Combined serum mesothelin and carcinoembryonic antigen measurement in the diagnosis of malignant mesothelioma

KAZUYA FUKUOKA[1,2], KOZO KURIBAYASHI[3], SHUSAI YAMADA[2], KUNIHIRO TAMURA[2], CHIHARU TABATA[2] and TAKASHI NAKANO[1,2]

[1]Cancer Center, [2]Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501; [3]Department of Respiratory Medicine, Murakami Memorial Hospital, Asahi University, Gifu, Gifu 500-8523, Japan

Received April 14, 2013; Accepted August 1, 2013

DOI: 10.3892/mco.2013.175

Abstract. Malignant mesothelioma (MM) is a highly aggressive tumor associated with asbestos exposure. The identification of a marker specific for MM may be of considerable value for the early detection of this tumor and may be used in particular to screen groups with a history of asbestos exposure. The aim of this study was to evaluate serum soluble mesothelin-related peptide (SMRP) levels as a diagnostic marker for MM and investigate whether its diagnostic value is enhanced by combination with other biomarkers. Serum SMRP levels were measured using a quantitative enzyme-linked immunosorbent assay in 96 patients with MM, 55 patients with lung cancer and 39 individuals with a history of asbestos exposure. Receiver operating characteristic curves were constructed for performance evaluation. Stepwise logistic regression analysis was used to select marker combinations (MCs). Serum SMRP levels in patients with MM were significantly higher compared to those in the other groups (P<0.001). The sensitivity of SMRP levels in diagnosing MM was 56% and its specificity for MM vs. lung cancer and individuals with asbestos exposure was 87 and 92%, respectively. The area under the curve (AUC) was 0.76 [95% confidence interval (CI): 0.68-0.83] for the differentiation between MM and lung cancer and 0.78 (95% CI: 0.71-0.86) for the differentiation between MM and individuals with asbestos exposure. For the MC of presence of effusion, SMRP and carcinoembryonic antigen (CEA) levels, the AUC for the differentiation between MM and lung cancer (0.92; 95% CI: 0.88-0.97) and the differentiation between MM and individuals with asbestos exposure (0.93; 95% CI: 0.87-1.0) was significantly higher compared to that for SMRP alone (P=0.0001 and 0.0058, respectively). While the specificity of this MC was comparable to SMRP alone, its sensitivity was ~20% higher compared to that of SMRP alone. Therefore, combining SMRP and CEA improves the diagnostic performance of SMRP alone. A combination of serum biomarkers, including SMRP, may facilitate the non-invasive diagnosis of MM.

Correspondence to: Dr Kazuya Fukuoka, Cancer Center, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan
E-mail: yumikofu@m3.kcn.ne.jp

Key words: malignant mesothelioma, mesothelin, soluble mesothelin-related peptide, Mesomark™, marker combinations

Introduction

Malignant mesothelioma (MM) is a tumor that develops from the serous membranes that line the body cavities and it may arise in the pleura, peritoneum and pericardium; in addition, although extremely rare, it may also develop in the tunica vaginalis testis. The most common form of this disease is the malignant pleural mesothelioma (MPM). MM was previously considered as being extremely rare; however, its incidence and associated mortality rate exhibited a sharp increase worldwide over the last 50 years, due to the close association of MM with asbestos exposure. The prognosis of MPM is poor, with a median survival of ~9-17 months (1). However, in selected patients with epithelioid tumor histology, early-stage disease, who undergo trimodality treatment (combination of chemotherapy, postoperative radiotherapy and extrapleural pneumonectomy), median overall survival of 51 months and 5-year survival rates of 46% have been reported (2). Recent phase II trials reported a median survival of ~30 months for the patients who completed the trimodality treatment (3,4). Therefore, early diagnosis may play a vital role in the improvement of therapeutic outcomes. Together with the advances in imaging studies and endoscopic examinations, the development of biomarkers useful for serum or effusion diagnosis is crucial for the early diagnosis of MM. Currently known biomarkers for diagnosing MM include cytokeratin 19 fragment (CYFRA) (5-7), tissue polypeptide antigen (TPA) (5,6,8), hyaluronic acid (8), carbohydrate antigen (CA125) (8,9) and osteopontin (10-15). However, these markers have low specificity for MM.

