RESEARCH ARTICLE

Editorial Process: Submission:06/14/2024 Acceptance:09/19/2024

Correlation of *CD 133* Biomarker with Outcomes and Therapy Response in Advanced-Stage Non-Small Cell Lung Carcinoma

Sri Mayasari[1,2], Arif Santoso[1,2]*, Irawaty Djaharuddin[1,2], Harun Iskandar[1,2], Nurjannah Lihawa[1,2], Harry Akza Putrawan[1,2], Imam Nurjaya[1,2], Rasiha Rasiha[3]

Abstract

Objective: This study aimed to analyze *CD 133* stem cell biomarker levels in serum and bronchial lavage, as well as correlate these levels with treatment responses and outcomes in patients with advanced-stage NSCLC. **Methods:** This is a cross-sectional, observational, and analytical study that analyzed data from NSCLC patients and control at Dr. Wahidin Sudirohusodo Hospital, Makassar. *CD 133* levels in serum and bronchial lavages were quantified using ELISA and correlated with treatment responses assessed by RECIST 1.1 criteria. **Result:** A total of 118 participants were used, with 66 being NSCLC patients and 52 as the control. The results showed significant differences in *CD 133* serum and bronchial lavage levels ($p<0.05$). Moreover, NSCLC patients with advanced tumors (T4) had higher *CD 133* levels in bronchial wash ($p<0.05$). The bronchial wash *CD 133* test showed higher sensitivity (83.6%) and specificity (79.2%) compared to serum *CD 133*. Even though *CD 133* levels were not significantly correlated with treatment response, they were higher in patients with advanced tumor stages. **Conclusion:** *CD 133* levels were significantly higher in bronchial wash samples from NSCLC patients with advanced tumors (T4). Also, there was a positive correlation between *CD 133* levels in bronchial wash and serum. These results emphasized the potential of *CD 133*, particularly in bronchial wash, as a valuable diagnostic tool for NSCLC.

Keywords: *CD 133*- RECIST- Bronchial lavage- Non-Small Cell Lung Cancer

Asian Pac J Cancer Prev, **25 (9)**, 3315-3325

Introduction

In the last three decades, lung cancer has become one of the predominant malignancies and a leading cause of cancer-related deaths globally. It was estimated that lung cancer accounted for 1.79 million cases worldwide, contributing to approximately 18% of all cancer-related mortalities [1]. Characteristically, the disease, rarely shows symptoms in its early stages, leading to majority of diagnoses at more advanced stages. Lung cancer metastasizes through intrapulmonary, intrathoracic, extrapulmonary, and extrathoracic routes. Extrathoracic metastases commonly affected distant organs including the brain, bones, liver, and adrenal glands through the lymphatic system [2,3]. In Indonesia, the disease is responsible for 30,843 deaths annually, representing about 13.2% of all cancer-related deaths per year [1].

Carcinoma Stem Cells (CSCs) are a small subset of cancer cells with self-renewal capabilities, playing a critical role in tumor metastasis, recurrence, and therapy resistance [4,5]. Cluster of Differentiation (CD) 44 is among the potential lung cancer CSC biomarkers and is important in pathological angiogenesis, and the production of radixin and moesin, which facilitates cell growth and membrane stability. Meanwhile, *CD 133* is another significant CSC biomarker, a potential therapeutic target and prognostic factor. Epithelial Cell Adhesion Molecule (EpCAM) or CD 326 is identified as a potential biomarker for epithelial-derived neoplasms and a homotypic calcium-independent cell adhesion molecule. Furthermore, the co-expression of ABCG2 (ATP-binding cassette subfamily G member 2), known as the breast cancer resistance protein, with *CD 133*, has been associated with increased cisplatin resistance and higher tumor recurrence rates [6,7].

CD 133, also known as prominin-1, is an antigen present on the cell surface, widely used to identify CSC populations across various cancer types [8]. A study by Weigmann et al. showed that *CD 133* was localized in the microvilli on the apical surfaces of several epithelial cells, such as the ependymal lining of the brain and the brush border membrane of kidney tubules in mice [9,10]. A study conducted in the year 2000 on the presence of *CD 133* in microvilli found cholesterol-based lipid microdomains on the apical plasma membrane, and

*[1]Pulmonology and Respiratory Medicine Department, Medical Faculty of Hasanuddin University, Makassar, South Sulawesi, Indonesia. [2]Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, Indonesia. [3]Medical Faculty of Hasanuddin University, Makassar, South Sulawesi, Indonesia. *For Correspondence: arifs777@gmail.com*

suggested it as a novel marker for cholesterol-based lipid formation [11,12]. It was necessary to investigate *CD 133* as a biomarker for therapy response in lung cancer. Therefore, this study aimed to analyze *CD 133* stem cell biomarker levels in serum and bronchial lavage, as well as correlate these levels with treatment responses (RECIST 1.1) and outcomes in patients with advanced-stage non-small cell lung carcinoma (NSCLC) at Dr. Wahidin Sudirohusodo Hospital, Makassar.

Materials and Methods

Study Design

The study used a cross-sectional observational analytic method, with retrospective observations. The data collection focused on pre-diagnostic lung cancer patients admitted for care at Dr. Wahidin Sudirohusodo Hospital. Clinical data necessary for the study were obtained during the patients care. In addition, blood serum and bronchial lavage samples were analyzed to evaluate *CD 133* levels.

