Diagnostic Importance of C-Reactive Protein (CRP) analysis in Tuberculosis Pleural Effusions.

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ABSTRACT:
One of the acute-phase biomarkers that have recently been investigated for its clinical utility in tuberculosis pleural effusion is C-reactive protein (CRP) which has already been commonly used as a marker of inflammation and tissue injury. Therefore, the present study was undertaken to analyze the viability of CRP as a diagnostic aid for tuberculosis in lymphocytic pleural effusions. Fifty two (n = 52) patients with lymphocytic pleural effusion with definite diagnosis of a disease condition, were taken into the test group and classified into non-tuberculosis (n = 28) group and tuberculosis pleurisy group where sputum culture was positive for Mycobacterium tuberculosis in pleural effusion (n = 24). CRP in pleural fluid was analyzed by automated turbidimetric immunoassay method as per description of the manufacturer and normal reference value in serum is ≤ 5.0 mg/L and that of pleural effusion in control group is = 20.45 mg/L. Twenty four patients (males = 19; Female = 5) were diagnosed with tuberculosis whereas 9 with pulmonary embolism, 5 with CABG and 14 with benign exudates of para-pneumonic origin. CRP of non tuberculosis effusions were noted to be relatively lower in levels (range 15.30 ± 5.10 to 32.10 ± 9.25 mg/L) as compared to those obtained in tuberculosis effusions (62.50 ± 12.75 mg/L). However, CRP of benign exudates of para-pneumonic origin showed a higher value, 32.10 ± 9.25 mg/L, than other non tuberculosis effusions. The level of significant difference was high with P<0.001 when CRP of tuberculosis pleural effusion was compared with non tuberculosis effusions, whereas with para-pneumonic exudates, the difference was moderately significant, P<0.01. The results clearly indicates a significant role of CRP for diagnostic facilitation of tuberculosis pleural effusion in comparison with non-tuberculosis effusions of para-pneumonic, CABG or pulmonary dysfunctions.

Key words: C-Reactive Protein (CRP), tuberculosis pleural effusion, para-pneumonic, Coronary Artery Bypass Grafting (CABG).

Short Title: CRP in tuberculosis pleural effusions

INTRODUCTION
It is well defined and documented that biochemical and microbiological analysis of the pleural fluid is useful in the diagnosis of tuberculosis pleuritis. In this context, several biochemical parameters are reported to be important in diagnosing tuberculosis pleural effusion, such as adenosine deaminase (ADA)1-4. However, the ADA test is not commonly available worldwide and for this reason, scientist and researcher are looking into alternative tests with lower cost and greater availability and reproducibility4. The necessity to develop newer test methods for this particular disease is also very significant keeping in view the dilemma that tuberculosis remains the most common cause of pleural effusion of all the distinctive pulmonary diseases5.

One of the biomarkers that have recently been investigated for its clinical utility in tuberculosis pleural effusion is C-reactive protein (CRP) which is an acute-
phase protein widely used as a marker of inflammation and tissue injury. Recent studies showed that in pleural fluid, CRP levels have been noted to be significantly elevated in tuberculosis and parapneumonic effusions than in other causes of pleural effusions. Therefore the aim of present study is to analyze the viability of CRP as a diagnostic aid for tuberculosis in lymphocytic pleural effusions.

**MATERIAL AND METHODS**

A total of 72 patients were included in the study. Twenty patients with lymphocytic pleural effusions in whom no diagnosis could be obtained was taken as controls, whereas 52 patients with consecutive lymphocytic pleural effusion with definite diagnosis of a disease condition, other than tuberculosis or positive *Mycobacterium tuberculosis* culture in pleural effusion (n = 28) and with +ve sputum culture for *M. tuberculosis* (n = 24) as described earlier were included into test group. According to description, pleural fluid was considered to be lymphocytic when the differential nucleated cell count revealed more than 50% lymphocytes. The diagnosed patients group consists of 28 men (53.80%) and 24 women (46.15%), with mean age 59.50 ± 15.25 years (46 yrs to 63 yrs). For present study the protocol detailed by Garcia-Pachon et al. was followed for all procedural steps to ensure standardization.

