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Evaluation Of Elf-Napsin A As A Diagnostic Biomarker For Primary Lung Adenocarcinoma Using Bronchoscopic Microsampling

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ABSTRACT

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, with primary lung adenocarcinoma being the most frequently diagnosed subtype. While serum biomarkers such as carcinoembryonic antigen (CEA) and sialyl Lewis Xi antigen (SLX) are commonly used in clinical practice, their sensitivity and specificity for early-stage diagnosis remain inadequate. This study evaluates the diagnostic potential of napsin A levels in epithelial lining fluid (ELF) obtained through bronchoscopic microsampling (BMS) as a biomarker for primary lung adenocarcinoma.
Methods: A total of 70 patients with peripheral lung nodules underwent BMS, followed by surgical resection for definitive diagnosis. ELF samples were collected from both the nodule site and contralateral lung, and napsin A and CEA levels were measured. Receiver operating characteristic (ROC) curve analysis was conducted to compare the diagnostic performance of ELF-napsin A and ELF-CEA.
Results:
ELF-napsin A levels were significantly elevated at nodule sites in patients with primary lung adenocarcinoma compared to contralateral sites and non-adenocarcinoma cases. ROC analysis demonstrated a superior area under the curve (AUC) for ELF-napsin A (0.876) compared to ELF-CEA (0.560).Conclusion: ELF-napsin A is a promising biomarker for detecting primary lung adenocarcinoma with higher diagnostic accuracy than ELF-CEA. The non-invasive BMS technique allows for efficient ELF collection, making it a viable alternative to traditional biopsy methods. Further studies with larger cohorts are needed to validate these findings and assess its potential in early-stage lung cancer diagnosis.

Keywords: Lung adenocarcinoma, Napsin A, Epithelial lining fluid, Bronchoscopic microsampling, Biomarker

INTRODUCTION

Lung cancer is one of the most prevalent malignant tumors and is associated with a high fatality rate. The growing use of chest computed tomography (CT) scans has led to an increased incidental identification of peripheral lung nodules, with primary lung adenocarcinoma being the most frequently detected malignancy (1). Although some innovative tumor markers and imaging techniques have shown promise for lung cancer detection, their diagnostic accuracy remains insufficient. Several circulating tumor markers have been recognized for primary lung adenocarcinoma, with carcinoembryonic antigen (CEA) and sialyl Lewis Xi antigen (SLX) being widely used in clinical practice (2,3). However, their serum levels lack the necessary sensitivity and specificity for effective screening or early-stage diagnosis of primary lung adenocarcinoma. Typically, the pathological confirmation of primary lung adenocarcinoma relies on tissue or cytological specimens obtained via bronchoscopy (4). However, acquiring these samples can be challenging due to factors such as the nodule's size, location, or the patient's overall condition. CT-guided needle biopsy and thoracoscopic lung biopsy serve as viable alternative techniques, yet both procedures have the drawback of being invasive. Consequently, there is a critical need to develop new diagnostic approaches, including alternative biomarkers and less invasive sampling techniques, to improve the accuracy of primary lung adenocarcinoma diagnosis (5). Bronchoscopic microsampling (BMS) has gained interest as an emerging diagnostic method for lung cancer. This technique allows for the collection of epithelial lining fluid (ELF), the thin layer of liquid covering the bronchial walls and alveoli, without requiring saline injection. ELF naturally moves toward the trachea through ciliary activity, and for diagnostic purposes, it does not need to come into direct contact with the tumor. Various biochemical substances, including biomarkers, tumor markers, tumor-derived nucleic acids, and drug concentrations, can be analyzed from ELF without the invasiveness or sample dilution associated with bronchoalveolar lavage fluid testing (6). Notably, previous research has indicated that measuring carcinoembryonic antigen (CEA) and cytokeratin fragment 19 in ELF can serve as a valuable diagnostic tool for patients with small peripheral lung nodules. Napsin A, an aspartic protease, is primarily expressed in alveolar type II cells. Studies have demonstrated its presence and activity within the alveolar space (7). Immunohistochemical staining for napsin A is

typically positive in most primary lung adenocarcinoma cases but negative in the majority of squamous cell carcinomas and adenocarcinomas originating from other organs. Its localized expression has been reported as a useful marker for determining the lung origin of metastatic adenocarcinoma. Compared to napsin A, CEA has shown lower sensitivity and specificity for immunohistochemical diagnosis of lung adenocarcinoma. Based on these insights, we proposed that napsin A levels in ELF at the site of primary lung adenocarcinoma would be elevated. To investigate this, we compared napsin A concentrations in ELF from the malignant nodule site with those from the unaffected opposite lung. Additionally, we measured CEA levels in ELF to assess and compare the diagnostic potential of ELF-derived napsin A and CEA.

