USEFULNESS OF PLEURAL FLUID CRP LEVEL IN DIFFERENTIAL DIAGNOSIS OF EXUDATIVE PLEURAL EFFUSION – A PILOT STUDY

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ABSTRACT

Background: *Pleural effusion is an abnormal collection of fluid in pleural space resulting from excess production or disruptions of homeostatic forces that regulate the flow of fluid in and out of the area. It is a frequent manifestation of serious thoracic disease whose specific diagnosis is a challenging task. Pleural effusion can be due to Infectious, malignant, parapneumonic disease and tubercular or other causes. Diagnosis of PE is not straight forward due to associated heart disease, malignancy or infection. A specific biomarker is therefore required for differential diagnosis of pleural effusion. C- Reactive Protein is an acute phase protein synthesized by hepatocytes and widely used to assess severity of infection and is directly associated to degree of inflammation in diseased state. The aim of this study is to assess specificity and sensitivity of Pleural Fluid CRP and other routine biochemical indices in diagnosis of exudative Pleural effusion arising due to varying etiologies.*

Material and Method: *The study involved 187 adult patients diagnosed with exudative pleural effusionand classified into 5 groups as follows: 1. malignant pleural effusion (MPE), 2. Chronic non-specific inflammation (CNI), 3. Parapneumonic pleural effusion (PPE), 4.Tubercular pleural effusion (TBPE) and 5. Others. After complete clinical evaluation, routine Pleural fluid analysis and CRP was analysed. Reciever Operating Characterstics (ROC) analysis established the cutoffs of CRP for discriminating between groups.*

Result and Discussion: *Pleural Fluid CRP level was significantly higher in infectious parapneumonic group, followed by Chronic non specific inflammatory group and Tubercular cases with lowest value in malignant group. ROC analysis of Pleural fluid CRP provided good sensitivity (97.05%), specificity (71.76%), NPV of 95.31% and PPV of 80.48 %for the differentiation of tubercular vs. non tubercular effusions. In ROC analysis of CRP for differentiation of Parapneumonic from non Parapneumonic pleural effusion (tubercular, chronic non-specific inflammation, malignant and others), sensitivity of 100%, specificity 98.88%, NPV of 100% and PPV of 81.82% was seen at cut off level of 90.8 mg/l. It provides largest Area under Curve I.e 1.000. CONCLUSION: Pleural fluid CRP can be used as a diagnostic aid specially differentiating between acute and chronic inflammation and also between infectious and non infectious inflammation with CRP value more than 30 mg/l almost excludes malignancy. Pleural fluid CRP can be used as specific biomarker for differential diagnosis of Parapneumonic pleural effusion with excellent sensitivity and acceptable specificity.*

Keywords: *Exudative Pleural Effusion, C - reactive protein, Parapneumonic, Malignant.*

INTRODUCTION

Pleural effusion (PE) is an abnormal accumulation of fluid in pleural space lined by mesothelial cells and found commonly in clinical practice. It may be induced by congestion, trauma, inflammation, infection and neoplasm affecting pleura, lungs, heart or mediastinum. Pleural effusion can be due to increased permeability of pleural membrane, Increased pulmonary capillary pressure, decreased negative intrapleural pressure, decreased oncotic pressure and obstruction of lymphatic flow (1). During infection, the mesothelial cells are the first which comes in contact with invading pathogen and trigger an appropriate immune response (2). A vigorous local and systemic inflammatory response are often seen in pleural infection. Development of pleural infection depends on balance between pleural immune response and virulence of the organism. While investigating the cause of PE, first step is to differentiate between exudative and transudative PE. According to a Meta analysis, exudative PE meet at least one of the following criteria i.e. PF protein >2.9g/dl, pleural fluid cholesterol > 45 mg/dl and lastly pleural fluid Lactate Dehydrogenase > 60% of upper limit for serum whereas transudative PE meet none (3,4). No further diagnostic investigation of fluid is required for transudative PE but subsequent determination of the cause of exudative PE is not only difficult but also crucial for accurate diagnosis of pleurisy. Various etiologies for exudative PE can be malignancy, bacterial infection like PTB or non-bacterial infections, chronic nonspecific infection, parapneumonic inflammation etc.

Classical diagnostic methods like radiological pulmonary infiltrates which characterizes pneumonia may be absent or concealed by pleural fluid. Further, clinical symptoms are much over lapping. Neutrophilia and leucocytosis lack specificity & pleural fluid cultures which are often time consuming and are frequently negative and thus further complicates the identification of the cause of

PE (5). Thus, development of a desired and specific biomarker for differential diagnosis for the cause of PE has been an area of active research. C-Reactive Protein (CRP) is an Acute phase protein synthesized by liver & is used to monitor changes in inflammation associated with many infectious diseases (6). Many fold rise in plasma CRP is due to increase in IL-6 produced predominantly by macrophages & Adipocytes as a result of immune stimulation. CRP binds to phosphocholine on microbes and assists in its intracellular killing by macrophages. It also activates the complement system via CIq complex. Hence CRP has been considered as an effector of innate immunity and can rise upto 1000 fold in response to infection, trauma and inflammatory condition which represents a huge range of illness seen in any hospital (7). It is also increased in inflammation of pulmonary origin that is being investigated in this study. Exudative PE of bacterial or non bacterial cause might be associated with high CRP level. CRP values are thus useful in determining disease progression and effectiveness of treatment. Role of CRP in diagnosis and prognosis of different cause of PE has been explored in this study. Despite the fact that there are many causes of PE, it is estimated that 90% of all pleural effusion are the result of only 5 conditions, congestive heart failure (CHF), pneumonia, malignancy, viral infection and pulmonary embolism (8). CHF cause almost all transudative PE whereas malignancy, pneumonia, pulmonary embolism and tuberculosis are the main cause of exudative PE. Bacterial pneumonia is associated with PE in 40% of cases (9).