Study Population

The population consisted of all NSCLC patients who received therapy and were admitted to Dr. Wahidin Sudirohusodo Hospital, Makassar. The inclusion criteria were patients treated at Dr. Wahidin Sudirohusodo Hospital, adults aged ≥18 years, pre-diagnostic lung cancer patients undergoing bronchoscopy procedures, patients currently receiving therapy, and those who consent to participate in the study. Meanwhile, the exclusion criteria comprised patients unwilling to participate, those suffering from acute/chronic lung diseases, patients with non-NSCLC lung cancer, and patients who had previously received cancer therapy.

Data and sample collection

The participants were lung cancer patients who registered from September to November 2023. We monitored for signs of metastasis after the third cycle of chemotherapy, which corresponds to a period of 60-90 days following the initial assessment. The outcome status, specifically whether participants were deceased or alive, was evaluated three months after the initial assessment.

Body Mass Index (BMI) is used to determine nutritional status according to the World Health Organization (WHO), calculated by dividing weight in kilograms by the square of height in meters, and classifying individuals as underweight (<18.5), normal (18.5 – 22.99), overweight (23 – 24.99), or obese (>25). Performance status estimates a patient's ability to perform daily activities without assistance, using the ECOG scale, ranging from 0 (full activity without restrictions) to 4 (completely bedridden and unable to perform any self-care) [13]. The TNM staging system, based on the 8th edition, is a globally recognized standard for classifying the extent of cancer spread, categorizing patients into stages with or without metastasis [14].

The Brinkman Index (BI) quantifies smoking exposure by multiplying the number of cigarettes smoked daily by the number of years the subject has smoked. It categorizes smokers into light (1-200), moderate (200-600), and heavy (>600) categories [15]. The RECIST 1.1 criteria evaluates patient status based on clinical symptoms, weight changes, and imaging results. Measurements are taken before chemotherapy and compared at the third-month follow-up, categorizing outcomes into complete response, partial response, progressive disease, or stable disease [16].

Sample collection was carried out in the Pulmonology and Respiratory Medicine Department of the hospital. The samples were stored at -80°C in the Sample Bank and *CD 133* level analysis was conducted at the Hasanuddin University Medical Research Unit (HUM-RC). The samples included bronchial lavage and serum obtained from 3 ml of venous blood from the participants. In this case, the samples were stabilized for 24 hours at 2-8°C. Equipment such as refrigerators, deep freezers (-80°C), spectrophotometers, incubators, ELISA plates, readers, centrifuges, and sterilization devices were used, along with distilled water, ice, and a Human ELISA kit (No. LS-F10515) from LifeSpan BioSciences Inc. The assay was based on the sandwich enzyme-linked immunosorbent assay (ELISA) principle.

Data Analysis

The data obtained were recorded in forms and categorized according to purpose and type. Appropriate statistical methods were selected for univariate analysis to describe frequencies and percentages of participants characteristics (median, minimum, and maximum values), and bivariate analysis to test the data normality using Kolmogorov-Smirnov/Shapiro-Wilk tests. Moreover, categorical data were analyzed using unpaired T-tests, numeric categorical data using Mann-Whitney/Kruskal-Wallis tests, and numeric data using Spearman correlation tests. A significance level of $\alpha = 5\%$ or 0.05 and $\beta = 95\%$ was used, where $p < 0.05$ indicated a significant relationship or difference between two variables.

Results

The flowchart in Figure 1 illustrates the selection of 118 eligible participants from an initial 134 pro-diagnostic tumor patients, excluding 16 who had received chemotherapy. The participants were divided into 66 NSCLC patients and 52 controls. Out of the NSCLC patients, 24 died and 42 survived. Among those who died, treatment types included systemic chemotherapy (6), immune checkpoint inhibitor (1), ALK inhibitor (1), tyrosine kinase inhibitor (7), systemic chemotherapy with radiotherapy (3), and tyrosine kinase inhibitor with radiotherapy (1). Additionally, 4 patients stopped chemotherapy due to adverse events, and 19 refused chemotherapies.

Study Characteristics

Based on the data in Table 1, this study used 118 participants, with 66 diagnosed with Non-Small Cell Lung Carcinoma (NSCLC) and the remaining 52 served as control. The NSCLC patients group predominantly comprised males (69.2%) aged 50 years and above (84.8%), and the majority being of Bugis ethnicity (72.7%). Meanwhile, the control group primarily consisted

