PLEURAL FLUID TO SERUM PSUEDOCHOLINESTERASE RATIO AND ITS VALIDATION WITH LIGHT'S CRITERIA.

Prakash Kikkeri Gowdaiah[1], Amrutha Avati[2], Priya Prakash Bhate[3], Akkamahadevi V. Nippanal[4]

HOW TO CITE THIS ARTICLE:
Prakash Kikkeri Gowdaiah, Amrutha Avati, Priya Prakash Bhate, Akkamahadevi V. Nippanal."Pleural Fluid to Serum Psuedocholinesterase Ratio and its validation with Light's Criteria". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 01, January 06; Page: 115-126.

ABSTRACT: 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 number of cases. This study was carried out to analyze the levels of pseudocholinesterase (ChE) in various types of pleural effusions and comparing ChE levels and the pleural fluid to serum ratio of ChE with Light's criteria to differentiate between transudates and exudates. **METHODS:**This study was conducted at Victoria Hospital and Bowring & Lady Curzon Hospitals attached to Bangalore Medical College and Research Institute. Study included 62 patients with pleural effusion, who met the inclusion and exclusion criteria. Duration of the study was 18months.**RESULTS:**31 cases (50%) had transudative effusion and 31 cases (50%) had exudative effusion. The mean PChE levels in transudate was 385+142U/L and in case of exudates it was 2074+660U/L. The difference between the two groups was statistically significant (p=<0.001). The average value of pleural fluid to serum ratio of PChE was 0.09+0.05 among transudates and among exudates it was 0.58+0.12. This difference was found to be statistically significant (p=<<0.001). The ratio 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%. **CONCLUSION:** Light's criteria has a good sensitivity and specificity, but pleural fluid to serum psuedocholinesterase ratio was the most efficient parameter in differentiating between transudates and exudates with a sensitivity of 100% and specificity of 96.7%.

KEY WORDS: Pleural effusion, Transudate, Exudate, Pseudocholinesterase (ChE), Light's criteria.

INTRODUCTION: Pleural effusion is a very common clinical presentation of diseases. A correct diagnosis of the underlying disease is essential for the management of pleural effusion. Transudative pleural effusions are caused by a limited number of diseases, whereas exudative effusions require more extensive diagnostic investigations. Therefore, the first step is to classify pleural effusions as transudates or exudates. Many criteria have been used to distinguish them, but none of the criteria have been found to be satisfactory. Light's criteria[1] is the most commonly used method and it has been found that even Light's criteria misclassifies a large number of effusions[2, 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. But there is no one parameter which is proved to be the best. Hence, there is a need to investigate into new parameters which may prove to be superior to the present available array of tests. Cabrer et al[8] estimated 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. Hence, this study was undertaken to evaluate the diagnostic efficacy of pseudocholinesterase levels in pleural fluids and the fluid to serum ratio of pseudocholinesterase in differentiation between transudates and exudates and compare it with Light's criteria.

In 1972, Light et al[1] developed a set of criteria for the diagnostic separation of pleural fluids into transudates and exudates. They used a pleural fluid to serum total protein ratio > 0.5, a fluid lactate dehydrogenase (LDH) value > 200 U/litre, or a fluid to serum LDH ratio > 0.6 to diagnose exudates, with the remaining fluids being classified as transudates.

In 1996, Garcia-Pachon et al[9] conducted a study on 153 patients with pleural effusion and applied 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 to each case. 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%
- Pleural fluid to serum cholinesterase ratio - 1.3%

In their study, pleural fluid to serum pseudocholinesterase ratio was the most accurate criterion.

MATERIALS AND METHODS

SOURCE OF DATA: Source of data were in-patients of Victoria Hospital and Bowring & Lady Curzon Hospital, Bangalore who were found to have pleural effusion.

