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Combined Biomarkers and Their Serum-to-Pleural Fluid Ratios for Differentiating Multiple Types of Pleural Effusion: A Single-Centered Study

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ABSTRACT

Background: Pleural effusion is a common clinical manifestation arising from diverse etiologies, including tuberculosis, malignancy, parapneumonic infection, and connective tissue disorders. Accurate differentiation between these types is essential for timely and targeted treatment but remains challenging due to overlapping clinical, radiological, and biochemical features. Conventional diagnostic methods, including microbiological assays and cytology, have limited sensitivity, while invasive procedures, although more accurate, are not always feasible. Biomarker-based approaches have emerged as promising noninvasive tools, but most studies have evaluated them in isolation rather than as combined diagnostic strategies. Objective: This study aimed to evaluate the diagnostic performance of a combined panel of serum and pleural fluid biomarkers and their ratios in distinguishing between tuberculous, malignant, complicated parapneumonic, uncomplicated parapneumonic, and connective tissue disease–related pleural effusions. Methods: A cross-sectional study was conducted over six months at Ayub Teaching Hospital, Abbottabad, involving 163 patients with confirmed pleural effusion. Serum and pleural fluid samples were analyzed for adenosine deaminase (ADA), lactate dehydrogenase (LDH), C-reactive protein (CRP), total protein, glucose, albumin, white blood cell count, pH, and pleural fluid–to–serum ratios. Statistical analysis included the Kruskal–Wallis H-test, Mann–Whitney U test with Bonferroni correction, and receiver operating characteristic (ROC) curve analysis. Results: Tuberculous effusion was most common (79.8%), followed by malignant (12.3%), complicated parapneumonic (4.3%), uncomplicated parapneumonic (2.5%), and connective tissue disease–related (1.2%). Pleural fluid ADA was significantly higher in tuberculous effusion (median 73.9 U/L, $p < 0.00001$), whereas LDH and CRP were markedly elevated in malignant and complicated parapneumonic effusions (median LDH > 1300 U/L, CRP > 11.0 mg/dL). PF/serum ADA and LDH ratios further improved discriminatory accuracy, and pH < 7.20 strongly indicated complicated parapneumonic effusion. Combined biomarker analysis demonstrated superior diagnostic performance (AUC up to 0.94) compared to individual markers. Conclusion: A multi-biomarker approach integrating ADA, LDH, CRP, pH, and pleural fluid–to–serum ratios significantly improves the etiological classification of pleural effusion. This strategy enhances diagnostic accuracy, reduces the need for invasive procedures, and supports timely, targeted management.

Keywords
Pleural effusion, adenosine deaminase, lactate dehydrogenase, C-reactive protein, biomarkers, differential diagnosis, parapneumonic effusion, tuberculosis.

INTRODUCTION

Pleural effusion is a common clinical entity encompassing diverse etiologies—most prominently tuberculous, malignant, and parapneumonic causes—with connective tissue disease–related effusions contributing a smaller yet clinically relevant proportion (1). While Light's classic framework and subsequent clinical refinements have standardized the transudate–exudate distinction, accurate etiologic classification at first presentation remains difficult because radiographic features and routine chemistry often overlap across disease categories (2). This diagnostic ambiguity carries consequences for timely therapy, invasive procedure selection, and resource utilization in high-burden settings (1).

In regions with substantial tuberculosis prevalence, tuberculous pleural effusion is a leading exudative phenotype, whereas malignant effusion remains a major cause of cancer-related morbidity and parapneumonic effusions arise frequently alongside community-acquired pneumonia; connective tissue disorders add additional complexity to the differential (3,4). Epidemiologic data further underscore the magnitude of the problem: extrapulmonary tuberculosis accounts for a sizeable minority of tuberculosis presentations and a notable proportion of these involve the pleura; globally, malignant effusion affects large populations and parapneumonic effusions are common in routine medical practice (5-9). Local data from Pakistan similarly show exudative effusions predominating with tuberculosis and parapneumonic etiologies most frequent, malignancy less

common, and connective tissue disorders infrequent but present, highlighting the need for pragmatic diagnostic strategies tailored to this context (10).

