ORIGINAL ARTICLE

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Mutagenic damage among bronchiectasis patients attending in the pulmonology sector of a hospital in southern Brazil

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SUMMARY

OBJECTIVE: Bronchiectasis is a chronic respiratory disease characterized by inflammation, irreversible dilation of the bronchi, and recurrent pulmonary infections, with a high morbidity and mortality rate, but is less studied from the point of view of its prevalence and associated factors not directly related to respiratory prognosis. As it is a disease related to the exacerbation of the inflammatory process and oxidative stress, this study searched to investigate the micronucleus frequency in patients with and without bronchiectasis treated at a specialized pulmonology service in a hospital in the extreme south of Brazil.

METHODS: Patients with a confirmed tomographic diagnosis of bronchiectasis were defined as cases. Mutagenicity was evaluated by the micronucleus test in patients' oral mucosa cells. Data collection was performed through a questionnaire containing socioeconomic, demographic, lifestyle, and health condition information.

RESULTS: Of the 95 patients involved in this study, 21 (22.1%) were diagnosed with bronchiectasis aged between 12 and 89 years. There was no significant difference in the frequency of micronucleus between patients with and without bronchiectasis. There was a significant positive association between age and frequency of micronucleus among patients with bronchiectasis, but this association does not occur among patients without the disease.

CONCLUSION: This is the first study to investigate data on the prevalence and clinical and epidemiological aspects of this chronic disease in Brazil, especially those related to the genotoxicity outcome.

KEYWORDS: Fibrosis. Mutagenesis. Bronchiectasis. Morbidity.

INTRODUCTION

Chronic respiratory diseases (CRDs) affect hundreds of millions of people around the world, and their worsening is increasingly recognized as one of the main causes of loss of quality of life and mortality associated with the disease[1].

CRD affects both the upper and lower airways, with the most common morbidities being allergic rhinitis, chronic obstructive pulmonary disease (COPD), asthma[2], and bronchiectasis. The latter has been shown to be a more frequent pathology than previously considered but with few studies[3].

Bronchiectasis is characterized by an abnormal and irreversible dilation of the bronchi that results in bacterial colonization, impaired mucociliary clearance, and chronic inflammation of the bronchial mucosa with the consequent gradual destruction of lung tissue[4,5]. This inflammatory process is progressive and results in a cycle of worsening lung injury[6].

The biological mechanism for the epidemiological association of chronic respiratory diseases and the subsequent development of lung cancer remains unclear. However, the lungs are in direct contact with high concentrations of oxygen and pollutants[7]. In addition to these factors, an important component of lung disease is inflammation and activation of inflammatory cells that produce reactive oxygen species and can lead to oxidative stress[7-9], being this, one of the main factors responsible for direct damage, enhancing other participating mechanisms such as inflammation, changes in the balance between proteases and antiproteases in the lungs and apoptosis[10,11]. Thus, lung cancer can be attributed to chronic inflammation and recurrent infection of bronchial trees that can activate multiple oncogenic pathways and facilitate the development of tumors[12].

Considering that the evolution of bronchiectasis and many other chronic lung diseases involves a vicious cycle consisting of

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bronchial changes, which predispose to lower lung clearance, leading to recurrent infections and increasing the inflammatory process and genomic instability, and in turn, could act as a critical step for the worsening of the clinical picture of these patients and even induce mutations or epigenetic alterations that may be associated with irreversible damages such as the appearance of lung cancer[13,14].

Studies have shown that there is a significant association between the increased frequency of chromosomal damage measured by the micronucleus (MN) test and other chronic lung diseases such as COPD, highlighting this technique as a noninvasive, robust, and low-cost method to early predict phenotypic and biological changes in individuals at high risk of developing lung cancer[15].

Pathologies with similar conditions in terms of the inflammatory process and oxidative stress have been associated with the occurrence of mutagenesis, and among the most widely chosen biomarkers for the study is the MN test. Given the scarcity of information on bronchiectasis and triggering factors, this study aimed to investigate the frequency of micronuclei in patients treated at a pulmonology clinic in the extreme south of Brazil, in addition to studying possible factors associated with increased mutagenic damage.