Now days, malignancy is a common condition with approximately 50% of lung cancer patients developing PE at later stages (10). PE in neoplastic diseases is caused by blockade of lymphatic drainage of the serosa and secondary inflammation. Cytological finding vary depending on the type of neoplasm. A number of biomarkers including CRP have been evaluated to help in diagnosis of malignant PE. There exist synergistic relationship between inflammation and cancer. Reactive Oxygen Species & Reactive Nitrogen Intermediates produced as a result of chronic infection induce DNA damage is proliferating cells. Further proinflammatory cytokines (eg Interleukin-6 & Tumor Necrosis Factor- α) promote tumor growth & metastasis by adversely affecting tumor cells metabolism & activating stromal cells in tumor microenvironment (11). Malignant PE, occurring secondary to lung cancer is diagnosed by the demonstration of malignant cells on cytological examination or in biopsy specimen or by histologically proven primary lung malignancy with exclusion of other causes of PE.

Tuberculosis (TB) is highly contagious infectious disease mostly affecting apex of lungs & caused by acid fast bacilli Mycobacterium tuberculosis is particularly significant in developing countries like India. TB remains the most common cause of effusion in the absence of any demonstrable pulmonary disease. Diagnosis is based on traditional tuberculin skin test, sputum smear microscopic examination of AFB, chest X ray, pleural fluid culture or typical caseating granuloma or pleural biopsy. Most TB cases are associated with PE (12). Pleural fluid CRP can be a promising biomarker since its production is enhanced by IL-6 & TNF and directly indicates the level of tissue damage.

Parapneumonic PE (PPE) occurs in 10% of patients with community acquired pneumonia and is considered to be associated with increased likelihood of a poor outcome (13). PPE is identified by the presence of pulmonary infection associated with acute febrile illness, pulomanary infiltrates, purulent spectrum & response to antibiotic treatment, identification of the organism in fluid, or presence of emphyema with finding of frank pus in pleural cavity. Microbiological studies can provide definitive results but it is time consuming and has low yield of approximately 60%. All these process lack sensitivity & therefore a pleural fluid biomarker is required for effective disease management.

Sometimes, PE is associated to nonspecific pleuritis i.e. classified as chronic non specific PE. Among other causes of PE (eg amoebic liver abscess, clylothorax, sarcoidosis etc) pulmonary embolism is most common. Its diagnosis is made on clinical grounds & a high probability perfusion scan or abnormal angiogram.

AIM

The aim of the study is to evaluate the utility of CRP as a biomarker for differential diagnosis of various causes of exudative pleural effusion including malignant, tubercular, chronic nonspecific inflammation and parapneumonic pulmonary disease.

MATERIAL AND METHOD

This is a tertiary hospital based observational study conducted in the "Institute of Respiratory Disease, SMS Medical College", Jaipur during the year 2013-2014. 187 adult patients (both male and female) with exudative pleural effusion (both outdoor and indoor) who agreed to participate in the study were enrolled. After full explanation, written informed consent was obtained from all patients. Subject with transudative PE, those suffering from HIV, encephalopathy, renal disease, uncontrolled diabetes mellitus, cardiac disorder, major psychiatric illness, pregnant or lactating women were excluded from the study. Patients with hematological disease, respiratory failure and on treatment (including ATT) or any other therapy were also excluded from the study. All patients were

classified into 5 etiologic classes on the basis of specific diagnostic criteria as follows.

Group 1 (N=11): patients with malignant pleural effusion (MPE)
Group 2 (N=58): patients with chronic non-specific inflammation (CNI)
Group 3 (N=9): patients with parapneumonic pleural effusion (PPE)
Group 4 (N=102): patients with tubercular pleural effusion (TBPE)
Group 5 (N=7): patients with other causes of pleural effusion.

All subjects will be submitted for:

