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Plasminogen Activator Inhibitor-1 (PAI-1) Levels in Loculated and Non-Loculated Infectious Pleural Effusions: A Comparative Cross-Sectional Study

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ABSTRACT: Infectious pleural effusion may progress to loculation through impaired fibrinolysis, causing prolonged hospitalization. Plasminogen activator inhibitor-1 (PAI-1), a fibrinolysis inhibitor, may reflect the antifibrinolytic process associated with pleural loculation. This study compared pleural fluid PAI-1 levels in loculated and non-loculated infectious pleural effusions and evaluated associations with loculation, hospital stay, and microbiological profile in 61 patients treated at Dr. Wahidin Sudirohusodo Hospital and teaching hospitals, Makassar, Indonesia. Pleural fluid PAI-1 concentrations were measured using enzyme-linked immunosorbent assay (ELISA), loculation was assessed using ultrasonography and/or computed tomography, and analyses included Spearman correlation, ROC analysis, and multivariable logistic regression. Patients with loculated effusions had higher PAI-1 concentrations than those with non-loculated effusions (median, 120.62 vs. 5.83 ng/mL; p=0.007), and concentrations correlated with loculation degree (Spearman's rho=0.521; p=0.002). ROC analysis identified a PAI-1 cut-off of 46.525 ng/mL, with area under the curve 0.703, sensitivity 73.3%, and specificity 61.3%. After adjustment, elevated PAI-1 remained independently associated with pleural loculation (adjusted odds ratio, 4.12; 95% confidence interval, 1.18–14.41; p=0.027). Streptococcus spp. predominated, but loculated effusions and Gram-negative organisms were associated with longer hospitalization, and PAI-1 weakly correlated with hospital stay (Spearman's rho=0.275; p=0.044). Elevated pleural fluid PAI-1 was associated with pleural loculation and greater septation and may serve as an adjunctive biomarker interpreted with imaging and microbiological findings and other clinical parameters.

1. INTRODUCTION

Pleural infection remains a major cause of morbidity worldwide and is characterized by progressive inflammation of the pleural space, frequently resulting in parapneumonic effusion and empyema that require prolonged hospitalization and invasive interventions [1,2]. Infectious pleural effusions evolve through exudative, fibrinopurulent, and organizing stages, during which fibrin deposition and pleural septation lead to loculation, impaired fluid drainage, treatment failure, and poorer clinical outcomes [3,4]. Thoracic ultrasonography has become the preferred modality for detecting pleural loculations because it accurately

identifies septations that are often missed on conventional imaging and provides important guidance for therapeutic decision-making [5,6]. Despite advances in antimicrobial therapy and pleural drainage, loculated pleural infection continues to be associated with increased healthcare utilization and mortality [7,8].

The development of pleural loculation is primarily driven by disruption of the balance between coagulation and fibrinolysis within the pleural cavity [9,10]. Plasminogen activator inhibitor-1 (PAI-1), the principal endogenous inhibitor of tissue-type and urokinase-type plasminogen activators, suppresses plasmin generation, thereby promoting persistent fibrin accumulation and pleural septation [11]. Experimental and clinical studies have demonstrated that elevated pleural PAI-1 concentrations contribute to impaired fibrinolysis and are mechanistically linked to the formation of organized pleural adhesions [12]. A prospective biomarker study on patients with pleural infection identified pleural PAI-1 as the biomarker most significantly correlated with the severity of ultrasonographic septation, extended hospitalization, and elevated 12-month mortality. This finding underscores its potential prognostic importance [13].