METHODS

Study population

This is a prospective and observational study with a quantitative approach developed from July 2018 to August 2018, having as the study population all adult patients treated at a specialized outpatient clinic at the Doctor Miguel Riet Corrêa Junior University Hospital (HU-FURG), in Southern Brazil (Rio Grande/RS).

Patients with high-resolution computed tomography confirmed diagnosis and clinical history consistent with bronchiectasis were defined as cases, and the other subjects were considered to study controls.

Ethical aspects

This observational study was approved by the Research Ethics Committee in the Health Area of the Federal University of Rio Grande (CEP/FURG), process 23116.006049-2018-10.

Human samples

A smear of buccal cells (for MN assay) and the oropharyngeal (for the bacterial colonization) were collected from 95 patients, using a cytobrush and swab, respectively.

Micronucleus test

Mutagenicity was evaluated via the MN test in cells of the oral mucosa of the patients. Smears were made on the slides, and these were stained with eosin-methylene blue according to Leishman; the total number of cells were counted by two analyzers using an optical microscope at 400´ and 1,000´ magnifications; and the frequency of MN was expressed as the number of MN in 1000 cells[16]. All slide readings were performed blindly.

Microbiological evaluation

The oropharyngeal samples were made on a slide smear with the oropharyngeal samples and were stained with Gram coloration, classifying the microorganisms into Gram-positive and/or Gram-negative.

Data analysis

The volunteers who agreed to participate in this study signed an informed consent form and were asked to answer a semi-structured questionnaire for data collection containing information on socioeconomic, demographic, lifestyle, and health conditions.

Comparison of the frequency of MN between groups (with and without chronic diseases or bronchiectasis) was performed using the Mann-Whitney test, and the association between age and frequency of MN was tested using simple linear regression analysis. For both analyses, a critical p-value of 5% was considered.

The association between the MN frequencies in buccal cells and different confounding factors was investigated using multiple Poisson regressions (multivariate and bivariate) with a robust estimate. For this, individuals who had a micronuclei frequency ≤ 2.5 micronuclei in 1000 cells were considered "without risk" (Holland et al., 2008). Regression analysis was conducted from hierarchical levels: first level (age, ethnicity, gender, marital status, and income *per capita*); second level (living and working conditions); and third level (life habit: smoking, drinking, and drug use and clinical conditions: pulmonary function and oropharynx colonization). The goodness of fit was checked using the deviance statistics. The variables with a p-value < 0.2 remained in the model but were considered significant only if the p-value was <0.05. Data analyses were performed using the SPSS software.

The sample power was calculated using the website http://powerandsamplesize.com/, by comparing the means and standard deviations of each parameter in the groups (with or without non-cystic fibrosis bronchiectasis), considering a level of significance of 5% in the ANOVA model.

RESULTS

A total of 95 patients seen at the HU-FURG during the study period were included (Table 1), with a 22.1% prevalence of bronchiectasis (21/95). Most patients were female (68.4%), white (80%), non-smoker (72%), with a mean age of 59.91±15.9 years (from 12–89 years). Regarding respiratory compromise, as evidenced by respiratory disorders during spirometry, approximately half of the patients included in this study had the ratio between forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) below 70%, with the frequency of airflow limitation of 57 vs. 42.9% in patients with non-cystic fibrosis bronchiectasis (NFB) when compared with controls, respectively.

Chronic bacterial colonization of the oropharynx in NFB has been an important predictive factor with regard to the prognosis of these patients, and 66.3% of patients (63/95) had bacterial colonization, with *Staphylococcus aureus* being the pathogen most frequently isolated.

Regarding the evaluation of mutagenesis in the oral mucosa of these patients, the frequency range of MN was between 0–3‰, and only samples from older individuals (over 59 years) had frequencies equal to or greater than 2.5 ‰. There was no statistically significant difference in the frequency of MN between patients with a history of chronic respiratory disease, as well as no difference in the frequency of MN between patients with and without bronchiectasis (Figure 1), and the power of the sample was 0.33.

