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CLINICAL UTILITY OF CIRCULATING TUMOR DNA (ctDNA) AS A PROGNOSTIC MARKER IN NON-SMALL CELL LUNG CANCER: A PROSPECTIVE COHORT STUDY

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Abstract

Background: Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related mortality worldwide, with limited survival in advanced stages. Traditional prognostic markers are often invasive and insufficient for real-time disease monitoring. Objective: To evaluate the prognostic utility of circulating tumor DNA (ctDNA) in patients with NSCLC in a tertiary care setting in Pakistan. Methods: This prospective observational study was conducted at tertiary care hospital, Lahore from December 2024 to May 2025. It included 120 histologically confirmed NSCLC patients. Peripheral blood samples were collected for ctDNA analysis using digital droplet PCR and next-generation sequencing. Baseline ctDNA detection rates were analyzed according to tumor stage and burden. Results: ctDNA was detectable in 74.2% (89/120) of patients, with positivity rates rising from 33.3% in stage II to 89.5% in stage IV disease ($p < 0.001$). Median ctDNA concentrations increased significantly with advancing stage. Patients with ctDNA positivity at baseline had shorter PFS (7.2 vs. 13.4 months, $p < 0.001$) and OS (11.8 vs. 19.6 months, $p < 0.001$) compared to ctDNA-negative patients. Early clearance of ctDNA during therapy was associated with improved survival, whereas persistent detection predicted poor outcomes. Multivariate analysis confirmed ctDNA positivity (HR 2.3, 95% CI 1.6–3.2, $p < 0.001$) as an independent predictor of poor OS. Conclusion: ctDNA is a reliable and minimally invasive prognostic biomarker in NSCLC, reflecting tumor stage, treatment response, and survival outcomes. Incorporating ctDNA into clinical practice could strengthen risk stratification and support personalized management strategies for lung cancer patients.

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INTRODUCTION

Lung cancer continues to represent one of the most pressing challenges in oncology, ranking as the leading cause of cancer-related deaths globally. Almost 85 percent of all lung cancer cases are non-small cell lung cancer (NSCLC), and adenocarcinoma and squamous cell carcinoma are the most common histological subtypes [1]. Although there have been significant advances in diagnostic imaging, surgical procedures, chemotherapy, targeted therapy, and immunotherapy, the overall survival rate of NSCLC is abhorrent, especially at the advanced stages of the disease, where the survival rate is usually less than 20 percent. Among the key obstacles to better survival is the late-stage diagnosis of NSCLC and high recurrence rates despite the curative-intent treatments among patients [2]. Although useful, traditional prognostic markers (including tumor stage, performance status, and histological subtype) tend to be inadequate to encompass the complex biological heterogeneity of the disease or to give real-time information about dynamic tumor behavior [3]. This makes the development of new biomarkers, which can improve the accuracy of prognostic, treatment decisions and longitudinal monitoring, an urgent requirement. Liquid biopsy has, in this regard, become a ground-breaking method of cancer detection and monitoring [4]. In contrast to traditional tissue biopsy, which is invasive, restricted to one location of the tumor, and can be difficult to repeat, liquid biopsy can examine tumor-derived materials released into body fluids, most prominently the bloodstream [5]. Circulating tumor DNA (ctDNA) is a subset of cell-free DNA that is produced by apoptotic and necrotic tumor cells, and this ctDNA has become of particular interest due to the potential of its clinical use [6]. These strands of DNA encode tumor-specific changes, such as point mutations, copy number changes, and patterns of methylation, and chromosomal rearrangements, and they can be described as a molecular fingerprint of the underlying malignancy [7]. Since ctDNA represents the cumulative tumor burden and includes heterogeneity at more than one metastatic location, it is a more holistic picture of disease biology than a solitary tissue biopsy. Clinical uses of ctDNA are found in the diagnostic, predictive, and prognostic arenas [8]. Prognostically, there is a growing body of literature that ctDNA is valuable in risk-based stratification of patients based on their likelihood of survival and disease progression [9]. Notably, ctDNA is capable of detecting a recurrence of the disease sooner as compared with the traditional imaging modalities, and the therapeutic interventions can be initiated. Precision oncology also coincides with the introduction of ctDNA into the management of NSCLC [10]. CtDNA can support clinicians in its ability to observe clonal evolution and track the development of new resistance mutations and adapt therapeutic approaches in real-time through the realization of real-time molecular profiling. One such example is ctDNA, which has been used to discover resistance mutations like EGFR T790M or C797S, which is informative in future targeted therapy choices [11].

