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Immunohistochemical and Fluorescence in Situ Hybridization Profiling of Primary Lung Cancers in Senegal: Histological Characterization, ALK Rearrangement Status, and Comparison of Two Immunohistochemical Clones

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Abstract

Background: Lung cancer remains a major public health, yet data from sub-Saharan Africa on immunohistochemical and molecular profiling are scarce. The identification of actionable molecular alterations, particularly anaplastic lymphoma kinase (ALK) rearrangements, is critical for guiding targeted therapy. This study aimed to characterize the histological and IHC profiles of primary bronchopulmonary cancers diagnosed in Senegal, and to evaluate ALK rearrangement status using both IHC and FISH. ***Methods:*** A prospective, multicenter, cross-sectional study was conducted from 2018 to 2020 at three pathology centers. IHC was performed with differentiation markers (TTF-1, p40, p63, CK7, CK20, CK5/6, chromogranin A, synaptophysin, CD56, Ki-67), and two anti-ALK antibody clones (D5F3 and QR017). ALK rearrangement was assessed by FISH. ***Results:*** The mean age was 60.3 ± 6.7 years, with a male predominance (sex ratio 3.8). Non-small cell malignancies accounted for 88.6% (n = 39) of cases, with adenocarcinoma being the most frequent subtype (34.1% of all cases). TTF-1 was positive in 41% of tested cases; p40 and p63, in 23% each. ALK rearrangement was confirmed by FISH in 2 of 24 tested cases (4.5%). The D5F3 clone showed 100% sensitivity and specificity. The QR017 clone showed 100% sensitivity with 95.5% specificity. A significant association was found between ALK positivity and adenocarcinoma histology ($p < 0.05$). ***Conclusions:*** This study demonstrates the feasibility and clinical utility of implementing IHC and FISH techniques for lung cancer diagnosis in a sub-Saharan. Systematic ALK testing should be integrated into routine lung cancer diagnostics in Senegal to enable access to targeted therapies.

Keywords: Lung cancer; immunohistochemistry; FISH; ALK rearrangement; Senegal; sub-Saharan; targeted therapy.

1. INTRODUCTION

Lung cancer represents one of the most lethal malignancies worldwide, accounting for approximately 2.2 million new cases and 1.8 million deaths annually according to GLOBOCAN 2020 estimates [1]. Despite significant advances in the understanding of molecular oncogenesis and the development of targeted therapies, the overall five-year survival rate remains poor, largely due to late-stage diagnosis and limited access to molecular profiling, particularly in low- and middle-income countries [2].

In sub-Saharan Africa, lung cancer accounts for approximately 3.9% of all diagnosed cancers [3]. In Senegal, the reported prevalence is 3.8%, but precise incidence data remain unavailable due to the absence of a comprehensive population-based cancer registry and a diagnostic delay attributable to limited technical

infrastructure [4,5]. The lack of molecular diagnostic capabilities in this region means that many patients are deprived of the personalized therapeutic strategies that have transformed lung cancer management in high-income countries.

The histopathological classification of lung cancer has undergone substantial revision in recent decades. The 2021 World Health Organization (WHO) classification integrates morphological, immunophenotypic, and molecular data for accurate subtyping [6]. The distinction between adenocarcinoma and squamous cell carcinoma (SCC) is of paramount therapeutic importance, as it determines eligibility for specific chemotherapy regimens (e.g., pemetrexed), anti-angiogenic agents (e.g., bevacizumab), and molecular-targeted therapies [7,8]. Immunohistochemistry (IHC) has become an indispensable tool in this regard, using a minimal panel of antibodies—typically TTF-1 and p40—to reliably differentiate between glandular and squamous differentiation [9,10].

Beyond histological subtyping, the identification of oncogenic driver mutations has revolutionized the treatment of non-small cell lung carcinoma (NSCLC). Among the most clinically significant molecular alterations is the rearrangement of the anaplastic lymphoma kinase (ALK) gene, located on chromosome 2p23 [11]. The EML4-ALK fusion, first described in 2007 by Soda *et al.,*[12], results from small inversions within the short arm of chromosome 2, leading to constitutive activation of the ALK tyrosine kinase and consequent oncogenic signaling. ALK rearrangements are found in approximately 3–7% of NSCLC cases and define a clinically distinct subset of patients who are typically younger, non-smokers or light smokers, and have adenocarcinoma histology [12,13].

The clinical significance of ALK rearrangement lies in its status as a predictive biomarker for response to ALK tyrosine kinase inhibitors (TKIs), including crizotinib, ceritinib, alectinib, and lorlatinib [14,15]. Crizotinib, the first-generation ALK inhibitor, demonstrated a 60% response rate and superior progression-free survival compared to conventional chemotherapy in ALK-positive patients [14,15]. Subsequent generations of ALK TKIs have further improved clinical outcomes, including in patients with central nervous system metastases [17].

The detection of ALK rearrangement can be performed using several methodologies, including FISH, IHC, reverse transcription polymerase chain reaction (RT-PCR), and next-generation sequencing (NGS) [18]. FISH using a break-apart probe was initially the gold standard for ALK testing; however, IHC has emerged as a reliable, cost-effective screening method, and the VENTANA ALK (D5F3) CDx assay has received regulatory approval as a companion diagnostic test in several jurisdictions [19,20]. The comparative performance of different IHC antibody clones against FISH remains an area of active investigation, particularly in resource-limited settings where the choice of diagnostic method must balance accuracy with affordability.

In sub-Saharan Africa, studies on the immunohistochemical and molecular characterization of lung cancers remain extremely limited. To our knowledge, no prior study from Senegal has systematically evaluated the immunohistochemical profile of lung cancers using a comprehensive antibody panel or assessed ALK rearrangement status using both IHC and FISH. This diagnostic gap has significant implications for patient management, as the absence of molecular testing precludes access to targeted therapies that could substantially improve survival.