1. Thorough clinical history including smoking and occupational history, physical examination (fever, cough, hemoptysis, weight loss, appetite loss, night sweats, breathlessness etc) and signs such as cervical lymph node enlargement, clubbing, SVC obstruction were done.
2. **Radiographical Investigation:** Chest X-ray (PA view) with side involved, amount of fluid, parenchymal involvement, cavitation and presence of any other abnormalities were recorded. CT of thorax & abdomen was done if necessary and ultrasonography of thorax and abdomen if obligatory.
3. **Thoracocentesis and pleural fluid analysis:** PF was accumulated in detached vials for biochemical - Protein (Biuret method), glucose (GOD-POD method), Albumin (BCG-method), cytological-cell count, cell type and microbiological examination i.e. Gram staining and conventional Ziehl-Neelson's stain for acid fast bacilli. Culture of suspected tuberculous effusion was made by BACTEC rapid culture method for MTB.
4. **Pleural fluid CRP** was analyzed on Transasia EM-360 autoanalyzer by Immunoturbidimetric method. It is based on the principle that human CRP reacts upon a specific antibody for human CRP and turbidity produced by the immune complex is observed at 340nm. The measured turbidity is directly proportional to CRP concentration of the calibrator which can then be used for quantitative determination of pleural fluid CRP.
5. **Routine laboratory Investigations**: Hemoglobin, Total Leucocyte count, Differential count (on Adonis Axiom-19 plus cell counter), ESR (by Westergren method), Bleeding time (by Duke's method), clotting time (by Sabreze's capillary tube method).
6. Tubercular pleurisy was diagnosed by tubercular skin test (Mantoux technique), lymphocyte count in pleural fluid, sputum/pleural fluid smear for AFB and pleural biopsy showing caseating granuloma
7. Malignant effusion was confirmed by cytological examination of pleural fluid or by thoracoscopic pleural biopsy using rigid thoracoscope (KARL STORZ ENDOSKOPE TRICAM SL II 20223020). The procedure was carried out by method described by BOUTIN and coworkers. CT guided biopsy and Abram's needle pleural biopsy was also done for confirmation of MPE cases when required.
8. Parapneumonic PE was diagnosed on basis of clinical, biochemical and radiological signs suspected acute inflammation, positive gram staining, positive bacterial culture or predominance of neutrophil cells in pleural fluid.

DATA ANALYSIS

Data collected was smudged in MS excel sheet 2007. Qualitative data were expressed as percentage (%) and proportions while quantitative data as Mean±S.D. P value less than 0.05 was considered to be statistically significant. Comparison among various groups was assessed by chi-square analysis, using ANOVA and Multiple Comparisons Tukey test. For the choice of optimal cut off, Reciever Operating Characterstics (ROC) curves were constructed and Youden Index calculated. Furthermore, accuracy of pf CRP in distinguishing between tubercular and non-tubercular and between parapneumonic and non parapneumonic PE was established by calculating sensitivity, specificity, Negative predictive value (NPV) and Positive predictive value (PPV). The best cut off has the highest Youden Index. The commercial statistical software package used was SPSS 17.0 (SPSS, Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Pleural effusion is often a clinical problem in medical practice, as its differential diagnosis includes a wide variety of local and systemic symptoms. Although it is easy to establish the presence of PE clinically and radiologically, it is not always easy to determine its etiology. Diagnosis may not be ascertained for some patients despite performance of all diagnostic steps, such as imaging methods, cellular, microbiologic and biochemical methods (14). Microbiological methods do provide some definitive results, however yield rate is only approximately 60 % and have a long turnaround time may result in delayed diagnosis (15). Diagnostic difficulties have led to the search of new novel biomarkers of Pleural Effusion. Various parameters have been used by many researchers for differential diagnosis of exudative PE (16,17).

In this study, diagnostic efficiency of pf CRP was evaluated for different etiological causes of

PE. **Table 1** shows complete gender distribution, demographic characteristics, physical and clinical symptoms of patients for all 5 groups. Incidence of malignant and tubercular effusion was predominantly common in males (77.6% and 67 % respectively) than in females (22.4% and 33.3% respectively). Due to smoking and alcoholic habits being more common in males, incidence of malignancy is three times more prevalent in males (78%) than females (22%). Similarly, for PPE 67% cases were males while on other hand, for Chronic non specific PE 72.7% cases were females. The observations were consistent to other reports where male female ratio in exudative pleural effusion was 4.45 showing a significant predominance of males in lung infection cases (18). The mean age of all patients was 44.12±16.5 years (table 1) and mean age of malignant group was significantly ($p<0.001$) highest among all i.e. 59.8± 11.86 years. Malignant mesothelioma usually presents in fifth to seventh decade of life and pleurisy develops only in later stages of disease. No significant difference was seen in BMI and other general symptoms of patients. All etiological classes of PE are associated with cachexia, loss of appetite, generalized weakness, weight loss, respiratory insufficiency and fever. In the present study, chest pain was the most common complaint (80.74%) reported in study population followed by cough (77%), Loss of appetite (68.98%), fever (68.44%), and shortness of breath (60.8%) were seen in more than half of population. Weight loss (40.64%), expectoration (31.55%) and hemoptysis (10.69%) were less common symptoms. Majority of patients have more than one symptoms and none was without any chest symptoms. Other studies have shown comparable frequencies of these symptoms i.e. chest pain (86.8%), dyspnoea (81.6%), fever (68.4%) and loss of appetite (60.5%) while cough (44.7%) and weight loss (34.2%) were less commonly obsereved (19). Majority of patients had moderate amount of PE (59.9%) with right side predominance (54%). Presence of free fluid in pleural space (88.77%) was more common in contrast to loculated effusion (11.22%). Presence of loculi indicates an intense inflammatory response. Straw coloured PE were found in 68.9% cases followed by hemorrhagic effusion in 27.27% patients in our study population. Among all hemorrhagic effusions, 77.59% cases belong to malignant group since malignancy is one of the most common causes of hemorrhagic pleural effusion.

