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ORIGINAL ARTICLE

Diagnostic Role of Angiopoietin-2 and Vascular Endothelial Growth Factor in Malignant Pleural Effusion

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ABSTRACT

Background Malignant pleural effusion is a prognostic and diagnostic sign in cancer lung .Role of tumor biomarkers in pleural fluid has been considered, but their diagnostic ability remains undetermined, therefore the aim of this current work is to evaluate the diagnostic role of both pleural fluid Angiopoietin-2 and vascular endothelial growth factor levels in malignant pleural effusions. **Patients and methods**: A case-control study including 56 patients diagnosed as pleural effusion of known origin. Both pleural fluid Ang-2 and VEGF levels were measured with other biochemical markers. **Results:** Pleural fluid VEGF could differentiate malignant exudative from benign exudative pleural effusion at a cut-off value 1590 (pg/ml),with higher Sensitivity 96.2%, Specificity 98.7% , PPV 100%,NPV 95% and Accuracy 97.8%. Additionally it has the ability to differentiate mesothelioma from other causes of malignant pleural effusion at 2225.5(pg/ml) cut off value, with Sensitivity 80%, Specificity 75%, PPV 66.7%, NPV 85.7% and Accuracy 76.9%.While Pleural fluid Ang-2, could detect malignant pleural effusion at a cut off value 15.7 ng/ml that yield lower Sensitivity 69.2%, Specificity 42.1%, PPV 62.1%, NPV 50% and Accuracy of 57.8% than that of Pleural fluid VEGF. **Conclusion:** Pleural fluid VEGF is a useful biomarker in diagnosing pleural effusion of malignant type, especially mesothelioma, while pleural fluid Angiopoietin-2 has a limited role in ruling out malignant pleural effusion.

Key words: Malignant pleural effusion, vascular endothelial growth factor (VEGF), Angiopoietin-2(Ang-2), mesothelioma.

INTRODUCTION

Malignant pleural effusion (MPE) is a common condition, observed in patients with malignant disease affecting pleural fluid turnover, either directly or indirectly [1] accompanied with bad prognosis. Those patients were expected to live from four to nine months duration [2, 3]. Diagnosing MPE might be considered a challenge, due to insufficient sensitivity in the noninvasive methods of investigations [4]. As for, the cytological analysis, it yielded a sensitivity of only 60% in malignancies and 50% in mesothelioma per se [5, 6]. Fine needle biopsy has a sensitivity of 67–73 %.While, in case of medical thoracoscopy, it's diagnostic sensitivity reached 95%, however not all patients can withstand this invasive maneuver besides it is not available at all medical services [7].On the other side, tumor biomarkers have been investigated by many researchers in order to improve the diagnostic yield of pleural fluid examination, but still their role are debatable [8]. It was noted that vascular endothelial growth factor (VEGF) along with angiopoietins, are essential for the regulation of tumor angiogenesis [9]. As for Angiopoietin/Tie2 axis (Ang-2), it has a great role in the formation of MPE, augmenting pleural vascular permeability, potentiating tumor angiogenesis, increasing VEGF and IL-6 release, and enhancing tumor-associated pleural inflammation [10]

On the same line, VEGF enhances vascular leakage, permeability and angiogenesis which is crucial in malignant pleural fluid development [11-13]. Concurrently, inflammatory cells, malignant cells and mesothelial cells, all presented in the pleura, could stimulate the release of VEGF [14].VEGF up regulation is consistent with Ang□2 expression at the tumor periphery and correlated with robust angiogenesis. Ang□2 plays the earliest start□up, while VEGF plays a subsequent promoting role with its delayed increase [15].Accordingly; the aim of this current work is to evaluate the diagnostic role of both pleural fluid Angiopoietin-2(PF Ang-2) and pleural fluid vascular endothelial growth factor (PF VEGF) levels in malignant pleural effusions.

METHODS

This study was implemented at Chest, Cardiothoracic Surgery and Medical Biochemistry Departments, Faculty of Medicine Zagazig University hospitals, starting from March 2018 till September 2019. The study was done according to The Code of Ethics of the world medical association (Declaration of Helsinki) for studies involving humans. All patients had written informed consents.

This is a case-control study including 56 patients diagnosed as pleural effusion of known origin, after excluding patients with pleural effusion due to(suspected more than one cause, chylothorax or hemothorax.),and patients on medications (eg.: anticancer therapy or corticosteroids or anti-inflammatory drugs)[16].

