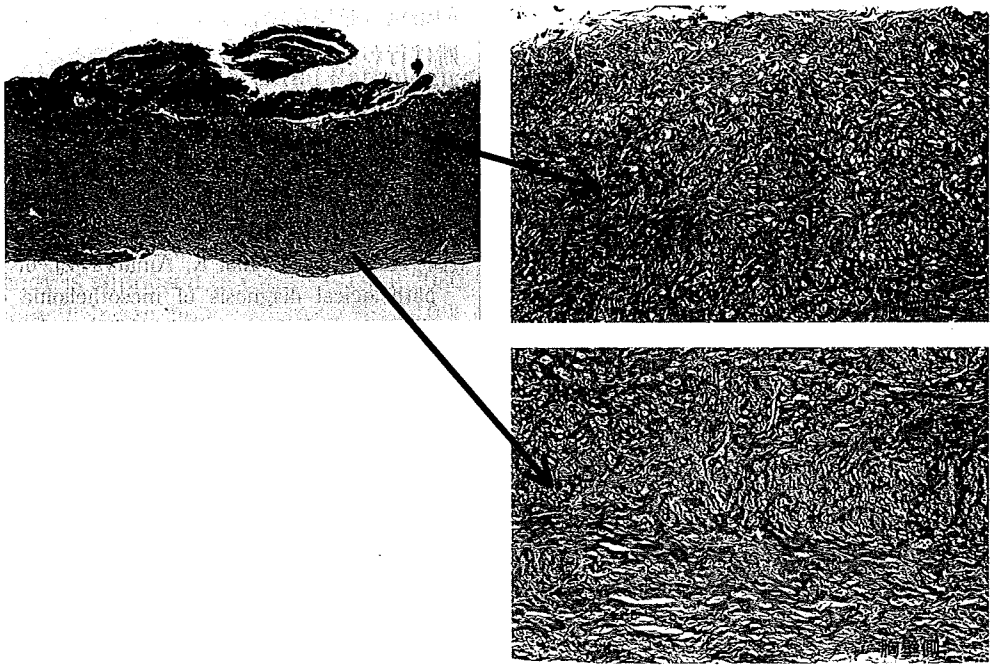


図5　線維性胸膜炎の組織像（HE染色，弱拡大と強拡大）

表7　線維性胸膜炎と線維形成型中皮腫との鑑別

線維性胸膜炎	線維形成型中皮腫
いわゆるzonation（+）	いわゆるzonation（−）
表層側→細胞密度が高い 細胞異型性を認める	
深部側→細胞密度が低い 細胞異型性に乏しい	
胸膜表面に垂直なcapillary（+）	Capillary（−）
Bland necrosis（−）	Bland necrosis（+）
Sarcomatous foci（−）	Sarcomatous foci（+）
Nodular expansion（−）	Nodular expansion（+）

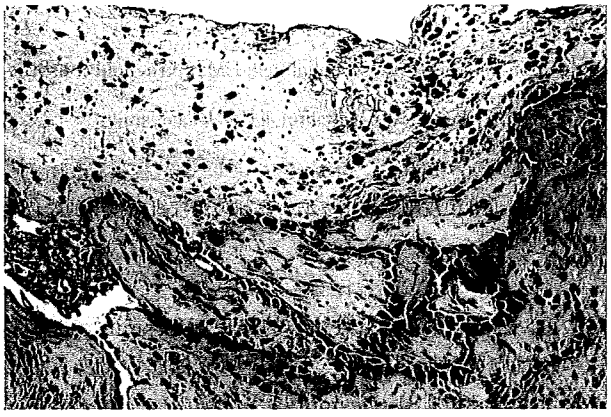


図6　異型的な中皮細胞過形成の組織像（HE染色，中拡大）

率が高く，これらを中皮腫の陰性マーカーとして用いる[10]．

肉腫型ではcytokeratin（CAM5.2，AE1/AE3）に対する抗体が陽性マーカーとなる．上皮型であげた中皮細胞マーカーであるcalretininなどの肉腫型での陽性率は低く，陽性マーカーとしては用いにくい．他の肉腫それぞれのマーカー，例えば，平滑筋肉腫におけるdesminやh-caldesmon，横紋筋肉腫におけるmyoD1やmyoglobin，SFTにおけるCD34などは，中皮腫での陽性率は低く陰性マーカーとなる[11]．

4. 中皮細胞の増殖病変の良悪性の鑑別

表6にあげる中皮細胞の増殖病変の良悪性の鑑別診断は，患者に適格な治療を決める意味できわめて重要である．早期の中皮腫例では，胸膜肺全摘術という侵襲の大きい手術が適応となる為，その重要性も増している．

まず，頻度の高い例として，線維性胸膜炎（図5）と線維形成型中皮腫の鑑別があげられる．この両者の鑑別ではとくに表層のみから得られた小さな生検材料の場合，きわめて難しい．両者の鑑別点としては表7に項目をあげるが，この中ではいわゆる“zonation”が信頼度が高い[12]．しかし，これも胸膜の表層から胸壁までの胸膜全層にわたる生検材料が提供されないと判断できない．この両者の鑑別に，紡錘形細胞の免疫組織化学的染色が有用な場合がある．中皮細胞マーカーであるcalretininやcytokeratin（CAM5.2あるいはAE1/AE3）は両者とも陽性であるが，desminは線維性胸膜炎では陽性であるが，線維形成型中皮腫では陰性である．α-SMAは両方とも陽性であることが多い．

次いで，早期の上皮型中皮腫と反応性の異型的な中皮細胞過形成（図6）との鑑別も重要である．この場合，浸潤像の有無，すなわち中皮細胞増殖がみられる範囲が最も重要な鑑別のポイントとなるが（図7），小さな生検で表層の組織しか得られていない場合，両者の鑑別には苦

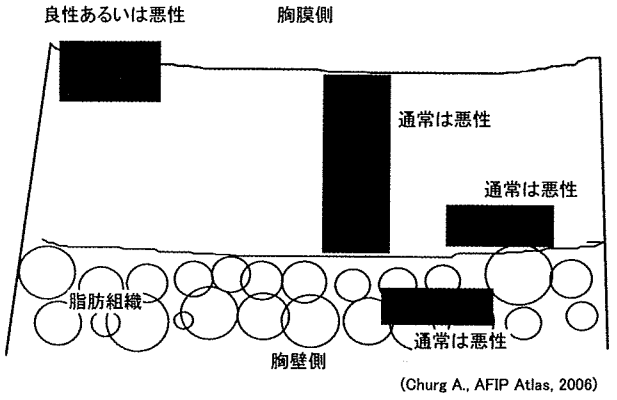
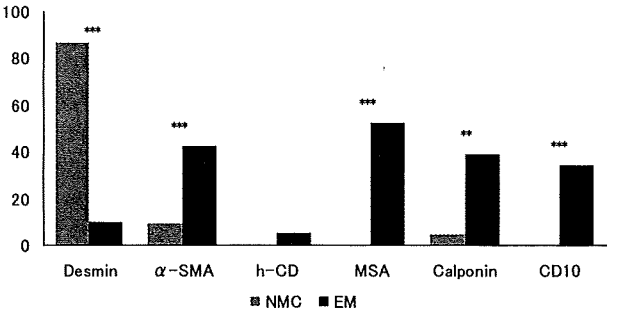