Basic characterstics of pleural fluid samples are shown in **Table 2.** Cytological and biochemical analysis of pleural fluid constitutes an important part of differential diagnosis of exudative PE. An increased White cell count I.e. more than 7000/ul is commonly found in most infectious exudates with majority of degenerate neutrophils in intense inflammatory conditions such as in Parapneumonic PE while lymphocytic predominance is commonly seen in Tubercular PE. Malignant PE is generally associated with moderate or slightly raised WBC count. Presence of Thrombocytes indicate acute haemorrhage or a contaimination during thoracocentesis. In the present study, Total Leucocyte Count was highest in PPE group, followed by tubercular and malignant groups, but the difference in TLC was insignificant ($p=0.70$). Significant difference was observed ($p<0.001$) in neutrophil count (%) and lymphocyte (%) level among all groups. Neutrophil count was highest in others group (57.7±29.6) and PPE group (53.2±35.2) and lymphocyte count was highest in CNI (80.0±8.1), followed by TBPE (79.3±14.7) and malignant PE group (64.7±17.4) (table2). Non-significant difference ($p=0.10$) was observed in platelet count and hemoglobin level among all classes of exudative PE (table 2). Comparable results have been shown by other workers with significantly higher level of neutrophils (%) in parapneumonic PE patients than in other categories of PE and level of lymphocytes was highest in Tubercular PE patients than in other cases of Pleural effusion (20). A predominance of neutrophil in pleural fluid is a simple marker of parapneumonic PE. Immune stimulation causes recruitment of large number of Polymorphonuclear cells locally that are further proliferated under the effect of cytokines and other inflammatory markers.

Pleural fluid glucose concentration was highest in MPE group (85.6±42.0), followed by CNI and others group (76.8±13.5 and 76.14±19.9) mg/dl respectively. Patients with PPE have lowest level of PF glucose i.e. 46.1±5.3 mg/dl. Glucose level < 60 mg/dl is often seen in complicated PPE. Various studies have shown low glucose level in infectious PE than non infectious or malignant condition (21). As regard to pleural fluid protein, significantly high level was obtained in CNI (5.5±0.9) and tubercular group (5.4±1.1) (g/dl). Mean PF Albumin level in CNI and PPE group were 3.1±1.1 g/dl and 3.8 ±1.3 g/dl respectively and were significantly higher than other classes ($p<0.05$). Exudative effusion mostly involves some types of inflammation which leads to increased leakage of fluid that has high protein concentration (22). Intense inflammation in CNI, PPE and TBPE groups accounts for increased level of PF protein and PF albumin in these patients than in malignant group due to intense inflammation that increases permeability of pleural membrane. Albumin being a low molecular weight protein enters from plasma to pleural space via inflamed pleura (table 2). **Table 3**. Shows mean value of PF CRP in all the groups. Difference is found to be significant ($p<0.001$) with highest level in parapneumonic PE (134.0±22.9) and lowest in Malignant group (26.8±18.7). In Chronic non specific infection and

tubercular PE, mean PF CRP level were 66.75± 9.7mg/l and 66.54±10.77 mg/l respectively while in others group it was 38.94±5.12 mg/l i.e. still higher than malignant group. When infectious and malignant conditions were compared, various authors have found increased PF CRP in parapneumonic PE and other infectious conditions than malignant effusion (23). They investigated pleural to serum CRP ratio and obsereved it to be significantly lower in malignant PE cases than Parapneumonic or Tubercular PE cases (23). Further, in another study PF to serum ratio of CRP was found to be highest in PPE, followed by TBPE than MPE. Mean PF CRP was highest in PPE compared to TBPE and MPE that may be twice in former than later groups (24,25). PF CRP less than 20 mg/l suggest malignant origin while value > 45mg/l eliminates probability of malignancy. Pf CRP > 80mg/l strongly argues for presence of Parapneumonic origin of PE (LR +7.4) whereas PF CRP less than 20mg/l rules out infectious origin of PE, whether bacterial or non bacterial in origin (LR – 0.22) (26).

CRP is an acute phase protein widely used as a marker of inflammation and tissue injury. Intense infectious state such as parapneumonia is associated with many fold rise in serum CRP. CRP in pleural fluid (PF) is produced from liver and arrives in pleural space due to increase in vascular permeability and leakage of plasma CRP via inflamed pleura in intense inflammatory condition as found in PPE and CNI patient in this study . In TBPE and CNI groups, PF CRP is less than PPE but as an indicator of inflammation, they are higher than non infectious states like malignancy. CRP in pleural space is high in TBPE group due to raised local production as a result of granuloma formation and as a defence mechanism against tubercle bacilli. Further, CRP in TB group may be due to maximum number of TB patients included in our study.

Mean PF CRP in CNI cases was near about TBPE group. These results are possibly due to misdiagnosis of early presentation of tubercular pleuritis. As all patients with CNI had same symptoms as TB with short duration of presenting complaints and approximately > 50% in this group were tuberculin positive and about 80% were relieved by Anti Tubercular Therapy, misdiagnosis may have occured and thus is responsible for comparable value if PF CRP. The mean PF CRP in group-v others category could not be compared to rest of study groups, as it contains both infectious (amoebic liver abscess with hepatopleural fistula) and noninfectious (pulmonary embolism, SLE etc.) diseases. CRP is directly associated to acute infection than chronic inflammatory condition like malignant PE. CRP value <20mg/l characterize chronic condition that may be tubercular or malignant in nature (27). This accounts for low PF CRP in MPE group as found in our study. Thus, PF CRP can be used as a simple, inexpensive and a reliable indicator of infection especially in PPE and TBPE and help to differentiate acute infection from chronic sub acute condition i.e. malignancy. (Figure 1)

