complete medical checkup did not lead to a diagnosis, a diagnosis was made subsequently in 13 patients during follow-up, the breakdown of whom was as follows: 11 patients with lung cancer; one patient with esophageal carcinoma (EC); and one patient

Table 2. Direct Views Available by Bronchoscopy in the First Complete Medical Checkup (n=31)

Lung cancer	24
Orders of branching Zero-order: 3; first-order: 6; second-order: 10; third-order: 5 Bronchoscopic findings hypertrophy: 14; node: 6; polyp: 4 Histopathology SCC: 22; adenocarcinoma: 1; double carcinoma (SCC at two sites): 1 Clinical stages of disease 0-IA: 13; IA: 9; IIIA: 1; IIIB: 1	
Upper respiratory tract carcinoma	7

SCC: squamous cell carcinoma

Table 3. Patients with Abnormal Chest CT Findings in the First Complete Medical Checkup (n=5)

CT Findings	Histopathology	Diagnostic Method	Clinical Stage	Treatment
Lung field mass shadow in rt-S^1	SCC	TBB	IB	S (lobectomy)
Lung field mass shadow in rt-S^2	SCC	CT-guide	IA	S (lobectomy)
Lung field mass shadow in lt-S^6	SCC	TBB	IB	S (lobectomy)
Lung field stripes	AC	Brushing	IV	BSC
Mediastinum lymphadenopathy	AC	TBAC	IIIB	EI

rt: right, lt: left, SCC: squamous cell carcinoma, AC: adenocarcinoma, TBB: transbronchial biopsy, CT-guide: Computed tomography-guided lung biopsy, TBAC: transbronchial aspiration cytology, S: surgery, BSC: best supportive care, EI: external irradiation

with URTC. In all the patients who had smoking history, the histopathological types of the tumors were identical between the first and second sputum cytologies. After conducting the first medical checkup, patients were followed by sputum cytology and chest X-ray. Bronchoscopy revealed abnormal findings in six patients. In six patients, chest CT indicated mass shadows. In one patient, a diagnosis was made under gastroendoscopy to closely examine hematemesis of unknown etiology. Regarding bronchoscopic findings in lung cancer, four and one had nodular and polyp tumors, respectively. The period until making a diagnosis was 2 to 47 months (median, 12). In 10 among 13 patients, a diagnosis was made within 1 year. Among 11 lung cancer patients in whom a diagnosis was made during follow-up, five and four had a stage 0-IA tumor and a stage IA tumor, respectively. In eight patients of the 11 lung cancer patients [PDT, four patients; and surgery, four patients (lobectomy: three patients and segmentectomy: one patient)], radical treatment was conducted successfully. Active treatment was not desired for one patient because of symptoms of dementia, and thus palliative care was provided (Table 4).

Currently, 13 patients are under follow-up because of failure to make a diagnosis. The median value for observation period is 28 months. The histopathological types of their tumors in sputum cytology were SCC in 10 patients and AC in three patients. Sputum cytology during follow-up was negative in most patients. However, the procedure was positive once in two patients during follow-up but was not positive thereafter. In one patient, furthermore, the procedure revealed suspicious findings only once. Among eight patients about whom follow-up was discontinued, the clinical course was difficult to investigate in two patients who failed to visit the hospital in order to undergo a medical examination immediately after the first complete medical checkup. However, the remaining six patients were followed for 3, 9, 17, 24, 61, and 83 months.

The contents of the examinations during follow-up were compared between 13 patients in whom a diagnosis was made during follow-up and 19 undiagnosed patients (13 patients under follow-up and six patients for whom follow-up was discontinued, except for two patients who failed to undergo a medical examination after the first complete medical

Table 4. Clinical Profiles of Patients Who Were Diagnosed during Follow-Up (n=13)

Diagnosis	Method	Period (month)	Order of Bronchus	Bronchoscopic Finding	Tumor Diameter	Clinical Stage	Treatment
Lung cancer (SCC)	Direct view	7	I	Node	-	0-IA	PDT
Lung cancer (SCC)	Direct view	8	III	Node	-	0-IA	PDT
Lung cancer (SCC)	Direct view	9	III	Node	-	0-IA	PDT
Lung cancer (SCC)	Direct view	27	IV	Polyp	-	0-IA	PDT
Lung cancer (SCC)	Direct view	47	I	Node	-	0-IA	BSC
Lung cancer (AC)	CT	3	-	-	28 mm	IA	S
Lung cancer (AC)	CT	4	-	-	20 mm	IA	S
Lung cancer (AC)	CT	6	-	-	12 mm	IA	S
Lung cancer (SCC)	CT	12	-	-	10 mm	Unknown	BSC
Lung cancer (AC)	CT	20	-	-	25 mm	IV	BSC
Lung cancer (SCC)	CT	33	-	-	15 mm	IA	S
URTC	Direct view	2	-	-	-	II	S + EI
EC	Gastroscopy	8	-	-	-	IIb	S

SCC: squamous cell carcinoma, AC: adenocarcinoma, CT: computed tomography, PDT: photodynamic therapy, S: surgery, EI: external irradiation, BSC: best supportive care, URTC: upper respiratory tract carcinoma, EC: esophageal carcinoma

Table 5. Follow-Up Period and Medical Examination of Patients under Follow-Up

	Diagnosis (n = 13)	Undiagnosis (n = 19)
Follow-up period (median), mth	2-47 (12)	3-83 (28)
Smoker, No.	10	12
Ex-smoker, No.	3	3
Nonsmoker, No.	0	3
Examination contents		
Chest X-ray (median), No.	0-8 (4)	1-11 (5)
Sputum cytology (median), No.	1-12 (3)	1-14 (4)
Sputum cytology-positive patients, %	20	4.7
Chest CT (median), No.	0-3 (1)	0-5 (1)
Bronchoscopy (median), No.	0-6 (2)	0-4 (1)

Chest CT: chest computed tomography

checkup). Consequently, a trend for difference was found only in the positivity rate of sputum cytology (Table 5). The clinical stages and treatment of 50 patients in whom a diagnosis could be made are shown in Table 6.

Discussion

In the course of mass screening for lung cancer, many patients are referred to a hospital for the purpose of undergoing a complete medical checkup because they were positive for sputum cytology and presented no chest X-ray findings. However, a definite diagnosis is difficult to make in such patients, they are frequently difficult to follow up, and there are a limited number of papers of reference (12-14).

