markers for differentiating between PM and SC.[24,25] The present study also indicated no or only focal Ber-EP4 or MOC-31 staining for PM and, on the other hand, very high intensity and dense staining for SC. Therefore, these antibodies are also recommended as negative markers.

Recently, ER has been reported as a significant negative marker for PM,[20,25] and it was reported that no case of PM but more than 85% of SC cases (87% by Ordonez[20] and 93% by Barnetson et al[25]) were positive for ER. The results for ER expression in the present study are similar to these previous reports.

The present study includes a relatively large percentage of cases among men; however, sex differences could be excluded based on the analysis of positive rates for each antibody (Table 3). Barnetson et al[25] also reported no significant differences in the expression pattern of various positive and negative markers, including hormone receptors, between peritoneal mesothelioma cases in males and females. Attanoos et al[19] reported mesothelial and epithelial markers in PM cases only in females. Ordonez[24] included 25 female cases among 40 PM cases; however, there was no discussion of sex related to the evaluation of the expression of various antigens, including ER and progesterone receptor.

CA19-9 is a classical adenocarcinoma marker.[36] Ordonez[21] reported that 31 (67%) of 45 SCs and 0 (0%) of 40 PMs were positive for this marker and concluded that the limiting factor for its practical use was its low sensitivity in SC. However, in the present study, only a weak staining pattern (1+) was observed in 3 (13%) of 23 PMs, compared with the relatively higher percentage of staining in 80% of SCs (16/20). These results indicate that CA19-9 could be used as a negative marker.

CD15, also known as Leu-M1, is also reported as a possible negative marker for PM or a positive marker for adenocarcinoma.[24] In the present study, no mesothelioma cases were positive; however, 12 (60%) of 20 SC cases were positive for this antibody, although most of the positive cases were in the 1+ staining category. This tendency was also observed in CEA immunostaining. The relatively lower specificity and lower staining degree of these adenocarcinoma markers may not be adequate for a differential diagnosis.

Recently, Comin et al[37] reported the usefulness of h-caldesmon for differential diagnosis of pleural epithelioid mesothelioma from pulmonary adenocarcinoma, as well as PM from SC involving the peritoneum.[22] Although this antibody was included in the present study, no mesotheliomas or SC showed positive staining. A preliminary study of 60 pleural epithelioid mesotheliomas in our department also showed no reactivity (data not shown). This discrepancy may be due to different immunohistochemical procedures or different case selection. Therefore, the usefulness of h-caldesmon could not be determined at this time.

Mesothelin is a known 40-kDa glycosyl-phosphatidylinositol–linked glycoprotein and a differentiation antigen[38] and is highly expressed in normal mesothelial cells and epithelioid mesotheliomas.[39] In this study, all PM cases and 19 (95%) of 20 SC cases were positive for mesothelin, suggesting no usefulness of this antibody for differential diagnosis. Ordonez[39] reported the same result.

Conclusions

Each of the antibodies evaluated in the present study has some weak points for differential diagnosis. A combination of positive and negative markers, such as calretinin and thrombomodulin as positive markers and Ber-EP4, MOC-31, CA19-9, and ER as negative markers, which showed relatively high sensitivity and specificity for differential diagnosis between PM and SC, may contribute to accurate diagnosis and adequate therapy. Recently, Davidson et al[40] reported differences in the gene expression profile by using microarray expression and the GeneChip technique between ovarian/peritoneal serous carcinoma and pleural and/or peritoneal mesothelioma. These genome-wide expression analyses will provide new potential markers for the differential diagnosis of PM and ovarian SC.

From the Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.

Address reprint requests to Dr Takeshima: Dept of Pathology, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.

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Pathology of mesothelioma

Kouki Inai

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Abstract The incidence of mesothelioma has been gradually increasing in Japan, and the underlying factor for this is considered to be the increase in the amount of asbestos imported into Japan between 1960 and 1975. Mesothelioma can be roughly divided into localized and diffuse types, but the former is extremely rare. In making a diagnosis of mesothelioma, it is important to confirm the location of tumor and the specific gross findings before histological examination. Mesothelioma can be categorized histologically as epithelioid type, sarcomatoid type, biphasic type, desmoplastic type, among others. It can take many forms; consequently, there are many diseases to be differentiated when the diagnosis of mesothelioma is based on histological analyses. Immunohistochemical stains are useful for making a diagnosis, but the correct combination of antibodies as positive or negative markers should be selected and a comprehensive assessment of the staining results is necessary. The accuracy of the pathological diagnosis is very important to the patients because they can be receive official compensation or relief when the diagnosis of mesothelioma is confirmed. Under present conditions, both clinicians and pathologists must make a concerted effort to improve the accuracy of the diagnosis of mesothelioma.

Keywords Antibody · Diagnostic accuracy · Differential diagnosis · Immunohistochemistry · Mesothelioma

K. Inai (✉)
Department of Pathology,
Graduate School of Biomedical Sciences,
Hiroshima University, 1-2-3 Kasumi,
Minami-ku, Hiroshima 734-8551, Japan
e-mail: koinai@hiroshima-u.ac.jp

Incidence of mesothelioma

Mesothelioma is a malignant tumor that originates in the pleura, peritoneum, pericardium and tunica vaginalis, all locations where a lining of normal mesothelial cell is present. According to the official data of Ministry of Health, Labor and Welfare in Japan, the number of death due to mesothelioma was 500 in 1995, increasing gradually to 953 in 2004. This increase appears to parallel the increase of the amount of asbestos imported into Japan between 1960 and 1975, given that the latent period from the beginning of exposure to asbestos and an initial diagnosis of mesothelioma is approximately 40 years.

More detailed examination of the 878 patients who officially died from mesothelioma in 2003 reveals that the male:female ratio was almost 3:1 and that the peak of age of patients was 60+ in males and 70+ in females. The location of the mesothelioma was in the pleura in 84% of the cases, in the peritoneum in 12% and in the pericardium in 1%; there were not cases of mesothelioma in the tunica vaginalis. A larger proportion of females had mesothelioma in the peritoneum and pericardium than in the pleura [1].

General findings on mesothelioma

Mesothelioma can be roughly classified into localized and diffuse types, with the incidence of the latter being much higher than that of the former [2]. The localized form of mesothelioma was more frequently diagnosed in the past; however, most of the localized forms are currently diagnosed as a solitary fibrous tumor [3], which is a separate entity from mesothelioma based on immunohistochemical

phenotyping (positive for CD34, a marker of primitive endothelial cell) and has no relation to asbestos exposure [4].

At the early stage of pleural mesothelioma, small nodules are found in the parietal pleura (not in the visceral pleura) that eventually extend along the pleural surface. Eventually, parietal and visceral pleurae show adhesion, and the tumor encloses the entire lung parenchyma. Very few cases of peritoneal mesothelioma have been reported at the early stage and, consequently, little is known in terms of pathology and disease progression during the early stage [5]. Most of peritoneal mesothelioma is found as a diffusely extensive tumor involving intestinal serosa or a large tumor located at the omentum or mesentery.

Following the initial diagnosis of mesothelioma, it is important to confirm the location of the tumor and its gross findings before histological examination. In the case of a pleural mesothelioma, a tumor in the lung parenchyma suggests lung cancer with a pleural extension. In females with peritoneal extension, the ovary should be carefully examined as the primary site of the tumor because the differential diagnosis between ovarian cancer and peritoneal mesothelioma is difficult and can only be made on the basis of histological analyses.

Histology of mesothelioma

Histological classification of mesothelioma is shown in Table 1 [6]. There are three major types—epithelioid type, sarcomatoid type and biphasic type—and the proportion of each is approximately 60, 20 and 20%, respectively (Figs. 1, 2, 3) [7]. The desmoplastic type is rare (probably 1–2%) (Fig. 4), and special variants only appear sporadically (several percentages). However, the proportion of each histological type varies among the reports because of the large variety of histological analyses used [8–11].