Table 1. Study Characteristic

Variable	Total (n = 118) %	NSCLC (n = 66) %	Control (n = 52) %	P-value
Age				0.000 [a]
<50	40 (33.9)	10 (15.2)	30 (57.7)	
≥50	78 (66.1)	56 (84.8)	22 (42.3)	
Sex				0.524 [a]
Male	78 (66.1)	42 (69.2)	36 (63.6)	
Female	40 (33.9)	24 (36.4)	16 (30.8)	
Ethnic				0.058 [b]
Makassar	18 (15.2)	5 (7.6)	13 (25.0)	
Bugis	73 (61.8)	48 (72.7)	25 (48.1)	
Toraja	8 (6.8)	4 (6.1)	4 (7.7)	
Tual	1 (0.8)	0 (0.0)	1 (1.9)	
Others	18 (15.2)	9 (13.6)	9 (17.3)	
Job				0.531 [b]
Farmer	23 (19.5)	15 (22.7)	8 (15.4)	
Laborer	4 (3.4)	2 (3.0)	2 (3.8)	
Employe	19 (16.1)	11 (16.7)	8 (15.4)	
Housewife	24 (20.3)	15 (22.7)	9 (17.3)	
Student	5 (4.2)	1 (1.5)	4 (7.7)	
Others	43 (36.4)	22 (33.3)	21 (40.4)	
BMI				1.000 [b]
Underweight	31 (26.3)	16 (24.2)	15 (28.8)	
Normal	83 (70.3)	47 (71.2)	36 (69.2)	
Overweight	3 (2.5)	2 (3.0)	1 (1.9)	
Obese	1 (0.8)	1 (1.5)	0 (0.0)	
Smoking History				0.281 [b]
Active Smoker	66 (55.9)	41 (62.1)	25 (48.1)	
Passive Smoker	21 (17.8)	13 (19.7)	8 (15.4)	
Non-Smoker	31 (26.3)	12 (18.2)	19 (36.5)	
Index Brinkman				0.520 [b]
Mild	10 (15.2)	3 (7.3)	7 (28.0)	
Moderate	24 (36.4)	16 (39.0)	8 (32.0)	
Heavy	32 (48.5)	22 (53.7)	10 (40.0)	
Comorbidity				
Tuberculosis	24 (20.3)	4 (6.1)	20 (38.4)	0.200 [c]
Other Infection	26 (22.0)	14 (21.2)	12 (23.1)	1.000 [c]
Diabetes mellitus	15 (12.7)	7 (10.6)	8 (15.3)	1.000 [c]
Hypertension	16 (13.6)	12 (18.2)	4 (7.7)	1.000 [c]
Symptoms				
Cough	31 (26.3)	15 (22.7)	16 (30.8)	0.989[b]
Hemoptysis	10 (8.5)	8 (12.1)	2 (3.8)	
Dyspnea	44 (37.3)	24 (36.4)	20 (38.5)	
Chest Pain	33 (28.0)	19 (28.8)	14 (26.9)	

Data reported as mean (SD) or n (%); [a], Chi-square test; [b], Kolmogrov-Smirnov; [c], Fisher's Exact test; BMI, Body ass Index; AE, Adverse Event

of individuals younger than 50 years (57.7%) with a significant proportion of Bugis individuals (48.1%). There was a significant age difference between the NSCLC and the control group (p=0.000), while other demographic variables such as gender, ethnicity, and occupation did not show significant differences.

Most participants in both groups had a normal Body Mass Index (BMI) (70.3%). Moreover, there was a high prevalence of active smokers in both NSCLC (62.1%) and control (48.1%) groups, with a predominant Brinkman

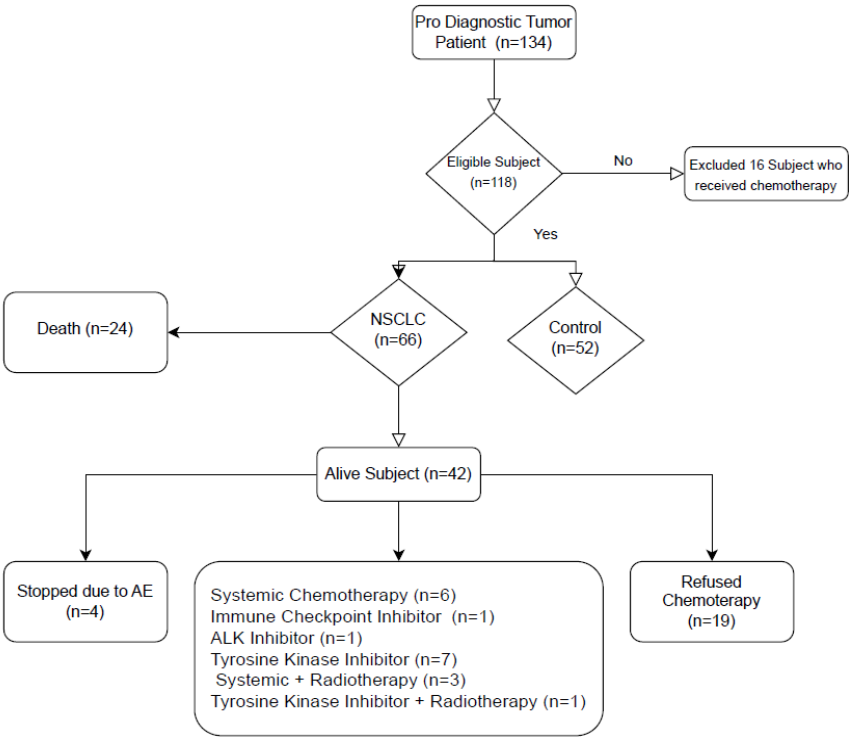

Figure 1. Study Flow Chart

Table 2. CD 133 Level Examination in NSCLC and Control Group

CD 133 Level	NSCLC (mean ± SD)		Control (mean ± SD)		P-value
Serum	0.550 ± 0.259	0.000 [a]	0.752 ± 0.171	0.000 [a]	0.000 [a]
Bronchial Lavage	0.330 ± 0.531		0.339 ± 0.762		0.000 [a]

