Original Article

Analysis of TGF-β1 Levels in Bronchoalveolar Lavage Fluid among Lung Cancer, Suspected, and Non-Cancer Patients

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Abstract

Background: Lung cancer remains the leading cause of cancer-related mortality worldwide and is often diagnosed at an advanced stage. TGF-β1 plays a pivotal role in carcinogenesis and may serve as a diagnostic biomarker. BALF represents a relevant medium for assessing biomarkers that reflect the tumor microenvironment. This study aimed to evaluate TGF-β1 levels in BALF and determine its diagnostic performance and optimal cut-off value for lung cancer.

Methods: This cross-sectional analytical study was conducted at Dr. Zainoel Abidin General Hospital, Banda Aceh, from April to June 2025, involving 64 patients (34 lung cancer patients, 9 suspected lung cancer patients, and 21 non-cancer patients). BALF samples were obtained via bronchoscopy, and TGF-β1 levels were measured using ELISA. Statistical analyses included the Mann-Whitney U test and ROC curve analysis.

Results: Median TGF-β1 levels were 66.24 pg/mL in the non-cancer group, 136.93 pg/mL in the suspected lung cancer group, and 200.25 pg/mL in the lung cancer group. The combined suspected/lung cancer group showed significantly higher levels than the non-cancer group (*P*=0.025). ROC analysis yielded an area under the curve (AUC) of 0.674 (95% CI=0.529-0.819; *P*=0.025). The optimal cut-off value was 105.63 pg/mL, with a sensitivity of 67.44%, specificity of 61.90%, positive predictive value of 78.38%, and negative predictive value of 48.15%.

Conclusion: TGF-β1 levels in BALF demonstrate potential as a diagnostic biomarker for lung cancer with moderate performance. It may serve as a supportive "rule-in" test to strengthen clinical suspicion but is insufficient as a stand-alone diagnostic tool. Combining TGF-β1 with other biomarkers in a multiparametric diagnostic panel is recommended to improve diagnostic accuracy.

Keywords: biomarker, bronchoalveolar lavage, lung cancer, TGF- β1

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INTRODUCTION

Lung cancer is among the most frequently diagnosed malignancies and remains the leading cause of cancer-related mortality worldwide, accounting for approximately 2.2 million new cases and 1.79 million deaths annually.[1] According to the World Health Organization, lung cancer contributed to 18.4% of all cancer deaths in 2018. In Indonesia, lung cancer is the most commonly diagnosed cancer in men, with 34,783 new cases reported in 2020 (14.1% of all cancers) and 30,843 deaths (13.2% of all cancer-related deaths).[2]

The disease poses a major public health and economic burden. In 2016, the Indonesian National Health Insurance Agency (*BPJS Kesehatan*) reported 1.3 million cancer cases, with a total cost of 2.2 trillion rupiah, ranking cancer as the second-largest healthcare expenditure after cardiovascular diseases.[1]

Lung cancer generally has a poor prognosis due to late-stage diagnosis.[3] Recent advances in targeted therapy have improved outcomes, largely driven by the identification of predictive and diagnostic biomarkers that guide personalized treatment strategies. Biomarkers—biological molecules measurable in tissue or body fluids—can indicate disease presence, progression, or treatment response.[4] One of the lung cancer biomarkers is Transforming Growth Factor Beta 1 (TGF-β1), which is found in Bronchoalveolar lavage fluid (BALF).

Bronchoalveolar lavage fluid is a valuable specimen for lung cancer investigation because it reflects the local tumor microenvironment. Although

BALF cytology alone has moderate sensitivity (29–69%), its specificity is high (90–100%), and diagnostic accuracy improves when combined with histological or molecular testing.[5,6]

Transforming Growth Factor Beta 1 is a multifunctional cytokine regulating cell proliferation, differentiation, apoptosis, and extracellular matrix synthesis.[7,8] Dysregulation of TGF-β1 signaling contributes to tumor initiation and progression through mechanisms such as epithelial–mesenchymal transition and immunosuppression.

Given its potential role in tumor biology, this study aimed to analyze TGF-β1 levels in bronchoalveolar lavage fluid among patients with lung cancer, suspected lung cancer, and non-cancer conditions, and to determine the diagnostic cut-off value of TGF-β1 as a potential biomarker for lung cancer detection.