Considering age as an important factor associated with MN, Figure 2 shows a significant positive association between age and frequency of MN among patients with bronchiectasis, but this association does not occur among patients without the disease. There is also no association between age and frequency of MN when considering patients with and without any chronic respiratory disease.

Comparing the results of MN frequency between men and women diagnosed or not diagnosed with NFB (Figure 3), there was no significant difference between the two groups. Furthermore, regardless of sex, age seems to remain a pillar in the increase in MN in patients with NFB (Figure 4).

The analysis of associated factors using Poisson regression analysis with robust variance did not show any significantly associated variable in both bivariate and multivariate analyses (Table 2).

DISCUSSION

The prevalence of NFB in Brazil has not been well characterized since this disease has been considered an orphan from an epidemiological point of view, which has remained underdiagnosed,

Table 1. Baseline characteristics of study population.

	Frequency (%)
Age (years)	
Average age (years)	59.91±15.9
Above 59	58.9
30–59	34.8
Up to 29	6.3
Gender	
Female	68.4
Male	31.6
Marital status	
Married	47.7
Single	12.8
Other	39.5
Skin color	
White	80
Non-white	20
Average family income*	2,123.97±1,876.59
Income >2 salaries	41.5
Between 1 and 2 salaries	29.8
Income ±1 salary	28.7
Average people residing in the house	2.91±1.99
Above 1	77.7
Up to 1	22.3
Average number of rooms in the house	5.16±1.74
More than 3	69.9
Between 2 and 3	9.7
1 room	4.3
Work	
Yes	89.5
Not	10.5
Cigarette	
Smoker	16.3
Ex-smoker	54.3
Non-smoking	29.3
Alcohol	
Addict	22.3
Do not drink anymore	22.3
Never drank	55.3
Drug user	
Already used	4.3
Never used	95.7
Altered pulmonary function**	
Not	44.9
Yes	55.1
Bacterial colonization	
Multicolonized (GPC+GNB)	16.8
Gram-Positive Cocci (GPC)	69.5
Gram-Negative Bacilli (GNB)	6.3
There was no bacterial growth	7.4

*Minimum Salary: R$954.00; **Altered spirometry if forced expiratory volume in one second/forced vital capacity (%) below 70%. GPC: Gram-positive cocci; GNB: Gram-negartive bacilli.

especially in developing countries[3,17,18]. Even with the high variability between different populations[19], some studies conducted in Europe and the United States of America reveal an increase in the prevalence of the disease in these locations[20-22].

Considering the scarcity of national studies and high variability in mortality rates related to this chronic disease (ranging from 2–35%), there seems to be an underestimation of Brazilian data. In this study, the prevalence of NFB found in

