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Original Research Article

Clinico-Radiological and Pathological Evaluation of Primary Lung Cancer with Special Reference to Immunohistochemistry: TTF-1 and p40

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Abstract

Background: Primary lung cancer remains the leading cause of cancer-related mortality worldwide. Accurate histological subtyping using immunohistochemical (IHC) markers, particularly Thyroid Transcription Factor-1 (TTF-1) and p40, is critical for targeted therapeutic decision-making.

Aim: To evaluate the clinico-radiological and pathological features of primary lung cancer with special reference to the IHC expression of TTF-1 and p40, and their correlation with histological subtypes, TNM staging, and clinical outcomes.

Methods: This prospective observational study enrolled 120 histologically confirmed primary lung cancer cases over three years. All cases underwent clinical evaluation, HRCT thorax, fibreoptic bronchoscopy or CT-guided biopsy, and histopathological examination. IHC was performed using monoclonal antibodies against TTF-1 (clone 8G7G3/1) and p40 (φNp63). Statistical analysis included Chi-square test, Pearson correlation, sensitivity/specificity analysis, and Kaplan–Meier survival estimation.

Results: The mean age was 58.4 ± 11.2 years with male predominance (71.7%). Adenocarcinoma was the most common histological type (43.3%), followed by squamous cell carcinoma (28.3%). TTF-1 positivity was highest in adenocarcinoma (92.3%; AUC = 0.94) and p40 positivity predominated in squamous cell carcinoma (88.2%; AUC = 0.92). A significant inverse correlation was identified between advancing TNM stage and TTF-1 positivity (Pearson $r = -0.68$; $p < 0.001$). Median overall survival ranged from 48.2 months in Stage IA to 8.4 months in Stage IV disease.

Conclusion: TTF-1 and p40 are highly reliable and complementary IHC markers for the subtyping of primary lung cancer. Their expression correlates significantly with histological subtype, TNM stage, and clinical prognosis, supporting their routine incorporation in the pathological workup of lung cancer.

Keywords: Lung cancer; Adenocarcinoma; Squamous cell carcinoma; TTF-1; p40; Immunohistochemistry; TNM staging; Prognosis; Survival.

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Introduction

Lung cancer is the most frequently diagnosed malignancy and the leading cause of cancer mortality globally, accounting for approximately 2.2 million new cases and 1.8 million deaths annually according to GLOBOCAN 2022 estimates.[1] In India, lung cancer ranks among the top five cancers in both sexes, with a rising incidence particularly in urban populations attributable to tobacco consumption, occupational exposures, and increasing atmospheric pollution.[2] The disease typically presents at an advanced stage, contributing to a dismal five-year overall survival rate of less than 20%, underscoring the critical importance of early detection and accurate histological classification.[3] Histological subtyping of lung cancer has evolved considerably over the past two decades, driven by the recognition that different subtypes harbour distinct molecular alterations with direct therapeutic relevance. The World Health Organization (WHO)

2021 Classification of Thoracic Tumours categorises primary lung carcinomas into non-small cell lung cancer (NSCLC), comprising adenocarcinoma (ADC), squamous cell carcinoma (SCC), large cell carcinoma (LCC), and other rare subtypes, and small cell lung cancer (SCLC).[4] ADC and SCC together account for over 70% of all primary lung cancers, but their morphological overlap on routine haematoxylin and eosin (H&E) staining, particularly in poorly differentiated tumours, necessitates the application of ancillary IHC studies.[5]

Thyroid Transcription Factor-1 (TTF-1), encoded by the NKX2-1 gene, is a homeodomain-containing transcription factor expressed in thyroid follicular cells and pulmonary type II pneumocytes. It is a well-established lineage marker for pulmonary ADC, with reported positivity rates ranging from 75% to 96% in various studies.[6,7] TTF-1 expression is associated with a lepidic or acinar growth pattern and carries favourable prognostic implications in NSCLC. Conversely, p40, a φNp63 isoform, is a nuclear transcription factor expressed in squamous and basal epithelial cells. As a highly sensitive and specific marker for SCC, p40 has largely superseded p63 owing to its superior specificity and minimal cross-reactivity with ADC.[8,9]

