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ORIGINAL ARTICLE

Current status of postoperative follow-up for lung cancer in Japan: questionnaire survey by the Setouchi Lung Cancer Study Group—A0901

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Abstract
Purpose. There is no recommended standard follow-up program after resection for lung cancer. Under these circumstances, each doctor establishes his or her own follow-up protocol. This questionnaire survey was conducted to grasp the current status of postoperative follow-up in Japan.
Methods. The questionnaire survey was aimed at determining what examinations were performed and at what frequencies in the setting of postoperative follow-up. Based on these results, examinations performed at a frequency of >50% and the time points after resection at which they were performed were selected and presented as components of an average follow-up program.
Results. Questionnaires were sent to 44 institutions, and 26 doctors responded to the questionnaire. All 26 of the doctors performed physical examinations, blood examinations, chest radiography, and computed tomography (CT) routinely, but their frequencies varied widely among the doctors. The average frequencies of the follow-up examinations as judged from this survey are as follows: Physical and blood examinations are performed three to four times a year for the first 3 years and twice a year during the next 2 years. CT is scheduled at 6 and 12 months after resection and is repeated annually thereafter. Chest radiography is performed three to four times a year for the first 3 years and once a year thereafter, between the CT examinations.
Conclusion. The follow-up programs used in clinical practice vary widely among institutions and doctors in terms of the types of examination performed and the frequencies at which they are performed.

Key words Lung cancer · Postoperative follow-up · Postoperative surveillance · Recurrence

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Introduction

Surgical resection with curative intent is selected for the treatment of localized non-small-cell lung cancer (NSCLC). The 5-year survival rate after complete resection for NSCLC is approximately 60%, and many patients develop recurrences after resection.[1] To detect recurrences, several examinations are performed periodically as part of the postoperative follow-up or surveillance. The purpose of postoperative follow-up is to detect recurrences and/or metachronous tumors, so adequate treatment can be offered in an attempt to improve the survival duration and the quality of life. Some investigators have suggested that the survival duration is greater in patients with asymptomatic recurrences detected by follow-up examinations than in those with symptomatic recurrences, and that follow-up examinations after resection are useful for detecting asymptomatic recurrences.[2,3] At the same time, several investigators have reported that the benefit of postoperative follow-up is questionable from the point of view of efficacy and cost-effectiveness.[4–8] Thus, the benefits and efficacy of postoperative follow-up remains controversial.

The board of the Japan Lung Cancer Society drew up a clinical practice guideline for lung cancer in 2005, and periodic follow-up after resection is not recommended in this guideline because there was still no clear persuasive evidence to support it.[9] Under these circumstances, each institution or each doctor establishes his or her own postoperative follow-up program in clinical practice. It is suspected that these postoperative follow-up protocols applied in clinical practice vary widely in terms of the examinations performed and their frequency. To grasp the current status of follow-up after resection for NSCLC in Japan, a questionnaire survey of the institutions affiliated with the Setouchi Lung Cancer Study Group was performed to determine the kinds of examination and the frequencies at which they are performed in the setting of postoperative follow-up. In addition, examinations that were performed frequently and the time points after resection at which they were performed were selected, and the average follow-up protocol based on the results of the questionnaire survey is presented.

Methods

A questionnaire designed to obtain information regarding the postoperative follow-up protocol adopted for NSCLC patients was sent by mail to 44 institutions affiliated with the Setouchi Lung Cancer Study Group. The questionnaire consisted of the following questions.

1. Is a standardized follow-up protocol followed at the institution?
2. Does the follow-up schedule differ depending on the disease stage?
3. What are the examination modalities chosen in the setting of postoperative follow-up? At what frequencies are these examinations performed? Please record your answers in Table 1.

Based on the information in this survey, the percentage of the 26 doctors who performed the examinations was calculated for each of the examinations at each time point after the resection. Then, examinations that were performed at a frequency of >50% and the time points after resection at which they were performed were selected and are presented as components of an average follow-up program in this study.

The TNM stage was determined according to the Union for International Cancer Control (UICC) TNM classification of pathological stage, 6th edition.[10]

Results

Questionnaires were sent to 44 institutions affiliated with the Setouchi Lung Cancer Study Group, 17 (38.6%) of which responded to the questionnaire. From these 17 institutions, 26 doctors, comprising 2 oncologists and 24 thoracic surgeons, responded to the questionnaire.

Of the 17 institutions, 7 reported that they followed a standardized institutional follow-up program, whereas the remaining 10 institutions did not (Table 2). Of the 26 doctors, 11 discontinued the follow-up 5 years after the resection, whereas the remaining 15 continued follow-up for >5 years after the resection. Among the 26 doctors, 15 arrange follow-up schedules based on the disease stage; for example, six doctors classified the patients into two groups based on the disease stage (stage IA and other stages), and four classified the patients into three groups based on the disease stage (IA, IB/II, and IIIA). Each of the 26 doctors performed blood examinations, chest radiography, and computed tomography (CT) routinely. Six doctors performed positron emission tomography (PET) or PET/CT, and nine doctors performed brain magnetic resonance imaging (MRI) or brain CT routinely. None of the doctors performed sputum cytology or abdominal ultrasonography (US) in the setting of postoperative follow-up.

Figure 1 shows the frequency of each of the examinations performed after the resection. The Y-axis shows the percentage of doctors who performed the examinations, and the X-axis shows the time of performance of the examination after the resection. More than half of

Table 1 Questionnaire

Procedure	1 Year				2 Years				3 Years				4 Years				5 Years				6 Years		7 Years		8 Years		9 Years		10 Years	
	1	2	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	6	12	6	12	6	12	6	12
Physical examination																														
Blood examination (tumor markers)																														
Sputum cytology																														
Chest radiography																														
Abdominal US																														
CT																														
Bone scintigraphy																														
Brain MRI/CT																														
PET/CT																														
Other examinations																														

The numbers underneath the 1–10 years are the months (1, 2, 3, 6, 9, 12) for that year
US, ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography

Table 2 Results of the questionnaire

Institutions that responded to the questionnaire	17
Doctors who responded to the questionnaire	26
Standardized follow-up program in the institution?	
Yes	6
No	11
Continued follow-up for more than 5 years?	
Yes	15
No	11
Arrange follow-up schedule by disease stage?	
Yes	15
1A/1B,2,3	6
1A/1B,2/3	4
1/2/3	2
1A/1B/2,3	1
1/2,3	1
1,2/3	1
No	11
Physical examination?	
Yes	26
No	0
Blood examination?	
Yes	26
No	0
Sputum cytology?	
Yes	0
No	26
Chest radiography?	
Yes	26
No	0
Abdominal US?	
Yes	0
No	26
CT?	
Yes	26
No	0
Bone scintigraphy?	
Yes	5
No	21
Brain MRI (CT)?	
Yes	10
No	16
PET/CT?	
Yes	7
No	19

the doctors performed physical examination four times a year during the first 3 years and twice a year during the next 2 years. After 5 years of postoperative follow-up, the frequency of the physical examination decreased gradually, although approximately half of the doctors continued the physical examinations once a year for up to 10 years after the resection. The blood examinations were performed almost at the same time points as the physical examination. CT was performed at 6 and 12 months after the resection and was repeated annually thereafter for the next 4 years. Chest radiography was performed at each visit during the first 2 years and repeated annually thereafter, between the CT

