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Research Article

The diagnostic value of C-reactive protein and adenosine deaminase biomarkers for differentiation of exudative pleural effusion

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ABSTRACT

Background: The aim of the present study was to investigate the roles biomarkers C-reactive protein (CRP) and adenosine deaminase (ADA) in the differentiation of exudative pleural effusion and tuberculosis from non-tuberculosis pleural effusion.
Methods: The present study enrolled 150 patients with pleural effusion that were admitted to hospital between 2012-2015. From the patients were obtained pleural fluid and serum which were administrated to test analyses within 24 hours. ADA and CRP levels were compared between the groups of exudative pleural effusion. Based on Light's criteria, biochemical, cytological etc. analyses were established the pleural effusion as exudative and the etiology of the effusion, 60 malign, 48 tuberculosis, 42 parapneumonic.
Results: ADA and CRP levels differ significantly between the different groups of exudative pleural effusion but ADA ratio, pleural fluid/serum, didn't contribute in this difference. As a better test for the difference of tuberculosis from non-tuberculosis was evaluated, ADA level in pleural fluid.
Conclusions: ADA and CRP are considerable biomarker for the differences in exudative pleural effusion groups and for the difference of tuberculosis from non-tuberculosis pleural effusion. It is recommended that that other markers need to be taken into consideration to assist the results.

Keywords: Adenosine deaminase, C reactive protein, Exudative pleural effusion, Tuberculosis, Non tuberculosis

INTRODUCTION

Pleural effusion is an abnormal accumulation of fluid in the pleural cavity influencing the respiratory process in causing difficulties in the normal movement of the lungs. In this case the pleural fluid formation is over passing its rate of absorption, and the pleural cavity has an exaggerated amount of pleural liquid in compare its normal state.[1] Based on Light's criteria and on biochemical, cytological and microbiological analyses can be possible in evaluating an exudative pleural effusion.[2] Once the possible exudative pleural effusion is set up it is needed to determine the etiology of the effusion. The common causes for an exudative pleural effusion are malign, parapneumonic and tuberculosis. Tuberculosis meanwhile is ranked as the second for the number of death worldwide, after HIV/AIDS, caused from a single infective agent, in 2014 the death worldwide from tuberculosis was 1.5 million people.[3] In Albania the incidence rate of tuberculosis is considered 19/100 000.[4] *Mycobacterium tuberculosis* grows very slowly and is needed from 2 to 6 weeks for the culture and the treatment often starts before the confirmation of the culture.[5] Further is needed a rapid and accurate diagnosis and the measurement of the biomarkers can provide a reliable information for the estimation of the etiology of the pleural effusion. Different biomarkers are proposed, that can facilitate the differentiation of the different groups of exudative pleural effusion and of tuberculosis from non-tuberculosis pleural effusion for an

earlier diagnose. From the Infection Disease Biomarker Database (IDBD) in this study will be focused on ADA and CRP as the biomarkers that have more reliable results in pleural effusion. Adenosine deaminase (ADA) is an enzyme associated with T lymphocyte activity and is produced from all the cells of human body but its level are higher in lymphocytes.[6] ADA plays an important role in the differentiation and maturation of the lymphoid system. C-reactive protein (CRP) is a protein of acute phase inflammation that is produced by liver and is present in the body before the antibodies.[7] CRP has the ability to recognize the pathogens and to eliminate them through the recruitment of the phagocytosis cells and the complement system. The aim of the present study was to measure the level of ADA and CRP biomarkers in pleural fluid and serum in patients with exudative pleural effusion and to evaluate the possibility for differentiating the different types of exudative pleural effusion and tuberculosis from non-tuberculosis pleural effusion.