METHOD OF COLLECTION OF DATA:

1. **Inclusion criteria:**
   - Male/Female patients
   - Age >18yrs
   - Presence of pleural effusion proved by clinical/radiological examination
   - Patients willing to give an informed consent

2. **Exclusion criteria:**
   - Patients having pleural effusion with suspected multiple etiologies
   - Patients having hepatic disease
   - Patients using any of the following drugs – OCPs, anti-cancer drugs, MAO inhibitors, neostigmine, chlorpromazine
   - Pregnant patients

3. **Sample size and design:**The study was a cross-sectional study and included 62 patients with pleural effusion who met the inclusion criteria with no reason for exclusion. The study was conducted from November 2011 to May 2013, for a period of 18months. The patients were assessed clinically and with laboratory investigations.

4. **Study method:** Patients were divided into two groups
   - Group I consisted of 31 patients with exudative effusions and
   - Group II consisted of 31 patients with transudative pleural effusion

   Pleural fluid analysis with protein, LDH and psudocholinesterase (ChE) estimation along with serum protein, LDH and ChE estimation were done and an analysis of the results so 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.

5. **Statistical methods:** The statistical analysis was done using the student t-test. The usefulness of the biochemical parameters was assessed using the Bayesian method in terms of sensitivity, specificity, positive predictive value and negative predictive value.

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. The following assumptions on data are made. Assumptions: 1.Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, cases of the samples should be independent.

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 for example) means that there is evidence to reject the null hypothesis in favor of the alternative hypothesis. Or, there is evidence that the difference between the two means is statistically significant.

Significant figures

+ Suggestive significance (P value:0.05<P<0.10)

* Moderately significant (P value: 0.01<P< 0.05)

** Strongly significant (P value: P < 0.01)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.,

RESULTS AND ANALYSIS

Study Population: This study enrolled 62 patients with pleural effusion among whom 42 were males (68%) and 20(32%) were females (Table 1; Fig 1).

Gender	Number of Patients	%
Males	42	68%
Females	20	32%
Total	62	100%

Table 1: Showing Gender Distribution of Patients

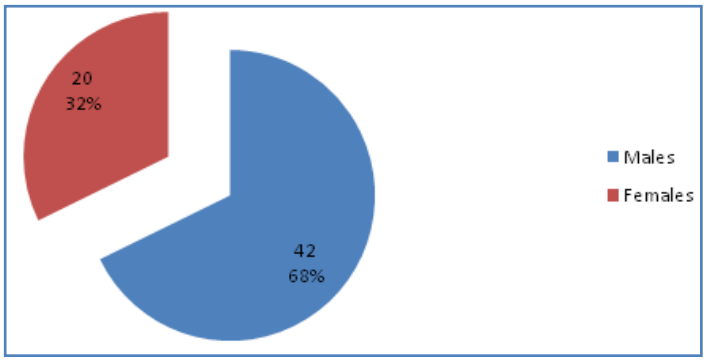

Fig. 1: Pie Chart showing Gender Distribution of Cases

AGE (in years)	Number of patients	%
20-30	16	25.8%
31-40	12	19.35%
41-50	9	14.5%
51-60	10	16.12%
61-70	9	14.5%
71-80	5	8%
81-90	0	-
91-100	1	1.6%