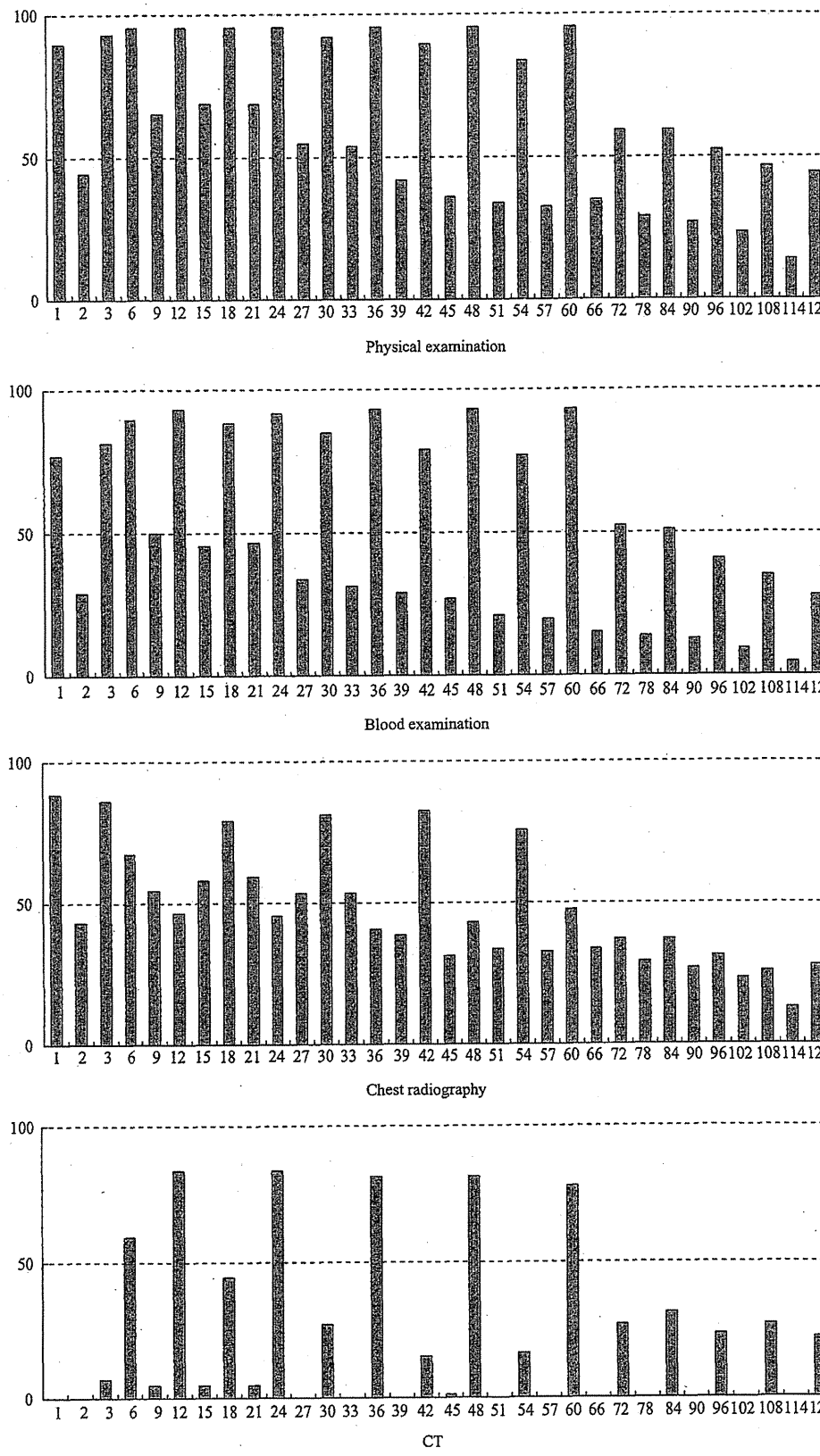
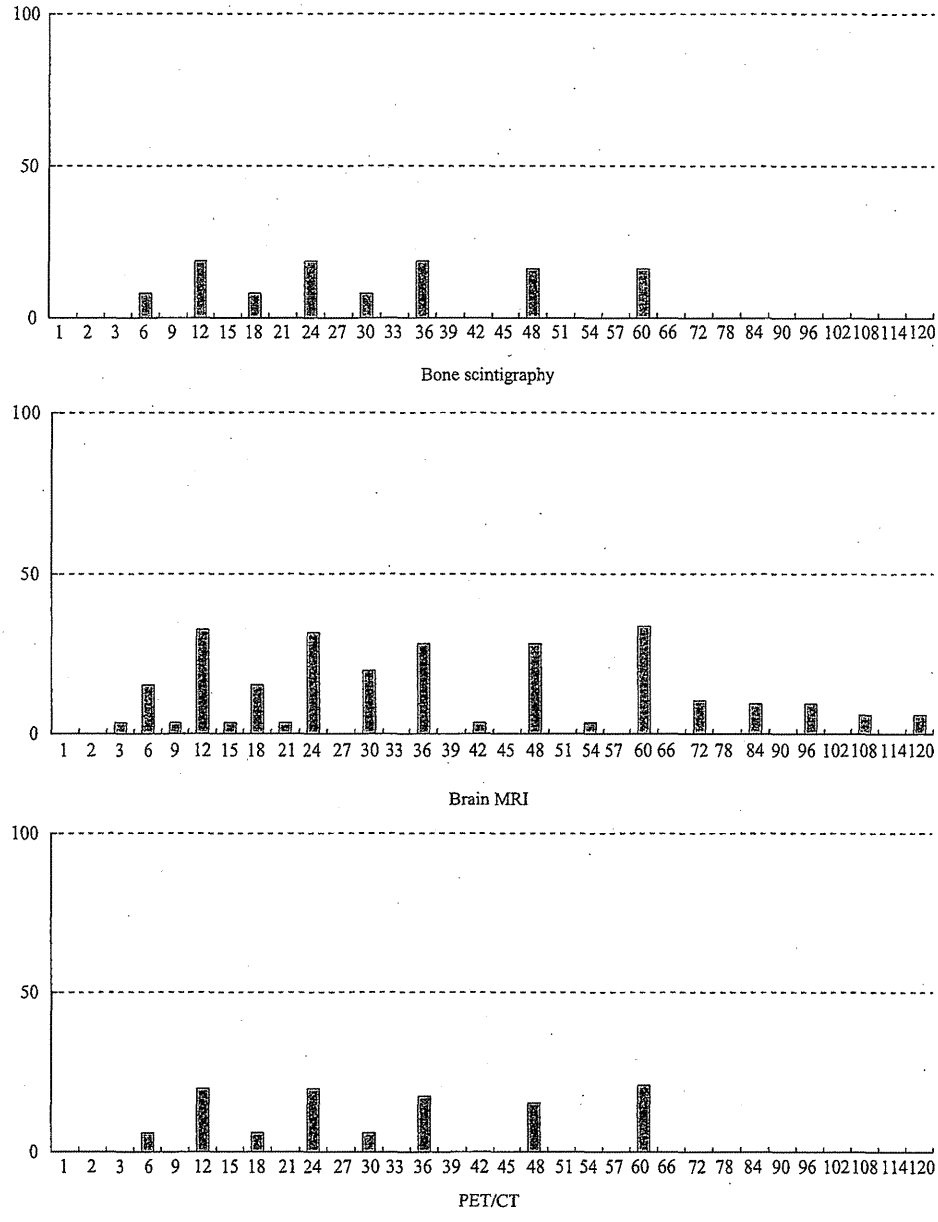


Fig. 1 Frequency of examinations at each time point after resection. *CT*, computed tomography; *MRI*, magnetic resonance imaging; *PET*, positron emission tomography

Fig. 1 Continued



examinations. A few of the doctors routinely performed bone scintigraphy, brain MRI or CT, and PET/CT.

Based on the results of the survey, the examinations that were performed at a frequency of >50% and the time points after resection at which they were performed were selected and are the components of an average follow-up program presented in this study (Table 3): Physical examination was scheduled three to four times a year for the first 3 years, twice a year for the next 2 years, then continued once a year for up to 8 years after the resection. Blood examinations were performed approximately at the same time points as the physical examination. CT examination was scheduled at 6 and 12 months after the resection and repeated annually thereafter for up to 5 years after the resection. Chest radiography was scheduled three or four times a year for the first 3 years and repeated once a year thereafter, between the CT examinations.

Discussion

Several issues need to be discussed in relation to the follow-up of NSCLC patients after resection. One of the

Table 3 Average follow-up program based on the results of this survey

Examination	1 Year						2 Years						3 Years						4 Years						5 Years						6 Years				7 Years		8 Years	
	1	2	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	6	12	6	12	6	12
Physical examination	○		○	○	○	○	○	○	○	○	○	○		○		○		○		○		○		○		○					○	○	○	○	○	
Blood examination	○		○	○	○	○		○		○		○		○		○		○		○		○		○		○										
Chest radiography	○		○	○	○		○	○	○		○	○	○			○				○				○												
CT				○		○		○		○				○				○				○				○										

issues is that optimal examination modalities for the postoperative follow-up have not yet been identified. Several guidelines have been proposed for the follow-up of NSCLC patients after surgery with curative intent, but there is a divergence among the guidelines, especially in relation to the recommendations for imaging examinations such as CT.[11–14] The study presented here demonstrated that follow-up programs applied in clinical practice in Japan also vary widely among institutions and/or doctors in terms of the examinations performed and the frequencies at which they are performed. No optimal or standard examination modalities in the follow-up setting have yet been established.

Another issue pertains to the efficacy and benefits of the follow-up examinations after resection. Several studies have suggested that the survival duration is greater in patients with asymptomatic recurrences than in those with symptomatic recurrences, and that follow-up is useful for detecting asymptomatic recurrences.[2,3] On the other hand, several investigators have reported that the survival benefit of postoperative follow-up is questionable.[4–8] The benefit of follow-up thus remains a controversial subject.

