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Ascertaining the diagnostic accuracy of biochemical markers in the identification of exudative pleural effusion

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Abstract
The diagnosis of a pleural effusion is often challenging as its formation can be an outcome of diverse diseases. A total of 53 confirmed cases of exudative pleural effusion with peripheral blood were collected, 40 with tuberculous pleurisy, and 13 with malignant etiology were included in the study. The diagnostic utility of three biochemical markers, i.e. protein, lactate dehydrogenase (LDH) and cholesterol were ascertained in the identification of exudative pleural effusion. The utility of the markers using both blood and pleural fluid were compared with pleural fluid alone. Light's criteria is the gold standard for identifying exudative pleural effusion and was able to identify 49/53 exudates in our samples. However, only pleural fluid biochemical markers are sufficient to reach the same sensitivity and if all three markers are used the sensitivity was found to be better (50/53) than the one achieved by Light's criteria. The limitation of collecting blood and pleural fluid needed for applying Light's criteria in high burden and low resource countries can be avoided by choosing more than two biochemical parameters from pleural fluid alone.

Keywords: Tuberculous pleurisy, LDH, cholesterol, exudative pleural effusion, Light's criteria

Introduction
The diagnosis of a pleural effusion is often challenging as its formation can be an outcome of diverse diseases. The first step to ascertain the cause for the accumulation of pleural effusion is to find out if the collected fluid is an exudate or a transudate. A transudative pleural effusion accumulates when the systemic factors alter the pleural pressure that leads to abnormal formation or less absorption of pleural fluid, for example in left ventricular failure, cirrhosis etc. On the other hand an exudative effusion results from the factors within the pleural space that alters the capillaries permeability and responsible for altered absorption [1]. Common causes of exudative pleural effusions are pleural tuberculosis and pleural malignancy.

A diagnosis of an exudative pleural effusion is often established on the basis of Light's criteria or total protein concentration greater or equal to 3g percent in pleural effusion. The sensitivity and specificity of Light's criteria is approximately 99% and 98% respectively [2]. Further studies have demonstrated that Light's criteria classified almost all exudates correctly but misclassified about 25% of transudates as exudates [3]. In recent years, other biochemical tests have been proposed for the differentiation of transudates and exudates. Most common biochemical marker used by researchers was pleural fluid cholesterol and found good results but without consistent data [4, 5]. In tertiary hospital setting, protein content of pleural fluid is the only biochemical parameter taken into account in addition to the history of patient along with symptoms, X-ray and cytology to reach a clinical decision. Identification of pleural fluid as transudate does not require any further investigations. Light's criterion is considered to be gold standard for identification of exudative/transudative pleural effusion. Its biggest limitation is the simultaneous collection of two biological samples i.e. pleural fluid/effusion and blood and is often a limiting factor in high burden and resource constraint countries.

There is no comprehensive study wherein a comparative analysis of fluid biochemical markers with that of pleural fluid/serum ratio of biochemical markers employed in the differentiating of exudates is available in our population. Hence the objective of this study was to specifically ascertain the utility of pleural fluid biochemical markers alone in identifying pleural exudates.

Material and Method

Study population

A total of 53 cases of confirmed exudative pleural effusion with blood were collected from TB and Chest hospital, Majitha road, Amritsar and Department of TB & Respiratory Diseases, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar. The study was approved by the Ethical committee of the participating institution.

Pleural fluid and blood processing

Thoracentesis was performed in all the patients to obtain pleural fluid and the collected fluid was centrifuged for 15 minutes at 10000 rpm; supernatant was separated in 8-9 aliquots. Serum was isolated by centrifuged coagulated blood at 3000 rpm, at 4 °C, for 15 minutes. Isolated serum was stored at -20 °C for further use. The samples were categorised into two groups: Group TPE had all the samples with Tuberculous etiology and the second group i.e. NTPE comprised of patients with pleural effusion due to malignancy.