Although accumulating evidence supports the biological role of PAI-1 in pleural fibrosis, comparative data regarding PAI-1 concentrations between loculated and non-loculated infectious pleural effusions remain limited, particularly in Southeast Asian populations [14]. Furthermore, the relationship between pleural PAI-1 levels, microbial etiology, and hospitalization outcomes has not been adequately characterized [15]. Therefore, this study aimed to compare pleural fluid PAI-1 levels between loculated and non-loculated infectious pleural effusions and to investigate their associations with pleural loculation, microbial groups, and length of hospital stay.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This cross-sectional observational analytical study compared pleural fluid Plasminogen Activator Inhibitor-1 (PAI-1) levels in patients with loculated and non-loculated infectious pleural effusions and evaluated associations with pleural loculation and length of hospital stay (LOS). Conducted from January to April 2026 at Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, and Hasanuddin University-affiliated teaching hospitals, the design and setting were consistent with the latest thesis methodology.

2.2 Study Population

The target population consisted of adult patients with infectious pleural effusion, including parapneumonic effusion and empyema. Patients admitted during the study period who met the eligibility criteria were included using a total sampling approach. The accessible population comprised adult patients with infectious pleural effusion treated at Dr. Wahidin Sudirohusodo Hospital and affiliated teaching hospitals of Hasanuddin University.

Patients were eligible if they were aged ≥18 years, had a diagnosis of infectious pleural effusion, had either loculated or non-loculated pleural effusion based on clinical and/or radiological assessment, underwent thoracentesis or pleural drainage, and had sufficient clinical and laboratory data for analysis. Patients were excluded if the pleural effusion was caused by malignancy or another non-infectious etiology, if severe coagulation disorders precluded pleural fluid sampling, or if essential clinical or examination data were incomplete.

2.3 Sample Size Determination

The sample size was calculated to compare the mean pleural fluid PAI-1 concentration between two independent groups, namely loculated and non-loculated infectious pleural effusions. Assuming a two-sided significance level of 0.05, a statistical power of 80%, standard deviations of 60 ng/mL and 50 ng/mL in the loculated and non-loculated groups, respectively, and an anticipated mean difference of 48 ng/mL, the minimum required sample size was 21 participants per group. After allowing for approximately 15% potential data loss, the target sample size was increased to 25 participants per group, resulting in a minimum total sample size of 50 participants. A total of 61 patients were ultimately included in the analysis.

2.4 Clinical Assessment and Data Collection

Baseline demographic characteristics, comorbidities, microbiological, laboratory, and radiological findings, pleural interventions, and hospitalization outcomes were obtained from medical records using a standardized form. Age, sex, diabetes mellitus, disease progression, and pleural management were recorded as clinical variables and potential confounders. Infectious

pleural effusion was diagnosed based on compatible clinical manifestations, thoracic imaging, and pleural fluid characteristics consistent with an exudative process by Light's criteria and/or evidence of infection. Microbiological investigations included Gram staining, acid-fast bacilli testing, culture, molecular testing, and other assessments from sputum or pleural fluid. Pleural loculation was assessed by thoracic ultrasonography and, when available, computed tomography. Effusions were classified as non-loculated or loculated and graded by septation number as mild (1–2), moderate (3–4), or severe (≥5 or multiloculated). LOS cut-off was ≥8 days.

2.5 Measurement of Pleural Fluid PAI-1

Pleural fluid samples were obtained aseptically during clinically indicated thoracentesis or pleural drainage. Samples were processed according to standardized laboratory procedures. After centrifugation to remove cellular components, the supernatant was stored until analysis. Pleural fluid PAI-1 concentrations were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. PAI-1 concentrations were expressed in ng/mL. For categorical analysis, the ROC-derived cut-off value of 46.525 ng/mL was used to classify patients into lower and higher PAI-1 groups.

2.6 Outcome Measures

The primary outcome of this study was the difference in pleural fluid PAI-1 concentrations between patients with loculated and non-loculated infectious pleural effusions. Secondary outcomes included the associations between pleural fluid PAI-1 concentrations and pleural loculation, including the degree of pleural loculation assessed by thoracic ultrasonography. The study also evaluated the association between pleural loculation and length of hospital stay (LOS), as well as the correlation between pleural fluid PAI-1 concentrations and LOS. In addition, the relationships of microbiological groups with PAI-1 concentrations and hospitalization duration were assessed. Finally, multivariable logistic regression was performed to determine whether elevated pleural fluid PAI-1 concentrations were independently associated with pleural loculation after adjustment for selected clinical variables.