[a], Mann Whitney Test

index classified as heavy (53.7% for NSCLC and 40.0% for control). Comorbidities like tuberculosis, infections, diabetes mellitus, and hypertension were also evaluated, with higher occurrences of tuberculosis and infections in the control group. The main symptomatic complaints among NSCLC patients included dyspnea (36.4%), chest pain (28.8%), cough (22.7%), and hemoptysis (12.1%), which were similar to the control group with dyspnea

Differences in AUC, Sensitivity, Specificity, and Cut-off Values between CD 133 in Bronchial Wash and Serum

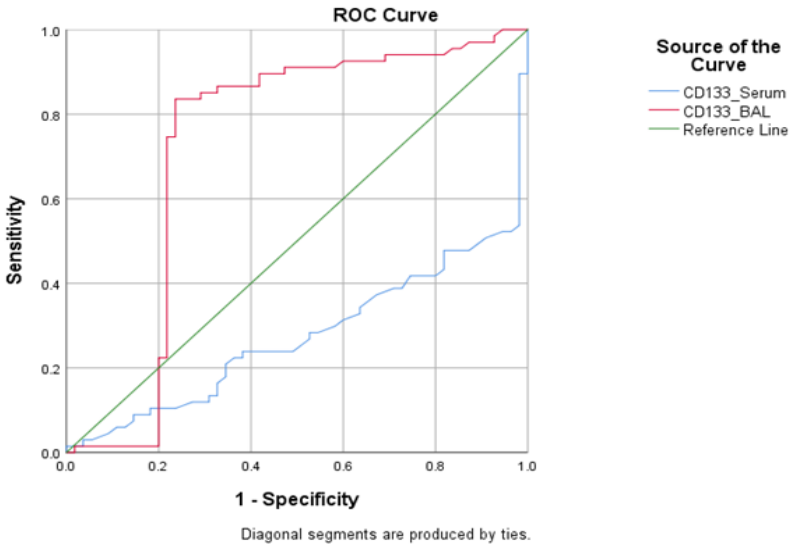

Figure 2. ROC Curve of Bronchial Wash CD 133 and Serum CD 133

Table 3. CD 133 Level NSCLC and Control Group Based on Clinical Characteristics

Variable	N = 66 (%)	CD 133 Serum Mean ± SD	P-Value	CD 133 Bronchial Lavage Mean ± SD	P-Value
Sex			0.805 [a]		0.480 [a]
Male	42 (69.2)	0.549 ± 0.260		0.352 ± 0.662	
Female	24 (36.4)	0.550 ± 0.263		0.292 ± 0.113	
Age			0.844 [a]		0.256 [a]
<50	10 (15.2)	0.527 ± 0.268		0.238 ± 0.114	
≥50	56 (84.8)	0.553 ± 0.259		0.347 ± 0.574	
Smoking History			0.135 [b]		0.426 [b]
Active Smoker	41 (62.1)	0.535 ± 0.262		0.359 ± 0.669	
Passive Smoker	13 (19.7)	0.659 ± 0.246		0.308 ± 0.122	
Non-Smoker	12 (18.2)	0.479 ± 0.247		0.256 ± 0.102	
Index Brinkman			0.902 [b]		0.962 [b]
Mild	3 (7.3)	0.529 ± 0.249		0.268 ± 0.056	
Moderate	16 (39.0)	0.525 ± 0.278		0.515 ± 1.059	
Heavy	22 (53.7)	0.544 ± 0.262		0.257 ± 0.150	
Symptoms			0.265 [b]		0.876 [b]
Cough	15 (22.7)	0.635 ± 0.236		0.288 ± 0.135	
Hemoptysis	8 (12.1)	0.569 ± 0.245		0.773 ± 1.494	
Dyspnea	24 (36.4)	0.375 ± 0.176		0.259 ± 0.108	
Chest Pain	19 (28.8)	0.593 ± 0.313		0.266 ± 0.147	
Histopathology			0.894 [b]		0.562 [b]
Squamous	52 (78.8)	0.551 ± 0.262		0.341 ± 0.597	
Non-Squamous	14 (21.2)	0.543 ± 0.256		0.290 ± 0.103	
Performance Status					0.950 [b]
1	27 (40.9)	0.547 ± 0.263	0.643 [b]	0.262 ± 0.143	
2	38 (57.6)	0.557 ± 0.261		0.379 ±0.689	
3	1 (1.5)	NA		NA	
Staging			0.779 [a]		0.472 [a]
Non-metastasis	7 (10.6)	0.482 ± 0.130		0.227 ± 0.158	
Metastasis	59 (89.4)	0.557 ± 0.270		0.342 ± 0.558	
T			0.676 [b]		0.013 [b]
T1	0 (0.0)	NA		NA	
T2	1 (1.5)	NA		NA	
T3	10 (15.2)	0.481 ± 0.276		0.190 ± 0.136	
T4	55 (83.3)	0.564 ± 0.258		0.361 ± 0.574	
N			0.412 [b]		0.511 [b]
N0	12 (18.2)	0.623 ± 0.246		0.652 ± 1.204	
N1	9 (13.6)	0.428 ± 0.219		0.236 ± 0.135	
N2	21 (31.8)	0.564 ± 0.284		0.261 ± 0.122	
N3	24 (36.4)	0.545 ± 0.255		0.264 ± 0.136	
M			0.220 [b]		0.927 [b]
M0	13 (19.7)	0.651 ± 0.214		0.244 ± 0.141	
M1a	23 (34.8)	0.503 ± 0.266		0.452 ± 0.879	
M1b	8 (12.1)	0.614 ± 0.298		0.252 ± 0.188	
M1c	22 (33.3)	0.514 ± 0.257		0.282 ± 0.121	
RECIST			0.708 [b]		0.708 [b]
Partial response	12 (18.2)	0.580 ± 0.247		0.293 ± 0.090	
Progressive disease	6 (6.0)	0.518 ± 0.290		0.273 ± 0.164	
Stable disease	1 (1.5)	NA		NA	

[a], Mann Whitney Test; [b], Kruskal-Wallis Test.

