

## A study of role of interleukin-8 in differentiating transudative and exudative pleural effusion

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### Abstract

**Background:** Differentiation between transudative and exudative effusion is considered the initial step in the etiological diagnosis of any pleural effusion. The differentiation is essential, since the treatment of underlying systemic cause is enough in transudative pleural effusion, whereas extensive investigations and treatment is required in exudative effusion. Recent studies have demonstrated the existence of a novel neutrophil activating peptide first named NAP-1, but subsequently renamed IL-8. Interleukin-8 being the mediator of local inflammation may help in the differentiation of transudates and exudates.

**Methodology:** This is a hospital based cross sectional study which included 70 patients with pleural effusion. After diagnosing pleural effusion, they were further divided into two categories, namely transudate and exudate based on Light's criteria.

The concentration of pleural fluid interleukin-8 was determined by an Enzyme Linked Immunosorbent Assay (ELISA) method by using commercially available assay kits.

**Statistical Analysis:** All results are expressed as mean  $\pm$  standard deviation (SD) for continuous variables and as frequencies for categorical variables. The difference in the age and gender between groups is disproved using independent student t-test and chi-square test, Mean pleural fluid Interleukin-8 level between the groups is analyzed using independent student t-test.

**Results:** The parameters in Light's criteria (pleural fluid protein and LDH) and Interleukin 8 were able to differentiate transudate from exudate and were found to be statistically significant with p value of  $< 0.001$ .

**Conclusions:** It is clear from our study that IL-8 can be used to diagnose and differentiate exudates from transudative pleural effusions with a good sensitivity and specificity.

**Keywords:** Pleural effusion, Interleukin 8, Exudative effusion, Transudative effusion, Empyema.

### Introduction

In cases of pleural effusion, identifying the type is very important to arrive at the etiological cause. Transudative effusions are due to an imbalance between the hydrostatic and oncotic pressures in the systemic or pulmonary circulation. Exudates are produced by increased vascular permeability. Transudates are most often caused by heart failure (80%) and, to a lesser extent, by hepatic cirrhosis. In 80% of cases, the exudate is secondary to malignancy (secondary or primary), pneumonia, tuberculosis or viral pleuropericarditis [1,2].

Pleural effusions are classified as transudates or exudates by Light's criteria which has been in use for a long time. The differentiation is essential, since the treatment of underlying systemic cause is enough in transudative pleural effusion, whereas extensive investigations and treatment is required in exudative effusion. The sensitivity of Light's criteria is between 94% to 100% and specificity is 57% to 85%. In case of improper diagnosis or partial treatment, the uncomplicated pleural effusions can become a complicated, characterized by deposits of fibrin and microorganisms in the pleural fluid. So there has been search for a novel biomarker which can differentiate between transudates and exudates more accurately [3,4].

Recent studies have demonstrated the existence of a novel neutrophil activating peptide first named NAP-1 but subsequently renamed IL-8. Interleukin-8 being the mediator of local inflammation may help in the differentiation of transudates and exudates. Levels in pleural effusion have been studied in very few researches. In view of achieving a

specific diagnosis as well as to differentiate between transudates and exudates we would like to assess the role of IL-8 in pleural effusion [5-7]. The cut off values of IL-8 levels with different aetiologies were detected from various studies as shown in table 1.

**Table 1:** Cut off values of IL-8 levels with different etiologies from various studies

Normal	5 pg/ ml
<b>Exudative</b>	3068.5 $\pm$ 1762.7
Parapneumonic effusion	1884.5 $\pm$ 366.7
Tuberculosis	1420 $\pm$ 1049
Malignancy	1574 $\pm$ 1079
<b>Transudates</b>	108.8 $\pm$ 92.0

### Materials and Methods

This is a tertiary care hospital based cross sectional study which included 70 patients with pleural effusion. Patients were included in the study after getting informed consent from the patient and patient's attender. Also, an ethical committee approval was obtained. Clinical history was elicited and physical examination was performed. Pleural effusion was confirmed based on the chest X-ray finding. USG thorax/CT thorax was taken if the effusion was loculated or minimal. After diagnosing pleural effusion, they were further divided into two categories, namely transudate and exudate based on Light's criteria. Pleural fluid was obtained by diagnostic pleural fluid aspiration (thoracentesis) after informed consent. Determination of biochemical

parameters (LDH, glucose & protein) was performed using micro 600 semi auto analyzer from MERC. Light's criteria for identifying exudate were as follows (atleast 2 of the 3 to be fulfilled)