2.7 Statistical Analysis

Data were analyzed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality with the Shapiro–Wilk test and are presented as mean ± standard deviation or median with interquartile range, as appropriate. Categorical variables are presented as frequencies and percentages. Groups were compared using the independent-samples t test or Mann–Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Correlations between PAI-1 concentration, pleural loculation, and LOS were assessed using Pearson's or Spearman's coefficients according to distribution. ROC analysis determined the discriminatory performance and optimal PAI-1 cut-off for loculated pleural effusion using the maximum Youden index. ROC analysis for LOS identified a prolonged hospitalization threshold of ≥8 days; AUC was 0.752, sensitivity 72.0%, and specificity 65.5%. Multivariable logistic regression identified factors independently associated with pleural loculation; adjusted ORs with 95% CIs were reported. The model included PAI-1, age, sex, diabetes mellitus, pleural management, and disease progression. All tests were two-sided, with $p < 0.05$ considered.

3. RESULTS AND DISCUSSION

3.1 Baseline Characteristics

A total of 61 patients with infectious pleural effusion were enrolled, including 30 (49.2%) with loculated effusions and 31 (50.8%) with non-loculated effusions. The median age was 51.5 years (IQR, 45.3–61.8) in the loculated group and 54.0 years (IQR, 44.5–60.5) in the non-loculated group (p=0.773) with no significant difference between groups. Patients aged ≥60 years accounted for 33.3% and 29.0%, respectively (p=0.717). Male patients were more frequent in the loculated group (66.7% vs. 38.7%; p=0.029). Diabetes mellitus was present in 6.7% and 19.4%, respectively, in the two groups (p=0.255). Disease progression occurred in 53.3% versus 32.3% of patients in the two groups, without statistical significance (p=0.096). Baseline demographic and clinical characteristics are summarized in Table 1.

3.2 Comparison of Pleural Fluid PAI-1 Levels According to Loculation Status

Pleural fluid PAI-1 concentrations differed by loculation status. Patients with loculated pleural effusions had higher PAI-1 concentrations than those without loculation. Median concentration was 120.62 ng/mL (IQR, 17.00–198.36) in loculated versus

5.83 ng/mL (IQR, 1.35–110.68) in non-loculated effusions (p=0.007). Mean concentration was higher in loculated effusions (298.12 ± 644.72 ng/mL vs. 55.04 ± 77.91 ng/mL), indicating variability (Table 2). PAI-1 concentrations increased with loculation. Mean PAI-1 concentration was 55.04 ± 77.91 ng/mL in non-loculated effusions, 78.45 ± 62.31 ng/mL in mild septation, 185.36 ± 168.42 ng/mL in moderate septation, and 653.78 ± 1,082.64 ng/mL in heavy septation. PAI-1 concentration correlated moderately with pleural loculation degree (Spearman's rho=0.521, p=0.002), with higher concentrations associated with more extensive septation (Table 7).

Table 1. Baseline demographic and clinical characteristics according to loculation status (n = 61)

Characteristic	Loculated (n = 30)	Non-loculated (n = 31)	Total (n = 61)	p-value
Loculation status, n (%)	30 (49.2%)	31 (50.8%)	61 (100.0%)	—
Age, years, median (IQR)	51.5 (45.3–61.8)	54.0 (44.5–60.5)	—	0.773[a]
Age ≥60 years, n (%)	10 (33.3%)	9 (29.0%)	19 (31.1%)	0.717[b]
Age <60 years, n (%)	20 (66.7%)	22 (71.0%)	42 (68.9%)	
Male, n (%)	20 (66.7%)	12 (38.7%)	32 (52.5%)	0.029[b]
Female, n (%)	10 (33.3%)	19 (61.3%)	29 (47.5%)	
Diabetes mellitus, n (%)	2 (6.7%)	6 (19.4%)	8 (13.1%)	0.255[c]
No diabetes mellitus, n (%)	28 (93.3%)	25 (80.6%)	53 (86.9%)	
Disease progression, n (%)	16 (53.3%)	10 (32.3%)	26 (42.6%)	0.096[b]
No disease progression, n (%)	14 (46.7%)	21 (67.7%)	35 (57.4%)	

Data are presented as median (interquartile range) or number (percentage). [a]Mann–Whitney U test. [b]Chi-square test. [c]Fisher's exact test.

