

# Mesothelin in Serum and Pleural Effusion in the Diagnosis of Malignant Pleural Mesothelioma with Non-positive Cytology

MARIA CRISTIANA FRANCESCHINI<sup>1</sup>, PAOLA FERRO<sup>1</sup>, PIER ALDO CANESSA<sup>2</sup>, ENRICO BATTOLLA<sup>3</sup>,  
PAOLO DESSANTI<sup>1</sup>, ALESSANDRO VALENTINO<sup>1</sup>, LAURA CASOLARI<sup>1</sup>, VINCENZO FONTANA<sup>4</sup>,  
RICCARDO PEZZI<sup>4</sup>, FRANCO FEDELI<sup>1</sup>, MARIA PIA PISTILLO<sup>5</sup> and SILVIO RONCELLA<sup>1</sup>

*Divisions of <sup>1</sup>Histopathology and Cytopathology, <sup>2</sup>Pneumology, <sup>3</sup>Clinical Pathology,  
Azienda Sanitaria Locale n° 5, La Spezia, Italy;*

*<sup>4</sup>Unit of Epidemiology and Biostatistics, <sup>5</sup>Tumor Epigenetics,  
IRCCS Azienda Ospedaliera Universitaria*

*San Martino-Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy*

**Abstract.** *Background/Aim: Mesothelin (SMRP) is regarded as a biomarker of malignant pleural mesothelioma (MPM). Herein, we analyzed the contribution of SMRP detection in pleural effusion and in serum to the diagnosis of MPM with non-positive cytology. Materials and Methods: The present study included 52 cases of MPM, 43 of pleural benign lesions and 25 of non-MPM pleural metastases. SMRP was measured by MesoMark ELISA (Cis-Bio International Gif/Yvette; France). Results: In non-positive cytology, effusion-SMRP showed higher diagnostic performance than serum-SMRP. We found 38 out of 52 (73.1%) cases of non-positive cytology MPM, out of which 27 (71.0%) were positive for effusion-SMRP (cut-off=12.70 nM) and 18 (47.4%) for serum-SMRP (cut-off=1.08 nM). When cytology, effusion- and serum-SMRP were used in combination, an overall sensitivity in detection of MPM of 78.9% was achieved. The same sensitivity was obtained by combining cytology with effusion-SMRP alone, whereas the combination of serum-SMRP with cytology led to a sensitivity of 61.5%. Conclusion: Detection of both effusion- and serum-SMRP can contribute to improve the diagnosis of MPM with non-positive cytology. However, the analysis of SMRP in effusion makes it unnecessary to test SMRP in the serum.*

Malignant pleural mesothelioma (MPM) is an asbestos-related tumor arising in the pleural cavity, with a poor prognosis and a worldwide incidence expected to increase in the next ten

years (1, 2). Diagnosis and treatment at an early stage of disease significantly improve patient survival (3).

Pleural effusion is often the primary manifestation of MPM and can be found in about 90% of patients with MPM at diagnosis (4). Effusion is obtained by performing pleural aspiration by thoracentesis and cytological examination is the most informative laboratory test for diagnosis. Unfortunately, in MPM, cytology displays a low sensitivity (about 30% using the Papanicolaou staining) and is not an accurate assay in differentiating MPM cells from reactive mesothelial cells or lung cancer cells (5, 6).

To avoid an invasive examination such as thoracoscopy, soluble tumor-related biomarkers are currently being investigated and frequently proposed as tools to support cytology in the diagnosis of MPM (7, 8). Among such biomarkers, soluble mesothelin-related peptide (SMRP) has recently received considerable attention (9, 10). SMRP originates from mesothelin, a 40-kDa cell surface glycoprotein with putative function in cell-to-cell adhesion (11). Mesothelin is expressed by normal mesothelial cells and overexpressed by various types of cancer, such as MPM (12), and may also provide the opportunity for novel mesothelin therapy (13).

The levels of SMRP has been found to be significantly increased in serum of patients with MPM (14-23) and, on this basis, it has been approved by the U.S. Food and Drug Administration for the diagnosis and monitoring of MPM.

In the present study, we assessed the SMRP levels in serum and effusion from patients with MPM by using an ELISA test detection system and analyzed their contribution to routine cytology in the diagnostic evaluation of suspected MPM.