Table 1 Histological classification of mesothelioma [6]

1. Epithelioid mesothelioma
2. Sarcomatoid mesothelioma
(1) Desmoplastic mesothelioma
3. Biphasic mesothelioma
4. Variants
(1) Lymphohistiocytoid mesothelioma
(2) Deciduoid mesothelioma
(3) Anaplastic mesothelioma
(4) Well differentiated papillary mesothelioma
(5) Others

Differential diagnosis

It is important to remember that there are many diseases to be differentiated when making a pathological diagnosis and that the tissue to be differentiated varies in terms of histological type (Table 2). Epithelioid types must be differentiated from lung adenocarcinoma for pleural mesothelioma [12], ovarian serous papillary adenocarcinoma or peritoneal serous carcinoma for peritoneal mesothelioma [13]. In terms of sarcomatoid types, sarcoma originating in the chest wall, lung, pleura, abdominal wall, peritoneum and intestine must be excluded. Sarcomatoid carcinoma (spindle cell sarcoma or pleomorphic carcinoma) of the lung is very difficult to differentiate from sarcomatoid pleural mesothelioma [14]. In terms of the biphasic type, carcinosarcoma or pulmonary blastoma of the lung, biphasic synovial sarcoma [15] of the pleural mesothelioma and carcinosarcoma of the female genital organs must be differentiated from their peritoneal counterpart. The desmoplastic type has a similar histology to fibrous pleuritis [16], and the differential diagnosis is very difficult, particularly if only a small biopsy specimen is available. Reactive mesothelial hyperplasia associated with pleuritis or other lung or pleural disease must be differentiated from early-stage epithelioid mesothelioma [17].

Usefulness of immunohistochemistry in an accurate diagnosis

The histochemical staining of hyarulonic acid and electron microscopic studies have been widely used in the past for making a differential diagnosis between mesothelioma and other tumors. However, immunohistochemical stains are currently the method of choice because of the simplicity and ease of these techniques. Many antibodies have been detected for use in immunohistochemical staining techniques aimed at diagnosing mesothelioma, but as yet there is no antibody that is completely specific for mesothelioma and on which a pathological diagnosis of mesothelioma can be singly based. Therefore, the combination of a number of antibodies as positive or negative markers is important, and an assessment of the results in a comprehensive manner is necessary (Table 3).

In the case of epithelioid mesothelioma, calretinin, WT1, thrombomodulin, mesothelin and D2-40 can be applied as a mesothelial cell marker [18–22]. CEA, TTF-1, Napsin A and surfactant apoprotein are used as markers for lung adenocarcinoma. In the case of ovarian serous papillary adenocarcinoma, we recommend CEA, Ber-EP4, MOC-31 and ER (estrogen receptor) as positive markers [23].

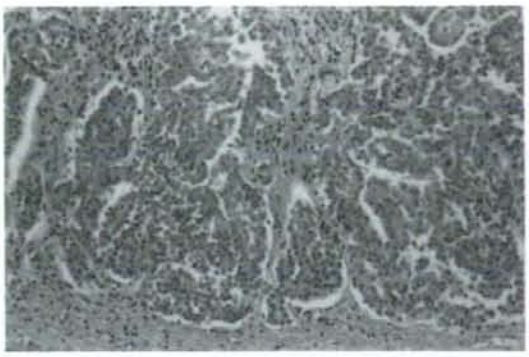

Fig. 1 Microphotograph of epithelioid mesothelioma (H&E stain). Papillo-tubular structure is prominent

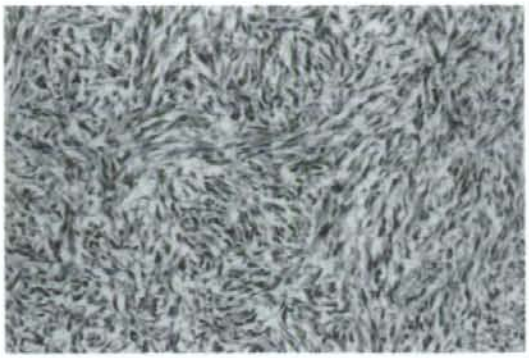

Fig. 2 Microphotograph of sarcomatoid mesothelioma (H&E stain). Proliferation of spindle cells mimies true sarcoma

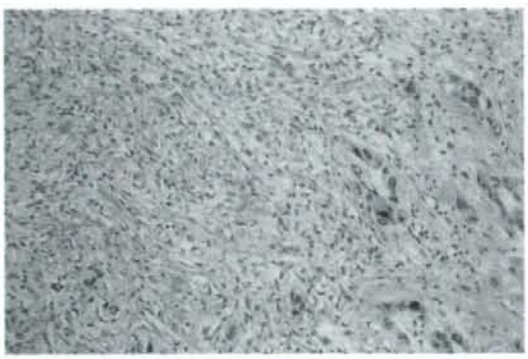

Fig. 3 Microphotograph of biphasic mesothelioma (H&E stain). The features of epithelioid mesothelioma and that of sarcomatoid mesothelioma are mixed within one tumor

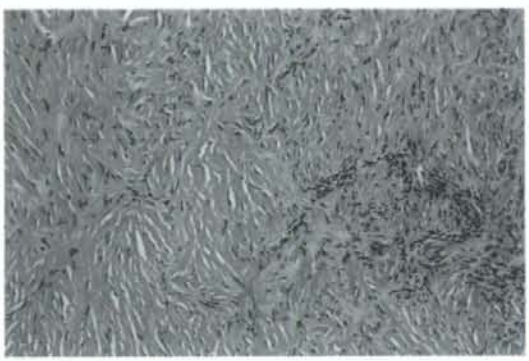

Fig. 4 Microphotograph of desmoplastic mesothelioma (H&E stain). The feature of granulation or fibrous pleuritis are dominant

The antibodies chosen for sarcomatoid mesothelioma are very different from those used for epithelioid mesothelioma. In the case of sarcomatoid mesothelioma, cytokeratin (AE1/AE3 or CAM5.2 as antibodies) exhibits a high specificity and is the most useful [24]. On the other hand, because the diagnosis for true sarcoma is based on the specific differentiation of tumor cells, mesothelioma is eliminated by making its differentiation clear. For example, the following antibodies are known to be useful: MyoD1, desmin and myoglobin for rhabdomyosarcoma; desmin, α-SMA and h-caldesmon for leiomyosarcoma; S-100p for malignant nerve sheath tumor; KP-1 for malignant fibrous histiocytoma [25].

The most difficult tumor to be differentiated from sarcomatoid mesothelioma of pleura is sarcomatoid carcinoma (spindle cell carcinoma, pleomorphic carcinoma) of the lung. When immunohistochemical stainings are used, both respond positively to cytokeratin [24]. In this case, therefore, the gross finding or the clinical diagnosis by imaging is very important, as already mentioned.

Immunohistochemical stains may be useful in differentiating between fibrous pleuritis and desmoplastic mesothelioma. Desmin is positive for spindle cells of the fibrous pleuritis, while desmin is negative for tumor cells of the sarcomatoid mesothelioma [26]. The combination of EMA, desmin and p53 is useful for differentiating between reactive mesothelial hyperplasia and early-stage epithelioid mesothelioma. Reactive mesothelial cells are positive for desmin and negative for EMA and p53 [27].