Microbiological confirmation from pleural fluid in tuberculous disease is notoriously insensitive, and while pleural biopsy increases yield, it is invasive and not universally feasible; cytology for malignant effusion has imperfect sensitivity in routine practice, and thoracoscopic biopsy—though accurate—is resource intensive (11). Consequently, clinicians rely on biomarker-informed algorithms to complement clinical judgment. The transudate–exudate paradigm leverages lactate dehydrogenase (LDH) ratios, but these indices alone do not reliably separate tuberculous, malignant, and parapneumonic subsets once exudation is established (13). Adenosine deaminase (ADA) is widely adopted for suspected tuberculous effusion, yet its specificity diminishes where inflammatory parapneumonic processes are prevalent, and its performance varies with pretest probability and laboratory thresholds (14). To improve discrimination, composite or ratio-based approaches—such as LDH/ADA or pairing ADA with inflammatory markers—have been proposed to better contextualize single-marker elevations within disease-specific biochemical patterns (15- 18). Emerging evidence suggests that integrating pleural fluid ADA with LDH, C-reactive protein (CRP), pH, and corresponding pleural fluid–to–serum ratios can enhance differentiation among tuberculous, malignant, and parapneumonic phenotypes compared with solitary markers, with decision-tree or ratio-guided models showing promise across heterogeneous cohorts (19). Notably, LDH/ADA thresholds have been evaluated to separate tuberculous from parapneumonic effusions, tumor marker panels may complement LDH to raise suspicion for malignancy when cytology is negative, and pleural CRP and pH align with inflammatory severity in complicated parapneumonic disease (20-22). Additional combinations—such as ADA indexed to systemic inflammatory burden—have been explored to mitigate false-positive ADA elevations in non-tuberculous inflammation, and scoring models continue to evolve toward pragmatic bedside applicability (23,24). Despite these advances, few studies from high-tuberculosis, resource-constrained South Asian settings have comprehensively evaluated a unified, multi-biomarker panel with serum–pleural ratios across all major effusion categories, creating a practical evidence gap for real-world algorithms that minimize invasive procedures without sacrificing diagnostic certainty (10,14,19).

Within this context, the present study evaluates a focused panel—pleural fluid ADA, LDH, CRP, pH, and key pleural fluid–to–serum ratios—to differentiate tuberculous, malignant, complicated and uncomplicated parapneumonic, and connective tissue disease–related effusions in a single-center cohort. The objective is to quantify the discriminatory performance of individual markers and ratios and to propose a parsimonious, clinically implementable diagnostic strategy for first-line categorization of exudative pleural effusions in a high-tuberculosis prevalence setting (19-4). The research question is: among adults presenting with pleural effusion, does a multi-biomarker approach incorporating pleural fluid ADA, LDH, CRP, pH, and pleural fluid–to–serum ratios improve etiologic discrimination between tuberculous, malignant, parapneumonic (complicated and uncomplicated), and connective tissue disease–related effusions compared with reliance on single biomarkers alone (19-24).

MATERIAL AND METHODS

This study was designed as a cross-sectional observational investigation aimed at evaluating the diagnostic performance of a combined biomarker panel in distinguishing between the principal etiological categories of pleural effusion. It was conducted in the Department of Pulmonology and Emergency Department of Ayub Teaching Hospital, Abbottabad, over a six-month period. The setting was selected due to its large catchment area and diverse case mix, ensuring inclusion of a representative spectrum of pleural effusion types in a high-tuberculosis prevalence region. The study adhered to standardized reporting and methodological practices to ensure reproducibility and external validity.

Adult patients aged 18 to 60 years presenting with radiologically confirmed pleural effusion were screened for eligibility. Inclusion criteria required fulfillment of the operational definition of pleural effusion, characterized by blunting of the costophrenic and costocardiac angles on posteroanterior chest radiography. Patients were excluded if pleural effusion was attributable to pulmonary embolism, left ventricular dysfunction, chronic kidney disease, or chronic liver disease, to minimize confounding from systemic causes unrelated to the biomarker profiles under investigation. Consecutive non-probability sampling was employed to recruit participants as they presented, reducing selection bias and approximating the underlying disease prevalence in the study population. Written informed consent was obtained from all participants after a detailed explanation of study objectives, procedures, and potential risks and benefits.

Upon enrollment, demographic variables (age, sex, residence, ethnicity, socioeconomic status) and relevant clinical data were recorded using a pre-structured proforma. Patients were classified into one of five etiological categories: tuberculous pleural effusion (TPE), malignant pleural effusion (MPE), complicated parapneumonic effusion (CPPE), uncomplicated parapneumonic effusion (UPPE), or connective tissue disease–related pleural effusion (CTD-PE), based on established diagnostic criteria. TPE was defined by the presence of caseating granulomas on pleural biopsy and/or culture positivity for Mycobacterium tuberculosis in pleural fluid or tissue, with corroborating clinical and radiological response to anti-tuberculosis therapy (11). MPE diagnosis was based on positive cytology or histopathology for malignant cells. PPE was defined as exudative effusion associated with bacterial pneumonia, lung abscess, or bronchiectasis without microbiological or histological evidence of tuberculosis; CPPE was differentiated from UPPE based on the requirement for invasive drainage. CTD-PE was confirmed by compatible histopathology or serology after excluding alternative causes.

