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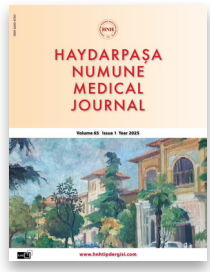

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ORIGINAL ARTICLE

Clinical Utility of Serum BRAF and PTEN Proteins in the Diagnosis of Non-Small Cell Lung Cancer: A Pilot Study

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Abstract

Introduction: Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality worldwide. Early and accurate diagnosis is essential for improving clinical outcomes. BRAF, a key kinase in the MAPK pathway, and PTEN, a negative regulator of PI3K/Akt signaling, are both implicated in tumorigenesis. This pilot study aimed to evaluate the diagnostic potential of serum BRAF and PTEN protein levels in patients with NSCLC.

Methods: In this cross-sectional pilot study, 60 histologically confirmed NSCLC patients and 20 age- and sex-matched healthy controls were recruited from Istanbul University Oncology Institution. Peripheral blood samples were collected prior to any systemic therapy. Serum BRAF and PTEN concentrations were measured using commercially available ELISA kits (CUSABIO). Statistical analyses included group comparisons, Pearson correlation, and receiver operating characteristic (ROC) curve analysis.

Results: Median serum BRAF and PTEN levels were significantly higher in NSCLC patients compared with controls ($p=0.001$ for both). A strong positive correlation between BRAF and PTEN was observed ($r=0.681$, $p<0.001$). ROC analysis indicated that PTEN (AUC=0.830) demonstrated superior diagnostic accuracy compared with BRAF (AUC=0.765).

Discussion and Conclusion: This pilot study, the first to analyze serum BRAF and PTEN simultaneously in NSCLC, suggests that PTEN may serve as a promising noninvasive diagnostic biomarker. These preliminary results warrant validation in larger, multicenter studies to confirm diagnostic performance and explore integration into clinical workflows.

Keywords: Biomarker; BRAF; ELISA; non-small cell lung cancer (NSCLC); PTEN.

Lung cancer is characterized by the uncontrolled proliferation of cells within lung tissue, driven by various genetic alterations. Key molecular events underlying lung carcinogenesis include the activation of oncogenes through mutations, the inactivation of tumor suppressor genes, disruptions in genes regulating the cell cycle and DNA repair mechanisms, as well as changes affecting growth factors and their receptors [1].

The BRAF protein serves a central role in regulating cellular proliferation. Mutations in the BRAF gene result in aberrant protein forms that activate signaling pathways leading to uncontrolled cell growth and tumor development. Functioning downstream in the MAPK pathway, BRAF acts in concert with MEK proteins to tightly control cell division and survival [2].

Among the various mutations identified within BRAF, the V600E substitution is of particular clinical importance in

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lung cancer, representing a key driver mutation for which targeted therapies have received FDA approval. This mutation alters BRAF activity and constitutively activates oncogenic signaling cascades [3].

Phosphatase and tensin homolog (PTEN), located on chromosome 10q23, is a well-established tumor suppressor gene encoding a dual-specificity phosphatase. PTEN exerts both lipid and protein phosphatase activities that are crucial for regulating cell proliferation, apoptosis, and the cell cycle. Loss or reduction of PTEN expression has been documented in multiple cancer types and is implicated in tumorigenesis. Even partial PTEN loss can initiate carcinogenic processes. While PTEN primarily functions to antagonize the PI3K/mTOR/Akt pathway, thereby restraining uncontrolled cell growth, it also inhibits key mechanisms involved in cell migration, survival, and tumor progression, underscoring its pivotal role in tumor suppression [4].

Inactivation of the PTEN gene is a common event in lung cancer; however, its precise role in modulating the metastatic behavior of lung cancer cells is not yet fully elucidated. Advances in technology, along with deeper insights into PTEN's structural characteristics and regulatory mechanisms, are expected to enhance our understanding of its biological functions. Such progress may pave the way for the development of novel and more effective therapeutic approaches for lung cancer patients [5].

The BRAF protein functions as a critical component of the epidermal growth factor receptor (EGFR) signaling cascade, which governs key cellular processes such as proliferation, differentiation, and survival. Positioned downstream of KRAS, BRAF serves as a pivotal regulator within this pathway. Activating mutations in the BRAF gene have been implicated in the pathogenesis of several malignancies, with the V600E point mutation representing the most prevalent variant, accounting for approximately 86% of BRAF mutations identified in tumor specimens [6].

