Prognostic value of pro-inflammatory cytokine and pro-angiogenesis factor in differentiating malignant from benign exudative effusion

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Abstract

Background and Aims: The precise mechanism of pathogenesis in exudation of effusions is uncertain. Released factors in inflammation and malignancy of pleura are related to incremented permeability of the micro-pleural vessels. Angiopoietins (Ang) take part in development of angiogenesis and pleural inflammation. Interleukin-8 (IL-8) influences proliferation and tumor angiogenesis and it is expressed in cancer. The aims of this study were to investigate the relationship between inflammation, angiogenesis and etiologies of exudative effusions, and to evaluate the diagnostic value in differentiating malignant from benign.

Methods: The study includes 49 pleural fluid (PF) samples. Ang-2 and IL-8 in PF and serum were estimated.

Results: Ten patients were transudative and 39 patients were exudative fluid, subdivided into 16 benign and 23 malignant effusion. Ang-2 and IL-8 either fluid level or ratio were in significantly high in exudative more than in transudative fluid ($P = 0.002$). Ang-2 and IL-8 in PF were in high level than in serum of exudative and transudative. Ang-2 fluid level and ratio were significantly high in benign exudative effusion ($P = 0.01$, $P = 0.05$, respectively), while IL-8 level was significantly high in malignant exudative effusion ($P = 0.04$). Cut-off points for PF Ang-2 and IL-8 in differentiating malignant from benign exudative were 15.67 ng/mL, 325.54 pg/mL, respectively.

Conclusion: Our results support the evidence that angiogenesis and inflammatory pathways are linked, and that inflammation and vascular permeability of pleura constitutes the pathogenic basis of the majority of exudative effusion.

Please cite this paper as: Elhefny RA, Shaban MM and Shaker OG. Prognostic value of pro-inflammatory cytokine and pro-angiogenesis factor in differentiating malignant from benign exudative effusion. *Clin Respir J* 2015; ••: ••–••. DOI:10.1111/crj.12302.

Summary at glance:

We studied the pleural fluid and serum levels of angiopoietin-2 and interleukin-8 in order to explore correlation between inflammation and angiogenesis with different etiologies of exudative effusions in order to diagnose, differentiate malignant from benign effusion and improve diagnosis decision through comprehensive tools that help with differential diagnosis.

Abbreviations:

ADA adenosine deaminase
Ang angiopoietins
EGF endothelial growth factor
ELISA enzyme-linked immunosorbent assay
IL-8 interleukin-8
LDH lactate dehydrogenase
ROC receiver operator characteristic
SPSS Statistical Package for the Social Science
VEGF vascular endothelial growth factor.

Key words

angiopoietin-2 – diagnosis – exudative effusions – interleukin-8 – malignant effusion

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Received: 09 March 2014
Revision requested: 08 March 2015
Accepted: 23 March 2015

DOI:10.1111/crj.12302

Authorship and contributorship

Contribution by all authors has been done during the planning, lay outing, analysis and clarification of data, outlining the paper or updating it critically for necessary rational content. All authors have approved the final version of the paper for submission. All authors had full access to all of the data in the study and take responsibility for the integrity of the data. All authors are responsible for the analysis of the data and its accuracy.

Ethics

This research was approved by the Scientific Research Ethics Committee [session No (1) on 25/3/2014]

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Introduction

Pleural effusion is correlated with both benign and malignant diseases. It is classified into exudative effusion and transudative effusion. Transudative effusion is noticed in patients with heart failure, renal failure and liver cirrhosis, whereas exudative effusion is noticed in numerous diseases including malignant diseases either primary including lung cancer and mesothelioma or secondary, pneumonia, pulmonary tuberculosis and collagen diseases (1).

Pleural effusion formation involves different mechanisms such as permeability of capillaries of pleural membrane is increased, pressure is increased of pulmonary capillary, negative pressure is decreased of intrapleural space, oncotic pressure is decreased and obstruction of lymphatic flow. The precise mechanism of pathogenesis in exudation of effusions is still uncertain (2).