Classification of Exudates and transudates

(a) Pleural fluid protein > 3g percent [6] was used to classify the exudative pleural effusion.
(b) Light's criteria[2] was used in classifying transudative and exudative pleural effusion, which include a pleural/serum protein ratio (PP/SP) > 0.5, a pleural/serum Lactate dehydrogenase (LDH) level > 0.6 and a pleural LDH level over 2/3 of the reference value (or 200 IU).

Protein, LDH and Cholesterol estimation

Total Protein, LDH and cholesterol levels were determined by the commercially available reagent kit (Erba Mannheim, Transansia biomed, Daman, India). Protein levels were estimated using Biuret method [7]. LDH estimation was based on the reduction of NADH to NAD in the presence of pyruvate and the activity was measured at 340 nm [8]. Cholesterol estimation was achieved as described by Allain *et al* [9] and the modification of Roeschlau [10].

Diagnostic criteria

The diagnosis of Tuberculous pleural effusion (TPE) is confirmed by positive AFB or culture positive or pleural effusion getting resolved after anti-tubercular therapy (ATT). On the other hand malignant pleural effusion (NTPE) was confirmed by cytology or histological changes in pleural biopsy involving pleura.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 statistical software. Continuous variables are expressed as mean ± standard deviation (SD). Differences in Age, Total Protein (TP), Lactate dehydrogenase (LDH), and cholesterol between two groups were analyzed using independent t-test. Cholesterol cut-off level was selected using the receiver operating characteristic (ROC) curve.

Results and Discussion

A total of 53 confirmed cases of pleural effusion with peripheral blood were collected and divided in two groups. Group TPE had all the samples with Tuberculous etiology and the second group i.e. NTPE comprised of patients with pleural effusion due to malignancy. There were 66% males and 34% females (table 1). The two groups revealed statistically significant differences with respect to the age and total fluid protein levels and were found to be statistically similar in terms of pleural fluid LDH and cholesterol levels (table 1). When males and females were compared within their own groups with respect to the age, pleural fluid protein, LDH and Cholesterol levels no statistically significant differences were observed (data not shown).

Table 1: Demographic and clinical characteristics of the study population.

	TPE (n=40)	NTPE (n=13)	p-value
Age (years), mean ± SD	37.38±20.7	64.92±3.97	0.000*
Gender Male, Female	27,13	8,5	-
Protein ±SD (g/dL)	5.0±1.4	3.16±1.3	0.000*
LDH ± SD (mg/L)	845.63±567.77	813.01±936.97	0.880
Cholesterol ± SD (mg/dL)	65.96±26.28	51.35±36.59	0.121

*statistically significant

Ratio of protein or LDH in pleural fluid and serum

The fluid/serum protein ratio and LDH ratio were measured and it was found that protein ratio is a better indicator when compared to LDH ratio, as the former could not identify 15.1% of exudates while the later failed to identify 30.2% of exudates. However, when both the ratios were taken into account only 11.3% cases could not be identified (table 2).

Table 2: Comparative analysis of biochemical parameters for the identification of exudative pleural effusion based on pleural fluid/serum ratios.

Physician diagnosis	Protein Ratio (n=53)		LDH ratio (n=53)		Protein Ratio or LDH ratio (n=53)	
	<0.5	>0.5	<0.6	>0.6	<0.5 or <0.6	>0.5 or >0.6
TB (40)	1	39	10	30	1	39
NTB (13)	7	6	6	7	5	8
Total (53)	8 (15.1%)	45 (84.9%)	16 (30.2%)	37 (69.8%)	6 (11.3%)	47 (88.7%)

Exudates = Protein ratio >0.5; LDH ratio >0.6; Transudates = Protein ratio <0.5; LDH ratio <0.6

The pleural fluid total cholesterol levels were studied and the cut-off values giving the best test performance were evaluated using ROC curve (Fig 1). The sensitivity and specificity are shown in Table 3.

Although, cholesterol is good marker in differentiating exudates from transudates but it is poor in differentiating TPE and NTPE patient groups (table 1).

