Original Article

# Use of pleural fluid ceruloplasmin in the differentiation of exudative and transudative pleural effusion

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## ABSTRACT

**Background:** Differentiating into transudate or exudate is the first step in the evaluation of effusions. Light's criteria is the standard but a significant number of transudates may not be differentiated based on these criteria. Acute phase proteins (APP) are present in plasma, which increase or decrease by about 25% during an acute inflammatory response. Ceruloplasmin (CP) is a positive APP. Hence, this study was done to know the diagnostic value of pleural fluid (pf) CP and pf to serum ceruloplasmin ratio (CPr) to differentiate the pleural effusion (PE) into exudate and transudate as compared to Light's criteria. **Materials and Methods:** Setting: Medical wards of St John's Medical College Hospital, Bangalore. **Design:** Cross-sectional descriptive study. Patients with PE were divided into exudate and transudate by definitive diagnosis. pfCP, CPr and Light's criteria were compared with definitive diagnosis for the differentiation of pf into exudate and transudate. **Results:** The mean value of the pfCP and CPr was found to be significantly different between exudates and transudates. Sensitivity and specificity of pfCP at ≥ 13.34 mg/dl is 89.7% and 83.3%, CPr at ≥ 0.37 is 91.4% and 83.3%, Light's criteria 94.82% and 83.3%, respectively. Light's criteria, pfCP and CPr have similar PPV (98%) with Light's criteria having higher NPV (62.5%) than pfCP (45%) and CPr (50%), respectively. CPr has higher NPV than pfCP. **Conclusions:** pfCP and CPr can differentiate pf into exudate and transudate with comparable PPV to Light's criteria.

**KEY WORDS:** Acute phase proteins, ceruloplasmin, exudates, pleural effusion, transudates

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## INTRODUCTION

Acute phase proteins (APPs) are the proteins present in plasma, which increase or decrease by about 25% during an acute inflammatory response.[1] Ceruloplasmin (CP) a positive APP is increased due to stimulation of macrophages and monocytes at the sites of inflammation by cytokines.[2] Pleural fluid (pf) accumulates in exudative effusions due to alteration of local factors influencing the formation and absorption of pf; in contrast, transudative effusion is due to alteration of systemic factors.[3] Studies have shown that CP is significantly increased in exudative PE compared to other APPs.[4] So the aim of the study was to know the diagnostic value of pfCP and serum ceruloplasmin ratio (CPr) in differentiating PE into exudate and transudate as compared to Light's criteria.[5]

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## MATERIALS AND METHODS

Patients were recruited from the medical wards, who were undergoing thoracocentesis for the definitive diagnosis of PE between February and July 2011. The study was approved by the Institutional Review Board. Based on previous studies[6] sensitivity of the Light's criteria was taken as 98% and sensitivity for CP in pf in one of the studies by Calikoglu *et al*.[4] was 92%. Expecting at least 85% sensitivity for CP in our setup and with 80% power and alpha error of 0.05, we need to study 71 cases. Seventy-one consecutive patients with pleural effusion (PE) who gave informed consent and fulfilled the inclusion and exclusion criteria were included in the study.

### Inclusion criteria

- All the patients admitted with PE for diagnostic thoracocentesis
- Patients who gave the informed consent.

### Exclusion criteria

- Patients on drugs like anticonvulsants[7] (carbamazepine, phenobarbital, phenytoin, valproic acid), oral contraceptive pills[8]
- Pregnant women
- Patients with previously diagnosed Wilson's disease.

The definitive diagnosis made by the clinicians was obtained from the patient medical records after prior approval. Effusions with malignant cells in pf cytology or biopsy specimen were considered malignant effusion. Para pneumonic effusion was considered when there was acute febrile illness with purulent sputum, pulmonary infiltrates, responsiveness to antibiotic treatment, or identification of the organism in the pf by culture. Tuberculous pleurisy was diagnosed with a positive acid fast stain in pf, pleural biopsy or sputum; presence of caseous granulomas in pleural biopsy or clearance of effusion in response to antitubercular therapy. PE due to pancreatitis was diagnosed based on the definitive diagnosis of pancreatitis and the absence of other causes of PE. Pleural effusion due to congestive heart failure was determined by an enlarged heart, pulmonary venous congestion on radiograph, peripheral edema, response to CHF treatment, and the absence of malignancy or pulmonary infiltrates associated with an inflammatory process or any other cause of PE. Renal failure was diagnosed when there was raised serum urea and creatinine values, signs of fluid overload and absence of any other causes of effusion.