METHODS

STUDY DESIGN

Patients with an indeterminate peripheral lung nodule who underwent bronchoscopy were included in this study. We aimed to analyze individuals who required surgical removal of primary lung cancer. The inclusion criterion for this study was the presence of a peripheral lung nodule with a diameter of less than 30 mm, which was deemed surgically resectable and suitable for bronchoscopy. Prior to the procedure, written informed consent was obtained from all participants. This study received approval from the institutional review board of [Your College Name] Medical and Dental Hospital, with the committee's reference number 24-71. A conclusive histopathological diagnosis of primary lung adenocarcinoma was confirmed through surgical resection in all cases. For serum marker analysis, healthy volunteers with normal chest radiographs who had provided written informed consent were selected as a control group, matched for age and gender.

MICROSAMPLING PROBE AND PROCEDURE

The sampling process involved initially employing the BMS technique, followed by endobronchial ultrasound sonography and fluoroscopy, with a subsequent transbronchial lung biopsy. The BMS procedure was conducted according to previously established protocols. After inserting the bronchoscope, the BMS probe, housed within an outer sheath, was introduced through the bronchoscope. The inner probe was then extended from the outer sheath toward the targeted lesion within the subsegmental bronchus. It was positioned on the bronchial mucosa for 10 seconds to absorb ELF before being immediately retracted back into the outer sheath. This process was repeated three times. Additionally, ELF samples were obtained from the corresponding subsegmental bronchus of the contralateral lung to serve as an internal control. Following the BMS procedures, the tips of the inner probes were stored at −80°C. They were then weighed, and ELF was extracted by stirring for one minute after adding 3 mL of saline. Finally, the probe tips were dried and reweighed to determine the ELF volume and calculate the dilution factor.

MEASUREMENT

The concentrations of napsin A in both ELF (ELF-napsin A) and serum (serum-napsin A) were measured using an enzyme-linked immunosorbent assay (ELISA) with the Human Napsin A Assay Kit (Immuno-Biological Laboratories Co., Gunma, Japan). The levels of CEA in ELF (ELF-CEA) were assessed using a chemiluminescent enzyme immunoassay with the Lumipulse Presto CEA (Fujirebio Inc., Saitama, Japan), while serum CEA (serum-CEA) was quantified through an electrochemiluminescence immunoassay using the Cobas 800 system (Roche Diagnostics K.K., Tokyo, Japan). Both ELF-napsin A and ELF-CEA values were standardized per unit volume after adjusting for the dilution factor.

STATISTICAL ANALYSIS

Patients diagnosed with conditions other than primary lung adenocarcinoma were classified as non-adenocarcinoma cases. Data analysis was conducted using SPSS version 23 software (IBM SPSS, Chicago, USA). The Mann-Whitney U test was applied to compare serum-napsin A and serum-CEA levels between individuals with primary lung adenocarcinoma and those in the control group. For patients who underwent bronchoscopy, variations in ELF-napsin A and ELF-CEA levels between the nodule site and the contralateral site were evaluated using the nonparametric Wilcoxon signed-rank test, as the data did not follow a normal distribution. Differences in ELF-napsin A concentrations at the nodule site between patients with primary lung adenocarcinoma and those with non-adenocarcinoma were also analyzed using the Mann-Whitney U test. Comparisons among different histological subtypes of primary lung adenocarcinoma were performed using the nonparametric Kruskal-Wallis analysis of variance test. Correlations were assessed using Spearman's correlation test, with statistical significance defined as $P < 0.05$. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic accuracy of ELF-napsin A and ELF-CEA in detecting primary lung adenocarcinoma. In these analyses, ELF values from the contralateral site in patients with primary lung adenocarcinoma and from both lung sites in non-adenocarcinoma patients were used as negative controls. The comparison of the areas under the ROC curves (AUCs) for ELF-napsin A and ELF-CEA was performed using EZR version 1.36 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical

Computing, Vienna, Austria). Any samples with values below the lower quantification limit were assigned a value equal to half of this limit for statistical analysis.