Inflammation and vascular hyper-permeability causing leakage of plasma are basic to the formation of exudative pleural effusion rich in protein (3). Released factors in inflammation and malignancy of pleura are related to incremented permeability of the micropleural vessels (3). Angiopoietins (Ang) act an essential part in development of angiogenesis in health and in disease (4). Ang-1 and Ang-2 act as binding molecule for tyrosine kinase with immunoglobulin-like and endothelial growth factor (EGF)-like domains 2, which is tyrosine kinase's receptor categorically shown on cell of endothelium (5). The instability of blood vessels is induced by Ang-2, which also facilitates angiogenesis in the presence of vascular EGF (VEGF) (6). Ang-2 measurements are raised in pleural effusions of exudative cause, recommending that Ang-2 as well as VEGF take part in inflammatory process and in the pathogenic mechanism of exudative pleural effusions (7).

Interleukin (IL-8) is a protein of non-glycosylated nature. Originally, IL-8 was purified from monocytes; however, numerous other cells can produce IL-8, for example, cells of endothelium and epithelium, hepatocytes, fibroblasts and chondrocytes (8). IL-8 was initially found as an effective attractor and activator of polymorphonuclear leukocytes. It likewise influences proliferation and migration of malignant cells, tumor angiogenesis and metastasis and that it is expressed in many cancerous cell types (9).

In order to investigate the relationship between inflammation, angiogenesis and etiologies of exudative effusions, the current study tries to search the role of both pro-inflammatory cytokine (IL-8) and pro-angiogenesis factor (Ang-2) in pleural effusions and further evaluation of their diagnostic value in differentiating malignant from benign exudative effusion.

Methods

A total of 49 pleural fluid samples, obtained from patients (32 men and 17 women) who underwent thoracocentesis in the Chest Department of Cairo University and Fayoum University Hospitals between January 2013 and August 2013.

The protocol was accepted by the Committee of Ethics of the Faculty of Medicine, Fayoum University, and samples were examined after written informed consent had been obtained.

All patients submitted to detailed history, through clinical examination, routine chemical and hematological blood analysis including liver and kidney functions tests, complete blood count, and coagulation profile, plain chest X-ray (postro-anterior and lateral views) and computed tomography scan of chest. All pleural fluid samples submitted to analysis in the form of biochemical, cytological and microbiological. Some diagnoses were established after flow cytometry and some after histopathologic results.

All samples of effusion of unknown origin were excluded.

Biochemical analysis

The next biochemical variables were done at same time in all samples (serum, pleural fluid and pleural fluid/serum ratio) including total protein, lactate dehydrogenase (LDH), glucose, adenosine deaminase (ADA) were calculated.

They further divided into exudates or transudates according to light's criteria (10).

Pleural fluid and serum Ang-2 levels estimation

Human Ang-2 levels were measured in both serum and fluid effusion using Quantikine enzyme-linked immunosorbent assay (ELISA) kit provided by R&D system Inc. (Minneapolis, MN, USA).

This assay applies a technique of quantitative sandwich enzyme immunoassay. A monoclonal antibody specific for Ang-2 has been applied onto a microplate in coating layers. Standards and samples are pipetted into the wells and any Ang-2 present is bound by the immobilized antibody. After any unbound substances washes away, an enzyme-linked monoclonal antibody specific for Ang-2 is put in to the wells. This is followed by the addition of a substrate solution to the wells;

development of color is proportioned to the amount of Ang-2 then development of the color is stopped and the intensity is measured.

Pleural fluid and serum IL-8 levels estimation

IL-8 ELISA is an enzyme-linked immunosorbent assay for the quantitative identification of human IL-8 in cell culture supernatants, plasma (heparin and citrate), serum and other body fluids. The assay recognized both natural and recombinant human IL-8.

This assay recruited an antibody specific for human IL-8 coated on a 96-well plate. Standards, samples and biotinylated anti-human IL-8 are pipetted into the wells and IL-8 present in a sample is caught by the antibody immobilized to the wells and by the biotinylated IL-8-specific detection antibody. After unbound biotinylated antibody is washed away, horseradish peroxidase-conjugated streptavidin is pipetted to the wells. The wells are washed again. Following this step of second wash, the addition of tetramethylbenzidine substrate solution to the wells, resulting in development of color proportional to the amount of IL-8 bound. The color of the stop solution changes from blue to yellow, and the measurement of the intensity of the color is done at 450 nm.