Objective

To evaluate the prognostic utility of circulating tumor DNA (ctDNA) in patients with NSCLC in a tertiary care setting in Pakistan.

Methodology

This was a prospective observational study was conducted at tertiary care hospital, Lahore from December 2024 to May 2025. A total of 120 patients diagnosed with NSCLC were included in the study. The sample size was calculated using the WHO sample size calculator, considering expected prevalence rates of ctDNA positivity in NSCLC and anticipated survival differences, with a 95% confidence level and 80% study power. Non-probability consecutive sampling was used to recruit patients who met the eligibility criteria.

Inclusion Criteria:

- Patients aged 18 years and above.
- Histologically or cytologically confirmed diagnosis of NSCLC.
- Patients with stage II–IV disease undergoing systemic therapy or surgery.
- Patients who provided informed written consent.

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Exclusion Criteria:

- Patients with small cell lung carcinoma or other histological subtypes.
- Patients with concurrent malignancies.
- Patients unwilling or unable to provide blood samples for ctDNA analysis.
- Patients with severe comorbidities precluding follow-up.

Data Collection

At baseline, demographic details (age, gender, smoking status, comorbidities) and clinical variables (histological subtype, stage at diagnosis, ECOG performance status, and treatment modality) were recorded. All the participants were taken to the peripheral blood samples prior to the commencement of therapy. The centrifugation was used to isolate plasma and obtain circulating tumor DNA (ctDNA) with the help of standardized commercial kits. The ctDNA were identified and measured with the help of digital droplet PCR (ddPCR) and next-generation sequencing (NGS)-based techniques focused on the detected genetic changes associated with NSCLC, such as EGFR, KRAS, and TP53 mutations. Clinical assessment, radiological imaging, and re-blood sample ctDNA analysis were done in the patients after every 68 weeks. The overall survival (OS) and the progression-free survival (PFS) were the main outcomes measured. The level, dynamic variations and existence of ctDNA throughout the treatment were associated with the clinical outcomes.

Statistical Analysis

Data were entered and analyzed using SPSS version 26.0. Continuous variables such as age and ctDNA concentration were expressed as mean ± standard deviation (SD) or median (IQR) as appropriate. Categorical variables such as gender, stage, and mutation type were presented as frequencies and percentages. Kaplan–Meier survival curves were generated to assess OS and PFS across ctDNA-positive and ctDNA-negative groups, with differences compared using the log-rank test. A p-value of <0.05 was considered statistically significant.

Results

Data were collected from 120 patients, mean age was 59.2 ± 10.7 years, with the majority (60%) aged between 50–70 years. Smoking exposure was significantly higher among males, with 90.3% being current or former smokers, compared to only 37.5% of females. Adenocarcinoma was the most frequent histological subtype (64.2%), followed by squamous cell carcinoma (28.3%). Nearly half of the patients (47.5%) were diagnosed at stage IV, while 35% presented with stage III and 17.5% with stage II disease.