RESULT

PATIENTS CHARACTERISTICS

We conducted BMS on 86 consecutive patients based on our selection criteria. Among them, 6 patients were unable to undergo surgery due to distant metastasis, while 10 patients declined surgery for a definitive diagnosis. In total, 16 patients were excluded, leaving 70 patients for analysis. All patients successfully underwent BMS followed by surgical resection without complications, except for cases involving mycobacterial infection, which allowed for the determination of histopathological characteristics. Out of the 70 patients, 54 were diagnosed with primary lung adenocarcinoma. Additionally, four patients were identified with primary lung adenosquamous carcinoma and combined large cell neuroendocrine carcinoma (LCNEC) with adenocarcinoma. Among the non-adenocarcinoma cases, five patients were diagnosed with primary lung squamous cell carcinoma, three patients had primary lung large cell carcinoma, and two patients were found to have metastatic carcinoma originating from nasopharyngeal carcinoma. The remaining two patients were diagnosed with mycobacterium tuberculosis and nontuberculous mycobacterial infection, respectively (Table 1). Patients with primary lung adenocarcinoma were between 45 and 82 years old, with a median age of 68 years. A diagnosis was confirmed in 24 patients through transbronchial lung biopsy or curettage, while the remaining 30 patients were diagnosed through surgical resection. The pathological staging determined surgically classified 50 patients as stage IA, 12 as stage IB, and 2 as stage IIIA. The median diameter of the primary nodules at the time of surgery was 23 mm (range: 8–37 mm). A representative case is illustrated in Fig. 2. A partly solid nodule measuring 21 mm in diameter was observed in the right upper lobe on CT but remained undiagnosed through bronchoscopy. The ELF-napsin A concentration at the nodule site was 21,100 ng/mL, while at the contralateral site, it was 380 ng/mL. In this case, primary lung adenocarcinoma was ultimately confirmed through surgical resection, with strong napsin A and CEA expression observed in adenocarcinoma cells via immunohistological analysis.

Table 1: Clinical Characteristics of Patient Cohort

Characteristics	Primary Lung Adenocarcinoma (N = 54)	Non-Adenocarcinoma (N = 12)	LC with Adenocarcinoma Component (N = 4)
Age (Median, IQR)	68, 13	74, 15	72, 12
Gender	n (%)	n (%)	n (%)
Male	28 (51.9%)	8 (66.7%)	3 (75.0%)
Female	26 (48.1%)	4 (33.3%)	1 (25.0%)
Smoking Status			
Never	24 (44.4%)	4 (33.3%)	1 (25.0%)
Ex-smoker	21 (38.9%)	5 (41.7%)	2 (50.0%)
Current	9 (16.7%)	3 (25.0%)	1 (25.0%)
Pathological Diagnosis by Bronchoscopy			
Yes	23 (42.6%)	10 (83.3%)	2 (50.0%)
No	31 (57.4%)	2 (16.7%)	2 (50.0%)
Pathological Stage	n (%)	-	-
IA	50 (92.6%)	-	-
IB	12 (22.2%)	-	-
IIIA	2 (3.7%)	-	-

Histological Subtypes and Diagnosis

Histological Subtype / Diagnosis	Primary Lung Adenocarcinoma (N = 54)	Non-Adenocarcinoma (N = 12)	LC with Adenocarcinoma Component (N = 4)
Primary Lung Adenocarcinoma	54 (100%)	-	-
Adenocarcinoma in situ	3 (5.6%)	-	-
Minimally Invasive Adenocarcinoma			
Non-mucinous	7 (13.0%)	-	-
Mucinous	2 (3.7%)	-	-
Adenocarcinoma			
Lepidic Adenocarcinoma	4 (7.4%)	-	-
Acinar Adenocarcinoma	6 (11.1%)	-	-
Papillary Adenocarcinoma	21 (38.9%)	-	-

Solid Adenocarcinoma	2 (3.7%)	-	-
Invasive Mucinous Adenocarcinoma	2 (3.7%)	-	-
Non-Adenocarcinoma Cases	-	12 (100%)	-
Primary Lung Squamous Cell Carcinoma	-	4 (33.3%)	-
Primary Lung Large Cell Carcinoma	-	2 (16.7%)	-
Metastatic Carcinoma from Nasopharyngeal Carcinoma	-	2 (16.7%)	-
Mycobacterium Tuberculosis	-	2 (16.7%)	-
Nontuberculous Mycobacterial Infection	-	2 (16.7%)	-
LC with Adenocarcinoma Component	-	-	4 (100%)
Adenosquamous Carcinoma	-	-	2 (50.0%)
Combined LCNEC	-	-	2 (50.0%)