All patients were subjected to; thorough medical history taking, general and local chest examination. Radiological evaluation in the form of Chest X-ray PA view, ultrasound on the (chest, abdomen and pelvis), CT chest if needed. Sputum analysis: Culture and sensitivity for aerobic and anaerobic bacteria, ZN stain and cytopathological examination for malignant cells. Ultrasound guided thoracentesis was carried out with pleural fluid analysis after withdrawing 100-ml pleural fluid with observing the appearance of the fluid and sending it for; 1) Biochemical analysis: (Total protein, RBCs, WBCs, glucose, albumin, LDH, Cholesterol, PH, ADA levels). Ang-2 and VEGF pleural fluid levels were measured with the enzyme-linked immunosorbent assay (ELISA) method [17]. The differentiation between transudate and exudate was based on Light's criteria [18], 2) Bacteriological (aerobic and anaerobic culture and sensitivity, ZN stain) and 3) Cytopathological examinations. Laboratory investigations were requested including: CBC, ESR, CRP, KFT, LFT, serum LDH. Pleural biopsy were performed for undiagnosed patients through image-guided biopsies or medical thoracoscopy, and all specimens were sent for histopathological examination

Statistical analysis: Data were analyzed using IBM SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA) and NCSS 11 for windows (NCSS LCC., Kaysville, UT, USA). Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

RESULTS

This study included 56 patients diagnosed as pleural effusion ,they were further classified: 11 patients with transudative pleural effusion,19 patients with benign exudative pleural effusion and 26 patients were suffering from malignant exudative pleural effusion(7 patients diagnosed by pleural fluid cytology, 5 patients diagnosed by closed tissue core-needle biopsy under radiological guidance and 14 patients underwent medical thoracoscopy). 60.7% of the studied patients were males and 39.3% were females with mean age 49.9±11.2 years.

A highly statistical significant difference was detected between transudative and benign exudative PF Ang-2 levels, with a highly significant increase in malignant exudative than that of transudative one .While a non-significant difference was noted between malignant exudative and benign exudative type . On the other hand PF VEGF level was remarkably increased in benign exudative pleural effusion than transudative type and also shows a highly significant increase in malignant exudative pleural effusion when

compared with that of the benign exudative type (Table 1).

PF Ang -2 level among the three etiologies of malignant exudative effusion (Adenocarcinom , Mesothelioma and Metastasis) showed a statistical significant difference , also a statistical significant difference among causes of benign exudative PE , except in-between TB and empyema the difference was insignificant ,while in transudative pleural effusion PF Ang-2 has non-significant value(Table 2).

Our results clarified a statistical significant increase in PF VEGF level in mesothelioma when compared with adenocarcinoma, and the same between adenocarcinoma and metastasis, associated with increase of PF VEGF level in mesothelioma than in metastasis but not reaching significant changes. Besides, there were a variation of significant PF VEGF levels among the three etiologies of benign exudative effusion(Parapneumonic, Tuberculosis and Empyema), however in case of heart failure and hepatic hydrothorax it has a non-significant changes between them(Table 3).

Variations of statistical significant positive correlation between PF Ang-2 level with both ESR and pleural fluid (WBCs, RBCs, proteins, and LDH) were detected. Also a highly significant positive correlation was noted between pleural fluid VEGF level and that of pleural fluid (RBCs, proteins and LDH) and ESR as well, while there was a significant negative correlation between Ang-2 and VEGF with glucose level in the pleural fluid (**Data not shown**).

A cut off value of 15.7 ng/ml for PF Ang-2 was concluded with a yield of Sensitivity 69.2%, Specificity 42.1%, Accuracy 57.8% , PPV 62.1% and NPV 50% for differentiating malignant exudative from benign exudative PE. While as for PF VEGF ,it has a cut-off value 1590 (pg/ml),with higher Sensitivity 96.2% , Specificity 98.7% , Accuracy 97.8%,PPV 100% and NPV 95%(Table 4)(Figure 1).

The ability of PF VEGF to differentiate mesothelioma from other causes of malignant exudative pleural effusion was 80% at 2225.5(pg/ml) cut off value, while it could exclude 75% negative cases (non-mesothelioma) among truly negatives examined with PPV 66.7% and NPV 85.7%, while Ang-2 could diagnose 50% of mesothelioma and exclude only 12.5% of non-mesothelioma type, at cut off value 18.7(ng/ml)with PPV 26.3% and NPV 28.6% . Besides, overall accuracy of PF VEGF was 76.9% as a predictor for presence of mesothelioma,while for PF Ang-2 accuracy was only 43.8% (Table 5) (Figure 2).

