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Utility of pleural fluid biomarkers in differentiating malignant and benign pleural effusion: a cross-sectional study

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Abstract

As cytopathological examination of the pleural fluid (PF) has a low yield, it is difficult to differentiate benign from malignant pleural effusions (PEs). There is still a lack of biomarkers in PF that can reliably help in diagnosing malignant PEs. Diagnostic accuracy in diagnosing malignant PEs is enhanced by the presence of tumor markers in PE. This would raise the likelihood of diagnosing malignant PEs with an unclear cause and starting treatment interventions early to lower morbidity and death rates in the patient. Thus, in an effort to enhance the PE diagnosis, novel PF biomarkers such as cancer ratio, cancer ratio plus, carcinoembryonic antigen (CEA), cancer antigen 125 (CA-125), and calprotectin were evaluated. A total of 108 patients with PEs were taken, and after applying inclusion and exclusion criteria, 83 patients with exudative PE were evaluated. After clinical history and routine blood investigations, PF was sent for cytopathology, biochemical examinations, microbiological examinations for aerobic culture, fungal culture, mycobacterial culture, and biomarker (CEA, CA-125, calprotectin) analyses. Out of 83 patients with exudative PE, malignant effusion was diagnosed in 40 patients, while 43 had benign effusions. PF biomarkers adenosine deaminase (ADA), CEA, cancer ratio, calprotectin, age/ADA and serum lactate dehydrogenase (LDH)/calprotectin ratio showed a statistically significant ($p<0.0001$) difference between benign and malignant PE. PF CEA showed the highest specificity (97.67%) at the cut-off point of 1.96 ng/mL and age/ADA ratio showed the highest sensitivity (97.5%) at the cut-off point of 1.56 for prediction of malignancy. Cancer ratio, PL calprotectin and serum LDH/calprotectin ratio also showed good sensitivity and specificity for the prediction of malignancy. PF CA-125 showed poor sensitivity (50%) and cancer ratio plus did not reveal any statistically significant difference for prediction of malignancy from benign effusion ($p=0.486$). PF biomarkers (cancer ratio, CEA, age/ADA ratio, calprotectin, and serum LDH/calprotectin ratio) are reliable indicators of malignancy in exudative pleural effusions. Patients with exudative lymphocytic with unexplained pleural effusion can be addressed early using inexpensive and generally available biochemical indicators, such as pleural fluid CEA, calprotectin, cancer ratio, and serum LDH/calprotectin ratio.

Key words: biomarkers, cancer ratio, calprotectin, CEA, pleural effusion.

Introduction

The accumulation of fluid between the visceral and parietal pleura is known as pleural effusion. Pleural effusion happens when either the rate of production rises or the rate of absorption falls. The normal production of pleural fluid is 0.01 ml/kg/hr. Using Light's criteria, the pleural effusion must first be assessed and categorized as an exudate or a transudate[1].The three most frequent causes of exudative pleural effusions are tuberculosis, malignancy, and parapneumonic effusion [1,2]. According to some studies, 44-77% of exudative pleural effusions are linked to malignancy [2].

The presence of malignant cells in the pleural cavity, which must be verified by pleural fluid cytology or in pleural tissue recovered by thoracoscopy, thoracotomy, or blind pleural biopsy, is what defines malignant pleural effusion (MPE). An exudative effusion with a recognized main cause of malignancy is classified as a malignant effusion in the absence of non-malignant explanations [3]. When the tumour etiology has been ruled out using imaging methods, medical history, and patient follow-up, any pleural effusion is regarded as benign.

Because benign and MPE have different prognoses, it is critical to distinguish between the two in order to make the best treatment decisions. There are no trustworthy clinical criteria or biochemical indicators to distinguish between benign and malignant pleural effusion. A reliable biomarker for tubercular effusions is pleural fluid ADA. At a cut-off level of 40 IU/L, it has 85.7% sensitivity and 80.8% specificity for tuberculosis detection [4]. According to certain studies, immunodeficiency disorders and elderly people may experience decreased ADA levels in tubercular pleural effusions (TPEs). As a result, care should be taken when interpreting low pleural fluid ADA levels in patients who may have TPE. Several investigations have reported elevated serum LDH and low levels of ADA in the majority of malignant effusions [5,6]. To determine the etiology and course of treatment, a cautious effusion assessment is therefore required. Despite being the least invasive and most effective approach available today for diagnosing cancer, pleural fluid cytology's narrow sensitivity range (40–87%) makes it unreliable in many cases [7]. Therefore, new biomarkers, such as cancer ratio, CEA, CA-125, and calprotectin, are being studied in order to improve the diagnosis of PE. This would increase the possibility of making an MPE diagnosis of unknown etiology to initiate early therapeutic interventions in an effort to reduce patient morbidity and mortality [2,8,9]

Recently, two studies were utilized pleural fluid ADA and serum LDH to investigate a novel biochemical marker. There have been three terms used: age-to-pleural fluid ADA ratio, cancer ratio plus (cancer ratio: pleural fluid lymphocyte count), and cancer ratio (serum LDH: pleural

fluid ADA [2,10].In addition to demonstrating a high cancer ratio, their research also revealed strong MPE sensitivity and specificity. Furthermore, another study for the tumor marker concentrations in pleural effusions in individuals who had a history of cancer or a high pre-test probability of malignancy. They concluded that tumor marker concentrations in pleural effusions might be interpreted in cases of inconclusive cytology [8].One study also demonstrated how MPE may be identified with the help of the biochemical properties of pleural fluid as well as clinical and radiographic symptoms [11].