1. Pleural fluid: Serum total protein ratio more than 0.5
2. Pleural fluid: Serum LDH ratio more than 0.6
3. Pleural fluid LDH more than 200 IU/L

**Table 2:** Patient characteristics and etiology of pleural effusion (light's criteria based)

Patient characteristics (n=70)	Exudate group (n=39)	Transudate group (n=31)
Age distribution in years		
(21-40 years), n (%)	16 (41)	6 (19.5)
(41-60 years), n (%)	15 (38.5)	20 (64.5)
>60 years, n (%)	8 (20.5)	5 (16)
Total, n (%)	39 (100)	31 (100)
Sex wise distribution		
Male, n (%)	33 (84.5)	24 (77.5)
Female, n (%)	6 (15.5)	7 (22.5)
Etiology, n (%)		
Heart failure	10 (25.5)	18 (58)
Cirrhosis of liver	2 (5)	3 (10)
Chronic kidney disease	2 (5)	10 (32)
Abscess (lung, empyema, liver)	4 (10)	-
Parapneumonic effusion	2 (5)	-
Tuberculosis	12 (30.5)	-
Malignancy	5 (12.5)	-
Pancreatitis	1 (2.5)	-
Perforation peritonitis	1 (2.5)	-

**Table 3:** Comparison of Light's criteria biochemical parameters with Interlukin 8

Pleural fluid analysis	Transudate	Exudate	p value
Pleural Glucose (mgs%)	91.61 ± 27.41	53.49 ± 13.16	< 0.001*
Pleural LDH (IU/L)	240.35 ± 100.83	910.13 ± 387.42	<0.001*
Pleural Protein (gms%)	2.32 ± 0.60	5.22 ± 1.51	<0.001*
Pleural fluid protein/ Serum protein	0.34 ± 0.08	0.74 ± 0.18	<0.001*
Pleural fluid LDH/Serum LDH	0.45 ± 0.12	2.59 ± 1.03	<0.001*
IL-8 (pg/ml)	319.35 ± 382.28	3273.33±2767.43	<0.001*

**Table 4:** Mean values of IL-8 in various exudative pleural effusion

Diseases	n	IL-8 Mean ± SD	p value
Systemic Failure effusion (heart failure, Liver cirrhosis, CKD)	14	1397.14 ± 1721.81	< 0.01
Abscess (Empyema, Liver abscess, Lung abscess)	4	9522.50 ± 367.91	
Malignancy	5	3442 ± 546.59	
Surgical effusions (Pancreatitis, peritoneal perforation)	2	3310 ± 1428.36	
Parapneumonic effusions	2	1915 ± 417.19	
Tuberculosis	12	3529.17 ± 1872.37	

**Table 5:** Difference in IL-8 between different diseases in exudate sub group: (ANOVA)

Systemic failure effusion	Subgroup	Mean Difference	p value
	Abscess	-8125.36	< 0.001*
	Tuberculosis	-2132.02	0.023*
	Abscess	8125.36	< 0.001*
	Malignancy	6080.50	< 0.001*
	Surgical effusions	6212.50	0.001*
	Parapneumonic effusions	7607.50	< 0.001*
	Tuberculosis	5993.33	< 0.001*

### Diagnostic Tool

After differentiating exudates from transudates further diagnosis of exudative effusion – Tuberculous pleuritis were diagnosed if either the bacillus was isolated from the pleural fluid with predominant of lymphocytes and ADA>40. Malignant pleural effusion was diagnosed if malignant cells, either at cytologic examination or in biopsy specimens were obtained. Parapneumonic pleural effusion and empyema were identified when there was an acute febrile illness with purulent sputum, pleuritic chest pain, pulmonary infiltrates and respond to antibiotic treatment or identification of the organism in the pleural effusion. Transudates - congestive cardiac failure, cirrhosis of liver and chronic kidney disease were diagnosed by further clinical features and investigations.

### Pleural Fluid Interleukin 8 Level

The concentration of pleural fluid interleukin-8 was determined by ELISA (Enzyme Linked Immunosorbent Assay) method by using commercially available assay kits. To perform the test 50 micro liters of pleural fluid was needed. The pleural fluid samples were centrifuged immediately. The supernatant layers were frozen at  $-20^{\circ}\text{C}$ . Manufacturer's recommendation and instruction were followed during performance of the test. The minimum cut off for detection of IL8 in the sample according to the manufacturer is 5pg/ml. Values greater than these were taken as significantly elevated as normally pleural fluid could not be obtained.