Table 1. Values of both PF Ang-2 and PF VEGF among different types of pleural effusion

	Transudative PE N=11	Benign Exudative PE N=19	Malignant Exudative PE N=26	F test	P value
Ang-2(ng/ml) Mean(±SD)	6.8 ± 1.8	16.1 ± 2.4	17.1 ± 1.9	132.2	<0.001
VEGF(pg/ml) Mean(±SD)	273.3±42.1	905.1±197.9	2251.5±456	203.2	<0.001

Post hoc analysis for the difference in between groups.

(Ang-2) Multiple comparison		Mean Difference	Sig.	95% CI	
				Lower Bound	Upper Bound
Malignant Exudate	Benign Exudate	0.997	0.109	-0.23	2.2
	Transudate	10.2	<0.001	8.9	11.5
Benign Exudate	Transudate	9.2	<0.001	7.8	10.6
(VEGF)					
Malignant Exudate	Benign Exudate	1346.4	<0.001	1151.5	1541.4
	Transudate	1978.2	<0.001	1768.9	2187.6
Benign Exudate	Transudate	631.8	<0.001	408.7	854.8

NS: P-value>0.05 is not sig. S:P-value <0.05 is sig. HS:P-value<0.001 is high sig.

Table 2. PF Ang-2 Levels in Patients with pleural effusion of different etiologies.

Etiology of PE	No (%)	Ang-2(ng/ml) Mean(±SD)	P value of LSD
Malignancy Exudative PE(26)			
-Adenocarcinoma	9	16.5 ± 0.78	0.03^{1},$<0.001^{2}$
-Mesothelioma	10	14.8 ± 0.75	$<0.001^{3}$
-Metastasis	7	19.1 ± 1.11	
Benign Exudative PE(19)			
- Para-pneumonic	5	12.8 ± 1.3	$<0.001^{4}$,$<0.001^{5}$
- Tuberculosis	7	17.3 ± 1.7	0.661^{6}
-Empyema	7	16.98 ± 0.93	
Transudative PE(11)			0.953^{7}
-Heart failure	5	6.8 ± 2.7	
-Hepatic hydrothorax	6	6.9 ± 0.72	

P-value 1= dif. Bet. Adenocarcinoma & mesothelioma P-value2= diff. bet. Adenocarcinoma & metastasis
P-value 3= diff. bet. Mesothelioma & metastasis P-value4= diff. bet. Para-pneumonic & TB
P-value5= diff. bet. Para-pneumonic & empyema P-value6= diff. bet. TB & empyema
P-value7= diff. bet. Heart failure and hepatic hydrothorax LSD: Least significant difference
NS: P-value>0.05 is not sig. S:P-value <0.05 is sig. HS:P-value<0.001 is high sig.

Table 3. PF VEGF Levels in patients with pleural effusion of different etiologies

Etiology of PE	No (%)	VEGF (pg/ml) Mean(±SD)	P value of LSD
Malignancy Exudative PE(26)		(F test)	
-Adenocarcinoma	9	1879 ± 229.5	0.001[1], 0.03[2]
-Mesothelioma	10	2544.7 ± 473.2	0.208[3]
-Metastasis	7	2311.6 ± 323.4	
Benign Exudative PE(19)		(F test)	
- Parapneumonic	5	689 ± 104.3	<0.001[4], 0.02[5]
- Tuberculosis	7	1076.3 ± 165.97	0.03[6]
-Empyema	7	897 ± 113.6	
Transudative PE(15)		(t-test)	
-Heart failure	7	290.6 ± 35.4	0.0534[7]
-Hepatic hydrothorax	8	293.1 ± 38.8	

P-value 1= dif. Bet. Adenocarcinoma & mesothelioma P-value2= diff. bet. Adenocarcinoma & metastasis
P-value 3= diff. bet. Mesothelioma & metastasis P-value4= diff. bet. Para-pneumonic & TB
P-value5= diff. bet. Para-pneumonic & empyema P-value6= diff. bet. TB & empyema
P-value7= diff. bet. Heart failure and hepatic hydrothorax LSD: Least significant difference
NS: P-value>0.05 is not sig. S:P-value <0.05 is sig. HS:P-value<0.001 is high sig.