Thus, using cancer ratio, cancer ratio plus, and additional tumor markers (CEA, CA-125, and Calprotectin) in pleural fluid analysis, we conducted a study to distinguish benign from malignant effusion in a sample of Indian population. So main objective of our study was to determine the level of Pleural fluid biomarkers (ADA, LDH, lymphocyte count, CEA, CA-125, Calprotectin) and find their association with benign and malignant pleural effusion.

Materials and Methods

This cross-sectional observational study was approved by the our institute (AIIMS Jodhpur) ethical committee before it started.All patients aged more than 18 years with exudative pleural effusions and giving consent were included in study. Patients showing frank pus and hemothorax on the diagnostic thoracentesis were excluded. Total 108 patients with pleural effusion were taken between March 2022 and September 2023, and after applying inclusion and exclusion criteria, total 83 patients were enrolled.

Every exudative pleural effusion patient had undergone a clinical evaluation. A thorough clinical history and routine clinical examination were performed. Along with RBS and coagulation measures like PT//INR, blood tests like CBC, LFT, KFT, and serum LDH were performed. Each patient underwent radiography, a CECT thorax if necessary, and a chest ultrasound. Under ultrasound guidance, a thoracentesis was performed, and 50 cc of pleural fluid was sent for a physical examination that included cytology, lactate dehydrogenase (LDH), glucose, ADA, and total proteins. The pleural effusion was examined for Gram stain, ZN stain, bacterial culture and CBNAAT. Pleural fluid sample (around 10ml) was centrifuged at 4000rpm for 8 minutes. The supernatant obtained from each sample was aliquoted into two Eppendrof tubes of 1.5ml each. One aliquot was used for the evaluation of CEA and CA-125 whereas the other aliquot was preserved at -80 degree celcius refrigerator for the evaluation of calprotectin by a quantitative ELISA test. The research included Age: Pl ADA ratio, Serum LDH: PL calprotectin ratio, cancer ratio plus (cancer ratio: Pl lymphocyte count), and cancer ratio (Sr

LDH: Pl ADA) values were computed. The patients' ultimate diagnosis would be correlated with the values of the cancer ratio, cancer ratio plus (CR Plus), age/Pl ADA ratio, and S.LDH:calprotectin ratio.

The diagnosis of tuberculous effusion was made on the basis on the presence of Mycobacterium tuberculosis in the pleural fluid, sputum, or pleural biopsy, as well as the presence of distinctive caseating granulomas in the pleural biopsy. A clinical diagnosis of tuberculous pleural effusion was also made in the absence of positive biopsy features in patients with exudative pleural effusion, pleural lymphocytic-to-neutrophil ratio > 0.75 and ADA>40U/L, positive clinical history of fever, weight loss, and/or suggestive radiological picture.

The diagnosis of malignant pleural effusion was made on the basis of positive pleural fluid cell cytology for malignant cells or USG/CT guided closed pleural biopsy, thoracoscopic confirmation of malignancy, or known cancer cases presenting with recurrent pleural effusion. Of the 40 malignant pleural effusions, diagnosis was confirmed by pleural fluid cytology (n=3), medical thoracoscopy (n=9), pleural biopsy (n=4), CT/USG-guided lung biopsy (n=13), endobronchial biopsy or EBUS-guided procedures (n=8), cervical lymph node biopsy (n=2), and liver biopsy (n=1). No case was classified as malignant pleural effusion based on clinical suspicion alone.

Statistical analysis

The percentage (%) and numerical formats were used to display the categorical variables. Conversely, the quantitative data were displayed as median with the 25th and 75th percentiles (interquartile range) and as means ± SD. The Shapiro-Wilk test was used to verify the normality of the data. In instances where the data was non-normal, non-parametric tests were employed. ROC curve was used to assess cut off point, sensitivity, specificity, positive predictive value and negative predictive .A p-value of less than 0.05 was deemed statistically significant in terms of significance.

Results

In our study, diagnosis of malignant pleural effusion was 40(48.19%). Thirty-two (38.55%) cases were tubercular effusion, 9 (10.84%) cases were parapneumonic effusion, and 2 (2.41%) cases were rheumatoid effusion. Significant differences were observed in the age distribution

between benign and malignant cases. The distribution of gender was comparable between benign and malignant cases (Table 1)

In terms of smoking status, non-smokers and ex-smokers were significantly more prevalent in benign cases compared to malignant cases. Conversely, smokers were significantly high frequent in malignant cases compared to benign cases. The commonest symptom seen in both group were Loss of weight followed by Chest pain, cough and Shortness of breath. These findings highlight distinct demographic and clinical characteristics between benign and malignant cases. In term of comorbidities, most of the patients in both groups were noncomorbid. Eight patients in benign group and 7 patients in malignant group were found diabetes (Table 1)

There were no significant differences observed in various laboratory investigations, including Protein (g/dL) (p value=0.071), Albumin (g/dL) (p value=0.81), Sugar (mg/dL) (p value=0.103), and Cholesterol (g/dL) (p value=0.344), between benign and malignant cases. Similarly, no significant difference was observed in LDH (IU/L) (p value=0.093) between benign and malignant cases. The median (25th-75th percentile) of LDH in benign cases was 291 (188.5-578.5), and in malignant cases, it was 456 (241-919.5), with no significant difference between the groups.

