Diagnostic Utility of Thoracoscopy & Mesothelin in Malignant Mesothelioma

Rana El-Helbawy [1]; Nesreen El-Helbawy [2]; Safaa Tayel[2] and Ehab Shaltot [3]

[1]Chest , [2]Medical Biochemistry and [3]Clinical Oncology Departments, Faculty of Medicine, Minoufiya University, Egypt
ranaelhelbawy@yahoo.com

Abstract: Background Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin triggered by asbestos exposure. Mesothelin is a tumour differentiation antigen that is normally present on the mesothelial cells lining the pleura. Mesothelin is an epithelial marker highly expressed by cancer cells from diverse origins, including ovarian or pancreatic adenocarcinomas, and mesotheliomas. Early detection of mesothelioma can greatly improve the chances of survival. **Objective** Evaluating the utility of mesothelin quantification in serum or pleural fluid as useful adjunction to thoracoscopy in diagnosis of MPM and its additional value over pleural fluid cytology. **Methods** This study was carried out on 44 adult patients (24 males and 20 females) with exudative pleural effusion divided into three groups; malignant pleural mesothelioma (MPM) (n=16), pleural metastases of carcinomas (Mets) (n=13), and non malignant pleural effusions (n=15). Mesothelin levels were measured in serum & pleural fluid by enzyme- linked immunosorbant assay (ELISA). **Results** Diagnosis was confirmed by analysis of pleural fluid in 10 out of 44 patients (22.7%), by needle biopsy in 12 out of 34 patients (35.3%), while thoracoscopy had a diagnostic yield of 90.3%. Patients with MPM had significantly higher pleural effusion mesothelin level (107.01±44.16 ng/ml) than those with metastatic effusion of carcinoma (34.88±30.88 ng/ml) or non malignant pleural effusion (38.08±18.99 ng/ml). Serum mesothelin showed similar trends. Pleural fluid & serum mesothelin levels positively correlate in patients with MPM. The optimal discrimination of patients with MPM from non neoplastic group could be performed at a cut-off point of pleural fluid mesothelin 51.95 ng/ml with area under the curve (AUC) of 0.97 (sensitivity 94%, specificity 100%) and at a cut-off point of serum mesothelin 49.4 ng/ml with AUC of 0.98 and the same (sensitivity 94%, specificity 100%).Pleural & serum mesothelin had an accuracy of 97% in distinguishing between MPM and effusion of non neoplastic origin. **Conclusions** The pleural mesothelin is useful adjunction to thoracoscopy in the diagnosis of MPM and correlates with serum mesothelin in cases of MPM. Mesothelin can distinguish between MPM and benign pleural effusion. Pleural fluid mesothelin has better diagnostic accuracy than the serum mesothelin in cases of MPM and Mets.
[Rana El-Helbawy; Nesreen El-Helbawy; Safaa Tayel and Ehab Shaltot. **Diagnostic Utility of Thoracoscopy & Mesothelin in Malignant Mesothelioma**. Journal of American Science 2012; 8(8):794 -803]. (ISSN: 1545-1003). http://www.americanscience.org. 120

Key words: Pleural effusion, malignant pleural mesothelioma, Mesothelin, SMRP, thoracoscope,

1. Introduction:

Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin associated with asbestos exposure [(1)]. It strikes about 2,500 people in the U.S. each year. But its rarity-and its tendency to mimic other lung related diseases can make mesothelioma hard to diagnose [(2)]. Diagnosis begins with a review of the patient's medical history, including any history of asbestos exposure. Complete physical examination may be performed, including An X-ray of the chest and lung function test, CT scan or MRI may also be useful[(3)]. Analysis of pleural fluid yields a confirmed diagnosis in a relatively small percentage of MPM patients, and needle biopsy offers only slightly better results. Medical thoracoscopy is recommended in the investigation of patients with MPM, especially when pleural fluid analysis is uninformative. The procedure of choice is the VATS (video-assisted thoracoscopy) procedure, which has a diagnostic yield of >95%, and allows for pleural biopsy, drainage of fluid and pleurodesis[(4).]

Early detection is critical to survival with mesothelioma, the use of pleural or blood –based biomarkers might allow detection of MPM at an early stage. Tumor markers offer an attractive means of diagnosis, being less expensive and less invasive [(5)].

Mesothelin is a tumour differentiation antigen that is normally present on the mesothelial cells lining the pleura. Mesothelin is an epithelial marker highly expressed by cancer cells from diverse origins, including ovarian or pancreatic adenocarcinomas, and mesotheliomas [(6).] Human mesothelin is made as a 69 kDa polypeptide with a hydrophobic sequence at the carboxyl end that is removed and replaced by phosphatidylinositol. [(7).] After glycosylation at one or more of its four putative glycosylation sites, it is probably cleaved by the protease furin to yield a 32 kDa soluble protein

called megakaryocyte potentiating factor (MPF) and a 40 kDa cell membrane bound protein called mesothelin[8]. The measurement of mesothelin represents a cheaper, less invasive technique that can provide support for clinical judgment in patients with mesothelioma [9].