The objectives of this study were therefore threefold: (1) to characterize the histological subtypes and immunohistochemical profiles of primary lung cancers diagnosed in Dakar, Senegal; (2) to determine the frequency of ALK rearrangement using both IHC and FISH; and (3) to compare the diagnostic performance of two IHC antibody clones—VENTANA ALK (D5F3) and QUARTETT QR017—against FISH as the reference standard, with the aim of proposing an optimized diagnostic algorithm adapted to the Senegalese context.

2. MATERIALS AND METHODS

2.1. Study Design and Setting

This was a prospective, multicenter, cross-sectional, descriptive study conducted from January 1, 2018, to December 31, 2020. The study was carried out at the Laboratory of Histology-Embryology and Cytogenetics (HEC) of Cheikh Anta Diop University (UCAD), Dakar, in collaboration with the pathology departments of three referral centers: the UCAD pathology service, Fann University Hospital, and the Hôpital Principal de Dakar. The HEC laboratory is the principal national public center performing clinical cytology, cytogenetics, and cytospermiology, as well as experimental research activities. The laboratory has been operational since 1980, performing approximately 6,000 biological examinations annually.

2.2. Study Population

Inclusion Criteria: All new cases of histologically confirmed primary bronchopulmonary cancer diagnosed at the three participating pathology centers during the study period were included.

Exclusion Criteria: Cases of confirmed secondary (metastatic) lung cancer and suspected lung cancer cases without histological confirmation were excluded.

2.3. Data Collection

Data were collected using a standardized questionnaire capturing demographic variables (age, sex), smoking status (active smoking, duration in years,

pack-years), clinical presentation (symptoms, tumor localization), type of biopsy specimen, histological type and subtype, tumor architecture and differentiation, immunohistochemical profile, and ALK genetic status.

2.4. Histological Analysis

Biopsy specimens were processed according to standard histotechnological protocols. Tissues were fixed in 4% buffered formaldehyde, dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin at 58°C. Sections of 3 µm thickness were cut using a rotary microtome and stained with hematoxylin and eosin (HE) for morphological evaluation. Histological classification followed the 2021 WHO criteria [6].

2.5. Immunohistochemistry

IHC analyses were performed at the HEC laboratory on 3–4 µm formalin-fixed, paraffin-embedded (FFPE) tissue sections, following established methodological principles [44]. The protocol included deparaffinization in three xylene baths (5 min each), rehydration through descending ethanol series (100%, 95%, 70%), antigen retrieval by immersion in Tris-EDTA buffer (pH 9.0) at 95°C for 30 minutes in a water bath, endogenous peroxidase blocking with hydrogen peroxide (H_2O_2) for 10 minutes, and application of the primary antibody for 1 hour in a humidified dark chamber. Detection was achieved using an HRP-conjugated secondary antibody system with 3,3′-diaminobenzidine (DAB) as the chromogen. Counterstaining was performed with hematoxylin (2–4 min), followed by dehydration and mounting.

The following primary antibodies were used:
Differentiation markers:

- TTF-1 (clone QR046, QUARTETT) — nuclear staining
- p40 (clone QR020, QUARTETT) — nuclear staining
- p63 (clone QR007, QUARTETT) — nuclear staining
- Chromogranin A (clone LK2H10, QUARTETT) — cytoplasmic granular staining
- Synaptophysin (clone QR054, QUARTETT) — cytoplasmic diffuse granular staining
- CK7 (clone C-35, QUARTETT) — cytoplasmic staining
- CK20 (clone Ks20.8, QUARTETT) — cytoplasmic staining
- CK5/6 (clones D5 and 16B4, QUARTETT) — cytoplasmic staining
- CD56/NCAM (clone 123C3.D5, QUARTETT) — cytoplasmic and membranous staining

Proliferation marker:

- Ki-67 (clone QR015, QUARTETT) — nuclear staining

ALK markers:

- VENTANA ALK (D5F3) — cytoplasmic and nuclear staining
- QUARTETT ALK (QR017) — cytoplasmic and nuclear staining

2.6. ALK Immunohistochemical Scoring

ALK immunoreactivity was evaluated using modified semi-quantitative criteria based on the intensity and distribution of cytoplasmic staining [21]: score 3+ = strong, granular cytoplasmic staining in >75% of tumor cells with diffuse homogeneous distribution; score 2+ = moderate, smooth cytoplasmic staining (may include partially strong staining) in ≥50% of tumor cells; score 1+ = weak, focal cytoplasmic staining below the criteria for score 2+; score 0 = no staining. Cases scored as 1+, 2+, or 3+ were considered IHC-positive. IHC evaluation was performed independently by two pathologists.

2.7. Fluorescence In Situ Hybridization (FISH)

The ALK genetic status was assessed by FISH on representative tumor areas from FFPE tissue sections using a bicolor break-apart LSI ALK probe (ALK Split FISH Probe, Abnova) consisting of separate red (3′) and green (5′) fluorescent probes. FISH was performed on cases for which sufficient residual tumor tissue was available after IHC analysis (n = 24 of 39 non-SCLC cases).

Pretreatment: Slides were deparaffinized in xylene (3 × 10 min), dehydrated in 100% ethanol (2 × 5 min), pretreated in a pretreatment solution at 80°C for 10 minutes, followed by protease digestion at 37°C for 10–60 minutes (depending on specimen condition), and dehydrated through graded ethanol (70%, 90%, 100%, 3 min each).

Hybridization: 10 µL of probe was applied to each hybridization area, covered with a 22 × 22 mm coverslip, and sealed with rubber cement. Co-denaturation was performed at 72°C for 5 minutes, followed by hybridization at 37°C for 16 hours in a humidified chamber.

Post-hybridization washing: Slides were washed in 2× SSC/0.3% NP-40 at 72°C for 2 minutes, dehydrated through graded ethanol, air-dried in darkness, and counterstained with DAPI.