However, a notable difference was observed in lymphocyte count (%) between benign and malignant cases ($p < 0.05$). The median (25th-75th percentile) of lymphocyte count (%) in benign cases was 90 (80-95), which was significantly higher compared to malignant cases [79(70-90)] (p=0.041). These findings highlight specific variations in lymphocyte count between benign and malignant conditions, while other investigated parameters did not show significant distinctions.

A substantial disparity was evident in various pleural fluid biomarkers between benign and malignant cases, revealing significant differences (p value < 0.05). The median (25th-75th percentile) and mean of pleural fluid biomarkers, including PL.ADA (IU/L) and PL. Calprotectin (ng/mL) was markedly elevated in benign cases at 51 (33.7-68.5) and 459(395-567), respectively compared to malignant cases (PL.ADA: 21.5 (15-28.025), p value < 0.0001; PL. Calprotectin: 215.95(168.612-295.16), p value < 0.0001).

Conversely, in malignant cases, the median (25th-75th percentile) of pleural fluid biomarkers such as serum LDH (IU/L), PL. CEA (ng/mL), PL. CA -125 (U/mL), cancer ratio, Age/ADA, and S.LDH/PL. Calprotectin was significantly higher than in benign cases In CR plus, there is no revealing significant difference found between benign and malignant pleural effusion(p

value=0.486)These findings underscore the diagnostic significance of these biomarkers in differentiating between benign and malignant pleural conditions (Table 2)

In prediction of malignancy, Age/ADA had highest sensitivity followed by PL.CEA and cancer ratio. PL. CA-125 had lowest sensitivity. On the other hand, PL. CEA had highest specificity followed by cancer ratio, S.LDH/PL. Calprotectin, PL. Calprotectin. Age/ADA had lowest specificity. Highest positive predictive value was found in PL.CEA (97.40%) and highest negative predictive value was found in Age/ADA (96.8%) (*Supplementary Table 1* and Figure 1).

Statistically significant difference between tubercular and malignant pleural effusion was observed in various pleural fluid biomarkers, including serum LDH (IU/L), PL.ADA (IU/L), PL.CEA (ng/mL), PL.CA-125 (U/mL), cancer ratio, CR Plus, PL.Calprotectin (ng/mL), Age/ADA, and S.LDH/PL. Calprotectin (p-value < 0.05). In tubercular effusion, the median (25th-75th percentile) values of PL.ADA and PL.Calprotectin were significantly higher compared to malignant cases. Conversely, in malignant cases, the median values of serum LDH (IU/L), PL. CEA (ng/mL), PL. CA-125 (U/mL), cancer ratio, CR Plus, Age/ADA, and S.LDH/PL. Calprotectin were significantly higher compared to tubercular effusion ($p < 0.0001$). These findings emphasize the significant differences in these biomarkers between tubercular effusion and malignant cases, underscoring their potential diagnostic and prognostic utility in distinguishing between these conditions (Table 3).

The CR Plus had best sensitivity of 97.5% for prediction of malignancy and PL.CA-125(U/mL) had lowest sensitivity of 57.50%. On the other hand, Cancer ratio, CR plus and PL.CEA (ng/mL) had 100% specificity for prediction of malignancy from tubercular effusion. PL calprotectin had lowest specificity of 84.6%. Among all the parameters, CR Plus was the best predictor of malignancy at cut off point of 10.21 with area under curve of 0.997 for correctly predicting malignancy from tubercular effusion (*Supplementary Table 2*, *Supplementary Figure 1* and Figures 2).

Discussion

Pleural effusions consequently present with a wide range of differential diagnoses. The first step is to distinguish transudative from exudative, which reduces the range of possible diagnoses. Pleural inflammation is the most prevalent cause of exudative effusions. Infections are the most frequent cause, followed by cancer.

On the other hand, it takes time to diagnose malignant effusion. People's life expectancy is lowered as a result. A trustworthy biomarker to detect malignant effusion does not exist. Therefore, in order to distinguish between benign and malignant pleural effusions, the following parameters are being assessed: age/ADA ratio, calprotectin/LDH ratio, pleural fluid (PL) CEA, PL CA-125, and PL Calprotectin.