Table 3. CD 133 Level NSCLC and Control Group Based on Clinical Characteristics

Variable	N = 66 (%)	CD 133 Serum Mean ± SD	P-Value	CD 133 Bronchial Lavage Mean ± SD	P-Value
Outcome			0.052 [a]		0.709 [a]
Alive	42 (63.6)	0.499 ± 0.253		0.273 ± 0.130	
Death	24 (36.4)	0.637 ± 0.250		0.429 ± 0.866	

[a], Mann Whitney Test; [b], Kruskal-Wallis Test.

Table 4. AUC, Sensitivity, Specificity, and Cut-off Value of Bronchial Wash and Serum CD 133

Variable	AUC	Sensitivity	Specificity	Cut off Point
CD 133 Serum	0.277 (CI 95% 0.186-0.369)	34.80%	36.50%	0.731 [a]
CD 133 Bronchial Lavage	0.743 (CI 95% 0.638-0.849)	83.30%	78.80%	0.174 [b]

[a], Graphic Method; [b], Youden Index

being the most common (38.5%). Histopathological examination showed a predominance of squamous cell carcinoma in NSCLC patients (78.8%), and a significant majority were in advanced metastatic stages (89.4%). Also, therapy response based on RECIST criteria showed that partial response was observed in 63.1% of the patients, progressive disease in 31.6%, and stable disease in 5.3%. The outcomes for NSCLC patients were death (36.4%), continuation of chemotherapy (28.8%), refusal of chemotherapy (28.8%), and cessation of chemotherapy due to adverse events (6.1%).

CD 133 Levels Examination in NSCLC and Control Group

The examination of *CD 133* serum levels in the NSCLC and control groups showed mean values of 0.550 ± 0.259 ng/mL and 0.752 ± 0.171 ng/mL, respectively. Meanwhile, the examination of *CD 133* levels in bronchial lavage in the NSCLC and the control groups showed mean values of 0.531 ± 0.330 ng/mL and 0.762 ± 0.339 ng/mL, respectively. These results indicated a significant difference between the *CD 133* serum levels in both groups (p=0.000). The same significant difference was observed in the *CD 133* levels in bronchial lavage between the NSCLC and the control group (p=0.000). Additionally, there was a significant difference between the *CD 133* serum levels (0.550 ± 0.259 ng/mL) and the *CD 133* levels in bronchial lavage (0.330 ± 0.531 ng/mL) in the NSCLC group, with a p-value of 0.000. In the control group, the p-value was 0.000 for the difference between *CD 133* serum levels (0.171 ± 0.752 ng/mL) and *CD 133* levels in bronchial lavage (0.762 ± 0.339 ng/mL). (Table 2).

CD 133 Level Based on Clinical Characteristics

CD 133 levels in serum tended to be higher across different characteristics, but there were no statistically significant differences for all characteristics except for T staging in *CD 133* Bronchial Lavage. Specifically, T4 stage showed the highest level and statistically significant difference in *CD 133* levels in bronchial lavage (p=0.013), emphasizing that tumor size or volume was associated with *CD 133* expression in bronchial lavage fluid. This showed a potential correlation between tumor size and *CD 133* expression, which could be significant for understanding disease progression and prognosis in NSCLC patients as presented in Table 3.

The ability of *CD 133* in bronchial lavage and serum to detect advanced-stage NSCLC versus control (non-malignant conditions) was analyzed using the receiver operating characteristic (ROC) curve, which graphically represents the relationship between sensitivity and 1-specificity (Figure 2). The area under the curve (AUC) was assessed from the ROC curve to determine the diagnostic strength. It was shown that *CD 133* in bronchial wash was better at predicting advanced-stage NSCLC compared to serum *CD 133* (0.743, 95% CI 0.638-0.849 vs. 0.277, 95% CI 0.186-0.369) as presented in Table 4. The *CD 133* bronchial wash test with a cut-off point of 0.174 ng/mL showed higher sensitivity (83.3%) and specificity (78.8%) compared to the serum *CD 133* test with a cut-off point 0.731 ng/mL, which had a sensitivity of 34.8% and specificity of 36.5% as presented in Table 4.

