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ALK Gene Rearrangement Screened by Immunohistochemistry and FISH in Non-Small Cell Bronchopulmonary Cancer (NSCLC) in Senegalese Subjects

Diop Ndiaga[1, 2*], Gueye M. V[1, 2], Faye, O[1, 2], Ngom, A. I[1, 2], Sy, M[1, 2], Diallo, A. S[1, 2], Gueye, F. D[2], Diallo, A. D[2], Ndiadé, A[3], Dieng, M[1], Thiam, I[4]

[1]Histology-Embryology-Cytogenetics Laboratory, Department of Biology and Functional Explorations, Faculty of Medicine, Pharmacy and Odontology, Cheikh Anta Diop University, Dakar, Senegal
[2]Clinical Cytology, Cytogenetics, Reproductive Biology and Human Development Laboratory, Aristide Le Dantec Hospital, Dakar, Senegal
[3]Histology-embryology-cytology-cytogenetics laboratory, Alioune Diop University, Bambey, Senegal
[4]Histopathology Laboratory, Cheikh Anta Diop University, Dakar, Senegal

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***Corresponding author:** Diop Ndiaga
Histology-Embryology-Cytogenetics Laboratory, Department of Biology and Functional Explorations, Faculty of Medicine, Pharmacy and Odontology, Cheikh Anta Diop University, Dakar, Senegal

Abstract **Original Research Article**

Introduction*:** Bronchopulmonary cancer is a worldwide scourge. In Senegal, the prevalence is 3.8%. The main objective of this work is to study ALK gene rearrangement, by immunohistochemistry and FISH, to propose a more targeted therapy to our patients. ***Patients and Methods: A multicenter, cross-sectional, descriptive, prospective study between 2018 and 2020 of bronchopulmonary cancer cases. Histology, immunohistochemistry, and FISH were performed. ***Results/Discussion*:** The mean age of patients was 60.29 ± 6.7 years. Non-small-cell lung cancer (NSCLC) predominated (92% of patients). In NSCLC, adenocarcinoma was the most frequent histological entity (34.2%). The ALK-antibody was searched in all NSCLC. Immunohistochemistry, confirmed by FISH, yielded positive results for ALK expression in 4.5% of cases. The majority of ALK-positive patients were male (66.6%) and non-smokers (66%). Significant associations between ALK gene rearrangement and histological type, notably adenocarcinoma (p=0.001) and squamous cell carcinoma (p=0.002), were found. Age (p=0.5), gender (p=0.4), and smoking (p=0.4) were not significantly associated with ALK rearrangement. Despite the relatively low incidence, *ALK* rearrangement should be routinely investigated in NSCLC, particularly in patients with adenocarcinoma. The discovery of the molecular anomaly should lead to the prescription of targeted therapies with tyrosine kinase inhibitors, which will considerably improve our patients' survival. ***Conclusion:*** IHC analysis confirmed by FISH yielded positive results for ALK expression in 4.5% of patients. Progress in understanding oncogenesis has led to the development of targeted therapies. Therefore, the prescription of these drugs is conditioned by the presence of the target molecular anomaly, which must be routinely sought in our patients.

Keywords: Cancer, lung, rearrangement, ALK, Senegalese.

INTRODUCTION

Bronchopulmonary cancer represents a global scourge in terms of morbidity and, above all, mortality. In sub-Saharan Africa, bronchopulmonary cancer accounts for 3.9% of all cancers diagnosed [1]. However, there is a delay in diagnosis due to obsolete technical facilities. In Senegal, the prevalence is 3.8% [2], and exact incidence data are not available. Despite major advances in therapeutic management and understanding carcinogenesis's molecular mechanisms, survival remains very poor.

Advances in our understanding of oncogenesis, particularly in non-smoking lung adenocarcinomas, have led to the development of therapies targeting abnormally activated signaling pathways responsible for oncogenic addiction: EGFR, ALK, but also KRAS, BRAF, PIK3CA, HER2, MET, RET and ROS1, or surface proteins involved in the antitumor immune response, such as PD-L1 [3]. Currently, certain targeted therapies have marketing authorization (MA) for the treatment of NSCLC. Prescription of these drugs may be conditional on the presence of a target molecular abnormality

(biomarker) that needs to be investigated, either routinely or in clinical trials.

