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BRAF Mutation Status and Histopathological Differentiation Patterns in Non–Small Cell Lung Cancer: A Tertiary Referral Center Study in Indonesia

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ABSTRACT

Introduction: Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer and a major cause of cancer-related mortality worldwide. BRAF mutations have emerged as clinically relevant molecular alterations associated with tumor behavior and targeted therapy responses. However, evidence regarding their relationship with histopathological differentiation remains limited, particularly in the Indonesian population. This study aimed to evaluate the association between BRAF mutation status and histopathological differentiation in patients with NSCLC at a tertiary referral center in Indonesia.

Methods: This retrospective cross-sectional study included patients diagnosed with NSCLC at a tertiary hospital in Medan, Indonesia. Clinical and demographic data were obtained from patients' medical records. Histopathological differentiation was classified as well-, moderately, or poorly differentiated. BRAF mutation analysis was performed using real-time polymerase chain reaction on formalin-fixed paraffin-embedded tissue samples. Statistical analyses were performed to determine the association between BRAF mutation status and histopathological differentiation.

Results: Most patients were men (76.7%), aged >40 years, and had a history of smoking (74.2%). Adenocarcinoma was the predominant histological subtype (75%), and most patients were diagnosed at stage IVA (60%). BRAF mutations were identified in 3.3% of the patients. Histopathological evaluation revealed that 20.8%, 37.5%, and 41.7% of the tumors were well-, moderately-, and poorly differentiated, respectively. A significant association was observed between BRAF mutation status and histopathological differentiation (P = 0.001), with mutations being more frequently detected in poorly differentiated tumors.

Conclusion: BRAF mutations were identified in a small proportion of NSCLC patients and were significantly associated with poor histopathological differentiation, suggesting a potential role in aggressive tumor biology.

Keywords

BRAF Mutation, Histopathological Differentiation, Non-Small Cell Lung Cancer, Molecular Oncology.

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INTRODUCTION

Lung cancer is the leading cause of cancer incidence and mortality worldwide, accounting for an estimated 2.5 million new cases and approximately 1.8 million deaths in 2022, representing 12.4% of all newly diagnosed cancers and 18.7% of global cancer-related mortality [1]. Non-small cell lung cancer (NSCLC) represents nearly 85% of all lung cancer cases and continues to impose a substantial clinical and socioeconomic burden

owing to its aggressive nature and frequent diagnosis at an advanced stage [2]. Despite advances in systemic therapy and molecular diagnostics, the overall prognosis of NSCLC remains poor, particularly in low- and middle-income countries, where late presentation and limited access to targeted treatment are common [3].

In Indonesia, lung cancer is one of the most prevalent malignancies and a major contributor to cancer-related mortality, particularly among men [4,5]. The persistently high prevalence of tobacco consumption, environmental pollution, occupational exposure, and delayed cancer detection continues to drive the increasing burden of pulmonary malignancies in Indonesia [6]. Nevertheless, lung cancer in never-smokers has gained increasing attention, especially in Asian populations, highlighting the complex molecular and biological heterogeneity underlying NSCLC development [7,8]. At the molecular level, oncogenic driver mutations have transformed the diagnostic and therapeutic landscapes of NSCLC. Among these alterations, mutations in the v-Raf murine sarcoma viral oncogene homolog B (BRAF) gene are relatively uncommon but clinically significant [9]. BRAF mutations are detected in approximately 1–4% of NSCLC cases, with the V600E mutation representing the most therapeutically relevant subtype because of its responsiveness to targeted BRAF and MEK inhibitor therapy [10-12]. Beyond their therapeutic implications, BRAF mutations may also reflect distinct biological behaviors, tumor progression patterns, and histopathological characteristics [13]. Histopathological differentiation remains an important prognostic parameter in NSCLC and is closely associated with tumor aggressiveness, metastatic potential, and clinical outcomes [14]. Poorly differentiated tumors generally exhibit more aggressive biological behavior and worse survival outcomes than well-differentiated neoplasms [15]. However, evidence regarding the association between BRAF mutation status and histopathological differentiation in NSCLC remains limited and inconsistent, particularly in Southeast Asian populations, where molecular epidemiological data are still scarce [16].