Microscopy and interpretation: Slides were examined using an Olympus BX-51 fluorescence microscope equipped with Leica Cytovision Genus v7.1 software and DAPI, FITC, and TRITC filter sets. For each case, 50 non-overlapping nuclei were scored by each of two independent readers.

Interpretation criteria: ALK was considered not rearranged when signals were adjacent or fused (appearing yellow under Orange/Green V2 filter) or

when only a single green signal was present. ALK was considered rearranged (positive) when red and green signals were separated or when an isolated red (3′) signal was present.

Quantitative thresholds: <10% positive cells (<5/50) = negative; >50% positive cells (>25/50) = positive. For borderline results (10–50%), a second reader evaluated the slide, and a combined percentage from 100 cells was calculated; ≥15% positive cells was considered positive.

2.8. Quality Control

All IHC reactions were validated using positive tissue controls (tissues known to express the target antigen), negative tissue controls (tissues not expressing the target antigen), and negative reagent controls (serial sections with the primary antibody replaced by diluent). FISH results were read independently by two operators, each scoring at least 50 nuclei.

2.9. Ethical Considerations

As analyses were performed as part of routine diagnostic workup, formal ethics committee approval was not required. Confidentiality and medical secrecy were maintained throughout data collection and analysis.

2.10. Statistical Analysis

Data were entered using Epi Data 3.2.0.0 and analyzed using Epi Info™ 7.2.5.0 (CDC/WHO). Descriptive statistics included means, standard deviations, and percentages. Comparative analyses were performed using Fisher's exact test, as expected cell counts were below 5 for most contingency tables. Sensitivity, specificity, PPV, and NPV of IHC were calculated using FISH as the reference standard. A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1. Epidemiological Characteristics

Over the three-year study period, 44 cases of primary bronchopulmonary cancer were identified. The mean age was 60.3 ± 6.7 years, with the 61–65-year age group being the most represented (29.5%) and extremes ranging from 27 to 79 years. Males were predominant, accounting for 79.5% of cases (n = 35), yielding a sex ratio of 3.9:1.

Active smoking was reported in 56.8% of patients, with a mean pack-year consumption of 33.3 ± 14 and a mean smoking duration of 34.5 ± 8.3 years (range: 20–50 years). Non-smokers accounted for 30.2%, while 13.0% were former smokers. Among men, 64% were smokers compared to only 25% of women.

3.2. Clinical Presentation

The diagnosis was primarily prompted by the identification of a pulmonary mass (right-sided in 44.5%, left-sided in 35.0%, bilateral in 5.0%, and mediastino-hilar in 4.5%), thoracic pain (38.6%), hemoptysis (22.7%), cough (18.2%), dyspnea (9.1%), alteration of general condition (9.1%), chronic pneumopathy (6.8%), mediastinal lymphadenopathy (2.0%), pleurisy (2.0%), and subpleural nodules (2.0%).

3.3. Histological Findings

Biopsy was the sole specimen type in all 44 cases (100%), with concomitant cytology available in 10 patients (22.7%). No surgical resection specimens were available.

Non-small cell malignancies predominated, accounting for 88.6% (n = 39) of cases. These comprised 37 non-small cell carcinomas—adenocarcinoma (n = 15, 34.1%), SCC (n = 9, 20.5%), undifferentiated NSCLC (n = 7, 15.9%), large cell carcinoma (LCC; n = 5, 11.4%), and neuroendocrine carcinoma not otherwise specified (NOS; n = 1, 2.3%)—as well as one pulmonary T-cell lymphoma (2.3%) and one mesenchymal tumor (2.3%). Small cell lung carcinoma (SCLC) accounted for 11.4% (n = 5).

Among adenocarcinomas, the acinar pattern predominated (33.3%), followed by mixed patterns (26.7%), papillary (20.0%), micropapillary (6.7%), solid (6.7%), and invasive non-mucinous (6.7%). Among squamous cell carcinomas, 62.5% were keratinizing and 37.5% were non-keratinizing.

3.4. Immunohistochemical Results

3.4.1. Differentiation Markers

The results of the IHC panel are summarized in Table 1.

Table 1: Summary of immunohistochemical markers tested in the study population (n = 44)

Antibody	Patients tested, n (%)	Positivity, n (%)
TTF-1	25 (56.8)	18 (40.9)
p40	18 (40.9)	10 (22.7)
p63	18 (40.9)	10 (22.7)
Synaptophysin	10 (22.7)	6 (13.6)
Chromogranin A	10 (22.7)	7 (15.9)
CD56	5 (11.4)	0 (0.0)
CK7	11 (25.0)	9 (20.5)
CK5/6	8 (18.2)	6 (13.6)
CK20	2 (4.5)	0 (0.0)
Ki-67	3 (6.8)	1 (2.3)
ALK—D5F3 (IHC)	39 (88.6)	2 (4.5)

Antibody	Patients tested, n (%)	Positivity, n (%)
ALK—QR017 (IHC)	39 (88.6)	3 (6.8) *
ALK—FISH	24 (54.5)	2 (4.5) †

*Includes one equivocal case (score 1+) not confirmed by FISH.
†Percentage among the 24 cases tested by FISH; corresponds to 4.5% of the total cohort (n = 44).

TTF-1 was tested in 25 cases and was positive in 41% of tested patients (figure 1). p40 and p63 were tested in 18 patients with identical positivity rates of 23% and equivalent sensitivity and specificity. CK7 was positive in 20.5% and CK5/6 in 13.6% of tested cases. CK20 showed no positivity.