**Diagnostic Criteria:**

The final diagnoses of the pleural effusions were established on the basis of well-defined diagnostic criteria documented in the literature and detailed by earlier studies. According to that criteria, 24 patients (males = 19; Female = 5) were diagnosed with tuberculosis whereas 9 with pulmonary embolism, 5 with CABG and 14 with benign exudates of para-pneumonic origin. In brief the diagnosis of tuberculosis pleural effusion was based on the presence of caseous granulomata in the pleural biopsy and/or positive culture for *Mycobacterium tuberculosis* in pleural fluid or biopsy material, or positive sputum culture for *M. tuberculosis* with an exudative pleural effusion.

**Biochemical and Microbiological analysis:**

CRP in pleural fluid was analyzed on Hitachi 912 (Roche, Basel) by the turbidimetric immunoassay method as per description of the manufacturer. The normal value of CRP in serum is ≤ 5 mg/L, whereas value in pleural effusions of control group was = 20.45 ± 5.60 mg/L. All the biochemical and microbiological analysis was performed according to previously established techniques and the subsequent pleural effusions and fluids was analyzed for cell count, protein, and glucose concentrations and cultures for bacteria and viruses.

**Statistical analysis:**

Statistical analysis was made by a two-tailed unpaired t-test for the differences between CRP pleural fluid levels in tuberculosis and non-tuberculosis pleuritis as well as Pearson's correlation using SPSS ver 13.0 (USA). A P level of <0.05 was considered statistically significant.

**RESULTS:**

In present study a total of 72 patients were included in the study, out of which twenty patients with lymphocytic pleural effusions with negative diagnosis were taken as controls, whereas 52 with consecutive lymphocytic pleural effusion with definite diagnosis of a disease condition, other than tuberculosis or positive *Mycobacterium tuberculosis* culture in pleural effusion (n = 28) and with +ve sputum culture (n = 20) were included in test group. As per description detailed earlier that a pleural fluid was considered to be lymphocytic when the differential nucleated cell count revealed more than 50% lymphocytes. In categorization, the diagnosed patients group consists of 28 men (53.80%) and 24 women (46.15%), with mean age 59.50 ± 15.25 years (46 yrs to 63 yrs). According to that criteria, 24 patients (males = 19; Female = 5) were diagnosed with tuberculosis whereas 9 with pulmonary embolism, 5 with CABG and 14 with benign exudates of para-pneumonic origin.
CRP of all non-tuberculosis effusions were analyzed separately and noted to be relatively lower in levels (range 15.30 ± 5.10 to 32.10 ± 9.25 mg/L) as compared to those obtained in tuberculosis effusions (62.50 + 12.75 mg/L). However, CRP of benign exudates of para-pneumonic origin showed a higher value, 32.10 ± 9.25 mg/L, than other non-tuberculosis effusions (Table 1). The level of significant difference was P< 0.001 when CRP of tuberculosis pleural effusion was compared with non-tuberculosis effusions, except para-pneumonic exudates which showed level of significance of P< 0.01 when compared with tuberculosis group. Fig 1 and Fig 2 depicts the graphic representation of CRP levels in individual groups as well as gender-wise distribution in tuberculosis group. The results clearly suggest a significant role of CRP with diagnostic facilitation of tuberculosis pleural effusion in comparison with non-tuberculosis effusions of para-pneumonic, CABG or pulmonary dysfunctions.