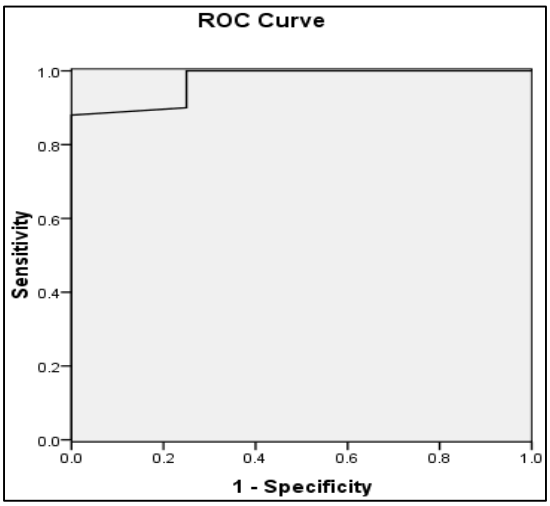

Fig 1: ROC curve of pleural fluid cholesterol levels

Table 3: AUC, cut-off, sensitivity and specificity of Cholesterol.

Marker	Cut-off	Sensitivity %	Specificity %	AUC
Fluid Cholesterol level	33 mg/mL	88	100	0.927

AUC = Area under curve

Pleural fluid biochemical markers

The cut-off value of cholesterol i.e. 33 mg/dL was used for identification of exudative pleural effusion and comparison was done with fluid total protein and fluid LDH levels (Table 4). It was found that 17% of exudative pleural effusions could not be identified if only fluid total protein was considered. However, LDH could identify 90.6% of exudates and performed better than fluid protein.

Table 4: Comparative analysis of biochemical parameters from fluid alone to identify exudative pleural effusion.

Physician diagnosis	Protein (g/dL) (n=53)		LDH (IU/L) (n=53)		Cholesterol (mg/dL) (n=53)	
	< 3	> 3	< 200	> 200	< 33	> 33
TB (40)	2	38	3	37	3	37
NTB (13)	7	6	2	11	4	9
Total (53)	9 (17%)	44 (83%)	5 (9.4%)	48(90.6%)	7 (13.2%)	46(86.8%)

Exudates = Protein >3 g/dL; LDH>200 IU/L; Cholesterol>33 mg/dL
Transudates = Protein <3 g/dL; LDH<200 IU/L; Cholesterol<33 mg/dL

Combination of Protein and LDH Ratios with Fluid cholesterol

We also tested various combination of pleural fluid/ serum ratio of protein, LDH and fluid cholesterol and found combination of LDH ratio with cholesterol gives better result than the combination of protein and LDH ratio; protein ratio and fluid cholesterol (table 5).

Table 5: Identification of exudates based on the combinations of pleural fluid/serum ratio of protein, LDH and pleural fluid cholesterol.

Physician diagnosis	Protein Ratio or LDH ratio (n=53)		Protein Ratio or Cholesterol(mg/dL) (n=53)		LDH ratio or Cholesterol(mg/dL) (n=53)	
	<0.5 or < 0.6	>0.5 or >0.6	<0.5 or <33	>0.5 or >33	<0.6 or < 33	>0.6 or >33
TB (40)	1	39	0	40	0	40
NTB (13)	5	8	4	9	2	11
Total (53)	6 (11.3%)	47 (88.7%)	4(7.5%)	49 (92.5%)	2(3.4%)	51 (96.6%)

Exudates = Protein ratio >0.5; LDH ratio >0.6,

Combination of three biochemical markers

Similarly when the combination of fluid protein, LDH and cholesterol were used to identify exudate, best result (51/53) was obtained when all three biochemical markers were taken together (table 6).

Table 6: Identification of Exudate based on the combination of three biochemical parameters in pleural fluid alone.