Statistical analysis

Data were statistically explained in terms of mean ± standard deviation, median and range, or frequencies (number of cases) and percentages when needed. For comparing numerical variables between the study groups; Mann–Whitney *U*-test for independent samples was used. Chi-squared (χ^2) test was performed for comparison of categorical data. When the expected frequency is less than 5, we used exact test as another option. Various variables were correlated using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal. The terms sensitivity and specificity were used to express accuracy. Determination of the optimum cut-off value for the markers in the study was done using receiver operator characteristic (ROC) analysis. *P* values less than 0.05 was regarded statistically significant. Computer program SPSS (Statistical Package for the Social Science; SPSS, Inc., Chicago, IL, USA) (version 15 for Microsoft Windows) were used for calculation of all statistical data.

Results

In the 49 patients, 10 had transudative pleural effusions (five hepatic, two renal and three hypoalbuminemia) and 39 had exudative pleural effusions. The 39 patients were subdivided into 16 benign and 23 malignant effusion. Malignant effusion were diagnosed as 10 bronchogenic carcinoma, four metastatic (two cases from breast cancer one from renal adenocarcinoma and one from cancer colon), one multiple myeloma, three non-Hodgkin lymphoma, and five mesothelioma, while benign effusion were diagnosed as seven para-pneumonic empyema, seven tuberculosis, one systemic lupus erythematosus and one hemothorax, as shown in Table 1.

Table 1. Various etiologies of pleural effusion in the present study

Etiology	Patients number (% within the group) $N = 49$
Exudative effusion	39 (100.0)
Bronchogenic carcinoma	10 (25.6)
Metastatic	4 (10.3)
Multiple myeloma	1 (2.6)
Non-Hodgkin lymphoma	3 (7.7)
Mesothelioma	5 (12.9)
Para pneumonic empyema	7 (17.9)
Tuberculosis effusion	7 (17.9)
Systemic lupus erythematous	1 (2.6)
Hemothorax	1 (2.6)
Transudative effusion	10 (100.0)
Hepatic hydrothorax	5 (50.0)
Renal hydrothorax	2 (20.0)
Hypoalbuminmia	3 (30.0)
P value	0.002*
further classification of exudative effusion:	39 (100.0)
Benign effusion	16 (41.0)
Malignant effusion	23 (59.0)

*$P < 0.05$ significant.

As regards demographic and pleural fluid data of the studied cases, the age and gender of the study cases showed no significant difference ($P = 0.872$, $P = 0.459$, respectively). There were 24 men and 15 women, with mean age 52.51 ± 13.22 years, in exudative effusion cases, while that of transudative effusion; eight were men and two were women, with mean age 53.80 ± 5.61 years, as shown in Table 2. Values of the biochemical analysis of pleural effusion showed differences between transudative and exudative effusion, which is significant ($P = 0.002$) as shown in Table 2.

Both Ang-2 in fluid and fluid : serum ratio were in significantly high level in exudative than in transudative pleural effusion ($P = 0.002$). Ang-2 in fluid was in a higher level than Ang-2 in serum in both of exudative and transudative pleural effusions

Table 2. Demographic and laboratory data of pleural fluid of the studied cases

Characters	Exudative effusion	Transudative effusion	*P* value
Age	52.51 ± 13.22	53.80 ± 5.61	0.872
Sex (male/female)	24/15	8/2	0.459
Pleural fluid protein (g/L)	5.65 ± 1.03	2.53 ± 0.75	0.003*
Pleural fluid/serum protein	0.82 ± 0.22	0.34 ± 0.08	0.003*
Pleural fluid LDH (IU/L)	699.9 ± 479.1	130.5 ± 26.18	0.001*
Pleural fluid/serum LDH	1.79 ± 1.41	0.35 ± 0.09	0.004*
Pleural fluid glucose (mg/dL)	72.26 ± 16.6	106.7 ± 49.58	0.002*
Pleural fluid/serum glucose	0.67 ± 0.25	0.85 ± 0.11	0.008*
Pleural fluid adenosine deaminase (U/L)	23.97 ± 19.59	9.09 ± 1.51	0.001*

Data are expressed as mean ± standard deviation.
*$P < 0.05$ significant.
LDH, lactate dehydrogenase.