Table 2: Showing Age Distribution of Patients Studied

Mean age in years (SD) = 47+19.31

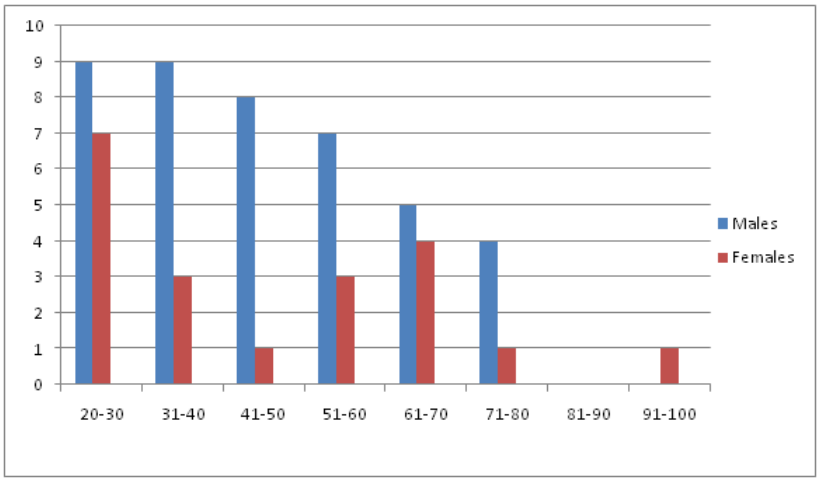

Fig. 2: Showing Age/Sex Distribution of Cases

In this study, the majority of patients belonged the age group of 20-30years (25.8%) followed by 31-40years (19.35%). The youngest patient was 20years old and oldest patient was 95years old (Table2; Fig 2). Mean age of the patient population was 47+19.31yrs.

Among the studied subjects, 31 (50%) had pleural exudates and 31 (50%) had pleural transudates (Table 3).

ETIOLOGY	NUMBER	%
TRANSUDATES		
Congestive heart failure	24	38.7%
Nephrotic Syndrome	5	8%
Non-Cirrhotic Portal Hypertension	2	3.2%
TOTAL	31	50%
EXUDATES		
Tubercular	16	25.8%
Parapneumonic	9	14.5%
Malignant	4	6.4%
Subphrenic Pus Collection	2	3.2%
TOTAL	31	50%

Table 3: Showing Causes of Pleural Effusion in This Study

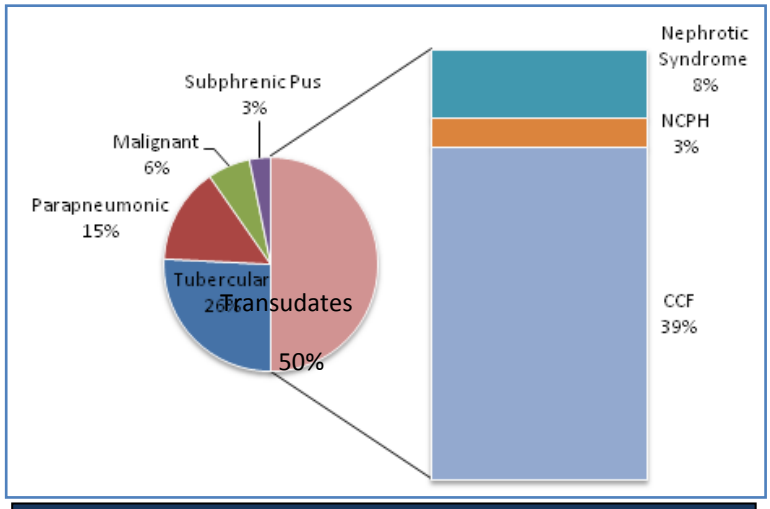

Fig. 3: Pie Chart Showing the Etiological Distribution

Among exudates, the most common etiology was tuberculosis (26%) and among transudates it was congestive heart failure (39%) (Table 3; Fig 3).

Light's criteria and its test parameters of (1) Pleural fluid to serum protein ratio (2) Pleural fluid LDH and (3) Pleural fluid to serum LDH ratio were applied to each of these cases and the results were analyzed individually and collectively. This was compared with (4) Pleural fluid pseudocholinesterase and (5) Pleural fluid to serum ratio of pseudocholinesterase.

(1) **Pleural fluid to serum protein ratio:** The pleural fluid to serum ratio of protein misclassified 3 cases (4.8%). A cut-off value of 0.5 was used[1].All these were exudates which were misclassified as transudates. The average value of pleural fluid protein in transudates was 1.27g/dl with a SD of 0.86 and in exudates it was 4.8g/dl with a SD of 1.2. The average value of pleural fluid to serum ratio of protein was 0.19+0.11g/dl for transudates and 0.67+0.15g/dl for exudates (Table 4).

