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Molecular profile of non-small cell lung cancer in northeastern Brazil

Ana Claudia da Silva Mendes de Oliveira[1,a], Antonio Vinicios Alves da Silva[2,b], Marclesson Alves[3,c], Eduardo Cronemberger[3,d], Benedito Arruda Carneiro[4,e], Juliana Carneiro Melo[5,f], Francisco Martins Neto[6,g], Fabio Tavora[1,7,h]

1. Programa de Pós-Graduação em Patologia, Universidade Federal do Ceará, Fortaleza (CE) Brasil.
2. Laboratório Argos, Fortaleza (CE) Brasil.
3. Unidade de Oncologia, Pronutrir Oncologia e Nutrição, Fortaleza (CE) Brasil.
4. Lifespan Cancer Institute, Warren Alpert Medical School, Brown University, Providence (RI) USA.
5. Programa de Pós-Graduação em Saúde Coletiva, Universidade de Fortaleza – UNIFOR – Fortaleza (CE) Brasil.
6. Unidade de Cirurgia Torácica, Hospital de Messejana Dr. Carlos Alberto Studart Gomes, Fortaleza (CE) Brasil.
7. Departamento de Patologia, Hospital de Messejana Dr. Carlos Alberto Studart Gomes, Fortaleza (CE) Brasil.

a. http://orcid.org/0000-0001-6035-8846
b. http://orcid.org/0000-0002-6266-3366
c. http://orcid.org/0000-0002-1662-3398
d. http://orcid.org/0000-0001-8493-7287
e. http://orcid.org/0000-0002-5468-1126
f. http://orcid.org/0000-0003-1734-3352
g. http://orcid.org/0000-0003-2565-365X
h. http://orcid.org/0000-0003-2361-0613

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ABSTRACT

Objective: To investigate the histological subtypes and mutational profiles of non-small cell lung cancer in Brazil, looking for correlations among histological subtypes, expression of anaplastic lymphoma kinase (ALK), EGFR mutation status, and programmed death-ligand 1 (PD-L1) expression. **Methods:** We evaluated 173 specimens obtained from patients with lung adenocarcinoma in northeastern Brazil. Expression of PD-L1 and *ALK* was evaluated by immunohistochemistry; *EGFR* mutation status was evaluated by sequencing. We categorized the histological subtypes in accordance with the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. **Results:** The most common histological subtypes of lung adenocarcinoma were solid predominant (in 46.8%), acinar predominant (in 37.0%), and lepidic predominant (in 9.8%). *ALK* expression was detected in 10.4% of the samples, and 22.0% of the tumors harbored *EGFR* mutations. The most common *EGFR* mutation was an exon 21 L858R point mutation (in 45.5%), followed by an exon 19 deletion (in 36.3%). The tumor proportion score for PD-L1 expression was ≥ 50% in 18.2% of the samples, 1-49% in 32.7%, and 0% in 49.5%. The solid predominant subtype was significantly associated with wild-type *EGFR* status (p = 0.047). Positivity for PD-L1 expression was not found to be significantly associated with *ALK* expression or *EGFR* mutation status. **Conclusions:** Our results suggest that the molecular profile of non-small cell lung cancer in northeastern Brazil differs from those of populations in other regions of the country, with *ALK* positivity being higher than the other biomarkers. Further studies including clinical and genetic information are required to confirm these differences, as well as studies focusing on populations living in different areas of the country.

Keywords: Anaplastic lymphoma kinase; ErbB receptors; B7-H1 antigen; Carcinoma, non-small-cell lung; Brazil.