Diagnostic Efficiency of PF CRP: The diagnostic efficiency of PF CRP with its sensitivity, specificity and AUC for differential diagnosis of different causes of PE is fully investigated in this study. CRP level varies widely in inflammatory conditions of infectious and non infectious origin and also between sub-acute and chronic infection (table 4). In the present study, ROC analysis of PF CRP provided good sensitivity (97.05%), specificity (71.76%), NPV of 95.31% and PPV of 80.48% for the differentiation of tubercular vs. non tubercular effusions (parapneumonic, CNI, malignant and others). At cut off level i.e. 51.5mg/l, AUC for pf CRP was 0.796(95 % CI was 0.718-0.875 with standard error of 0.040) (table 4) (**Figure 2a**.). Some authors have reported that at a cut off for PF CRP > 50mg/l sensitivity and specificity was 45% and 95% respectively. It was strongly suggestive of tubercular pleuritis. Though we found high sensitivity and low specificity than above, it may be due to selection of lymphocytic exudative PE in the above study (28).

In ROC analysis of PF CRP for differentiation of PPE from non PPE (tubercular, chronic non-specific inflammation, malignant and others) , sensitivity of 100%, specificity 98.88%, NPV of 100% and PPV of 81.82% was seen at cut off level of 90.8 mg/l (table 5). It provides largest AUC (1.000) shown in **figure 2b** for differentiation of PPE vs non PPE. Hence, PF CRP provides an excellent diagnostic and screening tool for the disease. Further, when several biomarkers (CRP, sTREM, ESR etc) were measured in pleural fluid to diagnosis infectious effusion, pf CRP level show high sensitivity (93.7%), specificity (76.5%) and PPV of 98.4% at cut off level >30 mg/l for diagnosis of PPE (29). Among various biomarkers CRP provides largest AUC of 0.92 for discriminating PPE from MPE and TBPE. Other studies have generated an AUC of 0.87 for PF CRP with cut off at >20 mg/l for discriminating PPE from PE due to other aetiologies (30). For accurate clinical diagnosis of PPE, PF neutrophilic predominance and CRP have highest accuracy as measured by area under ROC curve (AUC=0.85 and 0.82 respectively). Thus, in patients with PPE, finding of >50% of PMN in differential WBC count along with pf CRP > 45 mg/l is strong argument for Parapneumonic PE (30). Further studies have shown pf CRP as a better marker to assess diagnostic accuracy than procalcitonin and neutrophil count. It gives better sensitivity (73.3%) but neutrophil count gives better specificity (91.1%). The parameter with largest area under ROC curve was the

product of Total neutrophil count and pf CRP where AUC of 0.836 and sensitivity of 64.3% and specificity of 93.4 % was obtained for diagnosis of PPE (31). CRP is more sensitive and accurate reflection of acute phase response than ESR i.e. it appears and disappears more quickly than changes in ESR.

In the present study, ROC analysis of PF CRP for differentiation of chronic nonspecific pleuritis (CNI group) from other than CNI showed 100 % sensitivity at cut off level 49.59mg/l, but has low specificity of 35.23%. Though this cut off is excellent to use as screening tool due to high sensitivity and 100% NPV but PPV was only 8.8%. These finding may be due to close cut off level of CNI and Tubercular pleuritis, which is again possibly due to misdiagnosis of early presentation of tubercular pleuritis as CNI. AUC for pf CRP in reference to CNI was 0.64 (95% CI with range of 0.718-0.875 and standard error of 0.055) (Table 6) (**figure2.c**).

ROC analysis of pf CRP for differentiation of malignant from non-malignant effusion showed AUC=0.056 (Table 7) (**figure 2.d**) means that the test incorrectly classify all subjects with disease as negative and all subjects with non diseased as positive that is extremely unlikely to happen in clinical practice. Thus, PF CRP can be used as a diagnostic aid specially differentiating between acute and chronic inflammation and also between infectious and non infectious inflammation. CRP more than 30mg/l almost excludes malignancy and narrows options of lymphocytic pleural effusion. Thus, the present study indicated significant role of Pleural fluid CRP in differential diagnosis of Exudative PE. CRP has particularly high specificity and sensitivity in PPE cases than non infectious cases further confirming its role as a marker of inflammation.

Limitation of the study was the use of single biomarker to differentially diagnose PE. Other markers of inflammation individually or in combinations can also be used to determine specificity and sensitivity in different forms of exudative effusions. Further, studies in large population is required to establish a biomarker to be routinely used as screening tool in all cases of effusion to ascertain its underlying cause. This will help in accurate diagnosis, proper prognosis and effective monitoring of patients thus providing social benefit.

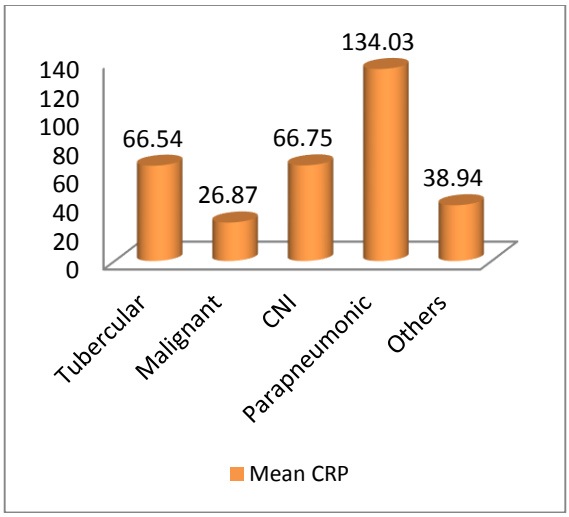

Fig. 1: Mean value of pleural fluid CRP (mg/L) in different etiological groups of pleural exudates.