The pf was collected by thoracocentesis for the estimation of total protein (TP), lactate dehydrogenase (LDH) and CP. Venous blood sample was collected from the patient into a vacutainer BD (Becton Dickinson), was allowed to clot for 20 to 30 min and centrifuged at 3000 rpm for 10 minutes. Pleural fluid and serum was used to measure TP, LDH and stored at –20°C degrees for measurement of CP.

TP was measured in both serum and pf using the modified Biuret method using bichromatic end-point technique.[9] LDH was measured using the modified Wacker's method with bichromatic rate technique.[10] Both the analytes were measured in Dade Dimension Rxl Max-Siemens health care diagnostics limited. The CP was measured manually using copper oxidase method[8] and the absorbance was measured at 530 nm using a spectrophotometer and calculation was done to give the results in mg/dl.[11,12] After the measurement of TP and LDH, pf was divided into exudate and transudate by Light's criteria[5] (TP ratio > 0.5, LDH ratio > 0.6, LDH greater than 2/3 of the upper limit of normal for serum LDH).

ROC curve was used to determine the cut-off value with best sensitivity and specificity for the pfCP and CPr, and divide the pf into exudates and transudates. The number of patients diagnosed as exudate and transudate by the Light's criteria, pfCP and CPr was compared with the definitive diagnosis. Ethical clearance was obtained from the ethical review board.

### Statistics

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean ± 1SD, median and quartile (25, 75), and results on categorical measurements are presented in number and percentage (%). A $P < 5\%$ was considered significant. Definitive diagnosis was considered as gold standard to differentiate the two groups. The Mann-Whitney U test has been used to find the significance of study parameters on continuous scale between the two group's exudates and transudates. A cut-off value to differentiate the pf into exudate and transudates depending on the highest sensitivity and specificity for the pfCP and CPr was determined by the ROC curve. Sensitivity, specificity, PPV and NPV were calculated. All the statistical analyses were done using the SPSS software version 16.

## RESULTS

A total of 71 patients were included in the study of which 7 patients were excluded as a definitive diagnosis was not reached. The remaining 64 patients consisted of 45 male and 19 females. Patient's pf were classified into exudate (58 patients) and transudate (6 patients) by definitive diagnosis, which was obtained from the patient health records. The mean age of the population was found to be 50.96 yrs and the mean value of the TP, CP and the median values of the LDH in serum and pf were 6.22 g/dl, 46.28 mg/dl, 285 IU/L and 3.55 g/dl, 24.31 mg/dl, 326.5 IU/L, respectively. The mean age of exudates and transudates was 50.15 yrs (range 18-82 years) and 58.83 yrs with age range 34-69 years, respectively. The demographic characteristics of the two groups are given in Table 1.

Shown in Table 2 are the mean values of TP, CP and median values of LDH in both exudates and transudates in serum and pf. Pleural fluid to serum CP ratio is also shown in Table 2. There was a significant difference between the two groups in the mean values of pfTP, pfCP, pfLDH and CPr by the Mann-Whitney U test as shown in Table 3. Compared to definitive diagnosis the sensitivity of Light's criteria was 94% and 83%, respectively. PPV and NPV were found to 98.2% and 62.5%, respectively.