図7　肥厚した胸膜における中皮細胞の分布による良悪性の鑑別



NMC: non-neoplastic mesothelial cell, EM: epithelioid mesothelioma
α-SMA: alpha-smooth muscle actin, *h*-CD: *h*-caldesmon
MSA: muscle specific actin
**: $p<0.01$, ***: $p<0.001$

図8　非腫瘍性中皮細胞と上皮型中皮腫の免疫組織化学的染色の比較

慮する．この際，免疫組織化学的染色で desmin が非腫瘍性中皮細胞では陽性，上皮型中皮腫では陰性であることが参考となる(図8)．Desmin は平滑筋のマーカーであるが，他の平滑筋マーカー(h-caldesmon など)は鑑別に使えない[13]．この事実は，desmin が中皮細胞に発現する生物学的意義を探る必要性を感じさせる．

5. おわりに

中皮腫の病理診断における他の悪性腫瘍との鑑別，中皮細胞増殖病変の良悪性の鑑別について述べてきたが，これらの鑑別に際して免疫組織化学的染色が有用であることを強調したい．かつ，患者の治療を始める前に生検材料による診断が求められる場合には，可能な限り，胸膜や腹膜などの漿膜組織を広くかつ深部まで含めた材料の採取を行うことを臨床医に望みたい．さらにこうした材料について，迅速診断による判断が求められることがあるが，浸潤の有無の判断は容易でなく，免疫組織化学的染色も必要な場合が多いことから，迅速診断による判断は行わないことが原則である．

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Differential Pathological Diagnosis of Mesothelioma

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Recently, mesothelioma, which is a malignant tumor related to asbestos-exposure, has rapidly increased, and this tendency is supposed to continue until 2030. The patients with mesothelioma have been compensated or reliefed by law in Japan, however, more than 10% of applicants have been refused from compensation or relief. The reason is mainly the accuracy of pathological diagnosis, and therefore, the importance of differential diagnosis is pointed out now.

The occurrence sites of mesothelioma are limited to serosal membrane, that is, pleura, peritoneum, pericardium and tunica vaginalis, and the proportion of pleural case is extremely large. Grossly, diffuse type is dominant compared to localized type, however, pleural invasion of lung cancer rarely mimic the characteristics of mesothelioma. Histologically, mesothelioma is divided to epithelioid type, sarcomatoid type, desmoplastic type and biphasic type and others. Its histological varieties cause the difficulties of differential diagnosis, and on the pathological diagnosis, the immunohistochemical stainings using adequate antibodies are useful. Especially the differentiation between benign and malignant lesion is important for avoiding the excessive invasion as treatment to the patients.

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Value of immunohistochemistry in the differential diagnosis of pleural sarcomatoid mesothelioma from lung sarcomatoid carcinoma

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Value of immunohistochemistry in the differential diagnosis of pleural sarcomatoid mesothelioma from lung sarcomatoid carcinoma

Aims: The differential diagnosis of pleural sarcomatoid mesothelioma (SM) from lung sarcomatoid carcinoma (LSC) invading parietal pleura and chest wall is a challenging issue. The aim of this study was to identify useful antibodies that can be used for the differential diagnosis of pleural SM from LSC.
Methods and results: Forty-five cases of pleural SM and 27 cases of LSC were immunohistochemically analysed by using 15 commercially available antibodies, including D2-40 and antibodies to calretinin, thrombomodulin, Wilms' Tumour 1, carcinoembryonic antigen (CEA), Napsin A, thyroid transcription factor (TTF)-1, pan-cytokeratin, CAM5.2, epithelial membrane antigen, Ber-EP4, MOC-31, α-smooth muscle actin, h-caldesmon and desmin. The results revealed that D2-40 positivity was significantly higher in pleural SM (86.7%) than in LSC (25.9%). The positivity of the adenocarcinoma markers, including CEA, Napsin A, and TTF-1, was low even in LSC.
Conclusions: Evaluating the positivity and degree of staining of the well-known mesothelial marker D2-40 could be applied to differentiate pleural SM from the sarcomatoid component of LSC, in addition to assessing clinical and radiological information.

Keywords: asbestos, calretinin, CEA, D2-40, differential diagnosis, immunohistochemistry, sarcomatoid carcinoma, sarcomatoid mesothelioma

Abbreviations: α-SMA, α-smooth muscle actin; CEA, carcinoembryonic antigen; CGH, comparative genomic hybridization; CK, cytokeratin; EMA, epithelial membrane antigen; H&E, haematoxylin and eosin; LSC, lung sarcomatoid carcinoma; SM, sarcomatoid mesothelioma; TM, thrombomodulin; TTF, thyroid transcription factor; WT1, Wilms' Tumour 1

Introduction

Mesothelioma is a rare, aggressive tumour involving the mesothelial cells of the pleura, peritoneum, pericardium, and testicular tunica vaginalis. It is very well correlated with occupational and environmental asbestos exposure.[1–4] In many countries, the incidence of mesothelioma has been increasing, so that pathologists increasingly encounter and diagnose it.

The pathological diagnosis of mesothelioma is occasionally difficult because of its rarity and its heterogeneous morphological and immunohistochemical findings.[5–7] The predominant histological subtypes of mesothelioma include epithelioid, sarcomatoid, biphasic and desmoplastic types.[5,8–10] There are many comprehensive immunohistochemical studies concerning the differentiation between epithelioid mesothelioma and pulmonary adenocarcinoma.[9–15]

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However, there are few reports on the differential immunohistochemical diagnosis of pleural sarcomatoid mesothelioma (SM) and tumours with spindle cell morphology, such as 'true' sarcoma, pulmonary sarcomatoid carcinoma (LSC) and fibrous and/or fibrosing pleuritis.[15–20] Usually, pleural SM grows along the parietal and visceral pleurae;[6] however, occasional cases of localized mesothelioma have also been observed.[21] On the other hand, LSC types, including pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma, grow more aggressively in pulmonary parenchyma than other non-small cell carcinomas, sometimes involving the parietal pleura and the chest wall.[5] In the event that epithelial components in pleomorphic carcinoma, such as adenocarcinoma, squamous cell carcinoma and large cell carcinoma, cannot be detected, and tissue obtained by needle biopsy is submitted for pathological diagnosis, the differential diagnosis between pleural SM and LSC is difficult even if immunohistochemistry is used. Therefore, clinical and radiological information is necessary to diagnose pleural SM definitively.[6,7,22]

Immunohistochemical analyses were using 15 performed, commercially available antibodies to evaluate the usefulness of immunohistochemistry in the differential diagnosis of pleural SM from LSC. In particular, we discussed the usefulness of D2-40 (anti-podoplanin antibody) was assessed in such differential diagnosis.