2. Patients and Methods:

Eighty-four consecutive patients with pleural effusion were recruited from Chest Department in Minoufiya University hospital, Egypt. During the period from July 2011 to April 2012. The most important step in narrowing the differential diagnosis was to distinguish a transudate from an exudate. To do this, Light's original criteria (ratio of pleural fluid/serum protein >0.5, ratio of pleural fluid/serum lactate dehydrogenase (LDH) >0.6 or pleural fluid LDH more than two-thirds of the upper limit of normal serum value) were appropriate. To discriminate true transudates from pseudo-exudates measurement of the pleural protein gradient or the pleural fluid albumin gradient can be applied: if serum protein level minus pleural protein level is >3.1 g/dL, or serum albumin level minus pleural albumin level is >1.2 g/dL, it was transudate. In case of possible misclassification by the use of Light's criteria, Heffner's criteria (pleural fluid LDH more than 0.45 of the upper limit of normal serum value, Pleural fluid protein more than 2.9g %, and pleural fluid cholesterol level more than 45mg%) may appear to be a better tool to differentiate a transudate from exudates. Searching to define diagnostic criteria for exudative pleural effusion all patients had undergone diagnostic thoracentesis and venipuncture with measurement of total proteins, LDH and albumin levels in serum and chemical quantification of pleural fluid LDH, TP, albumin, glucose, bilirubin and cholesterol. Blood sampling was done at the same time that thoracocentesis was performed. In patients submitted to more than one thoracentesis during the hospitalization period, only the results of the first tap were considered. In Forty- four out of 84 cases of a proven exudative pleural effusion, cytopathological and bacteriological examination of pleural fluid were done. With non-conclusive cytology after thoracocentesis, an additional procedure to obtain pleural histology tissue was the next step. Percutanous access to the pleural space using the Abram's needle and multiple specimens technique without repetition of the biopsy procedure were undergone in 34 out of 44 subjects. Medical thoracoscope was operated on 22 cases in which the prior diagnosis based on closed pleural biopsy (CPB) was nonspecific inflammation. Thoracoscopy also performed for precise staging, and specified the histological type in cases of MPM or Metastatic carcinoma that were diagnosed with thoracentesis or CPB. An extended video-assisted thoracoscopic surgery procedure is indicated in case of difficult thoracoscopy with adhesions and layers of fibrin. Tuberculous pleuritis was defined as one or more of the following criteria: Positive culture of M. tuberculosis; visualization of acid-fast bacilli from a clinical specimen including histopathology. Para-pneumonic effusion (PPE) was diagnosed if the pleural effusion was accompanied by community-acquired pneumonia, but the effusion was not grossly purulent, no bacteria were detected on the Gram stain or culture of the pleural fluid, also pleural fluid PH and glucose exceeded 7.2 and 60mg/dl respectively. Eligibility criteria included confirmed diagnosis of exudative pleural effusion. All patients provided informed written consent. Subjects are ineligible to participate in this study if any of the following criteria are met: Lack of the pleural space, transudative pleural effusion, prior treatment for MPM cases, evidence of active serious systemic diseases including renal, hepatic, or cardiac diseases, uncorrected coagulopathy, and hemodynamic instability.

Collection of blood samples and pleural effusion fluid ; Ten ml of whole blood and 10 ml of pleural fluid were withdrawn from all subjects into plain tubes and centrifuged at 3000 rpm for 5 min. serum and pleural fluid clear supernatant were isolated and kept in Eppindorfs at -20 ^{0}C until analysis.

Measurement of mesothelin**:** The mesothelin concentrations in pleural fluid and serum were determined using ELISA. The test required 2–3 hrs and only 20 µL of serum or pleural fluid. The assay used two monoclonal antibodies, one directed against part of the mesothelin sequence of mesothelin as the capture antibody, and the second, recognized part of the mesothelin sequence as the tracer antibody. During incubation, both antibodies reacted with mesothelin in a sandwich-like manner. After several washing procedures, the tracer remaining in the test tube was measured using a luminometer; the intensity of the luminescent signal was directly proportional to the mesothelin concentration of the serum or pleural fluid sample. The mesothelin concentration was quantified by comparison with a standard curve.

Measurement of serum and pleural laboratory parameters: Serum & pleural fluid LDH levels were determined by kinetic colorimetric method (Biosystems,Spain)[10]. Serum & fluid albumen levels measured by using brom cresol green colorimetric method (Diamond diagnostic ,Germany)[11].Serum & pleural fluid total protein levels were measured by colormetric method (Diamond diagnostic ,Germany)[12].Pleural fluid

glucose levels were measured by GOD-POD liquid colorimetric method (Diamond diagnostic ,Germany)[13]. Pleural fluid cholesterol levels were measured by colorimetric method (Spinreact, Spain)[14].Pleural fluid bilirubin levels were measured by colorimetric method (Diamond diagnostic ,Germany)[15].

Statistical methodology:

The data collected were tabulated & analyzed by SPSS (statistical package for the social science software) statistical package version 11 on IBM compatible computer. Quantitative data were expressed as mean & standard deviation (X±SD) and analyzed by applying student t-test for comparison of two groups of normally distributed variables and mann whiteny U test for non normally distributed ones. ANOVA test for analysis of variance (f-test) was used for comparison of more than two groups of normally distributed variables; and krauskal wallis test was used for comparison of more than two groups of non normally distributed variables. For comparison of the same group with normally distributed variables before and after intervention, paired t-test was used where wilcoxon signed ranks test was used for comparison of the same group with non-normally distributed variables before and after intervention. Qualitative data were expressed as number and percentage (No & %) and analyzed by applying chi-square test (X2). Whenever the expected values in one or more of the cells in a 2x2 tables was less than 5, fisher exact test was used instead. To compare between two proportions, z-test was used. Pearson correlation (r) was used to detect association between quantitative variables, while spearman correlation was used to detect association between qualitative and quantitative variables . Logistic regression test was used to detect relation between independent variable and another dependant ones. The ROC (receiver operating characteristic) curve was used to detect the cutoff value with highest sensitivity and specificity. Sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy were calculated according to the following formula:

