# Is the ratio of pseudocholineaterase levels in pleural fluid to serum a new parameter

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*Background and objectives: Pleural effusion is a common clinical condition faced in everyday practice. The first step in the management of pleural effusion is its differentiation into transudates and exudates. Light's criteria is the most widely used parameter to differentiate pleural effusions but studies have shown that Light's criteria misclassifies a significant amount of cases. Objectives: To study the levels of Cholinesterase in pleural fluids of diverse etiologies, To evaluate the validity of pleural fluid cholinesterase (ChE) and its ratio with serum cholinesterase in order to differentiate between transudates and exudates, To compare this diagnostic efficacy with Light's criteria Methods: The study was conducted in Hangal Shri Kumareshwar Hospital attached to S. Nijalingappa Medical College, Bagalkot. Study included 125 patients who had pleural effusion who met the inclusion and exclusion criteria. Duration of the study was 12 months. Results: 62 cases (50%) had transudative effusion and 63 cases (50%) had exudative effusion. The mean PChE levels in transudates was 806.26 + 516.28 U/L and in case of exudates it was 3476.11 + 1250.17 U/L. The difference between the two groups was statistically significant (p-value < 0.05). Accordingly the mean value of this ratio was 0.10 + 0.05 in the transudates group and 0.39 + 0.14 in the exudates group. This difference was found to be statistically significant (p-value <0.001). This ratio misclassified 5 cases. Among them 2 (3.2%) were transudates that were misdiagnosed as exudates and 3 (4.8%) were exudates that were misdiagnosed as transudates. In this study Light's criteria misclassified 13 cases in total with a sensitivity of 91.9% and a specificity of 87.3%. Conclusions: Light's criteria has a good sensitivity and specificity but P/S ChE was the most efficient parameter in differentiating between transudates and exudates in this study.*
*Key words: Pleural effusion, Pseudocholinesterase, Light's criteria*

## I. Introduction

Pleural effusion is the abnormal accumulation of fluid in the pleural space. A pleural effusion is always abnormal and indicates the presence of an underlying disease.[1] The first step in management of pleural effusion is to classify pleural fluid into a transudate or exudates even if this differentiation does not contribute to the etiological diagnosis. Transudative pleural effusion is caused by limited number of diseases. However exudative effusions might require extensive diagnostic investigations. For this purpose many criteria have been used but they weren't satisfactory.

In 1972, Light et al[2], developed a set of criteria for the diagnostic separation of pleural fluids into transudates and exudates. These included- a. pleural fluid to serum total protein ratio > 0.5, b. pleural fluid to serum LDH ratio > 0.6 and c. Pleural fluid LDH more than two thirds the normal upper limit for serum. However these criteria misidentify ~25% of transudates as exudates.[3] Many biochemical parameters like pleural fluid cholesterol, bilirubin, albumin, alkaline phosphatase, adenosine deaminase [4-7], malondialdehyde (MDA) and their ratio with serum values have been used to differentiate the type of pleural effusion. In 1990, in a study of 46 patients with pleural effusions, Meisel et al evaluated the usefulness of the pleural fluid to serum bilirubin concentration ratio. With this criterion 3 of 23 transudates and 6 of 23 exudates were misclassified. This result was not superior to that obtained with criteria of Light et al.[8] Paramothayan et al[9] conducted a study on 54 patients in 2002 and found that pleural fluid LDH and fluid to serum protein ratio measurements were equally good at differentiating between exudates and transudates, with a sensitivity of 90%, a specificity