The third issue related to follow-up is cost-effectiveness, which cannot be ignored nowadays when evaluating the efficacy of a certain modality. Several investigators have analyzed the cost-effectiveness of postoperative follow-up and concluded that it is inefficient and that the survival benefit accruing from the follow-up did not justify its cost.[4–6,8] However, these articles were all published from Western countries; and the medical cost for follow-up, social acceptability of the medical cost, and the patients' needs might be different in Japan. Therefore, the cost-effectiveness of the follow-up should be evaluated independently in Japan.

To answer these questions, it would be ideal to conduct a randomized controlled study. However, the lack of standard follow-up modalities makes to it difficult to design a randomized trial. Regarding this point, our survey might give some helpful information for designing such a trial as the survey showed what modalities were commonly used in clinical practice. Another factor that probably makes the randomized trial more difficult to conduct in Japan is ethics. As the first step to evaluate the efficacy of the follow-up, it would be ideal to conduct the randomized trial between a follow-up group and a no follow-up or minimal follow-up group. Follow-up after resection, however, is already commonly performed in clinical practice and no or minimal follow-up would not be acceptable from an ethical point of view—even though there are no recommended follow-up programs and no proven efficacy of follow-up. Considering the present circumstances in Japan, a possible

trial might be comparison between a follow-up group with average modalities and a follow-up group with more intensive modalities, such as the average follow-up modalities + periodical PET/CT.

The follow-up programs identified in this survey seem relatively intensive compared with those that are commonly accepted worldwide. A possible reason might be related to our medical insurance system. All Japanese citizens are covered by public medical insurance, and the cost of the follow-up examinations is not a burden on the patients. This circumstance makes access to hospitals easy and makes the postoperative follow-up examinations relatively intensive. We do not have information about the follow-up programs adopted in other areas of Japan, but we assume that their programs are similar to those presented in this study.

In this survey, one question pertained to "blood examinations (tumor markers)", and it is uncertain what kinds of tumor markers were measured. In the preoperative setting and in cases of advanced/recurrent lung cancer, tumor markers such as carcinoembryonic antigen (CEA) and cytokeratin 19 fragments (Cyfra), among others, are commonly measured as parameters of the tumor aggressiveness or for evaluating the effectiveness of the treatment. In the follow-up setting, therefore, it is assumed that similar tumor markers would be measured.

^{18}F-Fluorodeoxyglucose (FDG)-PET/CT enables examination of the whole body, excluding the brain, in a noninvasive manner; it also can differentiate, if not always definitively, between malignant and benign lesions. Because of these advantages, FDG-PET/CT was applied as one of the follow-up examinations at six of the institutions. Several investigators have reported the usefulness of FDG-PET/CT in the setting of postoperative follow-up for NSCLC.[15–18] However, FDG-PET/CT cannot be recommended commonly in the postoperative follow-up setting because of limitation of availablility in Japan, cost-effectiveness and unknown efficacy.

A total of 15 of the 26 doctors based their follow-up schedules on the disease stage. They performed the examinations less intensively in patients with an early stage of the disease and more intensively in those with more advanced disease. These schedule changes based on the disease stage might be reasonable because recurrence develops more frequently in patients with advanced disease.

Conclusion

A questionnaire designed to obtain information on the follow-up program adopted for NSCLC patients after complete resection was conducted to grasp the current status in our area. The follow-up programs vary widely among institutions and doctors in terms of the examinations performed and the frequencies at which they are performed. The efficacy of the follow-up for NSCLC patients after resection is still unclear, and further studies are needed to answer questions about it.

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None of the authors has any financial or other potential conflicts of interest to declare.

References

1. Asamura H, Goya T, Koshiishi Y, Sohara Y, Eguchi K, Mori M, et al. A Japanese Lung Cancer Registry study: prognosis of 13,010 resected lung cancers. J Thorac Oncol 2008;3:46–52.
2. Chiu CH, Chern MS, Wu MH, Hsu WH, Wu YC, Huang MH, et al. Usefulness of low-dose spiral CT of the chest in regular follow-up of postoperative non-small cell lung cancer patients: preliminary report. J Thorac Cardiovasc Surg 2003; 125:1300–5.
3. Westeel V, Choma D, Clement F, Woronoff-Lemsi MC, Pugin JF, Dubiez A, et al. Relevance of an intensive postoperative follow-up after surgery for non-small cell lung cancer. Ann Thorac Surg 2000;70:1185–90.
4. Egermann U, Jaeggi K, Habicht JM, Perruchoud AP, Dalquen P, Soler M. Regular follow-up after curative resection of non-small cell lung cancer: a real benefit for patients? Eur Respir J 2002;19:464–8.
5. Walsh GL, O'Connor M, Willis KM, Milas M, Wong RS, Nesbitt JC, et al. Is follow-up of lung cancer patients after resection medically indicated and cost-effective? Ann Thorac Surg 1995;60:1563–70; discussion 1570–2.
6. Younes RN, Gross JL, Deheinzelin D. Follow-up in lung cancer: how often and for what purpose? Chest 1999;115: 1494–9.
7. Virgo KS, Naunheim KS, McKirgan LW, Kissling ME, Lin JC, Johnson FE. Cost of patient follow-up after potentially curative lung cancer treatment. J Thorac Cardiovasc Surg 1996;112:356–63.
8. Virgo KS, McKirgan LW, Caputo MC, Mahurin DM, Chao LC, Caputo NA, et al. Post-treatment management options for patients with lung cancer. Ann Surg 1995;222:700–10.
9. Japanese Lung Cancer Society. Japanese clinical practice guideline for lung cancer. Kanehara Shuppan, 2005.
10. Sobin L, Wittekind, C. UICC TNM classification of malignant tumors. 6th edn, New York: Wiley-Liss, 2002. p. 272.
11. Anonymous. Follow-up of non-small lung cancer. American College of Radiology appropriateness criteria, 2005–2007. Available at: www.acr.org.
12. Rubins J, Unger M, Colice GL. Follow-up and surveillance of the lung cancer patient following curative intent therapy: ACCP evidence-based clinical practice guideline (2nd edition). Chest 2007;132(suppl):355S–67S.

13. Felip E, Stahel RA, Pavlidis N. ESMO Minimum clinical recommendations for diagnosis, treatment and follow-up of non-small-cell lung cancer (NSCLC). Ann Oncol 2005; 16(suppl 1):i28–9.
14. Pfister DG, Johnson DH, Azzoli CG, Sause W, Smith TJ, Baker S Jr, 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.
15. Takenaka D, Ohno Y, Koyama H, Nogami M, Onishi Y, Matsumoto K, et al. Integrated FDG-PET/CT vs. standard radiological examinations: comparison of capability for assessment of postoperative recurrence in non-small cell lung cancer patients. Eur J Radiol 2010;74:458–64.
16. Hicks RJ, Kalff V, MacManus MP, Ware RE, McKenzie AF, Matthews JP, et al. The utility of (18)F-FDG PET for suspected recurrent non-small cell lung cancer after potentially curative therapy: impact on management and prognostic stratification. J Nucl Med 2001;42:1605–13.
17. Hellwig D, Groschel A, Graeter TP, Hellwig AP, Nestle U, Schafers HJ, et al. Diagnostic performance and prognostic impact of FDG-PET in suspected recurrence of surgically treated non-small cell lung cancer. Eur J Nucl Med Mol Imaging 2006;33:13–21.
18. Keidar Z, Haim N, Guralnik L, Wollner M, Bar-Shalom R, Ben-Nun A, et al. PET/CT using ^{18}F-FDG in suspected lung cancer recurrence: diagnostic value and impact on patient management. J Nucl Med 2004;45:1640–6.