INTRODUCTION

Approximately 1.8 million new cases of lung cancer are diagnosed annually, accounting for 13% of all cancer cases worldwide. In the United States, there were an estimated 150,000 deaths from lung cancer in 2018.[1] For that same year, data from the Brazilian National Cancer Institute indicate that in Brazil, there were 27,200 deaths from lung cancer, as well as 31,270 new cases of the disease.[2]

Non-small cell lung cancer (NSCLC) accounts for more than 80% of all cases of lung cancer, and this broad category (NSCLC) encompasses a number of subtypes, the most prevalent of which is adenocarcinoma. According to the World Health Organization (WHO) classification, the most common histological patterns of growth in adenocarcinomas are acinar, solid, papillary, micropapillary, and mucinous.[3]

Most lung cancer patients present with metastatic disease and receive chemotherapy, targeted therapies, immunotherapies, or a combination of those modalities. The standard of care for advanced NSCLC was transformed by the identification of oncogenic drivers and the development of tyrosine kinase inhibitors targeting such drivers, including the epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) oncogenes. The more recent development of immune checkpoint inhibitors, such as anti-programmed death-ligand 1 (anti-PD-L1) and anti-cytotoxic T lymphocyte antigen 4, has also led to major therapeutic advances in this disease as demonstrated by the results of several clinical trials documenting improvements in overall survival.[4,5]

The PD-L1 checkpoint inhibitor (also known as B7 homolog 1) is the major ligand of PD-1, and its expression on the surface of tumors cells upregulates and inhibits the immune response. Some clinical trials in patients with NSCLC have demonstrated a correlation between increased PD-L1 expression on NSCLC cells and enhanced efficacy of single-agent anti-PD-1 or anti-PD-L1 inhibitors,[6,7] as well as of combinations of those with ipilimumab.[8]

Correspondence to:
Fabio Tavora. Avenida Santos Dumont, 5753, apto. 1607, CEP 60175-047, Fortaleza, CE, Brasil.
Tel.: 55 85 3486-3150. E-mail: ftavora@gmail.com
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The results were independent of molecular alterations in *ALK* or *EGFR*. These advances not only represent major therapeutic breakthroughs but also amplify the importance of identifying the molecular features of tumors in order to guide the therapy and maximize its benefits. In low- and middle-income countries, the enthusiasm for these novel treatments is tempered by the limited access to molecular tools to characterize tumors, as well as by the high costs of targeted therapies and immunotherapies.[9] As a result, a large proportion of patients continue to receive conventional (i.e., non-targeted) chemotherapy, which, in many circumstances, is associated with limited efficacy and significant adverse effects.

Only a few studies have documented the molecular features of NSCLC, including the prevalence of *ALK* rearrangement, *EGFR* mutation status, and PD-L1 expression, in Brazil.[10-12] There is even more limited information regarding patients in underserved regions such as the northeastern region of the country. Here, we report the findings in a sample of 173 specimens of lung adenocarcinoma evaluated at a major referral laboratory of pathology, describing the histological subtypes, *EGFR* status, *ALK* status, and PD-L1 expression.

METHODS

Sample selection

We conducted a retrospective analysis of NSCLC specimens evaluated at a regional referral laboratory for surgical pathology in Fortaleza, Brazil (the Argos Laboratory), between 2015 and 2016. All specimens were fixed in formalin, after which they were stained with hematoxylin and eosin in a routine manner. Cases were reviewed by two independent pathologists with experience in pulmonary pathology and were classified according to the WHO classification system.[3] The morphological patterns, predominant histological subtypes, and available clinical data were recorded. Only non-small cell carcinomas were included in the study, those with sarcomatoid or neuroendocrine differentiation therefore being excluded. The study was approved by the Institutional Review Board of Messejana Heart and Lung Hospital, also located in the city of Fortaleza, and was registered with the National Commission for Ethics in Research (CAAE protocol no. 65315317.0.0000.5039).