(12.63 ± 5.61 vs 1.45 ± 0.93 ng/mL, 5.26 ± 3.76 vs 5.24 ± 2.68 ng/mL, respectively). While IL-8 in fluid and in serum were in significantly higher level in exudative than in transudative pleural effusion ($P = 0.003$), IL-8 in pleural fluid was in a higher level than serum IL-8 level in both exudative and transudative pleural effusions (325.9 ± 77.34 vs 21.68 ± 10.34, 149.7 ± 50.76 vs 6.26 ± 1.71 pg/mL, respectively), as shown in Table 3.

Ang-2 and IL-8 levels were further assessed in benign and malignant exudative effusion. Both pleural fluid Ang-2 and pleural fluid : serum ratio were in a higher significance level in benign than in malignant effusions ($P = 0.011$, $P = 0.046$, respectively). While IL-8 in pleural fluid was in a significantly higher level in malignant than in benign exudative fluid ($P = 0.041$), as shown in Table 4.

Comparison of different causes of exudative pleural fluid as regards Ang-2 and IL-8 levels was done. As pleural fluid Ang-2 value was of high significance in benign than in malignant effusions, as shown in Table 4, patients with tuberculosis effusion had higher pleural fluid Ang-2 level (19.53 ± 4.88) than other etiologies, as shown in Table 5. As IL-8 in pleural fluid was in significantly high level in malignant more than in benign exudative fluid, as shown in Table 4, the mean value was higher in multiple myeloma (527.32) than in other etiologies, as shown in Table 5.

Ang-2 in pleural fluid showed negative correlation with age and pleural fluid glucose, which was significant, while it showed positive correlation with ADA in pleural fluid. Pleural fluid/serum ratio of Ang-2 was also significantly correlated positively with ADA in pleural fluid. Regarding pleural fluid, IL-8 was of significant positive correlation with pleural fluid glucose while it was correlated significantly and negatively with ADA in pleural fluid. The ratio of IL-8 in pleural fluid/serum showed significant negative correlation with ADA in pleural fluid, as shown in Table 6.

We used ROC curve to determine cut-off values for pleural fluid, Ang-2, pleural fluid : serum ratio of Ang-2 and pleural fluid IL-8 in differentiating malignant from benign exudative fluid, which were 15.67 ng/mL, and 12.45 and 325.54 pg/mL, respectively. Predictive values for diagnosis of malignant effusion showed pleural fluid Ang-2: sensitivity 91.3%,

Table 3. Ang-2 and IL-8 levels of the studied cases

Character	Exudative effusion ($N = 39$)	Transudative effusion ($N = 10$)	*P* value
Pleural fluid Ang-2 (ng/mL)	12.63 ± 5.61	5.26 ± 3.76	0.002*
Serum Ang-2 (ng/mL)	1.45 ± 0.93	5.24 ± 2.68	0.002*
Pleural fluid/serum Ang-2	11.43 ± 9.21	1.08 ± 0.63	0.001*
Pleural fluid IL-8 (pg/mL)	325.9 ± 77.34	149.7 ± 50.76	0.003*
Serum IL-8 (pg/mL)	21.68 ± 10.34	6.26 ± 1.71	0.003*
Pleural fluid/serum IL-8	17.18 ± 6.44	23.8 ± 3.65	0.005*

Data are expressed as mean ± standard deviation.
*$P < 0.05$ significant.
Ang-2, angiopoietin-2; IL-8, interleukin-8.