METHODS

Patients admitted with pleural effusion at the University Hospital 'Shefqet Ndroqi', between 2012-2015 were enrolled in the present study. The patient agreed to be part of the study and their health history was taken in consideration. The patients underwent thoracentesis and for each subject at least 30 ml of pleural fluid was collected in syringe. Venous blood samples and pleural fluid were collected under aseptic conditions, simultaneously, and all patients underwent serum and pleural fluid measurements within 24 hours. Based, on Light's criteria, on cytological, on biochemical analyses, etc it was possible to determine three types of exudative pleural effusion. For the categorizations of the different types of exudative pleural effusion was based on the following conditions; malign when were detected malignant cells on cytological examination; tuberculosis when a) *Mycobacterium tuberculosis* is isolated from the pleural fluid or the pleural tissue sample, b) Necrotic granulomas were found in pleural biopsy tissue samples; parapenumonic when chest radiographs revealed pulmonary infiltrates. To measure the level of CRP in blood and liquid were used the test of CRP with COBAS 6000, Roche company. To measure ADA level was used the colorimetric method of Giusti and Galanti, and the result of the test were measure on 628 nm. To carry out statistical analyses and to present the results were used the program of Microsoft office Excel, SPSS version 20 (IBM statistics 2011). The data were presented as mean±standard deviation (SD). The tests used in this study for the intergroup comparison of more than two groups of non-parametric data was Kruskal-Wallis H. Post-hoc tests were used in the assessment of data established to be significant in Krsukal-Wallis H. Receiver operating characteristic (ROC) were used to investigate the diagnostic value. For the tests used in this study, the differences are considered significant for $p<0.05$, $\alpha=5\%$.

RESULTS

The study was carried out in 150 patients (91 females (60%) and 59 male (40%); mean age 47.28±17.98). Based on Light's criteria and on cytological, biochemical data were identified 60 patients malign, 48 patients with tuberculosis and 42 patients with parapneumonic effusion. The distribution of the 150 patients according to the groups of the exudative pleural effusion and the respected value of ADA and CRP in liquid, serum and pleural fluid/serum was presented in (Table 1).

Table 1: The mean pleural fluid, serum and pleural fluid/serum ADA, CRP values in patients classified as exudative pleural effusion.

Type of exudative effusions	ADA pleural fluid (IU/L)	ADA serum (IU/L)	ADA pleural fluid/serum (IU/L)
Malign (68)	54.11±20.15	31.76± 14.45	2.23±1.76
Parapneumonic (42)	89.29±22.93	51.14± 16.12	1.89±0.68
Tuberculosis (48)	108.65±44.14	59.77± 23.73	2.16±1.29
Type of exudative effusion	**CRP liquid (mg/L)**	**CRP serum (mg/L)**	**CRP pleural fluid/serum (mg/L)**
Malign (68)	12.21±8.14	36.30± 12.10	0.37±0.26
Para pneumonic (42)	56.58±19.73	88.97± 25.71	0.68±0.29
Tuberculosis (48)	33.60±5.12	61.76± 8.65	0.55±0.12

ADA levels are higher in pleural fluid and in tuberculosis group and CRP levels and higher in serum and in parapneumonic group (Figure 1 and Figure 2). The biomarker ADA has higher mean in pleural fluid and tuberculosis reflects the highest level for ADA in pleural fluid. Meanwhile CRP has higher mean in serum and parapneumonic reflect the highest level for CRP in serum. The markers were evaluated for the differences of their value between the different types of exudative pleural effusion using the Kruskal Wallis H test (Table 2). The mean of biomarkers ADA and CRP has a significant difference between the different groups of exudative pleural effusion ($p<0.05$). No significant difference was observed in terms of pleural fluid/serum ADA for the differentiations of the different types of exudative pleural effusion ($p>0.05$). That was conducted as well the post hoc test in showing the difference that exists between the groups in pairs (Table 3). Parapneumonic and tuberculosis do not differ significantly between them; this means that their values

are quite close to each other for ADA in pleural fluid and serum. In this study the comparison of the groups between them it is not possible to be applied in the case of ADA pleural fluid/serum as its results were considered insignificant for the means difference of the different types of exudative pleural effusion. In the case of the ADA biomarker pleural fluid and serum the major differences were seen between the malign and tuberculosis type of exudative pleural effusion and in the case of CRP biomarker test the major differences were seen between the malign and tuberculosis type of exudative pleural effusion.

Table 2: The differences of biomarkers ADA and CRP values in the different types of exudative pleural effusion.

Kruskal wallis test	ADA pleural fluid	ADA serum	ADA pleural fluid/serum
Chi-square	63.297	46.655	0.003
df	2	2	2
p	0.000*	0.000*	0.998
Kruskal wallis test	**CRP pleural fluid**	**CRP serum**	**CRP pleural fluid/serum**
Chi-square	110.558	111.282	35.872
Df	2	2	2
p	0.000*	0.000*	0.000*

p<0.05 significant, *significant test

ROC analysis was carried out to evaluate the diagnostic value the ADA and CRP tests for the difference of the tuberculosis and non-tuberculosis pleural effusion, based on the AUC is possible to evaluate the quality of the test (Table 4). ROC curve of ADA pleural fluid and serum for the differentiation of tuberculosis and non-tuberculosis pleural effusion (Figure 3).