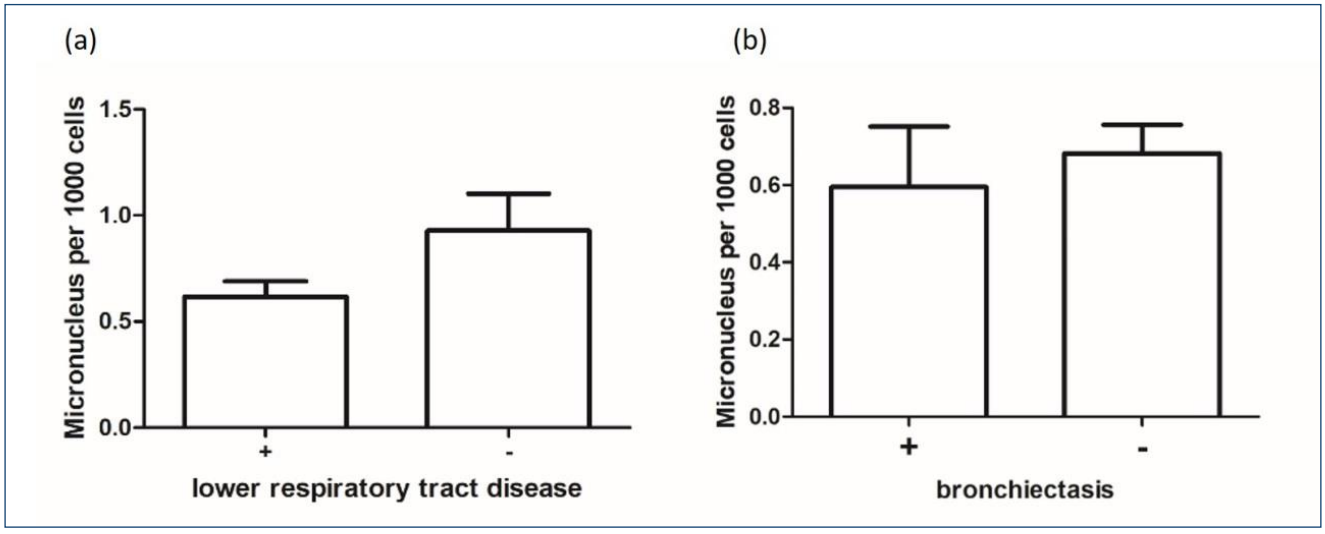

Figure 1. Frequency of micronucleus/1000 cells in patients (A) with and without a diagnosis of lower respiratory tract diseases (asthma, bronchitis, rhinitis, sinusitis, tuberculosis, emphysema, and cystic fibrosis) and (B) with or without non-cystic fibrosis bronchiectasis.