Blood and pleural fluid samples were collected simultaneously before initiating disease-specific therapy. Serum biomarkers included white blood cell (WBC) count, C-reactive protein (CRP), albumin, lactate dehydrogenase (LDH), glucose, and adenosine deaminase (ADA). Pleural fluid assays measured total protein, glucose, ADA, albumin, LDH, total cell count, and pH. Laboratory analyses employed validated instruments: Coulter DxH800 for WBC count, LABOSPECT 008 for CRP, total protein, glucose, ADA, albumin, and LDH, AU5800 for serum albumin and LDH, Neubauer counting chamber for pleural fluid cell counts, and calibrated pH paper for pleural pH. Samples with missing ADA measurements were excluded from ADA-related analyses to prevent bias in diagnostic accuracy estimates. All assays were performed under standardized quality-controlled laboratory protocols to ensure reproducibility and minimize measurement error. The sample size of 163 was calculated using the WHO sample size calculator, assuming a prevalence of malignant pleural effusion of 12%, a 95% confidence level, and a 5% margin of error (10). This sample size provided adequate power to detect clinically meaningful differences in biomarker distributions across effusion types. To reduce misclassification bias, diagnostic categorization was performed by a blinded multidisciplinary panel using standardized criteria. Confounding was addressed through strict exclusion criteria and stratified analyses of biomarkers against demographic and clinical covariates.

Statistical analyses were conducted using SPSS version 21 (IBM Corp., Armonk, NY, USA). Data distribution was assessed with the Shapiro–Wilk test. Continuous variables were summarized as mean ± standard deviation or median with interquartile range, depending on normality, while

categorical variables were expressed as frequencies and percentages. Biomarker levels across the five effusion categories were compared using the Kruskal–Wallis H-test, with post-hoc pairwise Mann–Whitney U tests and Bonferroni correction. Categorical associations were examined with the chi-square or Fisher's exact test as appropriate. Correlation between biomarkers and their pleural fluid-to-serum ratios was assessed using Pearson correlation coefficients. A two-sided p-value of <0.05 was considered statistically significant. No imputation was applied for missing data, as complete case analysis was used. Ethical approval for the study was obtained from the Institutional Review Board (Ref. No. RC-EA2024/2293) and from the College of Physicians and Surgeons Pakistan. All procedures adhered to the principles of the Declaration of Helsinki. Data confidentiality was maintained through anonymization and secure storage, and all analyses followed a pre-specified protocol to support reproducibility and facilitate future meta-analyses.

RESULTS

The demographic and clinical profile of the study population (Table 1) shows that out of 163 patients with pleural effusion, males constituted the majority at 60.7% (n = 99), while females accounted for 39.3% (n = 64). Most participants belonged to the middle socioeconomic class (58.3%, n = 95), followed by the rich (23.9%, n = 39) and poor (17.8%, n = 29) categories. The majority of patients resided in rural areas (63.2%, n = 103), indicating potential disparities in healthcare access and exposure to infectious diseases such as tuberculosis. Tuberculous pleural effusion (TPE) was by far the most common etiology, observed in 79.8% (n = 130) of cases. Malignant pleural effusion (MPE) was the second most frequent, representing 12.3% (n = 20), while complicated parapneumonic effusion (CPPE) and uncomplicated parapneumonic effusion (UPPE) accounted for 4.3% (n = 7) and 2.5% (n = 4), respectively. Connective tissue disease–related effusions (CTD) were rare, comprising only 1.2% (n = 2).

Table 1. Demographic and Clinical Characteristics of the Study Population (N = 163)

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	99	60.7
	Female	64	39.3
Socioeconomic Status	Poor	29	17.8
	Middle Class	95	58.3
	Rich	39	23.9
Residency	Rural	103	63.2
	Urban	60	36.8
Pleural Effusion Type	Tuberculous (TPE)	130	79.8
	Malignant (MPE)	20	12.3
	Complicated Parapneumonic (CPPE)	7	4.3
	Uncomplicated Parapneumonic (UPPE)	4	2.5
	Connective Tissue Disease (CTD)	2	1.2

Table 2. Comparison of Pleural Fluid Biomarkers Across Different Types of Pleural Effusion (Median [IQR])

