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Serum and Pleural Fluid Megakaryocyte Potentiating Factor: A Promising Marker for Malignant Pleural Mesothelioma Diagnosis

AMIRA I. MOUSTAFA, M.D.*; RANA K. ELSAYED, M.D.*; HEBAT-ALLAH MOSA AHMED, M.D.*; KHALED M. KAMEL, M.D.*; ALAA-ELDIN O. SHALABY, M.D.*; WALAA A. ATEYA, M.D.** and AHMED SAYED MAHMOUD, M.D.***

The Department of Chest, Clinical Pathology Department** and Cardiothoracic Department***, Faculty of Medicine, Cairo University*

Abstract

Background: The Malignant pleural mesothelioma (MPM) is a tumor which originates from the mesothelium, connected to asbestos exposure. Early diagnosis is a pivotal element in managing MPM. Serum biomarkers associated with these tumors, including megakaryocyte potentiating factors (MPF) and soluble mesothelin (SM), are crucial in managing MPM.

Aim of Study: We investigated the MPF levels in both malignant effusion (ME) and non-ME in serum and pleural Fluid (PF).

Patients and Methods: The study comprised 50 subjects, who were categorized into: 17 MPM subjects; 17 with non-mesothelioma ME; 16 with benign exudative effusion. Each underwent a comprehensive clinical examination and history-taking, along with standard laboratory testing, chest computed tomography (CT) scan, aspiration of PF, biopsy from the pleura, and collection of both PF and serum samples for measuring MPF levels.

Results: Compared with various diagnoses, such as inflammatory and metastatic effusions, MPF concentrations showed a significant increase within the pleural effusion (PE) and serum of MPM patients. The mean MPF concentrations detected in serum and PF from patients with epithelial and biphasic mesothelioma showed no significant difference. In serum and PF of lung cancer individuals, the mean MPF levels detected were not significantly different from those of individuals with malignancies of various origins in the metastatic group. In the tuberculous effusion group, the mean MPF levels detected did not significantly differ from those in the nonspecific inflammatory group within the inflammatory groupin PE and serum. Compared to the inflammatory type, the mean MPF levels in serum and PF exhibited a significant elevation in malignant PE.

Conclusion: MPF marker has the potential to be utilized in distinguishing MPM from PEs of inflammatory or metastatic nature.

Key Words: *Benign exudative effusion – Megakaryocyte potentiating factor – Non-mesothelioma malignant effusion – Malignant pleural mesothelioma.*

Correspondence to: Dr. Amira Ismail Moustafa, The Department of Chest, Faculty of Medicine, Cairo University

Introduction

MALIGNANT pleural mesothelioma (MPM), forms in the mesothelial lining, correlated with asbestos exposure **[1].**

In developing countries, the persistent use of asbestos and the protracted interval between asbestos exposure and the manifestation of tumor, MPM is anticipated to continue being a significant health issue worldwide for the foreseeable future **[2].**

Early diagnosis is a crucial element in managing MPM **[3].** Diagnosis may be postponed owing to non-specific presenting symptoms and challenges in distinguishing it from reactive mesothelium, benign pleural lesions, and adenocarcinoma **[4].**

With multiple histological forms, MPM might be challenging to differentiate from other malignancies. Epithelioid mesotheliomain particular closely resembles metastatic adenocarcinoma. in histological appearance, though immunohistochemistry offers a dependable method for differentiating mesothelioma from adenocarcinoma **[5].**

Serum biomarkers associated with tumors, including megakaryocyte potentiating factor (MPF)

and soluble mesothelin (SM), are involved in managing MPM. The precursor protein, the mesothelin gene product, is the source of these two markers and underwent cleavage into a soluble [31-kD N-terminal fraction] which is the MPF that is also named [N-ERC/mesothelin], and a membrane bound 40-kD C-terminal glycoprotein (mesothelin). Consequently, the membrane bound mesothelin is cleaved, discharging this fraction into the circulation as SM, called as "soluble mesothelin-related protein" or "C-ERC/mesothelin **[6]**". SM is now regarded as the standard serum biomarker indicator for MPM, utilizing the FDA-approved Mesomark ELISA kit **[7]**. The newly designed MPF ELISA kits necessitate additional validation **[8]**.

