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The importance of the biomarkers, ADA, CRP and INF-γ, in diagnosing pleural effusion etiologies

(The importance of the biomarkers ADA, CRP and INF-γ in facilitating the diagniosis of pleural effusion etiologies, for differentiation of transudative and exudative pleural effusions and for the differentiation of exudative pleural effusions)

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Abstract— *The aim of the present study is to investigate the clinical utility of biomarkers Adenosine deaminasa (ADA), C-reactive protein (CRP) and Interferon gamma (INF-γ) in the differentiation of exudative and transudative pleural effusion, and in the differentiation of the three types of exudative pleural effusion. The study enrolled 250 patients with pleural effusion that were admitted in hospital, from 2012-2015. The patients with pleural effusion were classified based on Light's criteria, on biochemical and on cytological analyses, as exudative (130), and transudative (120). The patients with exudative pleural effusion were categorized as: malignant, tuberculosis and parapneumonic. The patients went thoracentesis and venous blood samples, under aseptic conditions, and from each subject were collected in syringe at least 30 ml of pleural fluid. The measurement of pleural fluid and venous blood were done within 24 hours. To measure the levels of CRP in blood and liquid were used the test of CRP with COBAS 6000 Roche company. To measure the levels of ADA was used the colorimetric method of Giusti Gallant and for INF-γ was used the commercial enzyme-linked immunosorbent assay (ELISA) test. The Mann-Witney U statistical test for non-parametric data was used for the role that ADA and CRP plays in the differentiation of exudative and transudative pleural effusion. The values of ADA and CRP differ significantly between the two types of effusion ($p<0.05$). For the accuracy of the test was used the ROC curve analyses, and based on the area under the curve, ADA biomarker in pleural fluid is a better test for the differentiation of exudative from transudative pleural effusion. The Kruskal-Wallis H statistical test for non parametric data demonstrated that the values of ADA and CRP in serum and pleural fluid differ significantly between the three groups of exudative pleural effusion, with $p<0.05$. The values of ADA differ significantly when comparing malign with tuberculosis and tuberculosis with parapneumonic pleural effusion. The major differences for CRP biomarker were seen in the comparison of malign and parapneumonic pleural effusion. The Chi-square statistical test for the nominal data of INF-γ test demonstrated that, INF-γ in pleural fluid is a significant test for the differentiation of the three types of exudative pleural effusion and INF-γ in serum plays a less important role for this differentiation. As a conclusion, for the differentiation of exudative and transudative pleural effusion ADA biomarker is a better test for this differentiation. The biomarkers ADA, CRP in serum and pleural fluid, and INF-γ in pleural fluid, plays a significant role for the differentiation of the three types of exudative pleural effusion.*

Keywords—Pleural effusion, Exudative, Transudative, Biomarkers

I. 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. The pleural fluid formation is over passing its rate of absorption, and the pleural cavity has an exaggerated amount of pleural liquid in comparison to its normal state [1]. The diagnosis and the management of pleural effusions remains a clinical challenge having significant cost to patients and to the health care system [2]. Pleural effusion is classified in two main groups as exudative and transudative and based on Light's criteria the 2 groups can be distinguished from each other. If at least one of the Light's criteria is present the fluid is classified as exudative. The two types of effusion have different content of the fluid and the osmotic and oncotic pressure that influence their formation act different in both cases. 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 tuberculosis, malign and parapneumonic. A variety of laboratory tests are in use for the differential diagnosis of pleural effusions; nevertheless, a significant proportion remains undiagnosed. Tuberculosis meanwhile is ranked as the second for the number of death worldwide, after HIV/AID, 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 people [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]. Several biomarkers are being proposed for facilitating the differentiation of transudative and exudative pleural effusions and the differentiation of the different types of exudative pleural effusions. In this way it will be possible to have result in due time to start an earlier treatment of the patient. From the Infection Disease Biomarker Database (IDBD) in this study will be focused on ADA (Adenosine deaminasa), CRP (C Reactive protein) and INF—γ (Interferon gamma) [6]. (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 [7]. 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 [8]. CRP has the ability to recognize the pathogens and to eliminate them through the recruitment of the phagocytosis cells and the complement system. INF-γ is secreted from T cells and natural killer mostly and is influencing in augmenting the microbial function of macrophages, it stimulates the differentiation of native T helper in Th1, activate the polimorphonucleare leucocytes and T cell cytotoxic. INF- γ is the crucial factor for the activation of the macrophages [9],[10],[11]. The aim of the present study is to investigate the clinical utility of biomarkers Adenosine deaminasa (ADA), C-reactive protein (CRP) and Interferon gamma (INF-γ), in the pleural fluid and serum of patients with strictly characterized pleural effusions, in the differentiation of exudative and transudative pleural effusion, and in the differentiation of the three types of exudative pleural effusion.

