Diagnostic Significance of Serum Pleural Fluid Albumin Gradient in Classification of Pleural Effusion

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

ABSTRACT

Introduction: Classification of Pleural Effusion into transudative and exudatives is the initial step in management of effusions. Light’s criteria remains the most used method. But 20-30% of transudates may be misclassified as exudates according to Light’s criteria leading to unnecessary interventions. Serum Pleural fluid Albumin Gradient (SPAG) is considered as a sensitive indicator for transudate. The present study is to determine the diagnostic value of SPAG compared to Lights criteria and calculate its specificity and sensitivity

Materials & Methods: Prospective measurement of the SPAG and Light's criteria done in 234 consecutive patients with pleural effusion undergoing thoracentesis in Pulmonology wards. Serum and pleural effusion albumin, protein, Lactate dehydrogenase (LDH) measured in order to determine the SPAG and Light's criteria. Clinical diagnosis with histopathology and/or culture along with treatment response and outcome as transudate and exudate done. Specificity, sensitivity, PPV, NPV of Lights criteria and SPAG for exudative and transudative effusion determined.

Results: 200 cases were included in final analysis of which 51(25.5%) were transudates, 149 (74.5%) were exudates. Sensitivity and specificity of lights criteria obtained to be 78.43% and 100% for transudates. SPAG had 100% sensitivity and 99.3% specificity in identifying transudates. 11 of the transudates were misclassified as exudates by Light’s criteria whereas SPAG could identify all transudates correctly. They were on diuretic therapy.

Conclusions:
- SPAG is more sensitive than Light’s criteria for classifying transudates.
- SPAG is easy to practice, compared to Light’s criteria

Keywords: SPAG, LDH, ROC

INTRODUCTION

Pleural effusion is the result of fluid accumulation in the pleural space as a result of a number of different pathological mechanisms and occurs as a manifestation of primary lung pathology as well as other systems or immunological or infective involvement. The identification of etiology of pleural
effusion aids in treatment of the underlying condition and gives symptomatic relief, as well as timely intervention prevents complications of pleural effusion such as empyema, pleural thickening and pneumothorax.

The initial step in identifying the etiology is to classify pleural effusion into transudates and exudates. Transudates are secondary to alterations in the physiological factors affecting pleural fluid formation and absorption while exudates are secondary to increased permeability of pleura. Transudates are caused by increased hydrostatic pressures (e.g., heart failure), decreased oncotic forces (e.g., hypoproteinemia), increased negative intrapleural pressure (e.g., atelectasis), or movement of ascitic fluid through the diaphragm (e.g., hepatic hydrothorax). In contrast, exudates are due to the increased capillary permeability and/or impaired lymphatic drainage which results from the proliferative (e.g., malignancy) or inflammatory (e.g., parapneumonic effusions). It is clinically important to classify pleural and ascitic fluids into exudates and transudates because this is indicative of the underlying pathophysiological process involved and Such a distinction allows appropriate investigations to be instigated, enabling better patient management.

Clinical history and features elicited, followed by radio graphical, blood investigations and diagnostic thoracocentesis for analysis of pleural fluid for various biochemical, microbiological and pathological tests help in this.

The classification is classically done based on the Light criteria, which has a high sensitivity for exudative effusions. An effusion is considered exudative when any one of the following three criteria is met: pleural/serum total protein ratio > 0.5, pleural/serum lactate dehydrogenase (LDH) > 0.6, or pleural LDH > two-thirds the upper limit of normal. This has been reported as the best method for discriminating between exudates and transudates.1 But Light's criteria have high sensitivity for exudative but lower specificity and therefore may misclassify transudative effusions as exudates.2 Approximately 25% of pleural effusions due to CHF may be misclassified as an exudate, and hepatic hydrothoraces may also present with high proteins.

Misclassifying transudates as exudates may have serious adverse consequences, i.e., patients might undergo unnecessary invasive interventions such as thoracentesis, pleural biopsy.

Many alternative parameters have been proposed and analysed to improve the diagnostic accuracy. Among these alternative criteria are pleural fluid cholesterol level,3 serum to pleural fluid albumin gradient,4 effusion amylase activity5 and cholesterol and lactate dehydrogenase.6 The performance of these individual parameters is inconsistent throughout the different studies.