More recently, *ALK* gene rearrangement has been routinely sought in NSCLC. The ALK gene, located on chromosome 2, also codes for a receptor tyrosine kinase not normally expressed in the lung. This enzyme was originally identified in anaplastic large-cell lymphoma as a chimeric protein encoded by an open reading frame that spans the breakpoint of a chromosomal (2;5)(p23;q35) rearrangement with the nucleophosmin gene. Fusions of ALK with another upstream partner, notably EML4, were discovered in lung adenocarcinoma in 2007 [4]. EML4-ALK fusions result from various small inversions in the short arm of chromosome 2. At least nine different variants have been identified. EML4-ALK fusions result in protein oligomerization and constitutive gene activation [5]. The frequency of ALK rearrangement varies from 3% to 7% in NSCLC [4, 5]. Detection methods include PCR, fluorescence in situ hybridization, and immunohistochemistry.

This is an oncogenic alteration for which several TKIs are available as first-line treatment in the ALK+ NSCLC population [6], and other TKIs have been validated after the first line. The development of these therapies is therefore closely linked to the detection of the mutation or rearrangement (or translocation) in question. As a result, immunohistochemistry, in situ hybridization, and molecular biology techniques now play a key role in the morphological, phenotypic, and molecular diagnosis of bronchopulmonary cancers.

The main objective of this work is to study the ALK gene rearrangement in our cohort of patients, by immunohistochemical and Fluorescent In situ Hybridization (FISH) methods, to propose a more targeted therapy to our patients.

I. PATIENTS AND METHODS

Type of study and study period

We conducted a cross-sectional, descriptive, multicenter, prospective study by collecting at the Histology Embryology and Cytogenetics laboratory, January 2018 to December 2020, specimens from new cases of bronchopulmonary cancers diagnosed histologically in 3 follow-up centers in Dakar: the UCAD anatomy-pathology department, Fann Hospital and Dakar Main Hospital.

Study Population

Inclusion Criteria

Included in this study were cases with a formal conclusion of primary bronchopulmonary cancer on histology from the anatomy pathology departments of UCAD, Fann Hospital, and Dakar Main Hospital, between January 2018 and December 2020.

Non-Inclusion Criteria

- Cases of bronchopulmonary cancer of confirmed secondary origin
- Suspected cases of bronchopulmonary cancer without histological confirmation

METHODS

We collected bronchopulmonary biopsy specimens fixed by the operator in a tube containing 10% formalin for 48 hours. After macroscopic examination, histological sections were taken for each case. Histological sections were obtained after the following steps:

- Dehydration of samples by successive baths in alcohol of increasing strength.
- Inclusion of fragments in kerosene melted at 58°C
- Embedding: the sample is cast into a kerosene block
- The microtome can be used to make thin 3-micron cuts.
- And finally, the sections were stained using Hematoxylin Eosin staining for histological study to determine the histological type of tumor.

Immunohistochemical Analyses

Were performed in the Histology-Embryology and Cytogenetics laboratory for the various mutation-specific markers: Anti-ALK antibodies. Two ALK antibody clones, VENTANA (D5F3) and QUARTETT (QR017), were tested in our study.

Procedure

Preparation of IHC slides; Cut 3-4 µm sections of 4% formalin-fixed, paraffin-embedded tissue.

- Dewaxing of cuts in 3 xylene baths.
- Rehydration in decreasing alcohol baths: 100%, 95%, and 70%.
- Rinse with distilled water.
- Antigen unmasking: immerse the slide in buffer, pH 9.0, and incubate at 95°C in a water bath for 30 minutes.
- Remove and cool to room temperature in Buffer.
- Framing fabric cuts with a waterproof pen
- Block endogenous peroxidases by incubating tissue in hydrogen peroxide
- Rinse with Buffer
- Inhibitor for 5min then Buffer
- Apply the primary antibody to the tissue for 1 hour in a dark, humid chamber.
- Rinse with Buffer.
- Apply secondary antibody (HRP-Peroxide-DAB).
- Rinse with Buffer.
- Add chromogen (DAB)
- Rinse with distilled water.

- Hematoxylin counterstaining
- Wash with water
- Dehydrate in 2 alcohol baths
- Wash in 2 xylene baths
- Mount the slide for observation.

Control measures

It is essential to validate immunohistochemical methods by checking reagents and procedures.

All IHC reactions are checked with control material.