Table 4. Validity data of both PF Ang-2 and VEFG in the differentiation of malignant exudative pleural effusion from benign exudative type.

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Ang-2	15.7(ng/ml)	69.2%	42.1%	62.1%	50%	57.8%
VEGF	1590(pg/ml)	96.2%	98.7	100%	95%	97.8%

Table 5. Validity data of both PF Ang-2 and VEFG in the differentiation of the malignant mesothelioma from other malignant pleural effusions.

	Cut-off	Sensitivity	Specificity	PPV	PPN	Accuracy
VEGF	2225.5(pg/ml)	80%	75%	66.7%	85.7%	76.9%
Ang-2	18.7(ng/ml)	50%	12.5%	26.3%	28.6%	43.8%

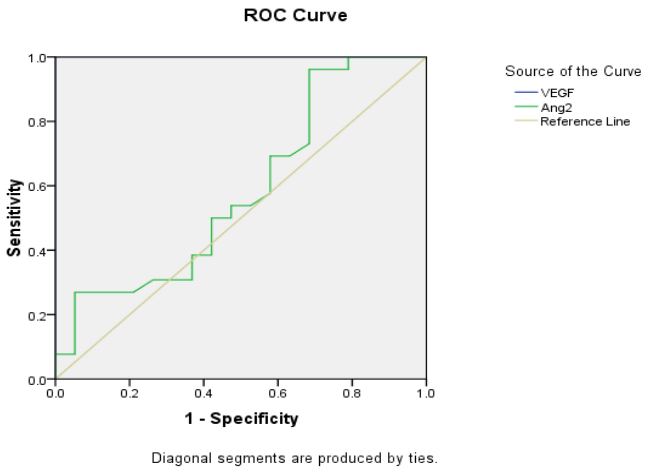

Figure 1. The area under the curve (AUC=0.584, P-value=0.34 & AUC=1.0, P-value<0.001) in the receiver operating characteristic (ROC) curve of angiotensin-2 and VEGF respectively in the differentiation of the malignant exudate and benign exudate group

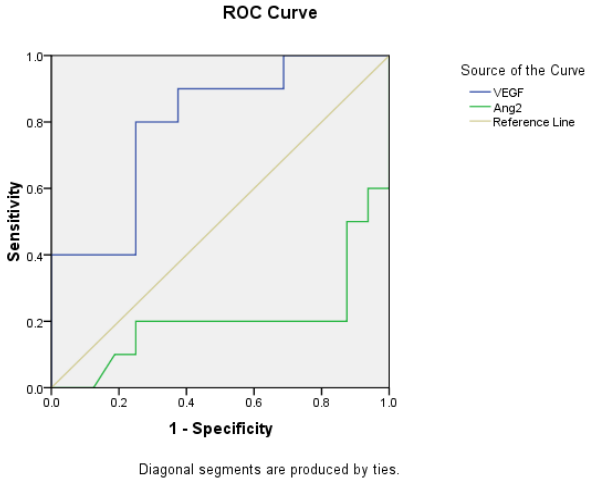

Figure 2. The area under the curve (AUC=0.204, P-value=0.01 & AUC=0.794, P-value=0.01) in the receiver operating characteristic (ROC) curve for PF Ang-2 and PF VEFG levels respectively in the differentiation of mesothelioma from other malignant pleural effusions.

DISSCUSION

Malignant pleural effusions (MPEs) had a great role in the disabilities associated with cancer patients [14]. Angiogenic process, associated with enhanced vascular permeability and pleural inflammation were accused to be the main pathophysiologic mechanisms for its development [19, 20]. Many studies have demonstrated that Ang-2, and VEGF, were vital in the tumor neovascularization [21]. In spite of conducting multiple studies on VEGF and Ang-2 in pleural fluid and serum ,but still their role in the differentiation of different etiologies of MPE especially mesothelioma were not investigated yet. On the light of the above hypothesis, this current work was performed to evaluate the diagnostic role of both pleural fluid Ang-2 and VEGF levels in malignant pleural effusion. It included 56 patients diagnosed as pleural effusion, they were further classified: 11 patients with transudative pleural effusion, 19 patients with benign exudative pleural effusion and 26 patients were suffering from malignant exudative pleural effusion

In this current work a highly statistical remarkable difference was observed between transudative and benign exudative PF Ang-2 levels ,with a highly significant increase in malignant exudative than that of transudative one .While there was unremarkable difference noted between benign exudative and malignant exudative types (Table 1).