There were no significant differences observed in pleural fluid investigations, including Protein, Albumin, Sugar, LDH and Cholesterol, between benign and malignant cases. However, a notable difference was observed in lymphocyte count (%) between benign and malignant cases (p value < 0.05). The median (25th-75th percentile) of lymphocyte count (%) in benign cases was 90 (80-95), which was significantly higher compared to malignant cases (79 (70-90)) (p value=0.041). In previous study which showed same result with no significant difference of pleural fluid protein, albumin, sugar and LDH in malignant and benign pleural effusion [11]. In other study showed high pleural cholesterol level seen in malignant effusion [12], but in our study did not show any significance difference. Other studies also showed that median lymphocyte count percentage was higher in TPE than MPE [2,13,14]. In our study, serum LDH showed significant difference between benign and malignant cases (p value=<0.001).This correlates with the values in the study done by Akash Verma [2].

The pleural fluid (PL) biomarker ADA, CEA, CA-125, Cancer ratio, Calprotectin, Age/ADA and Calprotectin ratio showed statistically significant (p value-<0.0001) difference between benign and malignant pleural effusion, but CR plus did not reveal any statistically significant difference (p value=0.486).This is might be due to low lymphocyte counts in parapneumonic effusion of benign cases.

Statistically significant difference was observed in various pleural fluid biomarkers, including PL. ADA (IU/L), PL. CEA (ng/mL), PL. CA-125 (U/mL), cancer ratio, CR Plus, PL. Calprotectin (ng/mL), Age/ADA, and Calprotectin ratio in between tubercular effusion and malignant cases ($p < 0.05$). Similar result showed by different studies for PL. ADA, Cancer ratio and cancer ratio [2]. The ADA levels of our study correlates with the ADA levels of other studies [4,10,15]. Statistically significant differences ($p < 0.001$) were observed in PL CEA and PL CA-125 levels, which effectively differentiated between benign and malignant pleural effusions [8]. Similarly, the Age/ADA ratio showed consistent results ($p < 0.001$) in distinguishing benign from malignant effusion [10]. Furthermore, PL calprotectin levels demonstrated significant differences in two separate studies ($p < 0.001$), aiding in the differentiation between benign and malignant pleural effusions [16,17].

Interpretation of the area under the ROC curve showed that discriminatory power of PL. ADA(IU/L) , PL. CEA (ng/mL), cancer ratio, Age/ADA and S.LDH/PL. Calprotectin was outstanding for predicting malignancy. Discriminatory power of serum LDH (IU/L) and PL. Calprotectin (ng/mL) was excellent and discriminatory power of PL. CA-125(U/mL) was acceptable.

Among all the parameters, PL. CEA (ng/mL) was the best predictor of malignancy at cut off point of >1.96 with area under curve of 0.984 for correctly predicting malignancy from benign effusion.

One previous study showed PL ADA had 97.3% of sensitivity and 68.2% of specificity at cut off point of 29.6 IU/L for predicting malignancy from tubercular effusion [10]. Similar result was found in our study with 92.5 % of sensitivity and 79.07% of specificity at cut off point of 30.6IU/L for predicting malignancy from benign effusion and 92.5% and 93.7% of sensitivity and specificity at cut off point of 30.75 IU/l for predicting malignancy from tubercular effusion, respectively.

The CEA provided the greatest diagnostic specificity for malignant effusion (97.5%) at a cut-off point of 5.2 ng/mL with low sensitivity (65.09%) for predicting malignancy from tubercular effusion [8]. While in our study CEA provided high sensitivity(95%) and highest specificity(97.67%) at cut off point of 1.96 ng/ml for predicting malignancy from benign effusion and 95% and 99.50% of sensitivity and specificity at cut off point of 1.99 ng/ml for predicting malignancy from tubercular effusion, respectively. In the same study [8], at a cut-off point of 345.65 U/mL, PL CA-125 provided a diagnostic sensitivity of 68.1% and specificity of 83.5% for predicting malignancy from tubercular effusion. While in our study, cut- off point was 842 U/ml with low sensitivity (50%) and high specificity (83.72%) for predicting malignancy from benign effusion and 57.5% and 88.10% of sensitivity and specificity at cut off point of 670 u/ml for predicting malignancy from tubercular effusion, respectively.

In the referenced study, the analysis of cancer ratio showed 97% and 95% sensitivity, and 26% and 85% specificity at cut-off points of 10 and 20, respectively, for differentiating between malignant and tubercular effusions[2]. In our study, we achieved 95% sensitivity and specificity at a cut-off point of 9.09 for distinguishing malignancy from benign effusion, and at a cut-off point of 9.21 for distinguishing malignancy from tubercular effusion.

An interesting finding in our study was that Cancer Ratio Plus did not demonstrate a statistically significant difference between the overall benign and malignant pleural effusion groups. However, when a separate analysis was performed between tubercular and malignant pleural

effusions, Cancer Ratio Plus showed excellent diagnostic performance. This finding is likely explained by the heterogeneity of the benign group, which included nine cases of parapneumonic effusion. Unlike tubercular pleural effusion, parapneumonic effusion is often associated with lower pleural fluid lymphocyte counts. Since Cancer Ratio Plus incorporates pleural fluid lymphocyte percentage in its calculation, inclusion of parapneumonic effusions may have reduced its discriminatory power in the overall benign-versus-malignant comparison. Therefore, Cancer Ratio Plus appears to be more useful for differentiating malignant pleural effusion from tubercular pleural effusion than from benign pleural effusions as a whole. In the cited study, CR plus was identified as a promising biomarker for distinguishing malignant effusions from benign cases [2]. A possible explanation is the difference in study populations. The benign group in the study by Verma et al. predominantly consisted of tuberculous pleural effusions, whereas our benign cohort was more heterogeneous and included parapneumonic and rheumatoid pleural effusions in addition to tuberculous effusions [2].