Mesothelin is a 40-kDa cell surface glycoprotein that is overexpressed in cells of pancreatic and ovarian cancer, mesothelioma and other malignancies. The mesothelin gene encodes a 69-kDa glycoprotein, the mesothelin precursor protein, which is cleaved by a furin-like protease and its N-terminal region is released in the blood as a 31-kDa protein, the megakaryocyte

potentiating factor (MPF). The 40-kDa C-terminal region of this glycoprotein binds to the cell membrane as mesothelin. Three distinct variants of mesothelin have been identified, one of which has a modified C-terminus and becomes detached from the cell membrane since it lacks a glycosylphosphatidylinositol (GPI) anchor. This soluble isoform corresponds to the soluble mesothelin-related peptide (SMRP) (16). SMRP and MPF may be highly specific biomarkers for MM and have an equivalent diagnostic performance (17-19). SMRP is currently the most extensively investigated and is considered to be the best available blood protein biomarker of MM (20).

However, the diagnostic performance of SMRP alone is not considered to be sufficiently high, as it appears to exhibit insufficient sensitivity for MM (20,21). In diagnosing malignant tumors, such as ovarian or prostate cancer, the diagnostic performance of individual serum biomarkers was improved by combining data obtained using multiple biomarkers (22,23).

In the present study, we evaluated the performance of serum SMRP levels in the diagnosis of MM and investigated whether its diagnostic value could be improved through its combination with other biomarkers.

Materials and methods

Study design. The subjects of this study were patients who satisfied the following inclusion criteria: i) age ≥20 years; ii) pathologically proven MM or lung cancer; and iii) except for ii), individuals with asbestos exposure proven on the basis of their history or from the medical viewpoint. Only patients who personally provided written informed consent for the measurement of their serum biomarkers were enrolled in this study. Subjects who satisfied the above inclusion criteria during the study period were retrospectively enrolled. The pathological diagnosis was based on standard histological and immunohistochemical criteria (24,25). The subjects were classified into three groups: individuals with a history of asbestos exposure, patients with lung cancer and patients with MM. This study was approved by the Institutional Review Board of the Hyogo College of Medicine.

Measurement of serum biomarker levels. At the time of confirmation of the diagnosis, blood samples were collected from the subjects and, following prompt separation of the serum, the samples were stored at -80˚C. The serum SMRP levels were measured using an ELISA kit (Mesomark™; Fujirebio Diagnostics Inc., Malvern, PA, USA) according to the manufacturer's instructions. The serum levels of CYFRA and carcinoembryonic antigen (CEA) were measured using commercially available immunoassay systems according to the manufacturer's instructions: the serum CEA levels were determined using a chemiluminescent immunoassay (Abbott Japan Co., Ltd., Tokyo, Japan) and the serum levels of CYFRA were determined using a solid-phase sandwich immunoradiometric assay (CIS Bio International, Gif-sur-Yvette, France). The manufacturer suggests 3.5 ng/ml for CYFRA and 5.0 ng/ml for CEA as the cut-off values to differentiate between non-malignant disease and malignant tumors.

Statistical analysis. Summary statistics were used (median and 25th and 75th percentiles) to evaluate the distribution of serum SMRP levels. The Steel's test, a non-parametric form of the Dunnett's test, was used for comparing MM to the other groups. The sensitivity and specificity of SMRP for diagnosing MM were calculated, along with the corresponding 95% exact confidence intervals (CIs). The above analyses were also performed for CYFRA and its performance was compared to that of SMRP by using the McNemar's test. To compare the serum SMRP levels between each histological subtype of MM, the Steel-Dwass test, a non-parametric form of the Tukey's test, was performed. Subsequently, a stepwise logistic regression analysis was used to select marker combinations (MCs) that were more effective for diagnosing MM. The criterion for assessing whether a difference was significant in the variable selection was 5%. The diagnostic performance of SMRP and the MC was assessed by constructing a receiver operating characteristic (ROC) curve and calculating the area under the curve (AUC). The AUC for SMRP and that for the MC were compared using the theory on generalized U-statistics to generate an estimated covariance matrix and the χ^2 test (26). For each test, two-sided $P<0.05$ was considered to indicate a statistically significant difference. Data were analyzed using the statistical software SAS, version 9.1.3 (SAS Institute Inc., Cary, NC, USA) and Stata, version 11.0 (StataCorp College Station, TX, USA). The GraphPad Prism software, version 4.00 for Windows (GraphPad Software, San Diego, CA, USA) was used to prepare the figures.