We conducted relatively inexpensive sputum cytology and chest X-ray as procedures for follow-up at 3- to 6-month intervals and performed bronchoscopy as required when sputum cytology was positive. Among 11 patients whose lung cancer was detected during follow-up at these 3- to 6-month intervals, five and four had stage 0-IA and IA tumors, respectively. The conceivable reasons why the first complete medical checkup failed to determine the presence of tumors

Table 6. Clinical Stages and Treatments at the Time of Diagnosis (n=49)

Lung cancer		
0-IA (n = 17)	Bronchoscopic treatment (PDT: 10; endobronchial irradiation: 5)	15
	External irradiation	1
	BSC	1
IA (n = 14)	Surgery (lobectomy: 9; segmentectomy: 3 sleeve lobectomy: 2)	14
IB (n = 2)	Surgery (lobectomy: 2)	2
IIIA (n = 1)	Chemotherapy + surgery	1
IIIB (n = 2)	Chemotherapy + external irradiation	1
	External irradiation	1
IV (n = 2)	BSC	2
Double cancer	PDT + external irradiation (0-IA at two sites)	1
Unknown	BSC	1
Upper respiratory tract carcinoma (n = 8)		8
Esophageal cancer (n = 1)		1

PDT: photodynamic therapy, BSC: best supportive care

are as follows: central lung cancers were possibly detected in a super early detection stage where they were unverifiable under bronchoscopy; and peripheral lung cancers were difficult to identify when present in a mixture of old tuberculosis of lung, pneumoconiosis, asbestosis, pulmonary fibrosis, and other disorders.

The NCCN guideline also recommends the repetition of bronchoscopy at 3-month intervals in patients who underwent surgery for lung cancer, for whom its recurrence is considered based on positive findings in sputum cytology and on negative chest X-ray and chest CT (17). Therefore, we deemed that follow-up at 3-month intervals during the clinical course observation period involved no concerns.

There are 21 patients whose follow-up is ongoing or discontinued. In advanced SCC, many cancer cells appear in specimens; therefore, cytodiagnosis is easy to make because these cancer cells present strong atypia. However, the number of appearing cancer cells is small in early SCC. Furthermore, these cancer cells present poor atypia and it may be difficult to differentiate them from atypical metaplasia of squamous cells attributable to the inflammation of bronchial epithelium or from atypical cells originating from atypical epithelium and other tissues (17, 26). Furthermore, a patient with adenocarcinoma of the lung who experienced pulmonary infarction provided false-positive results in sputum cytology (27). Two of three patients with undiagnosed adenocarcinoma in the present study had a history of chest pain, leading us to presume that they possibly had false-positive results in sputum cytology. Therefore, patients with concerns about the precision of sputum cytology are possibly included in 13 patients under follow-up. This possibility seems to denote the limitation of sputum cytology that makes a diagnosis by taking cell degeneration into consideration.

One issue regarding the patients for whom follow-up was discontinued is that they easily forgot to undergo a regular medical examination because they had no symptoms despite providing positive results in sputum cytology. When no definite diagnosis was obtained in the first complete medical checkup, we noted that they tended not to undergo a medical examination again unless receiving a full explanation about the need for regular follow-up.

In recent years, marked progress in medical devices has been noted. There are reports which have described that ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) is effective for the early detection of lesions in the lower respiratory tract (18, 19). However, FDG-PET is difficult to include in routine medical tests due to its high costs. As compared with FDG-PET, sputum cytology and chest X-ray remain attractive because they are inexpensive.

Regarding therapeutic outcomes in the present study, 33 patients with lung cancer [0-IA + IA+ IB, excluding patients receiving best supportive care (BSC)] eventually underwent radical treatment; 15 of them, in whom early lung cancer had been detected bronchoscopically, could be radically treated by endoscopic procedures (PDT and endobronchial irradiation). Fujimura et al recommended the adoption of their strategies for patients with roentgenographically occult lung cancer and obtained favorable results (12, 20-22). Unlike surgical therapy, radical treatment without provoking a decrease in pulmonary function will be achieved as described above if treating eligible patients for whom PDT and endobronchial irradiation are expected to provide radical treatment (23-25).

Therefore, endoscopic treatment constitutes the best therapeutic modality. However, we believe that active search combining sputum cytology suitable for central lung cancer with chest X-ray suitable for peripheral lung cancer is important in such patients because they may include patients with a radically treatable tumor. In addition, we consider that sputum cytology serves not only for the early detection of central lung cancer—the original objective of sputum cy-

tology, but also for the detection of cancers of other organs (especially URTC) and of peripheral lung cancer.

Conclusion

Not only lung cancer but also URTC and EC were successfully detected in patients who showed positive results in sputum cytology and presented a negative chest X-ray. Radical treatment was possible in 38 (76%) of 50 diagnosed patients, thus indicating the importance of follow-up through these procedures.