Compensation or relief of patients

In the compensation system for occupational exposures to asbestos and in the new law for non-occupational exposure to asbestos, if the diagnosis of mesothelioma is certain, it

Table 2 Differential diagnosis of mesothelioma

1. Epithelioid mesothelioma	
←→ Pleura	Adenocarcinoma of lung
	Metastatic adenocarcinoma involving pleura
	Reactive mesothelial hyperplasia
←→ Peritoneum	Serous papillary adenocarcinoma of ovary
	Serous carcinoma of peritoneum
2. Sarcomatoid mesothelioma	
←→ Pleura	Sarcoma, chest wall, pleura or lung
	Sarcomatoid carcinoma of lung
←→ Peritoneum	Sarcoma, abdomical wall, peritoneum or intestine
3. Desmoplastic mesothelioma	
←→ Pleura	Fibrous pleuritis
4. Biphasic mesothelioma	
←→ Pleura	Carcinosarcoma or pulmonary blastoma of lung
	Biphasic synovial sarcoma of pleura
←→ Peritoneum	Carcinosarcoma of ovary, uterus

Table 3 Antibodies used in the immunohistochemical staining for differential diagnosis

Type of mesothelioma	Positive markers	Negative markers
Epithelioid type	Calretinin	CEA
	WT1	TTF-1
	Thrombomodulin	Napsin A
	Mesothelin	Surfactant apoprotein
	D2-40	Ber EP4
		MOC31
		ER
Sarcomatoid type	AE1/AE3	Myo D1, Myoglobin
	CAM5.2	Desmin, h-calredesmon
		S-100p, KP-1

can almost always be presumed to be related to asbestos exposure and the patients can receive compensation or relief. Therefore, the accuracy of the pathological diagnosis as mesothelioma is very important. However, a rough estimate is that approximately 10–15% of mesothelioma patients receive an inadequate diagnosis. In the committee for the judgement of patients' relief, 30% of applicants are judged as not having mesothelioma or the decision is deferred until additional evidence is provided. It is therefore essential that Japanese clinicians and pathologists make an effort to improve the accuracy of the diagnosis as mesothelioma. In particular, the pathologists must improve the accuracy of the pathological diagnosis using adequate immunohistochemical stains.

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別刷

Surgery Frontier

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7. 中皮腫の病理

Pathology of mesothelioma

広島大学大学院医歯薬学総合研究科病理学　井内　康輝
Kouki Inai
（教授）

Summary

アスベストへの曝露によって中皮腫に罹患した患者に対する補償・救済制度の運用の面から，中皮腫の病理診断の精度向上が求められている。中皮腫は正常で中皮細胞が存在する胸膜，腹膜，心膜，精巣鞘膜に発生するが，その中でも胸膜発生例が80～90％と圧倒的に多い。肉眼的には漿膜に沿ってびまん性の進展を示す例が特徴的であるが，限局型も存在する。組織学的には，上皮型，肉腫型，線維形成型，二相型に分けられるが，これら以外に特殊型があり，組織像は多彩である。したがって病理学的には多くの疾患との鑑別診断が必要であり，組織型別に選択された複数の抗体を用いた免疫組織化学的染色が有用となる。鑑別診断の中では特に良悪性の判断が臨床的には最も重要である。診断のための生検においては，浸潤の有無の判断が可能となる十分量の組織採取が必要であり，また迅速診断による良悪性の判断は避けるべきである。

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Key Words　アスベスト曝露，免疫組織化学，上皮型中皮腫，肉腫型中皮腫，線維形成型中皮腫，二相型中皮腫

はじめに

中皮腫とは，胸膜，腹膜，心膜，精巣鞘膜に存在する中皮細胞・組織に由来する腫瘍である。その成因としては，アスベスト（石綿）への曝露が重視され，中皮腫患者の80％以上に職業上あるいは一般生活環境下（例えば尼崎のアスベストを扱う工場付近に住居がある）におけるアスベストへの曝露が証明される。従来は，職業上の曝露による発生例が大半で，中皮腫患者の多くが職業癌として労災補償を受けてきたが，近年，一般生活環境下での曝露による中皮腫例の増加が著しく，それらの患者に対する救済制度もつくられるに至った[1]。

こうした補償や救済制度では，中皮腫の大半がアスベストへの曝露によるという事実から，中皮腫であるという診断が確かであれば補償・救済が受けられる。中皮腫の診断については，画像診断，血液診断などの臨床診断に不確実な要素が多いことから，病理診断が最終判断として重視される。しかしながら，中皮腫の病理診断にも問題がないとはいえない[1]。そこで本稿では，中皮腫の病理診断の実際を紹介し，その問題点を指摘したい。

◆メモランダム◆

免疫組織化学

免疫組織化学は組織標本などにおいて腫瘍細胞の示す分化を把握するために用いられるが，近年，ホルマリン固定パラフィン包埋された通常の病理検査用の組織に利用可能な抗体が数多く開発され，これらの適切な組み合わせは，的確な病理診断を可能にしている。抗体の認識する抗原は多岐にわたり，ケラチンなどの細胞骨格蛋白，細胞膜に発現するホルモンレセプター，p53蛋白に代表される癌関連遺伝子蛋白などがあり，特に分子標的治療の対象となる蛋白の発現の有無は治療効果の予測に直結する。

中皮腫の性別と発生部位

1995年から2006年までの12年間における中皮腫の死亡統計を性別，発生部位別にまとめると表1のようになる[2]。これによれば，胸膜が65％，腹膜10％，心膜1％ということになるが，部位不明が23％と多い。この不明部位の多くは胸膜，一部は心膜と推測されるので，これらを含めて発生部位を考えると，胸膜は80～90％，心膜は2～3％となると思われる。腹膜は10％程度とみるのが妥当であり，精巣鞘膜については，文献では世界中でこれまで70例程度が報告されているにすぎない[3]。

肉眼所見

中皮腫はびまん型と限局型に分けられるが，前者が圧倒的に多い。最も多い胸膜例についてその発生・進展様式をみると，まず壁側胸膜に小顆粒状ないし小結節状の病変が出現するが，すぐに播種性に臓側胸膜へ広がり，胸膜に沿って進展して，壁側・臓側胸膜の癒着を起こし，肺を取り囲む形態をとる。これが中皮腫の肉眼像の代表的所見とされているが，すでにこの時期は進行期と考えられる。できれば腫瘍の進展が壁側胸膜に留まる間に，あるいは臓側に広がっても胸膜癒着が生じる前に診断できれば切除可能例が増え，患者の予後の改善につながると思われる[4]。

限局型はその数は少ない[5]。従来，限局型とよばれてきた例の多くは，現在，solitary (localized) fibrous tumor (SFT)とよぶ別の腫瘍に属する。この腫瘍は後述する肉腫型中皮腫と組織像が類似する紡錘形細胞腫瘍であるが，免疫組織化学的染色によって腫瘍細胞にCD 34（未分化間葉系細胞のマーカー）が陽性であることで中皮腫とは区別される[6]。肉眼的にも肺内の腫瘤として存在するか，あるいは臓側胸膜から有茎性に胸腔内へ突出する腫瘤をつくることが多い。中皮腫の大半が壁側胸膜から生じることから，SFTは肉眼的にも鑑別が可能である。このSFTを除いて限局型中皮腫の報告をみると，びまん型中皮腫と比べて予後はやや良好である[5]。この増殖態度の違いが何によるものかはいまだ明らかではない。

組織所見

中皮腫の組織分類およびそれぞれが占める割合を表2に示す。中皮組織は本来，上皮細胞様の形態を示す一層の中皮細胞の被覆と，その下の疎な紡錘形細胞の増殖からなり，これら2種類の細胞は起源は同じと考えられる。したがって，中皮腫には上皮型（図1），肉腫型（図2），線維形成型（図3）の他に，この両者の混在からなる二相型（少なくとも一方が10％以上混在する例と定義する）が存在するが，二相型は発生学的にみて中皮細胞が本来もつ性格を具現する腫瘍かもしれない。特殊型としては，さまざま

表1　わが国における中皮腫死亡例（1995～2006年）

発生部位	男性（%）	女性（%）	死亡総数（%）
胸　膜	4,636 (79.1)	1,224 (20.9)	5,860 (65.3)
腹　膜	474 (60.9)	304 (39.1)	778 (8.7)
心　膜	41 (62.1)	25 (37.9)	66 (0.7)
その他	135 (73.4)	49 (26.6)	184 (2.1)
部位不明	1,487 (71.3)	599 (28.7)	2,086 (23.2)
計	6,773 (75.5)	2,201 (24.5)	8,974 (100)