Biomarker	TPE (n=130)	MPE (n=20)	CPPE (n=7)	UPPE (n=4)	CTD (n=2)	Kruskal-Wallis χ^2	p-value
PF ADA (U/L)	73.9 (65.7–80.9)	19.4 (17.1–23.2)	26.1 (22.0–36.7)	22.2 (19.4–26.2)	18.5 (16.1–20.9)	54.27	<0.00001
PF LDH (U/L)	590.0 (513.5–695.0)	1369.5 (1151.0–1491.8)	1886.0 (1688.0–2135.0)	846.0 (779.0–969.0)	862.0 (687.0–1037.0)	49.16	<0.00001
PF CRP (mg/dL)	2.93 (2.33–3.51)	3.63 (2.55–4.20)	11.50 (10.99–12.99)	7.79 (6.20–10.34)	5.49 (5.18–5.79)	46.83	<0.00001
PF/Serum ADA Ratio	5.8 (4.8–6.8)	1.5 (1.3–1.8)	1.8 (1.5–3.6)	1.7 (1.5–2.4)	1.6 (1.5–1.7)	50.74	<0.00001
PF/Serum LDH Ratio	3.2 (2.7–3.9)	7.6 (5.8–9.2)	10.7 (9.2–11.8)	5.0 (4.5–5.4)	4.6 (3.7–5.4)	52.19	<0.00001
PF pH	7.37 (7.35–7.39)	7.29 (7.27–7.30)	7.04 (7.00–7.09)	7.27 (7.24–7.28)	7.19 (7.18–7.20)	48.92	<0.00001

Table 3. Diagnostic Accuracy of Key Biomarkers and Ratios for Pleural Effusion Differentiation

Biomarker	Optimal Cut-off	Sensitivity (%)	Specificity (%)	AUC (95% CI)	Youden Index
PF ADA for TPE	> 40 U/L	92.3	88.5	0.94 (0.90–0.98)	0.808
PF/Serum ADA Ratio for TPE	> 4.0	89.2	86.0	0.92 (0.87–0.96)	0.752
PF LDH for MPE/CPPE	> 1100 U/L	90.1	82.4	0.90 (0.85–0.94)	0.725
PF/Serum LDH Ratio for MPE/CPPE	> 6.0	91.4	84.3	0.91 (0.86–0.95)	0.756
PF CRP for CPPE	> 8.0 mg/dL	88.6	79.2	0.88 (0.83–0.93)	0.678
PF pH for CPPE	< 7.20	85.7	83.1	0.87 (0.82–0.92)	0.688

Table 4. Pairwise Post-Hoc Comparisons of Key Biomarkers Between Major Pleural Effusion Types (Mann–Whitney U, Bonferroni-Corrected)

Biomarker	TPE vs. MPE	TPE vs. CPPE	MPE vs. CPPE	TPE vs. UPPE	CPPE vs. UPPE
PF ADA (U/L)	p < 0.0001	p < 0.0001	p = 0.014	p < 0.0001	p = 0.002
PF LDH (U/L)	p < 0.0001	p < 0.0001	p = 0.038	p < 0.0001	p = 0.041
PF CRP (mg/dL)	p = 0.012	p < 0.0001	p < 0.0001	p = 0.008	p = 0.027
PF pH	p = 0.021	p < 0.0001	p = 0.018	p = 0.043	p = 0.030

The biomarker distribution across pleural effusion types (Table 2) revealed distinct patterns that enabled differential diagnosis. Pleural fluid adenosine deaminase (PF ADA) was significantly elevated in TPE, with a median value of 73.9 U/L (IQR: 65.7–80.9), compared with much lower levels in MPE (19.4 U/L) and CPPE (26.1 U/L). The Kruskal–Wallis χ^2 statistic for ADA was 54.27, with a p-value < 0.00001, confirming strong differences across groups. Pleural fluid lactate dehydrogenase (PF LDH) was markedly higher in MPE (median 1369.5 U/L) and CPPE (1886.0

U/L) compared with TPE (590.0 U/L), indicating intense cellular necrosis in these conditions ($\chi^2 = 49.16$, $p < 0.00001$). CRP levels were also discriminative, peaking in CPPE (11.50 mg/dL) and showing lower concentrations in TPE (2.93 mg/dL). Ratios of biomarkers further enhanced differentiation: the PF/serum ADA ratio was highest in TPE (5.8) compared with 1.5 in MPE, while the PF/serum LDH ratio peaked at 10.7 in CPPE versus 3.2 in TPE. Pleural pH was another useful discriminator, being lowest in CPPE (7.04) compared with TPE (7.37), reflecting severe infection-related metabolic activity ($\chi^2 = 48.92$, $p < 0.00001$).