- The positive control tissue is used to evaluate the IHC reaction of the primary antibody.
- The negative control tissue is used to assess the specificity of the primary antibody.
- The negative reagent control is used to assess any parasitic (non-specific) staining unrelated to the application of the primary antibody.

All tissue controls should be fixed, circulated, and embedded in the same way as patient tissue.

Assessment of immunoreactivity

LK immunoreactivity was assessed according to a modified semi-quantitative criterion, grounded on the intensity and distribution of cytoplasmic staining [7]. IHC score 3+ for strong, granular cytoplasmic staining, strong, granular cytoplasmic staining; staining in most tumor cells in most tumor cells, at least more than 75% of tumor cells, diffuse homogeneity in distribution. Score 2+ for moderate, smooth cytoplasmic staining (may also show partly strong staining) in most tumor cells, at least more than 50% of tumor cells; score 1+ for weak, focal cytoplasmic staining below score 2+ criteria and score 0 for no staining at all [7]. IHC evaluation was performed by 2 pathologists.

FISH technique

The ALK genetic status of each patient was assessed by FISH in representative tumor areas, using a two-color, break-apart ALK LSI rearrangement probe (ALK Split FISH Probe Abnova). In each case, 50 non-overlapping nuclei were examined under a Leica fluorescence microscope. The kit included separate red (3') and green (5') probes. It was determined that ALK wasn't rearranged when the two signals were adjacent or merged (appearing in yellow) or when there was a single green signal. ALK FISH was considered positive (rearranged) when red and green signals were distant, or when a single red signal was present. A sample was considered negative for ALK if fewer than 10% of cells showed separate 5' and 3' ALK probe signals or isolated 3' signals. The sample was considered positive if more than 50% OF cells showed separate ALK 5' and 3' probe signals, or isolated 3' signals. In the case of borderline results, when the number of positive cells is between 10-50%, a second reader evaluates the slide. The first and second cell count readings were added together and a percentage out of 100 cells was calculated. The sample was considered positive, if the percentage of positive cells was ≥15%.

Procedure

Slide pre-treatment

1. Immerse slides in xylene room temperature (RT)
2. Repeat the operation twice with fresh xylene each time.
3. Dehydrate slides in absolute ethanol. Repeat once with fresh absolute ethanol.
4. Let the blades air-dry.
5. Immerse slides in pre-treatment solution pre-warmed to 80°C.
6. Immerse slides in purified water.

Protease pretreatment

1. Immerse slides in protease solution at 37°C and monitor cell condition under a light microscope.
2. Immerse slides in purified water.
3. Let the blades air-dry.

Blade dehydration

1. Immerse slides in ethanol to increasing degrees, 70%, 90%, and 100%.
2. Let the blades air-dry.

Probe preparation

1. Preheat the probe to RT.
2. Briefly vortex the probe.

Co-denaturation and hybridization

1. Apply 10µl of the probe to each hybridization zone and cover it with a coverslip.
2. Seal the strips with rubber cement.
3. Co-denature the slides with ALK probe at 72°C.
4. Place slides in a humidified, pre-warmed hybridization chamber and incubate at 37°C.

Post-hybridization washing

1. Mark each hybridization zone on the back of the slides with a diamond-tipped pen.
2. Carefully remove the rubber cement.
3. Immerse slides in post-hybridization wash solution at RT to loosen coverslips. Stir gently to remove the coverslips; do not tear them off.
4. Immerse slides in post-hybridization wash solution pre-warmed to 72°C.

Blade dehydration

1. Immerse slides in ethanol to increasing degrees, 70%, 90%, and 100%.
2. Let the slides air-dry in a dark place.

Visualization

1. Apply DAPI counterstain and cover the slides with coverslips.
2. Examine the slides under a fluorescence microscope set to the correct filters.

3. Samples were read by two independent operators.

Ethical considerations

As these analyses were carried out on our patients as part of the diagnostic process, validation by an ethics committee was not necessary. However, throughout the study, we respected confidentiality and medical secrecy when processing patient data.

Data capture and processing

Data were entered using Epi Data 3.2.0.0 and processed using Epi Info™ version 7.2.5.0 from CDC/WHO. Means and percentages were compared using the Chi 2 test, Student's test, and Fischer's exact test according to their conditions of applicability. Differences of less than 0.05 were considered statistically significant.

II. RESULTS

Distribution of cases according to epidemiological aspects

Over 3 years from January 1, 2018, to December 31, 2020, 44 cases of primary bronchopulmonary cancers were diagnosed in follow-up centers at Fann Hospital, Principal Hospital, and the UCAD Anatomy and Human Pathology Department.