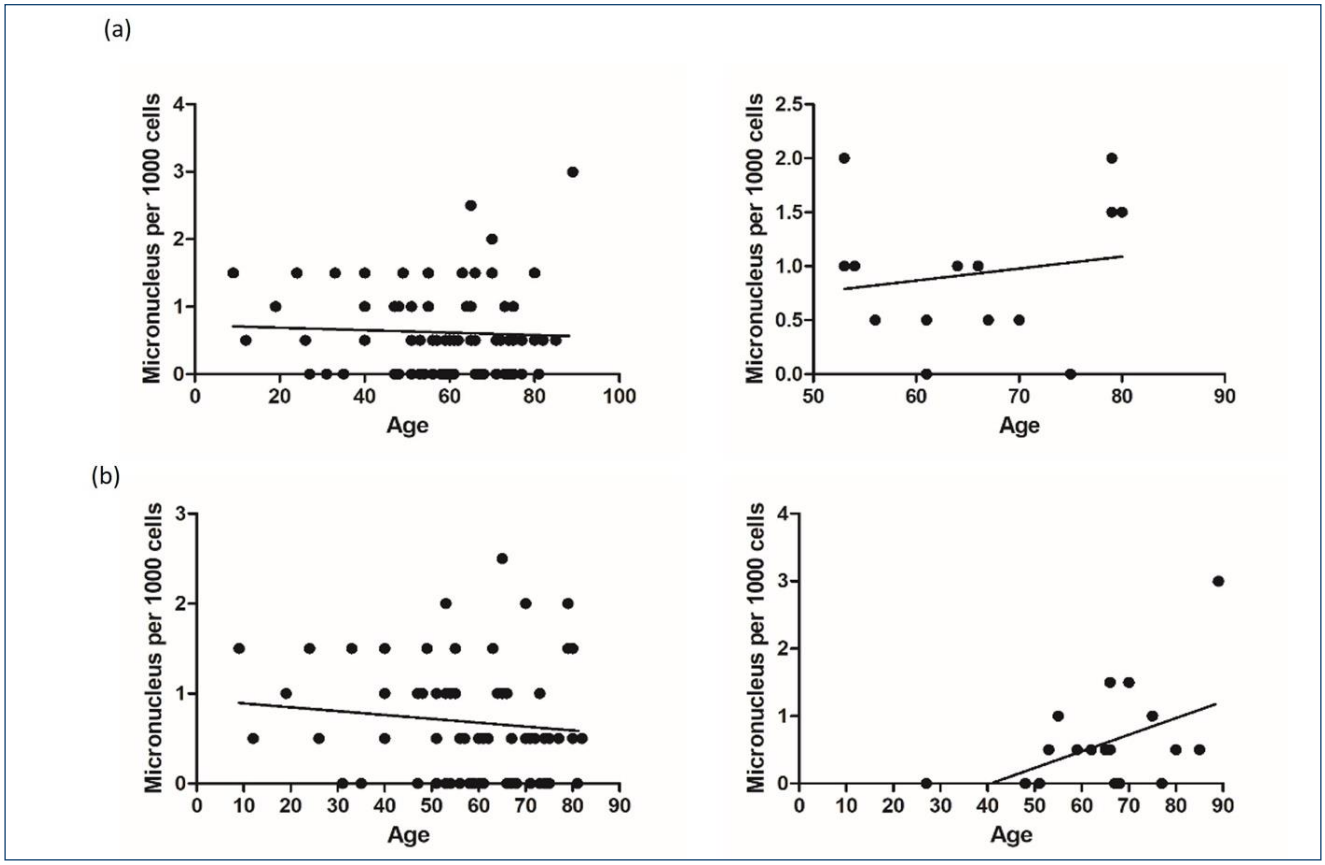

Figure 2. Association between age and micronucleus frequency (‰) in oral mucosa samples from patients (A) with (r^2=0.002; p=0.68) and without (r^2=0.03; p=0.53) diagnosis of respiratory diseases and (B) with (r^2=0.22; p=0.03) or without (r^2=0.01; p=0.35) diagnosis of non-cystic fibrosis bronchiectasis.

outpatients seen in a single month of 2018 at the regional reference center for pulmonology in the far south of the country was 22.1%. This prevalence tends to be higher proportionally to the increase in age of patients, corroborating other studies[23].

The data showed an association between age and frequency of MN among patients diagnosed with bronchiectasis, and this association did not exist between patients without the disease and patients with another chronic respiratory disease. Age has been identified as a factor related to mutagenesis, and even studies with healthy subjects have shown an increase in the frequency of age-related mutagenic damage[24]. In addition, the prognostic data from the NFB itself have highlighted age as an important factor for the increase in the prevalence of this disease, as well as an increase in hospitalization and mortality rates in this population[3,25].

The frequency of micronuclei has been widely studied in several pathologies. However, to date, no studies have been found that have shown the frequency of MN in elderly people with bronchiectasis as a possible biomarker for the evaluation of mutagenesis and cancer in these patients.

Studies have shown that bronchiectasis increases systemic inflammation and arterial stiffness and causes bone thinning, and the inflammatory response plays an essential role in tissue genotoxicity and consequently in tumorogenesis[5,26]. On the other hand, some authors have proposed that there is an intimate relationship between NFB and cancer, including proposing the tracking and follow-up of these patients in longitudinal studies[12,23]. In this sense, this is the first study that evaluates mutagenesis through the quantification of MN in mouth epithelial samples of individuals with NFB in Brazil, in order to validate this tool as a possible prognostic marker to be considered in the routine evaluations of these patients.

Although the frequency of MN in patients with bronchiectasis was similar to that in patients without the disease, this association reveals a scenario that highlights the need for additional patient care beyond those listed in the diagnostic and therapeutic management in order to prevent disease progression respiratory, indicating that the aging of the population with NFB associated with other risk factors, such as occupational exposure, constant imaging tests, exposure to chemicals, and