The Albumin gradient had been in use for classification of ascitic fluid into transudates and exudate.7 Pare and co-workers suggested that the serum-ascites albumin gradient (SAAG) is a better discriminator of portal hypertension than ascites protein concentration.8 Indeed, SAAG is now considered a useful physiological, clinical tool in the workup of ascites.9 The accuracy of such determinations is 97%.10 Infact “High-albumin gradient” (≥1.1 g/dL) and "low-albumin gradient" (<1.1 g/dL) have replaced the terms "transudative" and "exudative" in the description of ascites in most of the recent publications.11 The significance of pleural fluid to serum albumin gradient (>1.2 g/dl for transudates) in classification of effusions is also being validated now.

This study aims at determining the diagnostic value of serum pleural fluid albumin gradient in classifying pleural effusion compared to lights criteria. Being a simpler test commonly done in all laboratories with minimum calculation SPAG can be promising a simpler way to approach pleural effusion.

MATERIALS AND METHODS

Study setting

This study was conducted in Department of Pulmonology Government Medical College Thrissur, Kerala comprising the newly diagnosed patients with pleural effusion.

Study design

Prospective Observational Study

Study population

Patients who attended the Pulmonology OPD/admitted in Pulmonology or Medicine wards with pleural effusion who could be followed up until final etiology of pleural effusion could be made.
Inclusion criteria
1. All adult patients diagnosed clinically and radiologically with pleural effusion
2. All the patients who had given written informed consent in accordance with institutional guidelines

Exclusion criteria
1. Pediatric age group
2. minimal pleural effusion(<1cm detected by imaging studies)
3. contraindication for thoracocentesis
4. hemothorax (PCV>45%)
5. empyema
6. Patients lost to follow-up
7. Patients who do not consent to be a part of the study

Sample size calculation:
Sample size was calculated using the formula \((Z\alpha)^2 \times \frac{PQ}{d^2}\)

\(P=33\%\)
\(Z\alpha = 1.96, Q=100-P = 67, \text{Relative error } d = 20\% \text{ of } P = 6.6\)

Significance level of 0.05 and power 90%

So sample size = (1.96)^2 \times 33 \times 67/ (6.6)^2 = 101

So the minimum sample size needed was 101

Hence sample size for the study was 200

Study procedure
The step wise evaluation of pleural effusion was followed in diagnosing etiology of pleural effusion. All patients underwent thoracocentesis and pleural fluid analysis necessary blood investigations and radiological imaging such as CXR, USG, CT thorax; thorascopy or pleural biopsy/trucut biopsies were done accordingly. Cardiology, Nephrology, Gastroenterology/General Medicine consultataions were done in suspected transudative effusion for confirmation of diagnosis.

Initial workup:
Thoracentesis was performed in each case with due aseptic precautions. Care was taken not to let fluid to mix with blood. About 10mL to 15mL of pleural fluid was aspirated in each case and Specimen was stored at 2º -8º Celsius. Within half an hour 10ml blood was collected in vaccutainer and centrifuged and sera stored at 2º -8º Celsius.

The pleural fluid was analyzed as follows
1. Initially color of the fluid was noted.
2. Pleural fluid was sent for biochemical analysis for protein,albumin sugar, LDH
3. Pleural fluid was sent for microbiological analysis for Grams stain, AFB smear and Culture and NT culture in selected cases, also CBNAAT in selected cases.
4. Pleural fluid was sent for cytology for Malignant Cells.
5. The serum protein, sugar, albumin, LDH were tested

Case definition
- **Transudative effusion**: Effusions Associated with features of congestive cardiac failure/chronic liver disease/nephritic syndrome/chronic renal disease/hypoalbuminemia where other causes of exudative effusion was ruled out and responded to treatment for fluid overload / hypoprotenemia/management of ascites.
- **Tuberculous effusion**: Lymphocytic effusions with high ADA/Fluid AFB positive/culture positive for AFB/HPR suggestive of TB
- **Malignant effusion**: Malignant cells in fluid cytology/HPR positive for malignancy
- **Synpneumonic effusion**: Effusion developed in patients presented with high grade fever with respiratory symptoms of short duration and responded to appropriate treatment.

Classification for Transudates also done according to Light’s criteria - Pl Fluid LDH/Serum LDH Ratio >0.6, Pl Protein/Serum Protein Ratio >0.5, Serum LDH ≥ 200, any one criteria.