Table 4. Ang-2 and IL-8 levels in benign and malignant exudative effusion

Exudative effusion ($N = 39$)	Benign effusion ($N = 16$)	Malignant effusion ($N = 23$)	P value
Pleural fluid Ang-2 (ng/mL)	15.38 ± 6.33	10.73 ± 4.22	0.011*
Serum Ang-2 (ng/mL)	1.31 ± 0.26	1.55 ± 1.19	0.797
Pleural fluid/serum Ang-2	12.85 ± 6.27	10.43 ± 10.82	0.046*
Pleural fluid IL-8 (pg/mL)	297.29 ± 78.97	345.96 ± 71.16	0.041*
Serum IL-8 (pg/mL)	20.30 ± 6.77	22.63 ± 12.29	0.753
Pleural fluid/serum IL-8	15.64 ± 5.69	18.25 ± 6.82	0.253

Data are expressed as mean ± standard deviation.
*$P < 0.05$ significant.
Ang-2, angiopoietin-2; IL-8, interleukin-8.

specificity 56.2%; for pleural fluid : serum ratio of Ang-2: sensitivity 78.3%, specificity 50%; and for pleural fluid IL-8: sensitivity 52.2%, specificity 62.5%, as seen in Table 7 and Figs. 1–3, respectively).

Discussion

Pleural effusions can be associated with numerous pathologies, and the diagnosis generally needs a systematic assessment of the patients and a number of diagnostic procedures. Many helpful tests have been recommended to evaluate pleural effusions. Estimating different biomarkers is also recommended in differentiating para-pneumonic, tuberculosis and malignant pleural effusions. Regardless of the advances in biochemistry and invasive diagnostic procedures, the cause of pleural effusions remains unclear (11).

Permeability of the pleural microvasculature is increased and ascribed to the substances that are liberated in pleural effusions associated with inflammation and malignancy. Understanding the technique of fluid collection would expectantly help in developing more precise, potent and safe modality in treatment (12). Angiogenesis, increased permeability of vasculature of pleura and the process of inflammation are integrally recognized in the pathogenic mechanism of malignant pleural effusions (12).

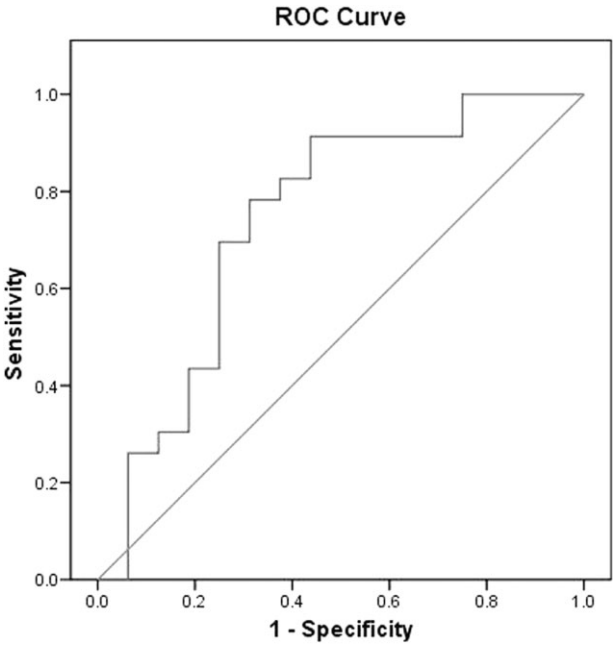

Figure 1. Receiver operator characteristic (ROC) curve for pleural angiopoietin-2 with 95% confidence.