Materials and methods

Fourty five pleural SM cases and 27 LSC cases were selected, including 22 pleomorphic carcinomas and five spindle cell carcinomas, diagnosed between 1987 and 2008, from the surgical archives of the Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University. Nine pleural SM and nine LSC cases had been included in a previous study.[22] The diagnosis of each case was confirmed by reviewing clinical information (especially chest computed tomography findings), main clinical and pathological tumour location and morphological slides including haematoxylin and eosin (H&E) and immunohistochemically stained slides. All LSC cases in this study were located in the pulmonary parenchyma. Questionable cases were omitted from the study. The average age of the patients with pleural SM was 63.2 years (range 35–85) and that of the patients with LSC was 65.3 years (range 56–89). All the patients were Japanese. The diagnosis in each case was based on the recommended criteria listed in the 2004 World Health Organization Classification.[5,23] In brief, pleural SMs consist of spindle cells arranged in fascicles or having a haphazard distribution, often resembling fibrosarcoma and malignant fibrous histiocytoma,[5] and LSC is a poorly differentiated non-small cell lung carcinoma that contains a component of sarcoma or sarcoma-like (spindle and/or giant cell) differentiation.[23] The subclassification of LSC is listed in Table 1. Biphasic and desmoplastic mesothelioma and pulmonary carcinosarcoma, pulmonary blastoma and giant cell carcinoma were excluded from the study.

Immunohistochemistry of sections from formalin-fixed paraffin-embedded tissues was performed using the Histofine Simple Stain MAX PO (MULTI) kit (Nichirei, Tokyo, Japan) with or without antigen retrieval. The list of primary antibodies, including the 15 antibodies, clone, source, dilution rate and antigen retrieval, is shown in Table 2. Based on the number of tumour cells observed following immmunohistochemistry, the tumours were scored using the following semiquantitative system: 0, no or trace reactivity; score 1+, <10% tumour cells; score 2+, 11–50% tumour cells; score 3+, >51% tumour cells. The definition of a 'positive case' in this study is a case with a score of 1+, 2+ or 3+.

When the expression of D2-40 was investigated, a high level of background staining was occasionally observed in the desmoplastic tissue surrounding infiltrating tumour cells, as pointed out by Chu *et al.*[24]

Table 1. Clinicopathological profile of pleural sarcomatoid mesothelioma and lung sarcomatoid carcinoma

	Sarcomatoid mesothelioma	Sarcomatoid carcinoma
Number of cases	45	27
Age (mean)	35–85 (63.2)	56–89 (65.3)
Male:female	40:5	24:3
Subtypes (WHO, 2004)		
Pleomorphic carcinoma		22
Epithelial components:		
Adenocarcinoma		19
Squamous cell ca.*		2
Large cell ca.*		1
Spindle cell carcinoma		5

*ca., carcinoma.

Table 2. Antibodies used in this study

Antibody to	Source	Clone	Pretreatment	Dilution
Podoplanin	Nichirei BioScience, Tokyo, Japan	D2-40	Autoclave	Pre-diluted
Calretinin	Zymed, San Francisco, CA, USA	Poly	Autoclave	1:50
WT1	Dako, Glostrup, Denmark	6F-H2	Autoclave	1:400
Thrombomodulin	Dako	1009	None	1:500
CEA	Nichirei BioScience	COL-1	Autoclave	Pre-diluted
Napsin A	IBL, Gumma, Japan	TMU-Ad02	Autoclave	1:200
TTF-1	Dako	8G7G3/1	Autoclave	1:50
CAM5.2	Becton Dickinson, Franklin Lakes, NJ, US	2A4	Autoclave	Pre-diluted
Pancytokeratin	Novocastra, Newcastle upon Tyne, UK	AE1/AE3	Autoclave	1:500
EMA	Dako	E29	Autoclave	1:100
Epithelial antigen	Dako	Ber-EP4	None	1:100
Epithelial related antigen	Dako	MOC-31	None	1:50
α-Smooth muscle actin	Dako	1A4	Autoclave	1:100
h-Caldesmon	Dako	H-CD	Autoclave	1:50
Desmin	Dako	D33	Autoclave	1:100

WT, wilms' tumour; CEA, carcinoembryonic antigen; TTF, thyroid transcription factor; EMA, epithelial membrane antigen.

Therefore, D2-40 was assessed in relatively cellular areas to prevent the background staining from interfering with the results. LSC was evaluated in the 'sarcomatoid' component, i.e. areas where the tumour cells exhibited spindle and/or pleomorphic morphology without clear epithelial tumour cell nest formations. Immunohistochemical evaluation for the 'epithelial' component of LSC was excluded from this study.

Statistical analyses were performed using Fisher's exact test and Mann–Whitney *U*-test. Sensitivity and specificity were calculated for each marker using a simple 2 × 2 table. A *P*-value of <0.05 was considered to be significant.

Results

POSITIVITIES OF ANTIBODIES FOR SARCOMATOID MESOTHELIOMA AND LUNG SARCOMATOID CARCINOMA

The positivities of each antigen for pleural SM and LSC are indicated in Table 3. Representative immunohistochemical reactivity for pleural SM and LSC is shown in Figures 1 and 2. The pattern of reactivity for each of the antibodies for the two tumour types is briefly described below.

D2-40

Of the 45 pleural SM cases, 39 (86.7%) were positive for D2-40. Of the 27 LSC cases, seven (25.9%) were positive for D2-40. Both the tumours exhibited a predominantly cytoplasmic pattern of immunoreactivity; however, both membranous and cytoplasmic patterns of reactivity were also noted in some cases. A high grade of reactivity (i.e. 2+ or 3+) was observed in 21 pleural SM cases (46.7% of the positive cases); however, most positive LSC cases had a low reactivity grade (i.e. 1+). The difference in reactivity grade was statistically significant between pleural SM and LSC. The intensity of reactivity in pleural SM and LSC cells in most cases was less than that in the normal lymphatic endothelium of the same specimens; however, some pleural SM cells were immunoreactive with the strong intensity similar to that of lymphatic endothelium. If a cut-off value of 10% was applied, the sensitivity of D2-40 would be 46.7% in pleural SM and 3.7% in LSC.

Table 3. Immunohistochemical findings for pleural sarcomatoid mesothelioma and lung sarcomatoid carcinoma

	Sarcomatoid mesothelioma					Sarcomatoid carcinoma						
		Reactivity grade					Reactivity grade					
Markers	*n* (%)	0	1+	2+	3+	*n* (%)	0	1+	2+	3+	*P*-value*	*P*-value†
D2-40	39/45 (86.7)	6	18	11	10	7/27 (25.9)	20	6	1	0	<0.001	<0.001
Calretinin	35/45 (77.8)	10	23	10	2	16/27 (59.3)	11	11	3	2	0.081	0.154
WT1	10/24 (41.7)	14	6	3	1	7/19 (36.8)	12	5	1	1	0.806	0.695
Thrombomodulin	9/23 (39.1)	14	8	0	1	10/23 (43.5)	13	9	1	0	0.500	0.790
CEA	0/27 (0)	27	0	0	0	4/27 (14.8)	23	4	0	0	0.055	0.039
Napsin A	0/15 (0)	15	0	0	0	4/20 (20.0)	16	4	0	0	0.093	0.965
TTF-1	0/15 (0)	15	0	0	0	3/20 (15.0)	17	3	0	0	0.174	0.122
CAM5.2	45/45 (100)	0	9	17	19	25/26 (96.2)	1	10	1	14	0.366	0.743
AE1/AE3	24/27 (88.9)	3	4	11	9	21/23 (91.3)	2	8	5	8	0.578	0.611
EMA	13/24 (54.2)	11	12	1	0	10/15 (66.7)	5	7	2	1	0.240	0.634
Ber-Ep4	0/15 (0)	15	0	0	0	2/21 (9.5)	19	2	0	0	0.333	0.225
MOC-31	0/15 (0)	15	0	0	0	0/19 (0)	19	0	0	0	NA‡	NA‡
α-SMA	14/24 (58.3)	10	6	6	2	9/21 (42.9)	12	6	2	1	0.231	0.193
h-Caldesmon	0/15 (0)	15	0	0	0	0/18 (0)	18	0	0	0	NA‡	NA‡
Desmin	4/36 (11.1)	32	4	0	0	2/25 (8)	23	1	1	0	0.523	0.172