### Statistical Analysis

All results are expressed as mean  $\pm$  standard deviation (SD) for continuous variables and as frequencies for categorical variables. The difference in the age and gender between groups is disproved using independent student t-test and chi-square test, respectively. Mean pleural fluid Interleukin-8 level between the groups is analyzed using independent student t-test. The relationship between the variables of pleural fluid was estimated using the correlation graphs. An ROC curve was created in order to determine the specificity and sensitivity of interleukin-8. p-value  $< 0.05$  is taken as being statistically significant. All analysis was done using Statistical Package for the Social Sciences (SPSS) version 16 for windows.

### Results

Based on Light's criteria, of the 70 patients, 39 patients had exudative pleural effusion and 31 patients had transudative effusion. The demographic features of the included patients are shown in table 2. In the transudates subgroup, the maximum number of patients were between 41 to 60 years, whereas in exudates, the bulk of the study population consisted of the age group 21 to 40 years and the difference in the mean age between these two subgroups was found to be insignificant. In both the subgroups, male patients contributed the bulk of the study population. Among the transudates subgroup, the maximum numbers (56.6%) were due to heart failure, followed by chronic kidney disease and

cirrhosis of liver. 30% of exudates were due to tuberculosis followed by heart failure, malignancy, abscess, parapneumonic effusion and abdominal causes.

The parameters in Light's criteria (pleural fluid protein and LDH) and Interlukin 8 were able to differentiate transudate from exudate and were found to be statistically significant with p value of  $< 0.001$ . Interleukin-8 had a significant positive correlation with LDH ( $r = 0.63$ ,  $p < 0.001$ ) and protein ( $r = 0.59$ ,  $p < 0.001$ ) in pleural fluid. Analysis of variance (ANOVA) was done to find out whether there was any difference in IL-8 concentrations seen among various diseases in patients with exudates. Our study results showed that there was significant difference seen in mean IL-8 concentrations in the abscess subgroup (empyema, liver and lung abscess) compared to rest of the diseases in exudates group. And there was also a significant difference in mean IL-8 concentrations seen between systemic failure subgroup and tuberculosis subgroup. Receiver operating characteristic (ROC) curve was created to identify the cut-off for IL-8 value above which exudate can be confirmed. Area under curve, which was calculated from the ROC curve was 0.94 for IL-8 which was found to be statistically significant,  $p < 0.001$ . In our study, we found that a cut off level of 430 pg/ml for IL-8 can able to detect exudates with a sensitivity of 76.9% and specificity of 90.3% and transudate with a sensitivity and specificity of 90.32% and 76.92% correspondingly. We had calculated the positive predictive value and the negative predictive value for this cut off level of IL-8 and it came out to be 90.9% and 75.68% correspondingly.

### Discussion

The differential diagnosis of pleural effusion present a dilemma frequently. The hallmark criteria identified for distinguishing exudates from transudate were given by Light, et al. However, several reports have found that Light's criteria miscategorised many cases of effusions, especially transudates [8-10]. Until now, measurements including bilirubin, cholesterol and ADA levels have been used with pleural fluid, but with no definite success [11]. Identifying new biochemical fluid markers is therefore suitable for differentiating transudates from exudates. The possible role of pleural fluid cytokines especially IL-8 in distinguishing transudates from exudates in pleural effusion has not been studied adequately, especially in our country. IL-8 levels were found to be higher in cases with infectious pleural effusions compared with the patients with non-infectious effusions. In our study, we evaluated 70 patients with pleural effusion and compared pleural fluid IL-8 levels with the gold standard Light's criteria, as IL-8 is also helpful in diagnosing the etiology among exudative pleural effusions [8,11,14].