Results

Patient characteristics. A total of 190 subjects were enrolled in this study. A summary of the clinical characteristics of these subjects, together with a breakdown of each group by age, gender, history of asbestos exposure and presence of effusion (pleural or peritoneal) is presented in Table I. Among the 39 individuals with asbestos exposure, pleural plaque was present in 16, benign asbestos pleurisy in 7, asbestosis in 3 patients, asbestosis plus benign asbestos pleurisy in 5, round atelectasis in 2 and no imaging abnormalities in 6 patients. The histological subtype in the 55 patients with lung cancer was adenocarcinoma in 24, squamous cell carcinoma in 14 and small-cell carcinoma in 17 patients. Among the 96 patients with MM, the primary tumor site was the pleura in 91 and the peritoneum in 5 patients (Table II). The histological subtype was epithelioid in 57 patients, sarcomatoid in 12, biphasic in 6, desmoplastic in 4 and unspecified in the remaining 7 patients (Table II). Of the 91 patients with MPM, 74 were diagnosed with clinical stage IV disease according to the staging classification proposed by the International Mesothelioma Interest Group (IMIG). Only 5 patients had either stage I or II disease (Table II).

Performance of serum SMRP in diagnosing MM. Fujirebio Diagnostics, Inc., the developer of the Mesomark assay, recommends a cut-off value of 1.5 nM, which was the 99th percentile of the normal serum SMRP concentration in a population of 409 healthy Americans (27). An investigation in a population of healthy Germans revealed a cut-off value of 1.5-1.6 nM, which was the 95th percentile of the serum SMRP concentration (28). In our study, we performed a preliminary investigation of the distribution of serum SMRP levels among

Table I. Characteristics of the study subjects.

Characteristics	AE (n=39)	LC (n=55)	MM (n=96)
Age (years)			
Mean ± SD	68.1±8.1	64.7±10.6	61.2±9.5
Range	44-90	39-84	33-83
Gender			
Male	36	45	75
Female	3	10	21
Asbestos exposure			
Occupational	26	1	55
Environmental	13	1	27
None	0	53	14
Presence of effusion	12	16	78

AE, asbestos exposure; LC, lung cancer; MM, malignant mesothelioma; SD, standard deviation.

Table II. Demographic data of MM patients.

Characteristics	Patient no. (%)
Primary site	
Pleura	91 (94.8)
Peritoneum	5 (5.2)
Histological subtype	
Epithelioid	57 (59.4)
Sarcomatoid	12 (17.4)
Biphasic	16 (16.7)
Desmoplastic	4 (5.8)
NOS	7 (7.3)
Staging classification[a]	
I	3 (3.3)
II	2 (2.2)
III	12 (13.2)
IV	74 (81.3)

[a]Proposed by the International Mesothelioma Interest Group (IMIG), peritoneal mesothelioma (n=5) was excluded. MM, malignant mesothelioma; NOS, not otherwise specified.

72 healthy individuals without a history of asbestos exposure. Since this investigation revealed that 69 individuals (96%) had serum SMRP levels of <1.5 nM, we selected 1.5 nM, the 96th percentile, as the cut-off value.

The distributions of serum SMRP levels in each group are shown in Fig. 1. The serum SMRP levels in MM patients were significantly higher compared to those in the other groups (P<0.001) (Table III). The sensitivity of SMRP for diagnosing MM was 56% (95% CI: 46-66%) and its specificity for MM vs. lung cancer and individuals with asbestos exposure was 87% (95% CI: 76-95%) and 92% (95% CI: 79-98%), respectively (Table IV). By contrast, the sensitivity of CYFRA for diagnosing MM was 63% (95% CI: 52-72%) and its specificity for MM vs. lung cancer was 49% (95% CI: 35-63%) (Table IV). The sensitivity of SMRP and CYFRA did not differ significantly (P=0.157), although the specificity of SMRP for MM vs. lung cancer was significantly higher compared to that of CYFRA (P<0.001). The serum SMRP levels in epithelioid disease [median, 2.47 nM; interquartile range (IQR): 0.97-4.86] were significantly higher compared to those in sarcomatoid disease (median, 0.8 nM; IQR: 0.38-1.15) (P=0.04). However, there were no significant differences when compared to the other histological subtypes. There was no significant association between the serum SMRP levels and MPM stages (data not shown).

The diagnostic performance of SMRP was evaluated using ROC curves (Fig. 2). For the differentiation between MM and lung cancer, the AUC was 0.76 (95% CI: 0.68-0.83) (Fig. 2A) and for the differentiation between MM and individuals with asbestos exposure, the AUC was 0.78 (95% CI: 0.71-0.86) (Fig. 2B). For CYFRA, the AUC for the differentiation between MM and lung cancer was 0.55 (data not shown). Therefore, the diagnostic performance of SMRP for differentiating between MM and lung cancer was superior to that of CYFRA.