In a particular study [10], the Age/ADA ratio demonstrated 93.2% sensitivity and 71.2% specificity in predicting malignancy from tubercular effusion, with a cut-off point of 2.62. Our study yielded comparable findings, with cut-off points of 1.56 and 1.85 for predicting malignancy from benign effusion and from tubercular effusion, respectively.

In previous studies using ELISA methods, demonstrated that calprotectin is a useful diagnostic biomarker in the diagnosis of malignant pleural effusion. The levels of the Calprotectin in pleural fluid in our study was carried out by the Krishgen Human Calprotectin GENLISA™ ELISA kit , in which two monoclonal antibodies are used instead of one monoclonal antibody which is usually used in other ELISA kits. Presence of double antibodies and sandwich technique leads to better sensitivity and specificity. Our study cut off corroborated well with two studies in which SEK504Hu ELISA kit (USCN Life Science Inc. Wu Han) and sandwich ELISA kit (Hycult Biotechnology Uden, The Netherlands) were used [16,17,18]. We believe that use of different ELISA kits and different antibodies used can lead to different Calprotectin levels and cut offs.

The cut-off value for Calprotectin levels in our study was ≤303 ng/ml, yielding an AUC of 0.87 in distinguishing malignancy from benign effusion. For predicting malignancy from tubercular effusion, the cut-off was 352.72 ng/ml, also achieving an AUC of 0.87. In two additional studies [9,18], cut-off values of <500.19 ng/ml and <730.5 ng/ml for Calprotectin were associated with AUCs of 0.6 and 0.9, respectively.

It is not a unique concept to combine biomarkers to increase test accuracy for pleural effusion diagnosis. So new biomarker S.LDH/Pl Calprotectin ratio was introduced as malignant effusion showed high serum LDH and low PL calprotectin. In our study, this ratio showed 87.5% sensitivity and 90.7% specificity at cut-off point of 0.73 for predicting malignancy from benign effusion and at cut off point of 0.75, sensitivity and specificity were 87.5% and 93.7% for predicting malignancy from tubercular effusion, respectively.

Among all the parameters, PL. CEA was the best predictor of malignancy for correctly predicting malignancy.

Hence overall in our study PL. CEA was best predictor of malignancy at cut off point of >1.96 with area under curve of 0.984 for correctly predicting malignancy from benign effusion, followed by cancer ratio and LDH/calprotectin ratio. While CR Plus was the best predictor of malignancy at cut off point of 10.21 with area under curve of 0.997 for correctly predicting malignancy from tubercular effusion followed by cancer ratio and PL CEA.

From a clinical perspective, pleural fluid CEA demonstrated the highest overall diagnostic performance and may be particularly useful for supporting a diagnosis of malignant pleural effusion. Cancer Ratio also showed excellent diagnostic accuracy and has the advantage of utilizing routinely available laboratory parameters, making it a simple and cost-effective biomarker. Age/ADA demonstrated high sensitivity and may be useful as an initial screening tool. Further studies are required to determine whether combining multiple biomarkers can improve diagnostic accuracy beyond that achieved by individual markers.

Limitations

The present study has several limitations. First, it was a single-center observational study with a relatively small sample size, which may limit the statistical power and generalizability of the findings. Second, tubercular pleural effusion constituted the majority of benign pleural effusions, which may have influenced the diagnostic performance of the evaluated biomarkers and limits the applicability of the findings to other benign etiologies. The benign group also included a small number of parapneumonic and rheumatoid effusions, and the inclusion of parapneumonic effusions with lower pleural fluid lymphocyte counts may have affected the performance of Cancer Ratio Plus in the overall benign-versus-malignant comparison. Third, some patients with known primary malignancy and pleural effusion were not subjected to pleural biopsy for histopathological confirmation of pleural involvement. Although alternative causes of pleural effusion were excluded through comprehensive clinical, radiological, and

pathological evaluation, the possibility of including a small number of paramalignant effusions cannot be completely excluded. Fourth, ROC-derived cutoff values and diagnostic performance measures were generated and evaluated within the same study cohort without external validation, which may have resulted in optimistic estimates of diagnostic accuracy. Finally, multivariable logistic regression analysis was not performed; therefore, the independent predictive value of individual biomarkers after adjustment for correlated variables could not be assessed. Larger multicentric studies including a broader spectrum of benign pleural diseases, external validation cohorts, and multivariable predictive modelling are required to confirm and extend the present findings.