Table 2. Comparison of PAI-1 levels according to infectious pleural effusion loculation status

Loculation status	n	Mean ± SD	Median (IQR)	Min–Max	p-value
Loculated	30	298.12 ± 644.72	120.62 (17.00–198.36)	0.32–3387.76	0.007[a]
Non-loculated	31	55.04 ± 77.91	5.83 (1.35–110.68)	0.07–320.32	

Data are presented as mean ± standard deviation (SD), median (interquartile range [IQR]), and minimum–maximum values. [a]Mann–Whitney U test.

3.3 Association Between PAI-1 Levels and Pleural Loculation

For categorical analysis using the median PAI-1 concentration of 46.525 ng/mL, high PAI-1 concentrations were more common in patients with loculated pleural effusions than those without loculation (66.7% vs. 35.5%; p=0.029). Patients with high PAI-1 concentrations had a 3.64-fold likelihood of pleural loculation (OR, 3.64; 95% CI, 1.26–10.47), indicating an association between pleural fluid PAI-1 concentrations and pleural loculation (Table 3). Receiver operating characteristic analysis showed moderate discriminatory performance, with an area under the curve of 0.703 and an optimal cut-off of 46.525 ng/mL. In multivariable logistic regression, PAI-1 ≥46.525 ng/mL remained independently associated with pleural loculation after adjustment for age ≥60 years, male sex, diabetes mellitus, invasive pleural management, and disease progression (adjusted OR, 4.12; 95% CI, 1.18–14.41; p=0.027), whereas no other adjusted variables showed a statistically significant association (Table 8).

Table 3. Association between PAI-1 category and infectious pleural effusion loculation status

PAI-1 category	Loculated (n = 30)	Non-loculated (n = 31)	p-value	OR (95% CI)
Low PAI-1 (<46,525 ng/mL)	10 (33.3%)	20 (64.5%)	0.029[b]	3.64 (1.26–10.47)
High PAI-1 (≥46.525 ng/mL)	20 (66.7%)	11 (35.5%)		

Data are presented as number (percentage). [b]Chi-square test. Abbreviations: OR, odds ratio; CI, confidence interval.

3.4 Length of Hospital Stay According to Loculation Status

Hospitalization duration was significantly longer among patients with loculated infectious pleural effusions than among those without loculation. The mean length of stay was 11.44 ± 5.49 days in the loculated group compared with 7.59 ± 2.86 days in the non-loculated group (p=0.001). When hospitalization duration was categorized according to the ≥8-day threshold, patients with loculated effusions were significantly more likely to experience prolonged hospitalization than patients without loculation (66.7% vs. 35.5%). Loculated pleural effusions were associated with a nearly five-fold increased likelihood of prolonged hospital stay (OR, 4.89; 95% CI, 1.53–15.61; p=0.013) (Table 4). Receiver operating characteristic analysis of hospitalization duration identified ≥8 days as the threshold for prolonged hospitalization, with an AUC of 0.752, sensitivity of 72.0%, and specificity of 65.5%.