EGFR mutation status

For each sample, we selected a representative formalin-fixed, paraffin-embedded block containing at least 10% viable tumor. After proteinase K digestion of the samples, we extracted DNA following standard protocols. Direct DNA sequencing of exons 18 through 21 of the *EGFR* gene was performed as previously described.[13] To detect gene mutations, we employed multiplex polymerase chain reaction in a next-generation sequencing instrument (MiSeq; Illumina, San Diego, CA, USA), as previously described in detail.[13]

ALK expression

For the evaluation of *ALK* expression, all specimens were processed in accordance with the well-established standard operating procedures adopted at the pathology laboratory. In brief, sections were stained in an automated slide staining instrument (Ventana Benchmark GX; Roche Diagnostics, Basel, Switzerland) and incubated with an approved anti-*ALK* rabbit monoclonal primary antibody (clone: D5F3, Cat. #: 790-4796; Roche Diagnostics), after which *ALK* was detected with an amplification kit (OptiView Amplification Kit, Cat. #: 760-099; Roche Diagnostics) and a diaminobenzidine immunohistochemical detection kit (OptiView DAB IHC Detection Kit, Cat. #: 760-700; Roche Diagnostics). Counterstaining was performed with hematoxylin, and negative controls were assessed. Samples were considered positive for *ALK* expression if any cells showed cytoplasmic staining, regardless of the proportion or intensity of staining.

PD-L1 expression

For the evaluation of PD-L1 expression, all specimens were processed in accordance with standard established protocols. Immunohistochemical staining for PD-L1 protein was carried out with the Ventana PD-L1 assay (clone: SP263, Cat. #: 740-4907; Roche Diagnostics) on the Ventana Benchmark GX system, PD-L1 being detected with the kits described for *ALK*. Counterstaining was performed with hematoxylin, and negative controls were assessed. In the interpretation of the results, PD-L1 expression was evaluated on tumor cells. Samples were considered positive for PD-L1 expression on the basis of the proportion of cells showing staining of any intensity, in 10% increments.[14]

STATISTICAL ANALYSIS

Correlations between categorical variables were analyzed with Fisher's exact test (when any cell in a contingency table had an expected count < 5) or Pearson's chi-square test (when none of the cells in a contingency table had an expected count < 5). All reported p-values are two-sided, and tests were conducted at a 0.05 level of significance. Statistical analysis was performed with the Statistical Analysis System, version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 173 patients with lung adenocarcinoma were evaluated. The general characteristics of the patients are shown in Table 1. The median age was 67 years (range, 36-93 years), and 103 (59.5%) of the patients were > 70 years of age. Eighty-one (46.8%) of the patients were male.

The most common sampling sites were the lung, pleura, and lymph nodes, which respectively accounted for 125 (72.2%), 23 (13.3%), and 12 (6.9%) of the 173 specimens collected. Most of the specimens were obtained by computed tomography-guided transthoracic biopsy, followed by lobectomy and transbronchial biopsy.

In accordance with the WHO classification of lung tumors,[3] we categorized the invasive adenocarcinoma growth patterns as follows (Table 1): solid predominant, in 81 (46.2%) of the specimens; acinar predominant, in 64 (37.0%); lepidic predominant, in 17 (9.8%); and papillary predominant, in 8 (4.6%).

We analyzed the *EGFR* mutation status in 149 patients. In 116 (77.9%), we detected no *EGFR* mutations (wild-type status). Thirty-three samples (22.1%) were found to harbor mutations in the *EGFR* kinase domain. As shown in Table 2, the main types of *EGFR* mutations were an L858R point mutation in exon 21, seen in 15 (45.5%) of the samples; a deletion in exon 19, seen in 12 (36.3%); and G719X point mutations in exon 18, seen in 3 (9.1%). The histological subtype mostly associated with wild-type EGFR status was the solid predominant subtype ($p = 0.0475$).

Samples were positive for protein expression of *ALK* in 18 (10.4%) of the 173 cases analyzed. Among the *ALK*-positive cases, the histological subtype was acinar predominant in 10 (55.6%), solid in 6 (33.3%), lepidic in 1 (5.6%), and papillary in 1 (5.6%). Cases in which the subtype was mucinous predominant did not display *ALK* rearrangements (Table 3). Figure 1 shows two lung adenocarcinoma samples of the acinar predominant subtype, of which one was negative for *ALK* expression (Figure 1A) and one showed strong, diffuse positivity for *ALK* (Figure 1B).

Table 1. Characteristics of patients with lung adenocarcinoma.