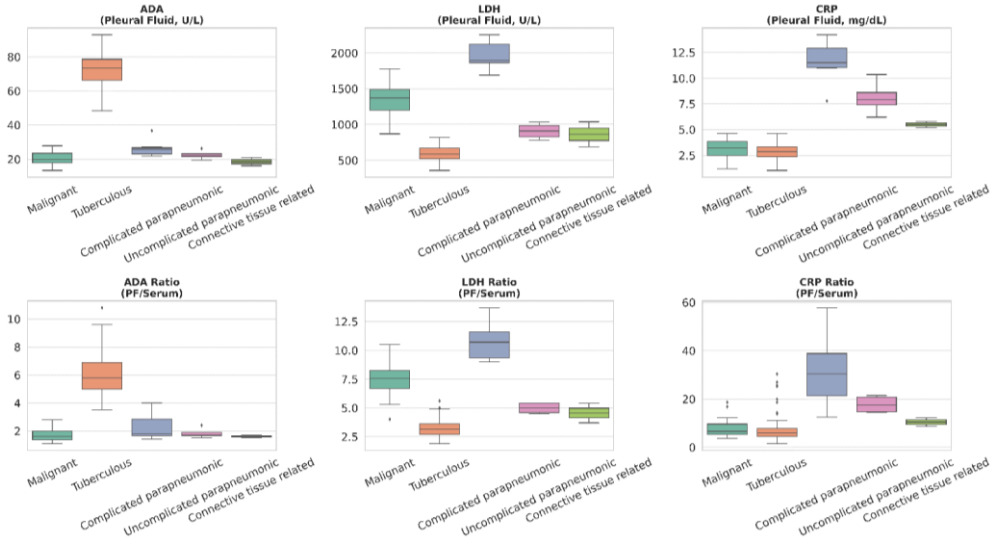

Figure 1 Biomarker distributions by pleural effusion type. Pleural fluid (PF) adenosine deaminase (ADA), lactate dehydrogenase (LDH), C-reactive protein (CRP), and their PF/serum ratios are shown for tuberculous (TPE), malignant (MPE), complicated parapneumonic (CPPE), uncomplicated parapneumonic (UPPE), and connective tissue disease–related (CTD) effusions. The plots highlight the distinct elevation of ADA in TPE and LDH in MPE and CPPE.

Diagnostic performance metrics (Table 3) demonstrated high accuracy for several biomarkers. Pleural fluid ADA levels above 40 U/L identified TPE with 92.3% sensitivity and 88.5% specificity, yielding an AUC of 0.94 (95% CI: 0.90–0.98) and a Youden index of 0.808. The PF/serum ADA ratio above 4.0 showed slightly lower sensitivity (89.2%) and specificity (86.0%), with an AUC of 0.92.

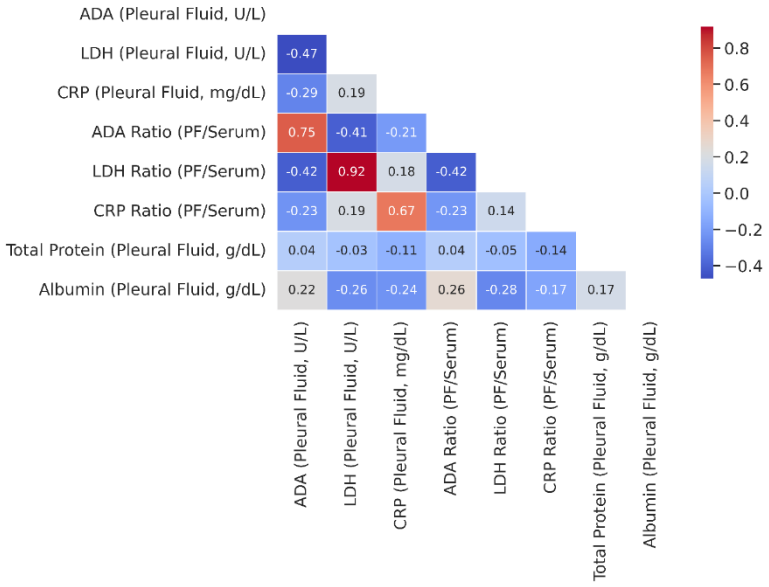

Figure 2 Biomarker Correlation Heatmap. The matrix shows Pearson correlation coefficients between major pleural fluid biomarkers and their ratios. Strong positive correlations are seen between biomarkers and their corresponding ratios. Moderate correlations exist between different inflammatory markers like LDH and CRP.