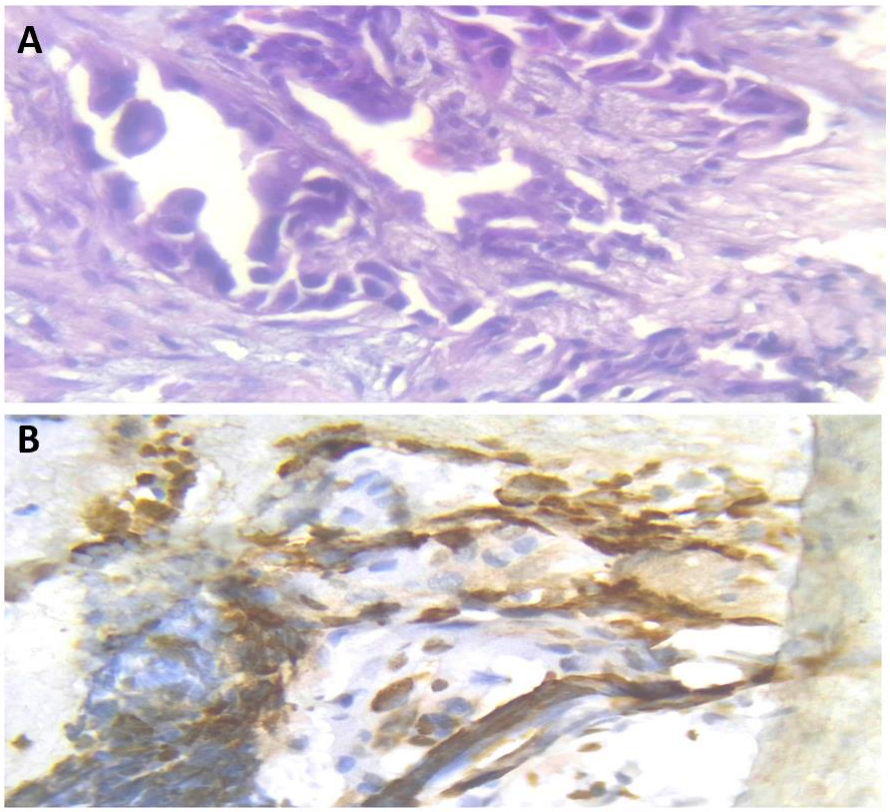

Figure 1: A: Histological appearance of a moderately differentiated adenocarcinoma (hematoxylin and eosin, G×100). B: TTF-1 positive with intense nuclear staining

3.4.2. Neuroendocrine Markers

Among the 5 SCLC cases, TTF-1 was positive in 4/5 (80%), chromogranin A in 4/5 (80%), synaptophysin in 2/5 (40%), and CD56 in 0/5 (0%). At least one neuroendocrine marker was positive in 80% of SCLC cases.

Among the 5 LCC cases, synaptophysin and chromogranin A were each positive in 25% of tested cases, while CD56 was negative in all cases. At least one neuroendocrine marker was positive in 40% of LCC cases, thereby identifying 2 cases (4.5% of the entire cohort) as large cell neuroendocrine carcinoma (LCNEC).

3.4.3. ALK Immunohistochemistry

ALK IHC was performed on all 39 non-SCLC cases (88.6% of total patients) using both clones.

VENTANA ALK (D5F3): Positive results (score 3+) were obtained in 2 cases (4.5% of all cases). No equivocal results (1+ or 2+) were observed. All negative cases showed score 0.

QUARTETT QR017: Positive results were obtained in 3 cases (6.8% of all cases). Of these, 2 cases (4.5%) scored 3+ and 1 case (2.3%) scored 1+ (equivocal).

3.5. ALK FISH Results

ALK FISH was successfully performed on 24 of the 39 non-SCLC cases (61.5%) for which sufficient residual tumor tissue was available. In the remaining 15 cases, insufficient tissue precluded FISH analysis.

ALK rearrangement was detected by FISH in 2 patients (8.3% of the 24 tested cases; 4.5% of the total cohort), both with adenocarcinoma. In both positive cases, clear separation of the red (3′) and green (5′) signals was observed, confirming *ALK* gene rearrangement (Figure 2).

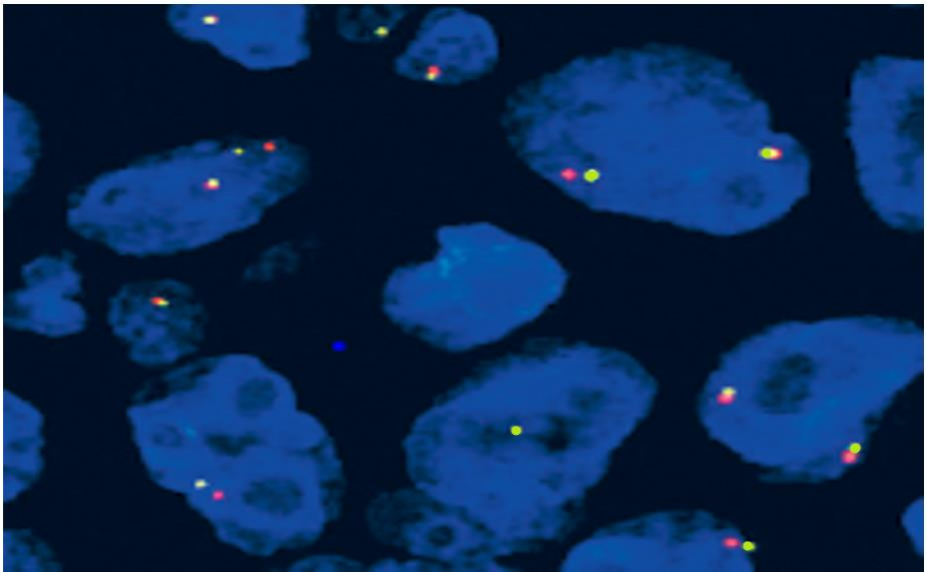

Figure 2: ALK FISH showing separation of red- and green-labeled probes, indicating *ALK* gene rearrangement in a patient with lung adenocarcinoma

3.6. Comparison of IHC Clones versus FISH

Of the 39 non-SCLC cases tested by IHC for ALK, 24 also had sufficient tissue for paired FISH analysis. The diagnostic performance of each IHC clone was calculated on these 24 paired cases (Tables 2 and 3).

Table 2: Diagnostic performance of VENTANA ALK (D5F3) versus FISH (n = 24).