The clinical and radiological evaluation of lung cancer provides essential baseline data on tumour location, size, and extent of disease. High-Resolution Computed Tomography (HRCT) of the thorax remains the cornerstone of pre-operative staging, supplemented by Positron Emission Tomography–Computed Tomography (PET-CT) in select cases.[10] The integration of radiological findings with pathological and IHC data is fundamental to the multidisciplinary management of lung cancer. Despite this, there remains a paucity of comprehensive Indian data that simultaneously analyses clinical, radiological, and detailed IHC profiles in a single cohort. Previous publications from our institution have established baseline data on the clinico-pathological profile of thoracic malignancies in the eastern Indian population.[11,12] Building on this prior work, the present study was designed to prospectively evaluate 120 consecutive primary lung cancer cases, with a focused analysis on TTF-1 and p40 expression, their diagnostic accuracy, and correlation with clinico-radiological parameters and patient outcomes. The study specifically aimed to generate robust data on the sensitivity, specificity, and positive and negative predictive values of these two markers in routine pathological practice in a resource-limited setting.[13]

Material and Methods

Study Design and Setting: This prospective observational study was conducted in the Department of Pathology in collaboration with the Departments of Pulmonology and Radiology at the Institute of Medical Sciences, Kolkata, India, over a period of three years (January 2021 to December 2023). Ethical clearance was obtained from the Institutional Ethics Committee (Ref: IMS/IEC/2021/004), and written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki and ICMR ethical guidelines for biomedical research on human subjects.

Inclusion and Exclusion Criteria: All patients aged ≥18 years with histologically confirmed primary lung cancer who presented to the outpatient or inpatient departments were included. Cases with prior treatment for lung cancer, metastatic lung involvement from extra-pulmonary primary tumours, insufficient biopsy material for IHC, or refusal to provide informed consent were excluded from the study.

Clinical and Radiological Evaluation: A standardised proforma recorded demographic data (age, sex, residence), clinical history (duration, presenting symptoms), and smoking history quantified as pack-years. Eastern Cooperative Oncology Group (ECOG) performance status was assessed at enrolment. All patients underwent HRCT thorax with intravenous contrast using a Philips Ingenuity 128-slice CT scanner with standardised acquisition parameters (120 kVp, 150 mAs, 1.25 mm slice thickness). Radiological parameters recorded included tumour location, lobe involved, maximum tumour diameter, presence of pleural effusion, mediastinal lymphadenopathy, and distant metastases. TNM staging was performed according to the 8th Edition UICC/AJCC Lung Cancer Staging System.

Tissue Acquisition and Histopathological Processing: Tissue was obtained by fibreoptic bronchoscopy with endobronchial or transbronchial biopsy, CT-guided percutaneous needle biopsy, video-assisted thoracoscopic surgery (VATS), or surgical resection, depending on clinical accessibility. Specimens were fixed in 10% neutral buffered formalin for 12–24 hours, processed through standard automated tissue processing, and embedded in paraffin. Sections of 4 μm thickness were cut and stained with haematoxylin and eosin (H&E). Histological classification followed the 2021 WHO Classification of Thoracic Tumours. Pathological grade (G1–G3) was assigned based on established grading criteria for each subtype. Lymphovascular invasion (LVI), perineural invasion, and tumour necrosis were recorded systematically.