Conclusions

Pleural fluid biomarkers (cancer ratio, CEA, Age/ADA ratio, calprotectin, and serum LDH/calprotectin ratio) are reliable indicators of malignancy in exudative pleural effusion. The best indicators of malignant pleural effusion were Pleural CEA, Cancer ratio, and serum LDH/calprotectin ratio Thus, patients with exudative lymphocytic with unexplained pleural effusion can be addressed early using inexpensive and generally available biochemical indicators, such as the pleural fluid CEA, cancer ratio, and Age/ADA.

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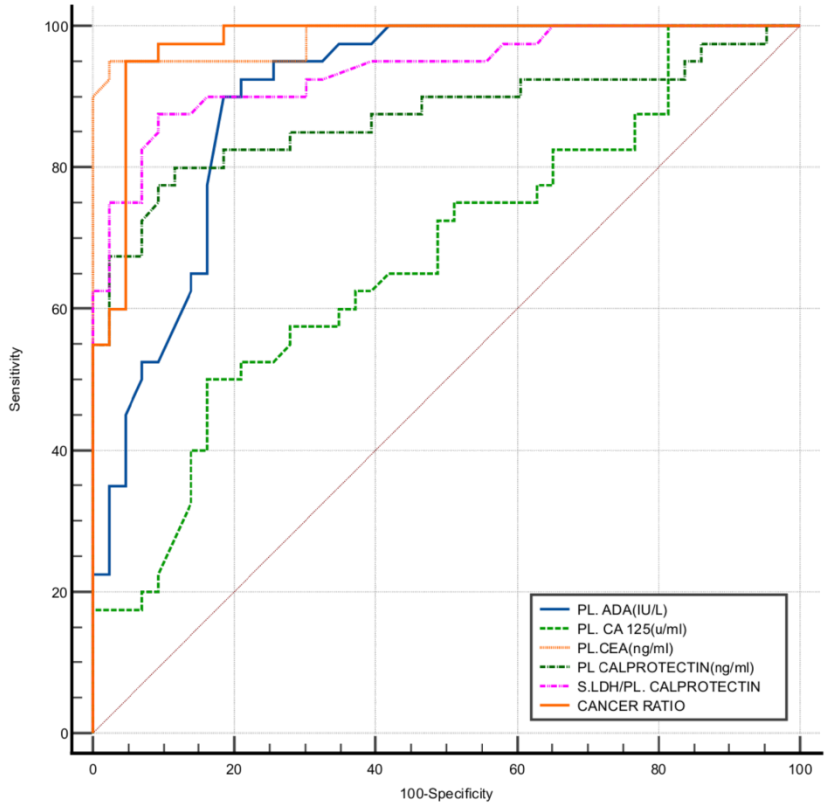

Figure 1. ROC curve of all biomarkers for predicting malignancy.