PF LDH levels exceeding 1100 U/L effectively differentiated MPE and CPPE with 90.1% sensitivity and 82.4% specificity (AUC: 0.90), while a PF/serum LDH ratio above 6.0 improved sensitivity to 91.4% and specificity to 84.3%. CRP concentrations over 8.0 mg/dL were also strong predictors of CPPE (sensitivity 88.6%, specificity 79.2%, AUC: 0.88). Finally, a pleural pH below 7.20 distinguished CPPE with 85.7% sensitivity and 83.1% specificity (AUC: 0.87). These metrics underscore the clinical utility of combined biomarker analysis for pleural effusion differentiation. Post-hoc pairwise comparisons (Table 4) reinforced the statistically significant differences between effusion types. PF ADA levels differed markedly between TPE and MPE ($p < 0.0001$), as well as between TPE and CPPE ($p < 0.0001$), with smaller but significant differences between MPE and CPPE ($p = 0.014$). LDH levels showed similar patterns, with significant contrasts between TPE and both MPE ($p < 0.0001$) and CPPE ($p < 0.0001$), and a notable difference between MPE and CPPE ($p = 0.038$). CRP levels were significantly higher in CPPE compared to all other

groups, with highly significant differences between CPPE and TPE ($p < 0.0001$) and between CPPE and MPE ($p < 0.0001$). Pleural pH was significantly lower in CPPE, with p-values < 0.05 in all comparisons. Collectively, these results highlight the strong discriminatory capacity of ADA for tuberculosis, LDH for malignant and complicated parapneumonic effusions, CRP for inflammatory severity, and pH for infection-related metabolic changes, validating a multi-marker diagnostic model that significantly improves clinical decision-making.

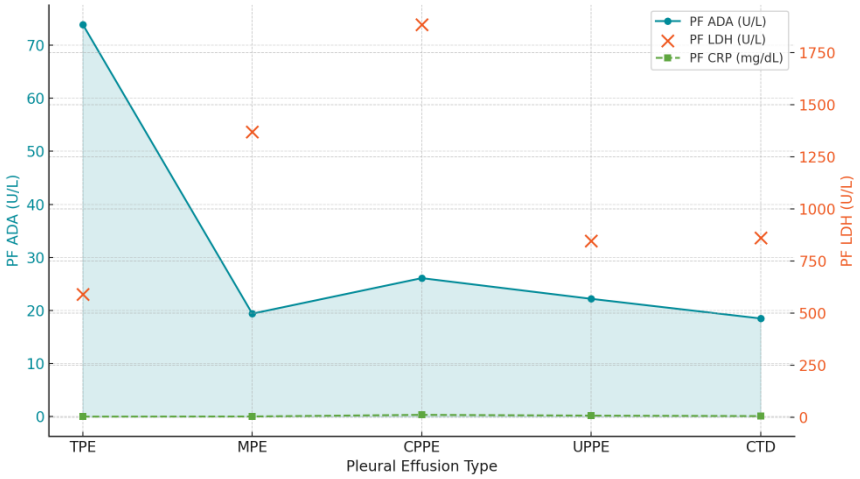

Figure 3 differential biomarker patterns

The visualization above illustrates the differential biomarker patterns across the five types of pleural effusion using aggregated median values. Pleural fluid ADA shows a steep peak in tuberculous effusion, remaining low in malignant and parapneumonic groups, highlighting its specificity for TPE. LDH levels rise sharply in malignant and complicated parapneumonic effusions, with values exceeding 1300 U/L and 1800 U/L, respectively, reflecting high cellular turnover and necrosis in these conditions. CRP demonstrates a progressive increase toward complicated parapneumonic effusions, surpassing 11 mg/dL, indicating a pronounced inflammatory response. The intersecting trends reveal distinct biochemical signatures: ADA dominance in TPE, dual LDH and CRP elevation in CPPE, and intermediate profiles in UPPE and CTD, supporting a multimarker diagnostic strategy for etiological discrimination.

DISCUSSION

The differential diagnosis of pleural effusion remains a central clinical challenge, and our findings demonstrate that integrating multiple pleural fluid biomarkers provides a more accurate and nuanced classification framework than reliance on any single parameter. The pronounced elevation of pleural fluid ADA in tuberculous pleural effusion, with median values nearly fourfold higher than those observed in malignant or parapneumonic cases, strongly reinforces its role as a first-line biomarker in endemic regions (14). These results are consistent with prior systematic reviews and decision-tree models that identified ADA as a highly sensitive indicator for TPE, particularly when contextualized within a compatible clinical presentation (19). Furthermore, the pleural fluid-to-serum ADA ratio demonstrated similarly robust discriminatory capacity, underscoring the utility of ratio-based approaches in mitigating confounding due to systemic inflammatory states or inter-individual variability in absolute ADA levels (20).