While BRAF and PTEN have been extensively studied as individual molecular targets in cancer research, their combined role in non-small cell lung cancer (NSCLC) remains underexplored. Building upon the existing knowledge of their independent contributions to oncogenic signaling, the present study was designed to investigate the simultaneous expression and potential interplay of BRAF and PTEN proteins in NSCLC. In addition to evaluating their individual and combined expression profiles, the study also aimed to assess the association between these proteins and specific clinicopathological parameters, as well as to examine the feasibility of using their levels—along with potential target miRNA combinations—as diagnostic biomarkers.

This work is a pilot study with a modest cohort (60 lung cancer patients; 20 healthy controls), which may limit power and generalizability. Furthermore, BRAF protein is not an established circulating biomarker; most validated liquid-biopsy applications assess BRAF mutations in plasma ctDNA rather than serum protein. For PTEN, circulating signals are plausibly EV-associated, but EVs were not isolated; therefore, EV-bound protein cannot be distinguished from free protein. Finally, ELISA-based measurements in serum are susceptible to pre-analytical and analytical variability (e.g., matrix effects, cross-reactivity), which should be addressed in larger, method-standardized studies. Unlike aminotransferases (AST/ALT), which rise in serum due to membrane injury and enzyme leakage during hepatocellular damage, BRAF and PTEN are intracellular signaling proteins that do not typically enter the circulation via passive leakage. Emerging data suggest that PTEN can be secreted and transported in extracellular vesicles (EVs)/exosomes, and EV-associated PTEN has been detected in human serum in oncologic contexts. In contrast, circulating BRAF is assessed clinically via tumor-derived cell-free DNA (ctDNA) mutations (e.g., V600E) rather than soluble protein quantification. Accordingly, any serum signal for these proteins likely reflects EV-associated cargo and should be interpreted cautiously.

The detection of intracellular signaling proteins such as PTEN and BRAF in the circulation can be partly explained by extracellular vesicle (EV) biology. Tumor cells actively release EVs, including exosomes (30–150nm vesicles of endosomal origin) and microvesicles (100–1000nm, shed from the plasma membrane), into the bloodstream. These vesicles encapsulate proteins, nucleic acids, and lipids, thereby reflecting the molecular composition and signaling status of the tumor.

PTEN, a tumor suppressor phosphatase, has been shown to be packaged into exosomes and secreted into the extracellular environment, where it can exert paracrine effects by being transferred to recipient cells [7,8]. This secretion not only facilitates intercellular communication but also provides a mechanism by which PTEN can be detected in patient serum. Similarly, oncogenic BRAF and its downstream signaling components can be incorporated into EV cargo. Recent studies have demonstrated that oncogenic kinases and MAPK pathway proteins are enriched in tumor-derived EVs, contributing to the modulation of the tumor microenvironment and metastatic niche formation [9,10].

Thus, elevated serum levels of PTEN and BRAF in NSCLC patients may represent both direct tumor cell turnover (e.g., necrosis, apoptosis) and active EV-mediated secretion. Importantly, exosomal release stabilizes these proteins in circu-

lation by protecting them from degradation, making them detectable through serum-based assays such as ELISA. This mechanism supports the potential of PTEN and BRAF as minimally invasive, serum-based biomarkers in NSCLC.

Despite extensive research into genetic and molecular biomarkers in NSCLC, the clinical applicability of serum-based protein measurements for early detection remains underexplored. BRAF and PTEN are two functionally distinct signaling proteins with established roles in oncogenic processes but with limited data on their circulating protein levels in lung cancer. We therefore designed this study to test the hypothesis that serum BRAF and PTEN concentrations are elevated in NSCLC and to assess their diagnostic accuracy for distinguishing NSCLC patients from healthy controls.