As a result, we conducted an analysis of the MPF concentration in the serum and the pleural Fluid (PF) from both malignant and non-malignant effusions (ME), to evaluate the diagnostic effectiveness of MPF for MPM and other exudative pleural effusion (PE).

Patients and Methods

Between January 2019 and October 2020, participants were enrolled from the Chest Department and outpatient clinic at Kasr Al-Ainy University Hospital. This study comprised 50 exudative PE patients. All subjects provided informed consent before enrollment. This study was conducted after obtaining Ethical Approval Committee letter, IRB: D-2-2019.

The study participants were individuals that were 16 years or older classified as experiencing exudative PE using chest computed tomography (CT), chest X-ray, clinical examination, and PE aspiration (including chemistry and cytology).

Exclusion criteria included patients receiving chemo- or radiotherapy, prior thoracic surgery related to malignant PE, and a diagnosis of transudative PE.

Each patient was exposed to a comprehensive history assessment, encompassing history of malignancy, occupation, residence, and symptoms, particularly chest pain and dyspnea.

Diagnostic procedures used are complete blood count (CBC), tests of liver functions and kidney functions, CT imaging of the chest, aspiration of PF for biochemistry and cytology, pleural biopsy through image guided techniques or thoracoscopy, and microscopic tissue examination.

Measurement of MPF concentrations in the human serum and PF:

By utilizing human MPF ELISA kits, the MPF concentrations in serum and PF were evaluated (Catalog #: I5863; Glory Science Co., Ltd).

Sample collection and storage:

Serum: Samples underwent centrifugation at approximately 3000×g for 10 minutes following the coagulation for 30 minutes in a serum separator tube. The samples taken were kept stored at either –20 or –80°C. The process of repetitive freeze-thaw cycles was circumvented.

PF: Samples were stored at –20 or –80°C, and Particulates were eliminated through centrifugation. The process of repetitive freeze-thaw cycles was ignored.

Assay procedure:

This process was executed per protocols. Measurement of optical density (O.D.) at 450nm was carried out with a micro-titer plate reader throughout a fifteen-minute timeframe.

Calculation of results:

The vertical (Y) axis was used to plot the average optical density (450nm) of each of the six measured standard concentrations against the corresponding measured concentrations on the horizontal (X) axis, and the results were measured in relation to a standard curve. This assay has a sensitivity of 1.0ng/ml.

Medical thoracoscopy and/or Ultrasound-guided pleural biopsy:

Histopathological microscopic examination:

The obtained biopsy specimens were subsequently fixed by formalin and submitted for histopathological microscopic examination. After being stained with hematoxylin and eosin, they were evaluated under light-microscopy.

Statistical methods:

All the data measured were formatted into tables and Minitab, 17.1.0.0 for Windows (Minitab Inc.-2013-Pennsylvania; USA) was utilized for:

1- Descriptive statistics:

Categorical data were introduced as numbers and percentages while continues data in mean and standard deviation.

2- Analytical statistics:

For comparing mean values between two independent groups, an independent *t*-test was deployed, whereas one-way ANOVA with the Turkey test was utilized for comparing means across multiple groups in this study.

3- Diagnostic utility and performance:

The (ROC) receiver operating characteristic curve was employed to assess the diagnostic efficacy of MPF for mesothelioma. An (AUC) area under the curve >0.9 is excellent; 0.8-0.9 is very good; 0.7-0.8 is good; and 0.6-0.7 is fair.

All the statistical tests used were conducted as two sided analyses, and a *p*-value below 0.05 was judged significant.