of 79%, a positive predictive value (PPV) of 84%, and a negative predictive value (NPV) of 86%. A combination of these parameters improved sensitivity to 100% and NPV to 100%, but lowered the specificity to 71% and PPV to 81%. This combination achieved a higher efficiency than Light's criteria. However they were not proved to be effective diagnostic tests. And Light's criteria misidentify ~25% of transudates as exudates. So there is a need to identify a parameter which is better than the present tests for differentiation. In 1978, Cabrer et al[10] conducted a study on pseudocholinesterase activity in pleural effusions of diverse aetiologies and concluded that there exists difference in the activity of pseudocholinesterase among different types of pleural effusions and it was possible to differentiate them into transudates and exudates with pseudocholinesterase levels. In 1996, Garcia-Pachon et al conducted a study on 153 patients and Light's criteria, the pleural fluid cholesterol level, the pleural fluid to serum cholesterol ratio, the pleural fluid cholinesterase level, and the pleural fluid to serum cholinesterase ratio were applied. The percentage of effusions misclassified by each parameter was as follows: Light's criteria, 7.8%; pleural fluid cholesterol, 7.8%; pleural fluid to serum cholesterol ratio, 6.5%; pleural fluid cholinesterase, 8.5%; and pleural fluid to serum cholinesterase ratio was just 1.3% making it the most accurate criterion.[11] A study was conducted on 80 patients by Ozer F et al in 2003 and it was found that the difference between the mean pleural fluid pseudocholinesterase (PChE) levels of transudates and exudates was statistically significant ($p < 0.001$). Similar significance was also obtained in the mean pleural fluid/serum pseudocholinesterase ratios between transudates and exudates ($p < 0.001$). In determination of exudative fluids, both sensitivity and specificity of the PChE level was 100%. Sensitivity and specificity of the pleural fluid/serum pseudocholinesterase ratio of 0.24 were 90 and 87%, respectively.[12] In 2004, Sharma et al conducted a study on 110 patients and observed that positive predictive value (PPV) and negative predictive value (NPV) for pleural fluid to serum ChE ratio was 98.075% and 96.67% respectively. And for Light's criteria PPV and NPV were 96.05% and 79.42% respectively.[13] In a study conducted by Naveen M et al[14] a comparison study of misclassification of pleural effusion by Light's criteria and by ratio of pleural fluid pseudocholinesterase and serum pseudocholinesterase was performed. The study showed that misclassifications were more due to Light's criteria as compared to pleural fluid pseudocholinesterase to serum pseudocholinesterase ratio. In 2014, Gowdaiah PK et al conducted a study on 62 patients with pleural effusions and observed that the ratio of pleural fluid to serum ChE misclassified only 1 case of pleural effusion among the analyzed 62 cases. The sensitivity of the ratio was 100% and specificity was 96.7% whereas, the Light's criteria misclassified 3 cases of pleural effusion (4.8%) with a sensitivity of 93% and specificity of 96%.[15]

Hence this study is being conducted so as to evaluate the level of pseudocholinesterase in pleural fluid and in serum; and to measure the ratio of its level in pleural fluid to serum and to compare its efficacy against Light's criteria.

**Objectives:** To study the levels of Cholinesterase in pleural fluids of diverse etiologies, To evaluate the validity of pleural fluid cholinesterase (ChE) and its ratio with serum cholinesterase in order to differentiate between transudates and exudates, To compare this diagnostic efficacy with Light's criteria

## II. Methodology

**Source of Data:** Data was collected from the patients admitted in Hanagal Shri umareshwar Hospital and Research Centre , S.Nijalingappa Medical College, Bagalkot and were found to have pleural effusion.
**Inclusion criteria:** Patients with Age > 18yrs, Presence of pleural effusion proved by clinical/ radiological examination, Patient willing to give an informed consent.
**Exclusion criteria:** Patients having pleural effusion with suspected multiple etiologies, Patients having hepatic diseases, Patients using any of the following drugs – OCPs, anti-cancer drugs, MAO inhibitors, neostigmine, chlorpromazine, Pregnant patients, Patients with OP compound poisoning.
**Sample size:** Sample size estimation done using Statstodo software.
According to a study done by Sharma M et a[16], Probability of Type I error (α) = 0.95, Power (1 - β) = 0.8, Sensitivity in group I (Pleural fluid cholinesterase) = 0.97, Sensitivity in group II (Light's criteria) = 0.92, Sample size require for per group for unpaired comparison= 61. Hence sample size is 61x2=122 = 1**25.**

**Study methods and design:** Patients were divided into two groups
• Group I consisted of 62 patients with transudative effusions and
• Group II consisted of 63 patients with exudative pleural effusion
Patients participating in the study were explained about the procedure, technique and complications of pleural fluid aspiration. Pleural fluid aspiration was performed under strict aseptic precautions after analysing the fluid levels by percussion and chest x-rays. In few of the cases, ultrasonography-guided pleural fluid aspiration was performed. Pleural fluid analysis with protein, LDH and ChE estimation along with serum protein, LDH and ChE estimation were done and an analysis of the results obtained was done. Protein levels

were estimated in serum and pleural fluid by Biuret method. LDH levels were estimated using the kinetic UV test for quantitative determination of LDH by measuring the decrease in absorbance of NADH at 340 nm. Cholinesterase levels were measured using the kinetic colorimetric method based on Ellman reaction using the Beckman Coulter Cholinesterase kit.

**Statistical analysis:** Data were entered in MS Excel and analysed in SPSS V22. Descriptive statistics for qualitative data was represented with percentages. Logistic regression was applied to find cut-off values. The usefulness of the biochemical parameters was assessed using the Bayesian method in terms of sensitivity, specificity, positive predictive value and negative predictive value. Results on continuous measurements are presented on Mean and SD, (Min- Max) and results on categorical measurements are presented in Number (%). Area under the curve and 95% confidence intervals were calculated. *p*-value of less than 0.05 was considered statistically significant. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis on metric parameters. A low p-value for this test (less than 0.05) means that there is evidence to reject the null hypothesis in favour of the alternative hypothesis.