WT, wilms' tumour; CEA, carcinoembryonic antigen; TTF, thyroid transcription factor; EMA, epithelial membrane antigen; α-SMA, α-smooth muscle actin.
**P*-value (the difference in the positive rate between pleural SM and LSC for each antibody calculated by Fisher's exact test).
†*P*-value (the difference in the distribution of reactivity scores between pleural SM and LSC for each antibody calculated by Mann–Whitney *U*-test).
‡Not available.

CALRETININ

Of the 45 cases with pleural SM (77.8%), 35 were positive for calretinin. Most of the positive cases fell in the 1+ reactivity grade. The pattern of immunoreactivity in the pleural SM as well as LSC cases was observed as reactivity in the nucleus and cytoplasm. Most of the positive cases had a lower reactivity score (i.e. 1+). There was no difference in reactivity grades between pleural SM and LSC.

WILMS' TUMOUR 1 AND THROMBOMODULIN

Approximately 40% of the cases of pleural SM (41.7%) and LSC (36.8%) were positive for Wilms' Tumour (WT) 1. Immunoreactivity was located in the cytoplasm in each tumour. Trace nuclear reactivity was observed in certain positive cases.

More than 30% of the pleural SM (39.1%) and LSC (43.5%) cases exhibited a primarily membranous pattern of reactivity for thrombomodulin (TM). However, most of the positive cases had a staining score of 1+.

CARCINOEMBRYONIC ANTIGEN, NAPSIN A AND THYROID TRANSCRIPTION FACTOR-1

None of the pleural SM cases was positive for antibodies to carcinoembryonic antigen (CEA), Napsin A and thyroid transcription factor (TTF)-1. A few LSC cases exhibited a weak cytoplasmic pattern of reactivity for CEA (cytoplasm), Napsin A (cytoplasm) and TTF-1 (nucleus).

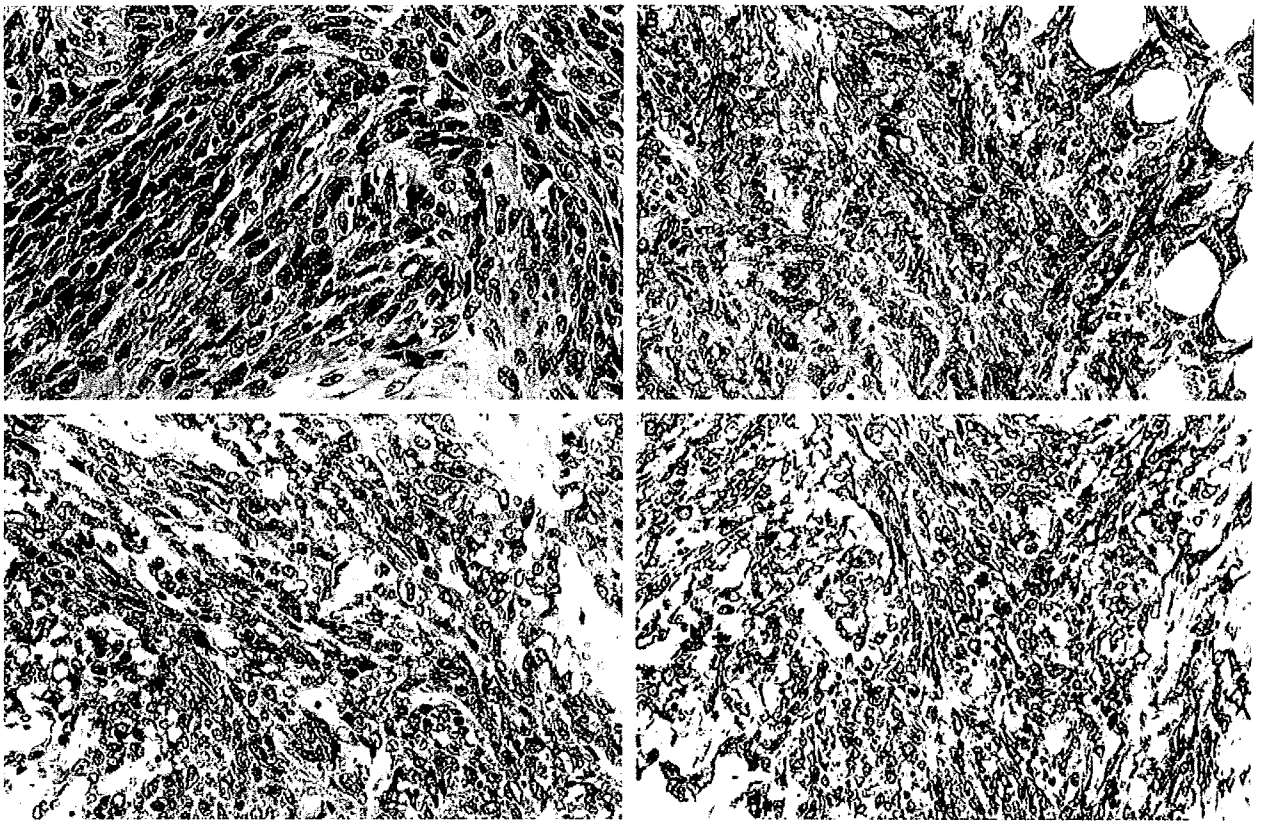

Figure 1. Histological and immunohistochemical results of a case of pleural sarcomatoid mesothelioma (59-year-old male patient). Atypical spindle cells arranged in fascicular pattern (A) (H&E). Tumour cells show cytoplasmic positivity for D2-40 (B) and nucleus and cytoplasmic positivity for calretinin (C). More than half of the tumour cells are immunoreactive for cytoplasmic CAM5.2 (D) (Streptavidin–biotin–peroxidase).

CYTOKERATIN (CAM5.2), CYTOKERATIN-MULTI (AE1/AE3), EPITHELIAL MEMBRANE ANTIGEN, BER-EP4 AND MOC-31

All the pleural SM cases and 96.1% of the LSC cases exhibited cytoplasmic patterns of reactivity for CAM5.2. Pan-cytokeratin (AE1/AE3) was also expressed in approximately 90% of the pleural SM and LSC cases. More than 50% of the pleural SM and LSC cases showed a primarily cytoplasmic and/or membranous pattern of reactivity for EMA. However, most of the positive cases had a 1+ reactivity score.

None of the cases of pleural SM was positive for Ber-EP4, and only two LSC cases exhibited a membranous pattern of reactivity. MOC-31 was not detected in either tumour.