Results

1- *Patients features:*

Table (1) presents the demographic data of the patients, along with radiological outcomes and PE cytology for each group.

Our study findings have manifested that inflammatory PE patients experienced significantly declined mean age (p=0.03), unlike all the disease groups. For any specific condition, no significant connection between smoking and sex status (p=0.06 & p=0.62, correspondingly) (Table 1).

Patients with metastatic PE exhibited a significantly higher prevalence of pleural nodules (p=0.01), whereas patients with inflammatory effusion exhibited the highest incidence of septations (p=0.08) but not significant (Table 1).

Inflammatory effusion patients manifested significantly greatest surplus neutrophil count (p=0.03). Mesothelioma and metastatic PEs patients revealed significantly raised malignant cells unlike inflammatory effusions (p<0.001). The three diagnostic groups did not manifest significant differences in the remaining cytological data (Table 1).

Table (1): The Demographic features of the patients in the current study, PF cytology, and radiological findings for each group.

Factors	Inflammatory (n=16)		Pleural mesothelioma (n=17)		Metastatic (n=17)		*p*
	Mean	SD	Mean	SD	Mean	SD	
Sex:	N	%	N	%	N	%	
Male	13	81.25	7	41.18	9	52.94	
Female	3	18.75	10	58.82	8	47.06	0.06#
Age	46.13	18.03	59.24	13.23	56.94	13.6	0.03$
Status of smoking							
Smoker	9	56.25	7	41.18	8	41.18	
Non smoker	7	43.75	10	58.82	9	58.82	0.62#
Site:	N	%	N	%	N	%	
Unilateral	14	87.5	17	100	17	100	*
Bilateral	2	12.5	0	0	0	0	
Cytology of pleural fluid							
RBCs	7	43.75	9	52.94	7	41.18	0.77#
Excess neutrophils	4	25	1	5.88	0	0	0.03#
Excess lymphocytes	12	75	8	47.06	11	64.71	0.24#
Mesothelial cells	14	87.5	17	100	14	82.35	0.21#
Malignant cells	0	0	12	70.59	10	58.82	<0.001#
Radiological finding							
Septations	7	43.75	2	11.76	3	17.65	0.08#
Pleural nodules	0	0	3	17.65	6	35.29	0.01#
Pleural thickening	4	25	9	52.94	6	35.29	0.24#

Data are expressed as mean, SD, number and%. $: One-way ANOVA test.
#: Chi square test. *p*-value is considered significant if it is <0.05.

2- *MPF concentrations across various diagnostic subgroups and categories:*

In the PE and serum, compared to metastatic or inflammatory effusions patients, MPM patients manifested significantly elevated MPF levels (p<0.001; Table 2).

In the context of Mesothelioma, the results for the investigated parameters were consistently elevated in epithelial mesothelioma than in biphasic type; however, the differences were insignificant. Across metastatic different tumors and bronchogenic carcinoma, no significant differences in metastatic PE were observed. Regarding MPF levels in serum or fluid, MPF ratio, and age, no significant differences were observed with in the inflammatory subgroup (nonspecific inflammation and TB) across any of the evaluated parameters (Table 2).

Table (2): MPF measurements in a variety of subgroups and diagnoses.

Factors	Inflammatory (n=16)		Mesothelioma (n=17)		Mets (n=17)		*p*$
	Mean	SD	Mean	SD	Mean	SD	
Pleural MPF (ng/ml)	8.38	1.23	11.47	1.39	9.18	0.66	<0.001
Serum MPF (ng/ml)	8.13	1.34	10.77	1.30	9.38	1.56	<0.001
Ratio	1.04	0.14	1.07	0.13	1.00	0.15	0.31