	FISH+	FISH−	Total
IHC D5F3+	2 (TP)	0 (FP)	2
IHC D5F3−	0 (FN)	22 (TN)	22
Total	**2**	**22**	**24**

Sensitivity = TP/(TP+FN) = 2/2 = 100%; Specificity = TN/(TN+FP) = 22/22 = 100%. PPV = TP/(TP+FP) = 2/2 = 100%; NPV = TN/(TN+FN) = 22/22 = 100%.

TP, true positive; FP, false positive; FN, false negative; TN, true negative.

Table 3: Diagnostic performance of QUARTETT QR017 versus FISH (n = 24).

	FISH+	FISH−	Total
IHC QR017+	2 (TP)	1 (FP)	3
IHC QR017−	0 (FN)	21 (TN)	21
Total	**2**	**22**	**24**

Sensitivity = TP/(TP+FN) = 2/2 = 100%; Specificity = TN/(TN+FP) = 21/22 = 95.5%.
PPV = TP/(TP+FP) = 2/3 = 66.7%; NPV = TN/(TN+FN) = 21/21 = 100%.

The single false-positive case with the QUARTETT QR017 clone was the equivocal (score 1+) case that was confirmed as negative by FISH.

3.7. Clinicopathological Associations with ALK Status

Among the three patients with any positive ALK IHC result (score ≥1+ by at least one clone), two were men (66.7%) and two were non-smokers (66.7%). All three had adenocarcinoma histology. Of note, only two of these three cases were confirmed as ALK-rearranged by FISH; the third (QR017 score 1+) was FISH-negative.

A significant association was found between ALK IHC positivity and adenocarcinoma histology (Fisher's exact test, $p < 0.05$), and an inverse association with SCC (Fisher's exact test, $p < 0.05$). Age ($p = 0.5$), sex ($p = 0.4$), and smoking status ($p = 0.4$) were not significantly associated with ALK status. Given the very small number of ALK-positive cases (n = 3 by IHC, n = 2 by FISH), these associations should be interpreted with caution and require validation in larger cohorts.

4. DISCUSSION

This study represents, to our knowledge, the first systematic evaluation of the immunohistochemical and molecular cytogenetic profile of primary lung cancers in Senegal, combining histological subtyping, a comprehensive IHC panel, and ALK rearrangement assessment by both IHC and FISH.

4.1. Epidemiological and Clinical Considerations

The mean age of 60.3 years and male predominance (sex ratio 3.9:1) in our cohort are consistent with previous reports from Senegal by Senghor *et al.,*[4] (59.3 ± 11.3 years) and Niang *et al.,*[22] (64.2 years), as well as international data from Algeria [23] and Guadeloupe [24]. The slightly lower mean age compared to European series likely reflects the younger demographic structure of the Senegalese population and potentially different risk factor profiles.

The low number of cases (44 over 3 years) across three major referral centers in Dakar underscores the significant underdiagnosis of lung cancer in Senegal. This can be attributed to several factors: financial barriers limiting access to histological diagnosis, late clinical presentation with death occurring before diagnostic biopsy, limited bronchoscopy facilities, and the absence of lung cancer screening programs. This observation is consistent with the broader challenge of cancer underdiagnosis in sub-Saharan Africa [3].

Active smoking was found in 56.8% of patients, comparable to rates reported by Niang *et al.,* in Senegal (57%) [22] and Cadelis *et al.,* in Guadeloupe (61.3%) [24]. The proportion of non-smokers (30.2%) was notably high, suggesting that non-tobacco-related risk factors—including occupational exposures, environmental carcinogens, genetic susceptibility, and indoor air pollution from biomass fuel combustion—may play a significant role in lung carcinogenesis in the Senegalese population [25,26]. The low smoking rate among women (25%) further supports this hypothesis and warrants dedicated investigation of non-tobacco risk factors in this population.

4.2. Histological Distribution

The predominance of non-small cell malignancies (88.6%) in our series is consistent with global epidemiological data [27,28]. The finding that adenocarcinoma was the most frequent histological subtype (34.1%), followed by SCC (20.5%), reflects a global epidemiological shift that has been documented over recent decades [29,30]. Earlier studies from West Africa, including Côte d'Ivoire [31] and Mali [32], reported SCC as the predominant subtype, suggesting that our findings reflect an evolving pattern in the region.

Several factors may explain this shift toward adenocarcinoma predominance. The 2021 WHO classification has introduced molecular data integrated with morphological and immunophenotypic characteristics, leading to reclassification of previously undifferentiated or large cell carcinomas [6]. Additionally, changes in tobacco product composition, including the use of filtered cigarettes and light tobacco, result in deeper inhalation of carcinogens to the peripheral lung parenchyma—the site of adenocarcinoma development—rather than the proximal bronchi where SCCs typically arise [29,30]. The increasing proportion of non-smokers with lung cancer, who predominantly develop adenocarcinomas, further contributes to this epidemiological trend.

4.3. Role of Immunohistochemistry in Histological Subtyping

Our study confirms the essential role of IHC in the accurate subtyping of NSCLC, particularly on small biopsy specimens where morphological features alone may be insufficient. The combination of TTF-1 and p40 has been recommended as the minimum IHC panel for distinguishing adenocarcinoma (TTF-1+/p40−) from SCC (TTF-1−/p40+) [7,9].

In our series, TTF-1 was positive in 41% of tested NSCLC cases, while p40 and p63 each showed 23% positivity. Notably, p40 and p63 demonstrated identical sensitivity and specificity in our cohort, which contrasts with literature suggesting that p40 has superior specificity due to the elimination of false-positive staining in adenocarcinomas [9]. However, the literature consensus generally supports p40 as the preferred squamous marker over p63 due to its greater specificity, and the equivalent performance observed in our series may be related to the relatively small sample size.