Investigation of MCs and their performance in diagnosing MM. To improve the performance of serum biomarkers in diagnosing MM, we investigated the optimal MCs. The measured variables common to patients with MM and lung cancer were age, gender, presence of effusion, clinical stage and the levels of SMRP, CYFRA and CEA. The measured variables common to patients with MM and individuals with a history of asbestos exposure were age, presence of effusion and the levels of SMRP, CYFRA and CEA. Since the distributions of all the biomarkers were significantly skewed to the right, the variables were logarithmically transformed using common logarithms. A stepwise logistic regression analysis was used to select the variables. To differentiate between MM and lung cancer, SMRP levels, presence of effusion and CEA levels were selected (Table V). From the signs of the estimates, we determined that the probability of a diagnosis of MM was higher for elevated SMRP levels, presence of pleural

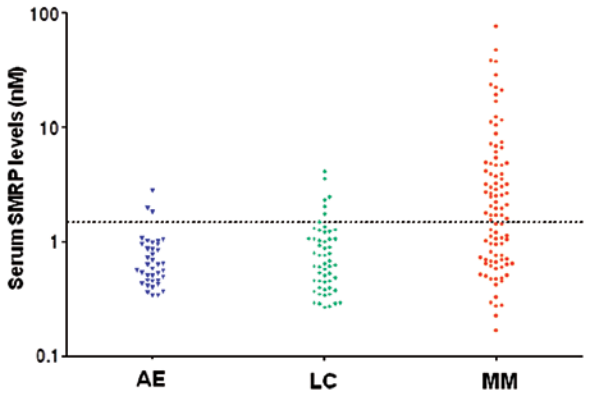

Figure 1. Distribution of serum soluble mesothelin-related peptide (SMRP) levels in each group. The serum SMRP levels in patients with malignant mesothelioma (MM) are compared to those in patients with lung cancer (LC) and individuals with a history of asbestos exposure (AE). The cut-off value is denoted by the horizontal dotted line.

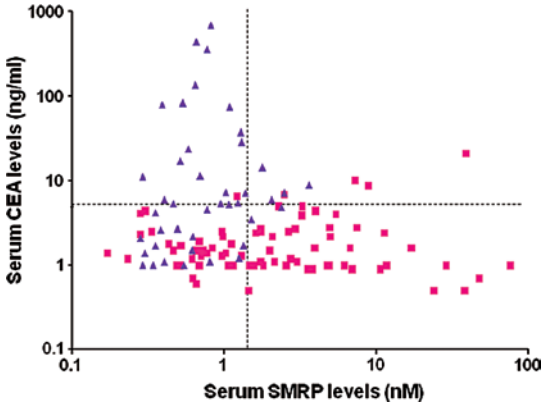

Figure 3. Scatter diagrams of serum biomarker levels in patients with malignant mesothelioma (■) and lung cancer (▲). Carcinoembryonic antigen (CEA) levels plotted against soluble mesothelin-related peptide (SMRP) levels. Each cut-off value is denoted by horizontal or vertical dotted lines.

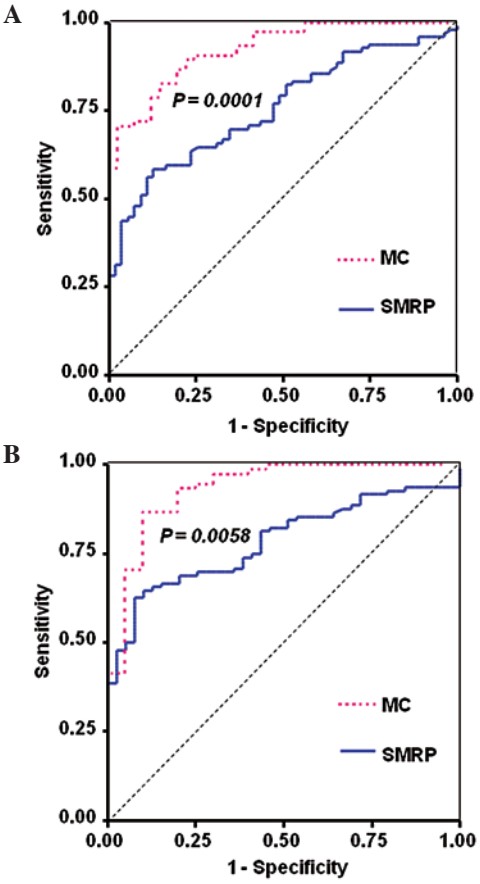

Figure 2. (A) Receiver operating characteristic (ROC) curves for soluble mesothelin-related peptide (SMRP) and the marker combination (MC) for differentiating between patients with malignant mesothelioma and lung cancer. The area under the curve (AUC) for the MC is significantly higher compared to that for SMRP alone (P=0.0001). (B) ROC curves for SMRP and the MC for differentiating between patients with malignant mesothelioma and individuals with a history of asbestos exposure. The AUC for the MC is significantly higher compared to that for SMRP alone (P=0.0058).