In concordance with these results, Taş and Köseler [1] concluded that PF Ang-2 level couldn't differentiate benign exudative from malignant exudative pleural effusions. While Koseler and colleagues [22] and Elhefny et al. [23] noted a higher PF Ang-2 levels ($p < 0.05$) in both benign and malignant exudative pleural effusion than that in transudative type, yet PF Ang-2 level in benign exudates were higher than that of malignant exudates without reaching a significant difference.

As regard PF VEGF level was remarkably increased in benign exudative pleural effusion than transudative type and also shows a highly significant increase in malignant exudative pleural effusion when compared with that of the benign exudative type (Table 1). In agreement with Hamed et al. [24] and Tomimoto and colleagues [17] who reported that PF VEGF were remarkably increased in pleural exudates than in pleural transudates.

It was hypothesized that bacterial infection or malignancy could enhance the release of both PF VEGF and PF Ang-2 inside the pleural cavity, emphasizing the main source of PF Ang-2 release is by pleural microvasculature (endothelial and perivascular cells), as it wasn't reported that Ang-2 is expressed by neither inflammatory nor mesothelial cells [25]. In exudative pleural effusion, the increased level of VEGF could be contributed to the increased permeability of pleural endothelium, as VEGF is considered a robust inducer of vascular permeability, more than histamine by 50,000 times [26,27,28].

In this study, PF Ang-2 level was remarkably different among three etiologies of malignant exudative effusion (Adenocarcinom , Mesothelioma and Metastasis), also it shows statistical difference among causes of benign exudative PE , except in-between tuberculous PE and empyema the difference was insignificant, while in transudative pleural effusion PF Ang-2 has non-significant value(Table 2). On the contradicted side, Sanad et al. [29] concluded that in tuberculous pleural effusion PF Ang-2 levels were higher than that in empyema and para-pneumonic pleural effusions .Similarly, Elhefny et al. [23], Taş and Köseler [1] found significantly higher PF Ang-2 levels in exudative effusions than in transudative one, suggesting that Ang-2 levels are higher in exudative effusions (mainly tuberculous origin) than in malignant PE.

As regard PF VEGF level a statistical significant increase in its level in mesothelioma when compared with adenocarcinoma, and the same between adenocarcinoma and metastasis, associated with increase of PF VEGF level in mesothelioma than in metastasis but not reaching significant values. Besides, there was a variation of significance in PF VEGF level among the three etiologies of benign exudative effusion (Parapneumonic, Tuberculosis and Empyema), however in case of heart failure and hepatic hydrothorax it has a non-significant changes between them (Table 3)

In concordance with results of former studies, malignant and para-pneumonic pleural effusions recorded a higher PF VEGF levels than that found in pleural effusion due to heart failure [24, 30, 31]. Düzkoprü et al. [16] results of their study revealed PF VEGF mean value was 3359 ±700 pg/ml in mesothelioma patients, 2175 ±435 pg/ml in non-mesothelioma patients, and 1092 ±435 pg/ml in bengin exudate group, accordingly it was concluded that, in mesothelioma group VEGF mean value was the highest than that observed in the benign exudative group with a statistically significant difference.

In contrary to our study, Thickett and colleagues [5] noted lower mean values of VEGF in malignant diseases than in empyema. Also, Verheul et al. [32] observed a high concentration of PE VEGF (1637 and 2167) pg/ml in empyema and tuberculosis, respectively. It was hypothesized by Fiorelli et al. [4] that in empyema, the exudated protiens is induced by bacterial pathogen causing release of VEGF which enhance vascular permeability.

Variations of statistical significant positive correlation were noticed in this study between PF Ang-2 level with both ESR and pleural fluid (WBCs, RBCs, proteins, and LDH). Also a highly remarkable positive correlation in PF VEGF level and that of pleural fluid

(RBCs, proteins and LDH) and ESR as well, while there was a remarkable negative correlation between Ang-2 and VEGF with glucose level in the pleural fluid (Data not shown)

On the same line with, Kalomenidis and colleagues [33], Sanad and others [29] who concluded that PF Ang-2 mean values correlated with , pleural fluid (WBCs count, RBCs count, LDH and total protein levels), but in case of pleural fluid pH and glucose levels, it was inversely correlated. It was postulated that Ang-2 in pleural fluid and PF VEGF levels were accompanied with intense pleural inflammation, vascular hyperpermeability and exudates formation, leading to accelerated metabolism, associated with decrease in both pleural fluid glucose levels and pleural fluid PH [33].