-Sensitivity = a/(a+c)
-Specificity = d/(b+d)
-Accuracy = (a+d)/(a+b+c+d)
-Negative value = d/(c+d)
-Positive predictive value = a/(a+b)

Where a = true positive cases; b = false positive cases; c = false negative cases; d = true negative cases. All these tests were used as tests of significance at $P<0.05$.[16]

3. Results:

The characteristic of the patients from whom serum or pleural fluid were obtained are seen in Table 1. Cytopathological examination of pleural fluid added to clinical and radiological results approached diagnosis in 10 out of 44 patients (22.7%). There were 3 cases of MPM, and 7 cases of pleural metastasis of carcinoma (Mets). Percutanous access to the pleural space using the Abrams needle proved definite histological diagnoses in 12 out of 34 subjects (35.3%). Among these 12 patients there were 6cases of tuberculous pleural effusion, 4cases of Mets and 2cases of MPM. Medical thoracoscope was operated on 22 cases that were undiagnosed after thoracentesis or closed pleural biopsy. Definite histological diagnosis was reached in 20 out of 22 cases (90.9%). There were 9 cases of MPM, 2 cases of Mets, 2cases of tuberculous pleural effusion, and 7 cases of parapeumonic effusion (PPE). In 2 and 3 patients in which the CPB and thoracentesis diagnosis respectively was MPM, thoracoscopy, performed for precise staging, challenged the diagnosis in these cases. In 4 and 6 patients of carcinoma diagnosed by CPB and thoracentesis respectively, thoracoscopy specified the histologic type in theses cases. The duration of the drainage by intercostals tube after thoracoscopy was in range between 3 and 15 days (mean = 7.54 and SD = ± 3.65). Thoracoscopic diagnoses were found to be erroneous in 2 of 22 cases, mainly owing to pleural adhesions that limited access to the pleural cavity. They were referred to surgeon and underwent VATS procedure, and they were diagnosed as MPM. There was one thoracoscopy-related death, one case of sepsis, and two cases of empyema. (The case of severe sepsis was due to a coagulase-negative *Staphylococcus aureus*. The empyemas were due to methicillin-resistant *S aureus* (one cases), *Pseudomonas aeruginosa(* another one case). The thoracoscopy-related death involved a 63-year-old man with no history of malignancy, in whom prior CPB yielded no pleural tissue; bleeding occurred during thoracoscopy and could not be controlled despite immediate conversion to open thoracotomy. The final diagnoses were classified into three groups: 16 cases of MPM (their histological subtypes were (8 epitheliod, and 8 biphasic subtypes), There were nine stage IV, four stage III, two stage II, and one stage I patients) , 13 cases of pleural metastases of carcinomas (8 cases of non small cell lung cancer,3 cases of breast cancer and 2 cases of lymphoma) and 15 patients with non malignant pleural effusion (8 tuberculous cases and 7 cases of PPE). There was significant statistical difference in different studied groups as regard age and gender Table (1). The prevalence of asbestos exposure was significantly higher among MPM than other groups Table (1). The difference in Pleural LDH, TP, albumin, and total bilirubin between Mets

group and non neoplastic group was significant. Also pleural albumin and serum LDH were significantly different in patients with MPM compared with non neoplastic effusion group Table (1). Patients with MPM had significantly higher pleural effusion mesothelin level (107.01±44.16 ng/ml) than those with metastatic effusion of carcinoma (34.88±30.88 ng/ml) or non malignant pleural effusion (38.08±18.99 ng/ml). Serum mesothelin showed similar trends. The mean serum mesothelin values for the 16 patients with MPM (102.41±43.24 ng/ml) was significantly higher than those for patients with Mets group (32.06±289 ng/ml) or from non-malignant pleural effusion group (35.16±17.65ng/ml) Table(2). The optimal discrimination of patients with MPM from Mets group could be performed at a cut-off point of pleural fluid mesothelin 50.45ng/ml with AUC of 0.91and (sensitivity 94%, specificity 77%).and at a cut-off point of serum mesothelin (54.4 ng/ml), with area under the curve (AUC) of 0.91 (sensitivity 88%, specificity 69%). The pleural and serum mesothelin had reasonable PPV 79% and 83% and the test was found to pick up the diseases with (86% and 79%) accuracy for pleural and serum mesothelin respectively (Table2): Using a pleural mesothelin cut-off of 50.45 ng/ml, 15 out of 16 patients (93.8 %) were positive in MPM group versus 3 out of 13 patients (23.1%) in the Mets group. The optimal discrimination of patients with MPM from non neoplastic group could be performed at a cut-off point of pleural fluid mesothelin 51.95 ng/ml with area under the curve (AUC) of 0.97 (sensitivity 94%, specificity 100%) and at a cut-off point of serum mesothelin 49.4 ng/ml with AUC of 0.98 and the same (sensitivity 94%, specificity 100%). Pleural and serum mesothelin had an accuracy of 97% in distinguishing between MPM and effusion of non neoplastic origin. Serum and pleural mesothelin (128.24±35.58 ng/ml and 132.99±35.02 ng/ml) showed significantly higher levels in epithelial mesothelioma compared with serum and pleural mesothelin level in biphasic type (76.58±34.87 and 81.03±37.54) respectively Table (4). However, the optimal discrimination of epithelial mesothelioma from biphasic subtype could be performed at a cut-off point of pleural fluid mesothelin 82.76ng/ml, AUC of 0.86 (sensitivity 100%%, specificity 75%) and at cut –off point of serum mesothelin 80.5ng/ml with the same sensitivity and specificity (Table5). Mesothelin was expressed in 8 out of 8 (100%) of epitheloid subtype and 2 out of 8 (25%) of the biphasic subtype of mesothelioma.