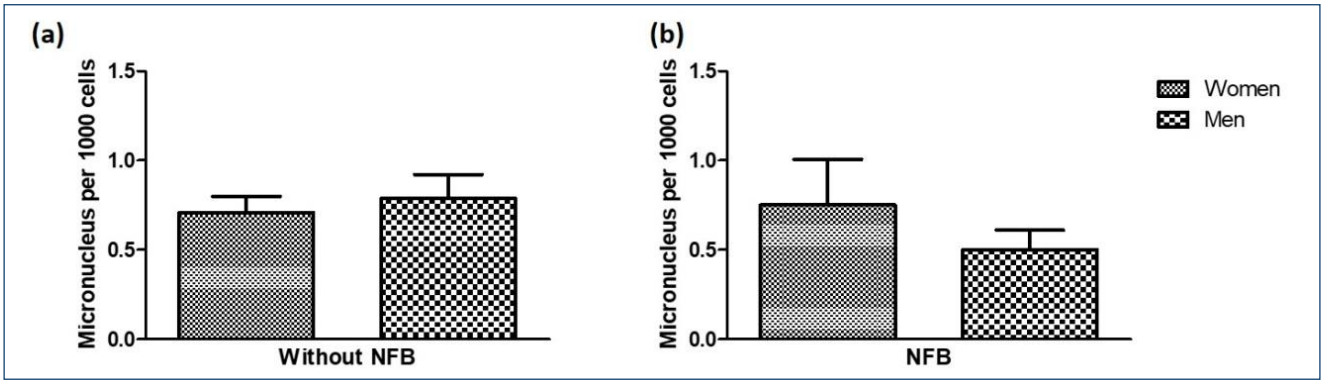

Figure 3. Frequency of micronucleus/1000 cells in men and women (A) without non-cystic fibrosis bronchiectasis (NFB) and (B) with non-cystic fibrosis bronchiectasis.

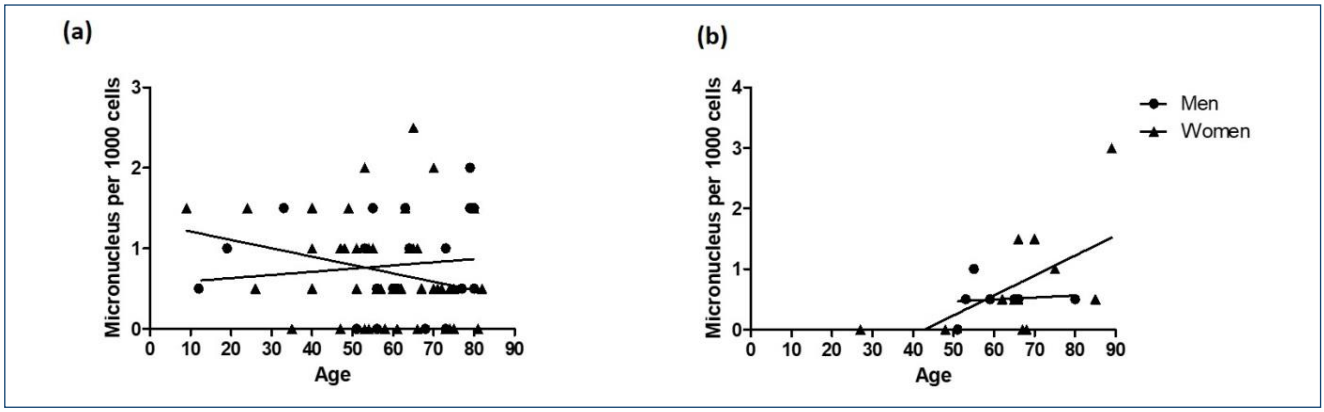

Figure 4. Association between age and micronucleus frequency (‰) in oral mucosa samples of (A) of men (r^2=0.014; p=0.60) and women (r^2=0.06; p=0.08) without diagnosis of non-cystic fibrosis bronchiectasis and (B) men (r^2=0.01; p=0.81) and women (r^2=0.35; p=0.04) with diagnosis of non-cystic fibrosis bronchiectasis.

Table 2. Bi- and multivariate analysis of associated factors using Poisson regression.