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悪性中皮腫の血清診断における可溶性メソテリン関連ペプチド (SMRP：Soluble Mesothelin-related Peptides) の有用性に関する多施設共同試験

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悪性中皮腫の血清診断における可溶性メソテリン関連ペプチド (SMRP：Soluble Mesothelin-related Peptides) の有用性に関する多施設共同試験

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要　旨

悪性中皮腫の血清診断における可溶性メソテリン関連ペプチド（soluble mesothelin-related peptides：SMRP）の診断性能を多施設共同試験によって検討した。対象は，悪性中皮腫85例，比較対照疾患としての肺癌240例，良性呼吸器疾患（石綿非関連良性疾患136例，石綿関連良性疾患157例），高血圧・慢性心疾患74例および石綿曝露歴のない健常者110名。Chemiluminescent enzyme immunoassay（CLEIA）法による血清SMRP濃度測定キット（ルミパルス® メソテリン）を用いて血清SMRP濃度を測定した。参考基準値を1.5 nmol/Lに設定した場合の各対象の陽性率は，悪性中皮腫66%，肺癌21%，石綿非関連良性疾患18%，石綿関連良性疾患15%，高血圧・慢性心疾患9%，健常者1%であった。また，悪性中皮腫における血清SMRP濃度は，比較対照群および健常者に比較して有意に高値であった。原発部位別の陽性率は，胸膜65%，腹膜86%であった。悪性胸膜中皮腫の各病期におけるSMRP陽性率は，I期（64%），II期（67%），III期（55%），IV期（69%）であった。また，各組織型におけるSMRP陽性率は，上皮型（65%），二相型（60%），肉腫型（75%），であった。今回のルミパルス® メソテリンを用いた試験結果では，悪性中皮腫の血清診断におけるSMRPの診断性能は，これまでの報告と同等であった。さらに，悪性胸膜中皮腫早期例，肉腫型および腹膜中皮腫における診断的有用性も示唆された。

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Evaluation of the usefulness of SMRP (Soluble Mesothelin-related Peptides) for the diagnosis of malignant mesothelioma

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Key words：malignant mesothelioma（悪性中皮腫），SMRP，soluble mesothelin-related peptides（可溶性メソテリン関連ペプチド），biomarker（バイオマーカー），early detection of mesothelioma（悪性中皮腫の早期診断）

はじめに

悪性中皮腫（malignant mesothelioma，MM）は体腔内面を広く覆う漿膜に発生する腫瘍で，胸膜，腹膜，心膜，および非常にまれではあるが，精巣鞘膜からも発生する。この中で，胸膜由来の悪性胸膜中皮腫（malignant pleural mesothelioma：MPM）が最も多い。これまで，MMは非常にまれな疾患とされてきたが，本疾患と石綿曝露との密接な関連性が報告されてから半世紀が経過した現在，その罹患者数および死亡者数は世界的に急激な増加を辿っている[1)2)]。MPMの予後は不良であり，生存期間中央値は9～17カ月とされている[3)]。MPMの早期発見は非常に困難であり，確定診断が得られた時点では，すでに進行期である症例が大多数を占める。MPMに対する標準的治療法はいまだに確立されておらず，手術療法によって腫瘍が肉眼的に完全切除されても治癒に至る症例は極めて少ない。したがって，治療成績の向上には，疾患の早期発見と手術療法に化学療法や放射線療法などを組み合わせた集学的治療を確立することが極めて重要な役割を果たす[4)]。

MPMの早期診断には，画像検査や胸腔鏡検査の進歩とともに，血清や体腔液診断に有用なバイオマーカーの開発が必要不可欠である。現在，MPMの血清診断における新規バイオマーカーとして最も注目されている分子が，可溶性メソテリン関連ペプチド（soluble mesothelin-related peptides：SMRP）である[5)]。SMRP本体の蛋白であるメソテリンは，膵臓癌，卵巣癌，中皮腫やその他の癌に過剰発現する40 kDaの細胞膜表面に存在する糖蛋白である。メソテリンは癌特異的抗原ではなく，胸膜，腹膜および心膜の正常中皮細胞にも発現する分化抗原と考えられている。メソテリンの遺伝子は69 kDaの前駆蛋白（mesothelin/megakaryocyte potentiating factor：MPF family proteins）をコードし，この糖蛋白はfurin-like proteinaseで切断後N末端側は31 kDaのMPFとして血中に放出される。C末端側40 kDaの糖蛋白はメソテリンとして細胞膜に結合している。メソテリンには3種類のvariant formが知られおり，そのうちのひとつは修飾されたカルボキシル基終末をもち，GPIアンカーを欠如するために細胞膜から遊離する。このsoluble isoformがSMRPに相当するとされる[6)]。2003年，Robinsonらは血清SMRPのMPMにおける診断的意義に関する最初の報告を行った[7)]。近年，SMRPを認識する2種類のモノクローナル抗体（OV569と4H3）を用いた新しい定量的enzyme-linked immunosorbent assay（ELISA）キットであるMESOMARK™の開発によって，血清SMRP濃度の測定が可能となった。このキットを用いて，SMRPのMMに対する診断性能を検討した研究が欧米や豪州から報告されている[8)～16)]。

今回，われわれは，MESOMARK™と同一の抗体を用いたchemiluminescent enzyme immunoassay（CLEIA）法による血清SMRP濃度測定キット（ルミパルス® メソテリン）を用いて，MM血清診断におけるSMRPの診断性能を多施設共同試験によって検討した。

Ⅰ．試験実施施設および対象

本試験は，愛知県がんセンター，大阪府立呼吸器・アレルギー医療センター，国立病院機構近畿中央胸部疾患センター，兵庫医科大学，兵庫県立成人病センター，労働者健康福祉機構岡山労災病院，国立病院機構呉医療センターの合計7施設にて，各施設の倫理委員会の審査・承認を受けて，2005年から2006年に多施設共同試験として実施した。

対象は，各施設を受診したMM患者および対照疾患患者のうち，試験参加に関して文書で同意が得られた被験者および健常者（石綿曝露歴のない院内ボランティア）の計802例である。MM85例のうち，MPMは77例で，7例が悪性腹膜中皮腫，1例が精巣鞘膜由来の中皮腫であった。比較対照疾患の内訳は，肺癌，良性呼吸器疾患（石綿非関連良性疾患および石綿関連良性疾患），石綿曝露歴のない高血圧・慢性心疾

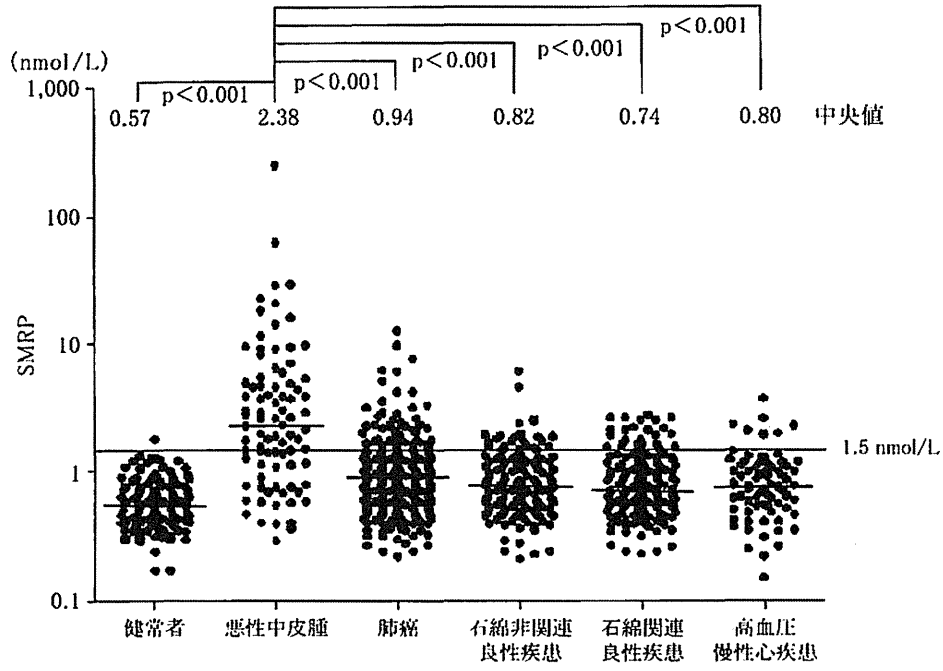

図 1　悪性中皮腫および対照疾患における血清 SMRP 濃度の分布

表 1　悪性中皮腫および対照疾患における血清 SMRP 濃度および陽性率

	健常者	悪性中皮腫	肺癌	石綿非関連良性疾患	石綿関連良性疾患	高血圧・慢性心疾患
症例総数	110	85	240	136	157	74
中央値（nmol/L）	0.57	2.38	0.94	0.82	0.74	0.80
SD（nmol/L）	0.30	28.57	1.36	0.74	0.58	0.62
陽性数	1	56	51	24	24	7
陽性率	1%	66%	21%	18%	15%	9%