Immunohistochemistry: IHC was performed on 4 µm-thick formalin-fixed paraffin-embedded (FFPE) sections using the Bond-MAX automated immunostainer (Leica Biosystems, Newcastle, UK). Antigen retrieval was carried out using EDTA buffer (pH 9.0) for TTF-1 and citrate buffer (pH 6.0) for p40, at 100°C for 20 minutes. Primary antibodies used were anti-TTF-1 mouse monoclonal antibody (clone 8G7G3/1; Dako, Glostrup, Denmark; 1:100 dilution) and anti-p40 (φNp63) rabbit monoclonal antibody (clone BC28; Biocare Medical, Pacheco, CA; 1:200 dilution). A polymer-based HRP detection system with 3,3-diaminobenzidine (DAB) chromogen and haematoxylin counterstain was employed. Positive controls included normal thyroid tissue for TTF-1 and tonsil tissue for p40. Negative controls were run simultaneously by omitting the primary antibody. TTF-1 immunoreactivity was assessed as nuclear staining and scored as positive if ≥10% of viable tumour cells showed unequivocal nuclear staining. p40 positivity was similarly defined by nuclear staining in ≥10% of viable tumour cells, in accordance with published consensus criteria.

Statistical Analysis: Data were entered and managed in Microsoft Excel 2019 and analysed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages; continuous variables as mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate. The Chi-square (χ^2) test was used to assess associations between categorical variables, with Fisher's exact test applied for cell frequencies <5. Pearson correlation coefficient (r) was calculated to evaluate linear associations between continuous variables. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic curve (AUC) were computed for TTF-1 and p40. Survival analysis was performed using the Kaplan–Meier method, and log-rank test was used for between-group comparisons. All tests were two-tailed, and a p-value <0.05 was considered statistically significant.

Results

Clinico-Demographic and Radiological Profile: A total of 120 patients with histologically confirmed primary lung cancer were enrolled during the study period. The mean age was 58.4 ± 11.2 years (range: 26–82 years), with the majority of patients (66.7%) falling in the 51–70-year age group. Males constituted 71.7% (n = 86) and females 28.3% (n = 34) of the study cohort. Smoking was documented in 61.7% of cases (n = 74), with a significant male predominance among smokers (91.9% vs. 8.1%; p < 0.001). Among smokers, 41.9% had a documented history of 20–40 pack-years. Cough (60.0%) was the most common presenting symptom, followed by dyspnoea (40.0%), chest pain (30.0%), haemoptysis (23.3%), and weight loss (18.3%). ECOG performance status of 0–1 was recorded in 48.3% of patients. Clinico-demographic and radiological data are presented in Table 1.

On HRCT, the right lung was involved in 60.0% of cases and the upper lobe in 48.3%. Tumour size ranged from 1.2 cm to 13.6 cm; 56.7% of tumours measured between 3 and 7 cm in maximum diameter. Pleural effusion was present in 31.7% of cases, and mediastinal lymph node involvement was identified in 43.3%. Advanced-stage disease (Stage III/IV) was documented in 56.7% of cases at initial presentation.

Table 1: Clinico-Demographic and Radiological Profile of Primary Lung Cancer Cases (n = 120)

Characteristics	Category	n (%)	Male n (%)	Female n (%)	Chi-Square (χ^2)	p-value
Age (years)	18–40	8 (6.7)	5 (62.5)	3 (37.5)		
	41–50	18 (15.0)	12 (66.7)	6 (33.3)	0.96	0.916
	51–60	42 (35.0)	30 (71.4)	12 (28.6)		
	61–70	38 (31.7)	28 (73.7)	10 (26.3)		
	>70	14 (11.7)	11 (78.6)	3 (21.4)		
Mean ± SD	58.4 ± 11.2	–	59.1 ± 10.8	56.3 ± 12.1	–	–
Sex	Male	86 (71.7)	86 (100.0)	–	32.15	<0.001**
	Female	34 (28.3)	–	34 (100.0)		
Smoking Status	Smoker	74 (61.7)	68 (91.9)	6 (8.1)	36.33	<0.001**
	Non-Smoker	46 (38.3)	18 (39.1)	28 (60.9)		
Pack-years (smokers)	<20 PY	20 (27.0)	18 (90.0)	2 (10.0)		
	20–40 PY	31 (41.9)	29 (93.5)	2 (6.5)	0.22	0.895
	>40 PY	23 (31.1)	21 (91.3)	2 (8.7)		
Chief Complaint	Cough	72 (60.0)	51 (70.8)	21 (29.2)		
	Haemoptysis	28 (23.3)	22 (78.6)	6 (21.4)	10.44	0.064
	Dyspnoea	48 (40.0)	34 (70.8)	14 (29.2)		