Even though *CD 133* levels in serum tended to be higher, there were no significant differences in *CD 133* levels in both serum and bronchial lavage across various therapy types administered to the 19 NSCLC patients

Table 5. CD 133 Serum and Bronchial Lavage Level to Therapy in NSCLC Patients

Variable	CD 133 Serum (mean ± SD)	P-value	CD 133 Bronchial Lavage (mean ± SD)	P-value
Systemic Chemotherapy	0.568 ± 0.290	0.515	0.272 ± 0.112	0.218
Immune Cek Point Inhibitor	NA		NA	
ALK Inhibitor	NA		NA	
Tyrosine Kinase Inhibitor	0.611 ± 0.217		0.276 ± 0.116	
Systemic + Radiotherapy	0.394 ± 0.491		0.174 ± 0.129	
Tyrosine Kinase Inhibitor + Radiotherapy	NA		NA	

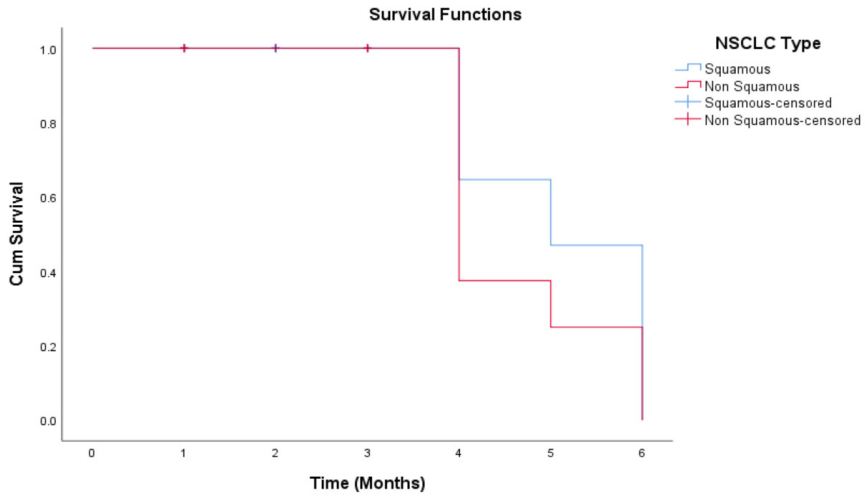

Figure 3a. Survival Analysis based on Cell Type in NSCLC Patients

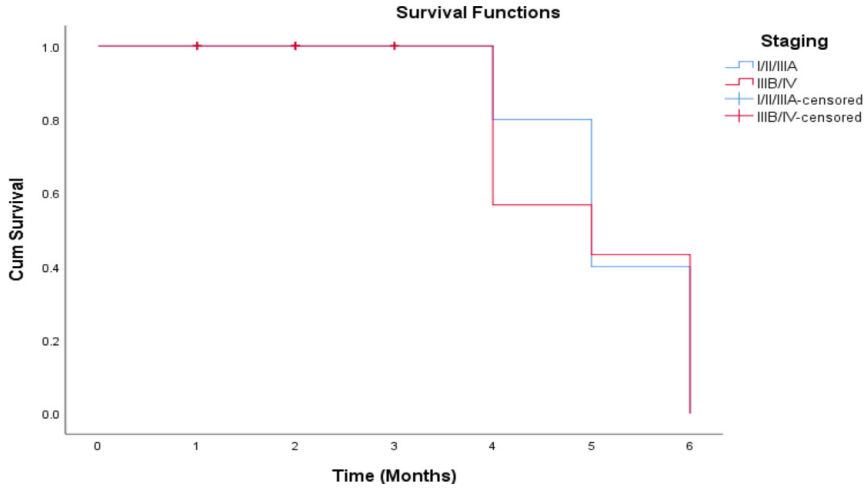

Figure 3b. Survival Analysis based on Cancer Stage in NSCLC Patients

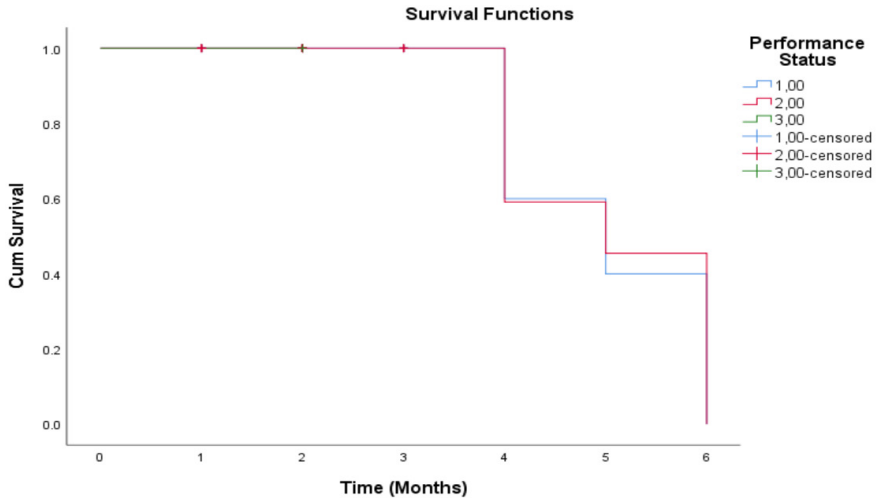

Figure 3c. Survival Analysis based on Performance Status in NSCLC Patients

(Table 5). The therapies included systemic chemotherapy, immune checkpoint inhibitors, ALK inhibitors, tyrosine kinase inhibitors, and combinations of systemic therapy with radiotherapy.