The utility of CK7 (positive in 20.5% of tested cases) and CK20 (uniformly negative) in confirming the pulmonary origin of adenocarcinomas is well established [33]. The CK7+/CK20− immunophenotype is characteristic of primary pulmonary adenocarcinoma and aids in the differential diagnosis with metastatic adenocarcinomas from colorectal (CK7−/CK20+) or other primary sites. The lack of CK20 positivity in our series is consistent with the primary pulmonary origin of the tumors.

These two antibodies (TTF-1 and p40) enabled reclassification of undifferentiated carcinomas in our series, consistent with published data showing that this pair reclassifies up to 80% of tumors previously diagnosed as large cell carcinoma [10].

4.4. Neuroendocrine Marker Expression

The identification of neuroendocrine differentiation in lung tumors has important diagnostic and therapeutic implications [34]. In our SCLC cases, the high positivity rate of TTF-1 (80%) and chromogranin A (80%) with lower synaptophysin expression (40%) is consistent with published data [36]. The absence of CD56 positivity in our series was unexpected, as CD56 is generally considered one of the most sensitive neuroendocrine markers in SCLC [37]. This finding may reflect technical issues with the specific antibody clone used or tissue fixation-related antigen degradation, and warrants further investigation.

The identification of LCNEC in 4.5% of our cohort (40% of LCC cases expressing at least one neuroendocrine marker) confirms the rarity of this entity,

whose frequency is estimated at approximately 3% of lung cancers worldwide [38]. The distinction between LCNEC, poorly differentiated NSCLC, SCLC, and atypical carcinoid remains challenging on small biopsies and underscores the importance of a comprehensive neuroendocrine marker panel.

4.5. ALK Rearrangement: Frequency and Clinicopathological Associations

The frequency of ALK rearrangement in our cohort (4.5%) falls within the internationally reported range of 2–7% [12,13,39,43]. This finding is clinically significant as it demonstrates that actionable ALK alterations are present in the Senegalese NSCLC population at rates comparable to those reported in European, Asian, and other African populations, thereby justifying the systematic implementation of ALK testing in routine diagnostics.

The clinicopathological profile of ALK-positive patients in our series—predominantly non-smokers with adenocarcinoma histology—is consistent with the classical description of ALK-rearranged NSCLC [12,13]. The exclusive association with adenocarcinoma and the inverse association with SCC confirm the well-established histotype specificity of ALK rearrangements. However, age, sex, and smoking status did not reach statistical significance, consistent with the findings of Sánchez-Ares *et al.,* in Spain [40] and Li *et al.,* in China [41]. These results indicate that demographic and lifestyle factors cannot reliably predict ALK status, reinforcing the need for universal molecular testing in NSCLC. The very small number of ALK-positive cases in our cohort limits the statistical power of these association analyses, and larger studies are needed to confirm these findings in the Senegalese population.

4.6. Comparative Performance of IHC Clones for ALK Detection

The comparison of two IHC antibody clones for ALK detection represents a key contribution of this study. The VENTANA ALK (D5F3) clone demonstrated perfect concordance with FISH (sensitivity 100%, specificity 100%, PPV 100%, NPV 100%), while the QUARTETT QR017 clone showed high sensitivity (100%) but slightly lower specificity (95.5%) and PPV (66.7%) due to one false-positive (score 1+) case.

These findings are consistent with large multicenter studies comparing different IHC clones against FISH. Marchetti *et al.,*[20] reported excellent performance of the D5F3 clone in a comprehensive comparison of IHC approaches, and Blackhall *et al.,*[42] confirmed high concordance between IHC and FISH in the European Thoracic Oncology Platform Lungscape project. Studies by Cabillic *et al.,*[45] and Vidal *et al.,*[46] have also demonstrated variable concordance between IHC and FISH depending on the clone used, underscoring the importance of clone selection and standardized scoring criteria. The presence of a false positive with the QR017 clone among equivocal (score 1+) cases highlights the importance of establishing clear interpretive criteria and confirms the recommendation for FISH confirmation of equivocal IHC results.

From a practical standpoint in the Senegalese context, where FISH equipment and expertise may not be universally available, our data support the use of the D5F3 clone as a stand-alone diagnostic test for ALK rearrangement, with FISH reserved for equivocal or discordant cases. This tiered approach is consistent with current international guidelines [18,20] and could be implemented in resource-limited settings where the cost and technical demands of FISH limit its routine use.

4.7. Implications for Clinical Practice in Senegal

The implementation of IHC and FISH at the HEC laboratory of UCAD represents a significant advancement in the diagnostic capabilities for lung cancer in Senegal. The demonstration that these techniques can be successfully performed in this setting, yielding results comparable to international standards, has several important implications.

First, accurate histological subtyping using IHC enables appropriate treatment selection, avoiding potentially harmful therapies (e.g., bevacizumab-related hemorrhage in SCC) and selecting optimal chemotherapy regimens.

Second, the identification of ALK-rearranged patients opens the possibility of targeted therapy with ALK TKIs. Crizotinib has shown a 60% response rate in first-line treatment, with superior progression-free survival compared to chemotherapy [14,15]. While access to these targeted therapies remains a challenge in sub-Saharan Africa due to cost considerations, the establishment of diagnostic capability is a prerequisite for future access programs, clinical trials, and advocacy for drug affordability.

Third, the successful comparison of two IHC clones provides practical guidance for laboratory selection of antibodies, particularly in settings where cost-effectiveness is a major consideration. The QUARTETT antibodies, which are generally less expensive than the VENTANA system, demonstrated acceptable performance for screening purposes, with the caveat that equivocal results require FISH confirmation.

4.8. Limitations

Several limitations of this study should be acknowledged. The small sample size (n = 44) limits the statistical power of clinicopathological association analyses and the precision of diagnostic performance estimates. FISH was performed on only 24 of 39 non-SCLC cases due to insufficient residual tissue in the remaining cases, potentially introducing selection bias. The absence of surgical resection specimens (all diagnoses were made on biopsies) may have affected the

accuracy of histological subtyping, particularly for tumors with heterogeneous architecture. The panel of molecular markers was limited to ALK, and other clinically important biomarkers (EGFR mutations, ROS1 rearrangement, PD-L1 expression, KRAS, BRAF) were not assessed. The three-year study period coincided in part with the COVID-19 pandemic (2020), which may have reduced diagnostic activity. Finally, follow-up data on treatment response and outcomes were not available.