References

1. Silverberg E, Boring CC, Squires TS. Cancer statistics, 1990. CA Cancer J Clin **40**: 9-26, 1990.
2. Frost JK, Ball WC Jr, Levin ML, et al. Early lung cancer detection: Results of the initial (prevalence) radiologic and cytologic screening in the John Hopkins study. Am Rev Respir Dis **130**: 549-554, 1984.
3. Flehinger BJ, Melamed MR, Zaman MB, Heelan RT, Perchick WB, Martini N. Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Memorial Sloan-Kettering study. Am Rev Respir Dis **130**: 555-560, 1984.
4. Fontana RS, Sanderson DR, Woolner LB, et al. Screening for lung cancer: a critique of the Mayo Lung Project. Cancer **67**: 1155-1164, 1991.
5. Kubik A, Polak J. Lung cancer detection results of a randomized prospective study in Czechoslovakia. Cancer **57**: 2427-2437, 1986.
6. Marcus PM, Bergstralh EJ, Fagerstrom RM, et al. Lung cancer mortality in the Mayo Lung Project. J Natl Cancer Inst **92**: 1308-1316, 2000.
7. Okamoto N, Suzuki T, Hasegawa H, et al. Evaluation of a clinic-based screening program for lung cancer with a case-control design in Kanagawa, Japan. Lung Cancer **25**: 77-85, 1999.
8. Tsukada H, Kurita Y, Yokoyama A, et al. An evaluation of screening for lung cancer in Niigata Prefecture, Japan: a population-based case-control study. Br J Cancer **85**: 1326-1331, 2001.
9. Sagawa M, Tsubono Y, Saito Y, et al. A case-control study for evaluating the efficacy of mass screening program for lung cancer in Miyagi Prefecture, Japan. Cancer **92**: 588-594, 2001.
10. Nishii K, Ueoka H, Kiura K, et al. A case-control study of lung cancer screening in Okayama Prefecture, Japan. Lung Cancer **34**: 325-332, 2001.
11. The Japan Lung Cancer Society. General Rule for Clinical and Pathological Record of Lung Cancer. 2nd ed. Kanehara & Co., Ltd., Tokyo, 2003: 177-186.
12. Fujimura S, Sakurada A, Sagawa M, et al. A therapeutic approach to roentgenographically occult squamous cell carcinoma of the lung. Cancer **89**: 2445-2448, 2000.
13. Bechtel JJ, Petty TL, Saccomanno G. Five year survival and later outcome of patients with X-ray occult lung cancer detected by sputum cytology. Lung Cancer **30**: 1-7, 2000.
14. Bechtel JJ, Kelly WR, Petty TL, Patz DS, Saccomanno G. Outcome of 51 patients with roentgenographically occult lung cancer detected by sputum cytologic testing: a community hospital program. Arch Intern Med **154**: 975-980, 1994.
15. NCCN Practice Guidelines in Oncology-v.2. 2008. (Non-Small Cell Lung Cancer) http://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf Accessed: 1 October 2007.
16. Ono R. Indications for bronchoscopic brachytherapy and conditions for irradiation. Ono R. Brachytherapy. Nakayama-Shoten Co., Ltd., Tokyo, 1995: 65-78.
17. Saccomanno G. Diagnostic Pulmonary Cytology. 2nd ed. American College of Clinical Pathologists, Chicago, IL, 1986.
18. Watanabe S, Tanaka D, Nakamura Y, et al. Occult cancer detected by positron emission tomography/computed tomography image fusion. Anticancer Res **25**: 459-461, 2005.
19. Pasic A, Brokx HA, Comans EF, et al. Detection and staging of preinvasive lesions and occult lung cancer in the central airways with 18F-fluorodeoxyglucose positron emission tomography: a pilot study. Clin Cancer Res **11**: 6186-6189, 2005.
20. Endo C, Sagawa M, Sato M, et al. What kind of hilar lung cancer can be a candidate for segmentectomy with curative intent?: Retrospective clinicopathological study of completely resected roentgenographically occult bronchogenic squamous cell carcinoma. Lung Cancer **21**: 93-97, 1998.
21. Sagawa M, Koike T, Sato M, et al. Segmentectomy for roentgenographically occult bronchogenic squamous cell carcinoma. Ann Thorac Surg **71**: 1100-1104, 2001.
22. Saito Y, Nagamoto N, Ota S, et al. Results of surgical treatment for roentgenographically occult bronchogenic squamous cell carcinoma. J Thorac Cardiovasc Surg **104**: 401-407, 1992.
23. Furuse K, Furoka M, Kato H, et al. A prospective phase II study on photodynamic therapy with Photofrin II for centrally located early-stage lung cancer. The Japan Lung Cancer Photodynamic Therapy Study Group. J Clin Oncol **11**: 1852-1857, 1993.
24. Sutedja G, Baris G, van Zandwijk N, Postmus PE. High-dose rate brachytherapy has a curative potential in patients with intraluminal squamous cell lung cancer. Respiration **61**: 167-168, 1994.
25. Edell ES, Cortese DA. Photodynamic therapy in the management of early superficial squamous cell carcinoma as an alternative to surgical resection. Chest **102**: 319-322, 1992.
26. Breuer RH, Pasic A, Smit EF, et al. The natural course of preneoplastic lesions in bronchial epithelium. Clin Cancer Res **11**: 537-543, 2005.
27. Kaminsky DA, Leiman G. False-positive sputum cytology in a case of pulmonary infarction. Respir Care **49**: 186-188, 2004.

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Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: An analysis of Japan Multinational Trial Organisation LC00-03

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ABSTRACT

We examined the impact of pretreatment neutrophil count on survival in patients with advanced non-small-cell lung cancer (NSCLC). A total of 388 chemo-naïve patients with stage IIIB or IV NSCLC from a randomised controlled trial were evaluated. The effects of pretreatment peripheral blood neutrophil, lymphocyte and monocyte counts and neutrophil–lymphocyte ratio on survival were examined using the proportional hazards regression model to estimate hazard ratios after adjustment for covariates. The optimal cut-off value was determined by proportional hazards regression analysis with the minimum P-value approach and shrinkage procedure. After adjustment for prognostic factors, the pretreatment elevated neutrophil count was statistically significantly associated with short overall ($P = 0.0008$) and progression-free survival ($P = 0.024$), whereas no association was found between prognosis and lymphocyte or monocyte count. The cut-off value selected for neutrophil count was 4500 mm^{-3} (corrected hazard ratio, 1.67; 95% confidence interval (CI), 1.09–2.54). The median survival time was 19.3 months (95%CI, 16.5–21.4) for the low-neutrophil group (<4500 mm^{-3}, $n = 204$) and was 10.2 months (95%CI, 8.0–12.3) for the

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high-neutrophil group ($\geq 4500\ mm^{-3}$, $n = 184$). We confirmed that pretreatment elevated neutrophil count is an independent prognostic factor in patients with advanced NSCLC receiving modern chemotherapy. Neutrophil count is easily measured at low cost, and it may be a useful indicator of patient prognosis.

1. Introduction

The prognosis for patients with advanced non-small-cell lung cancer (NSCLC) (TNM stage IIIB with a positive pleural effusion, or stage IV) has improved with recent advances in systemic chemotherapy, but still remains poor, with a median overall survival time between 4 and 15 months.[1] Prognostic factors identified in previous studies include tumour stage, performance status (PS), weight loss, sex, plasma lactate dehydrogenase (LDH) level and the presence of bone, liver or skin metastases.[2] Although novel immunological and histological biomarkers have been identified, these are often time-consuming to measure, and this is not part of the standard practice.

It is now evident that inflammatory cells in the tumour microenvironment have significant effects on tumour development.[3–6] Elevation in the pretreatment neutrophil count has been proposed as a prognostic factor for poor survival in patients with metastatic renal cell carcinoma,[7–9] and elevated neutrophil, monocyte or leucocyte count has been associated with poor survival in patients with metastatic melanoma.[10,11] A high-neutrophil–lymphocyte ratio may be related to poor prognosis in patients with colorectal cancer[12] and in those with advanced gastric cancer.[13] The European Lung Cancer Working Group found that the high-neutrophil count was an independent prognostic factor for poor survival in patients with unresectable advanced NSCLC[14] and in those with small-cell lung cancer.[15] A retrospective study found that neutrophil count was of prognostic value in patients with lung cancer.[16]

The aim of this study was to examine and confirm the impact of pretreatment peripheral blood neutrophil, monocyte and lymphocyte counts on overall and progression-free survival in a well-defined population of patients with advanced NSCLC being treated with regimens using newer chemotherapeutic agents in a randomised controlled clinical trial.