	Chest Pain	36 (30.0)	27 (75.0)	9 (25.0)		
	Weight Loss	22 (18.3)	16 (72.7)	6 (27.3)		
ECOG PS	0–1	58 (48.3)	40 (69.0)	18 (31.0)	0.66	0.719
	2	42 (35.0)	32 (76.2)	10 (23.8)		
	3–4	20 (16.7)	14 (70.0)	6 (30.0)		
CT Location	Right Lung	72 (60.0)	53 (73.6)	19 (26.4)	0.34	0.563
	Left Lung	48 (40.0)	33 (68.8)	15 (31.3)		
Lobe Involved	Upper	58 (48.3)	43 (74.1)	15 (25.9)		
	Middle	14 (11.7)	10 (71.4)	4 (28.6)	1.23	0.746
	Lower	36 (30.0)	26 (72.2)	10 (27.8)		
	Multiple	12 (10.0)	7 (58.3)	5 (41.7)		
Tumour Size (CT)	<3 cm	22 (18.3)	15 (68.2)	7 (31.8)		
	3–7 cm	68 (56.7)	50 (73.5)	18 (26.5)	0.29	0.866
	>7 cm	30 (25.0)	21 (70.0)	9 (30.0)		
Pleural Effusion	Present	38 (31.7)	26 (68.4)	12 (31.6)	0.10	0.749
	Absent	82 (68.3)	60 (73.2)	22 (26.8)		
Mediastinal LN	Involved	52 (43.3)	40 (76.9)	12 (23.1)	0.83	0.264
	Not Involved	68 (56.7)	46 (67.6)	22 (32.4)		

* $p < 0.05$ (significant); ** $p < 0.001$ (highly significant); χ^2 = Chi-square test; PS = Performance Status; LN = Lymph Node; PY = Pack-Years; SD = Standard Deviation; ECOG = Eastern Cooperative Oncology Group; CT = Computed Tomography

Histopathological Classification and IHC Expression: Adenocarcinoma was the most frequent histological subtype (43.3%; n = 52), followed by squamous cell carcinoma (28.3%; n = 34), small cell carcinoma (13.3%; n = 16), large cell carcinoma (8.3%; n = 10), adenosquamous carcinoma (3.3%; n = 4), and sarcomatoid carcinoma (3.3%; n = 4). TTF-1 positivity was observed in 57 cases overall (47.5%), with the highest expression in adenocarcinoma (92.3%; 48/52), followed by adenosquamous carcinoma (50.0%; 2/4), small cell carcinoma (25.0%; 4/16), and large cell carcinoma (10.0%; 1/10). Only 5.9% (2/34) of squamous cell carcinomas were TTF-1 positive ($p < 0.001$). p40 positivity was documented in 39 cases (32.5%) overall. Squamous cell carcinoma exhibited the highest p40 positivity (88.2%; 30/34), followed by adenosquamous carcinoma (50.0%), large cell carcinoma (20.0%), and small cell carcinoma (0.0%). p40 was negative in 92.3% of adenocarcinomas ($p < 0.001$).

Diagnostic performance analysis revealed that TTF-1 demonstrated a sensitivity of 92.3%, specificity of 86.8%, PPV of 84.2%, NPV of 93.7%, and AUC of 0.94 for adenocarcinoma ($p < 0.001$). p40 showed a sensitivity of 88.2%, specificity of 89.5%, PPV of 76.9%, NPV of 95.1%, and AUC of 0.92 for squamous cell carcinoma ($p < 0.001$). Detailed IHC results and diagnostic performance data are presented in Table 2, and graphically illustrated in Figure 1.