Physician diagnosis	Protein (g/dL) or LDH (IU/L) (n=53)		Protein (g/dL) or Cholesterol (mg/dL) (n=53)		LDH (IU/L) or Cholesterol (mg/dL) (n=53)		Protein (g/dL) or LDH (IU/L) or Cholesterol (mg/dL) (n=53)	
	< 3 or <200	> 3 or >200	< 3 or < 33	> 3 or > 33	< 200 or < 33	> 200 or > 33	<3 or <200 or<33	>3 or >200 or >33
TB (40)	1	39	1	39	1	39	0	40
NTB (13)	2	11	4	9	2	11	2	11
Total (53)	3 (5.7%)	50(94.3%)	5 (9.4%)	48(90.6%)	3(5.7%)	50(94.3%)	2(3.4%)	51(96.6%)

Exudates = Protein >3 g/dL; LDH>200 IU/L; Cholesterol>33 mg/dL
Transudates = Protein <3 g/dL; LDH<200 IU/L; Cholesterol<33 mg/dL

There is no single test which specifically differentiates transudates from exudates. In high burden countries like India, physician relies on fluid total protein estimation in addition to the history of patient along with symptoms, X-ray and cytology to reach a decision. On the other hand Light's criterion is considered to be the gold standard for identification of transudative/exudative pleural effusions. It is based on the total protein fluid/serum ratio of >0.5 or fluid/serum ratio of LDH >0.6 or a pleural LDH level over 2/3 of the reference value (or 200 IU) [2]. Using this criteria in our sample 50/53 samples could be identified as exudative in nature i.e. around 5.66% of samples could not be identified as exudate. The sensitivity and specificity reported by Light and his colleagues in their data could not be replicated in other studies and challenged by various investigators and some of them have suggested alternative markers to supplement the principal components or have recommended the use of additional markers as adjunct tools in the identification of exudates [11][12]. Cholesterol is a biochemical marker that has been used for the identification of pleural exudates [13, 14], however, the precise cut off values varied in different populations. Rufino *et al.* [5], reported a cut off value of a pleural cholesterol level >50 mg/dL for exudates with sensitivity of 97.22%, and specificity of 85.71%. Another study by Guleria *et al.* [15] reported a cut off value of 60 mg/dL with a sensitivity of 88% and specificity of 100% for exudates. Study by Ortega *et al.* [16] found pleural cholesterol cut off level of 40 mg/dl or higher that separated exudates and transudates, with a sensitivity of 96% and a specificity of 92% for exudates. In our study, a cut off value for the cholesterol levels was lower than above mentioned studies i.e. 33 mg/dL with sensitivity and specificity of 88% and 100%, respectively (Fig 1;Table 3). One possible reason for the variable cut off limits for the pleural fluid total cholesterol levels could be ethnic heterogeneity between the studied populations.

In light of this, the present study was undertaken to ascertain the role of three principal biochemical markers used by different investigators to identify exudative pleural effusion, i.e. total protein, LDH and cholesterol levels.

In this study, we found that the simultaneous analyses of blood and pleural fluid, as is required under Light's criterion, does not contribute significantly in the identification of exudative fluid. In fact, better level of sensitivity was achieved in our sample by studying pleural fluid alone but using atleast two or more than two biochemical markers (Table 6). Our findings clearly demonstrated that inclusion of three markers i.e. fluid protein, LDH and total cholesterol has the highest sensitivity that is better than the Light's criterion. Of the different combinations tried, the sensitivity of LDH and Cholesterol was better than the other two combinations, i.e. Protein & LDH and Protein & cholesterol (Table 5 and 6). Similar, findings have also been reported by other investigators [4, 11, 12]. Using only pleural fluid for the identification of exudative fluid also removes one of the limitations of Light's criterion, that it required two biological samples from the same patient i.e. pleural effusion and blood, which is often a limiting factor in utilizing this criterion especially in high burden and resource constraint countries. More studies are clearly warranted from other areas of India or other high burden countries to validate these findings or investigating other novel markers for the identification of exudative pleural effusions.

Conclusion

The limitation of two samples analyses for the Light's criterion for the identification of pleural exudate can be avoided as pleural fluid biochemical markers alone are sufficient for the diagnosing of exudative pleural effusion. The pleural cholesterol level can be used as independent variable to diagnose an exudative effusion but it has more diagnostic value when integrated with fluid total protein and fluid LDH levels.

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