effusion and lower CEA levels. It was concluded that the selected markers were reasonable from the clinical standpoint. Subsequently, the markers selected to differentiate between MM and individuals with a history of asbestos exposure were age and CYFRA (data not shown). However, this model was composed of a single marker rather than multiple markers. Therefore, it was excluded from further investigation.

To further evaluate the models in Table V, the association between SMRP and CEA was analyzed using scatter diagrams (Fig. 3). The scatter diagrams demonstrated that the majority of patients with high CEA levels were those with lung cancer. In addition, the majority of patients with high SMRP levels were those with MM. Therefore, the combination of SMRP and CEA resulted in only a minor overlap of the diagnostic findings of MM and lung cancer, suggesting that the diagnostic performance for MM was improved. By contrast, since the combination of SMRP and CYFRA resulted in a significant overlap of the diagnostic findings of MM and lung cancer, it was inferred that the diagnostic performance was scarcely improved (data not shown).

The MC was composed using the results of Table V. Since the ratio of the estimates for SMRP, presence of effusion and CEA was ~3:1:5, the following MC was selected: MC=1xI(presence of effusion) + 3 x $\log_{10}$(SMRP) - 5 x $\log_{10}$(CEA), where I (presence of effusion) was defined as an indicator function with a value of 1 when effusion was present and 0 when effusion was absent. Wherein -1 was selected as the cut-off value to maximize the sum of the sensitivity and specificity, the sensitivity of MC for diagnosing MM was 76% (95% CI: 64-85%) and its specificity for MM vs. lung cancer and individuals with asbestos exposure was 88% (95% CI: 74-96%) and 90% (95% CI: 68-99%), respectively. While the specificity of MC was comparable to SMRP alone, its sensitivity was ~20% higher compared to that of SMRP alone. In addition, three of the five MPM patients with stage I-II disease were above the cut-off value, although none exhibited elevated serum levels of SMRP alone. The ROC curves for MC are shown in Fig. 2. The AUC for the differentiation between MM and lung cancer was 0.92 (95% CI: 0.88-0.97), which was significantly higher compared to that for SMRP alone (P=0.0001) (Fig. 2A). The AUC for the differentiation between MM and individuals with a history of asbestos exposure was 0.93 (95%CI: 0.87-1.0), which was also significantly higher compared to that for SMRP alone (P=0.0058) (Fig. 2B). These results indicate that combining CEA with SMRP improves the performance

Table III. Diagnostic findings based on the serum SMRP levels.

Serum SMRP levels (nM)	AE (n=39)	LC (n=55)	MM (n=96)
Mean ± SD	0.78±0.50	0.93±0.77	5.77±11.1
Median	0.64	0.65	1.88[a]
QR25-QR75	0.49-0.96	0.40-1.08	0.71-4.79
Min-max	0.30-2.80	0.30-4.10	0.30-75.4

[a]P<0.001, MM vs. AE or LC (by Steel's test). SMRP, soluble mesothelin-related peptide; AE, asbestos exposure; LC, lung cancer; MM, malignant mesothelioma; SD, standard deviation; QR25, 25th percentile; QR75, 75th percentile; min, minimum; max, maximum.

Table IV. Sensitivity and specificity of biomarkers for diagnosing MM.

Biomarkers	AE (n=39)	LC (n=55)	MM (n=96)
SMRP (%)			
Sensitivity	8	13	56
95% CI	2-21	5-24	46-66
Specificity	92	87	
95% CI	79-98	76-95	
CYFRA (%)			
Sensitivity	8	51	63
95% CI	2-21	37-65	52-72
Specificity	92	49	
95% CI	79-98	35-63	
CEA (%)			
Sensitivity	64	57	9
95% CI	41-83	41-72	4-17
Specificity	36	43	
95% CI	17-59	28-59	

MM, malignant mesothelioma; AE, asbestos exposure; LC, lung cancer; SMRP, soluble mesothelin-related peptide; CYFRA, cytokeratin 19 fragment; CEA, carcinoembryonic antigen; CI, confidence interval.