## Materials and Methods

**Patients.** All the patients were recruited from the Division of Pneumology (Azienda Sanitaria Locale n° 5 La Spezia; Italy) between June 2008 and May 2013 and underwent thoracentesis and

*Correspondence to:* Dr. Silvio Roncella, Servizio di Anatomia ed Istologia Patologica Ospedale Sant'Andrea. Via Mario Asso n. 2, 19124 La Spezia, Italy. Tel/Fax: +39 0187604560, e-mail: silvio.roncella@asl5.liguria.it

**Key Words:** Mesothelioma, mesothelin, malignant pleural effusion, Mesomark assay, cytology.

Table I. Correlation between non-positive cytology for pleural effusion and pathology.

Pathology	Cases, n (%)	Cytology, n (%)	
		Negative	Suspicious
Mesothelioma	52 (43.3)	30 (57.7)	8 (15.4)
Epithelioid	33	17 (51.5)	6 (18.2)
Sarcomatoid	10	7 (70.0)	1 (10.0)
Biphasic	5	4 (80.0)	0 (0.0)
Papillary	2	0 (0.0)	1 (50.0)
Desmoplastic	2	2 (100.0)	0 (0.0)
Metastasis <sup>a</sup>	25 (20.8)	14 (56.0)	0 (0.0)
Benign <sup>b</sup>	43 (35.8)	43 (100.0)	0 (0.0)
All samples	120 (100.0)	87 (72.5)	8 (6.7)

Suspicious: Cases for which a precise diagnosis of mesothelioma could not be established (see Materials and Methods). <sup>a</sup>Lung (n=11), breast (n=5), ovarian (n=1), lymphoma/leukaemia (n=3), sarcoma (n=2), prostatic (n=1), unknown origin (n=2); <sup>b</sup>pneumonia (n=33), heart failure (n=2), tuberculosis (n=4), post-traumatic (n=1), asbestosis (n=3).

thoracoscopy. Pleural effusion and matched serum were drawn from each patient. The study protocol was approved by the local Ethics Committee (approval number 407 10/15/2008) and the informed consent was obtained from all patients. Diagnosis was on the basis of clinical signs, imaging data, cytological examination of pleural effusion and histology of pleural biopsies. Patients were defined as having benign disease when there were no signs of ongoing neoplasia and a minimum of one year of follow-up did not show any clinical evidence of tumour.

Fifty-two out of 120 (43.3%) samples were from patients with MPM (the majority being epithelioid, 33/52), 25 (20.8%) samples were from patients with non-MPM metastatic cancer and 43 (35.8%) samples were from patients with benign disease, as detailed in Table I. MPM patients were 46 (88.5%) males and six (11.5%) females, with a median age of 74.3 (range=61.1-88.9) years; of patients with metastases, 12 (48.0%) were male and 13 (52.0%) female, with a median age of 71.5 (range=43.1-86.2) years; patients with benign disease comprised of 36 (83.7%) males and seven (16.3%) females, with a median age of 77.4 (range=46.1-85.5) years.

**Cytology.** Cytology was assessed by standard protocols used in the Division of Histopathology and Cytopathology (Azienda Sanitaria Locale n° 5 La Spezia; Italy). Briefly, pleural effusion was obtained by thoracentesis, collected in sterile tubes without anticoagulant and rapidly brought to the laboratory. Part of the fluid was subjected to preparation of a conventional smear and the other part was used for cytopsin slide preparation.

For conventional smear, the effusion was centrifuged at 450 g for 10 min and the sediment stained with the Papanicolaou method and hematoxylin/eosin. To perform cytopsin preparations, 0.5 ml of the effusion was cytocentrifuged at 450 g for 10 min and stained with hematoxylin/eosin.