II. METHODS

The study included 230 patients with pleural effusion in a period from 2012 to 2015. 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. Pleural fluid samples of the patients were classified as exudative effusion based on Light's criteria, biochemical, and cytological analyses [12]. Samples were analyzed for total and differential cell count, glucose, total protein and LDH. The determination of the etiology of pleural effusions was based on the following conditions, *malign* (45) when were detected malignant cells on cytological examination; tuberculosis (48) 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; parapneumonic (37) 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 INF- γ was measured using commercial enzyme linked immunosorbent assay (ELISA) kits. To carry out statistical analyses and to present the results were used the program of Microsoft office Excel (2007), SPSS version 20 (IBM statistics 2011). The tests used in this study for the intergroup comparison of more than two groups of non-parametric data was Kruskal-Wallis H, for intergroup comparison of two groups was Mann–Whitney U test [13],[14]. Receiver operating characteristic (ROC) was used to investigate the accuracy of the tests. For the tests used in this study, the differences are considered significant for $p<0.05$, $\alpha=5\%$.

III. RESULTS (*Heading 1*)

After the determination of the type of pleural effusion ADA and CRP biomarkers were statistically analyzed for their values. Both biomarkers have different distribution of their values in pleural fluid and serum in transudative and exudative pleural effusion. In (Tab.1) are demonstrated the mean ± SD of ADA and CRP in serum and pleural fluid in transudative and exudative pleural effusions. ADA biomarker has higher value in exudative pleural effusion for its values in pleural fluid and CRP has higher value in exudative pleural effusion for its values in serum. Lights criteria in several studies it is indicated that it has low specificity and alternative tool have been proposed for this differentiation. The statistical test used for the differences between the two groups, transudative and exudative, for ADA and CRP biomarkers in serum and pleural fluid, for non parametric data is Mann-Witney U test. Based on the results of the Mann- Witney U statistical test, ADA and CRP values differ significantly between the two groups of pleural effusions, $p<0.05$ (Tab.2). The two biomarkers were evaluated for the accuracy of their test in this differentiation and ROC curve analysis was used in this regard. The area under the curve for each of the biomarkers, AUC, is a crucial indicator for the identification of the best biomarker that facilitates the differentiation. Based on the AUC, the test of ADA in pleural fluid is a better test for the differential between exudative and transudative than ADA in serum and CRP in serum is a better test for the differentiation than CRP in pleural fluid. In (Fig.1, Fig.2) it is demonstrated the comparison between both groups for CRP and ADA in pleural fluid and serum

TABLE 1. MEAN±SD OF ADA AND CRP BIOMARKERS IN SERUM AND PLEURAL FLUID IN EXUDATIVE AND TRANSUDATIVE PLEURAL EFFUSIONS

Biomarkers	**Exudative pleural effusion**	**Transudative pleural effusion**
	Mean±SD	***Mean±SD***
ADA pleural fluid (IU/L)	74.69±41.93	31.41 ±8.04
ADA serum (IU/L)	41.23±22.96	26.84 ±4.3
CRP pleural fluid (mg/L)	30.58 ±21.64	7.22±2.92
CRP serum (mg/L)	53.77±29.75	10.92±3.56

TABLE 2. MANN WITNEY U STATISTICAL TEST RESULT FOR ADA AND CRP BIOMAKERS IN SERUM AND PLEURAL FLUID FOR TRANSUDATIVE AND EXUDATIVE PLEURAL EFFUSIONS.

Biomarker	**ADA pleural fluid (IU/L)**	**ADA serum (IU/L**	**CRP pleural fluid (mg/L)**	**CRP serum (mg/L)**
Mann-Whitney U	1972.500	4545.500	2123.000	581.000
p	0.000*	0.000*	0.000*	0.000*

**significance*

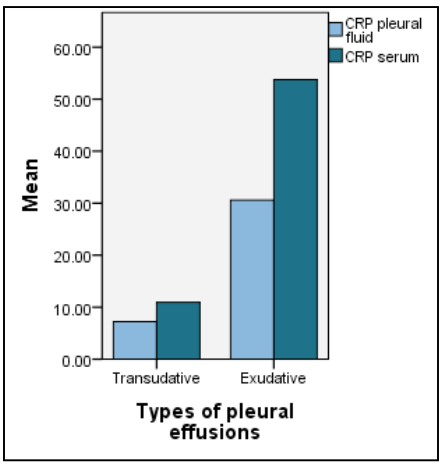

Fig.1 Serum and pleural fluid CRP levels' differences between transudative and exudative