α-SMOOTH MUSCLE ACTIN, H-CALDESMON AND DESMIN

Approximately half of the pleural SM and LSC cases exhibited cytoplasmic patterns of reactivity for α-smooth muscle actin (α-SMA). The distribution of expression score was variable. None of the pleural SM and LSC cases exhibited any immunopositivity for h-caldesmon. Four of the 36 pleural SM cases and one of the 25 LSC cases exhibited a weak cytoplasmic pattern of reactivity for desmin.

Table 3 lists the *P*-values for the difference in positivity rates (cases with staining grade >1+) between pleural SM and LSC determined by Fisher's exact test. Only D2-40 showed a significantly higher expression rate in pleural SM than in LSC.

The *P*-values according to the Mann–Whitney *U*-test for the difference in distributions of reactivity grades between pleural SM and LSC for each antibody are also shown in Table 3. Only D2-40 and CEA exhibited a significant difference in the proportions of reactivity grade.

SENSITIVITY AND SPECIFICITY OF EACH ANTIBODY FOR DIFFERENTIAL DIAGNOSIS BETWEEN MESOTHELIOMA AND LUNG SARCOMATOID MESOTHELIOMA

The sensitivity and specificity of each antibody for the differential diagnosis between pleural SM and LSC are

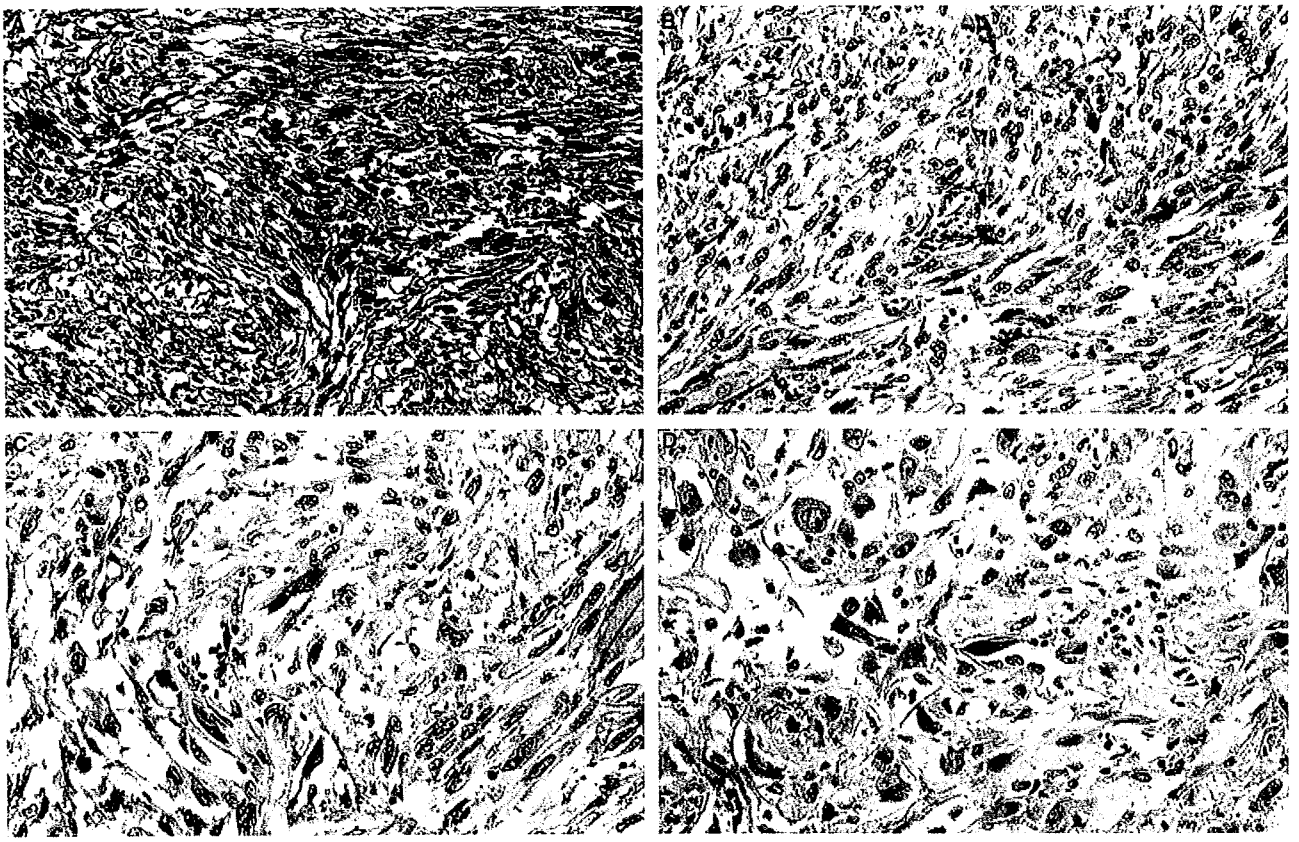

Figure 2. The histological immunohistochemical features of a lung pleomorphic carcinoma with foci of adenocarcinoma (78-year-old male patient). Atypical spindle and/or polygonal cells proliferating in the lung parenchyma (A) (H&E). No tumour cells show focal positivity for D2-40. Lymphatic endothelium in the tumour tissue positive for D2-40 (B). Some tumour cells exhibit nuclear/cytoplasmic positivity for calretinin (C). The focal carcinoembryonic antigen positivity is also illustrated (D) (Streptavidin–biotin–peroxidase).

indicated in Table 4. Only D2-40 exhibited a relatively high sensitivity and specificity for differential diagnosis. The other antibodies did not show a good combination of both high sensitivity and specificity.

Discussion

The accurate diagnosis of pleural SM and exclusion of other tumours with spindle cell morphology from the diagnosis is difficult to achieve based on the limited observation of histological features using techniques such as H&E immunohistochemistry, even though associated with clinical information.[6,7] It is especially difficult to differentiate between pleural SM and LSC invading pleural tissue and the chest wall due to their morphological similarities.

Recently, many 'mesothelial markers' including calretinin, D2-40, WT1, TM, mesothelin and cytokeratin (CK) 5/6 have been developed.[10,12,25] It is recommended that a set of antibodies should be used for the differential diagnosis of mesothelioma from other tumours and tumour-like lesions.[5,7,10] However, there are limited reports on the immunohistochemical differentiation of pleural SM and LSC.[15,18–20,26,27] Attanoos *et al.*[18] have described how the combination of a broad-spectrum CK with calretinin increases both the sensitivity and specificity for the diagnosis of pleural SM. Lucas *et al.*[19] conducted a comparative immunohistochemical study of mesothelioma and true sarcoma, including malignant fibrous histiocytoma, leiomyosarcoma, synovial sarcoma and LSC. They concluded that CK and calretinin had the greatest diagnostic value in the differential diagnosis of pleural SM from true sarcoma. However, immunohistochemical analyses with antibodies such as pan-cytokeratin, CK5/6, calretinin, WT1, TM and α-SMA play a limited role in differentiating between pleural SM and LSC. Our previous study indicated that a combination of CAM5.2, WT1 and pan-cytokeratin is useful for differentiating between SM and true sarcomas, but of the 10

Table 4. Sensitivity and specificity of each antibody for differential diagnosis between pleural sarcomatoid mesothelioma and lung sarcomatoid carcinoma