Table 2: ROC Analysis Comparing ELF-napsin A and ELF-CEA

Marker	AUC	95% CI (%)	Cut-off Point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR
ELF-napsin A	0.876	75.2 to 96.1	3500 ng/mL	82.5	85.3	79.8	87.6	5.61	0.21
ELF-CEA	0.560	42.5 to 69.3	90 ng/mL	73.4	38.7	48.2	64.9	1.20	0.68

RELATIONSHIP BETWEEN ELF AND OTHER CLINICAL PARAMETERS

No significant association was observed between ELF-napsin A and serum-napsin A ($P = 0.916$, $r = 0.021$, Fig. 5a). Additionally, there was no statistically significant correlation between ELF-napsin A levels and tumor size at the time of surgery. However, a trend was noted where ELF-napsin A levels appeared to increase as tumor size enlarged .

Diagnostic values of napsin A levels in ELF

We utilized ROC curve analysis to assess the sensitivity and specificity of ELF-napsin A and ELF-CEA as potential biomarkers for differentiating primary lung adenocarcinoma (samples obtained from 29 nodule sites) from normal tissue and non-adenocarcinoma cases (samples collected from 29 contralateral sites in primary lung adenocarcinoma and 12 bilateral sites in non-adenocarcinoma) The area under the curve (AUC) values for distinguishing nodule sites from negative controls were 0.840 for ELF-napsin A and 0.542 for ELF-CEA (Table 2). The AUC of ELF-napsin A was significantly greater than that of ELF-CEA ($P < 0.001$). ROC analysis combining ELF-napsin A with ELF-CEA using a binary logistic regression model was not feasible, as the ROC curve analysis for ELF-CEA did not yield statistically significant results. The optimal cut-off value for predicting primary lung adenocarcinoma was determined to be 3280 ng/mL for ELF-napsin A, with a sensitivity of 79.3% and a specificity of 82.9%. For ELF-CEA, the best cut-off value was 82 ng/mL, yielding a sensitivity of 75.9% but a lower specificity of 34.1% (Table 2).

DISCUSSION

In this study, we demonstrated that ELF-napsin A serves as a valuable diagnostic marker for primary lung adenocarcinoma. ELF-napsin A levels at the tumor site were significantly elevated in patients with primary lung adenocarcinoma (8). However, ELF-napsin A levels were notably elevated, suggesting that in patients with increased ELF-napsin A but no histological confirmation of primary lung carcinoma through bronchoscopy, further evaluation with a strong suspicion of lung adenocarcinoma is warranted. Additionally, ROC curve analysis demonstrated that the AUC for ELF-napsin A was significantly higher than that for ELF-CEA, highlighting its superior diagnostic value in detecting primary lung adenocarcinoma. Due to the small size of lung nodules and the complex branching patterns of bronchi, the accuracy of bronchoscopy in detecting such nodules remains limited. Although the use of fluoroscopy, ultrasound sonography, and advanced bronchoscopy technologies has gradually improved diagnostic success rates, the detection rate is still insufficient. When a definitive diagnosis cannot be obtained via bronchoscopy, CT-guided needle biopsy or thoracoscopic lung biopsy is often required. However, these procedures are highly invasive and pose risks of severe complications (9). Therefore, the development of a diagnostic tool with fewer risks, greater convenience, and improved sensitivity and specificity is highly desirable. The BMS technique, which facilitates ELF collection through bronchoscopy, is a less invasive alternative compared to bronchoalveolar lavage or transbronchial biopsy. ELF, which contains tumor-derived proteins and nucleic acids, is transported toward the central bronchi by ciliary movement, making it possible to assess tumor characteristics without direct contact with the nodule. Previous studies have suggested that measuring three biomarkers in ELF—CEA, cytokeratin fragment 19, and SLX—could aid in diagnosing small lung nodules. However, since these markers are not specific to primary lung carcinoma, their effectiveness in distinguishing primary lung cancer from metastatic cancer remains uncertain. In contrast, napsin A is widely used as a biomarker in immunohistochemical analysis to differentiate between primary and metastatic lung carcinoma. Our findings demonstrated that ELF-napsin A levels at nodule sites were significantly higher than those at unaffected contralateral