Table V. Results of stepwise logistic regression analysis (MM vs. LC).

Parameter	DF	Estimate	SE	Wald χ^2	P-value
Intercept	1	3.08	0.79	15.45	<0.001
SMRP[a]	1	2.83	0.92	9.48	0.002
Presence of effusion	1	1.28	0.42	9.15	0.003
CEA[a]	1	-5.52	1.46	14.20	<0.001

[a]The levels of SMRP and CEA were logarithmically transformed. MM, malignant mesothelioma; LC, lung cancer; DF, degree of freedom; SE, standard error of estimate; SMRP, soluble mesothelin-related peptide; CEA, carcinoembryonic antigen.

of SMRP alone in diagnosing MM and may facilitate early detection of MPM.

Discussion

The recent development of Mesomark, a quantitative ELISA kit using two monoclonal antibodies (OV569 and 4H3) that recognize SMRP, has enabled the measurement of serum SMRP levels. The findings of key studies on the performance of SMRP in diagnosing MM by using the Mesomark kit demonstrated that serum SMRP levels were significantly higher in MM patients compared to those in controls, such as healthy individuals, subjects with a history of asbestos exposure, or patients with asbestos-related benign pleural disease or lung cancer (9,11-21,27-35). In the present study, also undertaken using the Mesomark kit, the serum SMRP

levels were found to be significantly higher in MM patients compared to those in lung cancer patients and individuals with asbestos exposure. These findings are consistent with those first reported by Robinson *et al* (36), suggesting that the use of serum SMRP levels for diagnosing MM has excellent universality and reproducibility. Based on previous studies, including our own, SMRP is considered to be a highly specific biomarker for MM; however, its sensitivity, ranging from 48-80%, is moderate (9,11-21,27-35). To improve the performance of SMRP in diagnosing MM, there is a need to increase the sensitivity while maintaining a high degree of specificity.

One way of improving the sensitivity may be by lowering the cut-off value; however, this is not recommended, since it may result in a simultaneous reduction of specificity (26,28). Another approach may be to improve the diagnostic performance by combining data obtained using multiple biomarkers. The accuracy of the histopathological diagnosis of MM has markedly improved. One reason for this improvement has been the introduction of immunohistochemical analysis involving the combination of a positive marker that is highly expressed in MM and a negative marker that has a low frequency of expression in MM (37,38). A systemic review of markers for diagnosis of MM demonstrated that positive staining for CEA and epithelial antigen (clone Ber-EP4) and negative staining for epithelial membrane antigens and calretinin may confirm that a patient does not have MM (21). In addition, based on biomarker measurements in the pleural effusion, algorithms for the diagnosis of malignant pleural diseases were established. The CEA level achieved a greater accuracy in the differential diagnosis of MPM through its combination with other markers. For example, an elevated CYFRA level with a low CEA level in pleural effusion was shown to be highly suggestive of MPM (7).

To date, whether the combination of blood biomarkers, including SMRP, is able to improve the performance of SMRP alone in diagnosing MM remains controversial. A previous study by van den Heuvel *et al* (34) reported that the combination of two serum markers (CEA and SMRP) was the most accurate in differentiating MPM from non-small-cell lung cancer. The AUC of this marker combination demonstrated a significant improvement compared to the inverse levels of CEA alone. However, in that study, a direct comparison of diagnostic performance between this combination and SMRP alone was not performed.

Amati *et al* (31) evaluated the combination of two hematological biomarkers: 8-hydroxy-2'-deoxyguanosine (8-OHdG), an indicator of oxidative DNA damage and vascular endothelial growth factor β (VEGFβ), an angiogenic molecule. The results of that study indicated that the diagnostic performance of this combination in differentiating between healthy individuals and those with a history of asbestos exposure was superior to that of each biomarker alone. Although it was also mentioned that a combination of SMRP, 8-OhdG and VEGFβ was optimal for distinguishing between individual groups, including the MM group, that study provided no specific measures of diagnostic performance or any further details.