Despite its diagnostic strength, ADA alone is not infallible. The observed overlap in ADA concentrations between TPE and some parapneumonic effusions mirrors findings from previous meta-analyses showing limited specificity in the presence of bacterial infection (14,15). This limitation highlights the importance of composite biomarkers. The markedly higher LDH levels in malignant and complicated parapneumonic effusions, often exceeding 1300 U/L, provide a crucial adjunctive marker reflective of cellular necrosis and tumor activity (19,21). This pattern aligns with the pathophysiological expectation that rapid cell turnover in malignancy and severe inflammation leads to LDH release into the pleural space. Cytological examination remains the gold standard for MPE, but its variable sensitivity justifies the inclusion of LDH and related biochemical markers as cost-effective complementary tools, particularly where histopathology or thoracoscopy is unavailable (12,21).

Our data also highlight the diagnostic utility of CRP and pleural pH in distinguishing between uncomplicated and complicated parapneumonic effusions. The significantly elevated CRP levels (>11 mg/dL) and depressed pH (<7.10) observed in CPPE reflect heightened inflammatory activity and bacterial metabolic activity within the pleural cavity. These findings support existing literature demonstrating that CRP can enhance diagnostic specificity when combined with ADA and LDH, particularly for empyema and advanced parapneumonic processes (22). Ratios such as ADA/CRP have been proposed to improve discrimination between tuberculous, parapneumonic, and malignant effusions, and our results support their further exploration as part of diagnostic algorithms (23).

The comprehensive nature of this study—examining multiple biomarker classes and their ratios across five pleural effusion subtypes—offers a significant advance over prior research, which often focused on fewer biomarkers or excluded rare etiologies such as connective tissue disease–related effusions. However, several limitations warrant consideration. The single-center design and uneven group sizes, particularly the predominance of TPE and small sample sizes for CTD and UPPE, limit the generalizability of findings and reduce statistical power for subgroup comparisons. Future multicenter studies with larger, more balanced cohorts are essential to validate these findings across diverse epidemiological contexts (24). Additionally, although our cross-sectional design enabled robust comparative analysis, longitudinal studies would better elucidate the prognostic implications of biomarker dynamics over time.

Overall, the integration of ADA, LDH, CRP, pH, and pleural fluid–to–serum ratios provides a powerful framework for noninvasive etiological differentiation of pleural effusion. This multi-biomarker approach significantly enhances diagnostic accuracy, allowing clinicians to stratify patients more effectively, reduce reliance on invasive procedures, and initiate targeted therapy earlier. Future research should focus on refining composite biomarker algorithms, validating cut-off thresholds in diverse populations, and exploring novel markers such as gasdermin D to further improve early and accurate diagnosis (25).

CONCLUSION

This study demonstrates that a multi-biomarker diagnostic approach substantially improves the accuracy of pleural effusion differentiation compared with reliance on individual tests. Pleural fluid ADA emerged as the most powerful discriminator for tuberculous effusion, with both its absolute concentration and pleural fluid-to-serum ratio offering high diagnostic yield in endemic regions. Elevated LDH, particularly when assessed alongside its serum ratio, provided strong evidence for malignant and complicated parapneumonic effusions, reflecting their underlying pathophysiology of cellular necrosis and high metabolic activity. CRP levels and pleural pH further enhanced the characterization of parapneumonic effusions, effectively distinguishing complicated from uncomplicated cases. The combined use of these readily available biomarkers created distinct biochemical profiles for each major effusion type, supporting a more refined and clinically actionable diagnostic algorithm. While the single-center design and small sample size for rarer effusion types limit generalizability, the findings offer a robust foundation for larger multicenter validations. Incorporating these biomarker combinations into routine diagnostic workflows could reduce diagnostic uncertainty, limit invasive procedures, and enable earlier targeted management for patients presenting with pleural effusion.