Table (1): Comparison between three studied groups regarding all variables

Variables	Groups			ANOVA test	*P* value
	Malignant Pleural Mesothelioma (MPM) (n=16)	Metastatic Pleural Malignancy (n=13)	Non Malignant pleural effusion (n=15)		
	Mean ± SD	Mean ± SD	Mean ± SD		
Age	60.75±12.68	47.62±16.18	52.7935±14.08	4.71[*1]	<0.05
Pleural PH	8.03±0.38	7.97±0.28	7.88±0.51	0.59	>0.05
Pleural LDH U/L	482.31±403.32	366.85±259.18	574.67±305.13	3.13*	>0.05
Pleural Total protein gm/dL	4.71±2.48	3.94±1.31	4.21±1.66	6.22*[3]	<0.05
Pleural Albumin gm/dL	2.83±1.0	2.77±0.74	59.9±54.9	9.16*[2,3]	<0.05
Pleural Total bilirubin mg/dL	0.55±0.29	0.61±0.33	0.38±0.09	6.85*[3]	<0.05
Pleural Cholesterol mg/dL	62.31±29.88	56.0±15.34	50.4±17.72	0.93*	>0.05
Pleural Glucose mg/dl	79.5±37.31	71.31±23.76	66.27±35.43	1.04*	>0.05
Serum LDH U/L	715.13±376.81	573.62±289.92	517.27±525.51	5.55*[2]	<0.05
Serum Total protein gm/dL	8.29±1.64	7.86±1.44	7.75±0.76	0.51	>0.05
Serum Albumin gm/dL	4.42±0.91	4.32±0.95	4.07±0.7	0.52	>0.05
Serum mesothelin ng/ml	102.41±43.24	32.06±28.85	35.16±17.65	24.67*[1,2]	<0.001
Pleural mesothelin ng/ml	107.01±44.16	34.88±30.88	38.08±18.99	23.26*[1,2]	<0.001
Gender Male Female	13 81.3% 3 18.7 %	3 23.1% 10 76.9%	8 53.3% 7 46.7%	9.8	<0.05
Smoking(No-%) yes No	10 62.5% 6 37.5%	4 30.8% 9 69.2%	6 40 9 60	3.19	>0.05
Asbestos exposure Yes No	10 62.5% 6 37.5%	0 0 13 100%	0 0 15 100%	22.65	<0.001

* kruskal wallis test Chi-square (x_2)test

Significant difference is between MPM and Metastatic

Significant difference is between MPM and non-malignant

Significant difference is between metastatic and non-malignant

The prevalence of asbestos exposure & gender are significantly higher among MPM than other groups (*p*. <0.001).

Table (2): Comparison between Mesothelioma phenotypes (Epithelial & Biphasic) regarding studied variables

Variables	Epithelial Malignant Pleural Mesothelioma (n=8)	Biphasic Malignant Pleural Mesothelioma (n=8)	t-Test	P- value
	Mean ± SD	Mean ± SD		
Age	67.88±11.51	53.63±9.79	2.67	<0.05
Gender Male Female	7 87.5% 1 12.5 %	6 75% 2 25%	0.14	>0.05
Serum mesothelin	128.24±35.58	76.58±34.87	2.42*	<0.05
Pleural mesothelin	132.99±35.02	81.03±37.54	2.42*	<0.05

*Mann Whitney test Chi-square (x_2) test

Table (3): validity of mesothelin in detecting cases of MPM

	Cut off value (ng/ml)	AUC	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
Serum mesothelin	54.4	0.91	88%	69%	79	78	82
Pleural mesothelin	50.45	0.92	94%	77%	86%	83	91
Validity of pleural and serum mesothelin in detecting cases of mesothelioma in relation to metastatic pleural effusion							
Serum mesothelin	49.4	0.98	94%	100%	97%	100%	94%
Pleural mesothelin	51.95	0.97	94%	100%	97%	100%	94%
Validity of pleural and serum mesothelin in detecting cases of mesothelioma in relation to non malignant cases							
Serum mesothelin	80.5	0.86	100%	75%	88%	80%	100%
Pleural mesothelin	82.76	0.86	100%	75%	88%	80%	100%
Validity of pleural and serum mesothelin in detecting cases of epithelial mesothelioma in relation to biphasic Mesothelioma							

AUC: Area under the curve PPV: Positive predictive value NPV: Negative predictive value

Table (4): Pearson correlation between pleural and serum mesothelin

	Serum mesothelin					
Pl. mesothelin	**MPM (n=16)**		**Mets (n=13)**		**Non malignant PE (n=15)**	
	r	p-value	r	p-value	r	p-value
	.998	<0.001	.999	<0.001	.997	<0.001

When correlating pleural fluid & serum mesothelin levels, a highly significant positive correlation was found in patients with MPM (r=.998, p<0.001), Mets (r=.999, p <0.001) and Non malignant PE (r=.997, p =<0.001).