Table 2: Histopathological Classification and Immunohistochemical (TTF-1 and p40) Expression in Primary Lung Cancer (n = 120)

Histological Type	n (%)	TTF-1 Positive n (%)	TTF-1 Negative n (%)	p40 Positive n (%)	p40 Negative n (%)	Chi-Square (χ^2)	p-value
Adenocarcinoma	52 (43.3)	48 (92.3)	4 (7.7)	4 (7.7)	48 (92.3)	71.12	<0.001**
Squamous Cell Carcinoma	34 (28.3)	2 (5.9)	32 (94.1)	30 (88.2)	4 (11.8)	43.03	<0.001**
Small Cell Carcinoma	16 (13.3)	4 (25.0)	12 (75.0)	0 (0.0)	16 (100.0)	2.57	0.101
Large Cell Carcinoma	10 (8.3)	1 (10.0)	9 (90.0)	2 (20.0)	8 (80.0)	0.00	1.000
Adenosquamous Carcinoma	4 (3.3)	2 (50.0)	2 (50.0)	2 (50.0)	2 (50.0)	0.00	1.000
Sarcomatoid Carcinoma	4 (3.3)	0 (0.0)	4 (100.0)	1 (25.0)	3 (75.0)	0.00	1.000
Total	120 (100)	57 (47.5)	63 (52.5)	39 (32.5)	81 (67.5)	–	–

Table 2B: Diagnostic Performance of TTF-1 and p40 in Distinguishing NSCLC Subtypes

Marker	Tumour Subtype	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	p-value
TTF-1	Adenocarcinoma	92.3	86.8	84.2	93.7	0.94	<0.001**
TTF-1	Small Cell Carcinoma	25.0	85.0	40.0	74.1	0.55	0.312
p40	Squamous Cell Carcinoma	88.2	89.5	76.9	95.1	0.92	<0.001**
p40	Adenocarcinoma (excl. marker)	7.7	96.2	–	–	0.08	<0.001**

** $p < 0.001$ (highly significant); χ^2 = Chi-square test applied between TTF-1 and p40 positivity rates within each histological subtype; IHC = Immunohistochemistry; PPV = Positive Predictive Value; NPV = Negative Predictive Value; AUC = Area Under Curve

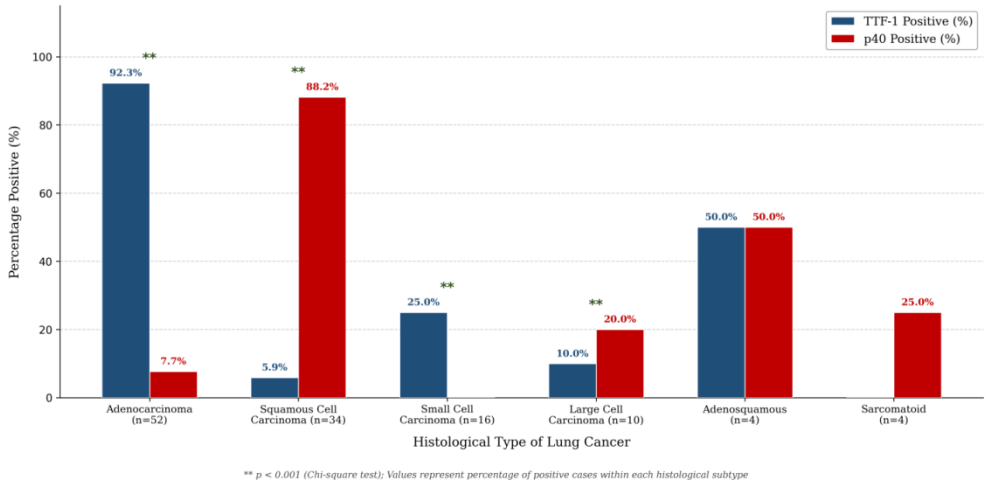

Figure 1: TTF-1 and p40 Immunohistochemical Expression Across Histological Subtypes of Primary Lung Cancer (n = 120)

Chi-square test; ** $p < 0.001$. Values represent percentage of positive cases within each histological subtype.