The mean age of the patients was 60.29 ± 6.7 years. The 61-65 age group was the most representative (29.5%), with extremes ranging from 27 to 79 years (Figure 1). Males predominated in 79.5% of cases, with a sex ratio of 3.88. Active smoking was found in 56.80% of cases, with an average number of packs/year of 33.29±14 (Table I). The duration of smoking was 34.46±8.31, with mean extremes of 20 years and 50 years. However, 30.2% of our patients were non-smokers. Smoking cessation accounted for 13%. The proportion of smokers was 64% among men and only 25% among women.

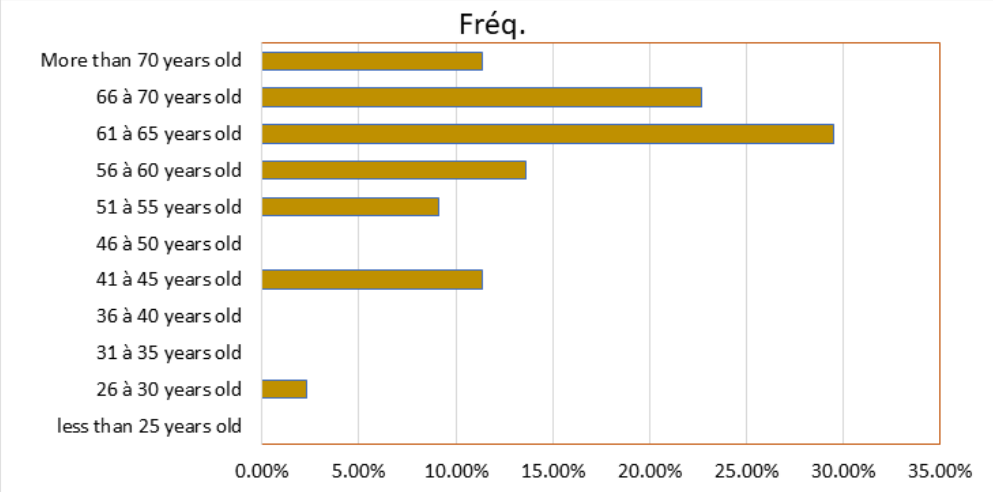

Figure 1: Age distribution of cases

Table I: Distribution of cases according to smoking status, duration of smoking, and number of pack years

ACTIVE SMOKING	FREQUENCY	DURATION OF SMOKING (YEARS)	FREQUENCY	NUMBER OF PACKS YEARS	FREQUENCY
YES	56,80%	Not applicable	30,2%	Not applicable	30,2%
NO	30,20%	Less than 22	10,8%	Under 14	2,3%
WEANING	13	From 22 to 24	6,4%	From 14 to 16	6,8%
TOTAL	100%	From 24 to 26	2,3%	From 16 to 18	2,3%
		From 26 to 28	3,3%	From 18 to 20	4,0%
		From 28 to 30	3,3%	From 20 to 22	9,1%
		From 30 to 32	9,1%	From 22 to 24	2,0%
		32 and more	34,6%	24 and more	43,3%
		TOTAL	100%	TOTAL	100%

Distribution of cases by clinical aspect

The diagnosis of bronchopulmonary cancer was mainly evoked by the presence of a lung mass (left 44.5% of patients and right 35%), followed by chest pain (38.63%), hemoptysis (22.72), cough (18,2%), dyspnea (9.1%), altered general condition (AEG) 9.1%, chronic pneumonitis (6.81%), mediastinal adenopathy (2%), pleurisy (2%) and left subpleural nodules (2%) (Table II).

Table II: Distribution of cases according to the circumstances in which the disease was discovered

Reasons for diagnosis		Frequency
Lung masses	Right	44,5%
	Left	35%
	Bilateral	5%
	Mediastino--hilar	4,5%
Chest pain		38,63%
Hemoptysis		22,72%
Cough		18,2
Dyspnea		9,1%
Altered general condition (AEG)		9,1%
Chronic lung disease		6,81%
Mediastinal adenopathy		2%
Pleuresis		2%
Subpleural nodule		2%

Distribution of cases according to histological data

Biopsy was the main type of sample taken (100%). It was associated with cytology in 10 patients (22.7%). There were no surgical specimens. Non-small-cell bronchial cancer (NSCLC) was predominant, diagnosed in 92% of patients. Adenocarcinoma was the most frequent histological entity, accounting for 34.2% of cases (Table III). Adenocarcinoma subtypes were predominantly acinar (34%), mixed (26.2%), and papillary (20%). Squamous cell carcinoma and undifferentiated carcinoma accounted for 20.5% and 15.2% respectively. Large cell carcinoma, small cell carcinoma, and neuroendocrine carcinoma without other indication were diagnosed in 11.4%, 11.4%, and 2.2% respectively.

