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Diagnostic value of angiopoietin-2 in the differentiation of malignant pleural effusions

Malign plevral efüzyonların farklılaşmasında anjiyopoietin-2'nin tanısal değeri

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Abstract

Aim: Angiopoietins play an important role in the regulation of inflammation, angiogenesis and increased vascular permeability, which are the main steps in the pathogenesis of malignant pleural effusions (MPEs). The present study investigates the diagnostic value of pleural fluid angiopoietin-2 (Ang-2) levels in the differentiation of malignant pleural effusions from other effusions.

Methods: This research was designed as case-control study in a single-center. The study included a total of 66 patients (13 had transudate, 28 had benign exudate and 25 had malignant pleural effusions). The patient group involved 25 patients diagnosed with MPE, based on the criteria of lung cancer and other organ malignancies, and malignant pleural effusion. The control group consisted of 41 patients, 13 with transudate according to the Light criteria and 28 with exudate other than MPE (parapneumonic, tuberculous pleurisy, embolism, etc.).

Results: Pleural fluid Ang-2 levels were found to be higher in both the benign and malignant exudates than in the transudative pleural effusions (P=0.001). Pleural fluid Ang-2 levels were higher in the benign exudate group than in the malignant exudate group, although the difference was not statistically significant (P=0.874). A patient with an exudative pleural effusion and a pleural fluid Ang-2 level of higher than 13.84 was found to be 1.87 times more likely to have a malignant pleural effusion.

Conclusion: Despite the use of Ang-2 levels in the differentiation of transudative and exudative pleural effusions, the present study found that Ang-2 level cannot be used to differentiate between malignant and benign exudative pleural fluids.

Keywords: Angiopoietin-2, Malignant pleural effusion, Exudative pleural effusion

Öz

Amaç: Anjiopoietinler, malign plevral efüzyon (MPE) patogenezinde ana basamaklar olan inflamasyon, anjiyogenez ve vaskuler permeabilite artışının düzenlenmesinde önemli bir rol oynamaktadır. Çalışmamızda, malign plevral efüzyonların diğer efüzyonlardan ayrımında plevra sıvı anjiopoietin 2 (Ang-2) seviyelerinin tanısal değerini araştırdık.

Yöntemler: Bu çalışma, tek merkezli vaka kontrol çalışması olarak tasarlandı. Çalışmamıza, 13 transüda, 28 benign eksüda ve 25 malign plevral sıvıya sahip (malign eksüda) toplam 66 hasta alındı. Çalışmaya hasta grubu olarak olarak MPE tanısı konulmuş 25 hasta alındı. MPE tanılı hastalar; akciğer veya diğer organ maligniteleri mevcut olan ve plevral sıvının maligniteye bağlı olarak olarak geliştiği hastalardan oluştu. Kontrol grubu olarak, Light kriterlerine göre transüda olduğu saptanan 13 hasta ve MPE dışındaki eksüda vasıflı (parapnomonik, tüberküloz plörezi, emboli vb) 28 hasta olmak üzere toplam 41 hasta dahil edildi.

Bulgular: Plevral sıvı Ang-2 seviyeleri, hem benign eksüda hem de malign eksüda vasfındaki sıvılarda transüda vasfındaki plevral sıvılara göre belirgin olarak yüksek bulundu (P=0,001). Benign eksüda grubunda plevra Ang-2 seviyeleri malign eksüda grubuna göre yüksek olarak saptandı ancak bu durum istatistiksel olarak anlamlı değildi (P=0,874). Eksüda vasıflı plevral sıvılarda Ang-2 düzeyi 13,84 değerinin üzerinde saptanmış olan bir hastada malign plevral efüzyon olma riski, 13,84 değerinin altında olan bir hastaya göre 1,87 kat fazla bulundu.

Sonuç: Ang-2'nin, transüda ve eksüda özelliğindeki plevral sıvıların ayrımında kullanılabilir olmasına rağmen, eksüdatif plevral sıvılarda, malign ya da benign ayrımı yapmada yetersiz olduğu saptandı.

Anahtar kelimeler: Anjiopoietin 2, Malign plevral efüzyon, Eksüdatif plevral efüzyon

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Introduction

Malignant pleural effusions (MPEs) are caused by a malignant disease affecting pleural fluid turnover, either directly or indirectly. Malignant pleural effusions constitute 28–61% of all pleural effusions, and lung cancer, breast cancer and lymphomas are held responsible for three-quarters of all malignant pleural effusions [1].