The differences of both these parameters were found to be statistically significant between transudates and exudates (p=<0.001).

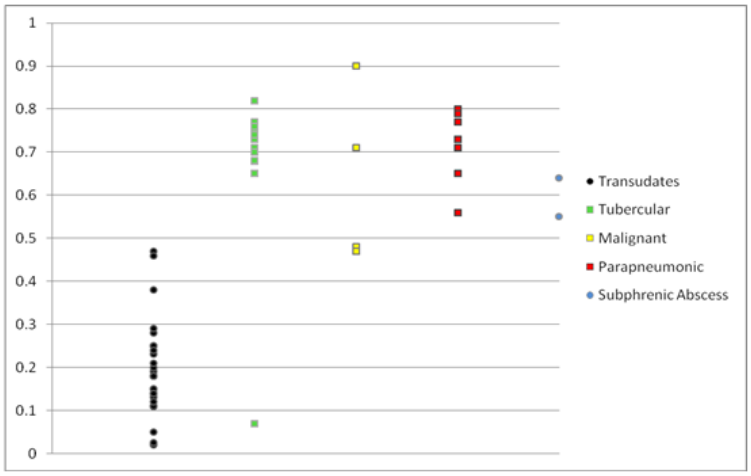

Fig. 4: Showing Pleural Fluid to Serum Ratio of Protein

(2) **Pleural Fluid LDH:** In case of LDH, two-thirds of the upper limit of normal was used as the cut-off[1]. In our hospital laboratory the cut-off value came up to 145U/L. The average levels of LDH in transudates was 108.7+72.4U/L and in exudates it was 1052U/L but the difference between the two groups was not statistically significant (p=0.06) (Table 4). The LDH levels misclassified 4 cases (6.4%). 2 transudates were misclassified as exudates and 2 exudates were misclassified as transudates (Fig 5).

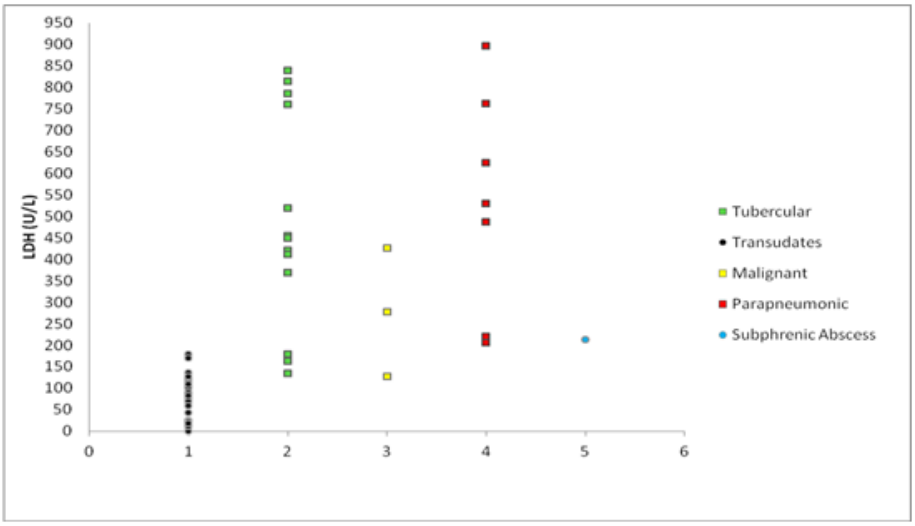

Fig. 5: Showing Pleural Fluid LDH

***1 value of subphrenic pus (18500U/L) and 2 parapneumonic effusions (1630 and 1645U/L) were excluded to avoid skewing of graph**

(3) **Pleural fluid to serum LDH ratio:** The cut-off value for pleural fluid to serum LDH level was 0.6[1] and the average value in this study was 0.27+0.14 in transudates and 2.13 in exudates and the difference between the two was statistically significant (p=0.003) (Table 4). The ratio misclassified 7 cases and all were exudates which were misclassified as transudates (Fig 6).

