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ORIGINAL RESEARCH

Relationships Between Bronchoscopy, Microbiology, and Radiology in Noncystic Fibrosis Bronchiectasis

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Background: Published data on the correlations of bronchoscopy findings with microbiological, radiological, and pulmonary function test results in children with noncystic fibrosis (CF) bronchiectasis (BE) are unavailable. The aims of this study were to evaluate relationships between Bronchoscopic appearance and secretion scoring, microbiological growth, radiological severity level, and pulmonary function tests in patients with non-CF BE.
Methods: Children with non-CF BE were identified and collected over a 6-year period. Their medical charts and radiologic and bronchoscopic notes were retrospectively reviewed.
Results: The study population consisted of 54 female and 49 male patients with a mean age of 11.7 ± 3.4 years. In the classification according to the bronchoscopic secretion score, Grade I was found in 2, Grade II in 4, Grade III in 9, Grade IV in 17, Grade V in 25, and Grade VI in 46 patients. When evaluated according to the Bhalla scoring system, 45 patients had mild BE, 37 had moderate BE, and 21 had severe BE. Microbial growth was detected in bronchoalveolar lavage fluid from 50 of the patients. Forced expiratory volume in 1 s (FEV_1) and functional vital capacity decreased with increasing bronchoscopic secretion grade ($P = 0.048$ and $P = 0.04$), respectively. The degree of radiological severity increased in parallel with the bronchoscopic secretion score ($P = 0.007$). However, no relationship was detected between microbiological growth rate and radiological findings ($P = 0.403$).
Conclusions: This study showed that bronchoscopic evaluation and especially scoring of secretions correlate with severe clinical condition, decrease in pulmonary function test, worsening in radiology scores, and increase in microbiological bacterial load in patients. Flexible endoscopic bronchoscopy should be kept in mind in the initial evaluation of non-CF BE patients.

Keywords: bronchiectasis, bronchoscopy, radiology

Introduction

Bronchiectasis (BE) is defined as permanent expansion of the bronchi, and is commonly associated with chronic cough, sputum, and recurrent lower respiratory infections. Radiological imaging and bronchoscopy can be useful for diagnosis of BE, particularly tests for cystic fibrosis (CF) and immunodeficiency panel tests, in some cases.[1] Bronchoscopy is an important tool in evaluating BE and especially used in the differential diagnosis of localized BE for underlying conditions such as foreign body and tumor. Bronchoscopy is used for microbiological and cytological examination of bronchial secretions in children who cannot produce sputum. Recently, it has been suggested that bronchoscopy be used as one of the first diagnostic tests for BE.[2] Increases in the amount of secretion and color changes, to various degrees, can be seen on bronchoscopy. Some patients show bronchoscopic findings of malacia and edema. There have been relatively few studies regarding the bronchoscopic findings and secretion in pediatric cases of non-CF BE.[3] There is little information in the literature regarding the correlations of bronchoscopy findings with microbiological, radiological, and pulmonary function test results.[2,4–7] This study was performed to evaluate relationships between bronchoscopic appearance and secretion scoring, microbiological growth, radiological weight level, and pulmonary function tests in patients with non-CF BE.

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Materials and Methods

The study population consisted of 103 patients followed up for non-CF BE at the Pediatric Pulmonology Department of Bezmialem Vakif University Faculty of Medicine, Istanbul, Turkey, between 2012 and 2018. Bronchoscopic appearance classification, bronchoscopic secretion scoring, bronchoalveolar lavage (BAL) culture, respiratory function tests, and radiological weight scores of the patients were recorded. CT evaluation, bronchoscopy, microbiological evaluation, and pulmonary function test were performed simultaneously in all patients. Patients who did not have pulmonary exacerbation and had not been treated with antibiotics in the 2 weeks before the bronchoscopy procedure were included in the study.