Several previous studies evaluated the diagnostic performance of combined SMRP and osteopontin measurements in MM. Creaney *et al* (12) demonstrated that the combination of SMRP, serum osteopontin and MPF did not exhibit increased sensitivity for detecting MM compared to that of SMRP alone. A recent study investigated serum SMRP and plasma osteopontin levels in 66 patients with MPM, 47 patients with non-malignant asbestos-related lung or pleural diseases, 42 patients with other benign pleural and lung diseases and 21 patients with lung cancer, as plasma osteopontin was proven to be more stable compared to serum osteopontin (14). A logistic regression analysis revealed that the combined marker model had an AUC of 0.912 and a sensitivity of 76%, with a 95% specificity (14). The AUC for this marker combination did not differ from that for serum SMRP alone. In previous studies, the majority of osteopontin-positive MM patients were also found to be positive for SMRP. This high degree of concordance may result in the finding that a combination of these two markers does not improve the performance of SMRP alone in diagnosing MM (12,14). Cristaudo *et al* (15) also measured serum SMRP and plasma osteopontin levels in 93 healthy subjects, 111 individuals with benign respiratory disease and 31 patients with MPM. That study was the first to demonstrate that a combination of these two markers was more efficient in MPM diagnosis compared to each marker used alone by means of the combined risk index, a new statistical approach of a logistic regression analysis. In that study, however, a small number of patients with MPM were enrolled and its histological subtype was limited to the epithelioid type. To confirm those findings, larger-scale studies are required. The combination of SMRP with CA125 (9), or MPF (12,18) has also been investigated. However, none of those studies demonstrated that the diagnostic performance of SMRP in combination with other markers outperformed that of SMRP alone.

The present study demonstrated that combining SMRP and CEA improved the diagnostic performance of SMRP alone, since these two markers act in a complementary manner. However, since we used the same data for selecting and assessing the performance of MC, it is possible that our evaluation of the MC may have been optimistic. Furthermore, in our study, data were collected from a single center; validation of the diagnostic performance of this particular MC by a multicenter study is recommended in the future.

It is difficult to determine whether pleural effusion developing in individuals with a history of asbestos exposure represents benign asbestos pleurisy or is an initial symptom of MPM and misdiagnosis at this stage may hinder the early detection of MPM. Future prospective research is required to confirm whether a combination of serum biomarkers, including SMRP, may be useful in diagnosing early-stage MPM.

Acknowledgements

This study was supported in part by the Special Coordination Funds for Science and Technology from Japan Science and Technology Agency and the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

1. Tsao AS, Wistuba I, Roth JA and Kindler HL: Malignant pleural mesothelioma. J Clin Oncol 27: 2081-2090, 2009.
2. Sugarbaker DJ, Flores RM, Jaklitsch MT, *et al*: Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 117: 54-63, 1999.