Table 4. Comparison and association of length of hospital stay according to loculation status

Length of hospital stay	Loculated (n = 30)	Non-loculated (n = 31)	p-value	OR (95% CI)
Continuous variable				
Mean ± SD, days	11.44 ± 5.49	7.59 ± 2.86	0.001[a]	—
Range, days	6.00–25.00	4.00–15.00		
Categorical variable				
<8 days	10 (33.3%)	20 (64.5%)	0.013[b]	4.89 (1.53–15.61)
≥8 days	20 (66.7%)	11 (35.5%)		

Data are presented as mean ± standard deviation (SD), range, or number (percentage). [a]Mann–Whitney U test. [b]Chi-square test. Abbreviations: OR, odds ratio; CI, confidence interval.

3.5 Association Between PAI-1 Levels and Length of Hospital Stay

Patients hospitalized for ≥8 days tended to have higher pleural fluid PAI-1 concentrations than those hospitalized for <8 days, with median concentrations increasing from 5.38 ng/mL (IQR, 1.36–109.57) to 76.46 ng/mL (IQR, 3.28–157.01). However, neither the categorical comparison using the ROC-derived PAI-1 cut-off of 46.525 ng/mL (p=0.104) nor the continuous comparison between hospitalization groups (p=0.113) was statistically significant for both group analyses. Across all participants, PAI-1 concentration and length of stay showed a weak positive correlation (Spearman's rho=0.275, p=0.044), indicating that higher PAI-1 concentrations were associated with longer hospital stays. These findings are summarized in Table 5.

Table 5. Association between PAI-1 levels and length of hospital stay

Variable	**Length of hospital stay <8 days (n = 30)**	**Length of hospital stay ≥8 days (n = 31)**	**p-value**
Low PAI-1 (<46.525 ng/mL), n (%)	20 (64.5%)	10 (32.3%)	0.104[b]
High PAI-1 (≥46.525 ng/mL), n (%)	10 (33.3%)	21 (67.7%)	
PAI-1 level, median (IQR), ng/mL	5.38 (1.36–109.57)	76.46 (3.28–157.01)	0.113[a]
PAI-1 and length of hospital stay, Spearman's rho	0.275		0.044[d]

[a]Mann–Whitney U test. [b]Chi-square test. **[d]Spearman correlation.**

3.6 Microbiological Profile and Its Association with PAI-1 Levels and Hospital Stay

The microbiological profile differed between loculated and non-loculated infectious pleural effusions. Streptococcus spp. was the most frequently identified microorganism, accounting for 41.0% of cases and was more common in non-loculated than loculated effusions (51.6% vs. 30.0%). Non-Klebsiella Gram-negative bacteria accounted for 21.3% and were more common in loculated effusions (26.7% vs. 16.1%). Mycobacterium tuberculosis accounted for 9.8%, Klebsiella pneumoniae for 3.3%,

mixed infections for 19.7%, and other or unspecified microorganisms for 4.9%. PAI-1 concentrations did not differ across microbiological groups (p=0.562), but hospital stay differed (p=0.003). Median hospitalization was longest with Klebsiella pneumoniae infection (15.00 days), followed by non-Klebsiella Gram-negative infections (14.00 days), and shortest with mixed infections (6.00 days). The distribution and associations with PAI-1 concentration and hospitalization duration are presented in Table 6.

Table 6. Distribution of causative microorganisms and their association with PAI-1 levels and length of hospital stay

Causative microorganism	Loculated (n = 30)	Non-loculated (n = 31)	Total (n = 61)	PAI-1, median (IQR), ng/mL	Length of hospital stay, median (IQR), days
Streptococcus spp.	9 (30.0%)	16 (51.6%)	25 (41.0%)	75.44 (1.59–151.12)	7.00 (6.00–10.00)
Non-Klebsiella Gram-negative bacteria	8 (26.7%)	5 (16.1%)	13 (21.3%)	77.49 (6.24–160.67)	14.00 (10.00–18.50)
Mixed (polymicrobial) infection	4 (13.3%)	8 (25.8%)	12 (19.7%)	29.83 (0.69–118.31)	6.00 (5.00–7.50)
Mycobacterium tuberculosis	5 (16.7%)	1 (3.2%)	6 (9.8%)	24.04 (1.37–251.87)	9.00 (7.25–10.00)
Klebsiella pneumoniae	2 (6.7%)	0 (0.0%)	2 (3.3%)	155.04 (155.04–155.04)	15.00
Other/unspecified microorganisms	2 (6.7%)	1 (3.2%)	3 (4.9%)	3.44 (1.75–101.25)	7.00 (7.00–8.50)
Overall p-value	—	—	—	0.562†	0.003[e]

Data are presented as number (percentage) or median (interquartile range [IQR]). Percentages were calculated within each loculation group. [e]Kruskal–Wallis test.