Markers	Sensitivity	Specificity
D2-40	86.7	74.1
Calretinin	77.8	40.7
WT1	41.7	63.2
Thrombomodulin	39.1	56.5
CEA	0	85.2
Napsin A	0	80
TTF-1	0	85
CAM5.2	100	3.8
AE1/AE3	88.9	8.7
EMA	54.2	33.3
Ber-Ep4	0	90.5
MOC-31	0	100
α-SMA	58.3	58.1
h-Caldesmon	0	100
Desmin	11	92

WT, wilms' tumour; CEA, carcinoembryonic antigen; TTF, thyroid transcription factor; EMA, epithelial membrane antigen; α-SMA, α-smooth muscle actin.

commercially available antibodies used, no useful antibodies were identified for differential diagnosis between pleural SM and LSC.[22] However, our previous study did not include D2-40 immunohistochemistry, and the sample size of our study as well as that of the previous reports comprised relatively few LSC cases (only nine and <10 cases, respectively).[18–20,26,27] Therefore, we tried to elucidate the useful antibodies to differentiate between pleural SM and LSC by increasing the number of cases analysed and the number of antibodies used. Our results indicate that significant efficacy of D2-40 was observed in the differential diagnosis between pleural SM and LSC. In other words, D2-40 showed a higher positivity (86.7%) in pleural SM than in LSC (26.1%) cells and a higher proportion of positivity among pleural SM cells.

Commercially available D2-40 has recently been used as an antibody that reacts with the 40-kDa antigen of the M2A oncofetal membrane antigen originally detected in germ cell neoplasia.[28] A recent study has demonstrated that this mucin-type glycoprotein is podoplanin.[29] This molecule is also expressed in the lymphatic endothelium; therefore, it is routinely used in the investigation of lymphatic invasion in tumours.[30,31] This antibody also reacts with normal mesothelial cells, and its use in the diagnosis of mesothelioma, especially the epithelioid type, has been emphasized.[24,32,33]

However, there are limited reports on D2-40 immunohistochemistry of SM. Ordonez[32] reported that D2-40 staining was positive in 86% of epithelioid mesothelioma cells, whereas no immunoreactivity was detected in six cases of SM and in five cases of biphasic mesothelioma (sarcomatoid component), and concluded that unlike calretinin, D2-40 is not effective in the diagnosis of these tumours. Muller *et al.* [33] have also reported that no D2-40+ cases were observed among 18 SM cases. Chu *et al.*[24] reported that D2-40 reactivity in sarcomatoid differentiation (either biphasic mesothelioma or pure sarcomatoid) was less frequent than in epithelioid mesothelioma [i.e. 33 of 33 epithelioid (100%), with reactivity observed in 10 of 16 biphasic (62.5%) and three of four sarcomatoid (75%) cases]. They further reported that the D2-40 pattern of reactivity in SM is less intense and is observed in the cytoplasm, as opposed to the intense and membranous pattern of reactivity observed in cases of epithelioid mesothelioma. They concluded that D2-40 was limited by its performance in areas of sarcomatoid differentiation and also mentioned that the interpretation of D2-40 reactivity in areas of sarcomatoid growth was complicated by the loss of the characteristic pattern of reactivity observed in epithelioid mesotheliomas and the high level of background staining present in both benign reactive pleural fibrous tissue and/or desmoplastic tissue surrounding infiltrating tumour cells.[24] Our present findings are consistent with the results reported by Chu *et al.*[24]; we selected a relatively cellular area for the immunohistochemical evaluation of D2-40, and excluded the desmoplastic type as a variant of SM, to prevent the above-mentioned problems in analysis.

On the other hand, Hinterberger *et al.*[34] described that 30% of SM or sarcomatoid components of biphasic mesothelioma, assessed using a tissue microarray technique from 341 mesotheliomas, were D2-40+, and the combination with calretinin increased the sensitivity in SM. However, they did not mention the specificity of D2-40 for other spindle cell tumours including LSC. Padgett *et al.*[27] also reported that D2-40 and anti-podoplanin antibody immunohistochemistry was more efficacious than calretinin for diagnosis of SM, i.e. 11 of 14 SM cases (79%) and one

of 13 sarcomatoid carcinomas from various organs (8%) were positive for D2-40. In the present study, we have demonstrated a sensitivity and specificity of more than 80% and 70%, respectively, for the differential diagnosis of pleural SM and LSC. The discrepancy between the results of these two reports[27,34] and our data may be due to the different interpretation of positivity. A different scoring system was applied in these studies, in which a case was defined as positive if >10% tumour cells were immunopositive. We applied this scoring system to our data and the results revealed D2-40 sensitivity of 46.7% (21/45) in pleural SM and 4% (1/23) in LSC. Therefore, D2-40 specificity is higher in the differential diagnosis of pleural SM and LSC.

Calretinin is strongly expressed particularly in epithelioid mesothelioma of the pleura or peritoneum.[35] On the other hand, reports on the positivity of calretinin in pleural SM and LSC have been limited and controversial. Attanoos *et al.*[18] reported that 12 of 31 SM (39%) and no LSC (0%) cases were positive for calretinin. Lucas *et al.*[19] reported calretinin reactivity of 60% of the sarcomatoid component of biphasic mesothelioma, 70% of SM and 60% of LSC. Kushitani[22] reported that 34 of 39 SM (87.2%) and six of nine LSC (66.7%) cases were positive for calretinin. In this study, the data indicate that the value of calretinin is limited due to its low specificity (41.7%) in the differentiation between pleural SM and LSC and a similar distribution of reactivity grades.

WT1 is expressed in rat and human mesotheliomas[36] and has been reported to be a very useful marker for differentiating between epithelioid mesothelioma and pulmonary adenocarcinoma.[9,15] In the present study, WT1 positivity in pleural SM and LSC was almost identical. Therefore, we consider that WT1 cannot be used for the differential diagnosis of pleural SM and LSC as opposed to its use in the diagnosis of pleural SM and true sarcoma.[22]

TM is a relatively old marker for epithelioid mesothelioma.[37] Reported TM positivity is 29% and [18] 70% [19] in SM, and 0%[18] and 40%[19] in SC. In this study, there was no significant difference in TM expression between pleural SM and LSC.

In this study, we included three so-called 'adenocarcinoma markers' including CEA,[9] TTF-1[9,38] and Napsin A.[39] As expected, none of these markers was positive for all pleural SM cases, although they were positive in some LSC cases. However, their degree of positivity was low. Nakashima *et al.*[40] reported that five of 37 LSC (13.5%) were positive for CEA, which is similar to our data. On the other hand, Rossi *et al.*[41] described a relatively high rate of positivity for TTF-1 in the sarcomatoid component of pleomorphic carcinoma and spindle cell carcinoma of the lung (43.1% and 55%, respectively). This high rate of positivity was not observed in the LSC cases in our study. However, in the event that no other specific carcinoma components are detected in LSC, these 'adenocarcinoma markers' may assist in accurate diagnosis.

Other epithelial cell markers such as CAM5.2 (CK7/8), pan-cytokeratin (AE1/AE3), EMA, Ber-EP4, MOC-31 and myogenic markers (α-SMA, h-caldesmon, and desmin) showed no significant differences between the two tumour types. Pathologists must be aware of this fact when diagnosing spindle cell lesions as intrathoracic neoplasms.