A better understanding of the relationship between molecular alterations and histopathological features may contribute to a more integrated molecular–pathological classification approach in NSCLC and potentially improve risk stratification and personalized therapeutic strategies [17]. Therefore, we aimed to evaluate the association between BRAF mutation status and histopathological differentiation in patients with NSCLC treated at a tertiary referral center in Medan, Indonesia.

METHOD

This retrospective cross-sectional study was conducted at a tertiary referral center in Medan, Indonesia. Consecutive patients diagnosed with primary non-small cell lung cancer (NSCLC) between January and December 2024 were included in this study. We included 120 eligible patients with histopathologically confirmed NSCLC and available formalin-fixed paraffin-embedded (FFPE) tumor specimens. Patients were eligible if they were aged ≥18 years, had a confirmed diagnosis of primary NSCLC, and had FFPE tissue blocks with sufficient tumor content for molecular analysis. Tissue specimens were obtained through bronchoscopy-guided biopsy, transthoracic biopsy, thoracotomy, or open surgical biopsy. Patients were excluded if the available tissue was insufficient for DNA extraction, the medical record was incomplete, or the tumor represented metastatic disease from an extrapulmonary primary malignancy. Demographic and clinicopathological variables, including age, sex, ethnicity, smoking history, histological subtype, clinical stage, and histopathological differentiation, were extracted from the medical records and pathology reports.

Histopathological diagnoses were established using hematoxylin and eosin-stained sections. The NSCLC subtype and tumor differentiation were assessed according to standard pathological criteria. Tumor differentiation was classified as well-, moderately, or poorly differentiated. When required, representative tumor areas were selected to ensure adequate viable tumor content for molecular testing. BRAF mutation analysis was performed using real-time polymerase chain reaction with a TaqMan probe-based detection in a certified molecular pathology laboratory. Genomic DNA was extracted from FFPE tumor tissues using a commercial extraction kit in accordance with the manufacturer's protocol. DNA concentration and purity were assessed before amplification to ensure analytical suitability. PCR amplification was performed under standardized conditions using BRAF-specific primers and probes with an internal amplification control. Internal controls were included to verify DNA quality, amplification efficiency, and reaction validity.

BRAF mutation analysis was performed using sequence-specific primers and fluorescent hydrolysis probes targeting the BRAF gene region. An internal amplification control was included in each reaction to ensure DNA integrity, amplification efficiency, and assay validity. The mutation-specific probe was labeled with the fluorescent reporter dye FAM and a minor groove binder (MGB) moiety to enhance hybridization specificity and detection sensitivity. The internal control probe was labeled with the VIC fluorescent reporter dye and MGB quencher to verify successful amplification and minimize false-negative results. Each PCR reaction was prepared in a final volume of 20 μL, consisting of a probe-based PCR master mix, BRAF-specific primer–probe mixture, internal control primer–probe mixture, nuclease-free water, and extracted template DNA. Amplification was performed with an initial denaturation step, followed by 40 cycles of denaturation and annealing/extension. Fluorescence signals were acquired during each cycle and automatically analyzed using real-time PCR system software. BRAF mutation status was determined based on the amplification curves and cycle threshold values. Samples showing specific amplification signals in the mutant detection channel were classified as BRAF mutation-positive.

Samples without mutant amplification but with successful internal control amplification were classified as BRAF mutation-negative. Samples with failed internal control amplification were considered invalid and were excluded from molecular interpretation. Data were analyzed using standard statistical software. Categorical variables are presented as frequencies and percentages. Continuous variables are presented as the mean ± standard deviation for normally distributed data or the median with interquartile range for non-normally distributed data. The association between BRAF mutation status and histopathological differentiation was assessed using the chi-square or Fisher's exact test, as appropriate. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