Characteristic	(N = 173)
Age (years), median (range)	67 (36-93)
> 70 years, n (%)	103 (53.5)
≤ 70 years, n (%)	70 (40.4)
Gender, n (%)	
Male	81 (46.8)
Female	92 (53.2)
Histological subtype, n (%)	
Acinar predominant	64 (37.0)
Solid predominant	81 (46.8)
Lepidic predominant	17 (9.8)
Papillary predominant	9 (4.6)
Mucinous predominant	3 (2.0)
Topography, n (%)	
Lung	125 (72.3)
Pleura	23 (13.3)
Lymph node	12 (6.9)
Bone	5 (2.9)
Brain	4 (2.3)
Liver	2 (1.2)
Other	2 (1.2)
Sampling procedure, n (%)	
Biopsy	130 (75.1)
Segmentectomy	21 (12.1)
Lobectomy	18 (10.4)
Other	3 (1.7)

PD-L1 expression was analyzed in 55 of the tumor samples. Of those, 27 (49.1%) were negative for PD-L1 expression and 28 (50.9%) showed some degree of PD-L1 expression. Using the tumor proportion score (TPS) cut-off values employed in clinical trials of atezolizumab,[6] we stratified PD-L1 expression by TPS, which was 0% in 27 (49.1%) of the 55 samples, 1-4% in 2 (3.6%), 5-49% in 16 (29.1%), and ≥ 50% in 10 (18.2%), as shown in Table 4. Figure 2 shows representative images of different extents of (i.e., TPS for) PD-L1 expression in lung adenocarcinoma: 0% (Figure 2A); 10% (Figure 2B); 50% (Figure 2C); and 100% (Figure 2D).

Table 3 shows the associations that gender, age, and histological subtype showed with PD-L1 expression, *ALK* expression, and *EGFR* mutation status. Neither PD-L1 positivity, *EGFR* mutation status, nor *ALK* expression was found to be significantly associated with gender or age. Fisher's exact test showed no significant relationship between positive PD-L1 expression and *EGFR* mutation status ($p = 0.407$) or between positive PD-L1 expression and *ALK* expression ($p = 0.408$).

DISCUSSION

Here, we have attempted to detect associations among PD-L1 expression, *ALK* expression, and *EGFR* mutation status in cases of NSCLC evaluated at a regional referral laboratory for surgical pathology in Brazil. Our findings show that the frequency of PD-L1 expression in patients with nonsquamous NSCLC was 50.9%, higher than the 37.9% reported in another study conducted in Brazil, in which the protocols were similar but different antibodies were used.[15] Previous studies have shown that approximately 30% of patients with advanced NSCLC have a high level of PD-L1 expression (defined as a TPS ≥ 50%).[16,17] In the present study, approximately half of the patients showed some degree of PD-L1 positivity, although only 18.2% had a TPS ≥ 50%. The relatively low proportion of patients with a high level of PD-L1 expression in our study sample could reflect variations in the antibodies, staining platforms, and assay methodologies across studies, as well as a certain degree of arbitrariness in the definition of PD-L1 positivity. There is therefore an urgent need for standardization of PD-L1 testing, which has yet to be addressed. In addition, given that the antibody used in the present study (clone SP263) exhibits staining characteristics for PD-L1 similar to those reported for other anti-PD-L1 antibodies, such as 22C3 and 28-8,[5] we can postulate that our findings are attributable to the unique molecular features of the population studied.

Table 2. Frequency of *EGFR* mutations in primary lung adenocarcinoma.

Mutation	n (%)
Exon 19 deletion	12 (36.3)
Exon 21 L858R point mutation	15 (45.5)
Exon 18 G719X point mutations	3 (9.1)
Exon 20 insertion	2 (6.1)
Exon 18 insertion	1 (3.0)

Knowing the proportional PD-L1 expression in lung tumors in any given population might be important not only for predicting responses to therapy but also for determining the overall prognosis. A recent clinical trial, known as the KEYNOTE-024 trial,[17] showed improved progression-free survival and overall survival in NSCLC patients whose tumors had a PD-L1 TPS ≥ 50%. A recent meta-analysis involving 47 studies and more than 11,000 patients showed a positive correlation between PD-L1 expression and a poor prognosis in lung cancer.[18] It is of note that the association with a poor prognosis was observed only in Asian populations. Given the lack of data for the population of Brazil, the present study might represent a first step toward identifying a specific prevalence.