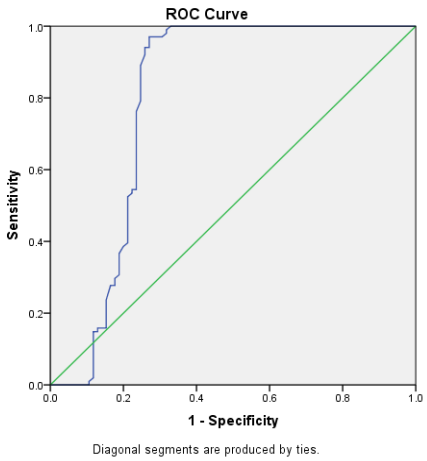

Fig. 2(a): ROC curve of pleural fluid CRP for the differentiation of Non Tubercular vs.Tubercular effusions (optimal cut-off point 51.5 mg/L).

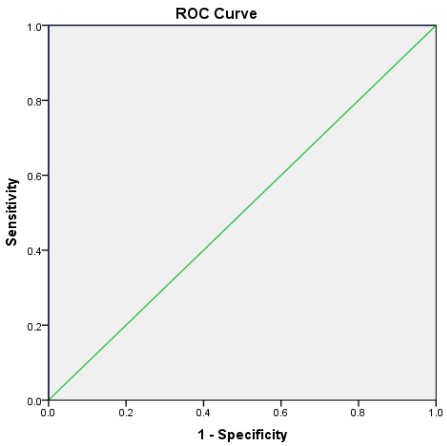

Fig. 2(b): ROC curve of pleural fluid CRP for differentiation of parapneumonic vs non parapneumonic effusions (optimal cut off point 90.8mg/L).

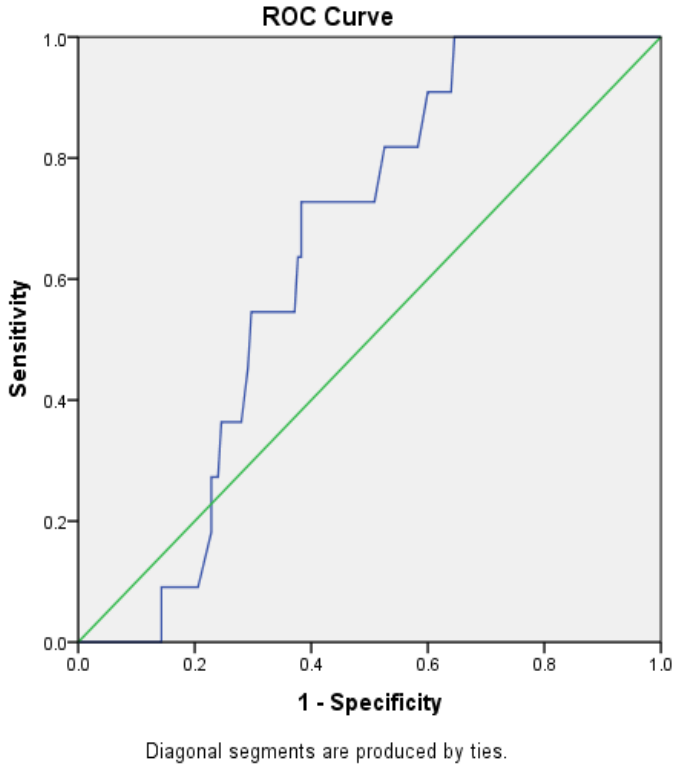

Fig. 2(c): ROC curve of CRP for the differentiation of CNI vs. Other than CNI effusions (optimal cut-off point 49.59 mg/L).

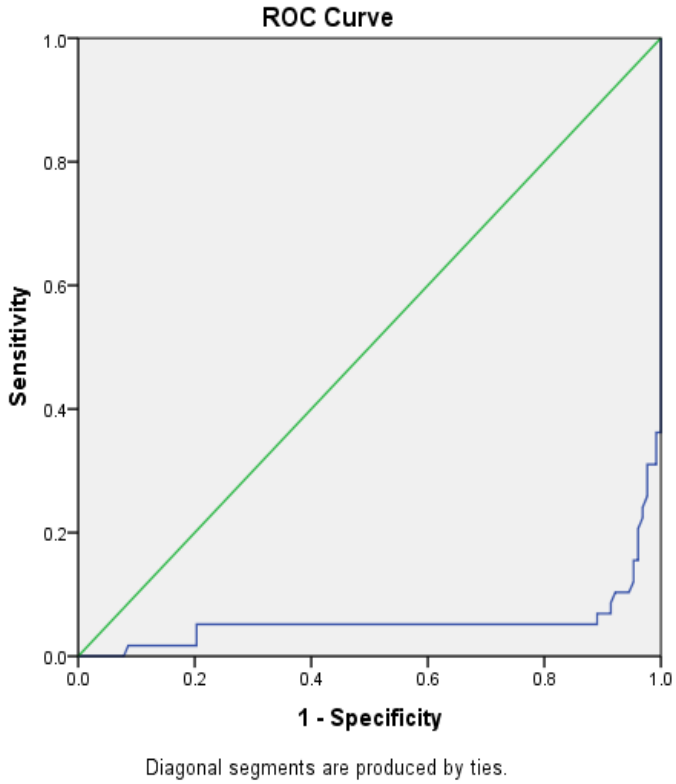

Fig. 2(d): ROC plot of pleural fluid CRP in reference to Malignancy.