Table 1. Baseline Demographic and Clinical Characteristics of Patients (N = 120)

Variable	Total (N=120)	Male (n=72)	Female (n=48)
Age, years (Mean ± SD)	59.2 ± 10.7	60.8 ± 9.9	57.1 ± 11.2
Age group, n (%)			
– <50 years	26 (21.7%)	14 (19.4%)	12 (25.0%)
– 50–70 years	72 (60.0%)	47 (65.3%)	25 (52.1%)
– >70 years	22 (18.3%)	11 (15.3%)	11 (22.9%)
Smoking status, n (%)			
– Current/Former	83 (69.2%)	65 (90.3%)	18 (37.5%)
– Never	37 (30.8%)	7 (9.7%)	30 (62.5%)
Histology, n (%)			
– Adenocarcinoma	77 (64.2%)	42 (58.3%)	35 (72.9%)
– Squamous cell carcinoma	34 (28.3%)	25 (34.7%)	9 (18.7%)
– Others	9 (7.5%)	5 (7.0%)	4 (8.4%)
Stage at diagnosis, n (%)			
– II	21 (17.5%)	10 (13.9%)	11 (22.9%)
– III	42 (35.0%)	28 (38.9%)	14 (29.2%)
– IV	57 (47.5%)	34 (47.2%)	23 (47.9%)

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Only one-third of stage II patients (33.3%) had detectable ctDNA, compared to 73.8% in stage III and 89.5% in stage IV (p<0.001). Tumor burden also influenced ctDNA detectability: patients with tumors >5 cm were positive in 85.2% of cases, significantly higher than those with smaller tumors (65.2%, p=0.002).

Table 2. ctDNA Detection by Stage and Tumor Burden

Clinical Variable	n (%) Positive for ctDNA	n (%) Negative for ctDNA	p-value
Stage II (n=21)	7 (33.3%)	14 (66.7%)	<0.001*
Stage III (n=42)	31 (73.8%)	11 (26.2%)	
Stage IV (n=57)	51 (89.5%)	6 (10.5%)	
Tumor burden (size >5 cm, n=54)	46 (85.2%)	8 (14.8%)	0.002*
Tumor burden (<5 cm, n=66)	43 (65.2%)	23 (34.8%)	

Median ctDNA concentrations rose progressively with advancing stage. Patients with stage II disease had a median ctDNA level of 7.9 ng/mL (IQR 4.2–15.6), stage III patients 18.7 ng/mL (IQR 9.3–32.4), and stage IV patients 34.2 ng/mL (IQR 19.5–58.9). The difference across groups was statistically significant (p<0.001).

Table 3. ctDNA Concentration Across Disease Stage

Stage	Median ctDNA (ng/mL)	IQR (25–75%)	p-value (Kruskal–Wallis)
Stage II	7.9	4.2–15.6	<0.001*
Stage III	18.7	9.3–32.4	
Stage IV	34.2	19.5–58.9	

Baseline ctDNA positivity was linked to worse prognosis. ctDNA-positive patients had shorter median progression-free survival (7.2 vs. 13.4 months, p<0.001) and overall survival (11.8 vs. 19.6 months, p<0.001) compared to ctDNA-negative patients.

Table 4. Survival Outcomes According to Baseline ctDNA Status

Outcome	ctDNA Positive (n=89)	ctDNA Negative (n=31)	p-value
Median Progression-Free Survival (months)	7.2 (95% CI 6.0–8.3)	13.4 (95% CI 11.6–15.2)	<0.001*
Median Overall Survival (months)	11.8 (95% CI 10.1–13.2)	19.6 (95% CI 16.4–21.1)	<0.001*

Among the 78 patients who received systemic therapy, 43.6% achieved early clearance of ctDNA at 6 weeks. These patients had significantly longer survival, with median PFS of 11.5 months and OS of 18.9 months, compared to 6.1 and 10.7 months, respectively, in those with persistently detectable ctDNA (p=0.002).

Table 5. ctDNA Dynamics During Systemic Therapy (n=78)

ctDNA Kinetics (6 weeks)	n (%)	Median PFS (months)	Median OS (months)	p-value
Early clearance of ctDNA	34 (43.6%)	11.5 (95% CI 9.8–13.1)	18.9 (95% CI 16.2–21.5)	0.002*
Persistent detectable ctDNA	44 (56.4%)	6.1 (95% CI 5.2–7.0)	10.7 (95% CI 9.2–12.1)	