2. Patients and methods

2.1. Study population

A total of 401 chemo-naïve NSCLC patients with stage IIIB with pleural effusion or stage IV without brain metastasis, who had Eastern Cooperative Oncology Group (ECOG) PS of 0 or 1, were enrolled from 45 institutions in Japan between March 2001 and April 2005 into Japan Multinational Trial Organisation LC00-03[17] (registered with ClinicalTrials.gov identifier NCT00079287). Patients underwent one of two treatment regimens: intravenous vinorelbine ($25\ mg/m^2$) plus gemcitabine ($1000\ mg/m^2$) on days 1 and 8 every 21 d for three cycles, followed by intravenous docetaxel ($60\ mg/m^2$) on day 1 every 21 d for three cycles [VGD arm, $n = 196$] versus intravenous paclitaxel ($225\ mg/m^2$) and carboplatin (area under the curve = 6) for 3 h on day 1, every 21 d for six cycles [PC arm, $n = 197$]). As there were no significant differences between treatment groups in terms of either overall (hazard ratio: 0.996, $P = 0.974$) or progression-free survival (hazard ratio: 0.966, $P = 0.742$), the combined data from the two arms were analysed in this study. Of 393 eligible patients, information regarding pretreatment neutrophils in peripheral blood was not available for five patients. Thus, data from 388 patients were included in the present study.

2.2. Statistical analysis

Overall survival was defined as the time from randomisation until death from any cause, and progression-free survival was defined as the time from randomisation until objective tumour progression or death. Survival curves were estimated with the Kaplan–Meier method. Associations between the factors and the prognosis were examined with the log-rank test in univariate analyses. The prognostic impact of pretreatment peripheral blood neutrophil, lymphocyte and monocyte counts, and neutrophil–lymphocyte ratio were examined using the proportional hazards regression model to estimate hazard ratios after adjustment for covariates without variable selection. Optimal cut-off points for continuous variables were selected using the minimum P-value approach with correction of the P-value.[18] The corrected hazard ratio and its 95% confidence interval (CI) were estimated using a shrinkage procedure with bootstrap resampling.[19] All statistical analyses were done using SAS version 9.1 (SAS Institute, Cary, NC).

3. Results

3.1. Patients' characteristics

Of 388 patients, 276 patients had died, and the median follow-up time for the 112 surviving patients was 567 d (range: 70–1711 d). The characteristics of the 388 patients (276 men [71%], 112 women [29%], median age 65 years [range, 33–81 years]) included in the present study are shown in Table 1. Median pretreatment counts of neutrophils, lymphocytes and monocytes were $4304\ mm^{-3}$, $1386\ mm^{-3}$ and $404.2\ mm^{-3}$, respectively. Spearman's rank correlations were 0.351 for neutrophils and monocytes, 0.034 for neutrophils and lymphocytes and 0.352 for monocytes and lymphocytes.

3.2. Relationship between pretreatment neutrophil, lymphocyte and monocytes counts and survival

In univariate analyses, pretreatment elevated counts of neutrophils were statistically significantly associated with short

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Table 1 – Baseline patients characteristics (n = 388).

Characteristics	No.	%
Age, years, median (range)	65 (33–81)	
Sex		
Male	276	71
Female	112	29
Smoking history		
Non-smokers	96	25
Former smokers	107	28
Current smokers	168	43
Unknown	17	4
Stage		
IIIB	68	18
IV	320	82
Histologic type		
Squamous cell	76	20
Adenocarcinoma	274	70
Others	38	10
ECOG performance status		
0	154	40
1	234	60
Weight loss (from 6 months before enrolment)		
<5%	317	82
⩾5%	71	18
LDH		
Normal (<ULN)	279	72
High (⩾ULN)	109	28
Bone metastases		
No	280	72
Yes	108	28
Liver metastases		
No	357	92
Yes	31	8
Skin metastases		
No	379	98
Yes	9	2
Neutrophils, mm^{-3}, median (range)	4304 (205–17,100)	
Lymphocytes, mm^{-3}, median (range)	1386 (243–4200)	
Monocytes, mm^{-3}, median (range)[a]	404.2 (0–1620)	
Red blood cells, $\times 10^4$ mm^{-3}, median (range)	420 (286–579)	
Platelets, $\times 10^4$ mm^{-3}, median (range)[b]	26 (11–380)	

ULN: upper limit of normal.
a One missing value.
b Two missing values.

overall (Fig. 1A, $P < 0.0001$) and progression-free survival (Fig. 1B, $P = 0.0001$). Although lymphocyte count did not correlate with survival, there were significant relationships between high-neutrophil–lymphocyte ratio and short overall ($P < 0.0001$) and progression-free survival ($P = 0.005$). The elevated monocyte count was also significantly associated with short overall survival ($P = 0.004$), and was moderately related to short progression-free survival ($P = 0.052$). We selected sex, smoking history, stage, ECOG PS, weight loss, plasma LDH and the presence of bone, liver or skin metastases as the known pretreatment prognostic factors.[2,14] Adjusted hazard ratios for the relationship between pretreatment neutrophil, lymphocyte and monocyte counts and neutrophil–lymphocyte ratio and overall and progression-free survival after adjustment for the known prognostic factors are shown in Table 2. There was a statistically significant association between elevated neutrophil count and short overall ($P = 0.0008$) and progression-free survival ($P = 0.024$), and between high-neutrophil–lymphocyte ratio and short overall ($P = 0.011$) and progression-free survival ($P = 0.040$), whereas no association was found between lymphocyte or monocyte count and prognosis. The relationship between neutrophil count and both overall and progression-free survival was linear, whereas the relationship between neutrophil–lymphocyte ratio and overall survival was to some degree non-linear.