SD：standard deviation

患であった（表1）。

II. 血清 SMRP 濃度の測定

すべての被験者に対して採血を実施し，速やかに血清を分離後，−80℃にて凍結保存した。

血清 SMRP 濃度は，体外診断用医薬品として2010年10月に認可されたルミパルス® メソテリン（富士レビオ株式会社製造）を用いて測定した。本キットは，米国，カナダ，ヨーロッパおよびオーストラリア等で販売されている ELISA 法による SMRP 測定キットである MESOMARK™ と同一の抗体を用いた，2ステップサンドイッチ法に基づく CLEIA 法による SMRP 測定試薬である。本試薬は，全自動化学発光酵素免疫測定システム用試薬であり，約30分間で測定が完了し，測定範囲は 0.1 から 100 nmol/L に及ぶ。MESOMARK™ との相関性は，ほぼ1：1であり，同等の測定結果の得られることが報告されており，参考基準値は両者ともに 1.5 nmol/L に設定されている[9)17)]。

統計学的解析には，statistical package for social science（SPSS）を使用し，2群間の有意

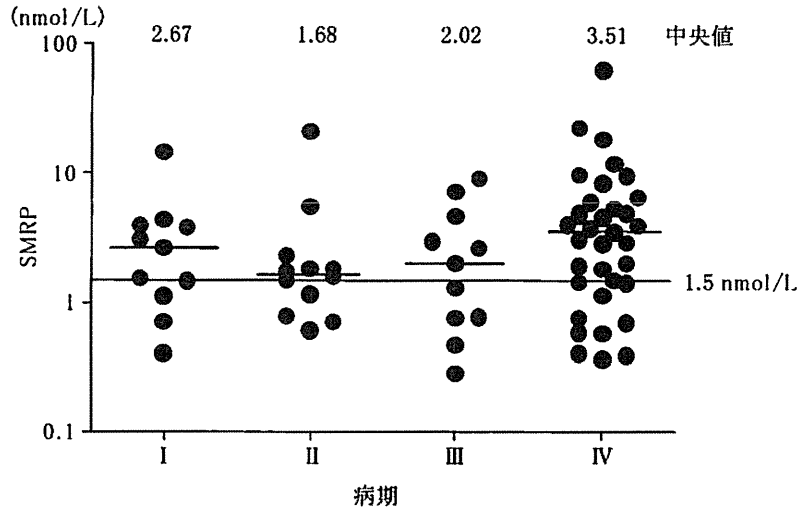

図 2　悪性胸膜中皮腫の各病期における血清 SMRP 濃度の分布

表 2　悪性胸膜中皮腫の各病期における血清 SMRP 濃度および陽性率

	I	II	III	IV
症例総数	11	12	11	35
中央値（nmol/L）	2.67	1.68	2.02	3.51
SD（nmol/L）	3.92	5.67	2.93	11.05
陽性数	7	8	6	24
陽性率	64%	67%	55%	69%

SD：standard deviation

差検定は Mann-Whitney 検定を用いて行った。各図中には測定値の中央値を示した（単位は nmol/L）。

III. 結　果

1．健常者における血清 SMRP 濃度

石綿曝露歴のない健常者 110 例の血清 SMRP 濃度を測定し，測定結果を対数変換した結果，SMRP 測定値の正規分布が確認された。SMRP 測定値は，1 例（1.86 nmol/L）を除き，すべて参考基準値（1.5 nmol/L）未満であった（陰性率 99.1%）。

2．MM および比較対照疾患における血清 SMRP 濃度および陽性率（図 1，表 1）

血清 SMRP 濃度の参考基準値を 1.5 nmol/L とした場合，この値よりも高値を示す症例の割合（陽性率）は，それぞれ MM66%（56/85 例），肺癌 21%（51/240 例），石綿非関連良性疾患 18%（24/136 例），石綿関連良性疾患 15%（24/157 例），高血圧・慢性心疾患 9%（7/74 例）であった。MM における血清 SMRP 濃度は，比較対照群および健常者に比較して有意に高値であった。MM の原発部位別の陽性率は，胸膜 65%（50/77 例），腹膜 86%（6/7 例）であった。

3．MPM 各病期における血清 SMRP 濃度および陽性率（図 2，表 2）

病期が確定した MPM69 例について，各病期における血清 SMRP 濃度および陽性率を検討した結果，各病期間での血清 SMRP 濃度に有意差はなく，陽性率は，それぞれ I 期 64%（7/

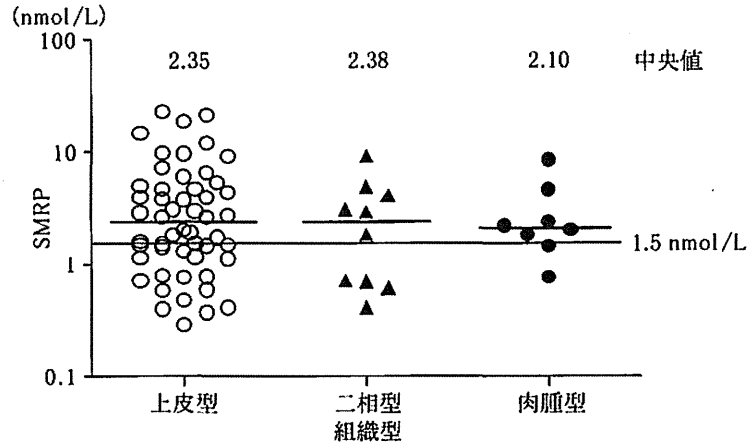

図 3　悪性胸膜中皮腫の各組織型における血清 SMRP 濃度の分布

表 3　悪性胸膜中皮腫の各組織型における血清 SMRP 濃度および陽性率

	上皮型	二相型	肉腫型
症例総数	52	10	8
中央値（nmol/L）	2.35	2.38	2.10
SD（nmol/L）	5.19	2.72	2.48
陽性数	34	6	6
陽性率	65%	60%	75%

SD：standard deviation

11例），II期67%（8/12例），III期55%（6/11例），IV期69%（24/35例）であった。

4．MPM各組織型における血清SMRP濃度および陽性率（図3，表3）

組織型の確定し得たMPM70例の内訳は，上皮型52例，二相型10例，肉腫型8例であった。各組織型間における血清SMRP濃度に有意差はなく，陽性率は，それぞれ上皮型65%（34/52例），二相型60%（6/10例），肉腫型75%（6/8例）であった。

IV．考　察

近年，SMRPを認識する2種類のモノクローナル抗体（OV569と4H3）を用いた新しい定量的ELISAキットであるMESOMARK™の開発によって，血清SMRP濃度の測定が可能となった[8)~16)]。これらの報告では，血清SMRP濃度は健常者および石綿曝露者，石綿関連良性胸膜疾患，肺癌などの対照疾患に比較して，MMにおいて有意に上昇していた[8)~16)]。MESOMARK™と同一の抗体を使用したCLEIAキットであるルミパルス® メソテリンを用いた本試験においても，血清SMRP濃度は，健常者および肺癌，良性呼吸器疾患，高血圧・慢性心疾患に比較してMMにおいて有意に上昇していた。この結果は，MM血清診断におけるSMRPの高い診断性能と測定結果の普遍性および再現性とを意味するものと考えられた。これまでのMESOMARK™を用いた測定報告では，MMにおける血清SMRP濃度中央値は0.79から2.39 nmol/Lの範囲に分布していた[8)~16)]。本試験におけるMMの血清SMRP

濃度中央値も2.38 nmol/Lであり，この範囲内に含まれていた。参考基準値（カットオフ値）に関しては，健常者を対象とした結果から，MESOMARK™およびルミパルス® メソテリンともに，1.5 nmol/Lに設定されている[9)17)]。本試験において，健常者110名のうち99%が，血清SMRP濃度1.5 nmol/L（参考基準値）未満であった。この参考基準値の条件下で，MMにおけるSMRPの陽性率（感度）は66%であった。一方，これまでのMESOMARK™を用いた測定報告では，SMRPの感度は48%から80%（中央値68.2%）と報告されており，ルミパルス® メソテリンの感度もこれらの測定報告に匹敵するものと考えられる。

MPMにおけるSMRP陽性率は65%であり，腹膜中皮腫を含むMM全症例の陽性率と概ね同等であった。MPM各病期間での血清SMRP濃度と陽性率に有意差はなく，I-II期においても，III-IV期に匹敵する陽性率が得られた。このことは，本キットが高感度の測定系であることから，早期例においても血清SMRPの検出が可能であることが推察された。Passらは[13)]，II期以上の症例はI期症例に比較して血清SMRP濃度が有意に上昇することを報告しているが，その他の報告では病期と血清SMRP濃度との明らかな関連性は指摘されなかった[9)10)14)]。今後，MPM早期診断マーカーとしてのSMRPの意義を明らかにするために，早期例を中心とした検討が必要になるものと考える。