Exudative pleural effusion with the three groups' tuberculosis, malign and parapneumonic diagnosed, was statistically evaluated with Kruskal-Wallis H statistical test. The statistical test was used for the differences of the biomarkers in facilitating the differentiation of the three groups. The test results for, ADA pleural fluid (2)=51.407, p=0.000; ADA serum (2)=57.946, p=0.000; CRP pleural fluid (2)=87.104, p=0.000; CRP serum (2)=82.281, p=0.000. The values of the biomarkers differ significantly in accordance with the type of exudative pleural effusions, $p<0.05$ for the two biomarkers in each of the diagnosed groups. In (Fig.3& Fig.4) are reflected the mean values of ADA and CRP in serum and pleural fluid at the three types of exudative effusions. The mean of ADA is higher in tuberculosis for pleural fluid and the mean of CRP in higher in serum for parapneumonic exudative pleural effusion. The post HOC analysis demonstrated that the major differences for CRP were seen when comparing malign and parapneumonic, and for ADA the major differences were seen when comparing malign with tuberculosis.

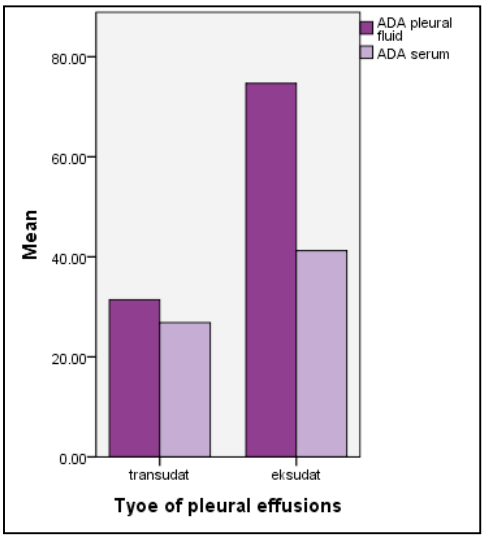

Fig. 2 Serum and pleural fluid ADA levels' differences between transudative and exudative

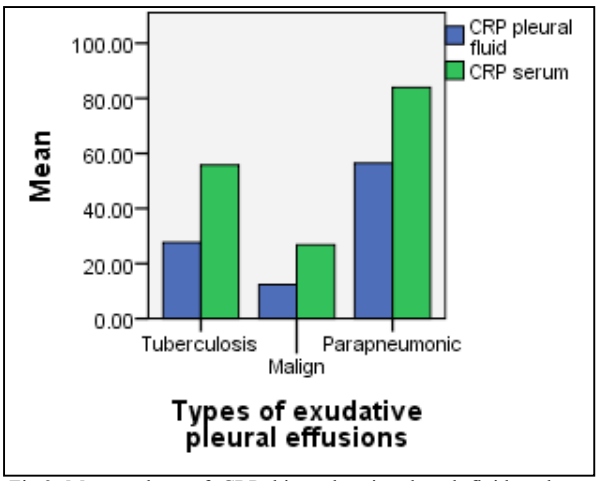

Fig.3 Mean values of CRP biomarker in pleural fluid and serum in exudative pleural effusions

INF- γ biomarker was as well evaluated for its results in exudative pleural effusion and for the possible differences of its results in accordance with the type of exudative pleural effusions. Positive and negative results in exudative pleural effusion where statistically evaluated with Chi square test [15]. Chi square statistical test result for INF- γ in pleural fluid (2)=37.552, p=0.000; INF- γ in serum (2)=66.540, p=0.000. The results of INF- γ pleural fluid and serum differ significantly between the different groups of exudative effusions, $p<0.05$. In (Fig.5) is demonstrated the positive and negative results of INF- γ according to the type of exudative pleural effusions. For the comparison tuberculosis and non tuberculosis Chi square statistical test result for INF- γ in pleural fluid (1)=13.769, p=0.000 have a significant result for this differentiation and for the identification of the tuberculosis group in compare to INF- γ in serum.

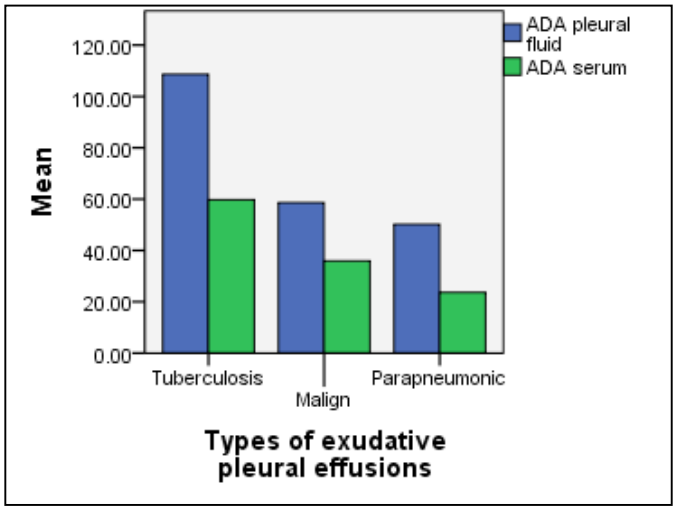

Fig. 4 Mean values of ADA biomarker in pleural fluid and serum in exudative pleural effusions