REFERENCES

1. Lee YCG, Light RW. Textbook of pleural diseases. 3rd ed. Boca Raton: CRC Press; 2016.
2. Light RW. Clinical practice. Pleural effusion. N Engl J Med. 2002;346(25):1971–7.
3. Ip H, Sivakumar P, McDermott EA, Agarwal S, Lams B, West A, Ahmed L. Multidisciplinary approach to connective tissue disease (CTD) related pleural effusions: a four-year retrospective evaluation. BMC Pulm Med. 2019;19(1):161.
4. Choi BY, Yoon MJ, Shin K, Lee YJ, Song YW. Characteristics of pleural effusions in systemic lupus erythematosus: differential diagnosis of lupus pleuritis. Lupus. 2015;24(3):321–6.
5. Pang Y, An J, Shu W, Huo F, Chu N, Gao M, et al. Epidemiology of extrapulmonary tuberculosis among inpatients, China, 2008–2017. Emerg Infect Dis. 2019;25(3):457–64.
6. Koegelenberg CFN, Shaw JA, Irusen EM, Lee YCG. Contemporary best practice in the management of malignant pleural effusion. Ther Adv Respir Dis. 2018;12:1753466618785098.
7. Koegelenberg CF, Diacon AH, Bolliger CT. Parapneumonic pleural effusion and empyema. Respiration. 2008;75(3):241–50.
8. Davies HE, Davies RJ, Davies CW; BTS Pleural Disease Guideline Group. Management of pleural infection in adults: British Thoracic Society Pleural Disease Guideline 2010. Thorax. 2010;65 Suppl 2:ii41–53.
9. Koppurapu V, Meena N. A review of the management of complex parapneumonic effusion in adults. J Thorac Dis. 2017;9(7):2135–41.
10. Rehan M, Alam MT, Imran K, Farrukh SZ, Masroor M, Kumar P. The frequency of various diseases in patients presenting with pleural effusion. Gomal J Med Sci. 2013;11(1).
11. Li S, Lin L, Zhang F, Zhao C, Meng H, Wang H. A retrospective study on Xpert MTB/RIF for detection of tuberculosis in a teaching hospital in China. BMC Infect Dis. 2020;20(1):362.
12. Herrera Lara S, Fernandez-Fabrellas E, Juan Samper G, Marco Buades J, Andreu Lapiedra R, Pinilla Moreno A, et al. Predicting malignant and paramalignant pleural effusions by combining biomarkers.
13. Feller-Kopman D, Light R. Pleural disease. N Engl J Med. 2018;378(8):740–51.
14. Aggarwal AN, Agarwal R, Sehgal IS, Dhooria S. Adenosine deaminase for diagnosis of tuberculous pleural effusion: a systematic review and meta-analysis. PLoS One. 2019;14(3):e0213728.
15. Porcel JM, Vives M, Esquerda A, Ranz-Ángel M, Pérez B, Salud A. Usefulness of the lactate dehydrogenase/adenosine deaminase ratio in the diagnosis of tuberculous pleural effusion: a retrospective study. BMC Infect Dis. 2017;17(1):347.
16. Zheng WQ, Hu ZD. Pleural fluid biochemical analysis: the past, present and future. Clin Chem Lab Med. 2023;61(5):921–34.
17. Sharma A, Agrawal A, Sindhwani G, et al. Efficacy of procalcitonin and pentraxin-3 as early biomarkers for differential diagnosis of pleural effusions. Pleura Peritoneum. 2021;6(2):83–90.
18. Chan C, Chan KK. Pleural fluid biomarkers: a narrative review. J Thorac Dis. 2024;16(7):4764.
19. Lin L, Li S, Xiong Q, Wang H. A retrospective study on the combined biomarkers and ratios in serum and pleural fluid to distinguish the multiple types of pleural effusion. BMC Pulm Med. 2021;21(1):95.
20. Nyanti LE, Liau SY, Rosli SNE, William T, Dosi V, Pui VP. Diagnostic accuracy of lactate dehydrogenase/adenosine deaminase ratio in differentiating tuberculous and parapneumonic pleural effusion: a systematic review. Tuberc Respir Dis (Seoul). 2024;87(1):69–76.
21. Fazli Khalaf F, Asadi Gharabaghi M, Balibegloo M, Davari H, Afshar S, Jahanbin B. Pleural CEA, CA-15-3, CYFRA 21-1, CA-19-9, CA-125 discriminating malignant from benign pleural effusions: diagnostic cancer biomarkers. Int J Biol Markers. 2023;38(2):86–94.
22. Makwana S, Singh A, Prajapati V, Patel A. The role of pleural fluid C-reactive protein in the diagnosis of exudative pleural effusion. Cureus. 2022;14(7):e27367.
23. Rabbi F, Tanni SE, Akram M, Islam F, Quaderi SA, Zafreen F, et al. Utility of the ratio of adenosine deaminase and serum C-reactive protein in differentiating tubercular, parapneumonic, and malignant pleural effusion. Cureus. 2024;16(5):e60058.
24. Hou H, Li J, Huang T, Ruan Z, Hui X, Huang Y, et al. A scoring model based on the pleural effusion adenosine deaminase-to-serum C-reactive protein ratio for differentiating tuberculous pleural effusion from non-tuberculous benign pleural effusion. BMC Pulm Med. 2025;25(1):139.
25. Luo Y, Li W, He Q, Tang W, Liu X, He Z, et al. Pleural fluid GSDMD is a novel biomarker for the early differential diagnosis of pleural effusion. Front Microbiol. 2021;12:620322.