**Table 1: Different causes of pleural effusion in exudates and transudates with their frequency and percentage**

| Types of pleural effusion | Frequency (*n*) | Percentage |
|---|---|---|
| Exudate | | |
| Tuberculosis | 21 | 31 |
| Para pneumonic effusion and empyema | 27 | 42 |
| Carcinoma | 9 | 14.1 |
| Others - | 2 | 3.1 |
| Transudate | | |
| Congestive cardiac failure | 4 | 6.2 |
| Renal failure | 2 | 3.1 |
| Total | 64 | 100 |

*n*=Frequency of patients with pleural effusion

Figure 1 is the ROC curve for the determination of pfCP cut-off values and Figure 2 is the ROC curve for the CPr. The area under the curve for pfCP was 0.914 and with a cut-off value of 13.34 mg/dl showed highest sensitivity and specificity to differentiate the pf into exudate and transudate. Similarly, the area under the curve for CPr was 0.94 and with cut off 0.33 having highest sensitivity and specificity to differentiate the two groups. Shown in Tables 4 and 5 is the sensitivity and specificity of the pfCP and CPr at various cut-off values. Sensitivity, specificity, PPV and NPV of the Light's criteria, pfCP and CPr is shown in Table 6.

The sensitivity and specificity of pfCP was 89% and 83% which was lower compared to Light's criteria. Similarly, the sensitivity and specificity of CPr were 91% and 83% which were better than pfCP alone.

## DISCUSSION

PE is abnormal collection of fluid in pleural space. The first step in the evaluation of a PE is to differentiate into exudate and transudate. The most commonly used criteria is the Light's criteria.[13] Light's criteria misidentify a transudative PE as exudative PE in as many as 25% of the cases[6] and the criteria include measurement of TP, LDH in both serum and pf to differentiate the two groups.

Several other parameters like pf cholesterol greater than 44.85 mg/dl showed a sensitivity and specificity of 97.1 and 100%, respectively.[14] Calikoglu *et al*.[4] studied on APPs and found that sensitivity and specificity of CP (92% and 84%) and transferrin (84% and 80%) was better than other APPs. Serum effusion albumin gradient greater than 1.2 g/l is useful in differentiation of exudates and transudates when the patient is on diuretic therapy.[15] Pleural fluid to serum bilirubin concentration ratio greater than 0.6 showed sensitivity and specificity of 96% and 83%, respectively.[16] Vives *et al*.[17] and Gazquez *et al*.[18] have concluded that Light's criteria is superior to pf bilirubin, cholesterol and albumin gradient in differentiation of exudate and transudates.

**Table 2: Mean and SD value of total protein, LDH, ceruloplasmin in serum and pleural fluid between two groups**

| Parameter | Exudate (58) | | Transudate (06) | |
|---|---|---|---|---|
| | Serum | Pleural fluid | Serum | Pleural fluid |
| Total protein g/l | 6.29±1.07 | 3.78±1.38 | 5.55±1.83 | 1.3±0.766 |
| LDH (IU)* | 291 (215,384) | 352.5 (217,719) | 212 (188,257) | 73.5 (29.5,163) |
| Ceruloplasmin mg/dl | 46.32±17.15 | 25.91±13.04 | 45.83±11.75 | 8.86±5.24 |
| Ceruloplasmin ratio (Pleural fluid to serum) | 0.58±0.29 | | 0.21±0.13 | |

LDH: Lactate dehydrogenase, *Lactate dehydrogenase=Median and quartile range

CP is a copper-containing protein with ferroxidase activity, which is responsible for the oxidation of $Fe^{2+}$ (ferrous iron) into $Fe^{3+}$ (ferric iron), therefore assisting in its transport in the plasma in association with transferrin, which can only carry iron in the ferric state.[19] It is synthesized mainly by the hepatocytes. It is also a positive APP, where the protein levels increase in response to inflammatory cytokines.[4]

Fleming *et al*.[20] studied rat lung and found that CP mRNA is expressed in rat lung on exposure to endotoxin and the specific site of production of CP is alveolar macrophages. They also found expression of CP mRNA in the lung on hyperoxic induction in rats. Mukhopadhyay *et al*.[21] have shown that the CP is secreted by human peripheral blood monocytes on specific induction by INF-γ. This explains the increased level of CP in exudative pf as seen in our study, where the cause is inflammatory injury of the pleura.

In our study we found increased CP levels in PE due to carcinoma. The mechanism is not well known. Studies done by Doustjalali *et al*.[22] in 2006 have patients with nasopharyngeal carcinoma showing enhanced serum and tissue CP expression and Pousset *et al*.[23] in 2001 showed high levels of CP in the serum of transgenic mice developing hepatocellular carcinoma.