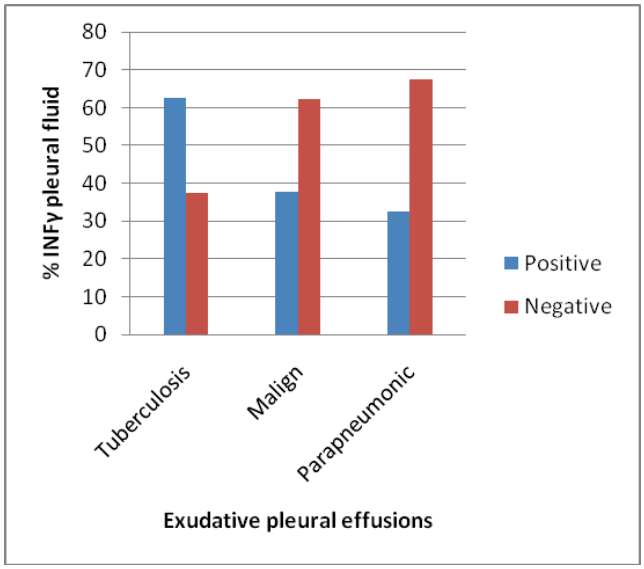

Fig.4 INF- γ pleural fluid results in exudative pleural effusions (tuberculosis, malign, parapneumonic)

IV. DISCUSSIONS

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. In consequence ADA, CRP and INF- γ biomarkers can come in help. ADA and CRP biomarker have lower value in transudative pleural effusion than in exudative pleural effusion; a similar result is evidence in other similar studies [16][17][18]. Several studies have been reported that the differentiation of exudative and transudative pleural effusion based on Lights criteria, proteins etc has low specificity [19]. In this way further tools are being proposed for facilitating the differentiation. For the difference between the two groups in the present study ADA in pleural fluid is a better biomarker than ADA in serum and CRP in serum is a better biomarker in comparison with CRP in pleural fluid for the differentiation. CRP as well is being proposed as useful biomarkers than can facilitate the differentiation of the two groups [20]. ADA and CRP cannot be used as the only possibility for the differentiation of exudative and transudative pleural effusions. Lights criteria are the basis for this differentiation and ADA, CRP biomarkers can be used in complementary as an added value with the Lights criteria for the differentiation of exudative and transudative pleural effusions. The ADA activity in the tuberculosis patients was significantly higher than in the other groups of exudative effusion, this is in line with other studies [21][22]. High levels of ADA can also be found in patients with neutrophilic effusions such as parapneumonic effusions or empyema. INF- γ test is relatively new in its implementation and it is estimated that INF- γ pleural fluid has more accurate results [23]. INF- γ pleural fluid is considered as an additional biomarker in tuberculosis diagnosis, but this test needs further scientific researches to be confirmed furthermore [24]. INF γ due to its cost it is not promoted as the only test available for tuberculosis diagnosis, in this was ADA test and CRP test is being proposed as well, due to the large availability and cost effectiveness. In other studies was evaluated the cost for performing and INF –γ test in comparison to ADA for pleural fluid and the cost for detecting one additional patient using INF –γ was equivalent of the cost to complete a tuberculosis treatment for six patients [25].

V. CONCLUSIONS

For the differentiation of transudative and exudative pleural effusions both ADA and CRP biomarkers have significant results for this differentiation. The two biomarkers can be used as an added value together with Lights criteria which are in basis and the main criteria of these differences. ADA in pleural fluid is a better test than ADA in serum for the differentiation of the two groups and CRP in serum is a better test than CRP in pleural fluid for the differentiation. Once the pleural effusion is diagnosed as exudative the biomarkers are evaluated for their significance for the differential diagnosis of exudative pleural effusions (tuberculosis, malign and parapneumonic). ADA and CRP in pleural fluid and serum have significant results for the differentiation of the three types of exudative effusions. INF- γ in serum and pleural fluid has a significant role in the differentiation of the three groups but INF- γ in serum plays a less important role in compare to INF- γ in pleural fluid which has a higher sensitivity and specificity. As well INF- γ in pleural fluid is a significant test for the differentiation of the tuberculosis groups from non tuberculosis pleural effusions. INF- γ test is an expensive test and it is recommended to not be used as the only test for the differential diagnosis but to be used together with other test as of ADA and CRP. However it is proposed that further studies might be conducted to verify more carefully the specificity and sensitivity of the biomarkers and further biochemical marker have to be developed and taken into consideration for assisting the differential diagnosis.

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