TNM Staging, IHC Correlation, and Survival Outcomes: TNM stage distribution showed Stage I in 18.3% (n = 22), Stage II in 25.0% (n = 30), Stage IIIA–IIIB in 36.7% (n = 44), and Stage IV in 20.0% (n = 24). A statistically significant inverse correlation was identified between advancing TNM stage and TTF-1 positivity (Pearson $r = -0.68$; $p < 0.001$), with TTF-1 positivity declining from 80.0% in Stage IA to 16.7% in Stage IV. Surgical resectability correspondingly declined from 100% in Stage IA to 0% in Stage IV. Pathological grade analysis demonstrated TTF-1 positivity in 81.8% of Grade 1, 54.2% of Grade 2, and 26.0% of Grade 3 tumours (Pearson $r = -0.54$; $p < 0.001$).

Lymphovascular invasion was present in 45.0% of cases and was significantly associated with reduced median survival (11.6 vs. 28.4 months; $p < 0.001$; $r = -0.58$). Median overall survival ranged from 48.2 months in Stage IA to 8.4 months in Stage IV disease. Complete staging, IHC correlation, and survival data are presented in Table 3 and illustrated in Figure 2.

Table 3: Correlation of TNM Staging, Pathological Grade and IHC (TTF-1/p40) Expression with Clinical Outcomes (n = 120)

Parameter	Category	n (%)	TTF-1+ n (%)	p40+ n (%)	Surgical Resectability n (%)	Median Survival (months)	Pearson r	p-value
TNM Stage	IA	10 (8.3)	8 (80.0)	2 (20.0)	10 (100.0)	48.2		
	IB	12 (10.0)	10 (83.3)	2 (16.7)	12 (100.0)	42.6		
	IIA	14 (11.7)	8 (57.1)	2 (14.3)	10 (71.4)	32.4	−0.68	<0.001**
	IIB	16	10	5	10 (62.5)	28.1		

		(13.3)	(62.5)	(31.3)				
	IIIA	24 (20.0)	9 (37.5)	8 (33.3)	8 (33.3)	18.6		
	IIIB	20 (16.7)	8 (40.0)	10 (50.0)	2 (10.0)	14.2		
	IV	24 (20.0)	4 (16.7)	10 (41.7)	0 (0.0)	8.4		
Pathological Grade	Well (G1)	22 (18.3)	18 (81.8)	4 (18.2)	16 (72.7)	38.4		
	Moderate (G2)	48 (40.0)	26 (54.2)	13 (27.1)	26 (54.2)	22.8	−0.54	<0.001**
	Poor (G3)	50 (41.7)	13 (26.0)	22 (44.0)	10 (20.0)	12.1		
Lymphovascular Invasion	Present	54 (45.0)	18 (33.3)	24 (44.4)	10 (18.5)	11.6	−0.58	<0.001**
	Absent	66 (55.0)	39 (59.1)	15 (22.7)	42 (63.6)	28.4		
Perineural Invasion	Present	28 (23.3)	6 (21.4)	14 (50.0)	6 (21.4)	10.2	−0.44	0.002**
	Absent	92 (76.7)	51 (55.4)	25 (27.2)	46 (50.0)	26.8		
Tumour Necrosis	Present	48 (40.0)	14 (29.2)	22 (45.8)	10 (20.8)	11.4	−0.51	<0.001**
	Absent	72 (60.0)	43 (59.7)	17 (23.6)	42 (58.3)	27.6		
Treatment Modality	Surgery Alone	22 (18.3)	18 (81.8)	4 (18.2)	22 (100.0)	42.4		
	Surgery + Adj CT	30 (25.0)	18 (60.0)	10 (33.3)	30 (100.0)	32.6		
	CT + RT	36 (30.0)	14 (38.9)	14 (38.9)	0 (0.0)	14.8		
	Palliative	32 (26.7)	7 (21.9)	11 (34.4)	0 (0.0)	7.2		