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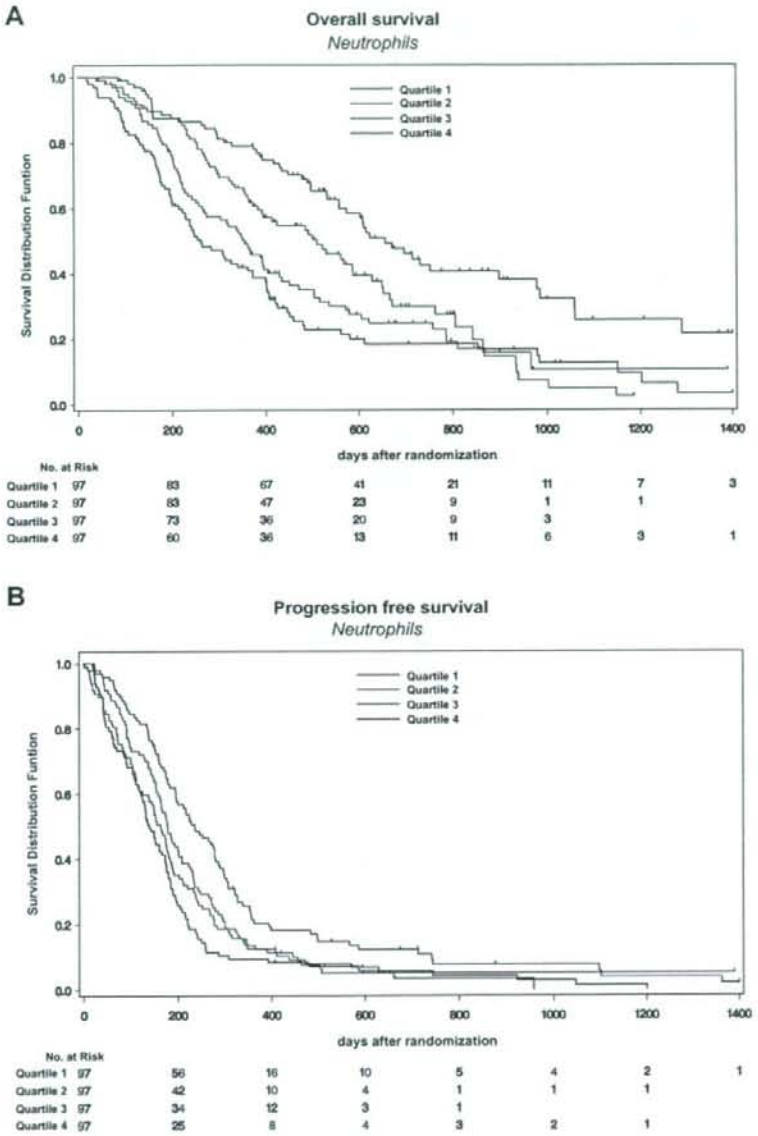

Fig. 1 – Kaplan–Meier estimates according to quartiles for the effect of pretreatment neutrophil count on (A) overall survival and (B) progression-free survival.

3.3. *Optimal cut-off value for pretreatment neutrophil count*

In selecting optimal cut-off values for the effect of neutrophil count on overall survival, the range between the 5th percentile (2205 mm^{-3}) and the 95th percentile (9657 mm^{-3}) for distribution of neutrophils was selected, and the possible cut-off points at intervals of 500 mm^{-3} from 2500 mm^{-3} to 9500 mm^{-3} were considered (giving 15 candidate cut-off points). Using the minimum *P*-value approach, the selected cut-off value for neutrophil count was 4500 mm^{-3} (corrected $P = 0.0009$) and the corrected shrunk hazard ratio was 1.67 (95%CI, 1.09–2.54, from 100 bootstrap samples; Table 3). The selected optimal cut-off value did not change even when we used the stratified proportional hazards model, stratified by the combination of all covariates. The median survival time was 19.3 months (95%CI, 16.5–21.4) for the low-neutrophil group (<4500 mm^{-3}, $n = 204$) and was 10.2 months (95%CI, 8.0–12.3) for the high-neutrophil group ($\geq$4500 mm^{-3}, $n = 184$) (Fig. 2). The results of prognostic factor analysis for overall survival are shown in Table 4. In terms of the relative order of significance, neutrophil count was one of the most important

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Table 2 – Multivariate Cox regression analysis for neutrophil, lymphocyte and monocyte counts.

Factors	Overall survival				Progression-free survival			
	Hazard ratio[a]	95%CI	P	P[b]	Hazard ratio[a]	95%CI	P	P[b]
Neutrophil count (mm^{-3})								
Quartile 1 (<3278)	1	–	–	0.0008	1	–	–	0.024
Quartile 2 (<4304)	1.25	0.86–1.82	0.251		1.19	0.88–1.61	0.258	
Quartile 3 (<5873)	1.76	1.22–2.53	0.002		1.32	0.97–1.78	0.076	
Quartile 4 (⩾5873)	1.94	1.35–2.79	0.0003		1.61	1.18–2.19	0.003	
Lymphocyte count (mm^{-3})								
Quartile 1 (<1082.3)	1	–	–	0.251	1	–	–	0.545
Quartile 2 (<1386.1)	1.14	0.81–1.61	0.438		1.10	0.82–1.47	0.535	
Quartile 3 (<1821.8)	0.83	0.58–1.19	0.303		0.88	0.65–1.20	0.424	
Quartile 4 (⩾1821.8)	1.13	0.80–1.59	0.495		0.95	0.70–1.28	0.732	
Neutrophil–lymphocyte ratio								
Quartile 1 (<2.093)	1	–	–	0.011	1	–	–	0.040
Quartile 2 (<2.914)	1.42	0.98–2.05	0.065		1.39	1.02–1.88	0.035	
Quartile 3 (<4.744)	1.83	1.27–2.62	0.001		1.50	1.09–2.06	0.012	
Quartile 4 (⩾4.744)	1.56	1.09–2.24	0.015		1.48	1.09–2.02	0.013	
Monocyte count (mm^{-3})								
Quartile 1 (<289.9)	1	–	–	0.381	1	–	–	0.969
Quartile 2 (<402.3)	0.93	0.65–1.32	0.674		1.05	0.78–1.41	0.755	
Quartile 3 (<550.4)	1.07	0.75–1.52	0.712		0.99	0.72–1.35	0.924	
Quartile 4 (⩾550.4)	1.26	0.89–1.78	0.203		1.04	0.76–1.42	0.792	

CI: confidence interval.

a Adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases.

b P-values for global association.

Table 3 – Cutpoint analysis for neutrophil count and overall survival.