Results of this study clarify the role of PF VEGF in differentiating malignant exudative from benign exudative pleural effusion, at cut-off value 1590 (pg/ml),with higher Sensitivity 96.2% ,Specificity 98.7% , Accuracy 97.8%, PPV 100% and NPV 95%. While in case of PF Ang-2 level at 15.7 ng/ml cut-off level, it has a lower Sensitivity 69.2%, Specificity 42.1%, Accuracy 57.8%, PPV 62.1% and NPV 50% in predicting malignant type (Table 4)(Figure 1).

Also, in the current study the ability of PF VEGF to differentiate mesothelioma from other causes of malignant pleural effusion was 80% at 2225.5(pg/ml) cut off value, while it could exclude 75% negative cases (non mesothelioma) among truly negatives examined with PPV 66.7% and NPV 85.7%, while PF Ang-2 could diagnose 50% of mesothelioma and exclude only 12.5% of non mesothelioma at cut off value 18.7(ng/ml)with lower PPV 26.3% and NPV 28.6% . Besides, overall accuracy of VEGF was 76.9% as a predictor for presence of mesothelioma, while for Ang-2 it was only 43.8% (Table 5) (Figure 2).

It was speculated that at the site of tumor growth, VEGF is able to induce vascular permeability, angiogenesis and more tumor advancement. Besides, it has a major role in the migration of tumor cells from the surrounding vessels inside the pleural cavity. [4]

This was more or less in agreement with Taş and Köseler[1]who demonstrated that pleural fluid Ang-2 cut-off value for differentiation of malignant and benign exudative effusions was found to be 13.84 ng/ml, with considerably lower Sensitivity (62.26%), and high specificity 92.31%. Additionally it has the ability to predict local control of MPE after treatment at cutoff level 25.57 pg/mL with sensitivity 90.40% and the specificity 81% [9].

On the other hand, Elhefny et al. [23] found remarkable higher mean value of Ang-2 in benign than malignant exudatives at cut-off level 15.67 ng/mL providing a sensitivity of 91.3% and a specificity of 56.2%.

In the literature there are different cut-off points as; Duysinx et al. [34] suggested a best threshold of PF VEGF for detecting malignant pleural effusion was at cutoff 382 pg/ml with sensitivity 69%, and specificity of 54%.Also, Shu et al. [35] concluded a cut-off value of PF VEGF at 959 pg/mL could determine presence of malignant pleural effusion with 47% sensitivity and 96% specificity. Fiorelli et al.[4]proposed VEGF cut off level 652 pg/ml which yielded a sensitivity 63% , specificity 83%, PPV=86% and NPV=58% for diagnosing malignant pleural effusion. Fafliora et al.[36] stated that PF VEGF levels among patients with malignant PE were increased by 1.93 ng/mL as compared to patients with benign PE

Bradshaw et al. [14] concluded that PF VEGF at 2000 pg/mL had an important role in diagnosing malignant pleural effusion especially mesothelioma type, associated with poor prognosis [37]. Moreover VEGF accompanied with angiogenesis participate in the pathophysiology and advancement of mesothelioma [38] .Besides, Düzkoprü et al.[16]postulated a significant increase of PF VEGF in malignant pleural effusions with higher values in mesothelioma than other causes of malignant pleural effusion.

However, Shen et al. [39] suggested a lower sensitivity and specificity (75% and 72%) respectively, in predicting malignant PE. Accordingly, it was suggested that VEGF should be accompanied with other pleural fluid biomarkers in the evaluation of MPE [16]. On the contradicted side, Fiorelli and colleagues [4] concluded that PF VEGF level couldn't differentiate lung cancer from other malignancies especially mesothelioma. Discrepancies in VEGF levels in malignant pleural effusion may be related to; 1) the fact that VEGF is mostly released by malignant tumors, but not all of them, and 2)its expression vary according to different histological types of malignant tumors [35]. Ratios and numerical differences in our study from other studied might be related to small number of our patients. Larger groups of patients were recommended for more emphasized results.

CONCLUSION

Pleural fluid VEGF is a useful biomarker in diagnosing pleural effusion of malignant type, especially mesothelioma, while pleural fluid Angiopoietin-2 has a limited role in ruling out malignant pleural effusion.

Conflict of Interest: Nothing to declare.
Financial Disclosures: Nothing to declare.

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