Table III: Distribution of cases by histological type of tumor

Histological types	Frequency	Histological subtype			Frequency
Adenocarcinoma	34,2%	***Adenocarcinoma***	Acinous		34%
Squamous cell carcinoma	20,5%		Papillary		20%
Undifferentiated NSCLC	15,9%		Micropapillary		6,6%
Large-cell carcinoma	11,4%		Solid		6,6%
Small cell carcinoma	11,4%		Invasive non-mucinous		6,6%
Neuroendocrine carcinoma SAI (†)	2,2%		MIXED	Acinous/solid	13%
Pulmonary T lymphoma SAI	2,2%			Acinux cribriforme	6,6%
Mesenchymal tumor SAI	2,2%			Solid /cribriform	6,6%
TOTAL	100,0%		TOTAL		100
		Squamous cell carcinoma	Keratinizing		62,5%
			Non-keratinizing		37,5%
			TOTAL		100%

(†) *without further indication*

Distribution of cases according to **immunohistochemistry and FISH data**

In our series, ALK antibody was detected in all NSCLC, i.e. 89.6% of patients. IHC analysis with the VENTANA ALK clone (D5F3) gave positive results (1+ 2+ or 3+) for ALK expression in 2 cases (4.5%) of patients. Results were 3+ positive in 2 cases, with no equivocal results of 1+ or 2+. When the FISH method was considered as the reference, the IHC test based on clone D5F3 showed a sensitivity of 100% and a specificity of 100%, with a positive predictive value (PPV) and a negative predictive value (NPV) of 100% each (Table IV).

IHC analysis with the QUARTETT QR017 clone yielded positive results (1+ 2+ or 3+) for ALK expression in 3 cases (6.5%). Definitive positive results (3+) were obtained in 2 cases (4.5%), and an equivocal result of 1+ was obtained (2.27%). When the FISH method was considered as the reference, the IHC test based on clone QR017 showed a sensitivity of 100% and a specificity of 95% ± with a PPV and NPV of 66% and 100% respectively (Table V).

Table IV: Comparison of immunohistochemical analyses with the VENTANA ALK (D5F3) clone versus FISH data taken as reference test

	FISH+	FISH-	TOTAL	
D5F3 +	2	0	2	PPV = RP∗ / (RP + FP∗) =100%
D5F3 -	0	22	22	NPV = RN∗ / (RN + FN∗) =100%
TOTAL	2	22	24	

∗: RP: Real Positive; RN: Real Negative; FP: False Positive; FP: False Negative

Sensitivity = RP / (RP+FN) = 2/2=100%.

Specificity = RN / (RN+FP) =22 /22=100%.

Table V: Comparison of immunohistochemical analyses with the QUARTETT QR017 clone versus FISH data taken as reference test

	FISH+	FISH-	TOTAL	
QR017 +	2	1	3	VPP = VP / (VP + FP) = 66% TOTAL VALUE
QR017 -	0	21	21	VPN = VN / (VN + FN) = 100% TOTAL VALUE
TOTAL	2	22	24	

Sensitivity = VP / (VP+FN) = 100%.

Specificity = VN / (VN+FP) = 95%.