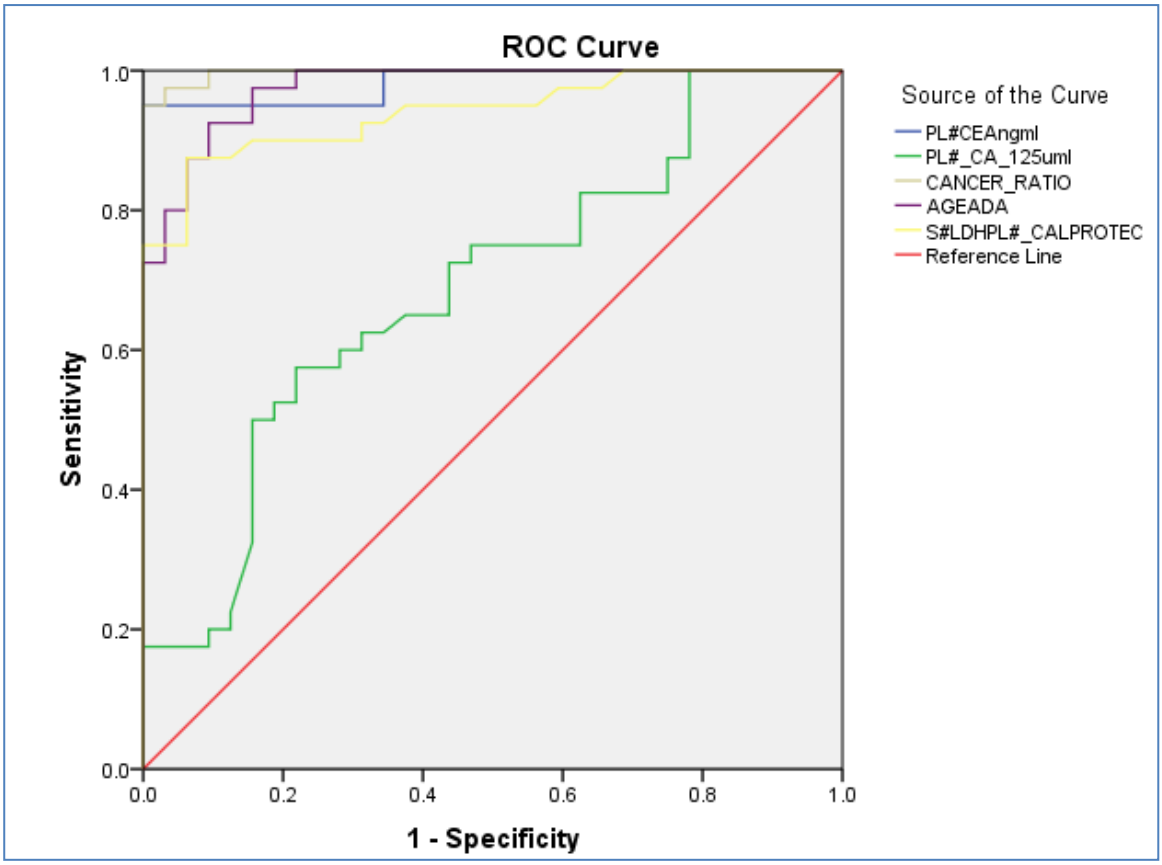

Figure 2. ROC curve of different biomarkers for predicting malignancy from tubercular effusion.

Table 1. Comparison of demographic characteristics between benign and malignant.

Demographic characteristics	Benign(n=43)	Malignant(n=40)	Total	p
Age(years)				
18-30	8 (18.60%)	0 (0%)	8 (9.64%)	0.009*
31-40	9 (20.93%)	4 (10%)	13 (15.66%)	
41-50	4 (9.30%)	5 (12.50%)	9 (10.84%)	
51-60	9 (20.93%)	6 (15%)	15 (18.07%)	
61-70	7 (16.28%)	15 (37.50%)	22 (26.51%)	
>70	6 (13.95%)	10 (25%)	16 (19.28%)	
Mean ± SD	49.05 ± 18.2	61.82 ± 12.82	55.2 ± 17	0.0004‡
Median(25th-75th percentile)	52(34.5-63.5)	64(54.75-70.25)	59(42-69)	
Range	18-78	32-87	18-87	
Gender				
Female	9 (20.93%)	10 (25%)	19 (22.89%)	0.659†
Male	34 (79.07%)	30 (75%)	64 (77.11%)	
Smokers				
Smoker	10 (23.26%)	22 (55%)	32 (38.55%)	0.01*
Non-smoker	27 (62.79%)	14 (35%)	41 (49.40%)	
Ex-smoker	6 (13.95%)	4 (10%)	10 (12.05%)	
Comorbidities				
Diabetes	8(18.60%)	7(17.5%)	15(18.07%)	>0.05*
hypertension	3(6.97%)	4(10%)	7(8.43%)	
COPD	2(4.65%)	3(7.50%)	5(6.02%)	
CAD	4(9.30%)	3(7.50%)	7(8.43%)	
CKD	2(4.65%)	1(2.5%)	3(3.61%)	
None	24(55.81%)	22(55%)	46(55.42%)	

‡Independent t test, * Fisher's exact test, † Chi square test

Table 2. Comparison of pleural fluid biomarkers between benign and malignant.

Pleural fluid biomarkers	Benign(n=43)	Malignant(n=40)	Total	p
Serum LDH(IU/L)				
Mean ± SD	218.88 ± 73.36	377.65 ± 176.08	295.4 ± 154.51	<.0001[§]
Median (25th-75th percentile)	198(172-238.5)	346(278-423.25)	250(192.5-369.5)	
Range	100-426	166-1088	100-1088	
PL. ADA(IU/L)				
Mean ± SD	51.44 ± 22.1	21.7 ± 8.67	37.1 ± 22.57	<.0001[§]
Median(25th-75th percentile)	51(33.7-68.5)	21.5(15-28.025)	29(21-53.6)	
Range	14-124	6.6-45	6.6-124	
PL. CEA(ng/mL)				
Mean ± SD	0.95 ± 0.5	4.21 ± 2.05	2.52 ± 2.19	<.0001[§]
Median(25th-75th percentile)	1(0.494-1.28)	3.71(2.625-5.5)	1.6(0.99-3.54)	
Range	0.2-2.22	1.21-9.66	0.2-9.66	
PL. CA 125(U/mL)				
Mean ± SD	482.5 ± 418.53	1564.57 ± 2667.56	1003.98 ± 1941.65	0.007[§]
Median(25th-75th percentile)	342(153.5-728.5)	822(294-1000)	539(167-974.5)	
Range	15-1590	92.9-13400	15-13400	
Cancer ratio				
Mean ± SD	5.07 ± 2.74	19.86 ± 9.86	12.2 ± 10.27	<.0001[§]
Median(25th-75th percentile)	4.32(2.97-6.66)	15.27(12.048-28.25)	8.8(4.29-15)	
Range	1.89-14.21	6.82-42.39	1.89-42.39	
CR Plus				
Mean ± SD	21.87 ± 23.53	29.17 ± 23.84	20.21 ± 25.09	0.486
Median(25th-75th percentile)	14.73(13.4-18.155)	21.9(16.782-31.265)	13.3(4.715-25.185)	
Range	1.98-141	7.17-146	1.98-146	
PL. Calprotectin(ng/mL)				
Mean ± SD	488.94 ± 153.62	267.18 ± 162.92	382.07 ± 192.72	<.0001[§]
Median(25th-75th percentile)	459(395-567)	215.95(168.612-295.16)	382(225.755-507.815)	
Range	235.69-811.78	55-784	55-811.78	
Age/ADA				
Mean ± SD	1.28 ± 0.95	3.6 ± 1.97	2.4 ± 1.92	<.0001[§]
Median(25th-75th percentile)	0.88(0.575-1.73)	3.01(2.17-4.165)	2.08(0.88-3.255)	
Range	0.15-4.42	1.48-10	0.15-10	
S.LDH/PL. Calprotectin				
Mean ± SD	0.49 ± 0.25	1.9 ± 1.44	1.17 ± 1.23	<.0001[§]
Median(25th-75th percentile)	0.47(0.31-0.6)	1.67(1.05-2.102)	0.65(0.425-1.625)	
Range	0.16-1.41	0.35-8.29	0.16-8.29	