Materials and Methods

This study included 60 patients diagnosed with lung cancer at the Istanbul University Oncology Institute and a control group of 20 healthy volunteers. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. This study was approved by the Clinical Research Ethics Committee of Istanbul Faculty of Medicine, Istanbul University (No: 2014/923). The study was performed according to the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Histological subtypes included adenocarcinoma (n=30) and squamous cell carcinoma (n=30). Molecular profiles were available for all 60 patients; no EGFR, ALK, KRAS, or BRAF mutations were detected. Comorbidity data (e.g., COPD, cardiovascular disease, diabetes) were recorded. This study has several limitations. First, all blood samples were obtained from treatment-naïve NSCLC patients, which standardizes the cohort but may limit generalizability to patients undergoing therapy. Second, pre-analytical variables such as the time from blood draw to processing, storage conditions, and the number of freeze–thaw cycles can affect serum protein stability and EV integrity. Although samples were handled under standardized laboratory protocols, subtle variations cannot be excluded. Third, hemolysis was carefully monitored and excluded; however, minimal undetected red blood cell contamination may still influence protein quantification. Finally, the modest cohort size warrants cautious interpretation, and larger, multicenter studies are required to validate these findings.

Inclusion criteria: Age≥18 years, histologically confirmed NSCLC, and an available serum sample prior to systemic therapy.

Exclusion criteria: Previous malignancy within 5 years, active infection, chronic liver disease, or concurrent inflammatory conditions.

Disease staging was conducted in accordance with the classification criteria established by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC). Prior to the initiation of treatment, each patient underwent a detailed clinical evaluation, including a comprehensive medical history, physical examination, and routine blood analyses. Only patients with an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 and who met the following hematologic criteria—absolute neutrophil count>1500/μL and platelet count>100,000/μL—were considered eligible for multidisciplinary treatment planning.

Peripheral blood samples were collected from all participants by venipuncture and allowed to clot at room temperature. Following clot formation, the samples were centrifuged at 4000rpm for 10 minutes, and the resulting sera were aliquoted and stored under appropriate conditions until analysis. Specifically, patient sera were collected prior to any therapeutic intervention, including adjuvant or metastatic treatments, to avoid confounding influences.

All serum samples were initially stored at −20°C and, for long-term preservation, subsequently transferred to −80°C until the time of biochemical analysis.

Serum BRAF and PTEN concentrations were measured using commercial ELISA kits (BRAF: CUSABIO, catalog no. CSB-E09973h; PTEN: CUSABIO, catalog no. CSB-206979). The detection range for BRAF was 0.031–2.0ng/mL (LOD: 0.0078ng/mL) and for PTEN was 0.125–8.0ng/mL (LOD: 0.0399ng/mL). Manufacturer-reported intra-assay and inter-assay coefficients of variation were <8% and <10%, respectively. Standard solutions were prepared by serially diluting a 48ng/mL stock standard using an appropriate standard diluent buffer. Microplate wells, pre-coated with specific antibodies, were loaded with 40μL of patient serum and 10μL of HGF antibody using an automated pipetting system. For the calibration curve, 50μL of each standard solution was added to the designated wells. Subsequently, 50μL of streptavidin–horseradish peroxidase (HRP) conjugate was dispensed into all wells, followed by incubation at 37°C for 1 hour to facilitate the formation of the antigen–antibody–enzyme complex.

After incubation, each well was washed five times with 300μL of wash buffer to remove unbound reagents. The wells were then dried thoroughly before the addition of 50μL of Chromogen Reagent A and 50μL of Chromogen Reagent B. The plate was incubated again at 37°C for 10 minutes to allow color development. The enzymatic reaction was terminated by adding 50μL of stop solution to each well.

Absorbance values were measured at 450nm using an ELISA microplate reader (ChroMate 4300). A standard calibra-

tion curve was constructed from the absorbance readings of the known concentrations, and sample concentrations were calculated accordingly. These values were then compared with software-generated concentration data to ensure accuracy and consistency.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows, version 30.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were categorized based on their median values to enable group comparisons. The Mann–Whitney U test was utilized to assess differences in non-normally distributed clinical and laboratory variables between NSCLC patients and healthy controls. Spearman's rank correlation coefficient was used to examine relationships between serum protein levels. Statistical significance was defined as $p<0.05$ for all tests. To evaluate the diagnostic performance of BRAF and PTEN serum levels, receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUC) values were calculated. Furthermore, the standardized effect size (Cohen's d) was computed using a pooled standard deviation of 13.45ng/mL. The analysis demonstrated a power value of 1.0, indicating that the study was highly powered to detect statistically significant differences in BRAF and PTEN levels between the groups at a significance threshold of $p=0.05$.