METHODS

This analytical observational study used a cross-sectional design to compare TGF-β1 levels in BALF among lung cancer, suspected lung cancer, and non-cancer patients. The study was conducted at the bronchoscopy unit of Dr. Zainoel Abidin General Hospital, Banda Aceh, from April to June 2025.

The population included all patients with suspected lung cancer based on clinical and radiological findings. Samples were selected by total sampling, yielding 64 subjects who met the inclusion criteria. The inclusion criteria were patients aged ≥18 years with suspected lung cancer who underwent bronchoscopy and provided informed consent, whereas the exclusion criteria were a history of prior chemotherapy or unsuccessful BALF collection.

Data collection involved two primary components: clinical and laboratory data. Clinical information, including demographic data, smoking status, clinical diagnosis, and radiological findings, was obtained from patient medical records. Radiological findings were obtained with bronchoscopy procedures for collecting BALF samples.

Bronchoscopy procedures were performed by trained pulmonologists following standard operating procedures under local anesthesia and conscious sedation. The bronchoscope was advanced into the target subsegmental bronchus corresponding to the radiologically affected area. Subsequently, sterile saline solution (0.9% NaCl) was instilled in aliquots of 20–50 mL, with a total volume of approximately 100–150 mL. After each instillation, the fluid was gently aspirated using low suction pressure to obtain BALF, which reflects the cellular and biochemical composition of the distal airways and alveolar space.[5]

The BALF samples were centrifuged at 3,000 rpm for 10 minutes at 4°C to separate the cellular and supernatant components. The supernatant was stored at −80°C until analysis. TGF-β1 concentration in the BALF supernatant was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. The optical density was read at 450 nm using a microplate reader, and concentrations were calculated using a standard curve generated from known TGF-β1 concentrations.

To ensure measurement reliability, all samples were analyzed in duplicate, and intra-assay and inter-assay coefficients of variation were maintained below 10%. Laboratory personnel performing the assays were blinded to patient diagnosis to minimize observation bias.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic and clinical characteristics of subjects. The Shapiro–Wilk test was applied to assess the normality of continuous data distributions.

For diagnostic analysis, patients were categorized into two groups: non-cancer and a combined group of suspected lung cancer and confirmed lung cancer. Differences in TGF-β1 levels between these two groups were analyzed using the Mann-Whitney U test.

Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the

diagnostic performance of BALF TGF-β1 in distinguishing the combined suspected/lung cancer group from the non-cancer group. The area under the curve (AUC), 95% confidence interval (CI), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. The optimal cut-off value was determined using the Youden index. A value of $P<0.05$ was considered statistically significant.

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Syiah Kuala, with ethical clearance number 045/ETIK-RSUDZA/2025. Permission to conduct the study was also obtained from Dr. Zainoel Abidin General Hospital.

RESULTS

This study included 64 patients who underwent bronchoscopy for suspected lung cancer at Dr. Zainoel Abidin General Hospital.

Table 1. Characteristics of study (n=54)

Characteristics	n	%
Sex		
Male	52	81.25
Female	12	18.75
Age		
18-25 years	1	1.59
26-44 years	6	9.38
45-59 years	30	46.88
≥60 years	27	42.19
Occupation		
Farmer	18	28,13
Trader/ Entrepreneuer/Private Sector	18	28.13
Housewife	9	14.06
Civil Servant	4	6.25
Others	15	23.44
Smoking Status		
Non-smoker	11	17.19
Passive smoker	3	4.69
Former smoker	3	4.69
Active smoker	47	73.44
Brinkman Index		
Non-smoker	13	20.31
Light smoker	2	3.13
Moderate smoker	4	6.25
Heavy smoker	45	70.31
Diagnosis		
Non-lung cancer	21	32.81
Suspected lung cancer	9	14.06
Lung cancer	34	53.13

Most patients were male (81.3%) and aged 45–59 years (46.9%). The majority were active smokers (73.4%), with 70.3% classified as heavy smokers by the Brinkman index. Based on final diagnosis, 53.1% had lung cancer, 14.1% were suspected lung cancer, and 32.8% were non-cancer (Table 1).