** p < 0.001 (highly significant); Pearson r = Pearson correlation coefficient; CT = Chemotherapy; RT = Radiotherapy; Adj = Adjuvant; LVI = Lymphovascular Invasion; G = Pathological Grade; Survival based on Kaplan–Meier estimation at time of study completion

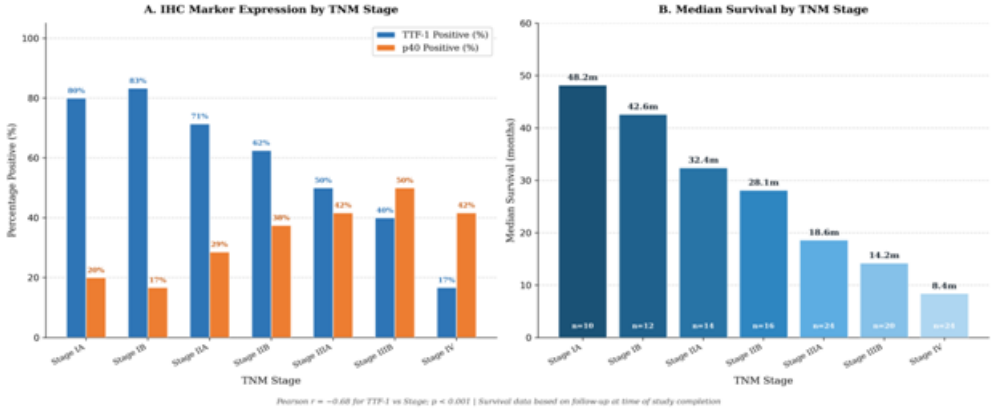

Figure 2: TNM Stage Correlation with TTF-1/p40 Expression (Panel A) and Median Survival in Months (Panel B) in Primary Lung Cancer Patients (n = 120)

Pearson r = −0.68 for TTF-1 vs. Stage (p < 0.001).

Discussion

The present study provides a comprehensive clinico-radiological and pathological assessment of 120 primary lung cancer cases with detailed IHC characterisation using TTF-1 and p40, representing one of the more sizeable prospective Indian series in the recent literature. The mean age of 58.4 ± 11.2 years and male predominance (71.7%) in the

current cohort are consistent with global and Indian epidemiological data.[14,15] The high proportion of smokers (61.7%) reinforces the well-established causal association between tobacco consumption and lung carcinogenesis, particularly in the Indian context where bidis and cigarettes contribute substantially to the overall tobacco burden.[16]

Adenocarcinoma was the most prevalent histological subtype (43.3%), concordant with the global shift observed over the past three decades wherein ADC has surpassed SCC as the most common NSCLC type, attributed partly to the rising prevalence of filtered cigarette use and increasing incidence among female non-smokers.[17] Moreira et al.[18] reported ADC as the predominant subtype in 46.2% of cases in a large multi-institutional series, while Navani et al.[19] from the United Kingdom similarly documented ADC in 44.1% of lung cancer cases. The proportion of SCC (28.3%) in our study aligns with the reported range of 20–35% in contemporary literature.[20]

The TTF-1 positivity rate for adenocarcinoma in the current study (92.3%) is at the upper range of published values, which span 75–96%.[6,21] Yatabe et al.[22] reported TTF-1 positivity in 89.6% of pulmonary ADC, while Pelosi et al.[23] documented 91.0% sensitivity in a meta-analysis of 27 studies. The marginally higher positivity in our series may reflect the predominance of acinar and lepidic ADC subtypes, as these architectural patterns are known to exhibit stronger TTF-1 expression compared to solid and micropapillary variants. Importantly, TTF-1 was expressed in only 5.9% of SCC cases, confirming its high specificity, consistent with earlier reports.[24]

p40 demonstrated high sensitivity (88.2%) and specificity (89.5%) for SCC in our series, with an AUC of 0.92. These results are in close agreement with Bishop et al.25 who reported p40 sensitivity of 89% and specificity of 98% for SCC in a cohort of 400 cases, and with the landmark study by Nonaka et al.[26] who established p40 as superior to p63 in distinguishing SCC from ADC. A comparative study by Rekhtman et al.[27] found p40 to be 100% sensitive for SCC without cross-reactivity in ADC, a slightly higher figure than our findings, possibly attributable to differences in antibody clones and scoring thresholds. The absence of p40 positivity in SCLC (0.0%) concurs with its established negative phenotype in this subtype.[28]