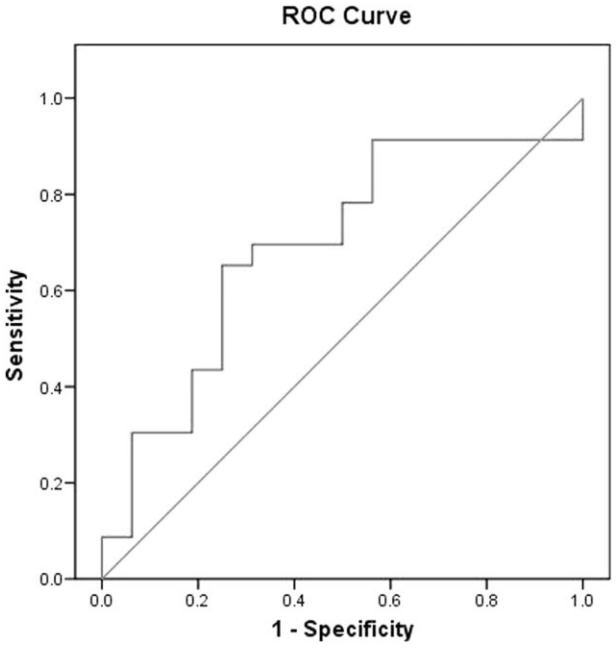

Figure 2. Receiver operator characteristic (ROC) curve for pleural fluid/serum ratio of angiopoietin-2 with 95% confidence.

Table 5. Ang-2 and IL-8 levels in patients with exudative effusion of different etiologies

Exudative effusion of different etiologies ($N = 39$)	Pleural fluid Ang-2 (ng/mL)	Serum Ang-2 (ng/mL)	Pleural fluid/serum Ang-2	Pleural fluid IL-8 (pg/mL)	Serum IL-8 (pg/mL)	Pleural fluid/ serum IL-8
Bronchogenic carcinoma ($N = 10$)	8.74 ± 2.15	1.16 ± 0.36	9.08 ± 6.44	328.42 ± 72.94	20.04 ± 9.94	18.58 ± 5.46
Metastatic ($N = 4$)	11.46 ± 6.84	3.01 ± 2.21	5.98 ± 5.85	333.66 ± 63.42	32.02 ± 4.88	10.64 ± 2.63
Multiple myeloma ($N = 1$)	10.00	1.62	6.17	527.32	60.96	8.65
Non-Hodgkin lymphoma ($N = 3$)	16.39 ± 4.42	1.19 ± 0.75	24.19 ± 26.15	364.2 ± 68.08	19.53 ± 3.11	18.93 ± 4.51
Mesothelioma ($N = 5$)	11.06 ± 3.25	1.38 ± 1.08	8.92 ± 3.76	344.83 ± 39.04	17.56 ± 1.72	21.47 ± 1.04
Para pneumonic empyema ($N = 7$)	13.49 ± 5.10	1.51 ± 0.188	8.73 ± 2.63	352.45 ± 79.84	21.61 ± 7.95	18.09 ± 6.49
Tuberculosis effusion ($N = 7$)	19.53 ± 4.88	1.08 ± 0.13	19.200 ± 1.65	233.98 ± 26.82	18.15 ± 5.24	13.35 ± 4.42
Systemic lupus erythematous ($N = 1$)	11.34	1.57	7.22	326.94	16.88	19.36
Hemothorax ($N = 1$)	3.62	1.25	2.89	324.67	29.68	10.93

Data are expressed as mean ± standard deviation.
Ang-2, angiopoietin-2; IL-8, interleukin-8.

Table 6. Correlation of Ang-2 and IL-8 levels with demographic and pleural fluid data within exudative cases

	Pleural fluid Ang-2 (ng/mL)		Serum Ang-2 (ng/mL)		Pleural fluid/ serum Ang-2		Pleural fluid IL-8 (pg/mL)		Serum IL-8 (pg/mL)		Pleural fluid/ serum IL-8	
Characters ($N = 39$)	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*R*	*P*
Age	–0.31	0.054*	0.07	0.632	–0.22	0.189	0.17	0.283	0.08	0.612	0.03	0.866
Pleural fluid protein (g/L)	0.04	0.799	–0.28	0.086	0.28	0.079	–0.08	0.605	–0.06	0.696	–0.06	0.712
Pleural fluid/serum protein	–0.20	0.214	0.09	0.549	–0.04	0.770	0.05	0.743	0.10	0.538	–0.10	0.507
Pleural fluid LDH (IU/L)	–0.09	0.560	0.16	0.323	–0.13	0.404	0.23	0.147	–0.00	0.984	0.18	0.263
Pleural fluid/serum LDH	–0.12	0.462	0.29	0.070	–0.19	0.236	0.16	0.312	0.00	0.999	0.12	0.466
Pleural fluid glucose (mg/dL)	–0.39	0.012*	0.05	0.733	–0.14	0.370	0.40	0.011*	0.16	0.305	0.23	0.155
Pleural fluid/serum glucose	–0.28	0.077	0.08	0.593	–0.10	0.513	0.29	0.073	0.28	0.081	0.06	0.681
Pleural fluid adenosine deaminase (U/L)	0.60	0.001*	–0.19	0.224	0.37	0.018*	–0.53	0.001*	–0.12	0.463	–0.33	0.038*