表2　中皮腫の組織分類と各組織型の占める割合

組織型	割合
上皮型（epithelioid type）	約60％
肉腫型（sarcomatoid type） 線維形成型（desmoplastic type）	約20％
二相型（biphasic type）	約15％
特殊型（variants） 脱落膜型（deciduoid type） リンパ組織球様型（lymphohistiocytoid type） 高分化乳頭状型（well differentiated papillary type） 小細胞型（small cell type）	数％

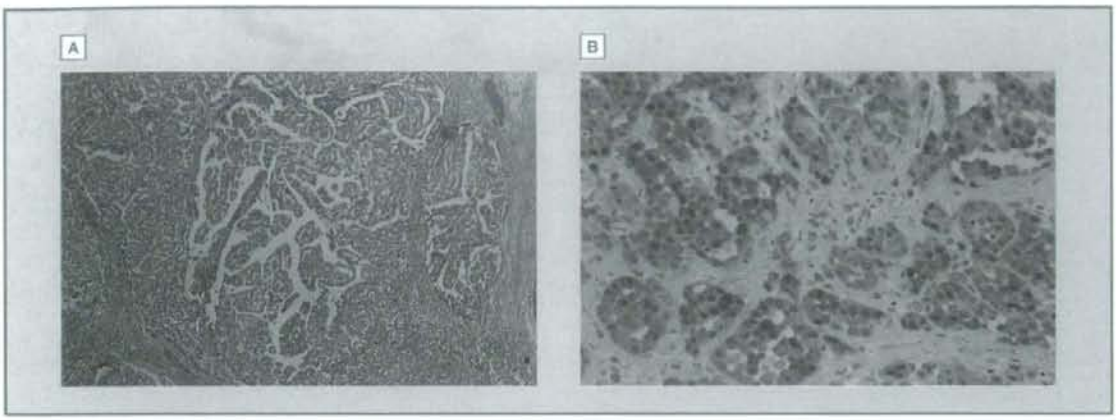

図1　上皮型中皮腫の組織像

A：上皮様細胞の乳頭腺管様構造をとる増殖を示す(H&E 染色，中拡大)。
B：図 1-A の calretinin の免疫組織化学的染色像。腫瘍細胞の核に強陽性，細胞質に弱陽性を示す(強拡大)。

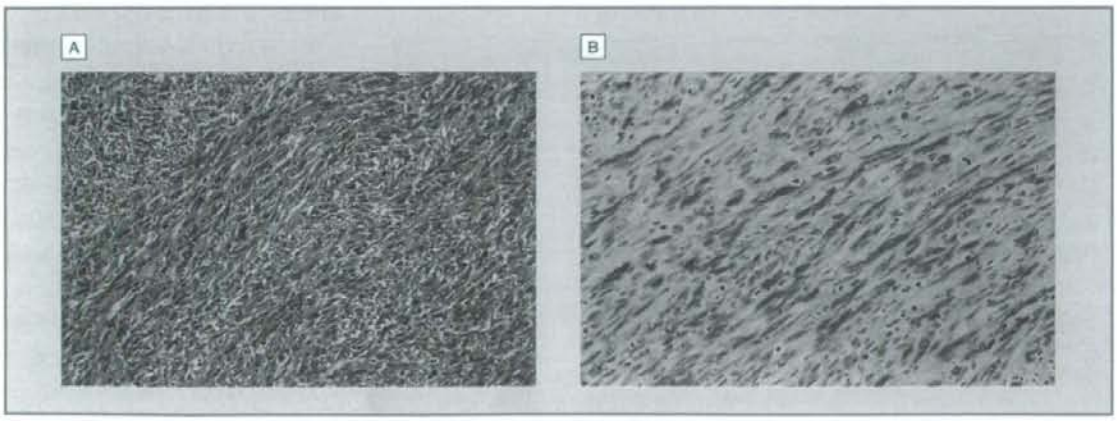

図2　肉腫型中皮腫の組織像

A：紡錘形細胞肉腫様の所見を示す(H&E 染色，中拡大)。
B：図 2-A の CAM 5.2 の免疫組織化学的染色像。腫瘍細胞の細胞質は強陽性を示す(強拡大)。

な組織像を示す例が報告されているが，これらについては中皮腫としての診断をより慎重に検討する必要がある症例が多い。

病理学的鑑別診断

前述のように，中皮腫の組織像は極めて多彩なので，H&E 染色組織標本のみでは鑑別すべき疾患あるいは病変は表 3 のようにあげられる。これらについて，画像所見を中心とした臨床診断を参考にしながら鑑別をすすめていく必要があるが，現状では表 4 にあげている抗体を用いた免疫組織化学

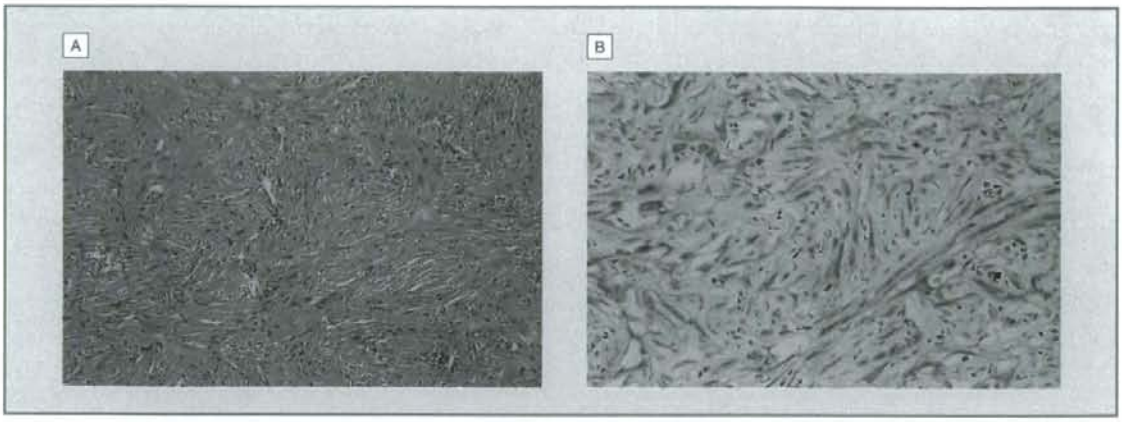

図3　線維形成型中皮腫の組織像

A：肉芽様の線維性結合組織の増殖内に異型の低い紡錘形細胞をみる(H&E 染色，中拡大)。
B：図3-A の CAM 5.2 の免疫組織化学的染色像。紡錘形細胞の細胞質は陽性を示す(強拡大)。

表3　中皮腫と鑑別すべき疾患・病変

組織型	発生部位	鑑別すべき疾患・病変
上皮型	胸膜	肺腺癌の浸潤 転移性腺癌 反応性中皮細胞過形成
	腹膜	卵巣の漿液性乳頭状腺癌，淡明細胞癌 腹膜の漿液性癌
肉腫型	胸膜	胸膜・胸壁の肉腫 肺肉腫様癌の浸潤
	腹膜	腹膜・腸管・子宮・附属器等の肉腫
線維形成型	胸膜	線維性胸膜炎
二相型	胸膜	滑膜肉腫 肺癌肉腫・肺芽腫
	腹膜	子宮・卵巣の癌肉腫

表4　中皮腫と他の悪性腫瘍の鑑別診断に必要な免疫組織化学的染色の抗体

組織型	発生部位	用いる抗体
上皮型	胸膜	calretinin (+)，WT-1 (+)，D 2-40 (+)，thrombomodulin (+) CEA (-)，TTF-1 (-)，SPA (-)，Napsin A (-)
	腹膜	calretinin (+)，D 2-40 (+)，ER (+) Ber EP 4 (-)，MOC 31 (-)
肉腫型	胸膜・腹膜	CAM 5.2 (+)，AE 1/AE 3 (+)，WT-1 (+)，D 2-40 (+) desmin (-)，S-100 (-)，CD 34 (-)，MyoD 1 (-)

