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INF-γ biomarker in differentiating tuberculosis pleural effusion

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Abstract
Pleural effusion is an abnormal accumulation of fluid in lungs influencing the normal process of breathing. The aim of the present study is to investigate the clinical utility of Interferon gamma (INF-γ) biomarker in pleural fluid and serum for the differentiation of tuberculosis pleural effusion. The study enrolled 33 patients with pleural effusion classified with exudative pleural effusions that were admitted in hospital, from 2012-2015. The patients with exudative pleural effusion were categorized as: tuberculosis (11), and non-tuberculosis (22). 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 24h. For measuring INF-γ was used the commercial enzyme-linked immunosorbent assay (ELISA) test. INF-γ in pleural fluid is a more accurate test for the differentiation of tuberculosis pleural effusion.

Keywords: Exudative pleural effusion, Biomarker, Tuberculosis, Non-Tuberculosis

1. Introduction
Pleural effusion is an abnormal accumulation of fluid in the pleural cavity influencing the respiratory process and causing difficulties in the normal movement of the lungs [1]. 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 to its normal state. Based on Light's criteria on biochemical, cytological and microbiological analyses can be possible in characterizing and exudative or transudative pleural effusions which have significant differences between them on the way the fluid is formed and the causes of the fluid formation [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 tuberculosis, malign, and parapneumonic effusions. Tuberculosis meanwhile is ranked as the second for the number of death worldwide, after HIV/AID, caused from a single infective agent [3-5]. 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 [6]. Further more 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. Detection of tuberculosis and differentiation of pleural effusion from non-tuberculosis effusion still poses diagnostic challenges. Alternative methods and biomarkers for diagnosis and differentiation of tuberculosis pleurisy from non-tuberculosis pleurisy effusion are being proposed, as INF- γ [7]. INF-γ is secreted from T cells and natural killer cells mostly and is influencing in augmenting the microbial function of macrophages, it stimulates the differentiation of naive T helper in Th1, activate the polimorphonucleare leucocytes and T cell cytotoxic [8-10]. INF- γ is the crucial factor for the activation of the macrophages. The measurement of INF- γ in pleural fluid and in serum is quite a new technique worldwide and this measurement is expensive.

2. Materials and methods
The study included 54 patients with pleural effusion in a period from 2012-2015. The patients underwent thoracentesis and venous blood samples that were analyzed within 24 hours. Pleural fluid samples of the patients were classified as exudative pleural effusion (33 patients) based on Light's criteria, biochemical, cytological analyses etc. 21 patients were not included in the categorization for exudative pleural effusion. The exudative pleural effusion is diagnosed as tuberculosis, malign and parapneumonic pleural effusion. Based on the following criteria was made available the possible diagnosis of; tuberculosis: Mycobacterium tuberculosis can be isolated from the pleural fluid of tissue and granuloma are present in tissue that shows the presence of Acid Fact Bacilli, malignant: the cytology of the pleural fluid is positive or it is known a malignant disease after the exclusion of alternative causes of pleural effusion, parapneumonic: patients with emphysema, as pus in the pleural cavity were included in the parapenumonic group. 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 data were presented as mean± standard deviation (SD). Chi square for nominal data was used to statistically analyze the differences of INF- γ result in dependence of gender and statistically analyze the differences of INF- γ results pleural fluid and serum in the different types of exudative pleural effusion. Mann Whitney- U statistical test was used for the results of INF- γ in pleural fluid. ROC curve analysis was used to evaluate the accuracy of the tests used for the biomarker.

3. Results
The present study was carried out in 33 patients with exudative pleural effusion, 21 female (63%); 12 males (37%), 11 tuberculosis and 22 non tuberculosis (12 parapneumonic and 10 malign), with mean ±SD of age 46.3±17. Figure 1

demonstrates the gender in accordance with tuberculosis and non tuberculosis groups.