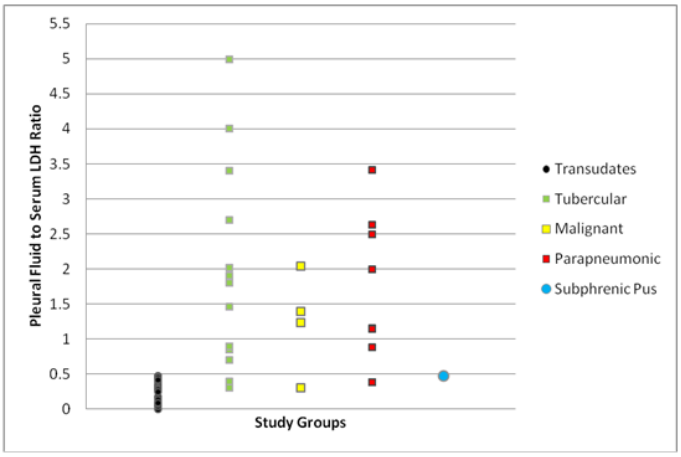

Fig. 6: Showing Pleural Fluid to Serum LDH Ratio

Overall the Light's criteria misclassified 3 cases of pleural effusion (4.8%). Of these one was an exudate (a case of malignant pleural effusion) and two were transudates (cases of CCF) (Table 5a).

(4) **Pleural fluid pseudocholinesterase:** The average Pseudocholinesterase (ChE) levels in transudative effusions was 385+142U/L and in case of exudates it was 2074+660U/L. The difference between the two groups was statistically significant (p=<0.001) (Table 4). The normal level of ChE varies between laboratories. The cut-off value is 1/10th of the upper limit of normal level of ChE in the respective laboratory[9]. In our study the cut-off value was 610U/L. In our study, the highest value among transudates was 700 U/L and lowest value seen in exudates was 1108U/L. With this cut-off value, 2 transudates were misclassified as exudates (Fig 7).

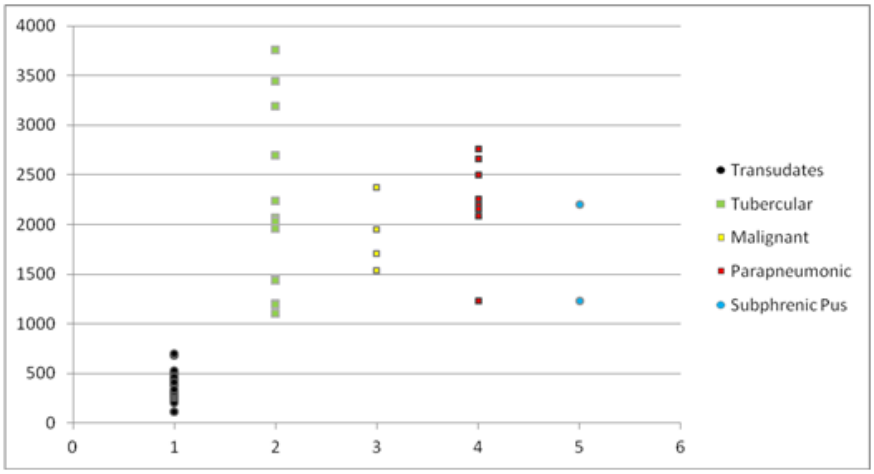

Fig. 7: Showing Pleural Fluid Pseudocholinesterase Levels (Units/L)

(5) **Pleural fluid to serum PChE ratio:**The cut-off value for pleural fluid to serum ratio of psuedocholinesterase (ChE) used in this study was 0.24[11]. In this study, the average value was 0.09+0.05 for transudates and 0.58+0.12 for exudates. This difference was found to be statistically significant (p=<<0.001) (Table 4). The ratio misclassified only one case of pleural effusion among the analyzed 62 cases and the case was a parapneumonic effusion (Fig 8).