的染色による鑑別が必須である[7][8]。

特に鑑別の困難な例として，肉腫型中皮腫と肺の肉腫様癌(sarcomatoid carcinoma)がある。肉腫型中皮腫と他の肉腫との鑑別では cytokeratin (抗体名 CAM 5.2 あるいは AE 1/AE 3) の陽性所見が有用であるが，肺原発の肉腫様癌が胸膜に進展した場合は cytokeratin 陽性となり，鑑別には使えない。陽性率において明確な差異のある他の抗体も見出されておらず，この両者については，画像所見において肺内の腫瘤があるか否かを決め手とすることが多い[8]。

病理診断において最も重要な鑑別は，中皮腫と良性病変との鑑別である[9]。浸潤がない，あるいは早期浸潤に留まる中皮細胞の増殖を上皮型中皮腫と診断できれば，患者の救命につながる可能性が大きい。臨床医が胸膜の肥厚が目立たない段階での早期例の発見に

表5　上皮型中皮腫と中皮細胞過形成の鑑別に用いる免疫組織化学的染色

抗体	上皮型中皮腫	反応性中皮細胞過形成
EMA	+(97.2%)	-
desmin	-	+(73%)
p53蛋白	+(52.9%)	-

表6　線維形成型中皮腫と線維性胸膜炎の鑑別に用いる所見

線維形成型中皮腫	線維性胸膜炎
(-)	zonation（胸膜表面近くで細胞密度が高い）あり
(-)	胸膜表面に垂直な毛細血管の増生
(-)	細胞の異型性あり
間質への浸潤	(-)
壊死	(-)
肉腫様増殖	(-)

努力している現状では，早期の中皮腫であるか否かの判断が病理医の重大な責務となる。

良悪性の判断については，脂肪組織等への浸潤のない例は中皮腫とよぶべきではなく「atypical mesothelial proliferation」と診断するという立場もあるが[10]，一方，免疫組織化学的染色もあわせて良悪の判断は可能とする立場もある。われわれは後者の立場に立つが，その鑑別において有用となる免疫組織化学的染色に用いる抗体を表5に示す。すなわち，EMAとp53蛋白が陽性でdesminが陰性であれば中皮腫である確率は高い[11]。

一方，線維形成型あるいは肉腫型中皮腫と線維性胸膜炎の間の鑑別については，しばしば誤った判断がなされている。その鑑別点は表6のようにあげられているが[10]，zonation（胸膜表面近くで細胞密度が高い）をみるためには，胸膜生検に際して胸膜表面から垂直に脂肪組織に達するような組織採取が求められる。表層のみの材料では，しばしば紡錘形細胞に異型があるという理由で中皮腫とされやすい。また，免疫組織化学的染色を行った場合，その解釈を誤っている場合も多い。胸膜炎に出現する紡錘形細胞はkeratin陽性，calretinin陽性である。さらにdesmin，α-SMAも陽性となり，中皮腫細胞ではdesminが陰性になることが多いという事実のみが鑑別に用いることができる[9]。

おわりに

中皮腫の病理診断は難しい。これまで多くの医療機関では中皮腫の診断を下す機会は少なく，1人ひとりの病理医の経験も限られてきたことが難しさを助長している。中皮腫への関心の高まりとともに病理診断講習会などによって少しずつ病理診断の精度は向上しつつあるが，判断に困る場合は適切なコンサルテーションが必要と思われる。

また，術中の迅速診断による中皮腫の判断は避けるべきである。前述のように，多種類の免疫組織化学的染色の組み合わせによって診断している現状では，迅速に判断できるのは明らかな浸潤性増殖を示す高分化な上皮型に限られる。迅速標本によるあいまいな診断より，前述した適切な生検材料を永久標本で可能な限り早く回答することを心掛けることが肝要であろう。

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Performance Evaluation of 4 Measuring Methods of Ground-Glass Opacities for Predicting the 5-Year Relapse-Free Survival of Patients With Peripheral Nonsmall Cell Lung Cancer: A Multicenter Study

Ryutaro Kakinuma, MD,† Ken Kodama, MD,‡ Kouzo Yamada, MD,§ Akira Yokoyama, MD,|| Shuji Adachi, MD,¶ Kiyoshi Mori, MD,# Yasuro Fukuyama, MD,** Keiko Kuriyama, MD, ††Junichi Oda, MD, ‡‡ Masayuki Noguchi, MD,§§ Yoshihiro Matsuno, MD,|||| Tomoyuki Yokose, MD,¶¶ Hironobu Ohmatsu, MD,* and Yutaka Nishiwaki, MD**

Objective: To evaluate the performance of 4 methods of measuring the extent of ground-glass opacities as a means of predicting the 5-year relapse-free survival of patients with peripheral nonsmall cell lung cancer (NSLC).

Methods: Ground-glass opacities on thin-section computed tomographic images of 120 peripheral NSLCs were measured at 7 medical institutions by the length, area, modified length, and vanishing ratio (VR) methods. The performance (Az) of each method in predicting the 5-year relapse-free survival was evaluated using receiver operating characteristic analysis.

Results: The mean Az value obtained by the length, area, modified length, and VR methods in the receiver operating characteristic analyses was 0.683, 0.702, 0.728, and 0.784, respectively. The differences between the mean Az value obtained by the VR method and by the other 3 methods were significant.

Conclusions: Vanishing ratio method was the most accurate predictor of the 5-year relapse-free survival of patients with peripheral NSLC.

Key Words: lung cancer, ground-glass opacity, 5-year relapse-free survival, GGO measurement

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The recent use of helical computed tomography (CT) clinically and for lung cancer screening has facilitated the detection of pure ground-glass opacities (GGOs) (nonsolid nodules) and mixed GGOs (part-solid nodules).[1–10] Most localized GGOs have been shown to represent neoplastic disease.[1–6] Several researchers have reported an improved outcome for patients with peripheral nonsmall cell lung cancer based on the extent of the GGO of lung cancer nodules on thin-section CT images.[7,11–14] A GGO is a finding on thin-section CT images of the lung that has been described as a hazy area of increased attenuation with preservation of the bronchial and vascular margins.[15] Most peripheral nonsmall cell lung cancers with a diameter of 2 cm or less and a GGO of 50% or greater of the length or volume of the lung cancer nodules are bronchioloalveolar carcinomas without lymph node involvement, lymphatic invasion, or vascular invasion.[3–7,16,17] These tumors are regarded as early lung cancers[17] and as candidates for limited resection.[16,17]

At the beginning of our study, only 3 GGO measurement methods had been reported: (1) measurement of the length of the GGO,[7] (2) measurement of the area of the GGO,[11,12] and (3) measurement of the vanishing ratio (VR) between the area of lung cancer nodules on thin-section CT images taken with the lung-field window setting and the mediastinal window setting.[14] However, the 3 methods had never been compared to determine which method most accurately predicts 5-year relapse-free survival of patients with peripheral nonsmall cell lung cancer.