Table 7. Pleural fluid PAI-1 levels according to the degree of pleural loculation

Degree of pleural loculation	Number of septations	n (%)	PAI-1, mean ± SD (ng/mL)	Min–Max (ng/mL)
Non-loculated / unseptated	0	31 (50.8)	55.04 ± 77.91	0.07–320.32
Mild septation	1–2	10 (16.4)	78.45 ± 62.31	0.32–196.26
Moderate septation	3–5	12 (19.7)	185.36 ± 168.42	7.17–734.53
Heavy septation	≥5	8 (13.1)	653.78 ± 1,082.64	46.53–3387.76
Spearman's rho			0.521	
p-value			0.002	

Data are presented as number (percentage) or mean ± standard deviation. Loculation severity was assessed according to the number of septations identified on thoracic ultrasonography. Kruskal–Wallis test and Spearman correlation were used as appropriate.

Table 8. Multivariable logistic regression analysis of factors associated with pleural loculation

Variable	Adjusted OR	95% CI	p-value
PAI-1 ≥46.525 ng/mL	4.12	1.18–14.41	0.027
Age ≥60 years	1.28	0.38–4.29	0.687
Male sex	2.31	0.71–7.49	0.164
Diabetes mellitus	1.56	0.39–6.24	0.528
Invasive pleural management	3.48	0.91–13.35	0.068
Disease progression	2.74	0.69–10.88	0.151

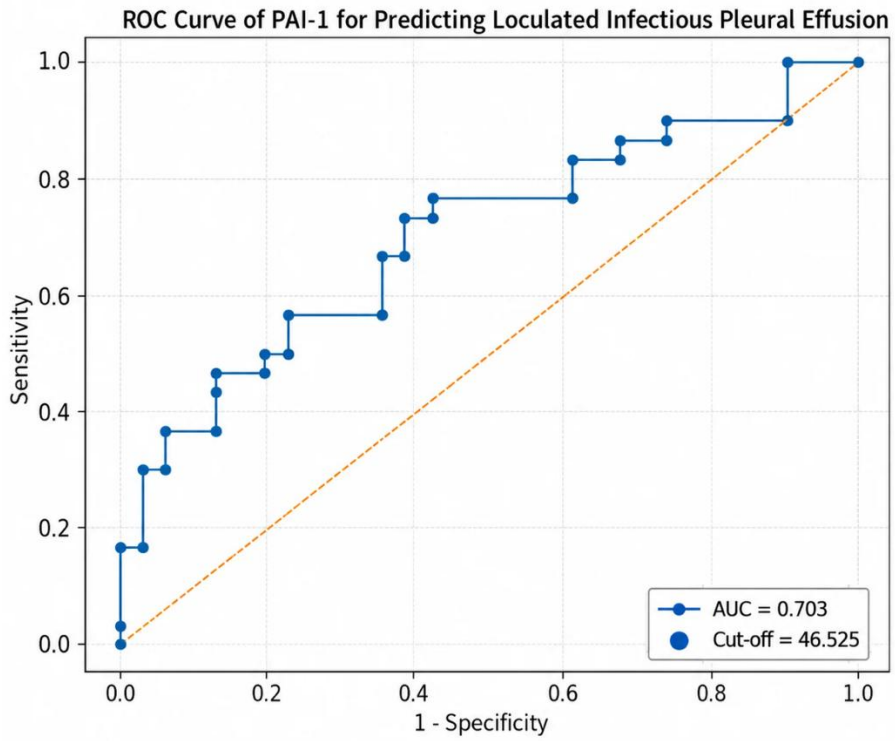


Figure 1. Receiver Operating Characteristic (ROC) Curve of Pleural Fluid Plasminogen Activator Inhibitor-1 Levels for Predicting Loculated Infectious Pleural Effusion