Although the exact pathogenesis of MPEs remains unknown, an increase in pleural vascular permeability, inflammation and angiogenesis are the main mechanisms in their development. Lymphatic obstructions caused by compression, and inflammatory and proangiogenic factors released from the tumor cells, are responsible for these mechanisms [1-3]. The most widely known of these factors is the vascular endothelial growth factor (VEGF), which is a growth hormone with proangiogenic and anti-inflammatory properties that plays a key role in increased vascular permeability [4,5].

Angiopoietins are glycoprotein molecules that possess regulatory effects on angiogenesis. To date, four angiopoietins have been identified, named Angiopoietin (Ang)) 1, 2, 3 and 4, all of which bind to the Tie-1 and Tie-2 receptors, which are members of the endothelium-specific tyrosine kinase family, and which exert their effects through the Tie-2 receptor [6]. By binding to the Tie-2 receptors, Ang-1 strengthens the connections between endothelial cells and with the surrounding supportive tissues (smooth muscle and extracellular matrix), thereby providing vessel stability and exerting negative effects on vascular permeability [7]. Ang-2 is competitive inhibitor of Ang-1 that destabilizes blood vessels by inhibiting the action of Ang-1 after binding to the Tie-2 receptor, and sensitizes the endothelium to inflammatory agents. Furthermore, it facilities VEGF-mediated angiogenesis and increases vascular permeability [8,9].

The role of Ang-2 in angiogenesis is mediated by VEGF-A, as in the presence of VEGF-A, Ang-2 destabilizes blood vessels and promotes vascular sprouting, but plays a suppressive role in accelerating vascular regression in the absence of VEGF-A [10]. The relationship between Ang-2 and VEGF-A is remarkable in tumor angiogenesis. The release of Ang-1 predominates in normal tissue, whereas the release of Ang-2 is more prominent in tumor tissue. This is considered to be a major step in tumor angiogenesis [11].

The Ang/Tie-2 pathway has been demonstrated to play an important role in the regulation of tumor-related angiogenesis, increased vascular permeability and inflammation, all of which are the main steps in the pathogenesis of MPE [11-13].

The present study investigates the diagnostic value of pleural fluid angiopoietin-2 levels in the differentiation of malignant pleural effusions from other effusions.

Materials and methods

This single-center case-control study was granted approval by the ethics committee. Between March 2012 and June 2013, Ang-2 levels were measured in patients with pleural effusion who were admitted to our chest disease clinic. The patient group involved 25 patients diagnosed with MPE, based on the criteria of lung cancer and other organ malignancies, and malignant pleural effusion. The control group consisted of 41 patients – 13 with transudate according to the Light criteria and 28 with exudate other than MPE (parapneumonic, tuberculous pleurisy, embolism, etc.). A diagnosis of pleural effusion was established by a physical examination, PA chest x-ray, computed tomography and thoracic ultrasound. Demographic characteristics, radiological findings, complete blood count, routine biochemistry, erythrocyte sedimentation rate (ESR) and C-reactive protein levels were recorded for all patients. A 50-ml pleural fluid was withdrawn by way of a pleural puncture. The appearance of the fluid, white blood cell count, glucose, total protein, albumin, and LDH and ADA levels were determined. The differentiation between transudate and exudate was based on Light's criteria. Patients with transudative pleural effusions underwent no further diagnostic procedure, while an inoculation into the Löwenstein-Jensen medium and nonselective medium was performed for exudative effusions. A cytologic examination was performed for all pleural effusions. Patients with an exudative effusion in whom the cytologic examination was non-diagnostic underwent a closed needle biopsy or video-assisted thoracoscopy (VATS).

Ang-2 measurement

The pleural fluid samples were centrifuged at 1,200 rpm for 7 minutes and stored at -80 degrees. The Human Ang-2 Enzyme-linked immunosorbent assay (ELISA) Kit (RayBiotech, New York, US) was used for the measurement of Ang-2 levels, with the results expressed as ng/ml.