Smear and cytopsin slides were reviewed by three cytopathologists (P.D, A.V and F.F) and the specimens were considered negative if there was no atypia and the number of mesothelial cells was not

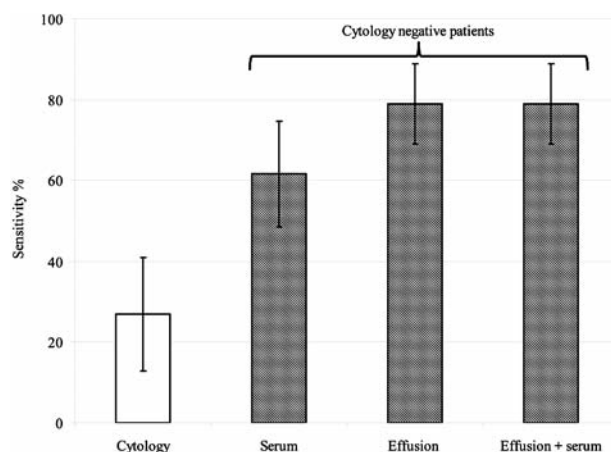


Figure 1. Sensitivity of cytology combined with serum-SMRP or effusion-SMRP, alone or together, in the diagnosis of mesothelioma. Mesothelin detection in both serum and pleural effusion increased the sensitivity of cytology (sensitivity=78%). The same sensitivity was found when cytology was combined with effusion-SMRP alone.

increased. In contrast, a positive cytology was defined as presence of hypercellularity and marked atypia of mesothelial cells, with enlargement of the cells, nuclei and the presence of nucleoli. Specimens were considered suspicious if the cells did not display any marked atypia as shown in cytology-positive samples, or in the presence of consistent atypia with too many cells to formulate a definitive diagnosis (Figure 1).

**Histology and immunohistochemistry.** Standard protocols at the Division of Histopathology and Cytopathology (Azienda Sanitaria Locale n° 5 La Spezia; Italy) were used to assess histology and immunohistochemistry. To perform immunohistochemistry, 5-µm-thick paraffin sections were mounted on slides and deparaffinized. Antigens were localized by means of a Ventana Medical System/view™ DAB detection Kit (Ventana Medical Systems, S.A. Strasbourg, France). Immunostaining was performed using an automated immunostainer, PathVision (Ventana Medical Systems) programmed for antigen retrieval. Strong reactivity for calretinin (CONFIRM rabbit monoclonal primary antibody to calretinin, clone SP65) and cytokeratin 5/6 (mouse monoclonal primary antibody to cytokeratin 5/6, clone D5/16B4,) associated with negative reactivity for carcinoembryonic antigen (CEA) (mouse primary antibody for CEA, clone TF3H8-1), epithelial membrane antigen (EMA) (CONFIRM mouse monoclonal primary antibody to EMA, clone E-29) and thyroid transcription factor-1 (TTF1) (CONFIRM mouse monoclonal antibody to TTF1, clone 8G7G3/1) (all from Ventana Medical Systems) is considered to be supportive of the diagnosis of MPM.

**Detection assay for SMRP.** Effusion and serum were processed by centrifugation and stored at -20°C. SMRP levels were measured by the MesoMark ELISA kit (Cis-Bio International Gif/Yvette, France; Fujirebio Diagnostic, Malvern, PA, USA) according to manufacturers' instructions. All effusion and serum samples were tested in duplicate. The cut-off level of mesothelin for MPM discrimination vs. all other pathologies (benign plus metastatic disease) was 12.70 nM and

1.08 nM for SMRP in effusion and serum, respectively, as reported in a previous study (14).

**Statistical analyses.** The diagnostic performance of SMRP was estimated through receiver operating characteristic (ROC) analysis and the area under the ROC curve (AUC) was used as a measure of accuracy for patients' classification. The Mann-Whitney test was applied to assess whether each AUC was statistically greater than 0.50 (level of non-discrimination or chance line). In addition, the exact binomial standard error of AUC was used to compute corresponding the 95% confidence interval (95% CI) (24). In order to provide a measure of association between the study biomarker and disease status, the diagnostic odds ratio (DOR) and corresponding 95% CI were also calculated (25).

All statistical analyses were performed using Stata (Stata Statistical Software Release 11.2; Stata Corporation, College Station, TX, USA).

## Results

**Contribution of effusion-SMRP and serum-SMRP to improving the diagnosis of MPM with non-positive cytology.** Cytology, effusion-SMRP and serum-SMRP detection were performed in 52 patients with MPM, 25 patients with metastases and 43 patients with benign disease. The origin of cancer in patients with MPM and metastases was ascertained by histology and immunohistochemistry on biopsy under thoracoscopy.