Level		Bivariate		Multivariate	
		95%CI	p-value	95%CI	p-value
1	Age (years)		0.95		0.71
	Over 59	1		1	
	30–59	0.86 (0.36–2.09)		0.84 (0.37–1.90)	
	Up to 29	0.95 (0.15–5.89)		0.47 (0.07–3.01)	
1	Gender		1.00		0.68
	Female	1		1	
	Male	1.00 (0.42–2.38)		1.22 (0.48–3.12)	
1	Marital Status		0.61		0.39
	Married	1		1	
	Single	1.24 (0.55–2.80)		1.39 (0.66–2.90)	
1	Skin Color		0.33		0.21
	White	1		1	
	Non-white	2.60 (0.38–17.8)		3.22 (0.51–20.27)	
1	Family income		0.30		0.26
	Above 2 minimum salary	1		1	
	Between 1 and 2 minimum salary	1.43 (0.61–3.35)		1.55 (0.71–3.35)	
	Below 1 minimum salary	0.56 (0.16–1.91)		0.58 (0.17–1.98)	
1	Education		0.55		0.55
	8 or more years	1		1	
	Up to 8 years old	0.46 (0.21–1.02)		0.46 (0.21–1.02)	
2	People per part of the house		0.07		0.09
	Above 1	1		1	
	Below 1	2.27 (0.93–5.50)		2.26 (0.89–5.73)	
2	Working time		0.64		0.80
		0.99 (0.96–1.03)		1.00 (0.97–1.04)	
3	Cigarette consumption		0.51		0.95
	Smoker	1		1	
	Ex smoker	0.57 (0.22–1.50)		0.81 (0.22–2.95)	
	Non-smoking	0.68 (0.24–1.90)		0.87 (0.25–3.07)	
3	Alcohol		0.71		
	Use	1			
	Don't drink anymore	1.14 (0.12–10.7)			
	Never drank	1.75 (0.26–11.53)			
3	Drug user		0.79		0.43
	Aready used	1		1	
	Never used	0.79 (0.14–4.54)		0.51 (0.10–2.70)	
3	Use of medications		0.38		0.53
	Use	1		1	
	Do not use	0.54 (0.14–2.12)		0.69 (0.22–2.17)	
3	Altered pulmonary function*		0.15		0.08
	Not	1		1	
	Yes	1.81 (0.80–4.09)		2.06 (0.91–4.65)	
3	Bacterial colonization		0.17		0.34
	GPC+GNB	1		1	
	GPC	0.40 (0.17–0.95)		0.46 (0.18–1.17)	
	GNB	0.89 (0.24–3.25)		0.95 (0.24–3.68)	
	NG	0.38 (0.56–2.60)		0.42 (0.06–2.96)	

*Altered spirometry if forced expiratory volume in one second/ forced vital capacity(%) below 70%. GPC: Gram-positive cocci; GNB: Gram-negartive bacilli; NG: No microbial growth.

comorbidities, and hospitalizations associated with recurrent infections due to the clinical condition of NFB may favor the increase in events genotoxic up to 4 times[27].

Advanced age appears to be associated with an increase in genomic instability resulting from reduced DNA damage repair capacity[28]. In addition, studies have shown that bronchiectasis seems to be directly related to the emergence of cancers, at a frequency ranging from 0.2–16%, and that age helps to increase these rates among bronchiectasis[23,29].

It should be noted that the MN test could be used as a biomarker as a screening method for genotoxicity in patients with chronic lung diseases such as bronchiectasis, even considering one of its main limitations: the background MN frequency in buccal cells is relatively low (approximately 0.1%)[27,30], which can influence the individual variability of the evaluated sample and, consequently, interfere in the statistical power found.

CONCLUSION

Therefore, this is the first study to investigate data on the prevalence and clinical and epidemiological aspects of this chronic disease in Brazil, especially those related to the genotoxicity outcome. We also emphasize that it was only in 2019 that the first Brazilian consensus was published[3] for the management of NFB and that due to the high impact of chronic lung diseases on human morbidity and mortality rates, and the severity and prognosis of these patients have emerged as an important point of clinical evaluation, having proposed several scores based on infection markers, the clinical status of patients, tissue involvement, and access to hospitalization services. However, mutagenesis markers that may predispose to lung cancer, for example, are not among the aspects evaluated to estimate the severity and prognosis of these patients. Therefore, using simple, easy-to-perform, and inexpensive tools for the early screening of genotoxic events in these patients could facilitate diagnostic and therapeutic management, including avoiding the negative outcome associated with the appearance of carcinogenic events and increasing the quality of life of patients.

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AUTHORS' CONTRIBUITION

DWVO: Visualization, Investigation, Writing – original draft. **KBM:** visualization, investigation, and writing – original draft preparation. **MMP:** Visualization, Investigation, Methodology. **CLFF:** Visualization, Investigation, Methodology. **FMRSJ:** Conceptualization, Data curation, Writing – review & editing. **DFR:** Conceptualization, Data curation, Writing – review & editing.

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