Statistical analysis

SPSS for Windows version 14.0.0 (SPSS Inc., Chicago, Illinois, US) software was used for all statistical analyses. Along with descriptive statistics (mean, standard deviation, frequency, percent distribution) in the analysis of data, a one-way analysis of variance (ANOVA) was used to compare data with normal distribution between the groups, a Tukey's multiple comparison test was used in the comparison of subgroups, an independent samples t-test was used for the paired comparison of the groups, a Chi-square test was used for the comparison of qualitative data, and Pearson's correlation coefficient was used to evaluate the relationship between variables. The area under the curve (AUC) in a receiver operating characteristic (ROC) curve analysis was calculated to determine the sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio (LR+) and cut-off value of ang-2 in the differentiation of malignant pleural effusions. The significance of the results was evaluated at an alpha level of 0.05.

Results

Demographic characteristics

The study included 25 patients with MPE and 41 control patients, 13 of whom had transudative and 28 of whom had exudative pleural effusion. Of the patients with MPE, 64% were male (n=16) and 36% were female (n=9). Of the patients in the transudative effusion group, 84.6% were male (n=11) and 15.4% were female (n=2), whereas 92.9% of the patients in the benign exudative effusion group were male (n=26) and 7.1% were female (n=2). The mean age was 64.96 (16.25) years in the patient group, 77.92 (11.04) years in the transudative effusion

group and 41.61 (24.82) years in the benign exudative effusion group (Table 1).

There was a statistically significant difference in terms of age between the transudate, benign exudate and malignant exudate groups (P<0.001). The mean age was significantly lower in the benign exudate group than in the transudate and malignant exudate groups (P=0.001, P=0.002, respectively), whereas the mean age did not differ significantly between the transudate and malignant exudate groups (P=0.06). The number of female patients was significantly higher in the malignant exudate group than in the transudate and benign exudate groups (P=0.03).

Patient characteristics

The distribution of patients with MPE was as follows: 12 patients (48%) had lung cancer, three (12%) had breast, colon or gastric cancer, two (8%) had multiple myeloma, two had chronic lymphocytic leukemia, one (4%) patient had non-Hodgkin lymphoma and one had ovarian cancer. Of the patients with a benign exudate, 11 (39.3%) had parapneumonic effusion, 13 (46.4%) had tuberculous pleurisy, three (10.7%) had an undiagnosed effusion and one (3.6%) had an embolism. The pleural biopsy was inconclusive in three patients in whom a diagnosis could not be established. All transudative effusions were secondary to congestive heart failure.

Ang-2 levels in the pleural fluid

Pleural fluid Ang-2 levels were found to be higher in both the benign and malignant exudates than in the transudative pleural effusions (P<0.001) (Table 2, Figure 1). The mean pleural fluid Ang-2 levels was higher in the benign exudate group [17.84 (2.99) ng/ml] than in the malignant exudate group [17.37 (3.88) ng/ml], although the difference was not statistically significant (P=0.87) (Table 3). The Ang-2 level was useful in differentiating between transudative and exudative pleural fluids, but was of no value in the differentiation of malignant and benign effusions.

Across the different etiologies, the highest pleural fluid Ang-2 levels were observed in the tuberculous pleurisy, pulmonary embolism, idiopathic benign exudate, malignant pleural effusion, parapneumonic effusion and congestive heart failure cases, in respective order (Figure 2).

Relationship between pleural fluid Ang-2 levels and other laboratory parameters

In the analysis of correlation, a positive correlation was identified between Ang-2 level and adenosine deaminase (ADA), and protein and albumin values (r=0.377, r=0.443, r=0.509, P=0.003, P=0.001 and P=0.001, respectively). Likewise, a significant positive correlation was identified between Ang-2 levels and erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels, and a negative correlation was found between Ang-2 and glucose levels (r=0.282, r=0.304, r=-0.298, P=0.03, P=0.02 and P=0.02, respectively) (Table 4).

Diagnostic value of Ang-2 in the differentiation of malignant exudate and benign exudate, exudate and transudate

The area under the curve in the ROC curve analysis for Ang-2 levels in the differentiation of exudative (malignant and benign exudates) and transudative effusions was 0.864 (0.758-0.936), which is above the desired threshold of 0.700. A cut-off value of >16.70 for Ang-2 yielded a sensitivity of 62.26%, a specificity of 92.31%, a positive predictive value of 97.1%, a negative predictive value of 37.5% and a likelihood ratio (LR) of 8.09 (Tables 5 and 6, and Figure 3).