MPMの各組織型と血清SMRP濃度との関連性に関して，本試験では，各組織型間での血清SMRP濃度と陽性率に有意差はなく，上皮型のみならず肉腫型においても75%が陽性を示した。これまでのMESOMARK™を用いた測定報告では，組織型と血清SMRP濃度との関連性については一定の見解が得られておらず，上皮型が肉腫型に比較して有意に高値であるという報告[7)8)10)15)]と，本試験と同様，各組織型間で有意差はないとする報告[9)11)13)14)]とに分かれている。

MMの診断には，石綿曝露歴，臨床症状，血液生化学検査，画像および内視鏡検査，体腔液細胞診，生検病理組織診などを駆使した総合的なアプローチが必要とされる[4)]。しかしながら，高齢者や全身状態不良例では，侵襲的検査の適応外となることも多く，診断に苦慮する場合も少なくない。このような症例では，MMに対して診断性能の高いバイオマーカーによる血清診断が重要な役割を担う。ルミパルス® メソテリンによる血清SMRP濃度測定は，一般の血液検査と同様，患者から採取した血清または血漿を用いて全自動分析装置にて約30分間で測定が完了する。したがって，患者への侵襲もなく，技術的にも容易に短時間で測定結果を得ることが可能であることから，今後，本キットは，MMを疑う患者に対して汎用されていくことが予想される。また，MM発症リスクの高い石綿曝露者を対象とした血液スクリーニング検査のひとつとして用いられることも想定される。

おわりに

現状では，石綿曝露者に発症した胸水貯留が良性石綿胸水であるのか，MPMの初期症状であるのかを鑑別することは困難である。しかし，この時期での診断機会を逸することは早期MPMの発見を見逃すこととなる。今回の検討では，SMRP測定がMPMの早期診断に有用なバイオマーカーとなることを示唆する結果が得られており，今後のさらなる研究の成果に期待が寄せられる。

文　献

1) 中野孝司．悪性胸膜中皮腫．呼吸 28：509-515, 2009.
2) 中野孝司．悪性胸膜中皮腫（びまん性悪性胸膜中皮腫）．別冊日本臨牀 呼吸器症候群（第2版）10：380-384, 2009.
3) Tsao AS, Wistuba I, Roth JA, Kindler HL：Malignant pleural mesothelioma. J Clin Oncol 27：2081-2090. 2009.
4) 福岡和也，田中文啓，辻村　亨，玉置知子，長谷

川誠紀，中野孝司：中皮腫に対する早期診断指標の探索的研究．日衛誌（Jpn. J. Hyg.）**66**：553-557, 2011.

5) Scholler N, Fu N, Yang Y, Ye Z, Goodman GE, Hellstrom KE, Hellstrom I：Soluble member (s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. Proc Natl Acad Sci USA **96**：11531-11536, 1999.
6) Hassan R, Bera T, Pasten I：Mesothelin：a new target for immunotherapy. Clin Cancer Res **10**：3937-3942, 2004.
7) Robinson BWS, Creaney J, Lake R, Nowak A, Musk AW, Klerk ND, Winzell P, Hellstrom KE, Hellstrom I：Mesothelin-family proteins and diagnosis of mesothelioma. Lancet **362**：1612-1616, 2003.
8) Scherpereel A, Grigorlu B, Conti M, Gey T, Gregoire M, Copin MC, Devos P, Chahine B, Porte H, Lassalle P：Soluble mesothelin-related peptides in the diagnosis of malignant pleural mesothelioma. Am J Respir Crit Care Med **173**：1155-1160, 2006.
9) Beyer HL, Geschwindt RD, Glover CL, Tran L, Hellstrom I, Hellstrom KE, Miller MC, Verch T, Allard WJ, Pass HI, Sardesai NY：Mesomark：a potential test for malignant pleural mesothelioma. Clin Chem **53**：666-672, 2007.
10) Cristaudo A, Foddis R, Vivaldi A, Guglielmi G, Dipalma N, Filiberti R, Neri M, Ceppi M, Paganuzzi M, Ivaldi GP, Mencoboni M, Canessa PA, Ambrosino N, Chella A, Mutti L, Puntoni R：Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. Clin Cancer Res **13**：5076-5081, 2007.
11) Creaney J, Van Bruggen I, Hof M, Segal A, Musk AW, de Klerk N, Horick N, Skates SJ, Robinson BW：Combined CA 125 and mesothelin levels for the diagnosis of malignant mesothelioma. Chest **132**：1239-1246, 2007.
12) Amati M, Tomasetti M, Scartozzi M, Mariott L, Alleva R, Pignotti E, Borghi B, Valentino M, Governa M, Neuzil J, Santarelli L：Profiling tumor-associated markers for early detection of malignant mesothelioma：an epidemiologic study. Cancer Epidemiol Biomarkers Prev **17**：163-170, 2008.
13) Pass HI, Wali A, Tang N, Ivanova A, Ivanov S, Harbut M, Carbone M, Allard J：Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. Ann Thorac Surg **85**：265-272, 2008.
14) Schneider J, Hoffmann H, Dienemann H, Herth FJF, Meister M, Muley T：Diagnostic and prognostic value of soluble mesothelin-related proteins in patients with malignant pleural mesothelioma in comparison with benign asbestosis and lung cancer. J Thorac Oncol **3**：1317-1324, 2008.
15) Creaney J, Yeoman D, Demelker Y, Segal A, Musk AW, Skates SJ, Robinson BWS：Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. J Thorac Oncol **3**：851-857, 2008.
16) Portal JAR, Becerra ER, Rodriguez DR, Michavila IA, Martinez AQ, Roza CD, Jimenez AL, Montes II, Rivas PC：Serum levels of soluble mesothelin-related peptides in malignant and nonmalignant asbestos-related pleural disease：Relation with past asbestos exposure. Cancer Epidemiol Biomarkers Prev **18**：646-650, 2009.
17) 中町　衛，桑原明子，村上　弘，皆川英孝，森山和重：ルミパルス　メソテリンの基礎性能評価．医学と薬学 **65**：261-267, 2011.

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Polymorphisms in intron 1 of the *EGFR* gene in non-small cell lung cancer patients

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Abstract. The epidermal growth factor receptor (*EGFR*) gene is highly polymorphic and its expression and activity may be affected by various polymorphisms. There have been several studies examining associations between *EGFR* polymorphisms and clinical outcome of lung cancer therapy; however, the underlying mechanism is largely unknown. The present study investigated *EGFR* polymorphism status and its correlation with clinicopathological features in Japanese non-small cell lung cancer (NSCLC) patients. We investigated 5 polymorphisms in the *EGFR* gene (-216G/T, -191C/A, 8227G/A, D994D and R497K) in 274 surgically-treated NSCLC patients. TaqMan single nucleotide polymorphism (SNP) genotyping assays and a PCR-based assay were used to analyze these polymorphisms. In our cohort of patients we did not find any evidence of the -191C/A polymorphism. Our results showed that the patients with the 8227GA or AA type in intron 1 had a significantly better prognosis with the anti-EGFR therapy than the patients with the GG type (p=0.0448) in terms of recurrence of lung cancer. No significant association was observed between 3 other SNPs (-216G/T, D994D and R497K) and clinicopathological features. The *EGFR* 8227G/A polymorphism in intron 1 may be associated with clinical outcome in NSCLC patients treated with EGFR tyrosine kinase inhibitors.

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Introduction

Lung cancer is a major cause of mortality from malignant diseases due to its high incidence, malignant behavior and lack of major advancements in treatment strategy (1). There is a large body of accumulated evidence that the epidermal growth factor receptor (EGFR) and its family members are heavily involved in the development and progression of numerous human tumors, including lung cancer (2,3). The EGFR tyrosine kinase inhibitor (TKI), gefitinib, was approved for the treatment of non-small cell lung cancer (NSCLC) in Japan in 2002. Two original studies have revealed that *EGFR* mutation status at the tyrosine kinase (TK) domain in NSCLC patients was correlated with a good response to gefitinib (4,5). From the results of the Iressa Pan-Asia Study (IPASS), *EGFR* mutations are the strongest predictive biomarker for progression-free survival (PFS) and tumor response to first-line gefitinib therapy for NSCLC (6).

The *EGFR* gene is highly polymorphic and its expression and activity are significantly affected by various polymorphisms (7-9). As for interethnic differences in CA repeat length in intron 1, a length of less than 17 in Japanese individuals is less frequent than in Caucasians (10). However, the frequency of *EGFR* mutations is higher in the Japanese population than in other ethnic groups. In intron 1, the -216G/T and -191C/A polymorphisms in the *EGFR* promoter are associated with altered promoter activity and gene expression (8). CA simple sequence repeats (CA-SSRs) in intron 1 (rs45559542) (8,12,13), -216G/T (rs712829) (8,12) and D994D (rs2293347) (11) polymorphisms have been reported to influence clinical outcomes in gefitinib-treated NSCLC patients. In addition, the 8227G/A polymorphism (rs763317) located in intron 1 has been reported to be associated with smoking status and gender in lung adenocarcinomas in the Taiwanese population (14).