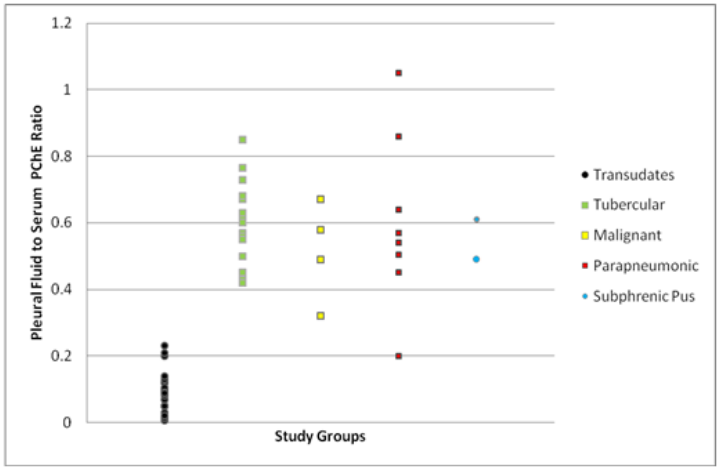

Fig. 8: Showing Pleural Fluid to Serum Pseudocholinesterase Ratio

Parameters	Transudative	Tubercular	Parapneumonic	Malignant	Subdiaphragmatic Pus
P Protein (g%)*	1.27+0.86	4.9+1.4	4.9+1.08	4.5+1.17	3.9
P/S Protein*	0.19+0.11	0.67+0.16	0.68+0.11	0.69+0.17	0.55
P LDH	108+72.4	511+240	778+336	496+210	7909
P/S LDH*	0.27+0.14	1.71+1.4	1.6+0.87	1.24+0.74	9.5
P ChE*	385+142	2080+319	2492+403	1893+363	1717
P/S ChE*	0.09+0.05	0.6+0.12	0.59+0.24	0.51+0.14	0.55

Table 4: Showing Average Values of Each Parameter Studied

*indicates that the p=<0.05 and the difference was statistically significant

In this study, most misclassifications were done by pleural fluid to serum(P/S) LDH ratio followed by pleural fluid protein levels (Table 5a). The pleural fluid to serum(P/S) ChE ratio accurately classified 98.4% of the cases, whereas Light's criteria correctly classified 95.2% of cases. In this study, the pleural fluid to serum cholinesterase ratio was the most efficient parameter with a sensitivity of 100% and specificity of 96.7% and was superior to the Light's criteria which showed a sensitivity of 93% and specificity of 96% (Table 5b).

	Total Number of Pleural Effusions (n=62)	
	Transudates Misclassified as Exudates (n=31)	Exudates Misclassified as Transudates (n=31)
P Protein (g%)	3 (4.8%)	3 (4.8%)
P/S Protein	-	3 (4.8%)
P LDH	2 (3.2%)	2 (3.2%)

P/S LDH	-	7 (11.2%)
Light's Criteria	2 (3.2%)	1 (1.6%)
P ChE	2 (3.2%)	-
P/S ChE	-	1 (1.6%)

Table 5a: Showing Number (n) and Percentage (%) of Misclassification

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV(%)
P Protein (g%)	90	90	90	90
P/S Protein	100	90	90	100
P LDH	93	93	93	93
P/S LDH	100	81	76	100
Light's criteria	93	96	96	93
P/S ChE	100	96.7	96.7	100

Table 5b: Diagnostic Efficacy of Each Parameter in this Study

DISCUSSION: 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(P/S) pseudocholinesterase ratio was compared with the Light's criteria in terms of efficacy.

In the original study by Light et al[1], 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 three cases of pleural effusion with a sensitivity of 93% and specificity of 96%(Table 5b). Two of these cases were CCF and one case was a malignant effusion (Table 5a). In the study done by Garcia-Pachon et al[9], Light's criteria misclassified 12 cases (9 transudate and 3 exudate) (Fig 9; Fig 10) with a sensitivity of 97.4% and specificity of 74.29% (Table 6).