The area under the curve in ROC curve analysis for Ang-2 in the differentiation of malignant and benign exudates was 0.515 (0.374-0.655), which is below the desired threshold of 0.700. A cut-off value of >13.84 for Ang-2 yielded a sensitivity of 20%, a specificity of 89.29%, a positive predictive value of 62.5%, a negative predictive value of 55.6% and a likelihood ratio (LR) of 1.87 (Tables 7 and 8, and Figure 4).

Table 1: Demographical characteristics of patients

		Transudate		Benign Exudate		Malignant Exudate		*P*-value [1]
Angiopoietin-2 ng/ml		77.92 (11.04)		41.61 (24.82)		64.96 (16.25)		<0.001
Gender	Male	11	84.6%	26	92.9%	16	64.0%	0.03
	Female	2	15.4%	2	7.1%	9	36.0%	

[1] One-Way analysis of variance, Chi-square test

Table 2: Angiopoietin-2 values in the transudate and exudate groups

	Transudate		Exudate		*P*-value [1]
	Mean.	SD	Mean.	SD	
Angiopoietin-2	11.86	3.49	17.62	3.41	<0.001

[1] Independent samples t-test

Table 3: Angiopoietin-2 values in the patient groups

	Transudate	Benign Exudate	Malignant Exudate	*P*-value
Angiopoietin-2 (ng/ml)	11.86 (3.49)	17.84 (2.99)	17.37 (3.88)	<0.001 [1]
	11.86 (3.49)	17.84 (2.99)	-	<0.001 [2]
	11.86 (3.49)	-	17.37 (3.88)	<0.001 [2]
	-	17.84 (2.99)	17.37 (3.88)	0.87 [2]

[1] One-Way analysis of variance, [2] Tukey's multiple comparison test

Table 4: Results of a correlation analysis

		Angiopoietin-2	ADA
Angiopoietin-2	r	1	0.377
	p		0.003
ADA	r	0.377	1
	p	0.003	
WBC	r	0.082	-0.079
	p	0.517	0.554
Glucose	r	-0.298	-0.482
	p	0.019	0.0001
Protein	r	0.443	0.637
	p	0.0001	0.0001
Albumin	r	0.509	0.556
	p	0.0001	0.0001
LDH	r	0.195	0.229
	p	0.129	0.083
ESR	r	0.282	0.198
	p	0.031	0.156
CRP	r	0.304	0.067
	p	0.021	0.641

Pearson's Correlation Coefficient, ADA: adenosine deaminase, LDH: lactate dehydrogenase, CRP: C-reactive protein

Table 5: The area under the curve (AUC) in the receiver operating characteristic (ROC) curve in the differentiation of the exudate (Malignant + Benign) and transudate groups

	AUC in the ROC curve
Angiopoietin-2	0.864 (0.758-0.936)

Table 6: Sensitivity, specificity and cut-off point for Angiopoietin-2 in the differentiation of exudate (Malignant + Benign) and transudate groups

Cut-off point	Sensitivity	Specificity	PPV	NPV	LR (+)
>16.70 (ng/mL)	62.26	92.31	97.1	37.5	8.09

PPV: positive predictive value, NPV: negative predictive value, LR (+): likelihood ratio

Table 7: The area under the curve (AUC) in the receiver operating characteristic (ROC) curve in the differentiation of the malignant exudate and benign exudate groups

	AUC in the ROC curve
Angiopoietin-2	0.515 (0.374-0.655)

Table 8: Sensitivity, specificity and cut-off point for Ang2 in the differentiation of the malignant exudate and benign exudate groups

Cut-off point	Sensitivity	Specificity	PPV	NPV	LR (+)
>13.84	20.00	89.29	62.5	55.6	1.87

PPV: positive predictive value, NPV: negative predictive value, LR (+): likelihood ratio