To determine the *EGFR* polymorphism status and its correlation with clinicopathological features in lung carcinoma in the Japanese population, we investigated *EGFR* gene status using TaqMan single nucleotide polymorphism (SNP) genotyping assays. These findings were analyzed in relation to the clinicopathologic features of lung cancer.

Materials and methods

Patients and treatment. The study group included 261 lung cancer patients who had undergone surgery at the Nagoya City University Hospital, Japan, between 1997 and 2011. Thirty-three patients were treated with gefitinib for the recurrence of lung cancer following surgery. We also investigated polymorphisms for 13 NSCLC patients who had been treated with gefitinib for the recurrence of cancer at the Kinki-chuo Chest Medical Center, Osaka Japan. The lung tumors were classified according to the general rule for clinical and pathological recording of lung cancer in Japan, as well as according to the WHO classification. All tumor samples were immediately frozen and stored at -80˚C until assayed.

The clinical and pathological characteristics of the 274 lung cancer patients were as follows: 194 (70.8%) were male and 80 were female; 192 were diagnosed as adenocarcinoma and 82 were diagnosed as other types of carcinoma (63 squamous cell carcinomas, 6 adenosquamous carcinomas, 6 large cell carcinomas, 3 carcinoids, 3 pleomorphic carcinomas, 1 adenoid cystic carcinoma and 1 carcinosarcoma); 187 (68.2%) were smokers (current or former smoker) and 87 were non-smokers (Table I). Written informed consent was obtained from the patients and the Institutional Ethics Committee of the Nagoya City University approved the study.

Genotyping assays for the EGFR polymorphism. Genomic DNA was extracted from peripheral blood (n=109) taken prior to surgery or from adjacent normal lung tissues taken at surgery using the Wizard SV Genomic DNA Purification system (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. *EGFR* mutation statuses at the kinase domain were investigated using the TaqMan PCR assay (Applied Biosystems, Foster City, CA, USA). The results of the TaqMan PCR assay have been previously reported (15).

TaqMan SNP genotyping assays (Applied Biosystems) were used for genotyping 4 polymorphisms in the *EGFR* gene (-216G/T, -191C/A, 8227G/A, assay ID: C_2310200_10; and D994D, assay ID: C_15970737_20; Table II) according to the manufacturer's instructions (16). The cycling conditions for the TaqMan SNP assays were as follows: 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min, with a 1-min extension at 25˚C following the last cycle. The R521K (rs11543848, also assigned as R497K in the literature) polymorphism was examined by the PCR-RFLP method as described previously (17). Sixty-four lung cancer samples were analyzed for *EGFR* gene amplification using fluorescence *in situ* hybridization (FISH) and the results have been previously reported (18).

Statistical analyses. Statistical analyses were carried out using the Mann-Whitney U test for unpaired samples and Wilcoxon's signed rank test for paired samples. Linear relationships between variables were determined by means of simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and the χ^2 test. The overall survival (OS) of lung cancer patients was examined using the log-rank test. All analyses were performed using the StatView software package (Abacus Concepts Inc, Berkeley, CA, USA) and differences were considered significant when $p<0.05$.

Table I. Clinical and pathological characteristics of the 274 lung cancer patients.

	Patients (n=274)	
	No.	%
Age (years)		
≤60	78	28.5
>60	196	71.5
Gender		
Male	194	70.8
Female	80	29.2
Smoking status		
Non-smoker	87	31.8
Smoker	187	68.2
Pathological subtype		
Adeno	192	70.1
Other	82	29.9
EGFR mutation		
Positive	81	29.9
Negative	190	70.1

Smoker, current or former smoker; Adeno, adenocarcinoma; *EGFR*, epidermal growth factor receptor.

Results

EGFR gene mutation and amplification statuses. Of the 274 patients, 42 had the deletion-type *EGFR* mutations in exon 19; 35 had the missense point mutations (5 G719S, 29 L858R and 1 L861Q) in exon 18 or 21; and 4 had exon 20 insertion mutations (15,20). Sixty-four samples were studied for *EGFR* gene amplification using FISH analyses. According to the criteria by Cappuzzo *et al*, 21 were FISH-positive and 43 were FISH-negative (19).

EGFR polymorphisms in Japanese lung cancers. In our Japanese cohort, there was no -191C/A polymorphism and we did not perform any further analyses for this polymorphism. For rs712829 (-216G/T), 255 patients were GG, 19 were GT and no TT was found. For rs2293347 (D994D), 125 patients were CC, 110 were CT and 39 were TT. For rs11543848 (R497K), 93 were AA, 135 were GA and 46 were GG. No correlation existed between these 3 SNPs (-216G/T, GG vs. GA+AA; D994D, CC+CT vs. TT; R497K, GG vs. GA+AA) and clinicopathological features of the lung cancers.

Of the 274 patients, 87 had the 8227G/A *EGFR* variant (9 AA and 78 GA). Of these, 64 were male and 23 were female, 24 were non-smokers, 59 were smokers and 4 were unknown. Adenocarcinomas were significantly more frequent in GG-type patients (139/187, 74.3%) than in the GA- or AA-type patients (53/87, 60.9%, p=0.0331). However, the polymorphism did not correlate with gender (p=0.5687), smoking (non-smokers vs. smokers, p=0.3325), or *EGFR* mutation (p=0.1539) statuses of lung cancer (Table III). *EGFR* gene amplification as identified by FISH positivity was not

Table II. Genotyping approach for polymorphism analysis of the *EGFR* gene.

Primer sequences	-216G/T (rs712829)	-191C/A (rs712830)
VIC-MGB	AGCCTCCGCCCCC	CCTCGGCCGCGTCG
FAM-MGB	CAGCCTCCTCCCCC	CCTCGGCCGCGGCG
Forward primer	CCCGCGCGAGCTAGAC	CCCCGCACGGTGTGA
Reverse primer	GGGCGCTCACACCTG	GGCTAGCTCGGGACTCC

VIC-MGB, VIC dye-labeled TaqMan MGB probe; FAM-MGB, FAM dye-labeled TaqMan MGB probe; *EGFR*, epidermal growth factor receptor.

Table III. Association of the *EGFR* 8227G/A polymorphism with clinicopathological data of 274 lung cancer patients.

	GG		GA+AA		
Factors	No.	%	No.	%	p-value
Age (years)					
≤60	52	27.8	26	29.9	0.7741
>60	135	72.2	61	70.1	
Gender					
Male	130	69.5	64	73.6	0.5687
Female	57	30.5	23	26.4	
Smoking status					
Non-smoker	63	33.7	24	27.6	0.3325
Smoker	124	66.3	59	72.4	
Pathological subtype					
Adeno	139	74.3	53	60.9	0.0331
Others	48	25.7	34	39.1	
EGFR mutation					
Positive	61	32.6	20	23.8	0.1539
Negative	126	67.4	64	76.2	

Smoker, current smoker or former smoker; Adeno, adenocarcinoma; *EGFR*, epidermal growth factor receptor.

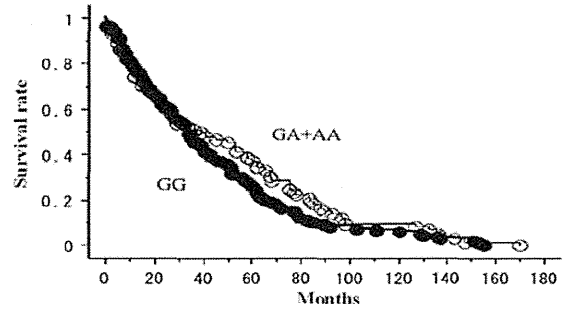

Figure 1. The overall survival of 225 lung cancer patients who were not treated with gefitinib was studied in reference to the *EGFR* polymorphism (8227G/A) status. The prognosis was not significantly different between the patients with 8227GG type (53/152 were deceased) and the patients with 8227GA or AA type (23/73 were deceased) (log-rank test, p=0.1753). *EGFR*, epidermal growth factor receptor.