For sample size justification and effect size estimation, G*Power software was used, referencing Cohen's established guidelines for small, medium, and large effect size classifications [11].

Results

In this study, serum concentrations of BRAF and PTEN proteins were measured in a cohort of lung cancer patients (n=60) and compared with healthy controls. Among the patients, the mean ($\bar{x}$), standard deviation (SD), and median (m) values for BRAF and PTEN levels were 14.5±15.7ng/mL and 14.2±14.4ng/mL (means), and 7.9ng/mL and 7.8ng/mL (medians), respectively. In the healthy control group, the corresponding values were 4.5±2.6ng/mL and 4.4±2.5ng/mL (means), and 4.7ng/mL and 4.6ng/mL (medians). Statistical analysis demonstrated that serum levels of both BRAF and PTEN were significantly elevated in NSCLC patients compared with healthy individuals ($p=0.001$) (Tables 1 and 2).

To evaluate the diagnostic performance of these markers, receiver operating characteristic (ROC) analysis was performed. The area under the curve (AUC) values were calculated as 0.765 for BRAF and 0.830 for PTEN, indicating that both biomarkers exhibit considerable diagnostic potential, with PTEN displaying a higher discriminative capacity than BRAF (Fig. 1).

Visual representations of serum protein distributions between NSCLC patients and healthy controls are provided as box plots. These plots clearly show statistically significant differences in BRAF and PTEN levels between the groups ($p<0.05$). The central lines in the plots represent median values for each dataset (Figs. 2 and 3).

Further analysis was conducted to examine associations between serum biomarker levels and demographic characteristics of NSCLC patients using the chi-square test, the results of which are summarized in Table 3.

No statistically significant associations were observed between serum BRAF or PTEN levels and demographic characteristics such as age, sex, or smoking history (Table 3). These findings suggest that the elevated biomarker levels in NSCLC patients are unlikely to be explained by these baseline variables. This study is limited by its cross-sectional design and lack of survival data, precluding analysis of the prognostic significance of serum BRAF and PTEN. Future studies should include progression-free survival and overall survival analyses to assess whether these biomarkers also hold prognostic value.

Table 1. Statistical summary of the concentrations of serum BRAF

		BRAF protein (ng/ml)				
		Mean (x)	**Standard Deviation (sd)**	**Median (m)**	**Minimum (min)**	**Maximum (max)**
Group	Patient	14.5	15.7	7.9	0.2	58.5
	Control	4.5	2.6	4.7	1.2	8.2

Table 2. Statistical summary of the serum PTEN concentration

		PTEN protein (ng/ml)				
		Mean (x)	**Standard Deviation (sd)**	**Median (m)**	**Minimum (min)**	**Maximum (max)**
Group	Patient	14.2	14.4	7.8	0.5	55.3
	Control	4.4	2.5	4.6	0.5	7.5

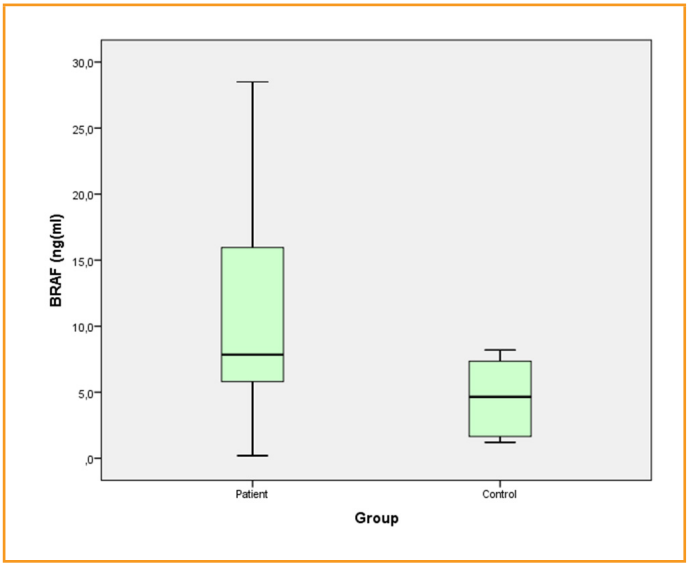

Figure 1. Box plot of BRAF protein levels in NSCLC patients (p<0.05).