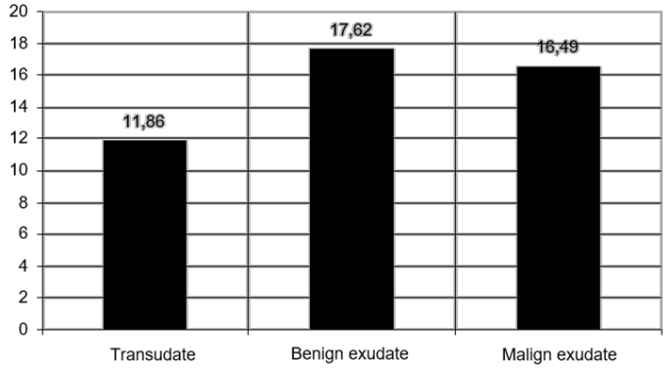

Figure 1: Distribution of Ang-2 values across the patient groups

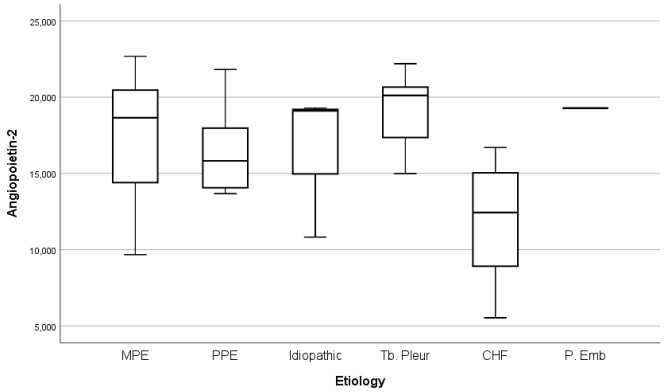

Figure 2: The highest pleural fluid Ang-2 levels according to the etiologies, MPE: Malignant pleural effusion, PPE: Parapneumonic effusion, Tb.Pleur: Tuberculosis pleuresy, CHF: Congestive heart failure, P. Emb: Pulmonary embolism

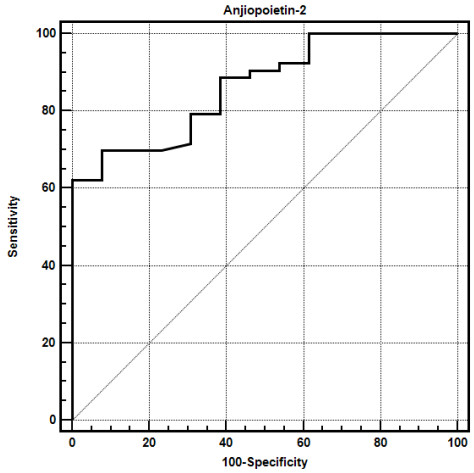

Figure 3: The ROC curve for the differentiation of the exudate (Malignant+Benign) and transudate groups

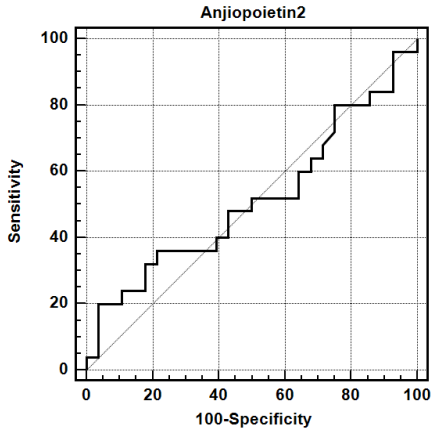

Figure 4: The ROC curve for the differentiation of the malignant exudate and benign exudate groups

Discussion

Ang-2 is known to affect the progression, invasion, metastatic characteristics and prognosis of a variety of tumors, and for this reason, recent studies have evaluated Ang-2 levels in such bodily fluids as serum, bronchial lavage fluid and pleural fluid, as well as in the tumor tissue, in order to identify any relationship between Ang-2 levels and tumor characteristics [14,15].

The present study investigates the diagnostic value of pleural fluid Ang-2 levels in the differentiation of malignant and benign pleural effusions. The results suggest that pleural fluid Ang-2 level was of no diagnostic value in the differentiation of malignant pleural effusions and benign pleural effusions, although it does have value in the differentiation of transudate and exudate.

Although it has been demonstrated that Ang-2 plays an important role in the regulation of tumor-related angiogenesis, increased vascular permeability and inflammation, all of which are important steps in the pathogenesis of MPE, no studies have found a significant difference in the pleural fluid Ang-2 levels of malignant pleural effusions and benign exudative effusions. Studies have even found higher pleural fluid Ang-2 levels in tuberculous pleural effusions and parapneumonic effusions than in malignant effusions [16-18].