*$P < 0.05$ significant.
Ang-2, angiopoietin-2; IL-8, interleukin-8; LDH, lactate dehydrogenase.

Table 7. Predictive values of pleural Ang-2, Ang-2 ratio and pleural IL-8 cut-off points in differentiating malignant from benign exudative effusion

Parameters	Pleural Ang-2 (ng/mL)	Pleural/serum Ang-2 ratio	Pleural IL-8 (pg/mL)
Area under receiver operator characteristic curve	0.74	0.69	0.69
Standard error	0.087	0.09	0.09
95% confidence interval	0.57–0.91	0.52–0.86	0.52–0.87
Significance level *P* value (area = 0.05)	0.01	0.05	0.04
Cut-off point	15.67	12.45	325.54
Sensitivity percentage	91.3	78.3	52.2
Specificity percentage	56.2	50	62.5

Ang-2, angiopoietin-2; IL-8, interleukin-8.

In order to investigate the relationship between inflammation, angiogenesis and etiologies of exudative effusions, the current study tries to explore the role of both pro-inflammatory cytokine (IL-8) and pro-angiogenesis factor (Ang-2) in pleural effusions and further evaluate their diagnostic value in differentiating malignant from benign exudative effusion.

The current study displays that Ang-2 in pleural fluid was in significant high level in exudative than in transudative, as shown in Table 3. This was in compatibility with previous studies (7, 13, 14), which found that the pleural fluid Ang-2 was in significant high level in patients with exudative more than transudative fluid.

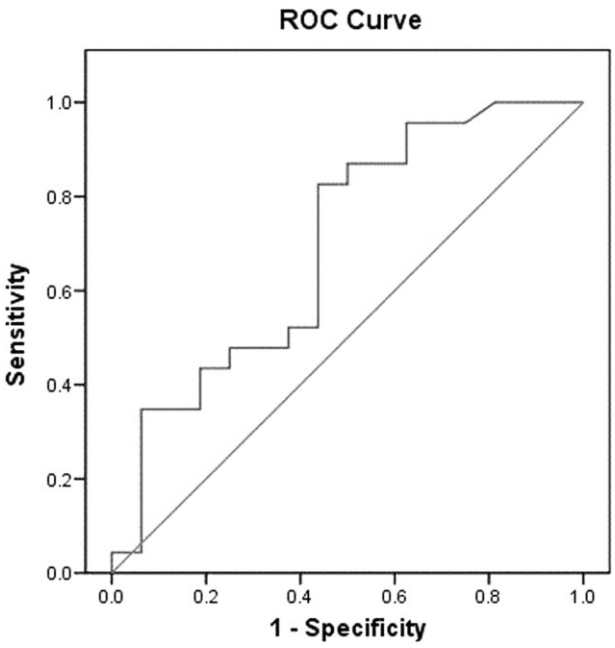

Figure 3. Receiver operator characteristic (ROC) curve for pleural interleukin-8 with 95% confidence.

These findings suggest that Ang-2 shares a cardinal role in the pathogenesis mechanism of various etiologies of exudative fluid (7).

Ang-2 in pleural fluid was in high level than Ang-2 in serum level in both exudative and transudative fluid as shown Table 3. That was coincided with Kalomenidis *et al*. (7), who proposed that local production of Ang-2 in pleural space occurs in diseases of pleura with malignancy and inflammation, and further production of Ang-2 is mainly by the cells of endothelium and surrounding cells of the microvessels of the pleura, as mesothelium and inflammatory cells have not been recorded to produce Ang-2 (7, 15).