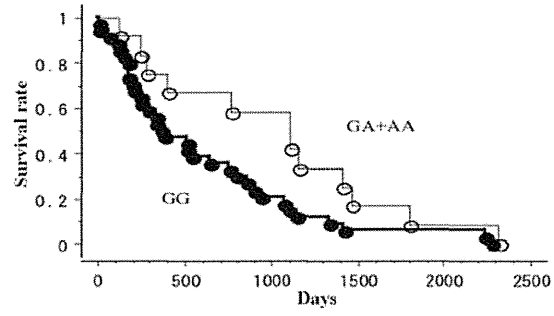

Figure 2. The overall survival of 46 gefitinib-treated lung cancer patients was studied in reference to the *EGFR* polymorphism (8227G/A) status. The patients with 8227GA or AA type (5/12 were deceased; median follow-up, 33.3 months) had significantly better prognosis than the patients with 8227GG type (26/34 were deceased; median follow-up, 20.0 months) (log-rank test, p=0.0448). *EGFR*, epidermal growth factor receptor.

correlated with polymorphism statuses, including D994D (p=0.5884), -216G/T (p>0.9999), R497K (p=0.2043) and 8227G/A (p>0.9999).

Correlation between clinical course of lung cancer patients and EGFR polymorphisms. The OS of the 225 lung cancer patients who did not receive gefitinib, with follow-up until June 30, 2011, was studied in reference to the *EGFR* polymorphism status. The prognosis was not significantly different between the *EGFR* 8227G/A types (GA+AA, 23/73 were deceased; GG, 53/152 were deceased; p=0.1753; Fig. 1). No significant association was observed between the other 3 SNPs (-216G/T, D994D and R497K) and disease outcome (data not shown).

Correlation between clinical course of gefitinib-treated lung cancer patients and EGFR polymorphism. The OS of 46 gefitinib-treated lung cancer patients, with follow-up until June 30, 2011, was studied in reference to the *EGFR* polymorphism status. In this analysis, 12 patients had *EGFR* 8227GA or AA types. Of the 46 patients, 31 had *EGFR* mutations and 11 were *EGFR* 8227GA or AA. There was a tendency towards higher *EGFR* mutation ratio in the 8227GA- or AA-type patients compared with GG-type patients (p=0.0702). Other clinical backgrounds, including gender (p=0.3071), smoking (p=0.4893) and pathological status (p=0.3059) were not correlated with 8227G/A polymorphism status. The prognosis following gefitinib therapy was significantly better for the *EGFR* GA- or AA-type patients (5/12 were deceased; mean survival, 1,014 days) when compared with the 8227GG-type patients (26/34 were deceased; mean survival, 607 days; log-rank test,

p=0.0448; Fig. 2). Using the multivariate analysis, *EGFR* mutation (p=0.0316; hazard ratio, 2.174) but not 8227G/A (p=0.2232; hazard ratio, 1.587) was the independent prognostic factor for gefitinib-treated patients.

There was no association between the other 3 polymorphisms (-216G/T, p=0.7599; D994D, p=0.1813; and R497K, p=0.885) and prognosis for the gefitinib-treated patients.

Discussion

In this study, gefitinib-treated patients with an A allele at the *EGFR* 8227G/A site were found to have a better prognosis compared with GG-type patients. However, there was no association between the other 3 polymorphisms (-216G/T, D994D and R497K) and prognosis following gefitinib therapy.

Previous studies have suggested that -216G/T (8,12) and D994D (11) polymorphisms are associated with clinical outcome of gefitinib therapy. In intron 1, CA-SSR of *EGFR* has been the most studied polymorphism. CA-SSR has been associated with *EGFR* gene expression and has been reported to correlate with clinical outcome of gefitinib therapy (8,12,13,21). Shorter CA repeats have been associated with higher transcription levels of *EGFR* and have been reported to be correlated with better clinical outcome of gefitinib therapy. Tiseo *et al* revealed that patients with the CA-16 genotype had a longer survival compared with those with other genotypes (13). Liu *et al* found that the -216G/T polymorphism and CA-19 genotype are found more frequently in patients with exon 19 deletions (22). On the other hand, Suzuki *et al* reported that the *EGFR* protein expression level was significantly higher in the shorter CA repeats group than in the longer allele group, but its length was not associated with *EGFR* somatic mutations (23). In a Japanese cohort, Ichihara *et al* reported that patients with a short CA-SSR1 had a prolonged OS as compared with those with a longer CA-SSR, but this difference was not significant in patients with a drug-sensitive *EGFR* mutation (p=0.13) (24). They found that FISH status, CA-SSR1 length and the SNP status in the promoter region (-216G/T or -191G/A) had no association with responsiveness to gefitinib in cases of lung cancer in Japanese individuals, similar to our results. One explanation for the results is that the variant forms of the SNPs, -216 G/T (6.6%) and -191G/A (0.6%), were less frequent in East Asians than in individuals of European descent (60.3 and 37%, respectively) (25). As for the D994D polymorphism, using direct sequencing, our group has previously revealed that the polymorphism did not affect the gefitinib sensitivity in Japanese individuals (26). This polymorphism is located in exon 25 and a synonymous SNP does not change the amino acid sequence of the protein, so it does not influence the biological function of the protein itself. Ma *et al* revealed that the D994D polymorphism did affect PFS but not OS following gefitinib therapy (11).

The 8227G/A polymorphism is also located in intron 1, but there have been few studies examining this SNP (14,27). Jou *et al* revealed that the *EGFR* 8227G/A polymorphism was associated with lung cancer, especially in non-smoking female lung adenocarcinoma patients in the Taiwanese population (14). Thus, this variation may lead to the different modifications of cancer genes, including *EGFR*, in tumorigenic pathways among different histological subtypes, gender and ethnicity. The 8227G/A SNP is located in intron 1, 6.9 kb downstream of the CA-SSR1 polymorphism. Additional functional analyses of this SNP are needed to better understand the mechanism by which the 8227G/A SNP of *EGFR* affects lung cancer. In our analysis, although the 8227G/A polymorphism in intron 1 was not correlated with *EGFR* somatic mutations, the GA or AA type was associated with longer survival of the gefitinib-treated patients. The underlying mechanisms remain unclear, but it may be that intron 1 of *EGFR* is associated with sensitivity to EGFR TKIs in lung cancer patients, and is correlated with certain biomarkers other than *EGFR* mutations. The sample size of the present study was too small to address this hypothesis. The extact effect of the polymorphism on survival time of patients treated with or without EGFR TKIs needs further clinical investigation with a larger sample size.

In summary, the 8227G/A polymorphism of *EGFR* may influence OS in gefitinib-treated lung cancer patients.

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References

1. Ginsberg RJ, Kris K and Armstrong G: Cancer of the lung. In: Principles and Practice of Oncology. DeVita VT, Hellman S and Rosenberg SA (eds). 4th edition. JB Lippincott Co., Philadelphia, PA, pp673-682, 1993.
2. Nicholson RI, Gee JM and Harper ME: EGFR and cancer prognosis. Eur J Cancer 37: S9-S15, 2001.
3. Onn A, Choe DH, Herbst RS, *et al*: Synchronous overexpression of epidermal growth factor receptor and HER2-neu protein is a predictor of poor outcome in patients with stage I non-small cell lung cancer. Clin Cancer Res 10: 136-143, 2004.
4. Paez JG, Jänne PA, Lee JC, *et al*: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304: 1497-1500, 2004.
5. Lynch TJ, Bell DW, Sordella R, *et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350: 2129-2139, 2004.
6. Fukuoka M, Wu Y-L, Thongprasert S, *et al*: Biomarker analyses and first overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 29: 2866-2874, 2011.
7. Liu W, Innocenti F, Wu MH, *et al*: A functional common polymorphism in a Sp1 recognition site of the epidermal growth factor receptor gene promoter. Cancer Res 65: 46-53, 2005.
8. Liu W, Wu X, Zhang W, *et al*: Relationship of EGFR mutations, expression, amplification, and polymorphisms to epidermal growth factor receptor inhibitors in the NCI60 cell lines. Clin Cancer Res 13: 6788-6795, 2007.
9. Brandt B, Meyer-Staeckling S, Schmidt H, *et al*: Mechanisms of EGFR gene transcription modulation: relationship to cancer risk and therapy response. Clin Cancer Res 12: 7252-7260, 2006.
10. Liu W, Innocenti F, Chen P, *et al*: Interethnic difference in the allelic distribution of human epidermal growth factor receptor intron 1 polymorphism. Clin Cancer Res 9: 1009-1012, 2003.
11. Ma F, Sun T, Shi Y, *et al*: Polymorphisms of EGFR predict clinical outcome in advanced non-small-cell lung cancer patients treated with Gefitinib. Lung Cancer 66: 114-119, 2009.