Table (5): Validity of serum or pleural mesothelin & cytology in detecting positive cases of MPM in relation to medical & surgical thoracoscopy results

Validity	Pleural mesothelin	Serum mesothelin	Cytology
Sensitivity	94%	88%	19
Specificity	100%	100%	100
Accuracy	98%	95%	70
PPV	100%	100%	100
NPV	97%	93%	68

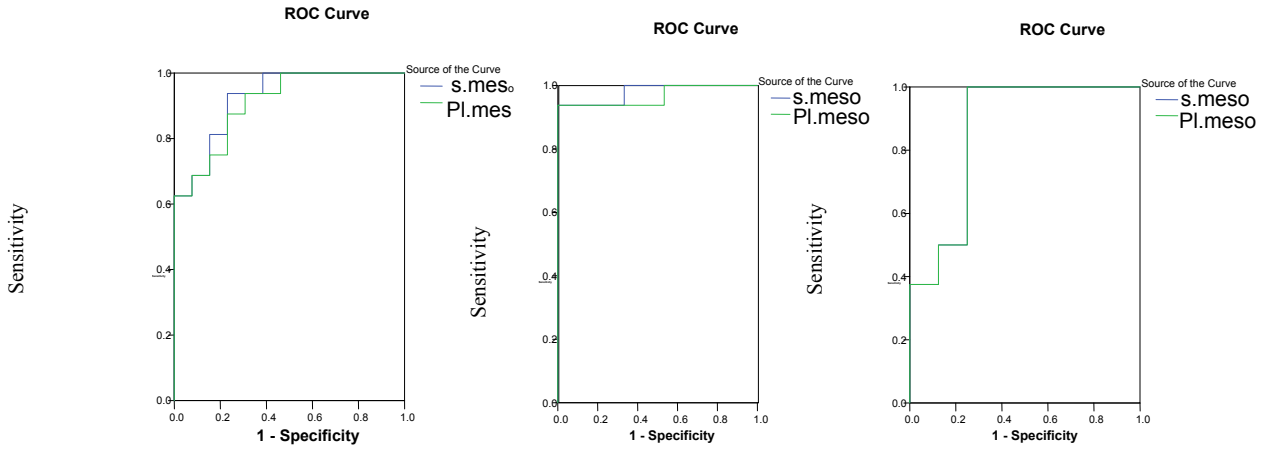

Figure 1 **Figure 2** **Figure 3**

Figure 1: validity of s.meso and pl.meso in detecting cases of MPM from metastatic PE
Figure 2: Validity of s .meso and pl.meso in detecting cases of MPM from non malignant PE
Figure 3: Validity of s.meso and pl.meso in detecting cases of Epith from Biphasic MPM

4. Discussion:

Malignant pleural mesothelioma is a relatively rare cancer, but its incidence is rapidly increasing on a global scale. Soluble mesothelin related peptide (SMRP) is currently biomarker of mesothelioma[17]. The goal of this study was to evaluate the diagnostic utility of mesothelin quantification in serum or pleural fluid as useful adjunction to thoracoscopy in MPM. We assessed its additional value over pleural fluid cytology in diagnosis of MPM. In this work, the differential diagnostic aspects of transudate versus exudate were further elaborated. Our study demonstrated that patients with MPM had significantly higher pleural effusion mesothelin level (107.01±44.16 ng/ml) than a population with non malignant pleural effusion (38.08±18.99 ng/ml)or metastatic effusion of carcinoma(34.88±30.88 ng/ml). and similar results were found in serum mesothelin levels . Helen and colleagues ([18] reported similar results in 167 patients presented with pleural effusion. Pleural fluid mesothelin concentration where significantly higher in patients with mesothelioma (n=24) vs. those with metastatic carcinomas (n=67) and benign effusion (n=75), median mesothelin concentration were 40.3 nM, 6.1 nM and 3.7 nM, respectively` (P<.0001). Our results was also compatible with Robinson *et al.,* [19] they measured the pleural fluid mesothelin concentration in 45MPM patients, 24individuals with non-malignant pleural effusions and 29 individuals with lung cancer involving malignant pleural effusion. They demonstrated that patients with MPM had significantly higher pleural effusion mesothelin levels than a population with non malignant pleuritis or lung cancer involving malignant pleural effusion. Our current results matches the results of workers[7] in 192 patients with exudative pleural effusion where mesothelin levels were measured in effusion and serum samples ,they found a significantly higher level of mesothelin in serum and effusion of patients with MPM, with specificity of 98% the assay had sensitivity of 67% comparing patients with mesothelioma and those with effusions of non neoplastic origin. In the present study The optimal discrimination of patients with MPM from non neoplastic group could be performed at a cut-off point of pleural fluid mesothelin 51.95 ng/ml with area under the curve (AUC) of 0.97 (sensitivity 94%, specificity 100%) and at a cut-off point of serum mesothelin 49.4 ng/ml with AUC of 0.98 and the same (sensitivity 94%, specificity 100%). SMRP had been studied by three independent groups on three different continents. The most promising data were originally presented by Robinson and colleagues [19] in which sensitivity of 84% was presented with close to a100% specificity using quantitative form of the MESOMARK assay. Multiinstituational studies performed by Scherpereel and colleagues (20), patients were recruited to donate serum with or without pleural effusion who either had mesothelioma, had pleural biopsy for lesion that were associated with asbestos exposure, or had a diagnosis of pleural metastasis. They were demonstrating AUCs between 0.693 to 0.872 for discriminating MPM patients from non-MPM cancer patients or from individual with asbestos related lesion. Cutoffs were defined in the study as being