**DISCUSSION**

In present study, tuberculosis pleurisy showed CRP > 60 mg/L, significantly (P < 0.01 to P < 0.001) higher than exhibited by effusion from pulmonary embolism, CABG, para-pneumonic and lymphatic pleural fluids with no diagnosis. The results clearly suggest a significant role of CRP with diagnostic facilitation of tuberculosis pleural effusion in comparison with non-tuberculosis effusions of para-pneumonic, CABG or pulmonary dysfunctions. In a recent study carried out in South Korea where the researchers have investigated the utility of serum C-reactive protein (CRP) and procalcitonin (PCT) for differentiating pulmonary tuberculosis (TB) from bacterial community-acquired pneumonia (CAP). The investigation was considered very significant because of its community which has an intermediate TB burden. The group which they have studied consists of more than 50 patients who had bacterial CAP and 30 had pulmonary TB. The analyzed CRP concentration was 14.58 mg/dL (range, 0.30 to 36.61) in patients with bacterial CAP and 5.27 mg/dL (range, 0.24 to 13.22) in those with pulmonary TB (P <0.001). They have concluded that concentrations of CRP differed significantly in patients with pulmonary TB and bacterial CAP and the high sensitivity and negative predictive value for differentiating pulmonary TB from bacterial CAP suggest a supplementary role of CRP in the diagnostic exclusion of pulmonary TB from bacterial CAP in areas with an intermediate prevalence of pulmonary TB. Recently, several studies have been focused on investigating newer biomarkers for diagnosing the presence of tuberculosis pleurisy in pulmonary effusion and differentiating it with non-tuberculosis pleurisy. The biomarkers that have been investigated were procalcitonin, ESR and CRP.

Similarly in a more recent study, the authors investigated the clinical significance of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in the treatment of spinal tuberculosis. They have included sixty-seven patients (41 males and 26 females, ranges 23 to 61 years) with active spinal tuberculosis. The clinical diagnosis revealed that the tuberculosis focus were located either in cervical spine, thoracic spine or in lumbar spine. All patients went through anti-tuberculosis chemotherapy and their blood was tested for ESR and CRP at different times before and after treatments. The average CRP was 44.3 ± 17.5 mg/L before chemotherapy, indicating active tuberculosis focus. After 4 to 6 weeks chemotherapy treatment, the average CRP was 26.7 ± 11.8 mg/L, the differences were statistically significant (P < 0.05), and the clinical symptoms of spinal tuberculosis relieved in all patients. Four weeks after operation, the average CRP declined to 23.8 ± 10.0 mg/L, which is again statistically significant (P < 0.05). Eight weeks after operation, the average values of CRP were at normal level in 47 cases, indicating inactive tuberculosis focus. They have concluded that the level of ESR and CRP are high in active spinal tuberculosis and low when focus controlled. The research group has suggested that CRP is reliable parameters in evaluation the treatment and prognosis of spinal tuberculosis.
A similar relevant and very significant study carried out earlier shows that the pleural fluid CRP level in lymphocytic pleural effusions has diagnostic value in tuberculosis pleuritis. They have noted high CRP levels and thus recommending that this diagnosis can be strongly suggested of tuberculosis pleuritis, if CRP is >50 mg/L; however, on the contrary, can be considered highly unlikely if a CRP pleural fluid level was <30 mg/L.4

It was argued that ideally, the diagnosis of tuberculosis pleuritis should be made by the demonstration of $M.\ tetuerculosis$ in pleural fluid or in pleural tissue. However, microbiological testing of pleural fluid for mycobacteria are frequently comes out negative and cultures, when give positive results, often take several weeks. It is for this reason that clinicians, physicians and scientists became more interested in newer cytological and biochemical findings, in addition to the clinical and radiological characteristics that facilitate in diagnosing tuberculosis pleuritis. At present, several tests are of great interest for diagnosing tuberculosis pleural effusion, such as ADA, interferon, lysozyme, the polymerase chain reaction or specific antibodies. However, as noted and evaluated by several studies, all these tests need specific and/or expensive instruments. Interestingly, it was evidently documented in a significant study, that the measurement of CRP level in pleural fluid can be useful in determining or differentiating tuberculosis pleurisy from other effusions. It has the advantage that it can be determined in conventional auto-analyzers with minimal over-head expense. As stated earlier the report noted that in patients with lymphocytic pleural effusion, value of CRP in the pleural fluid is >50 mg/L, strongly suggesting the possibility of tuberculous pleuritis. In contrast, low CRP levels, such as <30 mg/L in other conditions virtually ruled out this possibility.