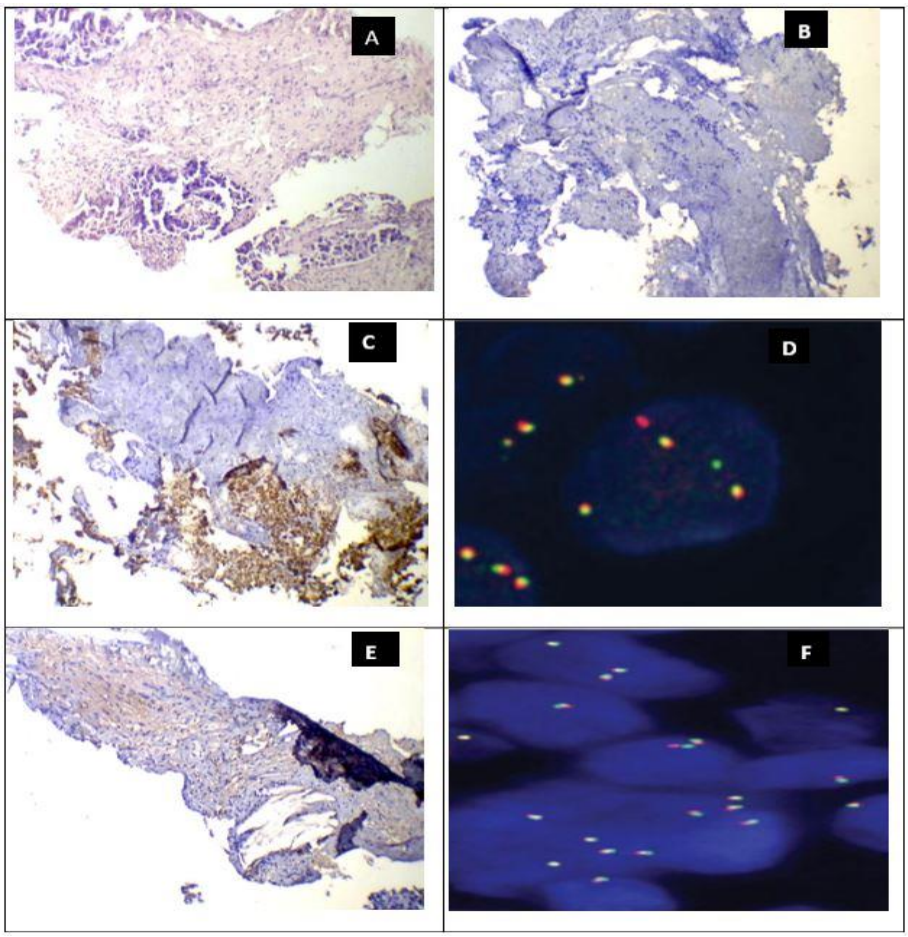

Figure 2: Histological analysis, immunohistochemistry with QUARTETT clone QR017, and FISH with ALK Split FISH Probe Abnova: A: histological appearance of a moderately differentiated adenocarcinoma (hematoxylin-Eosin, Gx100). B: a total absence of immunohistochemical staining (score 0). C: IHC positive for ALK with strong, granular, and diffuse cytoplasmic staining (score 3+). D: ALK-FISH positive with distant red and green signals. E: IHC positive (score 1+) marked by weak cytoplasmic staining. F: ALK-FISH negative showing both green and red signals fused intact, sometimes appearing yellow

Clinicopathologically, the majority of ALK-positive patients were male (66.6%) and non-smokers (66%). In terms of histological type, all ALK-positive patients had adenocarcinoma. Significant associations between ALK rearrangement and histological type were found for adenocarcinoma (p=0.001; chi2 = 20.16, ddl = 4) and squamous cell carcinoma (p=0.002; chi2 = 18.24, ddl = 4) (Table VI). Age (p=0.5), gender (p=0.4), and

smoking (p=0.4) were not significantly associated with ALK rearrangement.

Table VI: Comparison between histological type and ALK rearrangement

Adenocarcinoma / ALK	YES	NO	TOTAL
Positive	3	0	3
Negative	16	20	36
TOTAL	21	21	44
chi2 = 20.16, ddl = 4			
p = 0,001			

Squamous cell carcinoma / ALK	YES	NO	TOTAL
Positive	0	3	3
Negative	10	26	36
TOTAL	10	32	44
chi2 = 18.24, ddl = 4			
p = 0,002			

Active smoking / ALK	Yes	No
No answer	4	1
Positive	1	2
Negative	19	17
p = 0,4		

SEX / ALK	M	F
No answer	5	0
Positive	2	1
Negative	28	8
p = 0,4		

AGE / ALK	From 30 to 50	From 51 to 60	From 61 to 70
No answer	1	2	2
Positive	1	1	1
Negative	4	7	25
p = 0,5			