optimal at 0.93nM/L for distinguishing non-malignant pleural effusion from MPM with sensitivity of 80% and specifity of 82.6% .On the other hand , the research team in their meta-analysis of patients in 16 studies on the value of serum mesothelin as marker of mesothelioma, included data on 4,491 patients ,including 1,026 patients with MPM and various control group found that the sensitivities and specificities of mesothelin in the different studies ranged widely from 19% to 68% and 88% to 100%, respectively. This heterogeneity can be explained by differences in study population, because type of control group, mesothelioma stage, and histologic subtype significantly affected the diagnostic accuracy [21-23]. In our work the optimal discrimination of epithelial mesothelioma from biphasic subtype could be performed at a cut-off point of pleural fluid mesothelin 82.76ng/ml, AUC of 0.86 (sensitivity 100%, specificity 75%) and at cut–off point of serum mesothelin 80.5ng/ml with the same sensitivity and specificity. Mesothelin was expressed in 8 out of 8 (100%) of epitheloid subtype and 2 out of 8 (25%) of the biphasic subtype of mesothelioma. Which was compatible with the reports[24]. demonstrating that mesothelin was expressed in the epitheliod subtypes & the epitheliod part of the biphasic subtype and it wasn't found in the sarcomatous part of the biphasic type, making it poor biomarker for other types of mesothelioma. On the contrary it has been reported [25] that no statistically significant differences between pleural effusion mesothelin levels of the MPM histological groups.

The differences between our results and their might be explicable by differences in study population, and histologic subtype. In the present work pleural fluid & serum mesothelin levels positively correlate with MPM: Using a pleural mesothelin cut-off of 50.45 ng/ml, 15 out of 16 patients (93.8 %) were positive in MPM group versus 3 out of 13 patients (23.1%) in the Mets group. mesothelin could reliably define mesothelioma from metastatic effusion of carcinoma in almost of Mets group except in three cases. which is compatible with reports[26] they found that in patients mesothelin level at a cut–off of 20nM demonstrating 12 false positive results with metastatic adenocarcinomas accounting for over 90% of these cases. This could be explainable by the following data, the present study included eight patients with pulmonary and breast adenocarcinomas and mesothelin was found to be expressed at low level by a variety of adenocarcinomas, including pulmonary, breast and colorectal. On the other hand, our study revealed that mesothelin level was not elevated in stage I biphasic type of MPM. This could be explainable by the facts that Mesomark is not especially sensitive to early–stage mesothelioma or to type other than epitheliod variety [27]. Luo and his colleagues [23] demonstrated that the use of mesothelin in early diagnosis of early stages of MPM revealed low sensitivity, mesothelin was evaluated by differentiating 217 patients with stage I or II epithelioid and biphasic mesothelioma from 1,612 symptomatic or high-risk controls. The resulting area under the ROC curve was 0.77 (95% CI, 0.73 to 0.81). At 95% specificity, mesothelin displayed a sensitivity of 32%.

Also Elliot and his colleagues [27] reported that among stage III MPM patients (n=72), high mesothelin expression was observed in 26% of T2 tumors and 51% of T3 tumors. In the present work pleural fluid & serum mesothelin levels positively correlate with MPM(Table:4). On the other hand Harvey *et al.,*[28] study revealed that mesothelin tumor cell expression and serum levels do not strictly correlate ,although mesothelin serum levels are more frequently increased in MPM whose tumor expressed mesothelin, some patients without detectable mesothelin expression on tumor cell have elevated titer of serum mesothelin, the absence of detectable SMRP in pleural fluid of MPM could be due to technical artifact, or alternatively Elliot and his collage[27] reported that , in some cases SMRP might be released from normal mesothelial cell that are in contact with tumor microenvironment ,such as pleural effusion or peritoneal fluid.

In the present work, pleural fluid mesothelin level has been demonstrated to provide additional diagnostic value relative to cytological examination .The diagnostic yield of pleural fluid cytology (one specimen only and one pathological technique, cell spread) was 10 out of 44 cases (22.7%). However, cytopathological examination added to clinical and radiological results could reached diagnosis of MPM in 3 out of 16 cases (18.75%) of MPM .Mesothelin measurement was superior to cytological examination in the diagnosis and exclusion of mesothelioma (sensitivity, 94 vs.19%,specificity ,100% for both, accuracy, 94 vs., 19%) respectively. There are widely discordant views on the usefulness of pleural fluid cytology for the diagnosis of mesothelioma [29-30]. One can stated that the pleural fluid cytology is almost always negative in cases of mesotheliomas[30]. Current opinion favors the notion that a clear and unequivocal diagnosis of mesothelioma can not be established from examination of effusion fluid in isolation, because such sample do not allow histological assessment of invasion [29]. On the other hand, some mesotheliomas with an epithelial component are characterized by numerous atypical epithelioid cells, often with