Populations living in low- and middle-income countries face many challenges in order to gain access to new therapies. Not only are the prices of immune checkpoint inhibitors higher in Brazil but the implementation of biomarker selection also represents a barrier to access to the best immunotherapies.[19] In this context, health care systems are also penalized; in one study, a decision-analytic model showed that the use of PD-L1 expression as a biomarker increases the cost-effectiveness of immunotherapy.[20] Some studies have demonstrated that PD-L1 expression tends to be associated with smoking status, high pathologic grade, positive lymph nodes, and tumor size.[16,21]

The echinoderm microtubule-associated protein-like 4-*ALK* fusion gene was first identified in 2007 by Soda et al.,[22] who estimated its frequency to be 6.7% in patients with NSCLC. Since then, other studies, using immunohistochemistry, have estimated the frequency of *ALK* expression to be 3-7% among such patients.[23,24] The frequency of *ALK* expression in the present study (10.4%) was higher than the 3.2-4.8% previously reported for patients with NSCLC in Brazil.[10,12] To our knowledge, ours is the first study focusing on a population of patients in northeastern Brazil. The few previous studies reporting the prevalence of *ALK* and other biomarkers of NSCLC in Brazil have all focused on the population living in the southeastern region of the country. Socioeconomic disparities between the more developed southeastern region and the northeastern

Table 3. Expression of programmed death-ligand 1, *EGFR* mutation status, and expression of the anaplastic lymphoma kinase oncogene in patients with lung adenocarcinoma, by patient characteristic and histological subtype.

Characteristic	PD-L1 expression		*EGFR* mutation status		*ALK* expression	
	Negative n (%)	Positive n (%)	Wild-type n (%)	Mutated n (%)	Negative n (%)	Positive n (%)
Age						
≤ 70 years	10 (18.2)	8 (14.5)	47 (31.5)	18 (12.1)	65 (37.6)	5 (2.9)
> 70 years	17 (30.9)	20 (36.4)	69 (46.3)	15 (10.1)	90 (52.0)	13 (7.5)
Gender						
Female	17 (30.9)	15 (27.3)	61 (40.9)	20 (13.4)	82 (47.4)	10 (5.9)
Male	10 (18.2)	13 (23.6)	55 (36.9)	13 (8.7)	73 (42.2)	8 (4.6)
Histological subtype						
Acinar predominant	15 (27.3)	8 (14.5)	39 (26.2)	16 (10.7)	54 (31.2)	10 (5.8)
Lepidic predominant	2 (3.6)	1 (1.8)	11 (1.4)	3 (2.0)	16 (9.2)	1 (0.6)
Mucinous predominant	1 (1.8)	1 (1.8)	2 (1.3)	1 (0.7)	3 (1.7)	0 (0.0)
Papillary predominant	1 (1.8)	3 (5.5)	4 (2.7)	3 (2.0)	8 (4.6)	1 (0.6)
Solid predominant	8 (14.5)	15 (27.3)	60 (40.3)	10 (6.7)*	74 (42.8)	6 (3.5)

PD-L1: programmed death-ligand 1; and ALK: anaplastic lymphoma kinase. *p = 0.0475 vs. all other subtypes (chi-square test).