Cytology was unable to provide a diagnosis in 38/52 (73.1%) specimens, out of which 30 (57.7%) were cytology-negative and 8 (15.4%) were suspicious on cytology (Table I). Effusion-SMRP was positive (cut-off=12.70 nM) in 22/30 (73.30%) of the cytology-negative and in 5/8 (62.5%) of the cytology-suspicious specimens (Table II). Serum-SMRP was positive (cut-off=1.08 nM) in 16/30 (53.3%) and in 2/8 (25.0%) of the cytology-negative and -suspicious specimens, respectively (Table II).

**Diagnostic performance parameters of effusion-SMRP and serum-SMRP for MPM with non-positive cytology.** The performance of both effusion- and serum-SMRP tests for MPM with non-positive cytology maintained the same clinical significance found in a previous study (14) which analyzed a subset of patients included in the present study group. Indeed, effusion-SMRP had an overall diagnostic accuracy (AUC=84.0%) higher than that of serum-SMRP (AUC=71.3.0%) (Table III). Using the above mentioned biomarker thresholds, effusion-SMRP had a sensitivity of 71.1%, which was higher than that obtained for serum-SMRP (47.4%). In addition, comparable values for specificity were found for both biomarkers (87.7% for effusion-SMRP, 82.5% for serum-SMRP) and the DOR analysis confirmed the better diagnostic performance of effusion-SMRP as biomarker (effusion-SMRP: DOR=17.5; serum-SMRP: DOR=4.2) (Table III).

**Combined sensitivity of cytology, effusion-SMRP and serum-SMRP for MPM with non-positive cytology.** Patients with

Table II. *Mesothelin detection in serum and pleural effusion of mesotheliomas with non-positive cytology.*

Cytology	Mesothelin-positive	
	Effusion, n (%)	Serum, n (%)
Non-positive (n=38) <sup>a</sup>	27 (71.0)	18 (47.4)
Negative (n=30)	22 (73.3) <sup>b</sup>	16 (53.3) <sup>d</sup>
Suspicious (n=8)	5 (62.5) <sup>c</sup>	2 (25.0) <sup>e</sup>

Mesothelin-positive: Mesothelin level >cut-off=12.70 nM for effusion and 1.08 nM for serum; Cytology suspicious: cases for which a precise diagnosis of mesothelioma by cytology could not be established (see Materials and Methods). <sup>a</sup>See Table I; <sup>b</sup>epithelioid (n=14), sarcomatoid (n=4), biphasic (n=4); <sup>c</sup>epithelioid (n=4), papillary (n=1); <sup>d</sup>epithelioid (n=8), sarcomatoid (n=4), biphasic (n=4); <sup>e</sup>epithelioid (n=1), papillary (n=1).

Table III. *Estimate of diagnostic performance parameters for mesothelin levels in serum and pleural effusion of mesotheliomas with non-positive cytology.*

	Mesothelin	
	Effusion <sup>a</sup> Value (95% CI)	Serum <sup>b</sup> Value (95% CI)
AUC	84.0% (74.9-93.0%)	71.3% (60.3-82.2%)
Sensitivity	71.1% (54.1-84.6%)	47.4% (31.0-64.2%)
Specificity	87.7% (76.3-94.9%)	82.5% (70.1-91.3%)
DOR	17.5 (6.2-49.7)	4.2 (1.7-10.6)

AUC, Area under the curve; 95% CI, 95% confidence interval; DOR, diagnostic odds ratio. <sup>a</sup>Cut-off=12.70 nM, <sup>b</sup>cut-off=1.08 nM.

MPM were evaluated using all tests sequentially. In this context, cytology combined with serum-SMRP and effusion-SMRP had a global sensitivity of 78.9%. However, the contribution of serum-SMRP was practically negligible since effusion-SMRP by itself was able to reach the highest value for sensitivity of 78.9%, whereas cytology combined with serum-SMRP had a sensitivity of 61.5% (Figure 1). The combined values of serum and cytology, as well as those of effusion and cytology, had a higher sensitivity than that observed considering the two markers individually.