III. DISCUSSION

In 3 years, only 44 cases of primary bronchopulmonary cancer were diagnosed in the follow-up centers. The under-diagnosis may be explained by patients' lack of financial means for a histological diagnosis, or a delay in consultation, with death occurring before any specimen was taken for diagnosis. The mean age of patients was 60.29 ± 6.7 years. The 61-65 age group was the most representative (29.5%), with extremes ranging from 27 to 79 years. These results corroborate those obtained in Senegal by Senghor [8] and Niang [9] with 59.3 ± 11.3 and 64.2 years respectively. On the other hand, Thiam K [10] found a lower mean age of 54.6 years, but this was in a study population made up entirely of non-smokers. In Algeria, Marouani [11] found an average age of 61, and in Guadeloupe, Cadelis *et al*., [12] obtained an average age at initial diagnosis of 65 ± 12. These data confirm that primary bronchopulmonary cancer is more common in the elderly. This could be explained by the role of the duration of exposure to risk factors in the genesis of primary bronchopulmonary cancers. Indeed, the latency period before the effect of smoking habits becomes apparent is estimated at around 30 years [13].

Male predominance was noted, with a sex ratio of 3.88. Our results are consistent with figures observed in other studies, notably in Madagascar where Ramahandrisoa AVN [14] found a sex ratio of 3.81, in France where Coëtmeura D [15] found a sex ratio of 3.11, and in Morocco where Belmokhtar [16] found a sex ratio of 10.

Active smoking was found in 56.80% of cases, with an average number of packs/year of 33.29±14. The mean duration of smoking was 34.46 ± 8.31 years. On the other hand, 30.2% of our patients were non-smokers. The proportion of smokers among men was 64%, and among women only 25%. These results are similar to those obtained by Niang [8] in Senegal (57%), Cadelis [12] in Guadeloupe (61.3%), and Belmokhtar [16] in Morocco. These data confirm that smoking is the main risk factor for lung cancer in the Senegalese population, as elsewhere in the world. The low percentage of women smokers suggests the existence of other risk factors responsible for lung cancer. Indeed, several studies have implicated other risk factors in lung cancer, such as passive smoking, occupational and environmental exposure to carcinogens [17], and exposure to underlying lung diseases [18]. Several mechanisms have been proposed in an attempt to explain the relationship between underlying lung pathology and bronchopulmonary cancer; these mainly include the occurrence of genetic damage due to the accumulation of free radicals or toxic agents, as well as chronic inflammation leading to degeneration, deregulation of mitotic processes, and altered immune balance [19].

The diagnosis of bronchopulmonary cancer was mainly evoked by the presence of a lung mass (left 44.5% of patients, right 35%), followed by chest pain (38.63%),

hemoptysis (22.72), cough (18.2%), and dyspnea (9.1%). Similar results were found by Cadelis [12] in Guadeloupe and Refeno [20] in Madagascar. These data raise the question of the delay in diagnosis at the tumor stage, in our developing countries, which is a major cause of stress for the patient and leads to an altered prognosis. We therefore need to propose strategies for effective prevention and screening at a much earlier stage of lung carcinogenesis.

Non-small-cell lung cancer (NSCLC) predominated, being diagnosed in 92% of patients. Our results concur with those found in the literature [21-23]. In Senegal, Thiam [10] found a similar result, with 92% NSCLC. Among NSCLCs, adenocarcinoma was the most frequent histological entity, accounting for 34.2% of cases, followed by squamous cell carcinoma and undifferentiated carcinoma, which accounted for 20.5% and 15.2% respectively. Our results concur with those found in Morocco [16], where adenocarcinoma and squamous cell carcinoma were the most frequent histological types, accounting for 46.48% and 26.69% respectively. Cadelis found in his study 43.3% adenocarcinomas, 24.5% squamous cell carcinomas, and 10.4% undifferentiated carcinomas. The distribution of different NSCLC subtypes has changed in recent years. The adenocarcinoma histological type has become the most frequent NSCLC, in both smokers and non-smokers [23]. Several explanatory factors have been put forward, including changes in anatomopathological classifications. The World Health Organization (WHO) classification, updated in 2021 [24], underlines these evolutionary differences linked to the transformations of tobacco intoxication and introduces molecular data integrated with morphological and immunophenotypic characteristics, to reclassify these tumors, particularly for the most frequent histological types such as adenocarcinomas. In addition, the use of blond tobacco, the use of filters, and the increase in nitrosamines in cigarettes. These changes result in deeper inhalation of carcinogens in tobacco smoke [25], where adenocarcinomas develop, rather than being confined to the large bronchi (as with dark tobacco), where squamous cell carcinomas develop [23].