Subgroups		Serum MPF		Fluid MPF		MPF ratio		Age	
Pathology	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Biphasic Mesothelioma	3	10.00	0.50	10.50	1.00	1.05	0.09	46.00	19.00
Epithelial Mesothelioma	14	10.93	1.37	11.68	1.40	1.08	0.14	62.07	10.53
Mesothelioma	17	10.77	1.30	11.47	1.39	1.07	0.13	59.24	13.23
p-value§		0.07		0.18		0.68		0.29	
Lung cancer	8	9.50	1.51	9.13	0.58	0.98	0.14	60.13	12.06
Mets	17	9.38	1.56	9.18	0.66	1.00	0.15	56.94	13.60
Others	9	9.28	1.68	9.22	0.76	1.02	0.17	54.11	14.95
p-value§		0.77		0.76		0.58		0.37	
Inflammatory	16	8.13	1.34	8.38	1.23	1.04	0.14	46.13	18.03
TB	8	7.69	1.16	8.13	0.79	1.07	0.10	39.25	18.27
Nonspecific	8	8.56	1.43	8.63	1.58	1.02	0.18	53.00	15.97
p-value§		0.2		0.44		0.51		0.13	

Data are expressed as mean, SD. $: One-way ANOVA test. *p* deemed significant if <0.05.

3- *Serum and fluid MPF level sinnon-ME and ME, metastatic and mesothelioma as well as mesothelioma and the inflammatory group:*

The Serum and the fluid MPF levels were significantly higher in malignant PE than in inflammatory one ($p<0.001$); and in mesothelioma than in metastatic effusion ($p=0.009$ and $p<0.001$, respectively) (Table 3).

4- *Diagnostic efficacy of serum and PE MPF in mesothelioma:*

The AUC values of 94% and 97% (both $p< 0.001$) indicated high diagnostic efficacy of MPF in serum and PE for mesothelioma. In addition, at cut-off values were above 8.75 and 9.25ng/mLfor serum and PE, respectively, the PPV, NPV, sensitivity, and specificity were 37, 100, 100, 63% for serum, and 54,100,100, and 81% for effusion, respectively.

Table (3): Serum and fluid MPF levels in non-ME and ME, metastatic cases and Mesothelioma, and inflammatory groups.

Groups	N	Serum MPF		Fluid MPF		MPF ratio	
		Mean	SD	Mean	SD	Mean	SD
Malignant	34	10.32	1.58	10.07	1.58	1.036	0.144
Inflammatory	16	8.38	1.23	8.13	1.34	1.043	0.139
p-value§		<0.001		<0.001		0.86	
Mets	17	9.18	0.66	9.38	1.56	1.00	0.15
Mesothelioma	17	11.47	1.39	10.76	1.30	1.07	0.13
p-value§		<0.001		0.009		0.13	
Inflammatory	16	8.38	1.23	8.13	1.34	1.04	0.14
Mesothelioma	17	11.47	1.39	10.76	1.30	1.07	0.13
p-value§		<0.001		<0.001		0.53	

- Data are demonstrated as mean SD and analyzed statistically with the independent *t*-test (§); with a *p*-value below 0.05 is deemed significant.

Table (4): Diagnostic efficacy assessment of serum and PEMPF in Mesothelioma.

Factors (ng/ml)	Cutoff level	Specificity	Sensitivity	NPV	PPV
Pleural fluid MPF	>9.25	81%	100%	100%	54%
Serum MPF	>8.75	63%	100%	100%	37%

PPV: Positive predictive value. NPV: Negative predictive value.