squamoid cells, and cytoplasmic vacuoles. In another work [39], the researcher obtained high diagnostic yield of thoracentesis in MPM, possible reasons for this high yield included the use of multiple techniques: cell spreads, membrane filters and cell block. Also, was attributed to the examination of multiple specimens, as they obtained 53% positive results in the first cytology and 20% positive results in subsequent examination [30]. However more recent reports points out that the mesothelin test may not be as helpful in patients who have the rare sarcomatoid type of MPM to achieve 37% diagnostic rate. In the present work diagnostic yield of Medical thoracoscope was 20 out of 22 cases (90.3%) of exudative pleural effusion. few minor complication were faced in the course of this study in 3 cases, they were requiring medical supervision only. Our findings agree with *Harris,* and coworkers [35-36], used thoracoscope to explore the pleural cavity in 130 patients with malignant pleural effusion. The overall diagnostic accuracy was 91.5%. Their reports on the complications were minimal in the form of transient fever, local subcutaneous emphysema in 6 cases and tumor seeding in 4 patients. François and colleagues [34] in a 6-year retrospective study of 154 patients medical thoracoscopies were performed, 149 patients (96.7%) were diagnosed. Several studies [31-33], suggest that medical thoracoscopy increases the diagnostic yield in patients with malignant pleural disease when thoracocentesis and CPB are non-diagnostic. However Practice varies from one tertiary center to another [37-38]. In the present work a number of biochemical markers of pleural effusion have been identified, though the validity of their measurement in pleural fluid remains unclear [40].

Limitation to this study includes the small patient numbers.

Conclusion:

Serum SMRP have the advantage that it represent even less invasive studies than thoracentesis or serosal –surface biopsies , but it is beset with problems of specificity for mesothelioma and insensitivity for early stage disease and non epitheliod subtypes of mesothelioma . At present it cannot replace conventional cytological and biopsy diagnosis of mesothelioma, except as probability markers in unusual circumstances, when biopsy is contraindicated. A positive blood test for mesothelin at high specificity threshold is a strong incentive to urge further diagnostic steps. A useful recommendation to come out of this study is that whenever effusion fluid is sampled by a patient for whom mesothelioma falls within the differential diagnosis, at least 100-200 mL of effusion fluid should be submitted for cytological investigation, whenever possible. Cytocentrifugation can maximize the cell sample and aid in establishing diagnosis of mesothelioma. In addition, cytocentrifugation can produce a pellet from which cell- block sections can be prepared, with appropriate IHC studies. Pleural fluid mesothelin provides a valuable adjunct in the diagnostic assessment of patient presented with malignant pleural effusion, specially when cytological examination is not definitive. Serum or pleural mesothelin are useful adjunctions to medical thoracoscope in diagnosis of MPM. medical thoracoscope is safe and accurate diagnostic procedure which can be performed under local anesthesia with minimal or no complication .

Corresponding author
Rana El-Helbawy
Chest Department, Faculty of Medicine, Minoufiya University, Egypt

Reference

1. Fukuoka K, Tanaka F, Tsujimura T, *et al., (2011)*. Exploratory study in detection of markers for diagnosing early stage malignant mesothelioma. Nihon Eiseigaku Zasshi; 66(3): 553-7.
2. Kevin H, Krstiaan N, Joel T, *et al., (2010)*. .Diagnostic Performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. Am. J Respir. Crit. Care Med. 181 (6): 620-625.
3. Treasure T, Sedrakyan A. (2004). Pleural mesothelioma: little evidence, still time to do trials. Lancet; 1; 364(9440):1183-5.
4. Medford AR, Agrawal S, Bennett JA, *et al.* (2010). Thoracoscopic ultrasound prior to medical thoracoscopy improved pleural access and predicts fibrous septation. Respirology.; 15:804.
5. Ordonez NG. (2003). Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol.; 27:1418–28.
6. Argani P, Iacobuzio-D C, Ryu B, *et al.* (2001). Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res.; 7:3862–8.
7. Raffit Hassan, Alan T. Remaley, Maureen L. Sampson, *et al.* **(2006)**. Detection and quantitation of serum mesothelin, a tumor marker for patients with mesothelioma and Ovarian Cancer. Clin Cancer Res.**;** 12: **447.**