Study	Sensitivity	Specificity
Light et al[1]	99%	97.8%
Garcia-Pachon et al[9]	97.4%	74.29%
Sharma et al[11]	91.25%	90%
Present study	93%	96%

Table 6: Showing Efficacy of Light's Criteria in Different Studies

In this study, the ratio of pleural fluid to serum pseudocholinesterase with a cut-off value of 0.24 misclassified only one case of pleural effusion out of the total 62 cases giving it a sensitivity of 100% and specificity of 96.7% (Table 7). In studies done by Sharma et al[11] and Garcia-Pachon et al[9], the pleural fluid to serum cholinesterase ratio correctly classified effusions in 98.19% and 98.7% cases respectively. The percentage of misclassification of pleural effusions was lower with P/S ChE in all three studies when compared to Light's criteria (Fig 9; Fig 10). This is a very significant and consistent result making it the most efficient parameter studied.

Study	Sensitivity	Specificity
Garcia-Pachon et al[9]	100%	94.5%
Sharma et al[11]	98.75%	96.67%
Present study	100%	96.7%

Table 7: Showing Efficacy of P/S ChE in Various Studies

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 LDH levels were found to vary widely among exudates and the difference between the mean values of LDH among transudates and exudates was not statistically significant. 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[11], 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 6 cases in this study and in the study by Sharma et al[11], the same parameter misclassified the maximum number of cases.

Though this study had a small sample size of 62, it had two equally distributed study groups of transudates and exudates unlike the other studies. Other studies comparing pleural fluid to serum cholinesterase ratio had sample sizes of 110, 150 and 80 each[12]. Probably a study with a larger sample size would provide better results for analysis.

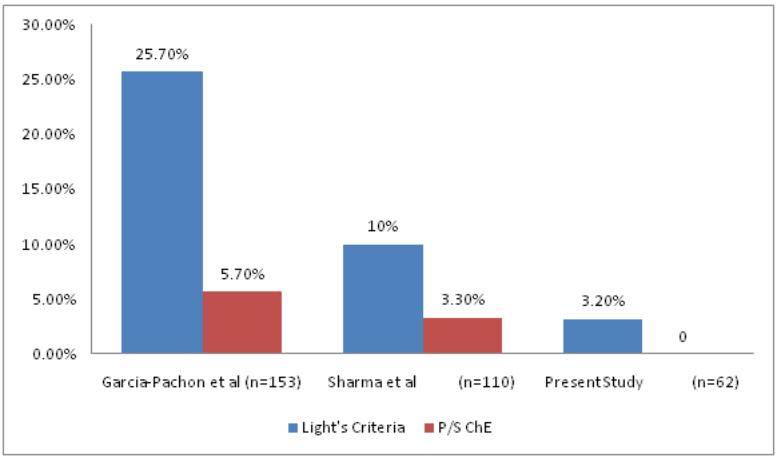

Fig. 9: Showing Percentage of Transudates Misclassified in Different Studies

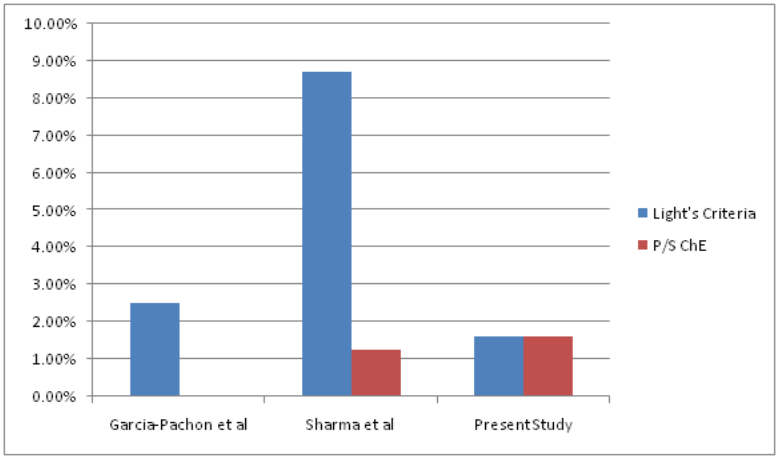

Fig. 10: Showing Percentage Misclassification of Exudates in Different Studies

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.