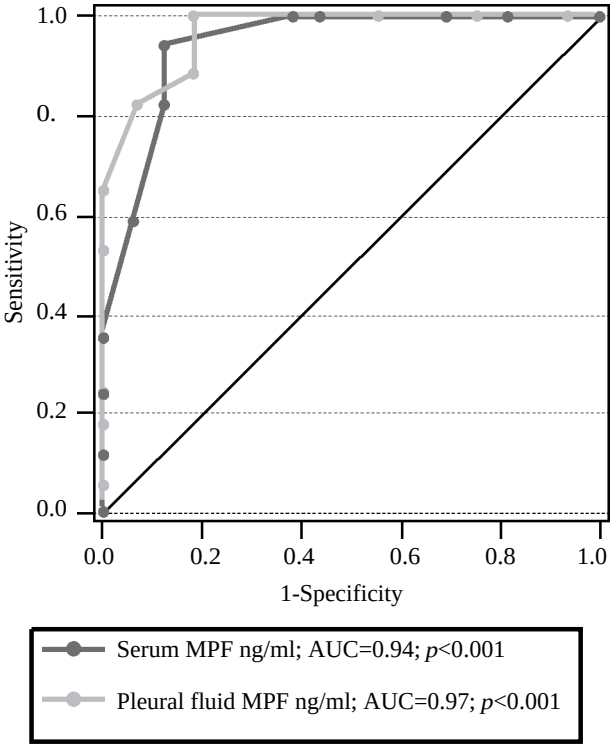

Fig. (1): ROC curve of MPF in serum and PF.

Discussion

Herein, we assessed the effectiveness of MPF in diagnosing various forms of exudative PE across three groups: MPM (n=17), benign exudative effusion (n=16), and non-mesothelioma ME (n=17).

Creaney et al. *[9]* evaluated MPF concentration in PF among 79 non-mesothelioma ME patients, 143 benign effusions patients, and 43 MPM patients. Herein, the mean (range) ages of these groups were 70 (37–96), 66 (19–96), and 71 (42–88), respectively. Here in, the mean ages of the three groups were all exceeded by these ages.

We observed no significant connections among smoking habits and sex for a specific diagnosis. Smoking did not experience a significant connection with MPM; however, exposure to asbestos *[10]* is a well-established cause.

Our findings on radiological presentation correlate with existing literature, indicating that CT scans cannot consistently distinguish MPM from metastatic pleural malignancy; nevertheless, MPM was demonstrated to have a higher incidence of mediastinal pleural involvement and circumferential pleural thickening *[11]*. Moreover, CT is not particularly proficient in distinguishing between MPM subtypes; however, the sarcomatoid subtype is more commonly associated with mediastinal pleural and interlobar fissure involvement, and ipsilateral volume loss *[12]*.

This study comprised patients diagnosed by pleural biopsy, with 44 cases (88%) undergoing thoracoscopic biopsy from the pleura with intercostal tube (ICT) insertion, whereas 6 (12%) had ultrasound-guided pleural biopsy.

The MPF levels demonstrated significant rise in PE and serum of MPM patients compared to metastatic or inflammatory effusions patients (p<0.001; Table 2).

Creaney et al. *[9]*, assessed MPF concentrations in PF, reported that MPM subjects had a median level (1464±219ng/mL). Unlike benign PEs and other malignancies patients, these MPF concentration was significantly higher (96±10 and 210±23ng/mL, respectively; p<0.0001).

Creaney et al. *[9]* employed two distinct commercial assays in their study of serum MPF levels. Based on the N-ERC assay (IBL), the serum concentrations of N-ERC/MPF in malignant mesothelioma (MM) patients varied from undetectable to 5.9ng/mL and the median concentration in MM patients was 0.16±0.05ng/mL, which did not significantly differ from levels in healthy individuals and those experiencing benign conditions (0.06±0.02ng/mL), or from patients with other malignancies (0.34±0.1ng/mL). In the same cohort, MPF levels were measured employing the MPF assay (MBL) and found to be variedfrom 2.6 to 622ng/mL in MM patients.

The MPF levels observed were significantly greater than those reported utilizing the IBL assay. In healthy subjects and those with benign conditions, the mean MPF concentration was 40.4±6.1ng/mL among MM patients.