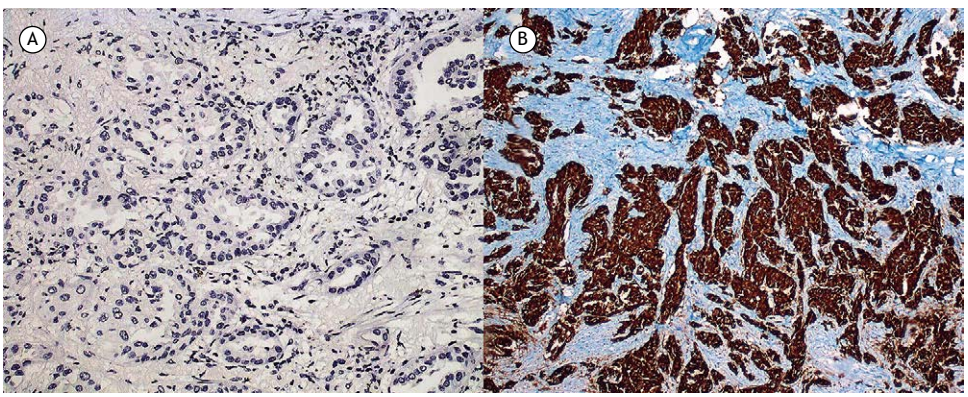

Figure 1. Anaplastic lymphoma kinase (*ALK*) expression in lung adenocarcinomas. In A, invasive lung adenocarcinoma of the acinar predominant subtype, staining negative for *ALK* expression (magnification, ×200). In B, invasive lung adenocarcinoma of the acinar predominant subtype, showing strong, diffuse positive staining for *ALK* (magnification, ×200).

region might have contributed to the higher frequency of *ALK* positivity found in our study. In fact, clinical variables and genetic information should also be considered in order to explain such differences, and further studies certainly will be required.

We found that 22.1% of patients with lung adenocarcinoma harbored *EGFR* mutations, which is similar to the 21.6% reported in another study conducted in Brazil,[12] albeit lower than the 26-33% reported for Latin America at large[25,26] and the 30-50% reported for Asia,[27] whereas it is higher than the 11-17% reported for White patients in the United States and the 8-13% reported for patients in Europe.[28] The most common *EGFR* mutations found in the present study were an exon 19 deletion and an exon 21 L858R point mutation, as has been reported for other populations.[12,21,29]

The associations between PD-L1 expression and *EGFR* mutations vary across studies. Some authors have shown a direct association between high PD-L1 expression and a positive *EGFR* mutation status.[30,31] However, Takada et al.[21] found that PD-L1 expression was significantly associated with a wild-type *EGFR* status. In the present study, we found no association between *EGFR* mutation status and PD-L1 expression. Our results are more akin to those described in a recent meta-analysis conducted by Yang et al.,[32] in which the authors concluded that the relationship between PD-L1 expression and *EGFR* mutation status was variable and not significant.

An association between *ALK* positivity and PD-L1 expression has been demonstrated in some clinical studies,[33,34] although not in others.[35,36] Although we identified a discrete trend toward such an association in the present study, it did not reach statistical significance. The unclear relationship between PD-L1 expression and the activation of oncogenic drivers (*EGFR* and *ALK*) in NSCLC, together with the discrepancies among studies, might be attributable to differences across studies in terms of the baseline clinical characteristics of the patients, heterogeneity among study populations, and the lack of standardization in the definition of PD-L1 positivity.

In attempts to determine whether molecular alterations are able to alter morphology, there have been several studies investigating the associations between *EGFR* mutations and the major histological

Table 4. Expression of programmed death-ligand 1, by tumor proportion score, in cases of primary lung adenocarcinoma (N = 55).

TPS	n (%)
0%	27 (49.5)
1-4%	2 (3.6)
5-49%	16 (29.1)
≥ 50%	10 (18.2)

TPS: tumor proportion score.