## Discussion

Every patient who has been directly exposed to asbestos and presents with pleural effusion may be suspected as having MPM. Indeed, pleural effusion is often the first presenting sign in MPM in a high percentage of patients (4). However, establishing the diagnosis of MPM through analysis of pleural effusion continues to be a clinical problem (5-6). When malignancy is suspected and the cytology is negative,

thoracoscopy should be performed. Thoracoscopy is the established method which provides the possibility of visualising the pleural cavity and directly obtaining biopsy specimens for histological and immunohistochemical analyses (26). Thoracoscopy is recognized as the gold standard for the diagnosis of MPM (27). However, it represents an invasive method which should be avoided whenever possible (26, 27). Indeed, the investigation of soluble tumor biomarkers in serum and effusion, which can be performed through a minimally invasive procedure, has been proposed as a tool to support cytology in the diagnosis of MPM (7, 8).

In the present study, we focused our attention on the contribution that serum- and effusion-SMRP can make to the diagnosis of MPM with non-positive cytology (negative and suspicious) and consequently to the choice of proceeding or not with thoracoscopy.

On our previous study, performed on a subset of the cohort analyzed herein, we assessed, by the Youden index, the best cut-off point to discriminate MPM from the other diseases (effusion-SMRP cut-off=12.70 nM and serum-SMRP cut-off=1.08 nM). In the present study, the same cut-off values were used to discriminate negative and positive SMRP samples (14).

In this regard, our findings confirm previous reports that the diagnosis of MPM using effusion by cytology has a low sensitivity (about 30%) and that the combined use with other biological markers is needed to improve the accuracy of final diagnosis (5-8).

Moreover, our results show that SMRP detection in serum and effusion may provide a means for reducing the discrepancies in diagnosing MPM. Indeed, SMRP detection allows diagnosis in about 73% for effusion and 53% for serum of specimens from patients with non-positive cytology. Similar conclusions were also reported by Filiberti *et al.* adopting a cut-off value of 12.0 nM, finding sensitivity of 76% (10); by Davies *et al.* with a cut-off=20.0 nM, finding sensitivity of 60% (20); and by Creaney *et al.* who, at a cut-off of 20.0 nM, found sensitivity of 77% (19). Furthermore, detection in serum reduced the discrepancy with cytology in diagnosing MPM. A similar conclusion was drawn by Creaney *et al.* who found sensitivity of 46% for serum-SMRP in MPM (19). In our study, we confirm that in patients with non-positive cytology for effusions, the diagnostic accuracy of effusion-SMRP is superior to that of serum-SMRP and that the lack of contribution of serum-SMRP to the overall diagnostic sensitivity makes this test unnecessary in the presence of effusion-SMRP.

The contribution of both tests (effusion- and serum-SMRP) to MPM diagnosis can also be important for confirming cases found to be suspicious by cytology. We recorded eight patients for whom cytology, performed on three recurrent effusion samples, was not diagnostic of MPM but was suspicious for MPM. In 2/8 serum and in 5/8 effusion

specimens, SMRP detection was positive. Although the number of suspicious cases in this study is small and therefore this finding warrants further validation, it suggests an additional clinical utility of SMRP for supporting the pathologist in making a diagnosis of MPM.

It is established that histological subtypes of MPM are associated with different sensitivity of cytology (6). Cytology has lower sensitivity for sarcomatoid and biphasic variants of MPM compared to the epithelioid variants (6). The findings of our study show that effusion- and serum-SMRP detection improves the final diagnosis by cytology of 100% for biphasic and of about 50% for sarcomatoid samples.

We also found that the sensitivity of cytology combined with serum/effusion-SMRP, was higher than that of serum and effusion alone. This finding confirms that SMRP detection in both fluids cannot be an alternative to cytology, but only additional to it for MPM diagnosis using effusions.

Other markers have been proposed for the indirect diagnosis of MPM by effusion. They include osteopontin (28), megakaryocyte potentiating factor (28) and fibulin-3 (29). However, none of these markers alone had, like mesothelin, the specificity and the sensitivity required for an accurate diagnosis. Therefore, we believe that a panel with combination of all (or some) of these markers may be a useful approach for the diagnosis of MPM. Recently, research in this area has pointed out that microRNAs, a class of short non-coding RNAs regulating gene expression post-transcriptionally, undergo characteristic alterations in MPM. Thus, many authors are proposing detection of these molecules in blood and effusions for diagnosis of MPM and promising studies are ongoing (30).