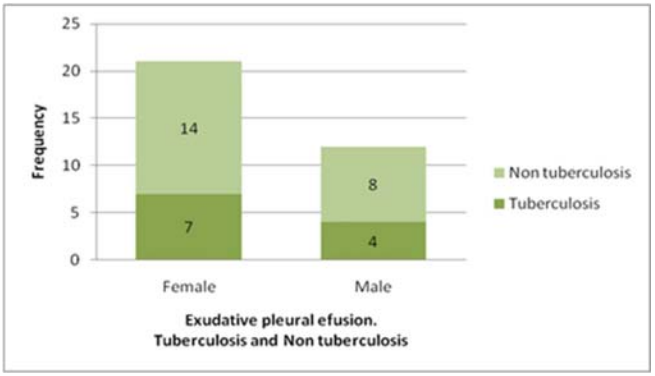

Fig 1: Exudative pleural effusions groups in accordance with gender

The biomarker INF-γ was statistically evaluated for their differences in dependence of gender. INF-γ pleural fluid Mann Witney –U test, U=116, p = 0.708, INF-γ in serum Chi-square (1) = 0.000, p = 1.000. The test is not significant demonstrating that INF-γ do not differ in accordance with gender. The results of INF-γ in pleural fluid for exudative pleural effusion has a mean ± SD (13.56±11.83). INF-γ in pleural fluid for tuberculosis pleural effusion has a mean ± SD (25.86±9.5/pg/mL) and INF-γ in pleural fluid for non tuberculosis pleural effusion has a mean ± SD (7.41±7.2/ pg/mL). In figure 2 are demonstrated the mean of INF-γ in pleural fluid in accordance of the type of exudative pleural effusions, and in figure 3 are demonstrated the negative and positive results for INF-γ in serum.

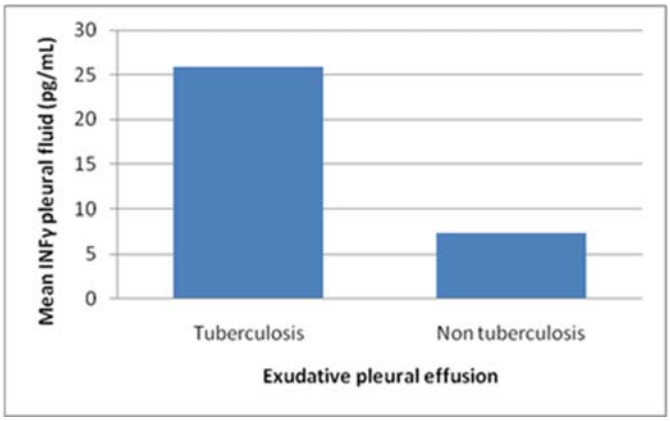

Fig 2: Mean of INF-γ in pleural fluid for tuberculosis and non-tuberculosis pleural effusion

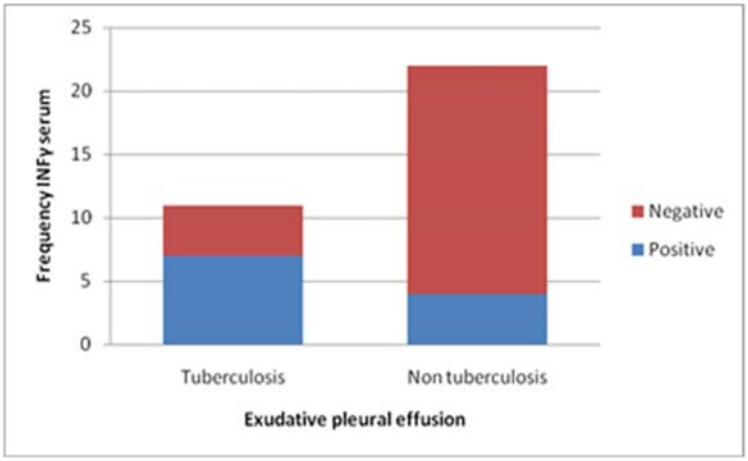

Fig 3: Negative and positive results of INF-γ in serum for tuberculosis and non-tuberculosis pleural effusion

INF-γ biomarker was statistically tested for its differences between the tuberculosis and non-tuberculosis exudative pleural effusions. In table 1 are demonstrated the results for the statistical tests used.