A total of 120 patients with histopathologically confirmed non-small cell lung cancer (NSCLC) were included in this study. Male patients predominated, accounting for 76.7% of the study population, whereas female patients represented 23.3% of the cases. Most patients were aged 41–60 years (47.5%), followed by those aged >60 years (42.5%), while patients aged 18–40 years constituted only a small proportion of the cohort. Ethnically, most patients were of Batak origin (60.0%), followed by Javanese (25.0%), Malay (11.7%), and Acehnese (3.3%). Adenocarcinoma was identified as the predominant histopathological subtype, accounting for 75.0% of all NSCLC cases, whereas squamous cell carcinoma accounted for 25.0% of the tumors. Histopathological evaluation revealed that poorly differentiated tumors constituted the largest subgroup (41.7%), followed by moderately differentiated (37.5%) and well-differentiated (20.8 %) tumors. These findings indicate that moderate-to-poor histopathological differentiation was the dominant morphological pattern in this cohort. Smoking exposure was documented in 74.2% of the patients. Among smokers, severe smoking intensity based on the Brinkman Index was observed in 51.7% of the patients, whereas moderate smoking intensity was identified in 22.5%. Most patients presented with advanced-stage disease, particularly stage IVA (60.0%), whereas early-stage disease was rarely observed. The distribution of the clinicopathological characteristics of the study population is summarized in Table 1.

BRAF mutations were detected in four patients (3.3%), whereas 116 patients (96.7%) were classified as BRAF mutation negative. Analysis of histopathological differentiation according to BRAF mutation status demonstrated that poorly differentiated tumors were more frequently observed among patients harboring BRAF mutations. Specifically, among BRAF mutation–positive cases, one patient (0.8%) demonstrated well-differentiated histology, one patient (0.8%) had moderately differentiated tumors, and two patients (1.6%) exhibited poorly differentiated tumors. In contrast, among patients without BRAF mutations, 24 patients (20.0%) were classified as well differentiated, 44 patients (36.6%) as moderately differentiated, and 48 patients (40.0%) as poorly differentiated.

Table 1. Clinicopathological Characteristics of the Study Population

Variables	n (%)
Sex	
Male	92 (76.7)
Female	28 (23.3)
Age	
18–40 years	12 (10.0)
41–60 years	57 (47.5)
>60 years	51 (42.5)
Ethnicity	
Javanese	30 (25.0)
Batak	72 (60.0)
Malay	14 (11.7)
Acehnese	4 (3.3)
Histopathological Differentiation	
Well differentiated	25 (20.8)
Moderately differentiated	45 (37.5)
Poorly differentiated	50 (41.7)
Histopathological Subtype	
Adenocarcinoma	90 (75.0)
Squamous cell carcinoma	30 (25.0)
Smoking Status	
Never-smoker	31 (25.8)
Smoker	89 (74.2)
Brinkman Index	
Never-smoker	31 (25.8)
Moderate	27 (22.5)
Severe	62 (51.7)
pTNM Stage	
IB	1 (0.8)
IIA	1 (0.8)
IIB	1 (0.8)
IIIA	16 (13.3)
IIIB	16 (13.3)
IIIC	7 (5.8)
IVA	72 (60.0)
IVB	6 (5.0)
BRAF Mutation Status	
Positive	4 (3.3)
Negative	116 (96.7)

A comparative statistical analysis demonstrated a statistically significant association between histopathological differentiation and BRAF mutation status ($p = 0.001$), indicating that BRAF mutations were more frequently associated with poorly differentiated tumor morphology than with well-differentiated tumors. The association between histopathological differentiation and BRAF mutation status is presented in Table 2.