8. Hassan R, Bera T, Pastan I. (2004). Mesothelin: a new target for immunotherapy. Clin Cancer Res.; 10:3937–42.
9. Ho M, Hassan R, Zhang J, *et al.,* (2005). Humoral immune response to mesothelin in mesothelioma and ovarian cancer patients. Clin Cancer Res.; 11:3814–20.
10. Fridman RB, Young DC. (1997). Effects of disease on clinical laboratory test .3rd, AACC press, Washington, D.C.
11. Young DS, (2001). Effects of disease on clinical lab .Test, 4th ed. AACC.
12. Kaplan A, Szalbo J., 1983. Clinical Chemistry interpretation and techniques, 2nd Ed: 157.
13. Rifai, N.and War/nick, G.R., (2006). Lipids,lipoproteins , apolipoprotiens, and other cardiovascular risk factors. In: Tietz text book of chemistry and molecular biology . eds: Burits, C.A.,Ashwood, E.R. and Bruns ,D.E. 4thed.Philadelphia: WB Saunders Company , 948- 951.
14. Higgins, T., Beutler, E., Doumas, B.T., (2005). Hemoglobin , iron, and bilirubin . In: Tietz Texbook of clinical chemistry and Mollecular Diagnosis, 4th ed Burtis, C.A., Ashwood, E.R., Bruns, D.E. (eds.). Saunders, Philadelphia, 1195-1198.
15. Porcel J M, Vives M, Cao G, *et al.(*2009). Biomarker of infection for the differential diagnosis of pleural effusions. Eur Respir J; 34:1383-1389.
16. Morton RF (2001). Medical statistics. In: A study guide to epidemiology and biostatistics 5th ed. By: Morton RF, Hebel JR and McCarter RJ .Aspen publication, Gaithersburg, Maryland, 71-74.
17. Yamada S, Tabata C, Tabata R, Fukuoka K, Nakano T. (2011). Clinical significance of pleural effusion mesothelin in malignant pleural mesothelioma. Clin Chem Lab Med; 49(10):1721-6.
18. Helen E, Jennie E, Najib M, *et al.* (2010). Outcome of pateints with non specific pleuritis/ fibrosis on thoracoscopic pleural biopsies. Cardiothorac J Surg; 38(4):472-477.
19. Robinson BW, Creaney J, Lake R, *et al.* (2003). Mesothelin- family proteins and diagnosis of mesothelioma .Lancet; 362: 1612-1616.
20. Scherpereel A,Grigoriu B, Conti M, *et al.* (2007). Soluble mesothelin related peptide in the diagnosis of malignant pleural mesothelioma. Am J Respir.Crit Care Med;; 173:1155-60.
21. Hollevoet K, Nackaerts K, Thimpont J, *et al.* (2010). Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma .Am J Respir Crit Care Med.; 181:620-5.
22. Bidlingmaier, (2009). Identification of MCAM/CD 146 as the target antigen of a human monoclonal antibody that recognized both epitheliod and sarcomatoid types of Mesothelioma. Cancer Research; 1570-77.
23. Luo L,Shi HZ, Liang Ql, Jiang J *et al.* (2010): Diagnostic value of soluble mesothelin related peptide for malignant mesothelioma: Ameta-analysis .Respir Med 104:149-156.
24. Janette C, Roslyn J, Ian M .*et al.* (2010). Serum soluble mesothelin concentration in malignant pleural mesothelioma: relation ship to Tumor volume, clinical stage and changes in tumor burden .Clin Cancer Res.; 17(5); 1181-9.
25. Nowak AK,Francis Rj, Phillips MJ, *et al.* (2010). A novel prognostic model for malignant mesothelioma incorporating quantitative FDG-PET imaging with clinical parameters. Clin Cancer Res.; 16:2409-17.
26. Robinson BW, Musk AW,Lake RA (2005): Malignant mesothelioma .Lancet, 366:397-408..
27. ELLOT l,Christos C, Luis A, *et al. (2012).* Mesothelin over expression promotes mesothelioma cell invasion and MMP-9 secretion in an orthotopic mouse model and in epitheliod pleural mesothelioma patients. Clinical Cancer Research,**;** 18: **44.**
28. Harvey I.,Alla.,Micheal H., Jeffrey A. (2008). Soluble mesothelin-Related apeptide LEVEL Elevation in mesothelioma serum and Pleural effusions. Ann Thoracic Surg; 85:265-272.
29. Hollevoet K, Reitsma JB, Creaney J, *et al.* (2012). Serum mesothelin for diagnosing malignant pleural mesothelioma: A n individual patient data meta-analysis. J Clin Oncol; Epub head of print.
30. Onda M, Willingham M, Nagata S, *et al.* (2005). New monoclonal antibodies to mesothelin useful for immuno-histochemistry, fluorescence-activated cell sorting, western blotting and ELISA. Clin Cancer Res.; 11: 5840–6.
31. Cataldi M, Bianchi M. (1999). Video thoracoscopy in the diagnosis of pleural effusions. Chest; 54: 11-4.
32. Epstein DM, Kline LR, Albelda SM *et al.* (1987). Tuberculous pleural effusion Chest. 91: 106.
33. Feinsilver SH, Barrows AA, Braman SS. (1986). Fiberoptic bronchoscopy in pleural effusion of unknown origin. Chest; 90: 516.

34. François X., Kinan A, Jean B, Bruno H (2002). Diagnostic Value of Medical Thoracoscopy in Pleural Disease A 6-Year Retrospective Study. Chest;; 121(5): 1677-1683.
35. Harris, RJ, Kavuru, MS, Mehta, AC, *et al.* (1995). The impact of thoracoscopy on the management of pleural disease. Chest; 107:845-852
36. Harris, RJ, Kavuru, MS, Rice, TW, *et al.* (1995). The diagnostic and therapeutic utility of thoracoscopy: a review. Chest**;**108:828-841.
37. Janssen. (2010). Why you do or do not need thoracoscopy. Eur Respir Rev,; 19 (117): 213-216.
38. Michaud G, Berkowitz DM, Ernst A. (2010). Pleuroscopy for diagnosis and therapy for pleural effusion. Chest; 138: 1242.
39. Wheatley P, Yang B, Patsios D, *et al.* (2010). Soluble mesothelin related peptide and osteopontin as marker of response in malignant mesothelioma .J Clin Oncol.; 28: 3316-22.
40. Seyhan EC, Altin S, Cetinkaya E, *et al.* (2009). The importance of pleural fluid and serum NT-proBNP levels in differentiating pleural effusion due to heart failure from other causes of effusion. Intern Med; 48: 287–293.

7/7/2012