Table 1: Statistical test used for the differences of INF-γ in tuberculosis and non-tuberculosis pleural effusion

Biomarker	Test used	Results	*p*
INF-γ in pleural fluid	Mann Witney U test	14	0
INF-γ in serum	Chi square test	6.818	0.009

The biomarker INF-γ has differences in its results in depends of the group tuberculosis and tuberculosis, and can serve as a useful biomarker for the differential diagnosis. To evaluate the accuracy of the test performed for the pleural effusion was used the ROC analyses curve for INF-γ in pleural fluid (figure 4)

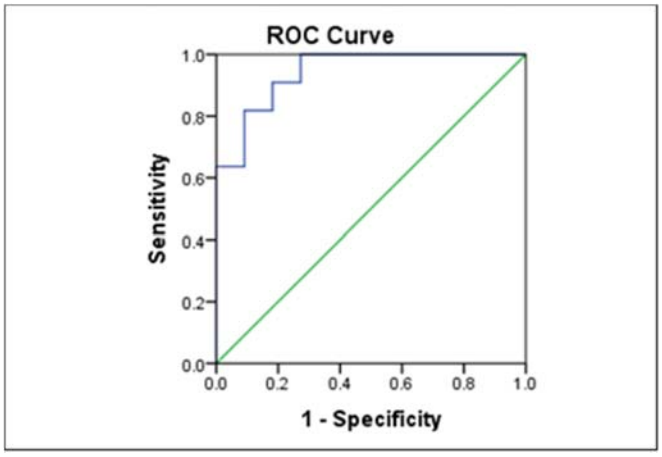

Fig 4: ROC curve analysis for INF-γ in pleural fluid

INF-γ in pleural fluid for a cut off value of 18.1 pg/mL, had a sensitivity and specificity respectively 81.8% and 90.9%. The AUC surface is 0.942, classifying it as a very good test that can facilitate the differential diagnosis of tuberculosis and non tuberculosis. Meanwhile INF-γ in serum has a sensitivity and specificity respectively 68.64% and 81.82%, and Positive predicted value and Negative predicted value respectively 63.64% and 81.8%. In this way from the test used for the INF-γ the test used in pleural fluid has better results that can be taken in consideration

4. Discussions

Several biomarkers has been proposed in facilitating the diagnosis of the exudative pleural effusions and INF-γ is one of the biomarker that is been taken into account especially for tuberculosis differentiation. INF is known for their role in inhibiting viral infections and in stimulating the entire immune system to fight disease, in response to pathogens. INF-γ is the principal macrophage-activating factor (MAF) and provides the means by which T cells activate macrophages to kill phagocytosed microbes. High levels of IFN-γ are found in patients with tuberculosis pleural effusions, while it is almost undetectable in pleural effusions of other [11].

Making a differential diagnosis between TPE and non-TPE is a critical clinical problem. The diagnosis of tuberculosis pleural effusions can be difficult as because *M.tuberculosis* requires approximately 2 to 6 weeks to grow, and a minimum of 10-100 viable bacilli are needed, in this way often the treatment is needed to start before the final confirmation of the diagnosis. In the present study were conducted test for INF-γ in pleural fluid and serum. In this study was statistically evaluated if the biomarker has a difference in depends of the gender, the test demonstrated that the biomarker do not have differences of its results in depends of the patients gender. INF-γ has higher positive result in tuberculosis pleural fluid and higher negative results in non-tuberculosis pleural effusion. The biomarker based on the results of the statistical test has a significant difference in dependence of the groups' tuberculosis and non tuberculosis.

INF-γ pleural fluid is characterized as a more accurate test for the differentiation of tuberculosis and non tuberculosis, with a higher sensitivity and specificity that the test for INF-γ in peripheral blood [12, 13]. A similar result is seen in the study where INF-γ pleural fluid is evaluated as a good diagnostic biomarker for tuberculosis [14]. In national studies was evaluated that INF-γ pleural fluid can be a useful biomarker for tuberculosis diagnosis with a sensitivity and specificity of 68.4% and 100% [15].

In the same time INF-γ is not a cheap test and its procedure it is not so easy to be executed. The present study is a broaden one taking in consideration other biomarker that can be supportive in the differentiation of tuberculosis and non tuberculosis pleural effusion like ADA (Adenosine deaminasa). INF-γ results can not define the stage of the infections, level of immune responses or progress of disease. A positive result of INF-γ should be followed by additional test to confirm the diagnosis.

5. Conclusions

INF-γ results do not differ in dependence of gender. INF-γ results demonstrates differences between tuberculosis and non tuberculosis pleural effusions. INF-γ in pleural fluid is a more accurate test for the difference of the groups. A positive result of INF-γ must be followed by other medical evaluation and other biomarkers test that are more cost effective can be taken into consideration for studying their accuracy.

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