Survival Analysis

Survival analysis (Figure 3) using the Kaplan-Meier curve showed that out of 66 NSCLC patients, 42 died, with 34 having squamous cell carcinoma and 8 with non-squamous cell carcinoma. The mean survival time was 5.1 months for squamous cell carcinoma and 4.6

months for non-squamous, and no significant difference between the two groups (p=0.190). When analyzing survival based on cancer stage, 5 patients without metastasis and 37 with metastasis died. Furthermore, the mean survival time was 5.2 months for non-metastatic patients and 5.0 months for metastatic, with no significant difference (p=0.914). Survival analysis based on performance status showed that out of 65 patients with performance status (PS) scores of 1-2, 42 died, with 20 having a PS of 1 and 22 having a PS of 2. In addition, the mean survival time was 5.000 months for PS 1 and 5.045 months for PS 2, with no significant difference (p=0.783).

Discussion

The results showed significant differences in *CD 133* serum and bronchial lavage levels (p<0.05). NSCLC patients with advanced tumors (T4) had higher *CD 133* levels in bronchial wash (p<0.05). The bronchial wash *CD 133* test showed higher sensitivity (83.6%) and specificity (79.2%) compared to serum *CD 133*. These results emphasized the potential of *CD 133*, particularly in bronchial wash, as a valuable diagnostic tool for NSCLC.

The NSCLC patients were predominantly males (69.2%) aged 50 years and above (84.8%). Ganti et al. (2021) reported similar findings in the US, where 53% of NSCLC patients were males and 67% were aged 65 years and above [1,17]. In Indonesia, Hanafi et al. (2024) found that 64.2% of lung cancer patients were males, with 51.1% aged between 40-60 years [18]. The higher proportion of male patients with NSCLC was attributed to various factors, including screening and diagnosis processes. Baiu et al. (2021) stated that lung cancer screening in the US was recommended for adults aged 55-80 years with a 30 pack-year smoking history. This focus on high-risk groups led to higher detection rates in men compared to women [19].

Smoking history in the NSCLC and control groups showed a high prevalence of active smokers (62.1% and 48.1%), with a heavy Brinkman index in both groups (53.7% for NSCLC and 40.0% for controls). Despite no significant differences in smoking history (p=0.281) and Brinkman index (p=0.520), tobacco use was associated to about 90% of lung cancer cases, with a 20-fold higher risk in heavy smokers. Smoking also worsened prognosis and increased mortality in NSCLC patients [20]. Sun et al. (2021) found that the immune microenvironment in smokers showed higher expression of chemokines and active immune cells compared to non-smokers, who had more immunosuppressive cells [21].

Common symptoms in NSCLC patients included dyspnea (36.4%), chest pain (28.8%), cough (22.7%), and hemoptysis (12.1%), similar to the control group. Xing et al. (2019) reported chronic cough, hemoptysis, dyspnea, weight loss, fatigue, and fever as common NSCLC symptoms. Moreover, squamous cell carcinoma was the most common histopathological type in this study (78.8%), followed by non-squamous (15.2%) and unspecified NSCLC (6.1%). Schabath and Cote (2019) identified adenocarcinoma (40%) and squamous cell carcinoma (25%) as the most common NSCLC types [22]. Most NSCLC patients in this study were at metastatic stages (89.4%). Therefore, early detection was challenging, leading to late-stage diagnoses, as shown by Olga et al. (2021), where 65.33% of male patients were diagnosed at stage III or IV [23].

The serum *CD 133* levels in the NSCLC and control groups showed mean values of 0.550 ± 0.259 ng/mL and 0.752 ± 0.171 ng/mL, respectively. The bronchial wash *CD 133* levels were 0.330 ± 0.531 ng/mL for the NSCLC group and 0.339 ± 0.762 ng/mL for the control. In addition, there were significant differences between the NSCLC and control groups in both serum *CD 133* (p=0.000) and bronchial wash *CD 133* levels (p=0.000).

Numerous studies support the cancer stem cell (CSC) hypothesis, which posits that CSCs are crucial in tumor initiation, metastasis, recurrence, and therapy resistance. *CD 133* is a widely used marker for CSCs in various malignancies, including glioblastoma, hepatocellular carcinoma, as well as ovarian, colon, and lung cancer [24–28]. Le et al. (2013) found a significant increase in *CD 133* expression in NSCLC tissues compared to normal lung tissues, with correlations to tumor stage, size, and differentiation. NSCLC patients often faced challenges such as early-stage diagnostic difficulties, high metastasis rates, recurrence, and poor prognosis. Hence, elevated *CD 133* expression in early-stage NSCLC could provide a valuable diagnostic method [29]. Active smokers had higher *CD 133* levels in bronchial wash (0.359 ng/mL) compared to passive smokers (0.308 ng/mL) and non-smokers (0.256 ng/mL), although the p-value did not indicate a significant difference (p=0.426). Sun et al. (2020) reported that smoking enhanced tumor formation capacity, increased CSC markers (*CD 133*), and promoted epithelial-mesenchymal transition (EMT) in bladder cancer [21].