3.7 Discussion

This study showed pleural fluid PAI-1 concentrations were higher in patients with loculated infectious pleural effusions than in those without loculation, supporting impaired fibrinolysis in pleural septation pathogenesis. Consistent with Bedawi et al. (2023), PAI-1 was the biomarker most strongly associated with ultrasonographic septation severity and adverse clinical outcomes in pleural infection [16]. Likewise, Idell (2021) emphasized that disruption of fibrin turnover is the principal mechanism underlying pleural organization and loculation, whereby excessive inhibition of fibrinolysis promotes persistent fibrin deposition and septal formation [16]. Similar observations by Komissarov et al. (2022) further demonstrated that increased antifibrinolytic activity contributes directly to pleural fibrosis rather than representing a simple consequence of inflammation [17]. Collectively, these findings reinforce that elevated PAI-1 reflects an active biological process driving pleural organization rather than merely a nonspecific inflammatory marker. In the present study, PAI-1 concentrations increased with pleural loculation and showed a moderate positive correlation with septation severity (Spearman's rho=0.521, p=0.002). This supports the biological relevance of PAI-1 in pleural organization progression, suggesting that greater local fibrinolysis impairment may accompany more extensive fibrin septation, consistent with persistent fibrin deposition promoting pleural compartment formation during infection.

Despite comparable demographic characteristics, patients with loculated effusions had longer hospital stays, indicating that pleural loculation reflects disease complexity rather than patient differences. This agrees with reports by Wrightson and Maskell (2012), who described loculated pleural infection as a determinant of treatment complexity requiring repeated drainage or intrapleural interventions [18]. Similarly, Bedawi et al. (2023) demonstrated that radiological evidence of pleural septation is associated with prolonged hospitalization and increased healthcare utilization [19]. The absence of significant associations between age, sex, and loculation in the present study also parallels findings from Bedawi et al. (2023), suggesting that local pleural biological processes are more influential than demographic factors in determining disease progression [9]. The present study identified a weak but significant positive correlation between pleural fluid PAI-1 concentration and length of hospital stay. Higher PAI-1 concentrations were associated with longer hospitalization, although the weak correlation suggests that PAI-1 alone does not explain variability in hospital stay. The absence of a statistically significant association when PAI-1 was analyzed using the ROC-derived cut-off suggests that the relationship with hospitalization duration may be continuous rather than dependent on a single biomarker threshold. This is plausible because increased PAI-1 may promote fibrin deposition and septation, making pleural fluid harder to drain and delaying infection resolution. However, length of hospital stay remains a multifactorial outcome influenced by disease severity, microbial characteristics, treatment response, adequacy of pleural drainage, comorbidities, and decision-making. This finding aligns with Addala et al. (2021), who emphasized multifactorial pleural infection outcomes rather than a single biomarker. [20].