Table 2. Association Between Histopathological Differentiation and BRAF Mutation Status

Histopathological Differentiation	BRAF Positive n (%)	BRAF Negative n (%)	Total, n (%)	p value
Well differentiated	1 (0.8)	24 (20.0)	25 (20.8)	
Moderately differentiated	1 (0.8)	44 (36.6)	45 (37.5)	
Poorly differentiated	2 (1.6)	48 (40.0)	50 (41.7)	
Total	4 (3.3)	116 (96.7)	120 (100)	0.001

DISCUSSION

In this study, NSCLC occurred predominantly in men, consistent with global and regional patterns that show a higher lung cancer burden among men [18]. This predominance is likely influenced by the high prevalence of tobacco exposure in Indonesian men. However, NSCLC should not be interpreted solely as a smoking-related disease because increasing evidence shows that lung cancer is biologically heterogeneous and may arise through complex interactions between environmental exposure, host susceptibility, and molecular

alterations [19]. Most patients were aged 41–60 years or older than 60 years, reflecting the cumulative effect of carcinogenic exposure and age-related accumulation of somatic mutations [20]. Nevertheless, the occurrence of oncogenic driver mutations across different age groups suggests that molecular profiling provides clinically relevant information beyond conventional demographic variables [21].

Adenocarcinoma was the predominant histological subtype in this cohort, consistent with the global shift in NSCLC epidemiology, in which adenocarcinoma has become the most frequent subtype [22]. This subtype is also strongly associated with targetable driver mutations, including BRAF alterations, reinforcing the importance of molecular testing in NSCLC [23]. Most tumors were moderately or poorly differentiated, and the majority of patients presented with advanced-stage disease. This pattern highlights the persistent challenge of delayed lung cancer diagnosis, particularly in settings where access to early detection, molecular diagnostics, and specialized referral pathways is limited [24]. Poor differentiation reflects the loss of normal cellular architecture and is commonly associated with aggressive tumor behavior, metastatic potential, and unfavorable clinical outcomes [25]. BRAF mutations were detected in 3.3% of patients, consistent with previously reported frequencies of approximately 1–5% in NSCLC populations [26]. Although uncommon, BRAF mutations are clinically important because they define a molecular subgroup with potential sensitivity to targeted therapy, particularly in tumors harboring BRAF V600E mutations [27]. The key finding of this study was the significant association between BRAF mutation status and histopathological differentiation of the tumor. BRAF mutations were more frequently observed in poorly differentiated tumors, suggesting a possible relationship between BRAF-driven signaling and aggressive morphological behavior. Biologically, aberrant BRAF activation enhances MAPK pathway signaling, promoting tumor proliferation, survival, invasion, and dedifferentiation [27].

These findings reinforce the value of integrating molecular pathology with conventional histopathological assessments in NSCLC. Histopathological grading remains clinically relevant, whereas molecular profiling provides an additional biological layer that may improve tumor characterization, risk stratification, and therapeutic decision-making. This study has several limitations. Its retrospective design may have introduced selection and information bias, and the single-center setting may have limited its generalizability. The number of BRAF mutation-positive cases was small, which is expected given the low prevalence of BRAF mutations in NSCLC. In addition, this study did not classify BRAF mutations into V600E and non-V600E subtypes, limiting the detailed genotype-specific interpretation. In summary, BRAF mutations were uncommon but were significantly associated with poor histopathological differentiation in this cohort. These findings suggest that BRAF alterations may contribute to aggressive tumor biology and support the implementation of integrated molecular–histopathological evaluation in NSCLC.

CONCLUSION

BRAF mutations were uncommon in this cohort of non-small cell lung cancer but demonstrated a significant association with poor histopathological differentiation, suggesting a potential link with aggressive tumor biology. The predominance of advanced-stage adenocarcinoma among middle-aged male smokers further reflects the persistent burden of late-presenting NSCLC in this population. These findings underscore the importance of integrating molecular profiling with conventional histopathological assessment to improve tumor characterization and support precision-based therapeutic strategies.

DECLARATIONS

This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia (approval number: 341/KEPK/USU/2025). Owing to its retrospective nature, the requirement for informed consent was waived.

CONSENT FOR PUBLICATION

The Authors agree to the publication in the Journal of Society Medicine.

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AUTHORS' CONTRIBUTIONS
L.K.A.P., N.N.S., and D.K. conceived the study. L.K.A.P. collected the data and drafted the manuscript. N.N.S., D.K., and P.E. contributed to the supervision, data interpretation, validation, and critical revision. All authors approved the final manuscript and are accountable for all aspects of the work.

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