[§]Mann-Whitney test

Table 3. Comparison of pleural fluid biomarkers between tubercular effusion and malignant.

Pleural fluid biomarkers	Tubercular effusion (n=32)	Malignant(n=40)	Total	p
Serum LDH(IU/L)				
Mean ± SD	222.03 ± 69.49	377.65 ± 176.08	308.49 ± 158.76	<.0001§
Median(25th-75th percentile)	197(180.5-236.75)	346(278-423.25)	272(194-376.75)	
Range	140-411	166-1088	140-1088	
PL. ADA(IU/L)				
Mean ± SD	58.04 ± 20.33	21.7 ± 8.67	37.85 ± 23.5	<.0001§
Median(25th-75th percentile)	60.95(44.5-70.475)	21.5(15-28.025)	29(20.75-56.325)	
Range	21-124	6.6-45	6.6-124	
PL. CEA(ng/mL)				
Mean ± SD	1.01 ± 0.46	4.21 ± 2.05	2.79 ± 2.23	<.0001§
Median(25th-75th percentile)	1.13(0.635-1.312)	3.71(2.625-5.5)	2.3(1.178-3.845)	
Range	0.24-1.96	1.21-9.66	0.24-9.66	
PL. CA 125(U/mL)				
Mean ± SD	465.89 ± 444.3	1564.57 ± 2667.56	1076.27 ± 2072.96	0.007§
Median(25th-75th percentile)	297.5(148-638.5)	822(294-1000)	528(171-999.25)	
Range	15-1590	92.9-13400	15-13400	
Cancer ratio				
Mean ± SD	4.2 ± 1.72	19.86 ± 9.86	12.9 ± 10.78	<.0001§
Median(25th-75th percentile)	3.83(2.8-5.142)	15.27(12.048-28.25)	9.85(4.145-17.25)	
Range	1.89-9.09	6.82-42.39	1.89-42.39	
CR Plus				
Mean ± SD	4.66 ± 1.84	29.17 ± 23.84	18.28 ± 21.54	<.0001§
Median(25th-75th percentile)	4.23(3.248-5.925)	21.9(16.782-31.265)	12.84(4.502-22.742)	
Range	1.98-9.57	7.17-146	1.98-146	
PL. Calprotectin(ng/mL)				
Mean ± SD	496.75 ± 157.55	267.18 ± 162.92	369.21 ± 196.51	<.0001§
Median(25th-75th percentile)	460.4(405.75-572.31)	215.95(168.612-295.16)	307.47(210.802-470.285)	
Range	235.69-811.78	55-784	55-811.78	
Age/ADA				
Mean ± SD	0.94 ± 0.57	3.6 ± 1.97	2.42 ± 2.01	<.0001§
Median(25th-75th percentile)	0.78(0.473-1.434)	3.01(2.17-4.165)	1.92(0.872-3.308)	
Range	0.15-2.31	1.48-10	0.15-10	
S.LDH/PL. Calprotectin				
Mean ± SD	0.48 ± 0.2	1.9 ± 1.44	1.27 ± 1.29	<.0001§
Median(25th-75th percentile)	0.45(0.338-0.6)	1.67(1.05-2.102)	0.8(0.467-1.758)	
Range	0.16-1.02	0.35-8.29	0.16-8.29	

§Mann-Whitney test

Online supplementary material

Supplementary Table 1. Receiver operating characteristic curve analysis of serum lactate dehydrogenase (IU/L), pleural fluid adenosine deaminase (IU/L), pleural fluid carcinoembryonic antigen (ng/mL), pleural fluid cancer antigen 125 (U/mL), Cancer Ratio Plus, pleural fluid calprotectin (ng/mL), age-to-adenosine deaminase ratio, and serum lactate dehydrogenase-to-pleural fluid calprotectin ratio for predicting malignant pleural effusion.

Supplementary Table 2. Receiver operating characteristic curve analysis of different parameters for differentiating malignant pleural effusion from tuberculous pleural effusion.

Supplementary Figure 1. Receiver operating characteristic curve of pleural fluid adenosine deaminase and pleural fluid calprotectin for differentiating malignant pleural effusion from tuberculous pleural effusion.