### IL-8 and Light's Criteria

The main objective of our study is the comparison of IL-8 levels with Light's criteria, especially in transudative patients who were in diuretic therapy, where Light's criteria misclassified transudates as exudates. In our study Light's criteria misclassified 14 out of 45 patients (diagnosed etiologically) with transudative pleural effusion on diuretic

therapy as exudates. Chakko et al., showed that diuretic therapy in cases of heart failure with pleural effusion leads to a concentration of pleural fluid protein, which erroneously falls in the exudative range. Whereas IL-8 misdiagnosed 8 cases out of 45 as exudates, among these 8 cases, 5 were misclassified with both Light's criteria and IL-8 while the remaining 3 cases were misdiagnosed with IL-8 alone. The explanation for this could be that the patient presented with an associated febrile illness due to respiratory infections, which caused inflammation of the pleural spaces. The increase in IL-8 concentration within inflammatory fluids was probably due to local production of the cytokine. Despite there being a few instances of misdiagnosis with IL-8, it has a much better sensitivity and specificity. The advantage of the reduction of misdiagnosing transudates following diuretic therapy using IL-8 over the Light's criteria gives an edge to use of IL-8 against the Light's criteria. IL-8 also has a good diagnostic value for exudates. Furthermore, it is helpful in classifying the etiology of exudative pleural effusions [15,19].

### IL-8 in Classifying Subgroups

A study by Zaki et al., reported that pleural IL-8 levels were significantly increased in exudative effusion when compared with transudate. Miller and Idell also found higher levels in exudates than transudates. Similarly, another study by Alexandrakis and his colleagues reported that concentrations of inflammatory cytokines namely IL-8, FER and TNF-alpha were significantly higher in serum and pleural effusion of patients with exudates than transudates. In our study, pleural fluid IL-8 levels were elevated in exudative group than in transudative group [16,17].

### IL-8 in Transudates and Exudates

In our study there was not much of a significant difference in IL-8 levels among transudates. Similar findings were reported by Pace et al, where large concentrations of interleukin-8 (IL-8) were detected in cancer and tuberculous effusions, but not in CHF. Ceyhan et al reported higher levels in empyema and parapneumonic effusion rather than the tuberculous effusion. Dlugovitzky et al., found higher levels of IL-8 in tuberculous group as compared to parapneumonic effusions. However Antony et al., detected higher levels in cases with parapneumonic effusions unlike those found in the group with tuberculous effusions. IL-8 concentrations in the empyema were noted to be higher than the parapneumonic group. Similarly, Ashitani et al and Broaddus et al found higher levels in the empyema group compared with the other groups. In accordance with these studies, our study revealed that the highest levels of IL-8 were detected in the empyematous and abscesses groups, pancreatitis and peritoneal perforation followed by tuberculous, malignant and parapneumonic pleural effusions in decreasing order. One of the objectives of our study, determining the aetiologies of pleural effusion was obtained based on IL-8 levels [5,16,17].

### Analysis of Variance (ANOVA)

Our study results showed that there was a significant difference seen in mean IL-8 concentrations in the abscess subgroup compared to rest of the diseases in exudates group. And there was also a significant difference in mean IL-8 concentration seen between systemic failure effusion subgroup and tuberculosis subgroup. Similar reports were demonstrated by Ceyhan et al, that in the empyema and parapneumonic group, the pleural fluid contained a significantly higher concentration of IL-8 than pleural fluids secondary to malignancy and tuberculosis [5,18,20].

ROC curves were used to assess the sensitivity and specificity of IL-8 for differentiating between the two patient groups. Unlike the study by Pace et al., conducted in Egypt and published in 2007, where the sensitivity of IL-8 was 100% and specificity 50 to 66.7%, PPV (from 94.4 to 94.7%). In our examination, area under curve, which was calculated from ROC curve was 0.94 for IL-8 which was noted to be statistically significant. The difference is the Pace et al compared serum and pleural fluid IL-8 levels with pleural fluid LDH and protein. IL-8 levels in pleural fluid showed a higher diagnostic accuracy compared to traditional criteria. The advantage of the reduction in misdiagnosing transudates following diuretic therapy using IL-8 over the Light's criteria gives an edge to use of IL-8. It may offer the basis for the introduction of novel anti-inflammatory agents in diagnosing and discriminating pleural fluid [20].

### Strength and Limitations of the Study

IL-8 is used as a single marker for diagnosing and classifying subgroups. IL-8 levels were independent of age and gender. There was a reduction in misdiagnosing transudates following diuretic therapy using IL-8. Since this is a cross sectional study, causal relationships cannot be established. A large cohort study is required to establish a causal relationship. A larger sample size might have explained the outcome in a better manner.

### Conclusions

It is clear from our study that IL-8 can be used to diagnose and differentiate exudates from transudative pleural effusions with a good sensitivity and specificity. The advantage of minimal misdiagnosis of exudates by IL-8 over the Light's criteria makes it a more effective biochemical marker to diagnose pleural effusion.

**Conflict of Interest:** None.

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