REFERENCES:

1. Light RW, MacGregor MI, Luchsinger PC. Pleural effusions: The diagnostic separation of transudates and exudates. AnnIntern Med 1972; 77:507-13.
2. Peterman TA, Speicher CE. Evaluating pleural effusions: A two-stage laboratory approach. JAMA 1984;252:1051-3.
3. Valdes L, Pose A, Alvarez D, Valle JM, Gude F. Biochemical discrimination of transudates and exudates (letter). Chest 1994;106:1634-5.
4. Reechaipichitkul W, Kawamatawong T, Teerajetgul Y, Patjanasoontorn B. Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion. Southeast Asian J Trop Med Public Health 2001;32:383-9.
5. Sharma SK, Suresh V, Mohan A, Kaur P, Saha P, Kumar A, Pande JN. A prospective study of sensitivity and specificity of adenosine deaminase estimation in the diagnosis of tuberculosis pleural effusion. Indian J Chest Dis Allied Sci 2001;43:149-55.
6. Gupta KB, Tandon S, Singh GP, Dhania OP, Jamneja AK. Pleural fluid cholesterol and serum cholesterol ratio as a parameter to differentiate between pleural transudate and exudate. Ind J Tub 1999;46:255-60.
7. Suay VG, Moragon EM, Viedma EC, Tordesa MP, Fabregas ML, Aldas JS. Pleural cholesterol in differentiating transudates and exudates. Respiration 1995;62:57-63.
8. Cabrer B Bofill D, Grau A. Valore de la colinesrasa en liquido plerual para su diagnostico etiolgico. Rev Clin Esp1978;150:183-4.
9. Garcia-Pachon E, Padilla-Navas I, Sanchez JF, Jimenez B, Custardoy J. Pleural fluid to serum cholinesterase ratio for the separation of transudates and exudates. Chest. 1996 Jul;110(1):97-101.

10. Heffner JE, Brown LK, Barbieri CA. Diagnostic value of tests that discriminate between exudative and transudative pleural effusions.Chest. 1997 Apr;111(4):970-8.
11. Sharma M, Gupta KB, Goyal KM, Nand N. Evaluation of cholinesterase to differentiate pleural exudates and transudates. J Assoc Physicians India. 2004 May;52:387-90.
12. Ozer F, Kanat F, Kalem A, Imecik O.Diagnostic efficiency of pseudocholinesteraselevel in discrimination of transudates-exudates Tuberk Toraks. 2003; 51(4):398-404.

AUTHORS:

1. Prakash Kikkeri Gowdaiah
2. Amrutha Avati
3. Priya Prakash Bhate
4. Akkamahadevi V. Nippanal

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Medicine, Bangalore Medical College and Research Institute, Bangalore.
2. Post Graduate Student, Department of Medicine, Bangalore Medical College and Research Institute, Bangalore.
3. Post Graduate Student, Department of Medicine, Bangalore Medical College and Research Institute, Bangalore.
4. Post Graduate Student, Department of Medicine, Bangalore Medical College and Research Institute, Bangalore.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Prakash Kikkeri Gowdaiah,
Associate Professor,
Department of Medicine,
Bangalore Medical College and Research Institute,
Bangalore.
Email- kikkeri47@yahoo.com

Date of Submission: 20/12/2013.
Date of Peer Review: 21/12/2013.
Date of Acceptance: 27/12/2013.
Date of Publishing: 02/01/2014