The following assumptions on data are made**:** 1. Dependent variables should be normally distributed, 2. Samples drawn from the population should be random; cases of the samples should be independent.

## III. Results

The study population comprised of total 125 patients. Out of them78 (62.4%) were males and 47 (37.6%) were females. In this study the majority of the cases belonged to the age group of 31-40 years (21.6%) followed by 41-50 years of age group (17.6%). The youngest patient was 18 years old and the eldest patient was aged 93 years old.

**Etiological distribution of pleural effusion**

The most common cause of pleural effusion was tuberculosis in this study (41.6%) among the exudates and congestive cardiac failure among the transudates (24%). Other significant causes included Dengue fever and Cirrhotic portal hypertension 7.2% and 8% respectively.

**Table-1: Pleural fluid to serum protein ratio**

| | Transudate | | | | | Exudate | | | | | t-value | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min | Max | Mean | SD | N | Min | Max | Mean | SD | | |
| **Serum Protein** | 62 | 4.10 | 8.10 | 5.96 | 0.77 | 63 | 4.10 | 8.30 | 6.43 | 0.89 | 3.19 | 0.002 |
| **Pleural Fluid Protein** | 62 | 0.60 | 3.30 | 1.24 | 0.62 | 63 | 1.10 | 5.00 | 3.37 | 0.79 | 16.79 | <0.001 |
| **PFP/SP** | 62 | 0.10 | 0.59 | 0.21 | 0.11 | 63 | 0.18 | 0.75 | 0.52 | 0.10 | 16.58 | <0.001 |

The mean value of serum protein among transudates group and exudates group were 5.96$\pm$0.77g/dL and 6.43$\pm$0.89g/dL respectively. And the mean pleuralfluid protein levels were 1.24$\pm$0.62g/dL and 3.37$\pm$0.79g/dL respectively.This ratio was found to be statistically significant in this study (p- value<0.05).

**Table-2: Pleural fluid LDH levels**

| | Transudate | | | | | Exudate | | | | | t-value | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min | Max | Mean | SD | N | Min | Max | Mean | SD | | |
| **Serum LDH** | 62 | 144.00 | 600.00 | 265.50 | 116.32 | 63 | 145.00 | 1778.00 | 350.98 | 218.86 | 2.72 | 0.007 |
| **Pleural Fluid LDH** | 62 | 20.00 | 228.00 | 80.24 | 38.77 | 63 | 45.00 | 667.00 | 260.29 | 141.53 | 9.67 | <0.001 |
| **F LDH / S LDH** | 62 | 0.13 | 0.79 | 0.31 | 0.12 | 63 | 0.18 | 3.24 | 0.83 | 0.54 | 7.47 | <0.001 |

The mean serum LDH levels were 265.5$\pm$116.32U/L among transudates group and 350.98 $\pm$218.86U/L among exudates group.The mean pleural fluid LDH levels among transudates and exudates were 80.24$\pm$38.77U/L and 260.29$\pm$141.53U/L respectively.This value was found to be statistically significant in this study(p- value<0.001).

**Table-3: Comparison of all the parameters based on Bayees method**

| Variable | Sensitivity | Specificity | PPV | NPV | Overall Accuracy |
|---|---|---|---|---|---|
| Pleural Fluid Protein (PFP) | 91.9% | 85.7% | 86.4% | 91.5% | 88.8% |
| PFP / Serum Protein | 90.3% | 88.9% | 88.9% | 90.3% | 89.6% |
| Pleural Fluid LDH (PF LDL) | 93.5% | 85.7% | 86.6% | 93.1% | 89.6% |
| PF LDH / Serum LDH | 91.9% | 82.5% | 83.8% | 91.2% | 87.2% |
| Pleural Fluid Choliesterase (PFC) | 93.5% | 92.1% | 92.1% | 93.5% | 92.8% |
| **PFC / Serum Cholinesterase** | **96.8%** | **95.2%** | **95.2%** | **96.8%** | **96.0%** |
| Light's Criteria | 91.9% | 87.3% | 87.7% | 91.7% | 89.6% |

In this study the most number of misdiagnosed cases were due to the pleural fluid to serum LDH ratio that misclassified 16cases. Pleural fluid to serum cholinesterase ratio misclassified 5 cases in the totalcases. In this study Light's criteria had a sensitivity of 91.9% and specificity of 87.3% incomparison to pleural fluid to serum cholinesterase ratio that had a sensitivity of 96.8% and specificity of 95.2% and hence was the most efficient parameter.