The purpose of this retrospective study was to evaluate the performance of these 3 different GGO measurement methods (length, area, and VR) and a fourth GGO measurement

From the *National Cancer Hospital East, Kashiwa, Chiba; †Research Center for Cancer Prevention and Screening, National Cancer Center, Tsukiji, Tokyo; ‡Department of Thoracic Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka; §Department of Thoracic Oncology, Kanagawa Cancer Center, Yokohama, Kanagawa; ||Department of Thoracic Oncology, Niigata Cancer Center, Niigata, Niigata; ¶Department of Radiology, Hyogo Cancer Center, Akashi, Hyogo; #Department of Thoracic Oncology, Tochigi Cancer Center, Utsunomiya, Tochigi; **Department of Thoracic Surgery, Nakatsu Municipal Hospital, Nakatsu, Oita; ††Department of Radiology, Osaka National Hospital, Osaka, Osaka; ‡‡Department of Radiology, Tsubame Rosai Hospital, Tsubame, Niigata; §§Department of Pathology, Tsukuba University School of Medicine, Tsukuba, Ibaragi; ||||Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Hokkaido; ¶¶Department of Pathology, Kanagawa Cancer Center, Yokohama, Kanagawa, Japan.

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Reprints: Ryutaro Kakinuma, MD, PhD, Cancer Screening Technology Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (e-mail: rkaki@ncc.go.jp).

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method, referred to as the modified length (ML) method in predicting the 5-year relapse-free survival of patients with peripheral nonsmall cell lung cancer.

MATERIALS AND METHODS

Subjects who met the following 4 criteria were selected: (1) peripheral nonsmall cell lung cancer resected before December 31, 1996; (2) tumors 2 cm or less in diameter on thin-section CT images; (3) outcome determined by December 31, 2001; and (4) reoutput of CT films with lung-field setting at a window width of 1800 and a window level of −700 and mediastinal setting at a window width of 400 and a window level of 40. Thin-section CT films of 187 cases obtained from 5 of the 7 medical institutions were submitted for measurement at all 7 medical institutions. Computed tomographic scans were performed with helical CT scanners (TCT-900S Superhelix; Toshiba Medical Systems, Tokyo, Japan; Somatom Plus; Siemens Medical Systems, Erlangen, Germany).

All surgical specimens were fixed in the inflated state by transpleural infusion of formalin. The specimens were sectioned and prepared for routine hematoxylin and eosin staining in each institution. Pathological specimens (hematoxylin and eosin stain) were reevaluated by 3 pathologists (M.N., Y.M., and T.Y.) using the criteria of the 1999 World Health Organization classification for lung cancer.[18] The pathologists were unaware of the thin-section CT findings in any of the cases.

Two readers at each of the 7 medical institutions measured the following parameters on the equators of the peripheral nonsmall cell lung cancer nodules: diameter, diameter of the solid component on the lung-field window and the mediastinal window settings, GGO area, and VR between the lung-field window and the mediastinal window settings of thin-section CT images. The equators of the lung cancer nodules were identified by one of the authors (R.K.) and confirmed by another author (H.O.). Readers consisted of 8 thoracic oncologists, 4 chest radiologists, and 2 thoracic surgeons. The 14 readers were specialists in diagnostic imaging with 6 to 24 years of experience (mean, 16.7 years). In Japan, diagnostic imaging of the chest is performed not only by radiologists but also by pulmonologists, thoracic oncologists, and some thoracic surgeons. They were blinded to the pathological diagnosis in each case. Diameters were measured with hand-held calipers according to the scales on the thin-section CT images.

Figure 1 shows all of the parameters of each measurement method. The GGO length (percentage) (ie, as a percentage of the maximal diameter of the nodule) was calculated using the formula: (L [maximal diameter of the nodule on the lung-field window setting] − Sl [maximal diameter of the solid component on the lung-field window setting]) / L × 100. The GGO ML (percentage) was calculated using the formula: (L [maximal diameter of the nodule on the lung-field window setting] − Sm [maximal diameter of the solid component on the mediastinal window setting]) / L × 100. The GGO area (percentage) (ie, as a percentage of the maximal area of the nodule) was quantified between the area (Al) of the lung cancer nodule on the lung-field

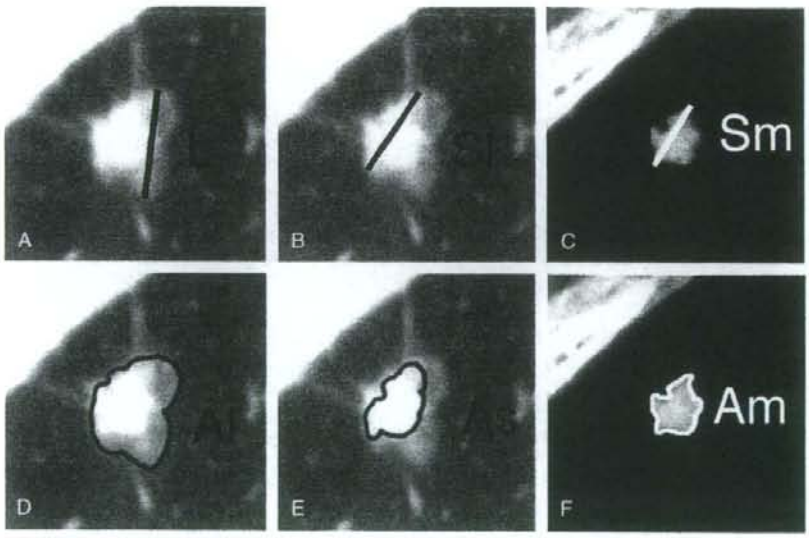

FIGURE 1. L, maximal diameter of the nodule on the lung-field window setting (A); Sl, maximal diameter of the solid component on the lung-field window setting (B); Sm, maximal diameter of the solid component on the mediastinal window setting (C); Al, area of the lung cancer nodule on the lung-field window setting (D); As, area of the solid component on the lung-field window setting (E); and Am, area of the solid component on the mediastinal window setting (F).

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window setting and the area (As) of the solid component on the lung-field window setting of thin-section CT images (ie, [Al − As] / Al × 100 [%]) based on subjective estimates made by visual inspection. The GGO VR (percentage) was also quantified between the area (Al) of the lung cancer nodule on the lung-field window setting and the area (Am) of the solid component on the mediastinal window setting of the thin-section CT images (ie, [Al − Am] / Al × 100 [%]) based on subjective estimates made by visual inspection.

A total of 131 peripheral nonsmall cell lung cancer cases in which there was a peripheral nonsmall cell lung cancer nodule(s) that was 2 cm or less in diameter were selected from the 187 originally submitted cases as follows: 2 readers in each institution measured and found a particular nodule's diameter to be 2 cm or less either independently or by consensus, and at least 8 of the 14 readers were in agreement that the diameter of the nodule was 2 cm or less. The 8 cases in which multiple lung cancers were diagnosed and 3 cases in which the pathology specimens were inappropriate for diagnosing lung cancer as determined by the pathological review were excluded from the final evaluation, and thus, the final number of cases analyzed in this study was 120.

Agreement between measurements made by the 2 members of each pair, among the total 14 readers, was assessed by κ statistics. The 5-year relapse-free survival rate based on values obtained by each method of measurement was calculated using the Kaplan-Meier method. Performance (Az) of the 4 measurement methods was evaluated using receiver operating characteristic (ROC) analysis. The average of the measurements made by the 2 readers at each institution was used to make the calculations using the Kaplan-Meier method and in the ROC analysis. κ statistics was analyzed with Microsoft Excel 2004 software (Microsoft Corp, Seattle, Wash). JMP software version 6.0 (SAS Institute Inc, Cary, NY) was used for the following analyses: evaluation of the normal distribution for the κ statistics and Student t tests for the κ statistics; Mann-Whitney U tests and Kaplan-Meier analyses for 5-year relapse-free survival; Mann-Whitney U tests for the mean numbers of 5-year relapse-free survival cases predicted by the length, area, ML, and VR measurement methods as having a GGO extent of 50% or more. The ROC analyses were performed with LABMRMC 1.0B3 software (University of Chicago). Differences were considered significant when P values were less than 0.05.