Iwahori et al. *[8]* analyzed serum MPF concentrations in 27 MPM patients: 13 epithelial, 3 sarcomatoid, 5 mixed, and 6 unclassified (by cytology). Controls comprised 47 lung cancer patients; 35 with various cancers (18 ovarian, 8 stomach, 9 colon); 9 asymptomatic asbestos-subjected individ-

uals; and 38 healthy adults lacking asbestos exposure history. The outcomes revealed that MPM patients experienced elevated mean serum MPF levels (68.7±101.1ng/mL) compared to those with healthy controls (9.0±2.9), asbestos-exposed but healthy individuals (9.7±5.3), lung cancer (16.6±15.3) and various cancers (15.1±9.7). Statistical analysis employing the Mann–Whitney U-test confirmed significantly differed median MPF values between the MPM and all control groups (p<0.001).

Hollevoet et al. [6] assessed serum MPF concentrations across various groups, encompassing 101 healthy controls, 46 patients experiencing benign respiratory diseases (primarily asthma, COPD, and pneumonia), 123 with benign asbestos-associated conditions (39 with diffuse pleural thickening, 69 with pleural plaques, 15 primarily with asbestosis, and 89 asbestos-subjected healthy subjects), 85 MPM instances (comprising 4 sarcomatoid, 73 epithelioid, and 8 biphasic histology cases), and 63 cancer lung patients (mostly non-small cell lung cancer). MPF concentration exhibited wide variation, ranging from 1.96 to 837.76ng/mL. Moreover, there were significant difference between MPM subjects (Mean ± SD; 50.83±98.71) and the other group (p<0.001).

"Jiménez-Ramírez et al. [13] analyzed serum MPF concentrations in 166 newly diagnosed MPM instances and 378 population-based controls. The outcomes revealed that median MPF levels were 50.24ng/mL in male patients versus 17.18ng/mL in male controls, and 56.07ng/mL in female patients compared to 17.63ng/mL in female controls—differences that were statistically significant (p<0.001). Variations in findings across investigations may be ascribed to differences in the MPF antibodies deployed, ethnic diversity among populations, and sample size disparities. Nonetheless, the present study's results are consistent with previous research, highlighting a significant in MPF concentrations in both serum and PF of MPM patientscompared toother diagnostic groups, despite inter-assay differences.

This study encompassed 14 instances of epithelial mesothelioma and 3 instances of biphasic mesothelioma, with no occurrences of the sarcomatoid instances due to its infrequency. Accordingly, the prevalence of the three primary histological variants of mesothelioma is around20% for the biphasic type, 20% for the sarcomatoid type, and 60% for the epithelioid type [14].

Table (2) indicates that the mean MPF levels in serum and PF for biphasic and epithelial mesotheliomawere negligible.

Iwahori et al. [8] similarly manifested no significantly differed MPF levels within MPM patients of various histological types.

Hollevoet et al. [6] demonstratedthat sarcomatoid MPM patients experienced significantly mitigated serum MPF levels, unlike individuals with biphasic histology (p<0.05) and epithelioid (p<0.01), with no significant difference found between the epithelioid and biphasic subtypes (p=0.51).

In metastatic PEs group, lung cancer was the initial diagnosis in 8 instances, while the remaining 9 cases included 5 with unidentified primaries, 1 cholangiocarcinoma, and 3 breast malignancies. No significant difference was found among metastatic bronchogenic carcinoma and other metastatic cancers concerning serum or fluid MPF levels, MPF ratios, or age.

Creaney et al. [9] manifested nosignificant difference in the mean MPF serum levels between 10 instances of various cancers with (13.07±2.3ng/ml), which comprised pancreatic (n=2), melanoma (n=2), breast (n=3), and one case each of melanoma, lymphoma, and thyroid cancer; and between 19 lung cancer cases (15.13±3.4ng/ml).

The inflammatory cohort encompassed 8 instances classified as non-specific inflammatory responses and 8 with TB granuloma. No significant differences between the two subtypes for fluid or serum MPF levels, MPF ratio, or age were found.