PE in acute pancreatitis is caused mainly due to transdiaphragmatic lymphatic blockage or pancreaticopleural fistulae and an increased concentration of IL-1, IL-6 and TNF-α in pancreatic secretion.[24] These inflammatory cytokines stimulate the APP synthesis.[4] This may be the reason for increase in pfCP in patients with pancreatitis.

Calikoglu *et al*.[4] studied 80 patients with PE on APPs like C-reactive protein, haptoglobin, transferrin, alpha-1acid glycoprotein and CP. CP concentrations were determined by immunoturbidometrical methods (Cobas Integra 700, Roche Diagnostics, Mannheim, Germany). They found the pfCP in exudates was 27 ± 10 mg/dl similar to our study values (25.91 ± 13.04 mg/dl). In transudates they found a higher value (16 ± 2 mg/dl) compared to our

**Table 3: Significance of test parameters between exudate and transudate**

| | Serum | | | Pleural fluid | | | pf/serum ceruloplasmin ratio |
|---|---|---|---|---|---|---|---|
| | sTP | Serum LDH | sCP | pfTP | pfLDH | pfCP | CPr |
| Mann-Whitney U | 133.000 | 91.000 | 173.000 | 17.000 | 32.000 | 30.000 | 21.000 |
| Wilcoxon W | 154.000 | 112.000 | 194.000 | 38.000 | 53.000 | 51.000 | 42.000 |
| *Z* | −0.945 | −1.912 | −0.023 | −3.618 | −3.271 | −3.317 | −3.524 |
| Asymp. Sig. (2-tailed) | 0.345 | 0.056 | 0.982 | 0.000 | 0.001 | 0.001 | 0.000 |

sTP: Serum total protein, sLDH: Serum lactate dehydrogenase, sCP: Serum ceruloplasmin, pfTP: Pleural fluid total protein, pfLDH: Pleural fluid lactate dehydrogenase, pfCP: Pleural fluid Ceruloplasmin, CPr: Pleural fluid to serum ceruloplasmin ratio, $P<0.05$=Statistically significant

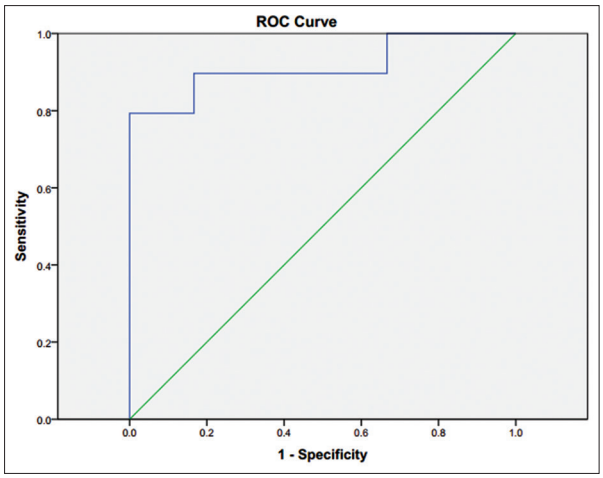


**Figure 1:** ROC curve of the pfCP for the detection of exudates in pf. ROC = Receiver operator characteristics curve, pfCP = Pleural fluid ceruloplasmin, pf = Pleural fluid. The area under the curve for the pleural fluid ceruloplasmin is 0.914

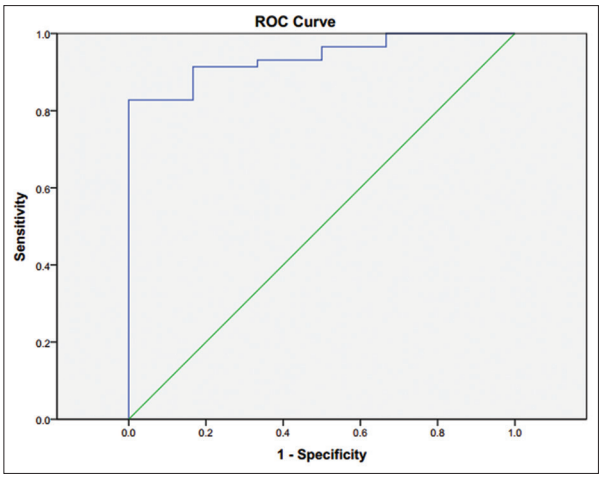


**Figure 2:** ROC curve of the CPr for the detection of exudates in pf. ROC = Receiver operator characteristics curve, CPr = Pleural fluid to serum ceruloplasmin ratio, pf = Pleural fluid, The area under the curve for the CPr is 0.94