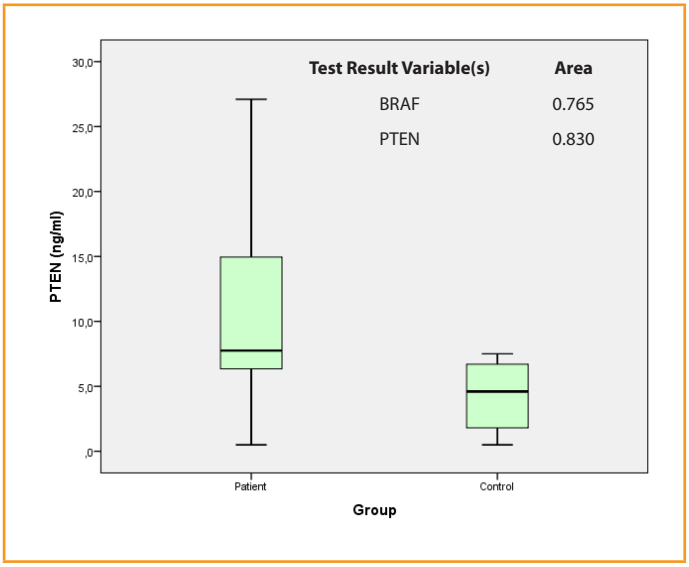

Figure 2. Box plot of PTEN protein levels in NSCLC patients (p<0.05).

Additionally, Spearman's rho correlation analysis revealed a strong positive correlation between BRAF and PTEN serum protein levels (r=0.681, p=0.000), suggesting potential co-regulation or parallel involvement in tumor biology.

Discussion

The molecular profiling of non-small cell lung cancer (NSCLC) has profoundly transformed therapeutic strategies, particularly for tumors exhibiting kinase alterations susceptible to targeted inhibition. This progress has marked a turning point in the management of lung cancer and contributed to notable clinical improvements. Beyond well-established targets such as EGFR, ALK, and ROS1, the BRAF gene has recently gained recognition as a clinically actionable molecular driver in advanced NSCLC.

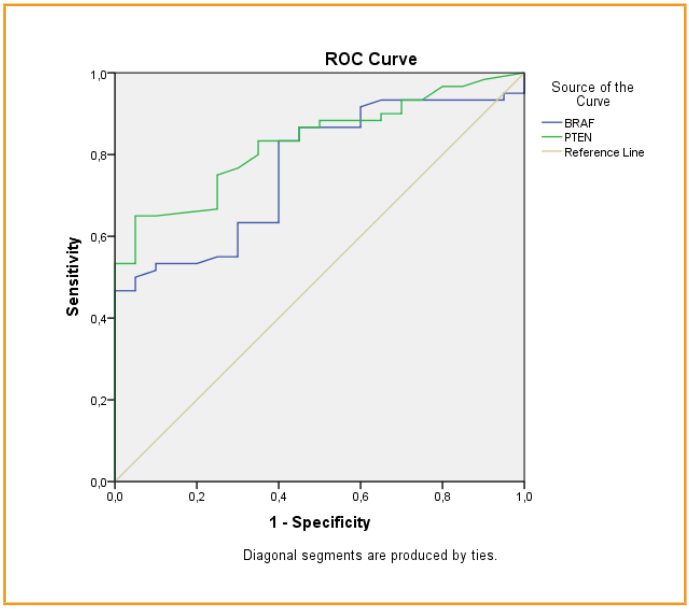

Figure 3. Receiver operating characteristic (ROC) curves for each test. (AUC with 95% CI values).