4.9. Future Directions

Future studies should expand the panel of molecular biomarkers tested, particularly EGFR mutations (which are the most frequent actionable alteration in NSCLC globally), ROS1 rearrangement, BRAF V600E mutation, and PD-L1 expression [47]. The implementation of NGS-based comprehensive genomic profiling should be considered as a long-term goal to enable simultaneous detection of multiple actionable alterations. Prospective studies correlating molecular profiles with treatment response and survival outcomes are needed to assess the clinical impact of molecular-guided therapy in the Senegalese population. Finally, multicenter collaborations across West Africa would increase sample sizes and enable more robust characterization of the molecular epidemiology of lung cancer in the region.

5. CONCLUSIONS

This study demonstrates the feasibility and clinical utility of implementing immunohistochemistry and fluorescence in situ hybridization techniques for lung cancer diagnosis at a university laboratory in Senegal. Our findings confirm that adenocarcinoma is now the predominant histological subtype of lung cancer in Senegal, consistent with global epidemiological trends. The IHC panel used in this study enabled accurate histological subtyping and identification of neuroendocrine differentiation, providing essential information for therapeutic decision-making.

ALK rearrangement was identified in 4.5% of cases, a frequency comparable to international reports, confirming that this actionable molecular alteration is present in the Senegalese population and justifying systematic testing. The VENTANA ALK (D5F3) clone demonstrated superior performance compared to the QUARTETT QR017 clone, with perfect concordance with FISH, supporting its use as a first-line screening tool. Cases with equivocal IHC results should be confirmed by FISH.

The integration of IHC and molecular cytogenetic techniques into routine diagnostic practice in sub-Saharan Africa is essential to improve cancer diagnosis, enable access to targeted therapies, and ultimately improve patient outcomes. Investment in laboratory infrastructure, technician training, and collaborative research networks is needed to sustain and expand these capabilities across the region.

Author Contributions

Diop N: study conception and design, data collection, laboratory work (IHC and FISH), data analysis, manuscript writing. Guèye MV, Ndiade A, Ibondou RK, Diatta AL, and Diallo AS: critical manuscript review. Sy M: study direction, laboratory supervision, critical manuscript review. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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REFERENCES

1. Deo SVS, Sharma J, Kumar S, *et al.,*GLOBOCAN 2020 report on global cancer burden: challenges and opportunities for surgical oncologists. *Ann Surg Oncol*. 2022; 29:6497–6500.
2. Stewart BW, Wild CP. *World Cancer Report 2014*. Lyon: International Agency for Research on Cancer; 2014.
3. Sung H, Ferlay J, Siegel RL, *et al.,*Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249.
4. Senghor F, *et al.,* Épidémiologie des cancers bronchopulmonaires primitifs des non-fumeurs au Sénégal. *Health Sci Dis*. 2020 ;21(12):14–19.
5. Thiam K, Touré NO, Ndiaye EM, *et al.,* Épidémiologie des cancers bronchopulmonaires primitifs des non-fumeurs au Sénégal. *Rev Mal Respir*. 2019 ;36 :15–21.
6. WHO Classification of Tumours Editorial Board. *Thoracic Tumours*. 5th ed. Lyon: IARC Press; 2021.
7. Couraud S, Westeel V, Ranchon F, *et al.,* Référentiel sur le cancer bronchique non à petites-cellules: actualisation 2021. Aristot ; 2021.
8. Vignaud JM. Carcinomes épidermoïde, basaloïde et adénosquameux pulmonaires. *Ann Pathol*. 2016 ;36(1):15–23.
9. Bishop JA, Teruya-Feldstein J, Westra WH, *et al.,* p40 is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol*. 2012 ;25 :405–415.
10. Kadota K, Nitadori J, Rekhtman N, *et al.,* Reevaluation and reclassification of resected lung carcinomas originally diagnosed as squamous cell