Classification into transudates and exudates also done based on SPAG (Serum Pl Fluid Albumin gradient >1.2 for transudate).
Follow up: patients presenting with pleural effusion were followed up for diagnosis and management, investigations were followed at every stage and necessary consultations were sent in case of transudative effusions. The clinical classification of transudate or exudate was reached independently by following up the patients and assessing the treatment outcome. Samples were only included in the final analysis if there was certainty about the clinical diagnosis, and excluded if uncertain etiology or more than one cause for effusion.

Study tools

- Informed consent
- Proforma

Study period

One year

Statistical analysis

Data were coded and entered into excel sheets and analyzed using Epi info/SPSS software. The qualitative data was expressed in proportions and quantitative data was expressed in means and standard deviation. The qualitative data was analyzed using standard error of proportions, standard error of difference between two proportions and chi-square test. The quantitative data was analyzed using standard error of the mean, the standard error of difference between two means and T-test. ROC curve was plotted in numerical variables to assess significant cut off values.

RESULTS

234 patients were initially included in the study, but due to uncertain diagnosis / clinical outcome /loss of follow up, 34 patients were excluded from final analysis

200 samples included for final analysis who had definite final diagnosis and nature of effusion confirmed by clinical outcome. 63% were males and the rest 37% were females. The male to female ratio was 1.70.

The majority of the study population were between 36-55 years of age accounting for 39.5%, 23% belonged to 15-35 years of age and the rest 37.5% were of 56-90 years of age. The mean age of the population was 50.75

Type of Effusion, Etiology of effusion, Etiology in Exudates According To Final Diagnosis

All the 200 patients were followed until final diagnosis was made and classification of pleural effusion was made for comparison with Lights criteria and SPAG and the transudative o exudative nature confirmed by clinical outcome. 51 patients (25.50%) had transudative effusion, 149 patients (74.5%) had exudative effusion according to the clinical outcome (Table 1).

The main etiology of exudative effusion was ca lung and tuberculosis with 34.50% and 34.0%. Parapneumonic effusion contributed to 6.0% of the exudative effusion. Among the transudative effusion the main etiologies were congestive cardiac failure and renal failure in 9.0% patients each. Pancreatitis was the cause in 2.0% and in 5.50% patients’ cirrhosis was the cause of transudative effusion.

By using protein ratio as 0.5 as cut off from Lights criteria, 40 (20%) of 51 transudates could be identified correctly with sensitivity of 78.43% whereas it could classify 147 (73.5%) of 149 exudates correctly with 98.65% sensitivity. The comparison with clinical outcome had a significant correlation with p value <.00001.

Using pleural fluid to serum LDH ratio to classify effusion using 0.6 as cut off from lights criteria, 42 (21%) transudates were classified correctly with sensitivity of 82.35%. 130 of 149 exudates were correctly classified with sensitivity of 87.24%. this criteria was compared to clinical outcome and had a significant relation with p value <.00001.

The pleural fluid LDH cut off was taken to be 200 U/L. This could classify 40 (20%) of the transudates correctly with a sensitivity of 78.43% .141 of 149 exudates were classified correctly with sensitivity of 94.6%.

Lights criteria when compared with clinical outcome identified all exudates correctly but 11 transudates were misclassified when all the three criteria were applied and analysed. The sensitivity of lights criteria was 78.43% whereas the specificity was found to be 100%. The positive predictive value of Lights’ criteria was found to be 100% and negative predictive value was 93.125% for identifying transudates.
10 of the misclassified transudates were all having CCF as diagnosis receiving diuretic therapy which explains the protein concentration effect and the failure of lights criteria in such cases. One patient with renal failure also had transudative effusion with high fluid protein, so was misclassified by Lights’. (Table 2)

**SPAG VERSUS CLINICAL OUTCOME**

<table>
<thead>
<tr>
<th>Area</th>
<th>Std. Error</th>
<th>Asymptotic Sig.</th>
<th>Asymptotic 95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>.998</td>
<td>.002</td>
<td>.000</td>
<td>.993 – 1.000</td>
</tr>
</tbody>
</table>

The test result variable(s): SPAG has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

- a. Under the nonparametric assumption
- b. Null hypothesis: true area = 0.5

The optimal cut off value of serum pleural fluid albumin gradient was 1.5 g/dl, above which all effusions were transudative.