In summary, the results of the current study confirm, in a cohort of Italian patients, that detection of both effusion- and serum-SMRP can be helpful but cannot be an alternative to cytology. Therefore, we suggest that SMRP, especially effusion-SMRP, may represent a useful initial test in patients with suspected MPM since it can provide additional information that may reduce the need for proceeding with thoracoscopy.

## Conflicts of Interest

The Authors attest that no potential conflicts of interest exist with any company/organization whose products or services may be discussed in this article.

## Acknowledgements

This work was supported by Ricerca Sanitaria Regione Liguria 2009, by Comitato Assistenza Malati e Lotta Contro i Tumori Sarzana and by AIL Sezione Francesca Lanzoni La Spezia. Italy.

The Authors thank A. Camaïora, V. Balestracci and R. Tome for collecting the serum and the pleural effusion samples and Dr G. Leto for critically reading of the manuscript.



## References

- Delgermaa V, Takahashi K, Park EK, Le GV, Hara T and Sorahan T: Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. *Bull World Health Organ* 89: 716-724, 2011.
- Gennaro V, Ugolini D, Viarengo P, Benfatto L, Bianchelli M, Lazzarotto A, Montanaro F and Puntoni R: Incidence of pleural mesothelioma in Liguria Region, Italy (1996-2002). *Eur J Cancer* 41: 2709-2714, 2005.
- Tsao AS, Wistuba I, Roth JA and Kindler HL: Malignant pleural mesothelioma. *J Clin Oncol* 20: 2081-2090, 2009.
- Boutin C, Schlessler M, Frenay C and Astoul P: Malignant pleural mesothelioma. *Eur Respir J* 12: 972-981, 1998.
- Renshaw AA, Dean BR, Antman KH, Sugarbaker DJ and Cibas ES: The role of Cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 111: 106-109, 1997.
- Rakha EA, Patil S, Abdulla K, Abdulkader M, Chaudry Z and Soomro IN: The sensitivity of Cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Diagn Cytopathol* 38: 874-879, 2010.
- Greillier L, Baas P, Welch JJ, Hasan B and Passiukov A: Biomarkers for malignant pleural mesothelioma: Current status. *Mol Diagn Ther* 12: 375-390, 2008.
- Ray M and Kindler HL: Malignant pleural mesothelioma: An update on biomarkers and treatment. *Chest* 136: 888-896, 2009.
- Canessa PA, Franceschini MC, Ferro P, Battola E, Dessanti P, Manta C, Sivori M, Pezzi R, Fontana V, Fedeli F, Pistillo MP and Roncella S: Evaluation of soluble mesothelin-related peptide as a diagnostic marker of malignant pleural mesothelioma effusions: Its contribution to Cytology. *Cancer Invest* 31: 43-50, 2013.
- Filiberti R, Parodi S, Libener R, Ivaldi GP, Canessa PA, Ugolini D, Bobbio B and Marroni P: Diagnostic value of mesothelin in pleural fluids: comparison with CYFRA 21-1 and CEA. *Med Oncol* 30: 543-551, 2013.
- Chang K and Pastan I: Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci USA* 93: 136-140, 1996.
- Ordóñez NG: Value of mesothelin immunostaining in the diagnosis of mesothelioma. *Mod Pathol* 16: 192-197, 2003.
- Tang Z, Qian M and Ho M: The role of mesothelin in tumor progression and targeted therapy. *Anticancer Agents Med Chem* 13: 276-280, 2013.
- Ferro P, Canessa PA, Battola E, Dessanti P, Franceschini MC, Chiaffi L, Morabito A, Fontana V, Pezzi R, Fedeli F, Pistillo MP and S Roncella: Mesothelin is more useful in pleural effusion than in serum in the diagnosis of pleural mesothelioma. *Anticancer Res* 33: 2707-2013, 2013.
- Beyer HL, Geschwindt RD, Glover CL, Tran L, Hellstrom I, Hellstrom KE, Miller MC, Verch T, Allard WJ, Pass HI and Sardesai NY: MESOMARK: A potential test for malignant pleural mesothelioma. *Clin Chem* 53: 666-672, 2007.