Neutrophil count (cut-off points, mm^{-3})	Uncorrected hazard ratio[a]	Uncorrected P-value
2500	1.95	0.016
3000	1.78	0.001
3500	1.40	0.021
4000	1.57	0.0007
4500	1.72[b]	<0.0001[c]
5000	1.49	0.002
5500	1.51	0.002
6000	1.46	0.008
6500	1.75	0.0004
7000	1.62	0.005
7500	1.59	0.015
8000	1.88	0.004
8500	1.86	0.007
9000	1.78	0.017
9500	1.89	0.009

a (Hazard of death in patients on or above the cut-off point) divided by (hazard of death in patients below the cut-off point), after adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases.

b Corrected hazard ratio: 1.67 (95%CI, 1.09–2.54).

c Corrected P = 0.0009.

prognostic factors along with ECOG PS ($P < 0.0001$), LDH ($P = 0.001$) and smoking history ($P = 0.002$). The adjusted hazard ratios for the relationship between neutrophil count (<4500 mm^{-3} versus ⩾4500 mm^{-3}) and survival according to the treatment groups were 1.62 (95%CI, 1.14–2.30) in the PC arm ($n = 195$) and 1.74 (95%CI, 1.22–2.48) in the VGD arm ($n = 193$). There was no interaction between the neutrophil count and the treatment arms (P for interaction = 0.437).

3.4. *Relationship between pretreatment neutrophil count and intensity of chemotherapy*

In order to evaluate the effect of neutrophil count on administration of chemotherapy and toxicity, we analysed the dose intensity of chemotherapeutic agents and the incidence of toxicity in each arm. In the VGD arm, there was no significant difference in the relative dose intensity of vinorelbine or

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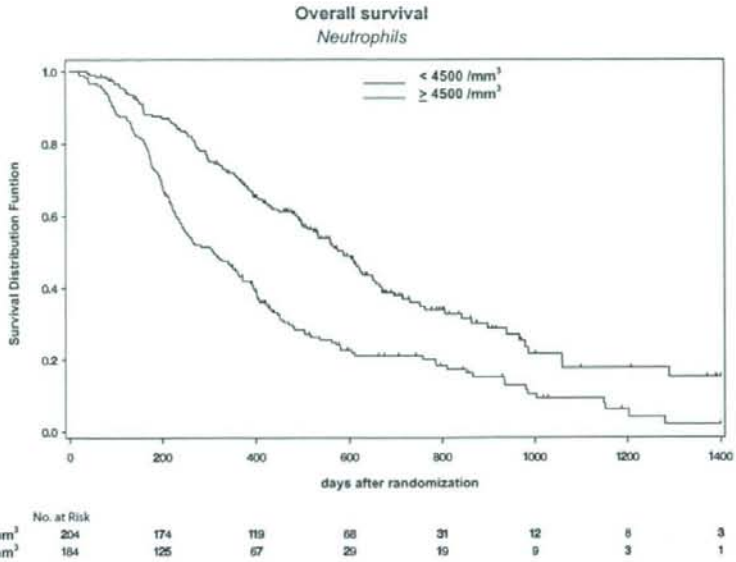

Fig. 2 – Kaplan–Meier estimates according to optimal cut-off point (4500 mm^{-3}) for the effect of pretreatment neutrophil count on overall survival.

gemcitabine between the low-neutrophil group (<4500 mm^{-3}) and the high-neutrophil group (≥4500 mm^{-3}). However, the relative dose intensity of docetaxel was significantly lower in the high-neutrophil group (median, 33%) than in the low-neutrophil group (median, 87%) ($P = 0.040$, Wilcoxon test).

The toxicity due to treatment was also analysed. In the VGD arm, the incidence of grade 3 or 4 non-haematological toxicity within the first three cycles of treatment was significantly higher in the high-neutrophil group than in the low-neutrophil group (26.5% versus 8.5%; $P = 0.002$, Fisher's exact test). Significantly fewer cycles were administered in the high-neutrophil group than in the low-neutrophil group (mean, 2.9 cycles versus 4.7 cycles; $P < 0.0001$, Wilcoxon test). None of the patients in the high-neutrophil group who experienced grade 3 or 4 non-haematological toxicity within the first three cycles completed the planned six cycles. The proportion of patients requiring reductions in the doses of vinorelbine or gemcitabine within the first two cycles of treatment was significantly higher in the low-neutrophil group (45.2%) than in the high-neutrophil group (26.4%) ($P = 0.007$, Fisher's exact test). No such differences in dose intensity or toxicity were seen in the PC arm.

4. Discussion

In multivariate analysis after adjustment for known prognostic factors, we found linear associations between pretreatment elevated neutrophil count and short overall and progression-free survival. As there was no such association for the lymphocyte count, the relationship between neutrophil-lymphocyte ratio and overall survival was also found, however, it was to some degree weak and non-linear. As a consequence, we consider that absolute neutrophil count may better serve as a prognostic factor. An optimal cut-off value for the relationship between neutrophil count and overall survival was identified as 4500 mm^{-3} (corrected hazard ratio, 1.67; 95%CI, 1.09–2.54). In the VGD arm, the low-neutrophil group (<4500 mm^{-3}) tended to have a lower incidence of severe non-haematological toxicity and tolerated longer administration of the chemotherapeutic agents compared with the high-neutrophil group. However, no such association was found in the PC arm, and pretreatment neutrophil count was equally predictive of prognosis in both treatment arms when analysed separately. We therefore do not consider it likely that the pretreatment neutrophil count serves as an indicator of intolerance to chemotherapy, rather than as an indicator of poor prognosis.

A number of studies in the last two decades have suggested an association between the neutrophil count or neutrophil–lymphocyte ratio and the prognosis of cancer patients,[7–16] although no acceptable explanations for the mechanisms underlying these observed associations have been proposed. Moreover, although neutrophilia often accompanies the diagnosis of cancer, the causes of neutrophilia in cancer patients are not fully understood, and are likely to be the result of a combination of factors. One obvious cause of neutrophilia is paraneoplastic production of myeloid growth factors by cancer cells themselves. Granulocyte-colony stimulating factor (G-CSF) is a growth factor that acts selectively on bone marrow granulocytic lineage cells, and is considered to play a central role in granulopoiesis. Administration of G-CSF was reported to increase bone marrow neutrophil precursors and shorten bone marrow transit time in mice and humans,[20–22] resulting in marked increases in the production of neutrophils. Granulocyte macrophage-colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor

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Table 4 – Prognostic factor analysis for overall survival using proportional hazards regression model without variable selection.