Table 2. Levels of TGF-β1 in BALF among Lung Cancer, Suspected Lung Cancer, and Non-Cancer Patients at Dr. Zainoel Abidin General Hospital (n=64)

Group	Median (pg/mL)	Min-Max (pg/mL)	*P*
Non-lung cancer (n=21)	66.24	6.11-3738.05	0.0001*
Suspected lung cancer (n=9)	136.93	22.4-779.35	0.018*
Lung cancer (n=34)	200.25	9.55-3544.69	0.0001*

Note: *Non-normal distribution ($P<0.05$)

Table 2 shows the median BALF TGF-β1 level differed among the groups, with 66.24 pg/mL (6.11–3738.05) in non-cancer, 136.93 pg/mL (22.4–779.35) in suspected cancer, and 200.25 pg/mL (9.55–3544.69) in lung cancer. Shapiro–Wilk tests indicated non-normal data distribution ($P<0.05$).

Table 3. Analysis of TGF-β1 levels in BALF among non-lung cancer and combined suspected/lung cancer patients

Group	Median	Min-Max	*P*
Non-lung cancer (n=21)	66.24	6.11-3738.05	0.025*
Suspected/lung cancer (n=43)	191.24	9.55-3544.69	

Note: *significant ($P<0.05$) with Mann-Whitney test

Table 3 presents the diagnostic analysis, in which the suspected and confirmed lung cancer groups were combined (n=43). Shapiro–Wilk tests indicated non-normal data distribution ($P<0.05$). The combined group had a significantly higher median TGF-β1 level (191.24 pg/mL) than the non-cancer group (66.24 pg/mL), with $P=0.025$.

Table 4. Diagnostic test of TGF-β1 as a biomarker for lung cancer among non-lung cancer and combined suspected/lung cancer patients

Area	*P*	95% CI
0.674	0.025	0.529-0.819

Receiver Operating Characteristic curve analysis revealed an AUC of 0.674 (95% CI=0.529–0.819; $P=0.025$) (Figure 1), indicating fair discriminatory ability of TGF-β1 to distinguish cancer from non-cancer (Table 4). The optimal cut-off value was 105.63 pg/mL, determined by the Youden Index.

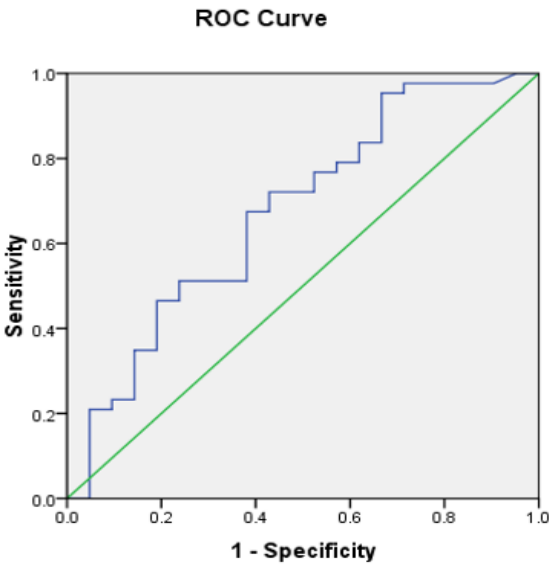

Figure 1. ROC curve and AUC of TGF-β1 as a biomarker for lung cancer

Using a cut-off value of 105.63 pg/mL, TGF-β1 demonstrated fair diagnostic accuracy for lung cancer (AUC=0.674; *P*=0.025). At this threshold, the biomarker showed a sensitivity of 67.4%, specificity of 61.9%, positive predictive value (PPV) of 78.4%, and negative predictive value (NPV) of 48.2%. These results indicate that higher BALF TGF-β1 levels are associated with lung cancer, supporting its role as an adjunct biomarker rather than a stand-alone diagnostic test (Table 5).

Table 5. Diagnostic performance of TGF-β1 as a biomarker for lung cancer (n=64)

Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
105.63	67.44	61.90	78.38	48.15

Note: PPV=Positive Predictive Value; NPV=Negative Predictive Value

DISCUSSION

This study demonstrated a progressive increase in BALF TGF-β1 levels from the non-lung cancer group (median 66.24 pg/mL) to the suspected lung cancer group (median=136.93 pg/mL) and the lung cancer group (median=200.25 pg/mL). This stepwise elevation reflects the biological progression from non-malignant to malignant pulmonary conditions and supports the role of TGF-β1 in the multistage process of lung carcinogenesis.[9,10]

The results are consistent with Chen et al, who reported significantly higher BALF TGF-β1 concentrations in lung cancer patients compared with benign pulmonary diseases (18.2 vs. 8.4 pg/mL; *P*=0.003).[11] Differences in absolute values may result from methodological variations, population differences, and assay standardization.