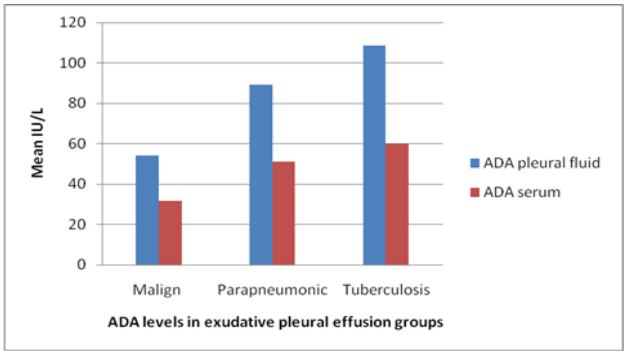

Figure 1: ADA levels in exudative pleural effusion groups.

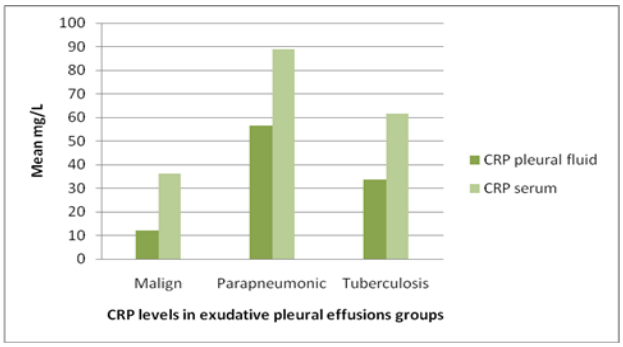

Figure 2: CRP levels in exudative pleural effusion groups.

The biomarkers values show significant result in the difference of the tuberculosis and non-tuberculosis pleural effusion. Since a high AUC was obtain for pleural fluid ADA in ROC analyses, we can conduct that the test of pleural fluid ADA is a better test for the differences of tuberculosis and non-tuberculosis pleural effusion, with a cut off value of 78.8 IU/L with a sensitivity and specificity respectively 75% and 67.65%.

Table 3: Post hoc test of Kruskal Wallis statistical test.

Biomarkers	Post hoc results	Malign parapneumonic	Malign-tuberculosis	Parapneumonic tuberculosis
ADA pleural fluid	Test statistic	-51.674	-61.765	10.091
	Adjusted p	0.000*	0.000*	0.815
ADA serum	Test statistic	-43.754	-53.356	9.063
	Adjusted p	0.000*	0.000*	0.887
CRP pleural fluid	Test statistic	-59.919	-87.742	-27.823
	Adjusted p	0.000*	0.000*	0.000*
CRP serum	Test statistic	-55.175	-89.8	-34.625
	Adjusted p	0.000*	0.000*	0.000*
CRP pleural fluid/serum	Test statistic	-37.317	-48.364	-11.048
	Adjusted p	0.000*	0.000*	0.000*

p<0.05 significant, *significant test

Table 4: The AUC of the ROC curve analyses of ADA and CRP.

Test result variable (s)	AUC	p	95% confidence interval	
			Lower bound	Upper bound
ADA pleural fluid	0.77	0.000*	0.69	0.85
ADA serum	0.74	0.000*	0.65	0.82
ADA pleural fluid/serum	0.50	0.982	0.40	0.61
CRP pleural fluid	0.66	0.000*	0.57	0.75
CRP serum	0.62	0.017*	0.53	0.71
CRP pleural fluid /serum	0.62	0.022*	0.53	0.70

p<0.05 significant, *significant test

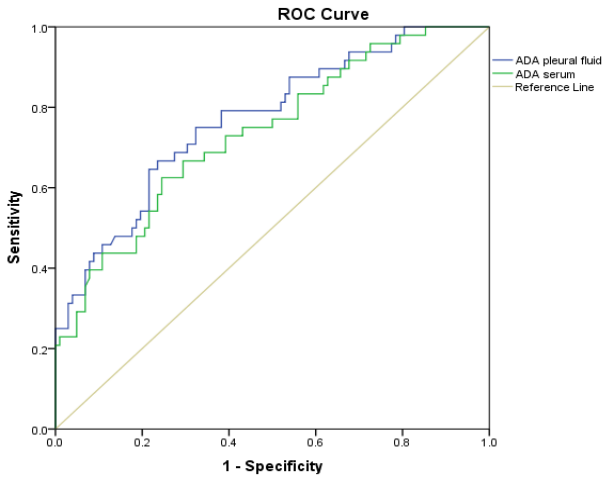

Figure 3: ROC curve of ADA in pleural fluid and serum.