Similarly, Sanad *et al*. (14) found that Ang-2 in pleural fluid was in significant high level than Ang-2 in serum in cases with exudative than transudative.

We also made a comparison between various etiologies of exudative fluid regarding different level of Ang-2, as Ang-2 in pleural fluid was in significant high level in benign more than in malignant effusions, as shown in Table 4. Patients with tuberculosis effusion had higher mean value than other etiologies as shown in Table 5. This findings support the evidence of hyper-permeability and pro-inflammatory function of Ang-2 and its participation in pleural inflammation (7, 16).

Similarly, Kalomenidis *et al*. (7) and Sanad *et al*. (14) who found that patients with tuberculosis pleuritis had higher pleural effusion Ang-2 level than other etiologies. In contrast, Tomimoto *et al*. (13) proved that Ang-2 was in significant high level in malignant effusion when compared with other etiologies (tuberculosis and heart failure).

In this study, pleural fluid Ang-2 was correlated significantly and negatively with pleural fluid glucose as shown Table 6. This in adjustment with Kalomenidis *et al*. (7) and Sanad *et al*. (14) who suggested that decreased level of glucose is mainly because of high

metabolic activity of pleural cavity, which occurs in pleural diseases associated with intense inflammatory reaction (7).

In this work, pleural fluid Ang-2 correlated significantly and positively with pleural fluid ADA, as shown Table 6, suggesting that Ang-2 share in pleural inflammation and in the pathogenesis of exudative tuberculous effusion.

In contrast, Kalomenidis *et al*. (7) and Sanad *et al*. (14) found that pleural fluid Ang-2 correlated significantly and positively with pleural fluid protein, LDH in pleural fluid, protein ratio of pleural fluid/serum and LDH ratio of pleural fluid/serum.

In the present study, IL-8 in pleural fluid was in high level than serum IL-8 in both exudative and transudative fluid as shown in Table 3. This indicates that this cytokine is collected at the site of the pathological process (8).

The present study showed that the pleural fluid IL-8 level was significantly higher in malignant than in benign exudates as shown in Table 4. These findings are in agreement with previous findings that carcinomatous pleural effusions contain high amounts of IL-8 (17, 18).

This supports the evidence that IL-8 is expressed in many cancerous cell types (9), is derived from tumor and may recruit inflammatory neutrophils, and promotes malignant and inflammatory cell interaction (19).

In contrast, Segura *et al*. (20) and Aleman *et al*. (21) observed an increase in pleural fluid IL-8 in infectious effusions. While Alexandrakis *et al*. (22) measured IL-8 level in benign and malignant pleural effusions, and found that IL-8 was high in benign pleural effusion, and stated that IL-8 can be found locally during the activity of benign and malignant disease with pleural effusion.

According to the ROC curve, pleural Ang-2 was more sensitive than pleural IL-8 in diagnosis of malignant effusion (91.3% compared with 52.2%); however, given higher percentage of false-positive (43.8%), the specificity decreases when compared with pleural fluid IL-8 (56.2% vs 62.5%) as shown in Table 7.

This study is in agreement with Costa *et al*. (23), who found that both angiogenesis and inflammation share common pathways with the same triggering molecular events. Our limitations for this study were the absence of follow-up for both level of mediators in order to observe their prognostic value, and the small size of the included samples of patients.

Conclusion

Our results of both pro-inflammatory cytokine (IL-8) and pro-angiogenesis factor (Ang-2) support the evidence that both angiogenesis and inflammatory pathways are closely linked, in addition both are in cooperated with infection and malignancy and that pleural inflammation and pleural vascular permeability constitutes the pathogenic base of huge and larger part of exudative effusion. Further studies are needed to detect if both mediators share an important role in the pathogenesis process of different etiologies of exudative effusion, and to find out their detected level locally in pleural fluid of significant or not. If this proves to be true, it will be helpful in designing novel therapeutic strategies to use locally in pleural space using nanotechnology (Ang-2 inhibitor and IL-8-targeted inhibitory therapy).

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