Previous studies on CRP in pleural fluid suggested, argued and concluded that this parameter can be used to differentiate transudates from exudates, or from para-pneumonic to pulmonary effusions. In one of the significant study, the authors noted that CRP levels were significantly higher in parapneumonic and tuberculous effusions, whereas another study reported that in pleural exudates, a CRP <20 mg/L suggests a malignant origin, whereas a level >45 mg/L ruled out this possibility. In yet another study CRP was analyzed in 55 patients with tuberculous pleuritis and in 60 patients with malignant pleural effusions. They have concluded that with a cut-off level of CRP ≤30 mg/L, the sensitivity was 72% and specificity 93%.

Through extensive literature survey, it was observed by our team that diagnostic importance of CRP has also been evaluated in other related areas such as whether it is a sensitive marker for discriminating between transudate and exudate and pleural effusions and or it can be used to distinguish inflammatory pleural effusions from other types of effusion or not. In this regard a previous study reported that pleural fluid’s CRP levels were significantly lower in the transudate group (14.9 ± 4.9 mg/L and 35.5 ± 4.9 mg/L, respectively). Also, the ratio of pleural fluid to serum was significantly lower in the transudate group (P<0.009; 0.8 ± 0.5 mg/L and 2.8 ± 0.7 mg/L, respectively). In the exudate group, 35 patients had neoplastic effusions, 10 chronic non-specific pleurisy, 19 tuberculous pleurisy, 16 parapneumonic effusions and one Dressler Syndrome. When these sub-groups were compared, the parapneumonic effusion of subgroup’s CRP levels was 89 ± 16.3 mg/L, and significantly higher than those in the other subgroups, exudate of neoplastic effusion, tuberculous pleurisy and chronic non-specific effusion and the transudate group. The ratio between pleural fluid and serum CRP was significantly higher in the parapneumonic effusion subgroup than in the neoplastic subgroup (P<0.0002) 6.6 ± 2.7 mg/L and 1.0 ± 0.2 mg/L, respectively. Furthermore, the pleural fluid CRP levels of >50 mg/L had a high sensitivity (93.7%) and specificity (76.5%) and a positive predictive value of 98.4%. They have concluded and thus suggested that in the
differential diagnosis of pleural effusions, higher CRP levels may prove to be a rapid, practical and accurate method of differentiating parapneumonic effusions from other exudate types and the pleural fluid’s CRP level may also be helpful in discriminating between exudative and transudative pleural effusions  

In conclusion of present study, determination of CRP in pleural fluids, whether from tuberculosis, para-pneumonic or other inflammatory conditions, is a simple and inexpensive test useful in the diagnostic workup of lymphocytic pleural effusions. As noted by previous studies as well, high CRP levels, such as greater than 50 mg/L, are very suggestive of tuberculous pleuritis, and low CRP levels make this diagnosis unlikely. However, the individual use of this parameter is not recommended for diagnosing tuberculous pleurisy, besides the fact that CRP pleural fluid level provides significant diagnostic information that should be considered together with all the other epidemiological, clinical, cytological and biochemical data.

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<tr>
<th>Diagnoses</th>
<th>Patients (n)</th>
<th>C-reactive protein, mg/L</th>
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<tr>
<td>I-Non-tuberculosis pleural effusion</td>
<td>(n = 28)</td>
<td>18.55 ± 4.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>(n = 9)</td>
<td>15.80 ± 5.10&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>CABG</td>
<td>(n = 5)</td>
<td>31.10 ± 9.25&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Para-pneumonic</td>
<td>(n = 14)</td>
<td>62.50 ± 12.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II-Tuberculosis pleuritis</td>
<td>(n = 24)</td>
<td>20.45 ± 5.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III-Lymphocytic fluids with no disease</td>
<td>(n = 20)</td>
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<sup>a</sup>P < 0.001, <sup>b</sup>P < 0.01

**Table 1:** Clinical Diagnoses and concentrations of C-reactive protein in various types of pleural fluids
Fig 1: Graphic representation of CRP concentration according to diagnoses

Fig 2: Genderwise CRP concentrations in tuberculosis effusion group