In Figure 1, ADA level in exudative pleural effusion, demonstrate the level of ADA biomarkers in pleural fluid (blue column) and in serum (red column), in the three types of exudative pleural effusion. The figure shows that ADA has higher value in pleural fluid than in serum and the type tuberculosis of pleural effusion has higher value than Para pneumonic and malign.

In Figure 2, CRP level in exudative pleural effusion, demonstrate the level of CRP biomarkers in pleural fluid (dark green) and in serum (light green), in the three types of exudative pleural effusion. The figure shows that CRP has higher value in pleural fluid than in serum and the type Para pneumonic of pleural effusion has higher value than tuberculosis and malign.

In Figure 3, ROC curve analysis of ADA in pleural fluid and serum. ROC analyses helps to evaluate the accuracy of a test used. The closer to the left corner the value of ADA appear the better the test is, as it shows a high specificity and high sensitivity. ADA in pleural fluid represents a larger area under the curve, making it a better test for the differentiation of tuberculosis and non-tuberculosis pleural effusion.

DISCUSSION

The etiology of the pleural effusion often it is difficult to be established even though the presence of the pleural effusion is easy to be established. The diagnostic steps as imaging methods, cellular, microbiologic and biochemical analyses etc. often are not enough to confirm the etiology of effusion in some patients. These difficulties in diagnostic have led to the search of new biomarkers that can facilitate the diagnostic process. As such ADA and CRP are evaluated as remarkable markers in this purpose to differentiate the different types of exudative pleural effusion and to differentiate tuberculosis and non-tuberculosis. In the present study the level of ADA pleural fluid differ more between the groups of exudative pleural effusion than ADA serum. The serum CRP differ more between the groups of exudative pleural effusion than the pleural fluid CRP (Table 3). CRP level were found higher in parapneumonic than tuberculosis or malign types of effusion. This may be connected to the fact that CRP has an important role in inflammation as an acute phase protein, at its level are raised significantly in the region where the inflammation happens.[8] The parapneumonic shows a significant difference of its value for CRP in serum and pleural fluid when compared with malign and tuberculosis pleural effusion. The value of CRP in tuberculosis are higher than in malign group and this is related with other similar studies.[9-11] This can be connected to the local production of the CRP or the leakage of CRP from the inflamed pleura.[8] CRP has higher value in parapneumonic group than in the other groups of exudative effusion, similar to other study.[12]

ADA in the present study showed elevated level for tuberculosis as its activity is strongly related with an activation of T lymphocytes. The second most common cause for elevated ADA in present study was parapneumonic effusion. Parapneumonic effusion is usually neutrophilic unlike tuberculosis which is lymphocytic.[13] Pleural fluid and serum ADA differ significantly between the types of exudative effusion. Meanwhile pleural fluid/serum ADA didn't show a contribution in this difference.

The estimation of ADA level in pleural fluid is very helpful in establishing the etiology of tubercular pleural effusion and to rule out non tubercular pleural effusion. Pleural fluid ADA can be utilized for differentiating tuberculosis effusions from those of non-tuberculosis etiology, this is confirmed in other studies.[14] ADA level in the present study more than 78 IU/L are interpreted for the difference of tuberculosis and non-tuberculosis

pleural effusion. In similar studies the ADA cut off value are proposed as above 40 IU/L, 72 IU/L ADA 77.5 IU/L.[12,14,15] Even more are proposed studied with cut off 100 IU/L.[16] ADA levels are seen to be increase in different body conditions not related strictly with pleural effusion, in this way this marker might be considered with reserves as a very good options for the difference.

CONCLUSION

The value of ADA and CRP biomarkers showed significances in different types of exudative pleural effusion. ADA in pleural fluid was evaluated as a better test for the differences tuberculosis from non-tuberculosis. However, it is proposed that further studies might be conducted to verify more carefully the specificity and sensitivity of ADA and further biochemical marker have to be developed and taken into consideration for assisting the differential diagnosis.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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