The inverse correlation between TTF-1 expression and TNM stage (Pearson $r = -0.68$; $p < 0.001$) is a clinically significant finding. Loss of TTF-1 expression with advancing stage may reflect progressive dedifferentiation of ADC as it transitions from early lepidic-predominant forms to invasive poorly differentiated variants. Hiyama et al.[29] observed a similar trend and postulated that epigenetic silencing of the NKX2-1 locus through promoter methylation may underlie this phenomenon. The inverse relationship between pathological grade and TTF-1 positivity ($r = -0.54$; $p < 0.001$) further supports the notion that TTF-1 expression serves not merely as a diagnostic IHC marker but as an independent prognostic parameter. In agreement, Myong [30] demonstrated that TTF-1-positive ADC patients had significantly longer disease-free survival compared to TTF-1-negative counterparts.

The median overall survival data in our cohort, ranging from 48.2 months in Stage IA to 8.4 months in Stage IV, are broadly consistent with published survival estimates.[31] The National Lung Screening Trial (NLST) and SEER registry data confirm a five-year survival of approximately 60–70% for Stage IA and less than 10% for Stage IV disease.[32] The substantially lower median survival in LVI-positive cases (11.6 vs. 28.4 months; $p < 0.001$) corroborates findings by Mollberg et al.[33] who identified LVI as an independent predictor of recurrence and reduced survival in NSCLC after curative resection. The presence of tumour necrosis in 40.0% of our cases and its association with adverse survival is consistent with data reported by Kadota et al.[34] demonstrating necrosis as a significant prognostic determinant in lung ADC.

Radiologically, upper lobe predominance (48.3%) and right-sided involvement (60.0%) are consistent with established anatomical observations related to differential bronchial airflow patterns and carcinogen deposition.[35] The rate of mediastinal lymph node involvement (43.3%) is reflective of the advanced stage at presentation that characterises most lung cancer diagnoses in India, underscoring the need for population-level lung cancer screening programmes among high-risk smokers. While low-dose CT screening is endorsed by USPSTF guidelines in the United States for eligible smokers,[36] comparable national policies remain lacking in India.

From a practical pathology standpoint, our data reinforce the complementary utility of TTF-1 and p40 as a two-marker IHC panel for the rapid and cost-effective subtyping of NSCLC, particularly in small biopsy and cytology specimens where morphological features may be equivocal.

This approach minimises tissue consumption and reduces diagnostic turnaround time in resource-constrained settings, which is particularly relevant for high-volume laboratories in tertiary referral centres of developing nations where tissue-sparing protocols have direct patient-management implications.

Conclusion

The present study demonstrates that TTF-1 and p40 are highly sensitive and specific IHC markers with excellent diagnostic performance for adenocarcinoma and squamous cell carcinoma of the lung, respectively. The significant inverse correlation of TTF-1 expression with TNM stage and pathological grade underscores its dual role as a diagnostic and prognostic biomarker.

Lung cancer in our cohort predominantly presented in older males with a smoking history at advanced stages of disease. The incorporation of a TTF-1 and p40 dual-marker panel into routine pathological practice is strongly recommended for accurate and resource-efficient subtyping of primary lung cancer, with direct implications for patient management and targeted therapeutic selection.

Declarations

Conflict of Interest: The authors declare no conflicts of interest.

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Ethical Approval: Obtained from the Institutional Ethics Committee (Ref: IMS/IEC/2021/004). Written informed consent was obtained from all participants.

Data Availability: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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