In our series, ALK antibody was detected in all NSCLC, i.e. 89.6% of patients. IHC analysis with the VENTANA ALK clone (D5F3) yielded positive results (1+ 2+ or 3+) for ALK expression in 4.5% of patients. Results were 3+ positive in all cases, with no equivocal 1+ or 2+ results. When the FISH method was considered as the reference, the IHC test based on clone D5F3 showed a sensitivity of 100% and a specificity of 100%, with a PPV and NPV of 100% each.

IHC analysis with the QUARTETT QR017 clone gave positive results (1+ 2+ or 3+) for ALK expression in 6.5% of cases. Definitive positive results (3+) were obtained in 4.5% of cases, and an equivocal result of 1+ was obtained (2.27%). When the FISH method was considered as the reference, the IHC test based on clone QR017 showed a sensitivity of 100% and a specificity of 95%, with a PPV and NPV of 66% and 100% respectively. These results confirm the low frequency of ALK rearrangements (4.5% in our study) in bronchopulmonary cancer. This result falls within the 2-7% range reported in the literature [25-27].

In this study, we compared the VENTANA (D5F3) test with an IHC analysis based on the use of the QUARTETT QR017 clone. The results obtained indicate that both IHC tests have the same level of sensitivity, while specificity was higher with the D5F3 clone (100% vs. 95%). Similarly, the NPV was similar for both tests, while a significant difference was observed for the PPV (100% with clone D5F3 versus 66% with clone QR017). The latter difference was mainly due to the presence of a false positive among cases with a score of 1+. Our data are consistent with those of large multicenter studies conducted using clone 5A4 [28, 29].

Clinicopathologically: the majority of ALK-positive patients (1+ 2+ or 3+) were male (66.6%) and non-smokers (66%). In terms of histological type, all ALK-positive patients had adenocarcinoma. Significant associations were found between ALK rearrangement and histological type, notably adenocarcinoma ($p=0.001$; $chi2 = 20.16$, $ddl = 4$) and squamous cell carcinoma ($p=0.002$; $chi2 = 18.24$, $ddl = 4$). Age ($p=0.5$), gender ($p=0.4$), and smoking ($p=0.4$) were not significantly associated with ALK rearrangement. These results are consistent with those obtained by Sanchez Ares M [30] in Spain and Li W [31] in China. This confirms that age and smoking are not reliable criteria for predicting or excluding ALK rearrangement. In our study, we found no relationship between ALK rearrangement and gender. However, it should be noted that Sanchez Ares found that ALK positivity was strongly associated with the female gender ($P<0.001$); however, contrary results have been reported [32, 33]. Despite the relatively low incidence (4.5% in our series), the fact remains that *ALK* gene rearrangement should be routinely investigated in non-small-cell bronchopulmonary cancers, particularly in those with adenocarcinoma as a matter of routine. The discovery of molecular abnormalities in these tumors should lead to the prescription of targeted therapies with tyrosine kinase inhibitors (TKIs) in our patients; this will considerably improve the survival of our patients. The development of ALK TKIs, in particular crizotinib, has shown a response rate of 60% in first-line treatment, and superiority in terms of progression-free survival compared with chemotherapy [34].

CONCLUSION

Bronchopulmonary cancer is a worldwide scourge**.** In Senegal, the prevalence is 3.8%. NSCLC accounts for 92% of lung cancers in our patients. In our series, ALK antibody was detected in all NSCLC, i.e. 89.6% of patients. FISH-confirmed IHC analysis was

positive for ALK expression in 4.5% of patients. Advances in our understanding of oncogenesis, particularly in non-smoking lung adenocarcinomas, have led to the development of therapeutics targeting abnormally activated signaling pathways responsible for oncogenic addiction. The prescription of these drugs depends on the presence of the target molecular anomaly (biomarker) that needs to be investigated. Thus, the presence of an *ALK* gene rearrangement should be routinely sought in NSCLC of Senegalese subjects. Two techniques were used in our patients to determine ALK status: immunohistochemistry, which detects overexpression of the protein in the form of granular cytoplasmic staining, and FISH, which highlights intrachromosomal rearrangement of the *ALK* gene.

DECLARATIONS SECTION

Ethical approval and consent to participate: All authors of this manuscript participated freely and ethically:

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