Ioannis et al. identified significantly higher VEGF and Ang-2 levels in exudative pleural effusions than in transudative effusions [16]. The authors noted lower Ang-2 levels in malignant pleural effusions than in parapneumonic and tuberculous pleural effusions, and the highest Ang-2 levels were recorded in the pleural tuberculosis group. Similarly, Elhefny et al. [18] found significantly higher pleural fluid Ang-2 levels in exudative effusions than in transudative effusions, suggesting that Ang-2 levels are higher in benign exudative effusions than in malignant pleural effusions, with the highest Ang-2 levels being observed in the pleural tuberculosis group. Their study also found higher interleukin-8 (IL-8) levels, a marker of inflammation, in malignant effusions, and reported a positive correlation between pleural fluid Ang-2 and ADA levels in tuberculous pleural effusions. Similar to these two studies, the present study reports the highest pleural fluid Ang-2 levels in the pleural tuberculosis group and a significantly positive correlation was identified between pleural fluid Ang-2 levels and pleural fluid ADA levels. The present study also found a positive correlation between pleural fluid Ang-2 levels and ESR and CRP, which are markers of inflammation. The presence of a positive correlation between pleural fluid Ang-2 levels and such inflammatory markers as IL-8, ESR and CRP, and findings of higher Ang-2 levels in parapneumonic and tuberculous pleural effusions, suggest that Ang-2 is increased in pleural effusions in which inflammation is prominent.

Although studies have yet to detect any significantly increased Ang-2 levels in malignant pleural effusions, a study of mice showed a remarkable decrease in the amount of pleural fluid and pleural tumor foci with the inhibition of Ang/Tie-2 [19]. Fang et al. [20] further demonstrated that the combined inhibition of Ang-2 and VEGF showed synergistic effects in reducing the production of malignant pleural effusions and tumor

growth, and claimed that this combination could be used in the treatment of MPE in the future.

Other studies into pleural fluid Ang-2 levels showed higher Ang-2 levels in exudative effusions than in transudative effusions. In a similar study, and consistent with a previous study, Tomimoto et al. [17] found higher VEGF and Ang-2 levels in exudative pleural effusions than in transudative effusions. In a study by Sanad et al. evaluating pleural fluid Ang-1 and Ang-2 levels in 40 patients with transudative pleural effusion and 40 patients with exudative pleural effusion, the Ang-2 levels were reported to be significantly higher in the exudative pleural effusion group than in the transudative pleural effusion group [21].

Elhefny et al. [18] found significantly higher Ang-2 levels in benign exudative effusions than malignant exudative effusions. In their study, the mean Ang-2 level was 15.38 (6.33) in the benign exudative effusion group and 10.73 (4.22) in the malignant exudative effusion group. The present study, however, found no statistically significant difference in the pleural fluid Ang-2 levels between the benign and malignant exudative effusion groups. In the study of Elhefny et al. [18], the area under the curve in a ROC curve analysis for Ang-2 levels in the differentiation of malignant and benign exudative effusions was found to be 0.704, which is above the desired threshold of 0.700. A cut-off value of 15.67 ng/mL yielded a sensitivity of 91.3% and a specificity of 56.2%. In the present study, the area under the curve in the ROC curve analysis in the differentiation of malignant and benign exudative effusions was 0.515, which is below the desired threshold of 0.700. The cut-off value was found to be 13.84 ng/ml. Sensitivity was considerably low (62.26%), and specificity was 92.31%. In the differentiation between malignant exudative pleural effusions and benign exudative pleural effusions, the present study found that a patient with a pleural fluid Ang-2 level greater than 13.84 was found to be 1.87 times more likely to have a malignant pleural effusion than a patient with a pleural fluid Ang-2 level below 13.84. The study concludes that Ang-2 is of low value in the differentiation of malignant and benign exudates due to the likelihood of being less than 2 and an AUC of 0.515 in the ROC curve analysis.

Conclusion

Similar to the results reported in literature, the present study shows that pleural fluid Ang-2 level is valuable for differentiating between transudate and exudate, but is of no value in the differentiation of malignant and benign pleural effusions. The present study also found that Ang-2 levels are higher in pleural tuberculosis and parapneumonic effusions where inflammation is more prominent, and that Ang-2 levels positively correlate with CRP and ESR, which are the markers of inflammation. Despite the small number of study patients, the similarity of the results of the present study with those reported in literature increases their value.

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