**Table-4: Area under the curve on basis of accuracy**

| Area Under the Curve | | | | | |
|---|---|---|---|---|---|
| Test Result Variable(s) | Area | SE | P-value | 95% Confidence Interval | |
| | | | | Lower Bound | Upper Bound |
| PFP/ Serum Protein | 0.957 | 0.018 | <0.001 | 0.921 | 0.993 |
| PleuralFluidLDH (PF LDH) | 0.940 | 0.022 | <0.001 | 0.897 | 0.983 |
| PF LDH / Serum LDH | 0.903 | 0.030 | <0.001 | 0.845 | 0.962 |
| PleuralFluidCholiesterase (PFC) | 0.971 | 0.013 | <0.001 | 0.945 | 0.997 |
| **PFC/SerumCholinesterase** | **0.986** | **0.010** | **<0.001** | **0.967** | **1.000** |
| Light'sCriteria | 0.896 | 0.032 | <0.001 | 0.834 | 0.958 |

PFC / Serum Cholinesterase cover maximum area (0.986) when compared with other methods.

## IV. Discussion

Pleural fluid is one of the most common presentations of a wide variety of the cases. The first step in the management of pleural effusion is its differentiation into a transudate or exudate. The most popular method used is the Light's criteria. But various studies have concluded that pleural effusions are misclassified by Light's criteria in a substantial number of patients. Hence, better and newer parameters with higher sensitivity and specificity are needed. In this study, a new parameter i.e. pleural fluid to serum pseudocholinesterase ratio was compared with the Light's criteria in terms of efficacy.

In the original study by Light et al[2], in a series of 150 patients, the authors correctly classified all but two of the pleural effusions, one transudate and one exudate. In this study Light's criteria misclassified 13 cases in total, whereas the new parameter misclassified only 5 cases. In the study done by Garcia-Pachon et al[11], Light's criteria misclassified 12 cases (9 transudate and 3 exudate) with a sensitivity of 97.4% and specificity of 74.29%  In the study by Naveen M et al[14], the levels of cholinesterase in the pleural fluids was found to be effective to differentiate between transudates and exudates. Among the 50 patients included in the above study, Light's criteria misdiagnosed 10% of the cases. And pleural fluid cholinesterase levels misdiagnosed 2 cases. However in the present study pleural fluid cholinesterase levels misdiagnosed 9 of the total cases.

**Efficacy of Light's criteria in different studies**

| Study | Sensitivity | Specificity |
|---|---|---|
| Light et al[2] | 99% | 97.8% |
| Garcia-Pachonet al[11] | 97.4% | 74.29% |
| Sharma et al[13] | 91.25% | 90% |
| GowdaiahPK et al[15] | 93% | 96% |
| Present study | 91.9% | 87.3% |

In this study pleural fluid to serum cholinesterase ratio misdiagnosed 5 cases. The observed sensitivity and specificity in this study was 96.8% and 95.2% respectively. In a study done in 2014 by Gowdaiah PK et al[15] the ratio of pleural fluid to serum cholinesterase ratio misclassified only one case. Similar findings were obtained in the study done by Garcia-Pachon et al[11] and Sharma et al[13]. Thus such a consistent observation can be considered as a significant parameter for evaluation of pleural effusion.

**Efficacy of pleural fluid to serum cholinesterase ratio in various studies**

| Study | Sensitivity | Specificity |
|---|---|---|
| Garcia-Pachonet al[11] | 100% | 94.5% |
| Sharma et al[13] | 98.75% | 96.67% |
| Gowdaiah PK et al[15] | 100% | 96.7% |
| Present study | 96.8% | 95.2% |

Cholinesterase is synthesized in the liver and its levels can be influenced by different disorders like acute hepatitis, cirrhosis, acute infections, pulmonary embolism, chronic renal disease, and after surgical procedures. Hence, the ratio of pleural fluid to serum cholinesterase is a better parameter than the absolute value of cholinesterase in the pleural fluid. The pleural fluid to serum ratio of LDH misclassified the maximum number of cases in this study. In the study done by Sharma et al[13] and Gowdaiah PK et al[15] the ratio of pleural fluid to serum LDH was not found to have a statistically significant difference between transudates and exudates. The pleural fluid protein levels misclassified 13 cases in this study. In the study done by Gowdaiah PK et al[15] it misdiagnosed 6 cases and in the study by Sharma et al[13], the same parameter misclassified the maximum number of cases.

## V. Conclusion

The levels of pseudocholinesterase in pleural fluid and its fluid to serum ratio are significantly higher in exudative pleural effusions than transudative ones. These two are better parameters that can be used to differentiate between transudates and exudates. The ratio of pleural fluid to serum pseudocholinesterase ratio is superior to Light's criteria in differentiating between transudates and exudates.

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