Table 1: Demographic & clinical characteristics of study subjects

General characteristics of subject		CNI PE	Malignant PE	Parapneumonic PE	Tubercular PE	Others	Total	P value
No of patients (n)	N	11 (5.9)	58 (31)	9 (4.8)	102 (55)	7 (3.7)	187	-
	Males (N)	3 (27.3 %)	45 (77.6%)	6 {66.67 %}	68 {66.67%}	6(85.7 %)	128 (68.4%)	0.017
	Female (N)	8 (72.7 %)	13 {22.4%}	3 {33.33 %}	34 {33.33 %}	1 (14.3%)	59 (13.55%)	
Average age (in years) mean ± SD		39.9 ±15.3	59.81 ± 11.86	34.44 ± 11.4	36.19 ± 12.65	48.71±13.7	44.12±16.49	P< 0.001
Location	Urban (n=99)	4 (4.04 %)	34 {34.34%}	4 {4.04 }	55 {55.56}	2 (2.02)	99 (52.94)	0.415
	Rural (n = 88)	7 (7.95%)	24 {27.27}	5 {5.68}	47 {53.41}	5 (5.68)	88 (47.05)	
BMI (kg/m^2) (mean ± SD)		19.96 ±4.32	20.47 ± 3.67	21.27 ± 2.43	20.00 ±3.17	19.86±0.8	20.20±3.31	0.771
Smoking Status	Ex-Smoker	0 (0.00)	4 {6.90 }	1 {11.11}	1 (0.98)	0 (0.00)	6 (3.20)	0.000
	Non Smoker	8 (72.7)	10 {17.24 }	5 {55.56 }	51 (50.0)	3 (42.86)	77 (41.17)	
	Smoker	3 (27.3)	44 {75.86 }	3{33.33 }	50 (49.02)	4 (57.14)	104 (55.61)	
Alcoholic status	Ex-alcoholic	0 (0.00)	2 {3.45 }	0{0.00 }	3 (2.94)	0 (0.00)	5 (2.67)	0.395
	Non Alcoholic	10 (90.91)	48 {82.76}	5{55.56 }	87 (85.29)	6 (85.71)	156 (83.42)	
	Alcoholic	1 (9.09)	8 {13.79 }	4 {44.44 }	12 (11.76)	1 (14.29)	26 (13.90)	
Side of effusion	Bilateral	0 (0.00)	3 {5.17 }	0 {0.00 }	1 (0.98)	0 (0.00)	4 (2.13)	0.609
	Left	6 (54.55)	24 {41.38 }	4 {44.44 }	44 (43.14)	5 (71.43)	83 (44.38)	
	Right	5 (45.45)	31 {53.45}	5 {55.56 }	57 (55.88)	2 (28.57)	100 (53.47)	
Amount of effusion	Massive	0 (0.00)	26 (44.83)	0(0.00)	3 (2.94)	0 (0.00)	29 (15.50)	P < 0.001
	Moderate	11 (100)	20 (34.38)	0 (0.00)	77 (75.49)	4(57.14)	112 (59.89)	
	Minimal	0 (0.00)	12 (20.69)	9 (100.0)	22 (21.57)	3 (42.86)	46 (24.59)	
Nature of Exudate	Free	9 (81.82)	58 (100.0)	9 (100.0)	84 (82.35)	6 (85.71)	166 (88.77)	P = 0.010
	Loculated	2 (18.18)	0 (0.00)	0 (0.00)	18 (17.63)	1 (14.29)	21 (11.22)	
Colour of exudate	Chocolate	0	0	0	0	1	1	P= 0.000
	Hemorrhagic	0	45	0	4	2	51	

	Milky	0	0	0	0	1	1	
	Straw	11	13	5	97	3	129	
	Turbid	0	0	4	1	0	5	
Respiratory symptoms	Cough	9	45	9	78	3	144	P= 0.113
	Expectoration	3	14	8	32	2	59	P= 0.004
	SOB	8	46	4	62	5	125	P=0.090
	Chest pain	6	46	8	85	6	151	P= 0.290
	Hemoptysis	0	11	1	8	0	20	P= 0.120
Non- Respiratory symptoms	Appetite loss	8	39	0	78	4	129	P= 0.001
	Weight loss	3	31	0	41	1	76	P=0.012
	Weakness	0	1	0	1	0	2	P= 0.900
	Fever	9	15	9	90	5	128	P=0.001