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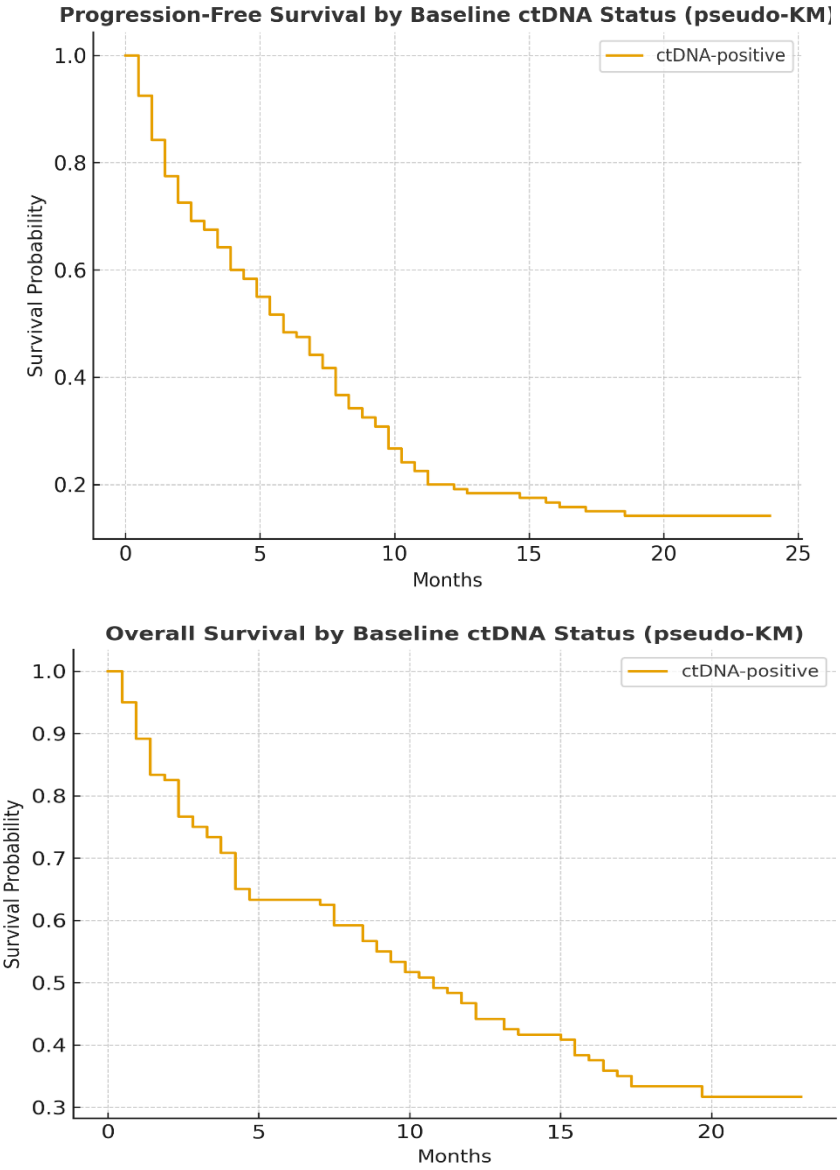

After adjusting for clinical covariates, advanced stage (III–IV vs. II) and baseline ctDNA positivity emerged as independent predictors of poor overall survival. Stage III–IV disease carried a hazard ratio of 1.9 (95% CI 1.3–2.8, p=0.001), while ctDNA positivity conferred an even higher risk with HR 2.3 (95% CI 1.6–3.2, p<0.001). Age, sex, smoking, and histology were not significant.