Desmoplastic mesothelioma was excluded from this study because it may have increased the difficulty in immunohistochemical evaluation with D2-40. However, immunohistochemical study of this tumour, which is difficult to diagnose is required, especially the differentiation of fibrous/fibrosing pleuritis.[6]

In conclusion, the well-known mesothelial marker D2-40 can be applied to differentiate between pleural SM and the sarcomatoid component of LSC. However, some pleural SM cases exhibit no reactivity for this antibody. Recently, Knuuttila *et al.*[26] have reported that specific loss of 4q11-p13/p15 and 4q and gain of 5p are detected by comparative genomic hybridization (CGH) analysis. Gordon *et al.*[42,43] have reported gene expression profile differences between malignant pleural mesotheliomas and lung carcinomas using microarray expression and GeneChip technique. These CGH and genome-wide gene expression profiling analyses are likely to provide new potential markers for the differential diagnosis of pleural SM and LSC in the near future.

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総説

肺がんCT検診の有効性評価のための無作為化比較試験計画

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要旨

肺癌死亡減少の重要な手段の一つとして低線量CT検診があるが，政策として採りあげるにはEBMに基づいた有効性の評価が必須である．欧米で遂行中のRCTの結果が「無効」となった場合には，CT検診は葬り去られる可能性がある．それを覆すには本邦におけるRCTで結果を出すしかなく、厚労省垣添班でRCT計画を策定した．

・40%の肺がん死亡減少効果を見込み，50-64歳の住民50000人を対象
・年齢・施設・喫煙状況で層別化
・研究群では低線量CTと喀痰細胞診を10年間行い（喫煙者では低線量CT検診を10回，非喫煙者では低線量CT検診は1,3,7年目の3回で残りは現行検診），対照群では現行検診を10年間行う

参加者の検診は無料とすると，研究費用の概算は15年間に38億5400万円で，年間2億5700万円にのぼった．膨大ではあるが，乳がん超音波検診のRCTでもかなりの額が投入されているので，不可能な額ではない．

キーワード：　低線量CT、肺がん検診、無作為化比較試験、有効性評価
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はじめに

胸部CTによる肺がん検診は、高い肺癌発見率（現行検診の5－20倍）、高い早期癌の割合（80%以上）、高い発見例の5年生存率（80%以上）が報告されており[1-7]、いずれも驚異的なレベルと言って良い状況である。しかしながら、検診における発見率・早期癌割合・生存率は、いずれもバイアスの関与が大きいとされており、それだけでは効果の有無は不明と考えるのが通説である。

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Overdiagnosis bias

バイアスの中でも問題視されているものの一つにOverdiagnosis biasがある。これは「ゆっくり大きくなり、他疾患で死亡するため、死亡に関係しない」肺癌を発見してしまうことであるが、CT検診にはこれがある程度の大きさで存在しているだろうと想定されている。たとえば、CT所見と剖検所見を比較した報告[8]によれば、1047例の剖検例中，死亡前2ヶ月以内に胸部CTを受検した例は187例あり、そのうち、139例は陰影がなかったが48例には陰影が存在した。対象としてCT検診でのみ見つかるような病変を想定しているため、16mm以上の陰影や10個以上存在するような陰影を除外すると、28例において15mm以下の陰影が1ないし9個、死亡の2か月以内の胸部CT写真上で指摘できることが判明した。その28例中19例が剖検時に初めてチェックされ、そのうち原発性肺癌が2例

存在した。187例中2例に「死亡の原因に関連しない、CTをしていなければ気付かなかったはずの原発性肺癌」が存在したことになる[8]。これは10万対比で1070にあたり、このことからでも、CT検診におけるOverdiagnosis biasは無視できない影響があると想定せざるを得ない。

有効性評価に基づく肺がん検診ガイドライン

厚生労働省の研究班である祖父江班（現：濱島班）では、有効性評価に基づくがん検診ガイドラインを、毎年臓器を変えながら作成している[1]。前述したようなバイアスの問題があるため、がん検診の有効性評価は、検診受診により受診者の死亡率が低下するかどうかによって評価するのが国際的な標準であり、祖父江班でもそのように行っている。それによれば、低線量CTによる肺がん検診に関しては、これまでのところ発見率と発見病期や組織型の報告が多く、生存率がわずかに報告されているのみであり、肺がん死亡について言及している論文は質の低いコホート研究が1つだけだが、その論文では肺がん死亡減少効果は認められていない[9]。その結果、肺がん死亡減少効果の有無は不明であり、対策型検診としては勧められない、とされている[1]。

効果評価に関する今後の動き

前述した祖父江班の報告は、すなわち肺がん死亡を評価するような研究がなされる必要がある、ということでもある。今後は、まず、短期的に研究をまとめられるStage Shiftなどの傍証に関して論文化が必要と考えられる。ただし、Stage Shiftとは進行癌の割合が減ることではなく（これのみではOverdiagnosis biasだけでも観察されてしまう）、受診が繰り返されるにつれて受診者あたりの進行癌の「数」が減少してくることが必要なのだが、その点に関する誤解は現在においても、残念ながら専門家の間でも存在している。

死亡率減少を直接評価する研究としては、国内では厚生労働省中山班のコホート研究がおこなわれており、海外ではNLST, NELSONなどのRCTが進行中である[10-12]。中山班の結果も期待されるが、RCTで効果が否定されると、CT検診はつぶれかねない状況が予想され、しかも、NLSTは効果に関して楽観視はできない状況と推定されている。

厚生労働省垣添班の研究

厚生労働省垣添班では、効果が確立していないがん検診の方法の効果を評価するための道筋を明らかにすることを目的の一つとしており、胸部CT、PSA、胃内視鏡によるがん検診に関しての効果評価研究の計画立案を行っている。

胸部CTに関しては、海外でのRCTで否定されても対抗できるような研究が必要であるため、2008年度には日本におけるRCT研究計画を作成した。RCTは、膨大な期間・参加者・研究費が必要であり、以前は日本では実現は不可能と考える向きもあったが、最近は乳がんエコーのRCTが12万人規模で進行中であるなど環境は変化しており、実現できる可能性はある。

どのようなRCTを組めば良いか？

実際にRCT計画案を作成するに当たっては、種々の要素を確定していく必要があった。以下にそれを列記する。

1．研究群は胸部CT、では対照群は？

「無検査」または「胸部X線」が考えられるが、日本ではX線（＋細胞診）を推奨しており、対照群を無検査にすることは不可能であることから、対照群は「胸部X線」とする。

2．喀痰細胞診は？

日本では推奨しているので、外すことは難しい。そのため、両群の喫煙者に併用する必要がある。

3．検診は何年行うべきか？

Lung Screening Studyなどの結果からは、3年程度の短期の検診では有意差は得られなさそうであり、ELCAPのデータでも5年ぐらい経ってから死亡率が開いてくる可能性があ