Table 2: Basic characteristics of pleural fluid samples of study groups

Pleural fluid (pf) markers	CNI PE	Malignant PE	Parapneumonic PE	Tubercular PE	Others	P value	Significance
Pf Glucose (mg/dl)	76.8± 13.5	85.6 ±42.0	46.1 ± 5.3	69.8 ± 16.5	76.1 ± 19.7	<0.001	S
Pf Protein (g/dl)	5.5 ± 0.9	4.6 ± 1.1	4.0 ± 0.4	5.4 ± 1.1	4.59 ± 0.69	<0.001	S
Pf Albumin (g/dl)	3.1±1.1	3.0 ± 0.78	3.8 ± 1.3	3.06 ± 1.3	2.9 ± 1.51	<0.05	S
A/G Ratio	1.29 ± 0.76	1.87 ± 0.96	1.42 ± 0.71	1.27 ± 1.0	1.32 ± 0.9	<0.05	S
Pf TLC (per cubic mm)	7.6 ± 2.8	11.6 ± 2.71	30.2 ± 10.2	18.4 ± 3.2	10.87 ± 4.55	P= 0.70	NS
Pf Neutrophil count (%)	16.8 ± 11.0	18.7 ± 5.31	53.2 ± 35.2	20.6 ± 14.5	57.7 ± 29.6	<0.001	S
Pf Lymphocyte Count (%)	80.0 ± 8.1	64.7 ± 17.4	26.7 ± 15.0	79.3 ± 14.7	42.3 ± 29.6	<0.001	S
Pf Platelet Count (per ul)	2.2 ± 0.4	2.6 ± 1.4	3.3 ±1.2	2.4 ± 0.8	2.23 ± 0.79	P= 0.10	NS
Hemoglobin level (g/dl)	9.5 ± 1.7	10.7 ± 1.7	11.0 ± 1.2	10.9 ±1.5	10.9 ± 1.77	P= 0.10	NS

Values are in mean ± Standard Deviation: S is Significant and NS is non-significant

Table 3: Mean value of Pleural fluid CRP (mg/L) in different etiological groups of pleural exudates

Diagnosis	No. of cases	Mean CRP	Std. Deviation	P value
Tubercular	102	66.54	10.77	< 0.001 Significant
Malignant	58	26.87	18.75	
Chronic nonspecific pleuritis	11	66.75	9.77	
Parapneumonic	9	134.03	22.91	
Others	7	38.94	5.12	
Total	187	56.41	28.99	

ANOVA - Analysis of Variance ---F =145.14; p<0.001 S, Multiple Comparisons - Tukey Test ---

Table 4: Diagnostic performance of pf CRP for differential diagnosis of tubercular and non-tubercular effusion at different cut off level on ROC curve

Positive if greater than or equal to	Sensitivity (SN)	1- Specificity	Specificity(SP)	Youden Index Y= (SN+SP)-1
44.3050	1.000	0.341	0.659	0.659
45.1250	1.000	0.329	0.671	0.671
45.625	0.990	0.318	0.682	0.672
47.000	0.980	0.318	0.682	0.663
49.595	0.970	0.306	0.694	0.664
51.590	0.970	0.282	0.718	0.688

Table 5: Diagnostic performance of pf CRP for differential diagnosis of parapneumonic and non parapneumonic effusion at different cut off level. Optimum cut off of 90.8 mg/l is excellent to use as screening test

Positive if greater than or equal to	Sensitivity (SN)	1- Specificity	Specificitym(SP)	Youden Index Y= (SN+SP)-1
81.345	1.000	0.036	0.964	0.964
83.465	1.000	0.018	0.982	0.982
90.815	1.000	0.000	1.000	1.000
104.500	0.889	0.000	1.000	1.889
117.350	0.778	0.000	1.000	0.778

Table 6: Diagnostic performance of pf CRP for differential diagnosis of CNI and non CNI effusion at different cut off level. Optimum cut off of 49.59 mg/l is excellent to use as screening test.

Positive if greater than or equal to	Sensitivity (SN)	1- Specificity	Specificity (SP)	Youden Index Y= (SN+SP)-1
45.125	1.000	0.674	0.326	0.326
45.625	1.000	0.663	0.337	0.337
47.000	1.000	0.657	0.343	0.343
49.59	1.000	0.646	0.354	0.354
51.590	0.909	0.640	0.360	0.269
52.005	0.909	0.634	0.366	0.275

Table 7: Diagnostic performance of pf CRP at optimum cut off for differential diagnosis of different etiologies of PE at 95% CI

Diagnosis	Cut off level of pf CRP (mg/l)	Sensitivity	Specificity	PPV	NPV	AUC	Interval Lower bound-upper bound
Tubercular PE	≥ 51.6	97.06	71.76	80.48	95.3	0.282	0.718-0.875
Parapneumonic PE	≥ 90.8	100	98.88	81.82	100.0	1.000	1.000-1.000
CNI	≥49.59	100	35.23	8.80	100.0	0.644	0.535-0.752
Malignant PE	-	-	-	-	-	0.056	0.007-0.104

CONCLUSION

The present study indicates utility of Pleural Fluid CRP not only as a marker of inflammation and acute infection but for differential diagnosis of Parapneumonic pleural effusion from other PE with excellent sensitivity, good NPV and acceptable specificity. High value of PF CRP is indicative of infectious origin of PE while value less than 30mg/l strongly supports malignant origin of PE.

The authors declare that they have no competing interest.

Author's Contribution: Dr Sanjay Gabhale carried out the experimental and diagnostic part of the study. He contributed significantly in designing the study, selection of study population and carrying out the microbiological and radiological investigations. Pallavi Taparia carried out all biochemical tests, interpretation and analysis of data and drafting of manuscript. Dr Dharamveer Yadav conceived the study and participated in design and coordination of the work. Dr S. P. Agnihotri, Senior Professor, Institute of Respiratory Disease, SMS Medical College, Jaipur provided with patients who agreed to participate in the study and also made required test available in the laboratory.

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Abbreviations: PE - Pleural Effusion, CRP - C - reactive protein, Pf – Pleural Fluid, CNI - chronic non-specific inflammation

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