Factors	Hazard ratio	95%CI	P-value
Performance status			
0	1.00	–	–
1	2.03	1.54–2.67	<0.0001
Neutrophil count			
<4500 mm^{-3}	1.00	–	–
⩾4500 mm^{-3}	1.72	1.34–2.19	<0.0001
LDH			
Normal	1.00	–	–
High	1.57	1.20–2.05	0.001
Smoking history			
Non/former smokers	1.00	–	–
Current smokers	1.56	1.18–2.06	0.002
Liver metastases			
No	1.00	–	–
Yes	1.62	1.08–2.43	0.020
Sex			
Male	1.00	–	–
Female	0.74	0.54–1.02	0.064
Weight loss			
<5%	1.00	–	–
⩾5%	1.30	0.96–1.76	0.092
Skin metastases			
No	1.00	–	–
Yes	1.78	0.85–3.72	0.124
Bone metastases			
No	1.00	–	–
Yes	1.21	0.90–1.63	0.204
Stage			
IIIB	1.00	–	–
IV	1.24	0.88–1.75	0.222

are the other examples of haematopoietic growth factors that cause neutrophilia by *in vivo* administration.[23,24] A variety of non-haematopoietic malignant tumours including mesothelioma,[25] squamous cell carcinoma of the oropharynx,[26] melanoma,[27] glioblastoma[28] and carcinoma of the lung[29] have been reported to secrete G-CSF or GM-CSF and cause significant leucocytosis. Although there have been several reports of the existence of autocrine growth loops for G-CSF and GM-CSF in non-haematopoietic tumour cells, implying G-CSF- and GM-CSF-producing tumours are more aggressive,[30,31] the relationship between paraneoplastic production of myeloid growth factors and prognosis remains unclear. Furthermore, considering the linear relationship we observed between pretreatment neutrophil count and survival in this study, ectopic production of myeloid growth factors, which often causes marked neutrophilia, does not seem to be the sole reason for the observed association between neutrophil count and prognosis.

Other possible factors that cause neutrophilia are coexistent infection and cancer-related inflammation. In this study, patients with active infection were excluded based on the eligibility criteria of the trial, and there is no clear reason to assume the existence of latent infection as the cause of neutrophilia and poor prognosis.

The association between cancer and inflammation was initially pointed out during the 19th century. However, recent advances in understanding of tumour biology have stimulated renewed interests in searching for links between cancer and inflammation.[3–6] Today, it is widely accepted that chronic inflammation contributes to the initiation and progression of cancer. Furthermore, it is now known that inflammatory processes almost always accompany cancer, and persistence of chronic inflammation-like processes within cancer tissue causes suppression of anti-tumour immunity by several mechanisms, such as activation of type 2 T-helper responses, recruitment of regulatory T cells and activation of the chemokine system, and results in promotion of cancer growth and metastasis. Thus, inflammation may result in the aggressive growth of a tumour. The cytokines interleukin (IL)-6 and tumour necrosis factor-alpha (TNFα), which are implicated in the pathogenesis of cancer-related inflammation as well as of acute inflammatory processes, are also known to induce neutrophilia.[32–34] It is possible that the neutrophil count at diagnosis indicates the severity or nature of inflammation occurring within the tumour, and thus reflects prognosis. In a recent report, a proportion of patients with metastatic cancer were shown to have IL-6-mediated elevation in serum cortisol levels. This may partly explain the neutrophilia of cancer

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patients, although its contribution to outcome is not yet known.[35]

We did not measure inflammatory markers such as C-reactive protein or haemogram of total white cell count in this study. However, we are investigating correlations between several cytokines and prognosis in a correlative study of another clinical trial (ClinicalTrials.gov identifier NCT00616031).

Besides inflammation in cancer tissue, host factors may influence the prognosis of cancer patients. It is now known that lifetime exposure to infectious diseases and other sources of inflammation not only is related to the pathogenesis of cancer, but also plays an important role in ageing and influences longevity.[36,37] Ageing is a complex process, and numerous genes are known to have associations with longevity.[38] Polymorphisms of the genes that encode proteins involved in inflammatory processes (e.g. IL-1, IL-6, IL-10 and TNFα) are suspected to affect ageing and longevity. Given the close relationship between cancer and inflammation, it is natural to speculate that genetic polymorphisms in inflammation-related genes may also influence host responses to cancer and prognosis; peripheral neutrophil count may be an indicator of this association.

Another possibility is that neutrophil directly down-regulates host cellular immunity against cancer, thereby affecting the prognosis. *In vitro* studies showed that neutrophils suppress the cytolytic activity of lymphocytes and natural killer cells when co-cultured with neutrophils and lymphocytes from normal healthy donors; the degree of suppression was proportional to the number of neutrophils added.[39–41] The clinical relevance of these effects seen in *in vitro* studies is currently unknown. The biological basis for the multi-factorial and complex association is also unknown, and merits further research.

5. Conclusion

Using the dataset from a randomised controlled trial, we have confirmed that pretreatment peripheral blood neutrophil count is an independent prognostic factor in patients with advanced NSCLC receiving modern chemotherapy. The results need to be investigated for generalisability in other populations. Since neutrophil count is easily measured at low cost, it may be a useful predictor of prognosis in clinical practice. Considering the strength of the association reported here, neutrophil count should be taken into account as a stratification factor in future randomised clinical trials of patients with advanced NSCLC.

Conflict of interest statement

Kaoru Kubota has received honoraria from Eli Lilly, Sanofi-Aventis, and Chugai. All other authors declared no conflicts of interest.