RESULTS

Subjects of the Evaluation

A total of 120 cases (men/women, 69/51) with lesions 2 cm or less in diameter were used in the final evaluation. Patient ages ranged from 26 to 83 years (mean, 63 years). The histological diagnoses were the following: bronchioloalveolar carcinoma (n = 11), adenocarcinoma (n = 93), squamous cell carcinoma (n = 9), adenosquamous cell carcinoma (n = 1), large cell neuroendocrine carcinoma (n = 1), large cell carcinoma (n = 1), carcinoid (n = 2), and nonsmall cell carcinoma (n = 2). Seventy-seven patients underwent lobectomy, 2 underwent lobectomy with wedge resection, 27 patients underwent segmental resection, and 14 underwent wedge resection. The pathological stages were the following: IA (n = 99), IB (n = 5), IIA (n = 2), IIIA (n = 9), IIIB (n = 4), and IV (n = 1). Of the 120 patients, 24 experienced a relapse by December 31, 2001. Scanning collimations and reconstruction intervals of thin-section CT scans in these cases were the following: 2 mm/2 mm (n = 70), 2 mm/3 mm (n = 30), 3 mm/2 mm (n = 7), 3 mm/3 mm (n = 5), 3 mm/1.5 mm (n = 4), 3 mm/1 mm (n = 3), and 1 mm/1 mm (n = 1).

Agreement on Measurement Results

The total for the combination of 2 readers, of the 14 readers, was 91 for each measurement method. The κ statistics for agreement between measurements made by the 2 members of each pair, among the total 14 readers, at the GGO cutoff value of 50% (ie, whether the extent of the GGO was ≥50% or <50%) by the length, area, ML, and VR measurement methods ranged from 0.52 to 0.91 (mean, 0.68; 95% CI, 0.66–0.69), from 0.51 to 1.0 (mean, 0.7; 95% CI, 0.68–0.72), from 0.61 to 0.91 (mean, 0.78; 95% CI, 0.77–0.79), and from 0.61 to 1.0 (mean, 0.78, 95% CI, 0.77–0.8), respectively. The κ statistics for each measurement method were normally distributed. Student t tests showed the differences in the mean κ statistics between pairs of methods, except between the ML and VR methods, to be significant (area vs length, $P = 0.025$; ML vs length, $P < 0.0001$; ML vs area, $P < 0.0001$; VR vs length, $P < 0.0001$; VR vs area, $P < 0.0001$; ML vs VR, $P = 0.86$).

ROC Curve Analysis

Figure 2 shows the results of the ROC curve analyses for performance in predicting the 5-year relapse-free survival of patients with peripheral nonsmall cell lung cancer. The Az values (area under the ROC curve) for the length, area, ML, and VR measurement methods ranged from 0.615 to 0.752 (mean, 0.683; 95% CI, 0.645–0.721), from 0.682 to 0.725 (mean, 0.702; 95% CI, 0.687–0.718), from 0.706 to 0.743 (mean, 0.728; 95% CI, 0.717–0.739), and from 0.748 to 0.825 (mean, 0.784; 95% CI, 0.757–0.81), respectively, with

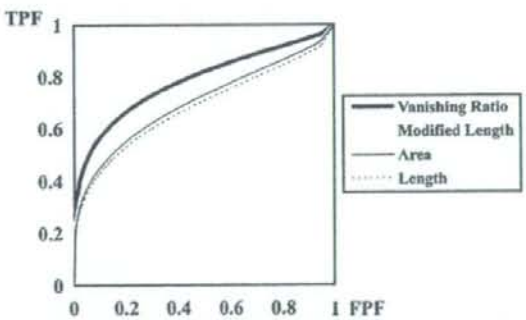

FIGURE 2. The ROC curves according to each measurement method. Mean Az values of the 7 institutions: VR, 0.784; ML, 0.728; area, 0.702; length, 0.683 (VR vs other 3 methods, statistically significant). FPF indicates false-positive fraction; TPF, true-positive fraction.

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TABLE 1. 5-Year Relapse-Free Survival Rates According to the Parameter Measured

	5-Yr Survival Rate, %							
	Institution							
	A	B	C	D	E	F	G	
Length method								
Group 1	100	100	100	100	93	100	95	98*
Group 2	79	78	79	78	80	78	78	78†
Area method								
Group 3	100	100	100	100	95	100	96	99*
Group 4	79	77	79	75	79	77	77	77†
ML method								
Group 5	100	97	100	97	100	100	97	99*
Group 6	76	75	77	76	77	77	76	76†
VR method								
Group 7	100	98	100	96	96	100	94	98*
Group 8	70	71	73	70	72	74	83	73†

Each group in which the extent of the GGO was 50% or more in each institution consisted of cases represented by gray boxes or black boxes in each horizontal row in Figure 3 according to the parameter measured. For example, when the length method was used, group 1 in institution A consisted of 14 cases without recurrence (gray boxes), as shown in the first horizontal row of the length method in Figure 3.

*Mean 5-year relapse-free survival rate in the group with a GGO extent of 50% or more.

†Mean 5-year relapse-free survival rate in the group with a GGO extent of less than 50%.

Group 1, ([L − Sl] / L × 100) ≥ 50%; group 2, ([L − Sl] / L × 100) < 50%; L, maximal diameter of the nodule on the lung-field window setting; Sl, maximal diameter of the solid component on the lung-field window setting.

Group 3, ([Al − As] / Al × 100) ≥ 50%; group 4, ([Al − As] / Al × 100) < 50%; Al, area of the lung cancer nodule on the lung-field window setting; As, area of the solid component on the lung-field window setting.

Group 5: ([L − Sm] / L × 100) ≥ 50%; group 6, ([L − Sm] / L × 100) < 50%; Sm, maximal diameter of the solid component on the mediastinal window setting.

Group 7, ([Al − Am] / Al × 100) ≥ 50%; group 8, ([Al − Am] / Al × 100) < 50%; Am, area of the solid component on the mediastinal window setting.

the VR method yielding the highest mean Az value. The differences in mean Az values between pairs of methods, except between the length and the area methods, were significant (ML vs length, $P = 0.031$; ML vs area, $P = 0.036$; VR vs length, $P = 0.0004$; VR vs area, $P = 0.0011$; VR vs ML, $P = 0.0028$; length vs area, $P = 0.297$).

5-Year Relapse-Free Survival Rates

Table 1 shows the 5-year relapse-free survival rates at the GGO cutoff value of 50% for each of the 4 measurement methods in each institution. The 5-year relapse-free survival rates of the cases with a GGO of 50% or more for the length, area, ML, and VR measurement methods ranged from 93% to 100% (mean, 98%), from 95% to 100% (mean, 99%), from 97% to 100% (mean, 99%), and from 94% to 100% (mean, 98%), respectively. None of the differences in the mean survival rates of the cases with a GGO of 50% or more between pairs of measurement methods were significant.

5-Year Relapse-Free Survival Cases With a GGO of 50% or More

Figure 3 shows cases in which the extent of the GGO was 50% or more according to each measurement method. The mean numbers of 5-year relapse-free survival cases predicted by the length, area, ML, and VR measurement methods were 17, 21, 27, and 44, respectively. The differences between the mean numbers of 5-year relapse-free survival cases obtained by the VR method and by the other 3 methods were significant ($P < 0.01$). The numbers of unanimous cases, those in which measurement by the length, area, ML, and VR methods yielded a GGO percentage of at least 50% in all 7 institutions, were 10, 12, 17, and 33, respectively, with the VR method yielding the highest number of accurately predicted cases.

DISCUSSION

This is the first multicenter study to retrospectively evaluate the correlation between the extent of GGO measured by 4 different methods in patients with peripheral nonsmall cell lung cancer and their 5-year relapse-free survival. The VR method yielded both the highest mean Az value and the largest number of unanimous 5-year relapse-free cases with a GGO of 50% or more.