3. Krug LM, Pass HI, Rusch VW, *et al*: Multicenter phase II trial of neoadjuvant pemetrexed plus cisplatin followed by extrapleural pneumonectomy and radiation for malignant pleural mesothelioma. J Clin Oncol 27: 3007-3013, 2009.
4. Van Schil PE, Baas P, Gaafar R, *et al*: Trimodality therapy for malignant pleural mesothelioma: results from an EORTC phase II multicentre trial. Eur Respir J 36: 1362-1369, 2010.
5. Bonfrer JM, Schouwink JH, Korse CM and Baas P: Cyfra 21-1 and TPA as markers in malignant mesothelioma. Anticancer Res 17: 2971-2973, 1997.
6. Schouwink J, Korse CM, Bonfrer JM, Hart AA and Baas P: Prognostic value of the serum tumour markers Cyfra 21-1 and tissue polypeptide antigen in malignant mesothelioma. Lung Cancer 25: 25-32, 1999.
7. Paganuzzi M, Onetto M, Marroni P, *et al*: Diagnostic value of CYFRA 21-1 tumor marker and CEA in pleural effusion due to mesothelioma. Chest 119: 1138-1142, 2001.
8. Hedman M, Arnberg H, Wernlund J, Riska H and Brodin O: Tissue polypeptide antigen (TPA), hyaluronan and CA 125 as serum markers in malignant mesothelioma. Anticancer Res 23: 531-536, 2003.
9. Creaney J, van Bruggen I, Hof M, *et al*: Combined CA125 and mesothelin levels for the diagnosis of malignant mesothelioma. Chest 132: 1239-1246, 2007.
10. Pass HI, Lott D, Lonardo F, *et al*: Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. N Engl J Med 353: 1564-1573, 2005.
11. Grigoriu BD, Scherpereel A, Devos P, *et al*: Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment. Clin Cancer Res 13: 2928-2935, 2007.
12. Creaney J, Yeoman D, Demelker Y, *et al*: 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.
13. Rai AJ, Flores RM, Mathew A, *et al*: Soluble mesothelin related peptides (SMRP) and osteopontin as protein biomarkers for malignant mesothelioma: analytical validation of ELISA based assays and characterization at mRNA and protein levels. Clin Chem Lab Med 48: 271-278, 2010.
14. Creaney J, Yeoman D, Musk AW, de Klerk N, Skates SJ, and Robinson BW: Plasma versus serum levels of osteopontin and mesothelin in patients with malignant mesothelioma - which is best? Lung Cancer 74: 55-60, 2011.
15. Cristaudo A, Bonotti A, Simonini S, *et al*: Combined serum mesothelin and plasma osteopontin measurements in malignant pleural mesothelioma. J Thorac Oncol 6: 1587-1593, 2011.
16. Hassan R, Bera T and Pasten I: Mesothelin: a new target for immunotherapy. Clin Cancer Res 10: 3937-3942, 2004.
17. Ray M and Kindler HL: Malignant pleural mesothelioma: an update on biomarkers and treatment. Chest 136: 888-896, 2009.
18. Hollevoet K, Nackaerts K, Thimpont J, *et al*: Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. Am J Respir Crit Care Med 181: 620-625, 2010.
19. Cristaudo A, Bonotti A, Simonini S, Bruno R and Foddis R: Soluble markers for diagnosis of malignant pleural mesothelioma. Biomark Med 5: 261-273, 2011.
20. Hollevoet K, Reitsma JB, Creaney J, *et al*: Serum mesothelin for diagnosing malignant pleural mesothelioma: an individual patient data meta-analysis. J Clin Oncol 30: 1541-1549, 2012.
21. van der Bij S, Schaake E, Koffijberg H, Burgers JA, de Mol BA and Moons KG: Markers for the non-invasive diagnosis of mesothelioma: a systematic review. Br J Cancer 104: 1325-1333, 2011.
22. Schorge JO, Drake RD, Lee H, *et al*: Osteopontin as an adjunct to CA125 in determining recurrent ovarian cancer. Clin Cancer Res 10: 3474-3478, 2004.
23. Etzioni R, Falcon S, Gann PH, Kooperberg CL, Penson DF and Stampfer MJ: Prostate-specific antigen and free prostate-specific antigen in the early detection of prostate cancer: do combination tests improve detection? Cancer Epidemiol Biomarkers Prev 13: 1640-1645, 2004.
24. Travis WD, Brambilla E, Müller-Hermelink HK and Harris CC (eds.): World Health Organization Classification of Tumors, Pathology and Genetics. Tumours of the Lung, Pleura, Thymus, and Heart. IARC Press, Lyon, 2004.
25. Husain A, Colby T, Ordonez N, *et al*: Guidelines for pathologic diagnosis of malignant mesothelioma: a consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med 133: 1317-1331, 2009.
26. DeLong ER, DeLong DM and Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristics curves: a nonparametric approach. Biometrics 44: 837-845, 1988.
27. Beyer HL, Geschwindt RD, Glover CL, *et al*: Mesomark: a potential test for malignant pleural mesothelioma. Clin Chem 53: 666-672, 2007.
28. Weber DG, Taeger D, Pesch B, Kraus T, Brüning T and Johnen G: Soluble mesothelin-related peptides (SMRP)-high stability of a potential tumor marker for mesothelioma. Cancer Biomark 3: 287-292, 2007.
29. Scherpereel A, Grigoriu B, Conti M, *et al*: Soluble mesothelin-related peptides in the diagnosis of malignant pleural mesothelioma. Am J Respir Crit Care Med 173: 1155-1160, 2006.
30. Cristaudo A, Foddis R, Vivaldi A, *et al*: Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. Clin Cancer Res 13: 5076-5081, 2007.
31. Amati M, Tomasetti M, Scartozzi M, *et al*: Profiling tumor-associated markers for early detection of malignant mesothelioma: an epidemiologic study. Cancer Epidemiol Biomarkers Prev 17: 163-170, 2008.
32. Pass HI, Wali A, Tang N, *et al*: Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. Ann Thorac Surg 85: 265-272, 2008.
33. Schneider J, Hoffmann H, Dienemann H, Herth FJ, Meister M and 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.
34. van den Heuvel MM, Korse CM, Bonfrer JM and Baas P: Non-invasive diagnosis of pleural malignancies: the role of tumour markers. Lung Cancer 59: 350-354, 2008.
35. Rodriguez Portal JA, Rodriguez Becerra E, Rodriguez Rodriguez D, *et al*: 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.
36. Robinson BW, Creaney J, Lake R, *et al*: Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 362: 1612-1616, 2003.
37. Ordonez NG: The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol 27: 1031-1051, 2003.
38. Ordonez NG: Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol 27: 1418-1428, 2003.