An additional finding of this study was the heterogeneous microbiological profile observed in loculated pleural infections, whereas Streptococcus spp. predominated in non-loculated effusions. Although microbial groups were not significantly associated with PAI-1 concentrations, Gram-negative organisms and Klebsiella pneumoniae tended to be associated with longer hospitalization, suggesting that pathogen-specific characteristics may contribute to clinical outcomes alongside local pleural fibrinolytic activity. These findings are in agreement with Koegelenberg et al. (2021), who reported that microorganism type primarily affects disease severity and prognosis rather than specific pleural biomarkers [21]. The significant difference in hospitalization duration across microbiological groups ($p=0.003$) supports the multifactorial nature of outcomes in pleural infection. However, because the study did not perform multivariable analysis examining the independent effect of microorganism groups on length of stay, these findings should be interpreted as associations rather than independent effects. Importantly, multivariable analysis identified elevated PAI-1 as an independent factor associated with pleural loculation after adjustment for age, sex, diabetes mellitus, invasive pleural procedures, and disease progression, strengthening its relevance. Consistent with Komissarov et al. (2022), impaired fibrinolysis appears to represent a central pathway leading to pleural organization irrespective of baseline patient characteristics [17]. The ROC analysis showed moderate discriminatory performance of PAI-1 (AUC=0.703), with an optimal cut-off of 46.525 ng/mL, sensitivity of 73.3%, and specificity of 61.3%. These findings indicate that PAI-1 has potential clinical value as an adjunctive biomarker for identifying patients at increased risk of pleural loculation, but its discriminatory ability is insufficient to support its use as a standalone diagnostic marker. This interpretation is consistent with the ERS/ESTS statement on pleural infection, which recommends an integrated diagnostic approach combining clinical, radiological, microbiological, and pleural fluid findings for optimal patient management [19]. Furthermore, the MIST2 trial showed that combined intrapleural tissue plasminogen activator and DNase therapy improves pleural drainage, reduces surgical referral, and shortens hospital stay in pleural infection. Given the association between PAI-1 and pleural septation, PAI-1 measurement may complement thoracic ultrasonography in identifying patients needing assessment of pleural complexity and drainage adequacy. Nevertheless, this application remains hypothesis-generating and requires validation before PAI-1 is incorporated into clinical decision-making. Future multicenter studies with cohorts and serial biomarker measurements should validate PAI-1 utility and biomarker-guided management for outcomes.

This study has limitations. Cross-sectional design and single-time-point biomarker measurement preclude causal inference and temporal assessment of pleural fluid PAI-1 levels. The single-center–predominant setting may limit generalizability. Residual confounding from infection severity, symptom duration, prior antibiotic use, drainage timing, comorbidities, and intrapleural fibrinolytic therapy cannot be excluded. Loculation assessment was limited by imaging quality and documentation. Small numbers limited power. Other biomarkers and outcomes were not assessed, restricting interpretation of relationships among PAI-1, loculation severity, and outcomes.

4. CONCLUSION

Elevated pleural fluid PAI-1 concentrations were significantly associated with pleural loculation in patients with infectious pleural effusion, supporting a role for impaired local fibrinolysis in pleural septation and organization. PAI-1 also increased with loculation severity and correlated with hospital stay length. Patients with loculated effusions had higher PAI-1 concentrations and longer hospital stays than those with non-loculated effusions. Using a cut-off of 46.525 ng/mL, PAI-1 showed moderate discrimination for pleural loculation (AUC=0.703) and remained independently associated after adjustment for clinical variables (adjusted OR=4.12, 95% CI 1.18–14.41; p=0.027). Microbial profiles and hospitalization duration differed, but microorganism type was not significantly associated with PAI-1 concentration. These findings suggest PAI-1 may be an adjunctive biomarker of pleural loculation and antifibrinolytic activity, complementing imaging and fluid assessment for risk stratification.

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AUTHOR CONTRIBUTIONS

Mohammad Haris (MH): Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **Nurjannah Lihawa (NL):** Methodology, Supervision, Validation, Writing – review & editing. **Muh. Ilyas (MI):** Supervision, Validation, Writing – review & editing. **Jamaluddin Madolangan (JM):** Writing – review & editing. **Arif Santoso (AS):** Writing – review & editing. **Harry Akza Putrawan (HAP):** Writing – review & editing. **All authors read and approved the final manuscript.**

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

The study protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Dr. Wahidin Sudirohusodo Hospital, Makassar (Approval No. 200/UN4.6.4.5.31/PP36/2026; Protocol No. UH26010111; approved on 2 February 2026). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment, and patient confidentiality was maintained by anonymizing identifiable information before analysis.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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