ると学会などで報告されている。そのため、最低でも5年は必要で、できれば8年ないし10年あったほうが望ましいと考えられた。

4．対象は喫煙者 or 非喫煙者

肺癌死亡は喫煙者に多いので死亡を減らすためには喫煙者にターゲットを絞るのは合理的である。一方、非喫煙者ではGGOなどが発見され、その予後は良い。日本のデータが良いのは、これらを含んでいるためかもしれない。中山班のデータでも喫煙者より非喫煙者のほうが効果が大きいようだ。また、今回の研究の対象を喫煙者に限れば、研究終了後も非喫煙者に対してのCT検診の効果のエビデンスは存在しないことになる。海外の研究はすべて喫煙者限定であるため、日本で非喫煙者のデータを出す必要があるということもあり、今回の研究では非喫煙者も含むことにした。

5．年齢は？

厚労省の「がん対策推進基本計画」での死亡率減少の目標は74歳以下（75歳以上は減少できなくても良いという意味ではなく、コントロールが困難ということ）としている。10年行うとすると、50-64歳をリクルートすることになり、その線が妥当であろうということになった。

6．性別は？

喫煙者 vs 非喫煙者の議論と同じであり、日本でやるなら男女とも行うべきだろうという結論になった。

7．胸部X線に比較して、肺がん死亡減少効果がどの程度あると見込むか？

中山班のデータからでも、非喫煙者ではかなりあるかもしれないが、喫煙者ではそれほど楽観できないように思われる。死亡者の割合は圧倒的に喫煙者が多いので、喫煙者での期待できる死亡減少効果を、全体で期待できる死亡減少効果として算出しなければならない。その点からは、毎年受診しても死亡率50%減少は難しいのではないか、という意見もあり、一方で、毎年受診して20%減少では費用に見合わない、という意見もあった。

8．毎年CTを撮らないとダメか？

喫煙者では半年に1回のCT検診でも死亡するケースがあることがわかっており、毎年必要だろうと思われたが、非喫煙者では10年に3－4回で充分ではないかと思われた。検診期間の後のフォローの期間は必要か、という問題もあったが、非喫煙者のようなゆっくりしたものにはあったほうが良いが、喫煙者のような速いものには必ずしも必要でないと思われた。

組み上げたRCT計画案

以上のような議論の末、費用的な面も加味して、以下のようなRCT計画案を組み上げた。

① 50-64歳の男女に対して、10年間の胸部CTが胸部X線に比べて40%の肺がん死亡減少効果があると見込んで必要症例数を算出
② 喫煙率、男女比などは現行検診のデータを使用
③ 妥当な応諾率，コンタミネーションを見込むと50000人必要
④ 研究群では低線量CTと喀痰細胞診を10年間
　(ア) 喫煙者では低線量CT検診（＋喀痰）を10回
　(イ) 非喫煙者では低線量CT検診（＋喀痰）は1, 3, 7年目の3回で，残りは現行検診
⑤ 対照群では現行検診を10年間
⑥ 当初計画としては3-4年程度で一段落させることを要請されたため、
　(ア) プライマリ・エンドポイントとして，追跡法による精度（感度・特異度）を2群間で比較
　(イ) セカンダリ・エンドポイントとして，発見時の病期の分布（特に進行がんの罹患数），腫瘍径の分布を比較し，肺がん死亡率の減少の程度を推定
　(ウ) 10年間（登録期間を5年と設定したので全部で15年）に延長できれば

肺がん死亡率減少効果も評価可能、
という枠組みにした

計画を実行する際に留意すべき問題

計画を実行する際には、その他にいくつかの検討すべき事柄がある。まず、研究群がCTを受けることも必要だが、対照群がX線を受けないと結果的に「CT vs. 非受診」となってしまい、「効果あり」という結果が出ても「CTとX線はどちらが効果大きいのか?」という疑問が解消できない。そのため、対照群にはX線を受けてもらうことが必須だが、日頃受けてない人を受けさせることはきわめて困難である。その点からは、ある年の肺がん検診受診者から対象を選べば、もともと受診する傾向のある集団なので、そういった面での問題は少なくなる可能性が高い。

また、CT検診の効果は不明なので研究群・対照群に損得はないのだが、リクルートのしやすさや、市町村の協力の受けやすさから、対照群への「advantage もどき」があったほうが良いのではないか、という問題がある。これは欧米ではあまり問題にならないのだが、現実に日本における検診が「行政サービス」のような位置づけで行われていたりするため、また「検診受診」を「良いこと」ととらえやすい国民性などのために、このような問題が生じる。そこで、研究参加を促す目的で，対照群では，内臓脂肪測定を初年度に行いその後の経過を観察するコホート研究へ参加することもできる，という追加研究を提案することにした。

研究費用

この研究計画に要する費用は以下の通りである。

1．検診費用：研究参加者は無料、ただし精密検査以降の費用は「医療」扱いとする。保険点数をベースして費用算定し、フォロー・事務費用等を含むものとした

(ア) 検診群喫煙者のCT
16千円×7500人×10回＝12億円

(イ) 検診群非喫煙者のCT
16千円×17500人×3回＝8.4億円

(ウ) 検診群非喫煙者のX線
2100円×17500人×7回＝2.6億円

(エ) 対照群のX線
2100円×25000人×10回＝5.3億円

(オ) 喀痰細胞診(受診者の3割)
3360円×7500人×10回＝2.5億円

(カ) 喀痰再検討(初回分のみ)
2000円×7500人×2施設＝0.3億円

受診率が90%と仮定すると
約27億9400万円/15年間
一律10%減で発注すると
約25億1500万円/15年間

2．データセンター費用
約8億8000万円/15年間

(ア) 文書作成・印刷・発送、説明要員(CRC)派遣・養成、

(イ) リクルート用パンフレット・ビデオ作成、

(ウ) コールセンター設置・運営、説明会開催、訪問監査、

(エ) 登録データ管理システム設計・運営、など

3．事務局費用　年間1200万円

4．総額
約38億5400万円/15年間(2億5700万円/年)
検診費用を10%減で発注すると、
約35億7500万円/15年間(2億3850万円/年)

おわりに

低線量CTによる肺がん検診のRCTを立案した。X線に比べ40%の死亡減少効果があると想定して、50-64歳の男女50000人を対象、喫煙者には喀痰も行い、研究群は喫煙者CT10回、非喫煙者CT3回X線7回の検診、対照群はX線10回として、研究総額は38億余で年間2億5700万円と算定された。高額ではあるが、年間2億程度の予算で行われている他の研究もあり、不可能な額ではない。他国でのRCTの結果で混乱をきたさないよう、一刻も早く本邦における研究をスタートさせるべきである。

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The study design of a randomized controlled trial to evaluate the efficacy of thoracic CT screening for lung cancer in Japan

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Low-dose thoracic CT screening for lung cancer can be an important measure to decrease lung cancer mortality. However, the evaluation of the efficacy based on EBM is indispensable to adopt as the public policy. The project team for thoracic CT screening in Kakizoe Team has made a plan of a randomized controlled trial to evaluate the efficacy of thoracic CT screening for lung cancer in Japan.
Fifty thousand people of 50-64 years old are required. They are stratified by age/institution/smoking situation. During next ten years, a half of them take CT (smokers: 10 CT, non-smokers: 3 CT and 7 roentgenogram), and the other half take 10 chest roentgenogram. Comparing with Roentgenogram Group, forty percent of mortality reduction is expected in CT Group. The rough estimate of the research cost reached three billion and eight hundred fifty-four million yen in 15 years.

Key words: low-dose CT, Lung Cancer Screening, Randomized Controlled Trial, Efiicacy
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