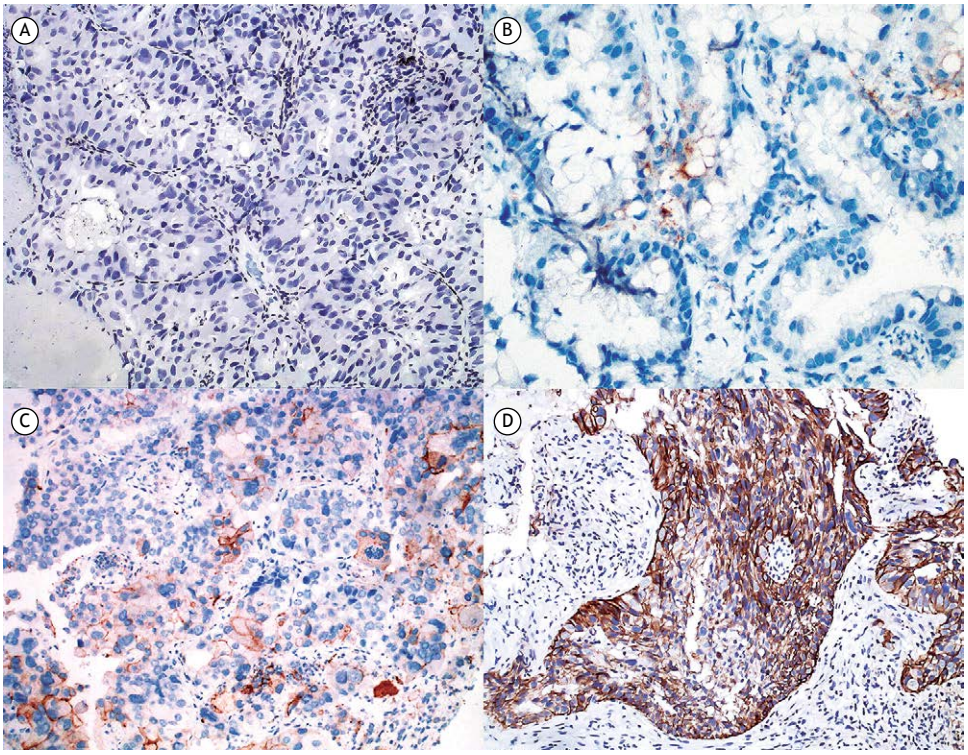

Figure 2. Programmed death-ligand 1 (PD-L1) expression in lung adenocarcinomas. In A, absence of PD-L1 expression in lung adenocarcinoma of the acinar (cribriform) predominant subtype (magnification, ×200). In B, focal positivity for PD-L1 (tumor proportion score [TPS] = 10%) in lung adenocarcinoma of the mucinous predominant subtype (magnification, ×200). In C, moderate positivity for PD-L1 (TPS = 50%) in lung adenocarcinoma of the solid predominant subtype (magnification, ×200). In D, diffuse, intense positivity for PD-L1 (TPS = 100%) in lung adenocarcinoma of the solid predominant subtype (magnification, ×200).

patterns. A study conducted in Japan showed that *EGFR* mutations were significantly associated with the papillary predominant and lepidic predominant subtypes.[37] In a study conducted in China, Song et al.[38] found that the micropapillary predominant and lepidic predominant subtypes were associated with *EGFR* mutations. In a population of patients in the United States, the lepidic predominant subtype was found to be the only histological subtype associated with *EGFR* mutations,[39] whereas the acinar predominant subtype was the only subtype found to be associated with *EGFR* mutations in a population of patients in Brazil.[12] Our finding that the solid predominant subtype was most strongly associated with wild-type *EGFR* status underscores the fact that the relationship between *EGFR* mutation status and the histological subtype of lung adenocarcinoma remains unclear.

Our study has several limitations. First, it was a single-center retrospective study, which makes it impossible to rule out the possibility of bias. Second, because we focused mainly on pathological findings, there is a lack of clinical data, which could have improved the study. Finally, the immunohistochemical analysis of PD-L1 involved the use of only one antibody, which might have been inappropriate if there was heterogeneity in the PD-L1 expression within a tumor sample.

In summary, we have reported the frequency of clinical biomarkers of NSCLC, together with the corresponding pathological findings, in a population of 173 patients in northeastern Brazil. We found no significant associations among those biomarkers. Despite the fact that the frequency of PD-L1 expression and *EGFR* mutation status were consistent with the few data available for Brazil, the frequency of *ALK* expression was higher than that previously reported for populations in Brazil. Further studies are encouraged in order to understand how such biomarkers are distributed throughout this heterogeneous population and, more importantly, how to translate that knowledge into better routine clinical practice.

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