Similarly, Xie et al. (2019) found that increased cancer-associated gene (ΔNp63α) in epithelial tissues promoted tumor formation and CSCs in lung cancer, correlating positively with *CD 133*. Higher levels of ΔNp63α and *CD 133* were observed in the lung cancer tissues of smokers compared to non-smokers. Tobacco smoke increased interleukin-6 (IL-6), which promoted ΔNp63α expression in transformed bronchial epithelium, leading to increased CSCs in the epithelium through the IL-6/ΔNp63α/notch axis [30]. There were significant differences between serum *CD 133* levels (0.550 ± 0.259) and bronchial wash *CD 133* levels (0.330 ± 0.531) in the NSCLC group, with a p-value of 0.000. Similarly, in the control group, significant differences were found between serum *CD 133* levels (0.752 ± 0.171) and bronchial wash *CD 133* levels (0.339 ± 0.762), with a p-value of 0.000.

Bronchoalveolar lavage (BAL) or bronchial wash as a diagnostic method for lung tumors has been reported since the early 1980s. Bronchial wash in lung cancer can be performed at any stage and is informative in depicting primary immune status, modulating immune response to therapy, and providing differential diagnoses [31]. Ghosh et al. (2013) stated that assessing biomarkers like carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and carbohydrate antigen 125 (CA125) in bronchial wash was clinically more useful compared to

serum. The receiver operating characteristic curve showed higher diagnostic accuracy for these markers in bronchial wash than in serum [32]. Similar findings were shown in this study, where *CD 133* levels in bronchial wash were better at identifying NSCLC compared to serum *CD 133* levels (AUC: 0.749, 95% CI: 0.645-0.853 vs. 0.283, 95% CI: 0.192-0.374). The *CD 133* bronchial wash test with a cut-off point of 0.174 ng/mL had higher sensitivity (83.6%) and specificity (79.2%) compared to the serum *CD 133* test with a cut-off point of 0.740 ng/mL, which had sensitivity of 32.8% and specificity of 37.7%.

This study found that participants with tumor size T4 had higher *CD 133* levels in bronchial wash (83.3%) compared to those with T3 (15.2%) and T2 (1.5%), with a significant difference (p=0.013). Moreover, Huang et al. (2014) reported that *CD 133* protein expression was higher in NSCLC tumor tissues compared to adjacent non-tumor tissues. It was also shown that *CD 133* was located in the tumor nucleus, and its high expression in NSCLC correlated with specific clinical pathological parameters, including tumor diameter, differentiation, and 5-year survival rates [33]. Similarly, this study found that *CD 133* levels in bronchial wash were higher in participants with T4 tumor sizes (mean 0.361 ± 0.574) compared to those with T3 and T2, indicating its potential as a diagnostic marker in NSCLC.

This study used a cross-sectional design, which had several limitations, such as not being able to observe the outcomes of all participants, being conducted over a short period, and being restricted to a centered hospital. Additionally, the control group included participants with other medical conditions, both related to lung diseases (non-cancer) and unrelated, which could influence the results. Some advanced NSCLC patients refused chemotherapy due to worsening recovery status or intolerable adverse events. Moreover, the cross-sectional design does not allow for the observation of disease progression and long-term outcomes. Future studies should consider larger sample sizes, longer follow-up periods, and cohort designs to better understand the longitudinal effects and outcomes of NSCLC treatments. Despite the limitations, this study provided valuable insights into the role of *CD 133* in NSCLC. The results emphasized the potential of *CD 133* as a diagnostic and prognostic biomarker, paving the way for further studies in this field. The results are significant and add to the growing body of evidence supporting the use of *CD 133* in the management of NSCLC.

In conclusion, therefore, it can be concluded that CD 133 levels were significantly higher in bronchial wash samples from NSCLC patients with advanced tumors (T4) compared to those with smaller tumors (T3 and T2). There was a positive correlation between *CD 133* levels in bronchial wash and serum, indicating an overall increase in *CD 133* in NSCLC patients. In this case, *CD 133* levels in bronchial wash were more effective in predicting advanced NSCLC than serum *CD 133*, with higher sensitivity and specificity, which emphasized its potential as a valuable diagnostic tool.

Although previous studies have correlated elevated *CD 133* levels with poor differentiation and metastasis, suggesting its role as a prognostic marker, this study did not find a significant correlation between *CD 133* levels and treatment outcomes or chemotherapy types. Patients with metastatic NSCLC generally have lower survival times, however, this study did not find a statistically significant difference between metastatic and non-metastatic patients. These results emphasized the importance of further studies to validate the clinical applications of CD 133 as both a diagnostic and prognostic biomarker in NSCLC.

Author Contribution Statement

SM, AS, ID, HI, NL, HAP: Conceptualization, Investigation, Methodology, Data curation, Validation. RR: Statistical analysis. Supervision. SM, IN, RR: Writing-original draft, Writing-review & editing. All authors read and approved the submitted version.

Acknowledgements

General

The authors are grateful to all participants who have consented to include the data for the study as well as Sulhidayah, a Hasanuddin University Medical Research Unit (HUM-RC) laboratory analyst, for the assistance in processing samples.

Approval

If it was approved student thesis at Pulmonology and Respiratory Medicine Department Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

Ethical Declaration

Ethical approval was obtained from the Health Research Ethics Commission of the Faculty of Medicine, Hasanuddin University, Makassar, and Dr. Wahidin Sudirohusodo Hospital (Recommendation number 78/UN/4.6.4.5.31/PP36/2024, protocol number UH24010027).

Data Availability

Data will be made available on request

Study Registration

The study does not require study registration.

Conflict of Interest

The authors declared that there are no conflicts of interests or personal relationships that could have influenced the work presented in this paper.

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