carcinoma using immunohistochemical analysis. *Am J Surg Pathol*. 2015 ;39:1170–1180.
11. Horn L, Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. *J Clin Oncol*. 2009 ;27(26):4232–4235.
12. Soda M, Choi YL, Enomoto M, *et al.,* Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature*. 2007 ;448(7153):561–566.
13. Inamura K, Takeuchi K, Togashi Y, *et al.,* EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. *Mod Pathol*. 2009 ;22(4):508–515.
14. Shaw AT, Kim DW, Nakagawa K, *et al.,* Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med*. 2013;368(25):2385–2394.
15. Solomon BJ, Mok T, Kim DW, *et al.,* First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med*. 2014 ;371 :2167–2177.
16. Doebele RC, Pilling AB, Aisner DL, *et al.,* Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin Cancer Res*. 2012;18(5):1472–1482.
17. Soria JC, Tan DSW, Chiari R, *et al.,* First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet*. 2017 ;389(10072):917–929.
18. Hamard C, Mignard X, Pecuchet N, *et al.,* IHC, FISH, CISH, NGS in non-small cell lung cancer: What changes in the biomarker era? *Rev Pneumol Clin*. 2018 ;74(5):327–338.
19. McLeer-Florin A, Moro-Sibilot D, Melis A, *et al.,* Dual IHC and FISH testing for ALK gene rearrangement in lung adenocarcinomas in a routine practice: a French study. *J Thorac Oncol*. 2012;7(2):348–354.
20. Marchetti A, *et al.,* ALK protein analysis by IHC staining after recent regulatory changes: a comparison of two widely used approaches, revision of the literature, and a new testing algorithm. *J Thorac Oncol*. 2016;11(4):487–495.
21. Zhou J, Zhao J, Sun K, *et al.,* Accurate and economical detection of ALK positive lung adenocarcinoma with semiquantitative immunohistochemical screening. *PLoS One*. 2014 ;9(3):e92828.
22. Niang A, Sagna MM, Diatta MBN, *et al.,* Profils épidémiologiques, clinique, paraclinique et évolutif des cancers bronchiques primitifs au Sénégal. *Rev Mal Respir*. 2018 ;35: A215–A216.
23. Marouani A, Abdellouche D, Khalfaoui M, *et al.,* Le cancer bronchopulmonaire primitif : à propos de 780 cas. *Rev Mal Respir*. 2016 ;33 :99–100.
24. Cadelis G, Kaddah S, Bhakkan B, *et al.,*Épidémiologie et incidence du cancer bronchique primitif dans une région à faible consommation tabagique : la Guadeloupe. *Rev Mal Respir*. 2013 ;30 :537–548.
25. Ketfi A, *et al.,* Cancer bronchique primitif et risques professionnels dans une population Nord-Africaine. *Pan Afr Med J*. 2020 ;37 :120.
26. Alberg AJ, Brock MV, Ford JG, *et al.,* Epidemiology of Lung Cancer: Diagnosis and Management of Lung Cancer. 3rd ed. *Chest*. 2013;143(5 Suppl):e1S–e29S.
27. Antoine M, *et al.,* Classification histomoléculaire des cancers pulmonaires. *Rev Mal Respir Actual*. 2018 ;10:236–247.
28. Debieuvre D, *et al.,* Vingt ans d'épidémiologie des cancers bronchopulmonaires diagnostiqués dans les hôpitaux non-universitaires français. *Rev Mal Respir*. 2022;14(1):24–25.
29. Quoix E, Lemarié E. Épidémiologie du cancer bronchique primitif: aspects classiques et nouveautés. *Rev Mal Respir*. 2011 ;28:1048–1058.
30. Travis WD, Brambilla E, Nicholson AG, *et al.,* The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. *J Thorac Oncol*. 2015;10(9):1243–1260.
31. Akouatia DGE, *et al.,* Étude de l'évolution de l'incidence des cancers bronchopulmonaires primitifs de 2007 à 2017 selon le registre des cancers de Côte d'Ivoire. *Rev Mal Respir*. 2022;14(1):212–213.
32. Soumaré DDN, *et al.,* Aspects épidémiologiques et histopathologiques des cancers bronchopulmonaires au Mali. *Rev Mal Respir*. 2017;34: A89.
33. Antoine M. Qu'apporte l'immunohistochimie à la prise en charge du cancer bronchique? De la morphologie au diagnostic et au traitement. *Rev Pneumol Clin*. 2007 ;63:183–192.
34. Pelosi G, Sonzogni A, Harari S, *et al.,* Classification of pulmonary neuroendocrine tumors: new insights. *Transl Lung Cancer Res*. 2017;6(5):513–529.
35. Genton CY. L'immunohistochimie : son principe, ses applications et ses limites. *Clin Mother Child Health*. 2006;3(1):477–481.
36. Van Meerbeeck JP, Fennell DA, De Ruysscher DKM, *et al.,* Small-cell lung cancer. *Lancet*. 2011;378(9804):1741–1755.
37. Pelosi G, Rodrigez J, Viale G, *et al.,* Typical and atypical pulmonary carcinoid tumor overdiagnosed as small cell carcinoma on biopsy specimens. *Am J Surg Pathol*. 2005;29:179–187.
38. Naidoo J, Santos-Zabala ML, Iyriboz T, *et al.,* Large Cell Neuroendocrine Carcinoma of the Lung: Clinico-Pathologic Features, Treatment, and Outcomes. *Clin Lung Cancer*. 2016;17(5):121–129.
39. Tang Z, Wang L, Tang G, Medeiros LJ. Fluorescence in Situ Hybridization (FISH) for detecting Anaplastic Lymphoma Kinase (ALK) rearrangement in lung cancer: clinically relevant technical aspects. *Int J Mol Sci*. 2019;20:3939.
40. Sánchez-Ares M, *et al.,* Fluorescence in situ hybridization analysis of the ALK gene in 2,045 non-small cell lung cancer patients from North-

Western Spain (Galicia). *Oncol Lett*. 2016;12(2):1403–1407.
41. Li W, *et al.,* Combinational analysis of FISH and Immunohistochemistry Reveals Rare Genomic Events in ALK Fusion Patterns in NSCLC that Responds to Crizotinib Treatment. *J Thorac Oncol*. 2017;12(1):94–101.
42. Blackhall FH, Peters S, Bubendorf L, *et al.,* Prevalence and clinical outcomes for patients with ALK-positive resected stage I to III adenocarcinoma: results from European Thoracic Oncology Platform Lungscape project. *J Clin Oncol*. 2014;32:2780–2787.
43. Zhao F, Xu M, Lei H, *et al.,* Clinicopathological characteristics of patients with non-small cell lung cancer who harbor EML4-ALK fusion gene: a meta-analysis. *PLoS One*. 2015;10:e0117333.
44. Ramos-Vara JA. Principles and Methods of Immunohistochemistry. *Methods Mol Biol*. 2017;1641:115–128.
45. Cabillic F, Gros A, Dugay F, *et al.,* Parallel FISH and immunohistochemical studies of ALK status in 3,244 non-small cell lung cancers reveal major discordances. *J Thorac Oncol*. 2014;9:295–306.
46. Vidal J, Clavé S, De Muga S, *et al.,* Assessment of ALK status by FISH on 1,000 Spanish non-small cell lung cancer patients. *J Thorac Oncol*. 2014;9:1816–1820.
47. Lantuejoul *S*, *et al.,* Tests immunohistochimiques PD-L1 dans les cancers du poumon non à petites cellules: recommandations par le groupe PATTERN de pathologistes thoraciques. *Ann Pathol*. 2018;38(2):110–125.