The wide variation in TGF-β1 values observed in the non-cancer group indicates biological heterogeneity influenced by inflammation, comorbidities, and individual immune responses.[12,13] Elevated TGF-β1 levels in lung cancer patients may be explained by several mechanisms. Tumor cells can actively secrete TGF-β1 in response to hypoxia and oxidative stress, while macrophages and activated fibroblasts in the tumor microenvironment also contribute to local production.[8] Furthermore, TGF-β1 promotes epithelial-to-mesenchymal transition (EMT), which enhances tumor invasion and metastasis through a self-amplifying feedback mechanism.[14]

In BALF, TGF-β1 concentrations reflect local pulmonary cytokine activity more accurately than serum measurements. Thus, BALF analysis provides better sensitivity for detecting early molecular changes within the alveolar microenvironment.

When the suspected and confirmed cancer groups were combined, the Mann–Whitney test revealed a significant difference (*P*=0.025). This grouping was clinically justified because both groups require similar diagnostic and therapeutic management. These findings support the hypothesis that elevated TGF-β1 levels can differentiate benign from malignant or pre-malignant pulmonary conditions, although it may not precisely distinguish between suspected and confirmed cancer cases.[15,16] The significant association between TGF-β1 and malignancy supports its role not merely as a secondary response but as an active participant in tumor initiation and progression.[17]

The ROC curve analysis revealed an AUC of 0.674 (95% CI=0.529–0.819; *P*=0.025), indicating fair discriminatory ability. The optimal cut-off of 105.63 pg/mL, determined by the Youden Index, yielded a sensitivity of 67.4% and specificity of 61.9%. These findings suggest a fair ability of the biomarker to differentiate between groups, with a balanced trade-off between sensitivity and specificity.[18]

Although moderate, these metrics suggest that TGF-β1 has diagnostic relevance. Sensitivity of 67.4% means that two-thirds of lung cancer cases could be identified, while the specificity of 61.9% indicates moderate accuracy in ruling out non-cancer cases. The PPV of 78.4% highlights TGF-β1's utility as a supportive "rule-in" biomarker, whereas the lower NPV of 48.2% shows limited ability to exclude malignancy. Therefore, TGF-β1 should be interpreted alongside clinical, radiologic, and cytologic findings rather than as a standalone diagnostic marker.

The gradual increase of TGF-β1 from non-cancer to cancer groups and its moderate diagnostic accuracy support its potential as an adjunctive biomarker in lung cancer diagnosis. Compared with conventional serum markers such as Carcinoembryonic Antigen (CEA) or Cytokeratin 19 fragment antigen 21-1 (CYFRA 21-1), TGF-β1 provides additional biological insight by reflecting tumor-promoting pathways like EMT, angiogenesis, and immune suppression.[19,20]

BALF measurement offers advantages such as proximity to the disease site, minimally invasive sampling during bronchoscopy, and broad assay availability. However, several factors, such as sampling variability, assay differences, and confounding pulmonary conditions (e.g., chronic obstructive pulmonary disease (COPD), fibrosis, and infection), can influence the results.

LIMITATION

The main limitations of this study include variability in BALF sampling technique, lack of standardized processing protocols, and potential confounding by pulmonary comorbidities that may influence TGF-β1 levels. Conditions such as pulmonary fibrosis, acute COPD exacerbations, or respiratory infections can elevate TGF-β1, potentially leading to false-positive results. Therefore, TGF-β1 interpretation should always be integrated within a comprehensive clinical and radiological context.

CONCLUSION

This study found a progressive increase in BALF TGF-β1 levels from non-lung cancer to suspected and confirmed lung cancer groups, consistent with its role in lung carcinogenesis. A significant association was observed between BALF TGF-β1 levels and lung cancer occurrence. The optimal cut-off value of 105.63 pg/mL demonstrated fair discriminatory ability. Overall, BALF TGF-β1 may serve as a supportive biomarker to reinforce clinical suspicion of malignancy but is not suitable as a stand-alone diagnostic test. Its clinical application should be integrated within a multiparametric diagnostic approach.

Future research should focus on larger and more diverse populations to validate these findings and establish standardized procedures for BALF collection and processing. Future research should also evaluate TGF-β1 performance across different histological subtypes of lung cancer and explore its integration into multimarker diagnostic panels to enhance diagnostic accuracy. In addition, longitudinal studies are warranted to assess the utility of TGF-β1 in monitoring therapeutic response and post-treatment surveillance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study or manuscript preparation.

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