Table 3. Comparison of serum BRAF and PTEN serum protein levels with demographic data in NSCLC patients

	Number of patients	BRAF p value	PTEN p value
Age			
≤65	48	0.475	0.846
>65	12		
Smoking			
Yes	35	0.310	0.420
No	25		
Gender			
Male	51	0.848	0.727
Female	9		
Stage			
III	28	0.323	0.986
IV	32		
Metastasis			
Yes	32	0.782	0.232
No	28		
Type			
Adeno	30	0.294	0.605
Squamoz	30		

BRAF mutations are detected in approximately 1.5–3.5% of NSCLC patients and are known to induce continuous activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling cascade, thereby

promoting uncontrolled cellular proliferation. Therapeutic strategies involving dual inhibition of the BRAF and MEK pathways—specifically the use of dabrafenib and trametinib in combination—have shown encouraging clinical efficacy in patients with metastatic NSCLC harboring the BRAFV600E mutation. Based on compelling clinical trial results, this therapeutic regimen has been granted approval by both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for use in this subset of patients [12].

In parallel with advances in molecularly guided therapies, the identification and validation of predictive and prognostic biomarkers remain central to optimizing individualized treatment strategies and minimizing therapeutic resistance. Among these, phosphatase and tensin homolog (PTEN) has been investigated as a potential prognostic marker across a variety of cancers, including NSCLC. Loss of PTEN function, which impacts the PI3K/AKT signaling axis, has been correlated with tumor progression and adverse clinical outcomes, suggesting its relevance in the stratification of patients and refinement of therapeutic approaches [13].

In multiple large-cohort investigations, PTEN protein expression has been evaluated primarily through immunohistochemistry (IHC), yielding valuable insights into tumor histology and prognostic relevance. IHC remains the most accessible and cost-effective approach for assessing PTEN levels in lung cancer tissue, and it is widely employed in clinical settings for this purpose. However, in the present study, PTEN expression was quantified using a noninvasive method—enzyme-linked immunosorbent assay (ELISA)—which was applied to serum samples rather than tissue sections. Although distinct from IHC, ELISA also utilizes antigen–antibody interactions to detect and quantify protein concentrations.

The biological function of PTEN in non-small cell lung cancer (NSCLC) has been explored through both in vitro and in vivo models. Experimental studies using NSCLC cell lines have demonstrated that overexpression of PTEN leads to suppressed tumor cell proliferation, often through mechanisms involving cell cycle arrest and induction of apoptosis. Correspondingly, in our study, both BRAF and PTEN serum protein levels were significantly elevated in the serum of lung cancer patients compared with healthy controls ($p=0.001$) [14].

A notable example is the study by Lu et al., [15] which utilized A549 lung adenocarcinoma cells to assess the interaction between PTEN, hTERT, and the PI3K/AKT signaling pathway. Their findings showed that wild-type PTEN, when overexpressed, inhibited cell proliferation, while PTEN silencing via siRNA promoted cell survival, highlighting its central role in tumor suppression [15,16].

Furthermore, meta-analyses and subgroup studies have strengthened the association between PTEN expression and clinical outcomes. For instance, reduced PTEN expression—assessed at both protein and mRNA levels—has been consistently linked to poor prognosis in NSCLC, with significant impacts on overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS). In a meta-analysis by Xiao et al., [17] which pooled data from 23 studies and approximately 2,500 patients, diminished PTEN levels measured by IHC were significantly associated with inferior survival metrics.

In our study, although PTEN expression levels were found to differ significantly between patient and control groups, survival analyses could not be performed due to limited follow-up data. Nevertheless, the consistent association between low PTEN expression and worse outcomes in the literature suggests its potential as both a prognostic indicator and a predictive biomarker in NSCLC.

Mutations in the BRAF gene play a critical role in informing therapeutic decisions for patients with advanced non-small cell lung cancer (NSCLC). A growing body of clinical evidence has demonstrated the clinical benefit of targeted therapies—such as selective BRAF inhibitors, combination treatments, and immunotherapeutic strategies—for patients carrying BRAF alterations. As such, determination of BRAF mutation status has become a key component in the personalized management of NSCLC. In the current analysis, BRAF mutational status was assessed in a cohort of 3,102 patients with advanced-stage NSCLC. In line with previous findings, BRAF mutations were identified in a relatively small subset of patients, accounting for 5.4% of the total cohort [18].

Lung cancer continues to rank as the leading cause of cancer-related mortality globally, primarily due to the high rate of distant metastases observed at the time of diagnosis. The presence of metastasis significantly limits therapeutic efficacy and contributes to persistently low overall survival rates among affected individuals.