**DISCUSSION**

Separation of exudates from transudates remains a useful initial step in determining the cause of a pleural effusion and the decision as to whether further and often more invasive, investigations should be carried out on the patient.13

In 1972, Light et al developed criterion for the diagnostic separation of transudates from exudates.12 Thereafter it was found that Light’s criteria misclassified a large number of effusions which may lead to unwarranted invasive interventions in about 20% to 30% of patients with transudates.16,14 However Lights criteria remains the most common method.

The present study was to determine the diagnostic significance of serum pleural effusion gradient (SPAG) in classification of pleural effusion. The objective was to compare SPAG with lights criteria and determine the accuracy of SPAG and lights criteria based on sensitivity, specificity and other statistical variables when equated against the final clinical diagnosis. The strength of our study is that we have utilized ROC analysis for comparing different tests. Furthermore, ROC analysis provides an opportunity to calculate likelihood ratios at various cutoff points of the diagnostic test.17,18

The total number of sample population included in final analysis is 200. The mean age of the study population was 50.75 ± 15.64 years. The male: female ratio was 1.70, with 63% males. These patient population characteristics were comparable with similar studies by Roth et al (61 yrs-mean age; 1.68-male to female ratio)4 and Sujatha G et al (51.3 – mean ag; 1.32-male female ratio).19

In this study, the main etiology of exudative effusion were tuberculosis (34%) and malignancy (34%) and parapneumonic effusion (6%) and transudative effusion were caused mainly by CCF (9%) and renal failure 9% each followed by cirrhosis in 5.5% patients.
In a similar study by Dhar et al, the commonest cause of exudates was tuberculosis (42%); others were neoplasm (22%), parapneumonic (4%) and rheumatoid arthritis (2%). The transudative effusions were due to heart failure (10%), cirrhosis of liver (12%), and nephrotic syndrome (8%).

The mean value of serum pleural fluid albumin gradient obtained was 1.60g/dl for transudate and 0.85 for exudates from our study which was comparable with mean value obtained from other studies. In a study by Chakko et al, the mean value of SPAG obtained was 1.74 for transudates and 0.85 for exudates.

In this study, the calculated sensitivity and specificity of Lights’ criteria was 78.43% and 100% for transudates whereas SPAG had a sensitivity of 100% and specificity of 99.30% in classification of transudative pleural effusion. The positive predictive value was 100% and negative predictive value was 98% in identifying transudates.

In a study conducted by Sujatha G et al, the sensitivity and specificity of Light’s criteria for the separation of transudates and exudates in pleural effusion, by Sunanda V et al, the mean value of SPAG obtained was 1.74 for transudates and 0.85 for exudates.

Roth et al studied serum pleural fluid albumin ratio in 59 patients in 1992. The sensitivity and specificity for identifying exudates with Light's criteria are 100 percent and 72 percent, respectively, from the data in 59 patients. The corresponding sensitivity and specificity for identifying exudates with the albumin gradient are 95 percent and 100 percent.

Burgess L et al in 1995 compared various biochemical parameters in pleural fluid and serum against lights criteria and the sensitivity and specificity of the serum-effusion albumin gradient was found to be calculated as 87% and 92%.

10 of the misclassified transudates according to Lights criteria in the present study were all having CCF as diagnosis receiving diuretic therapy which explains the protein concentration effect and the failure of lights criteria in such cases. One patient with renal failure also had transudative effusion with high fluid protein so was misclassified by Lights.

They all had higher pleural fluid protein value in the exudative range, with a mean pleural fluid protein of 3.97 g/dl in these patients.

Diuretics could lead to fluid resolution through multiple mechanisms. By decreasing left atrial pressure, less fluid would leak from the pulmonary microvasculature, leading to decreased fluid formation and eventual resolution by lymphatic drainage. Also, by decreasing systemic venous pressure, the lymphatic drainage would be increased. Finally decreasing systemic arterial pressure could lead to a pressure gradient that favoured fluid reabsorption via the pleural micro vessels.

Chakko et al showed that treatment of patients with congestive heart failure and pleural effusions with diuretics leads to a concentration of pleural fluid protein which can be in the exudative range.

A malignant effusion with cytology positivity was classified as transudative by SPAG, which had also a collapse lung which may have added on to the pleural effusion due to change in pressures.