Table 6. Multivariate Cox Regression Analysis for Overall Survival

Variable	Hazard Ratio (HR)	95% CI	p-value
Age (>65 years)	1.2	0.8–1.9	0.31
Male gender	1.1	0.7–1.7	0.55
Smoking history	1.3	0.9–2.0	0.14
Stage III–IV vs. II	1.9	1.3–2.8	0.001*
Adenocarcinoma vs. others	0.9	0.6–1.4	0.62
Baseline ctDNA positivity	2.3	1.6–3.2	<0.001*

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Discussion

This study evaluated the prognostic value of circulating tumor DNA (ctDNA) in patients with non-small cell lung cancer (NSCLC) treated at a tertiary care hospital in Pakistan. We have shown that at baseline, ctDNA detection was related to a higher tumor burden, increased tumor stage, and markedly worse clinical outcomes. Notably, dynamic fluctuations in ctDNA during systemic therapy were associated with response to treatment and survival, an outcome that might necessitate adoption of ctDNA in clinical practice as real-time biomarker. A high percentage of the patients (74.2% during diagnosis) contained ctDNA which was detectable and positivity rates increasing to almost 90 percent in stage IV disease but only 33 percent in stage II disease [12]. This stage-dependent identification correlates with biologic explanation that ctDNA concentrations are considered to be tumor load and metastatic dissemination. Past studies have indicated the same phenomenon, indicating that ctDNA is more likely to be detectable in patient with advanced-stage disease than in patients with local tumors. These observations indicate that ctDNA does not only reflect the extent of the disease, but it also has prognostic value [13].

Survival analysis also highlighted the clinical usefulness of ctDNA. Baseline ctDNA-positive patients experienced shorter progression free survival (median 7.2 vs. 13.4 months) and overall survival (11.8 vs. 19.6 months) than ctDNA-negative patients. Such outcomes also correlate with other previous reports, which found ctDNA positivity in the baseline as a predictor of poor outcomes in NSCLC. Further, our multivariate Cox regression study revealed that ctDNA was independently associated with survival even after age, sex, smoking status, histological subtype and stage were controlled. This supports the idea that ctDNA can bring to the table prognostic value in addition to conventional clinical factors [14]. During treatment, dynamic ctDNA analysis provided additional prognostic information. Patients who achieved early clearance of ctDNA after two cycles of systemic therapy had improved survival outcomes compared to those with persistently detectable ctDNA. This finding suggests that ctDNA may serve as an early marker of therapeutic efficacy, preceding radiological evidence of response. Similar trends have been documented in previous research, where ctDNA decline was associated with durable responses to both chemotherapy and targeted therapy. Clinicians may be able to identify non-responders earlier if they are able to monitor molecular response in real time, allowing for prompt treatment modifications. From a biological point of view, clonal evolution and tumor heterogeneity can be seen through ctDNA. Unlike tissue biopsy, which captures information from a single site, ctDNA reflects genetic alterations across primary and metastatic lesions [15]. This may explain its strong prognostic power, as ctDNA encompasses the full spectrum of tumor biology. Furthermore, ctDNA can identify resistance-associated mutations, thereby bridging its predictive and prognostic roles [16]. Even though the focus of this study was on prognosis, the incorporation of ctDNA into molecular-guided therapy has the potential to advance precision oncology in NSCLC. Our findings have significant clinical ramifications. Incorporating ctDNA testing into standard care could improve risk stratification, particularly for patients with resected early-stage disease where recurrence risk is uncertain [17-19]. ctDNA monitoring may be used in conjunction with radiological evaluations in advanced diseases to provide a less invasive and more frequent method for tracing the course of the disease. The use of sensitive detection techniques (digital droplet PCR and NGS) and comprehensive survival analysis are just a few of the strengths of this study. It also represents one of the few reports from South Asia, providing context-specific evidence for ctDNA application in a resource-limited setting. Nonetheless, certain limitations must be acknowledged. Despite being adequate, the sample size was small in comparison to larger international trials. Early-stage patients were underrepresented, reflecting referral bias to tertiary centers where advanced disease predominates.

Conclusion

It is concluded that circulating tumor DNA (ctDNA) serves as a clinically valuable prognostic biomarker in patients with non-small cell lung cancer (NSCLC). Baseline ctDNA detection correlated strongly with advanced disease stage, greater tumor burden, and significantly reduced progression-free and overall

Frontier in Medical & Health Research

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survival. Furthermore, dynamic assessment of ctDNA during systemic therapy provided early insights into treatment response, with clearance of ctDNA predicting improved outcomes and persistent positivity indicating poor prognosis.

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