- Cristaudo A, Foddìs R, Vivaldi A, Guglielmi G, Dipalma N, Filiberti R, Neri M, Ceppi M, Paganuzzi M, Ivaldi GP, Mencoboni M, Canessa PA, Ambrosino N, Chella A, Mutti L and Puntoni R: Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. *Clin Cancer Res* 13: 5076-5081, 2007.
- Luo L, Shi HZ, Liang QL, Jiang J, Qin SM and Deng JM: Diagnostic value of soluble mesothelin-related peptides for malignant mesothelioma: A meta-analysis. *Respir Med* 104: 149-156, 2010.
- Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE and Hellstrom I: Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet* 36: 1612-1616, 2003.
- Creaney J, Yeoman D, Naumoff LK, Hof M, Segal A, Musk AW, De Klerk N, Horick N, Skates SJ and Robinson BW: Soluble mesothelin in effusions: A useful tool for the diagnosis of malignant mesothelioma. *Thorax* 62: 569-576, 2007.
- Davies HE, Sadler RS, Bielsa S, Maskell NA, Rahman NM, Davies RJ, Ferry BL and Lee YC: Clinical impact and reliability of pleural fluid mesothelin in undiagnosed pleural effusions. *Am J Respir Crit Care Med* 180: 437-444, 2009.
- Hooper CE, Morley AJ, Virgo P, Harvey JE, Kahan B and Maskell NA: A prospective trial evaluating the role of mesothelin in undiagnosed pleural effusions. *Eur Respir J* 4: 18-24, 2012.
- Scherpereel A, Grigoriu B, Conti M, Gey T, Grégoire M, Copin MC, Devos P, Chahine B, Porte H and Lassalle P: Soluble mesothelin-related peptides in the diagnosis of malignant pleural mesothelioma. *Am J Respir Crit Care Med* 173: 1155-1160, 2006.
- Pass HI, Wali A, Tang N, Ivanova A, Ivanov S, Harbut M, Carbone M and Allard J: Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. *Ann Thorac Surg* 85: 265-272, 2008.
- Pepe MS: The Statistical Evaluation of Medical Tests for Classification and Prediction. Oxford, UK: Oxford University Press, 2003.
- Glas AS, Lijmer JG, Prins MH, Bonsel GJ and Bossuyt PM: The diagnostic odds ratio: A single indicator of test performance. *J Clin Epidemiol* 56: 1129-1135, 2003.
- Metintas M, Ak G, Dundar E, Yildirim H, Ozkan R, Kurt E, Erginel S, Alatas F and Metintas S: Medical thoracoscopy vs. CT scan-guided Abrams pleural needle biopsy for diagnosis of patients with pleural effusions: A randomized, controlled trial. *Chest* 137: 1362-1368, 2010.
- Canessa PA, Ferro P, Manta C, Ivory M, Franceschini MC, Fedeli F and Roncella S: Clinical value of mesothelin in pleural effusions vs. Histology by medical thoracoscopy. *Medical Oncology* 30: 649-653, 2013.
- Hollevoet K, Nackaerts K, Gosselin R, De Wever W, Bosquée L, De Vuyst P, Germonpré P, Kellen E, Legrand C, Kishi Y, Delanghe JR and van Meerbeeck JP: Soluble mesothelin, megakaryocyte potentiating factor, and osteopontin as markers of patient response and outcome in mesothelioma. *J Thorac Oncol* 6: 1930-1937, 2011.
- Pass HI, Levin SM, Harbut MR, Melamed J, Chiriboga L, Donington J, Huflejt M, Carbone M, Chia D, Goodglick L, Goodman GE, Thornquist MD, Liu G, de Perrot M, Tsao MS and Goparaju C: Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. *N Engl J Med* 367: 1417-1427, 2012.
- Kirschner MB, Cheng YY, Badrian B, Kao SC, Creaney J, Edelman JJ, Armstrong NJ, Vallely MP, Musk AW, Robinson BW, McCaughan BC, Klebe S, Mutsaers SE, van Zandwijk N and Reid G: Increased circulating miR-625-3p: a potential biomarker for patients with malignant pleural mesothelioma. *J Thorac Oncol* 7: 1184-1191, 2012.

Received July 23, 2014

Revised September 4, 2014

Accepted September 9, 2014