The expression of the PTEN gene is subject to precise regulatory control through both genetic and epigenetic mechanisms, including protein–protein interactions (PPIs). Although genetic alterations in PTEN are relatively rare in NSCLC, loss of PTEN protein expression is notably more frequent. Several studies have reported PTEN protein loss in over 40% of NSCLC cases, suggesting that post-translational and epigenetic dysregulation plays a major role in its inactivation. Additionally, associations have been reported between PTEN loss and clinicopathological features such as smoking status, squamous cell carcinoma (SQLC) histology, and reduced survival outcomes [19,20]. No statistically

significant association was observed between serum BRAF and PTEN protein expression levels and clinical variables such as age, disease stage, sex, presence of metastasis, or histological subtype. The present study revealed that both serum BRAF and PTEN protein levels were significantly increased in patients with lung cancer. BRAF and PTEN serum protein levels were not correlated with the stage of NSCLC, histology, sex, or metastasis. These findings suggest that both the BRAF and PTEN proteins might have diagnostic value for NSCLC (based on the results of the ROC analysis according to sensitivity and specificity).

We would like to clarify that this study is a pilot study aimed at exploring the diagnostic potential of serum BRAF and PTEN protein levels in NSCLC. The observed elevations in PTEN may reflect extracellular release from apoptotic or necrotic tumor cells or other biological mechanisms. These preliminary findings are intended to generate hypotheses and provide a basis for further mechanistic studies in future research.

As a pilot study, our focus was on serum protein detection rather than genetic profiling. Assessing serum BRAF protein levels together with mutational status would strengthen the clinical interpretation, and we plan to incorporate mutational analysis in subsequent studies.

To the best of our knowledge, the serum protein levels of PTEN and BRAF have not been previously investigated in patients with NSCLC. This study examined the combined protein levels of BRAF and PTEN in the serum of NSCLC patients. Further trials with larger patient populations are necessary to determine the clinical importance of these biomarkers in patients with lung cancer. This study is the first investigation in the literature to evaluate the combined serum levels of BRAF and PTEN proteins in NSCLC patients. Further studies with larger patient cohorts are needed to elucidate the clinical significance of these biomarkers in lung cancer.

Conclusion

Lung cancer management has undergone a paradigm shift with the integration of molecular profiling into routine clinical practice. The advent of targeted therapies has prompted various professional bodies to establish standardized protocols aimed at optimizing biomarker testing, including the selection of suitable patient cohorts and appropriate testing methodologies. Molecular profiling through targeted assays enables comprehensive interrogation of genomic regions, allowing for the timely identification of oncogenic alterations critical to informed therapeutic decisions.

Liquid biopsy techniques, particularly serum-based assays, offer practical advantages over traditional tissue biopsies. These include being minimally invasive, yielding rapid results, and enabling repeated sampling throughout the treatment course. However, the sensitivity of blood-based assays may be lower compared with tissue-derived analyses, limiting their role as standalone diagnostic tools in non-small cell lung cancer (NSCLC). A range of platforms exists for both tissue- and plasma-based testing, each with its own benefits and constraints, which must be carefully weighed in clinical decision-making. Importantly, molecular aberrations identified via plasma analysis have been associated with similar clinical responses to those found in tissue samples, supporting the complementary role of liquid biopsy in personalized therapy.

Moreover, serial plasma testing offers the ability to monitor therapeutic response in real time and to detect emergent resistance mutations prior to radiographic progression. Consequently, comprehensive biomarker assessment is indispensable for guiding optimal treatment strategies in lung cancer care.

Among emerging biomarkers, BRAF and PTEN have gained attention for their potential diagnostic and prognostic relevance. These molecules, when accurately detected, may contribute significantly to clinical assessment and personalized treatment planning. Immunohistochemistry remains a commonly used approach for the evaluation of BRAF and PTEN status, although recent advances have explored more rapid and sensitive analytical techniques. Despite this progress, a persistent challenge lies in improving the analytical sensitivity of these methods, particularly in distinguishing between indolent alterations and those predictive of malignant transformation.

Ethics Committee Approval: The study was approved by Istanbul University Ethics Committee (No: 2014/923, 16.04.2014).

Conflict of Interest: The authors declare that there is no conflict of interest.

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