**Table 4: Sensitivity and 1-specifity at various cut off value for pleural fluid ceruloplasmin as obtained by ROC curve**

| Cut off value mg/dl | Sensitivity | 1-specificity |
|---|---|---|
| 10.25 | 0.897 | 0.500 |
| 11.593 | 0.897 | 0.333 |
| 13.3438 | 0.897 | 0.167 |
| 14.0875 | 0.879 | 0.167 |
| 14.525 | 0.862 | 0.167 |
| 14.937 | 0.793 | 0.167 |
| 15.156 | 0.793 | 0.000 |

ROC: Receiver operator characteristics, pleural fluid ceruloplasmin cut-off value of 13.34 mg/dl has the sensitivity of 89.7% and specificity 83.3%

**Table 5: Sensitivity and 1-specifity at various cut-off values for pleural fluid to serum ceruloplasmin ratio as obtained by ROC curve**

| Cut off value CPr | Sensitivity | 1-specificity |
|---|---|---|
| 0.2718 | 0.931 | 0.500 |
| 0.2756 | 0.931 | 0.333 |
| 0.2958 | 0.914 | 0.333 |
| 0.3301 | 0.914 | 0.167 |
| 0.3447 | 0.897 | 0.167 |
| 0.3518 | 0.862 | 0.167 |
| 0.4041 | 0.724 | 0.000 |

ROC: Receiver operator characteristics, CPr: Pleural fluid to serum ceruloplasmin ratio, Cut-off value of 0.33 has 91.4% sensitivity and 83.3% specificity

**Table 6: Sensitivity, specificity, PPV, NPV of Light's criteria, pfCP and CPr**

| | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| Light's criteria | 94 | 83 | 98.2 | 62.5 |
| pfCP ≥13.34 mg/dl by ROC curve | 89 | 83 | 98.1 | 45 |
| CPr ≥0.37 by ROC curve | 91 | 83 | 98.1 | 50 |

PPV: Positive predictive value, NPV: Negative predictive value, pfCP: Pleural fluid ceruloplasmin, CPr: Pleural fluid to serum ceruloplasmin ratio, pfCP ≥13.34 mg/dl and CPr ≥0.37 is taken as the cut of value to differentiation exudates from transudates

study (8.86 ± 5.24). This may be due to lower percentage of the people with transudate and with different population group in our study. They had 26 transudative effusions with CCF.

Compared to Light's criteria, pfCP has less sensitivity and NPV but had similar specificity in differentiating exudative PE from transudative PE. Transudate PE are not related to local pleural pathology, but are produced by an imbalance between the hydrostatic and oncotic pressures, which does not lead to inflammation.[6] This explains why there is an increased CP levels in exudates compared to transudates.

## CONCLUSION

In this study we found that pfCP and CPr were able to differentiate exudates from transudates. The Light's criteria showed similar positive predictive value (98.2%) and better negative predictive value (62.5%) compared to pfCP (45%) and CPr (50%) respectively in differentiating exudates from transudates. The advantage over Light's criteria is that we need to measure only pfCP. It was also found that the pleural fluid to serum ceruloplasmin ratio was better than pfCp alone. Holmberg-Laurell factor[10] was used in the estimation of ceruloplasmin by ferroxidase method and crystalline CP was not used in standardization. In inflammation there may be increase in total ceruloplasmin, whereas ferroxidase method estimates only holo ceruloplasmin.[12] This study had a small sample size and few transudates. Pleural fluid from patients with no diagnosis was excluded from the study; these are the cases where there is a need for extensive investigations and a need for an effective marker to classify pf into exudate or transudate. A larger study with more transudates is required to define the role of this test.

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