In a study conducted by Joseph et al comparing albumin gradient or fluid to serum albumin ratio and pleural fluid lactate dehydrogenase in the diagnostic separation of pleural effusion in published in 2002, from ROC analysis it was clear that pleural fluid LDH performed better compared to SpAG as documented by the highest value for the AUC which were 0.9 and 0.81 for FLDH and SPAG respectively. The optimum cut-off levels were 163 IU/L for FLDH, 9 g/L for SEAG.

In our study the ROC analysis was performed for pleural fluid variables, lights criteria and SPAG, the AUC was found to be highest for SPAG 0.998 while pleural fluid LDH had AUC of 0.914. The ratio of pleural fluid protein and LDH to serum protein and LDH had AUC 0.894 and 0.873 respectively. The optimum cut off value in our study was 150 U/L for PFLDH and 1.5 g/dl for SPAG. This value of 1.5 g/dl above which all transudative effusions are identified may be at the expense of specificity for transudates.

Hence SPAG was found to be more efficient in identifying transudates especially in the clinical settings of congestive heart failure and renal failure. This is supported by various previous studies. Our study also proves SPAG can be used as a more sensitive diagnostic method to...
identify transudates especially those with high pleural fluid protein as in patients on diuretics. It can avoid multiple serum and pleural fluid tests and calculations because of ease in using a single parameter to identify and classify transudative pleural effusion.

Our present study certainly suffers several limitations in aspects like small sample size and lesser number of transudative effusions.

CONCLUSION

- It was found that SPAG is a good parameter to differentiate transudates and exudates. SPAG is more sensitive than Light’s criteria for classifying transudates and is more specific for exudates.
- The optimal cut off value for SPAG was found to be 1.5 g/dl after plotting ROC, above which pleural effusion can be classified as transudates.
- SPAG which uses a single parameter i.e albumin is easy to practice when compared to Light’s criteria where three parameters as well as serum calculations because of ease in using a single parameter to identify and classify transudative pleural effusion.

REFERENCES


Table1: Type of Effusion, Etiology of effusion, Etiology in Exudates According To Final Diagnosis

<table>
<thead>
<tr>
<th>TYPE</th>
<th>FREQUENCY</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transudate</td>
<td>51</td>
<td>25.50%</td>
</tr>
<tr>
<td>Exudate</td>
<td>149</td>
<td>74.50%</td>
</tr>
</tbody>
</table>

DIAGNOSIS
Table 2: Clinical outcome versus pleural fluid/serum protein ratio, LDH ratio, s.LDH and Lights criteria.

<table>
<thead>
<tr>
<th>Pleural Fluid Protein / Serum Protein Ratio</th>
<th>Transudate</th>
<th>Exudate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Outcome</td>
<td>51 (25.5%)</td>
<td>149 (74.5%)</td>
<td>.00001</td>
</tr>
<tr>
<td>Protein Ratio</td>
<td>40 (20%)</td>
<td>147 (73.5%)</td>
<td></td>
</tr>
<tr>
<td>Missclassified</td>
<td>11 (5.5%)</td>
<td>2 (1%)</td>
<td></td>
</tr>
</tbody>
</table>

| Pleural Fluid LDH                          |                   |               |           |
| Clinical Outcome                          | 51(25.5%)         | 149(70.5%)    | .0001     |
| LDH Ratio                                 | 40 (20%)          | 141(70.5%)    |           |
| Missclassified                            | 11(5.5)%          | 8(5.3%)       |           |

| Pleural Fluid LDH / Serum LDH Ratio        |                   |               |           |
| Clinical Outcome                          | 51 (25.5%)        | 149 (74.5%)   | .00001    |
| Ldh Ratio                                 | 42 (21%)          | 130 (65%)     |           |
| Missclassified                            | 9 (17.6%)         | 19 (37.25%)   |           |

| Lights Criteria                           |                   |               |           |
| Clinical Outcome                          | 51 (25.5%)        | 149 (74.5%)   | .00001    |
| Lights Criteria                           | 40 (20%)          | 160 (80%)     |           |
| Missclassified                            | 11 (5.5%)         | 0             |           |

Table 3: Clinical Outcome

<table>
<thead>
<tr>
<th>Spag Outcome</th>
<th>Transudate</th>
<th>Exudate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transudate</td>
<td>51 (25.5%)</td>
<td>1 (0.5%)</td>
<td>52</td>
</tr>
<tr>
<td>Exudate</td>
<td>0</td>
<td>148 (99.32%)</td>
<td>148</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>149</td>
<td>200</td>
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</table>
Figure 1: ROC plot of SPAG