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REFERENCES

1. Hotta K, Fujiwara Y, Kiura K, et al. Relationship between response and survival in more than 50,000 patients with advanced non-small cell lung cancer treated with systemic chemotherapy in 143 phase III trials. *J Thoracic Oncol* 2007;2:402–7.
2. Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol* 2004;22:330–53.
3. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *New Engl J Med* 1986;315:1650–9.
4. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539–45.
5. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860–7.
6. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454:436–44.
7. Negrier S, Escudier B, Gomez F, Reitz M, DGCIN – German Cooperative Renal Carcinoma Chemo-Immunotherapy Trials Group. Prognostic factors of survival and rapid progression in 782 patients with metastatic renal carcinomas treated by cytokines: a report from the Groupe Francais d'Immunotherapie. *Ann Oncol* 2002;13:1460–8.
8. Atzpodien J, Royston P, Wandert T, et al. Metastatic renal carcinoma comprehensive prognostic system. *Brit J Cancer* 2003;88:348–53.
9. Donskov F, Hokland M, Marcussen N, Torp Madsen HH, von der Maase H. Monocytes and neutrophils as 'bud guys' for outcomes of interleukin-2 with and without histamine in metastatic renal cell carcinoma – results from a randomised phase II trial. *Brit J Cancer* 2006;94:218–26.
10. Schmidt H, Bastholt L, Geertsen P, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. *Brit J Cancer* 2005;93:273–8.
11. Schmidt H, Suciu S, Punt CJA, et al. Pretreatment levels of peripheral neutrophils and leukocytes as independent predictors of overall survival in patients with American Joint Committee on Cancer Stage IV Melanoma: results of the EORTC 18951 biochemotherapy trial. *J Clin Oncol* 2007;25:1562–9.
12. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil–lymphocyte ratio as a prognostic factor in colorectal cancer. *J Surg Oncol* 2005;91:181–4.
13. Yamanaka T, Matsumoto S, Teramukai S, Ishiwata R, Nagai Y, Fukushima M. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. *Oncology* 2007;73:215–20.
14. Paesmans M, Sculier JP, Libert P, et al. Prognostic factors for survival in advanced non-small-cell lung cancer: univariate and multivariate analyses including recursive partitioning and amalgamation algorithms in 1052 patients. *J Clin Oncol* 1995;13:1221–30.
15. Paesmans M, Sculier JP, Lecomte J, et al. Prognostic factors for patients with small cell lung carcinoma: analysis of a series of 763 patients included in 4 consecutive prospective trials with a minimum follow-up of 5 years. *Cancer* 2000;89:523–33.
16. Ferrigno D, Buccheri G. Hematologic counts and clinical correlations in 1201 newly diagnosed lung cancer patients. *Monaldi Arch Chest Disorder* 2003;59:193–8.
17. Kubota K, Kawahara M, Ogawara M, et al. Vinorelbine plus gemcitabine followed by docetaxel versus carboplatin plus paclitaxel in patients with advanced non-small-cell lung cancer: a randomised, open-label, phase III study. *Lancet Oncol* 2008;9:1135–42.

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18. Altman DG, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" cutpoints in the evaluation of prognostic factos. *J Natl Cancer Inst* 1994;**86**:829–35.
19. Holländer N, Sauerbrei W, Schumacher M. Confidence intervals for the effect of a prognostic factor after selection of an 'optimal' cutpoint. *Stat Med* 2004;**23**:1701–13.
20. Lord BI, Bronchud MH, Owens S, et al. The kinetics of human granulopoiesis following treatment with granulocyte colony-stimulating factor in vivo. *Proc Natl Acad Sci USA* 1989;**86**:9499–503.
21. Uchida T, Yamagiwa A. Kinetics of rG-CSF-induced neutrophilia in mice. *Exp Hematol* 1992;**20**:152–5.
22. Price TH, Chatta GS, Dale DC. Effect of recombinant granulocyte colony-stimulating factor on neutrophil kinetics in normal young and elderly humans. *Blood* 1996;**88**:335–40.
23. Aglietta M, Piacibello W, Sanavio F, et al. Kinetics of human hemopoietic cells after in vivo administration of granulocyte-macrophage colony-stimulating factor. *J Clin Invest* 1989;**83**:551–7.
24. Ulich TR, del Castillo J, Watson LR, Yin SM, Garnick MB. In vivo hematologic effects of recombinant human macrophage colony-stimulating factor. *Blood* 1990;**75**:846–50.
25. Demetri GD, Zenzie BW, Rheinwald JG, Griffin JD. Expression of colony-stimulating factor genes by normal human mesothelial cells and human malignant mesothelioma cells lines in vitro. *Blood* 1989;**74**:940–6.
26. Nagata S, Tsuchiya M, Asano S, et al. Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. *Nature* 1986;**319**:415–8.
27. Lilly MB, Devlin PE, Devlin JJ, Rado TA. Production of granulocyte colony-stimulating factor by a human melanoma cell line. *Exp Hematol* 1987;**15**:966–71.
28. Tweardy DJ, Cannizzaro LA, Palumbo AP, et al. Molecular cloning and characterization of a cDNA for human granulocyte colony-stimulating factor (G-CSF) from a glioblastoma multiforme cell line and localization of the G-CSF gene to chromosome band 17q21. *Oncogene Res* 1987;**1**:209–20.
29. Asahi Y, Kubonishi I, Imamura J, et al. Establishment of a clonal cell line producing granulocyte colony-stimulating factor and parathyroid hormone-related protein from a lung cancer patient with leukocytosis and hypercalcemia. *Jpn J Cancer Res* 1996;**87**:451–8.
30. Tachibana M, Miyakawa A, Tazaki H, et al. Autocrine growth of transitional cell carcinoma of the bladder induced by granulocyte-colony stimulating factor. *Cancer Res* 1995;**55**:3438–43.
31. Oshika Y, Nakamura M, Abe Y, et al. Growth stimulation of non-small cell lung cancer xenografts by granulocyte-macrophage colony-stimulating factor (GM-CSF). *Eur J Cancer* 1998;**34**:1958–61.
32. Ulich TR, del Castillo J, Keys M, Granger GA, Ni RX. Kinetics and mechanisms of recombinant human interleukin 1 and tumor necrosis factor-alpha-induced changes in circulating numbers of neutrophils and lymphocytes. *J Immunol* 1987;**139**:3406–15.
33. Ulich TR, del Castillo J, Guo K, Souza L. The hematologic effects of chronic administration of the monokines tumor necrosis factor, interleukin-1, and granulocyte-colony stimulating factor on bone marrow and circulation. *Am J Pathol* 1989;**134**:149–59.
34. Ulich TR, del Castillo J, Guo KZ. In vivo hematologic effects of recombinant interleukin-6 on hematopoiesis and circulating numbers of RBCs and WBCs. *Blood* 1989;**73**:108–10.
35. Lissoni P, Brivio F, Fumagalli L, et al. Immune and endocrine mechanisms of advanced cancer-related hypercortisolemia. *In vivo* 2007;**21**:647–50.
36. Finch CE, Crimmins EM. Inflammatory exposure and historical changes in human life-spans. *Science* 2004;**305**:1736–9.
37. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. *Exp Gerontol* 2004;**39**:687–99.
38. Capri M, Salvioli S, Sevini F, et al. The genetics of human longevity. *Ann NY Acad Sci* 2006;**1067**:252–63.
39. Petrie HT, Klassen LW, Kay HD. Inhibition of human cytotoxic T lymphocyte activity in vitro by autologous peripheral blood granulocytes. *J Immunol* 1985;**134**:230–4.
40. el-Hag A, Clark RA. Immunosuppression by activated human neutrophils. Dependence on the myeloperoxydase system. *J Immunol* 1987;**139**:2406–13.
41. Shau HY, Kim A. Suppression of lymphokine-activated killer induction by neutrophils. *J Immunol* 1988;**141**:4395–402.

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