Pure GGOs represent the lepidic growth of lung cancer cells, especially in adenocarcinoma.[3] Higher attenuations or solid components in mixed GGOs are caused by cancer cells, collapse of alveolar spaces, regions of fibrosis, or severe narrowing of the alveolar spaces.[9] Noguchi et al[19] proposed a new classification for small adenocarcinoma into a replacement type (types A, B, and C) and nonreplacement type (types D, E, and F). The 5-year survival rate of types A (localized bronchioloalveolar carcinoma) and B (localized bronchioloalveolar carcinoma with alveolar collapse) is 100%. Based on the good prognosis, Noguchi et al[19] suspected that types A and B might be carcinoma in situ. Although limited surgery is 1 option for treating such tumors, the pathological diagnoses are made after resection. However, recent radiological-pathological correlations have revealed that GGOs in adenocarcinoma are a sign of noninvasiveness or minimal invasiveness.[1–5,7–10]

The length and ML methods are intended to be objective, whereas the area and VR methods are subjective in nature. Based on the results of this study, however, one of

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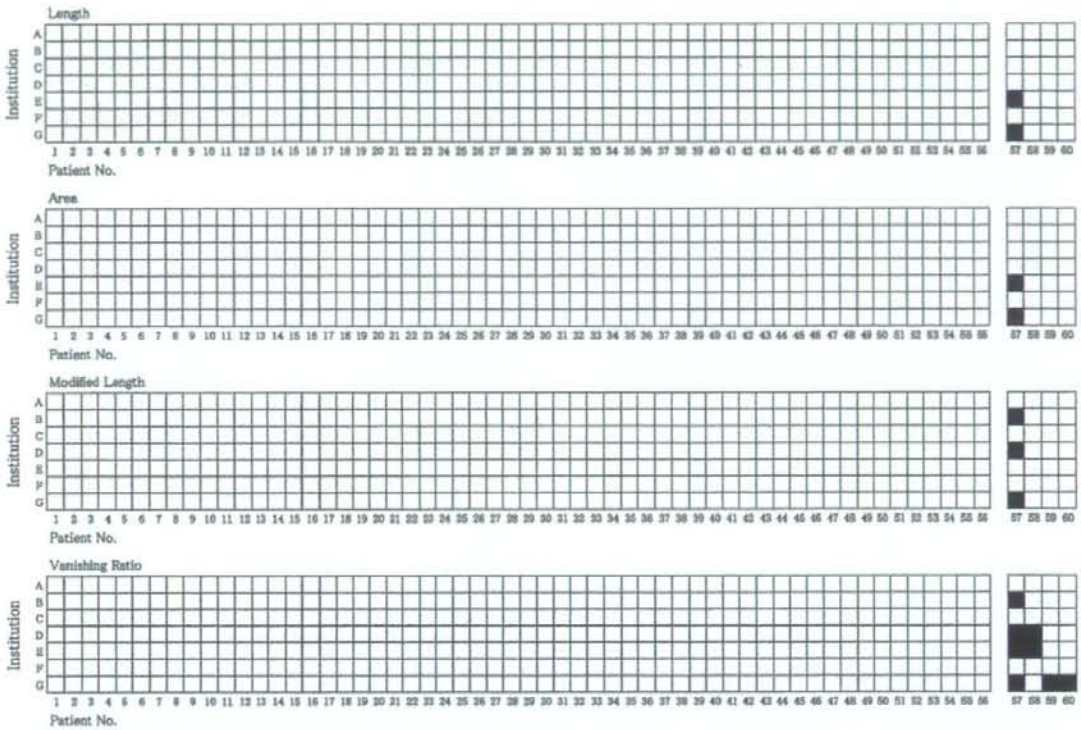

FIGURE 3. Gray and black boxes indicate cases measured by each participating institution as having a GGO extent of 50% or more. The vertical axis lists the 7 institutions that used the 4 different measurement methods. The horizontal axis shows whether a patient survived without recurrence (gray box) or had a recurrence (black box) within 5 years after surgery. Each vertical column of boxes represents the same patient. At 3 of the 7 institutions (A, C, and F), no cases with recurrence had a GGO extent of 50% or more by any of the 4 measurement methods. In institution G, the extent of the GGO was 50% or more by the length method in the 20 patients without recurrence (patient numbers 1–13, 15, 23, 26, 38–40, and 56) and 1 patient with recurrence (patient number 57), whereas in the 46 patients without recurrence (patient numbers 1–41, 43, 44, 48, 49, and 51) and 3 patients with recurrence (patient numbers 57, 59, and 60), the extent of GGO was 50% or more by the VR method.

the "subjective" methods (ie, the VR method) is superior to the "objective" methods in terms of identification of 5-year relapse-free cases and Az values in the ROC analysis. In this study, the length and ML methods were performed with hand-held calipers. Measurement with calipers involves inherent subjectivity in identification of the margin of the lesion and measurement of the diameter of the lesion.[16] Despite being a subjective and visual method of evaluation, the VR method identified the largest number of 5-year relapse-free survival cases with a GGO of 50% or more. This may have been because the margin of the solid component of peripheral nonsmall cell lung cancer nodules is clearer with the mediastinal window setting, and the interobserver measurement error may be reduced as a result.

After the measurements in our study had been completed, several other GGO measurement methods were proposed for predicting the outcome of peripheral nonsmall cell lung cancer.[20–25] A study of the different kinds of length method concluded that the ratio of the size of the maximum solid component to the size of the entire tumor is the best predictor for noninvasive peripheral adenocarcinoma.[20] Another different kind of approach to length measurement is the tumor shadow disappearance rate (TDR).[21–25] To calculate the area of a tumor by the TDR method, the readers measure the maximum dimension of the tumor and the largest dimension of the perpendicular axis in the lung-field window setting and measured them in the mediastinal window setting, and the area according to each setting is calculated by multiplying the maximum dimension of tumor by the largest dimension of the perpendicular axis. Therefore, the results obtained by the TDR method, as one kind of length method, may be affected by interobserver measurement error because the greatest tumor diameter cannot always be identified by visual inspection of CT images.[26]

National Institutes of Health Image software, which runs on a Macintosh computer (Apple Inc, Cupertino, Calif), and

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Scion Image software, the Windows version of National Institutes of Health Image (Scion Corp, Frederick, Md), were used in the other studies to measure the extent of GGOs in peripheral nonsmall cell lung cancer nodules objectively.[27,28] One study used the VR method, but measurements were performed quantitatively with a computer rather than visually.[27] That study concluded that future multicenter trials were needed to clarify the value of limited resection for clinical stage IA adenocarcinoma based on the extent of GGO determined with computer software. One limiting factor in that study that may affect interobserver measurement error is disagreement in the selection of the CT image that shows the nodule's largest cross-sectional area.[29] The other study used the area method,[28] and 1 limiting factor in that study that may affect interobserver measurement error is manual tracing of the areas of the tumor and solid component on the computer software.

Our study has several limitations. First, the scanning collimations and reconstruction intervals in the 5 medical institutions that actually provided thin-section CT images were not the same. The differences in scanning collimations and reconstruction intervals may have affected the evaluation of GGO and the solid component, thereby giving rise to interobserver measurement errors. Second, the lung-field window settings and mediastinal window settings may not have been optimized in the VR method. If the window width is set too wide or the window level is set too low in the Hounsfield units of the mediastinal window settings, the residual area of peripheral nonsmall cell lung cancer nodules becomes larger, resulting in a smaller VR percentage. If the VR method is to be used to select candidates for limited surgery, the most appropriate lung-field window and mediastinal window settings must be identified. Third, the visual measurements by the VR method cannot prevent intraobserver and interobserver measurement errors. One study found that tumor size was measured more accurately and consistently by readers who used an automated autocontour technique than by readers who used hand-held or electronic calipers.[26] Automated computer techniques need to be developed to reduce intraobserver and interobserver measurement errors.[30]

In conclusion, the VR method has the greatest ability to predict 5-year relapse-free survival in patients with peripheral nonsmall cell lung cancer. As a result of this finding, a prospective multicenter study of the VR method using automated computer techniques is warranted.

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