Creaney et al. [9] reported no significant differences in effusion MPF levels between those with other benign causes and those with benign effusions associated with infection.

The AUC for serum MPF was 0.94 and for pleural MPF was 0.97, which were utilized to differentiate MPM patients from other groups, encompassing metastatic and inflammatory effusion (Fig. 1). The sensitivity; specificity; PPV; and NPV were 100, 63, 37, and 100%, respectively, with a cut-off value of (8.75ng/ml) in serum and (9.25/mL) in effusion.

Creaney et al. [9] observed a specificity of 95% when comparing to healthy individuals or patients experiencing benign conditions (n=55). Serum N-ERC and MPF exhibited sensitivity values of 29% and 52% (cut-off=1.6 and 33.2ng/mL), respectively.

Iwahori et al. [8] determined that the AUCfor serum MPF was 0.879 in distinguishing MPM individuals from controls, which included healthy adults, asbestos-subjected subjects, and lung cancer patients (cut-off value=19.1ng/ml; specificity= 90.4%, sensitivity=74.1%).

Hollevoet et al. [6] reported AUCs spanned from 0.816–0.849 for distinguishing MPM patients from various groups at a cut-off of 13.46ng/mL. At this threshold, sensitivity and specificity were 68% and 97%, and positive and negative likelihood ratios were 22.67 and 0.33, respectively.

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عامل تعزيز الخلايا النواء فى المصل والسائل الجينى : علامة واعدة لتشخيص الورم المتعدد الاورمات الجينى الخبيث

أجريت هذة الدراسة فى مستشفى قصرالعينى جامعة القاهرة، بالتعاون بين قسم الصدر وقسم الباثولوجيا الاكلينكية فى الفترة ما بين يناير ٢٠١٩ الى أكتوبر ٢٠٢٠.

هدف الدراسة هو دراسة مستوى عامل تحفيز الخلية ذات النواة الكبيرة فى السيرم والسائل البللورى فى مرضى الارتشاح البللورى الخبيث وغير الخبيث.

وقداشتملت على:

- مجموعة ١ : تضم ١٧ مريضاً مصابون بورم المتوسطة الخبيث.
- مجموعة ٢ : تضم ١٧ مريضاً مصابون بالارتشاح البللورى الخبيث من غيراورام المتوسطة.
- مجموعة ٣ : تضم ١٦ مريضاً مصابون بالارتشاح البللورى النضحى الحميد.

تم عمل الاتى للمرضى: أخذ التاريخ المرضى والفحص وعمل تحاليل طبيه تشمل صورة د مكامله ووظائف كبد وكلى وتحاليل سيولة بالدم واخذ عينة من السائل البللورى وعمل اشعة عادية ومقطعيه على الصدر واخذ عينة من الغشاء البللورى عن طريق السونار او منظار التجويف الصدرى واخذ عينة من السيرم والسائل البللورى لقياس مستوى عامل تحفيز الخلية ذات النواة الكبيرة.

وقدوجدأنه:

مستوى عامل تحفيز الخلية ذات النواة الكبيرة كان اعلى وذو دلالة احصائية فى السائل البللورى والسيرم لمرضى ورم المتوسطة الخبيث عن مرضى الارتشاح البللورى الخبيث من غيراورام المتوسطة ومرضى الارتشاح البللورى النضحى الحميد.

مقارنة مستوى عامل تحفيز الخلية ذات النواة الكبيرة بين مرضى ورم المتوسطة الخبيث الطلائى وثنائى الطور فى السيرم والسائل البللورى أوضح عدم وجود اختلاف ذو دلالة احصائية.

مستوى عامل تحفيز الخلية ذات النواة الكبيرة كان اعلى وذو دلالة احصائية فى السائل البللورى والسيرم لمرضى الارتشاح البللورى الخبيث عن مرضى الارتشاح البللورى النضحى غيرالخبيث.