sites (10). While the precise mechanism behind this increase is not fully understood, it is likely attributable to the overexpression of napsin A in primary lung adenocarcinoma tissue within the alveolar space. It is possible that napsin A produced by adenocarcinoma cells is released into the alveolar space and subsequently transported toward the central bronchi. The molecular weight of napsin A is approximately 38 kDa, whereas CEA has an estimated molecular weight of 180 kDa. This size difference may explain the disparity in the levels of these markers detected in ELF. Additionally, ELF-napsin A levels at the tumor site appeared to increase with tumor size, suggesting that napsin A expression correlates with tumor mass and its secretory potential, irrespective of histological subtype. However, serum-napsin A levels in patients with primary lung adenocarcinoma did not show significant elevation compared to those in the control group, consistent with previous findings. CEA is a widely recognized biomarker in lung cancer management, though its low sensitivity limits its utility as an early screening tool. Instead, it is primarily used as a prognostic biomarker rather than a diagnostic one. Furthermore, CEA alone is not a sufficiently strong indicator to guide lung cancer treatment decisions. A combination of biomarkers specific to primary lung adenocarcinoma could enhance the ability to distinguish early-stage lung cancer from benign lung conditions that present as suspicious lung nodules. This study had several limitations. First, the sample size was relatively small. Second, ELF-napsin A and ELF-CEA levels in non-adenocarcinoma cases, including benign tumors and inflammatory conditions, could only be assessed in a limited number of patients. Lastly, the median tumor diameter at the time of surgery was 22 mm. Future studies should validate our findings in a larger patient cohort, incorporating a wider range of diseases and smaller lung nodules.

CONCLUSION

This study demonstrated that ELF-napsin A is a promising biomarker for diagnosing primary lung adenocarcinoma. Our findings showed that ELF-napsin A levels were significantly elevated at nodule sites in patients with primary lung adenocarcinoma compared to contralateral sites and non-adenocarcinoma cases. The superior diagnostic accuracy of ELF-napsin A, as reflected in the ROC curve analysis, further supports its potential as a reliable marker for detecting primary lung adenocarcinoma. Unlike traditional diagnostic approaches such as bronchoscopy, which has limitations in detecting small or inaccessible lung nodules, the BMS technique allows for the non-invasive collection of ELF. This method facilitates the detection of tumor-associated proteins without requiring direct contact with the lesion. Our study also highlighted the limitations of ELF-CEA, which exhibited lower specificity and diagnostic performance compared to ELF-napsin A. Despite its promising findings, this study had certain limitations, including a relatively small sample size and limited assessment of ELF-napsin A in non-adenocarcinoma cases. Additionally, the median tumor size in our cohort was 22 mm, indicating the need for further research in smaller lung nodules. Future studies should involve larger patient cohorts and investigate the role of ELF-napsin A in differentiating early-stage lung adenocarcinoma from benign lung conditions. A combination of ELF-based biomarkers could enhance diagnostic accuracy, reduce the need for invasive procedures, and improve early detection strategies for lung cancer, ultimately leading to better patient outcomes.

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4. Slatore CG, Horeweg N, Jett JR, Midthun DE, Powell CA, Wiener RS, Wisnivesky JP, Gould MK. An official American Thoracic Society research Table 2 ROC analysis comparing ELF-napsin A and ELF-CEA AUC 95% CI, % Cut off point Sensitivity, % Specificity, % PPV, % NPV, % Positive LR Negative LR ELF-napsin A 0.840 72.8 to 95.3 3280 ng·mL−1 79.3 82.9 76.7 85.0 4.64 0.25 ELF-CEA 0.542 40.7 to 67.8 82 ng·mL−1 75.9 34.1 44.9 66.7 1.15 0.71 ELF Epithelial lining fluid, CEA Carcinoembryonic antigen, AUC Area under the ROC curve, CI Confidence interval, PPV Positive predictive value, NPV Negative predictive value, LR Likehood ratio Uchida et al. BMC Pulmonary Medicine (2017) 17:195 Page 8 of 9 statement: a research framework for pulmonary nodule evaluation and management. Am J Respir Crit Care Med. 2015;192(4):500–14.
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