Bronchoscopic procedure

Bronchoscopy was performed with the same pediatric pulmonologist: a laryngeal mask airway using pediatric-type bronchoscopes according to the size of the patient (Pentax), with an outer diameter of 3.7 mm or 4.9 mm. An intravenous catheter was secured and, after initial examination, local anesthesia was applied to the vocal cords with 0.3–2.5 mL of 2% lidocaine solution (not exceeding a dose of 2 mg/kg). Anesthesia was first applied to the region of the vocal cords, and then to the trachea above the carina and the bronchi. The patients were sedated with intravenous midazolam (maximum dose of 1 mg/kg), and, in some cases, ketamine (1 mg/kg) was used if the above modalities failed to achieve appropriate sedation for the procedure. Peripheral perfusion and depth of respiration were closely monitored by the anesthetist. Respiration and blood oxygen saturation were also monitored electronically in all patients.

Bronchoscopy imaging scoring

The bronchoscopic appearance of the bronchi was classified into 1 of 5 main groups in accordance with the classification of Chang et al.[4]: Type 1, only mucosal anomaly/inflammation; Type 2, bronchomalacia; Type 3, obliterative-like lesion; Type 4, combination of malacia+obliterative-like lesion; and Type 5, normal structure.

Bronchoscopic secretion scoring

Bronchoscopic secretion scoring was performed in accordance with the classification of Chang et al.[7] Secretions were quantified according to the amount of mucus in the airways in relation to lumen size (Type 1, secretion covering <1/3 of the lumen; Type 2, rate of secretion between 1/3 and 2/3; and Type 3, secretion covering >2/3 of the lumen) and scored from the trachea to the level of the lobar bronchi (total of 9: trachea, right main stem, right upper lobe, right middle lobe, right lower lobe, left main stem, left upper lobe, left lingula, and left lower lobe). When segmental bronchi were seen, the worst segment (ie, the segment with the most secretions) was scored. These scores were used to obtain a final bronchoscopic secretion grade from grades I to VI: Grade I, nil secretions; Grade II, near dry, bubbles only in <1/2 total number of bronchi involved; Grade III, minimal, bubbles found in >1/2 total number of bronchi involved or secretion Type 1 in <1/2 total number of bronchi involved; Grade IV, mild, secretion Type 1, >1/2 total number of bronchi involved or secretion Type 2, <1/2 total number of bronchi involved; Grade V, moderate, secretion Type 2, >1/2 total number of bronchi involved or secretion Type 3, <1/2 total number of bronchi involved; and Grade VI, large, secretion Type 3, >1/2 total number of bronchi involved.

Microbiological analysis

BAL fluid was obtained from the macroscopically most abnormal lobe. When changes were generalized, BAL fluid was obtained from the right middle lobe and left upper lobe lingular segment. Sterile saline (1 mg/kg, maximum: 20 mL) was applied to the lobe, aspirated, and collected into closed containers for microbiological examination. Growth of 10^4 in BAL fluid was considered to be significant.[8]

Radiological evaluation

All high-resolution computed tomography (HRCT) scans were performed by the same radiologist who was unaware of the clinical condition of the patients. If more than 1 chest HRCT had been performed for a patient, only the last scan was scored.

The Bhalla scoring system was used to evaluate computed tomography (CT) findings.[9,10] This scoring system evaluates the following: (1) severity of BE; (2) extent of BE; (3) peribronchial thickening; (4) extent of mucus plugs; (5) abscesses/sacculation; (6) generality of bronchial division involvement; (7) number of bubbles; (8) emphysema; and (9) collapse/consolidation. Scores from 0 to 3 were assigned to each of the first 7 categories (severity of BE, extent of BE, peribronchial thickening, extent of mucus plugs, abscesses/sacculation, generality of bronchial division involvement, and number of bubbles) and from 0 to 2 for the last 2 categories (emphysema and collapse/consolidation). The total score had a maximum value of 25 and was calculated by adding individual scores for each item. The scores were classified as Grade 0 (none), Grade I (mild), Grade II (moderate), and Grade III (severe).[9,10]

Pulmonary function test

Radiology, bronchoscopy, and pulmonary function test were performed simultaneously as the initial evaluation. Spirometry was performed according to the published criteria (ATS/ERS Task Force Standardization of Lung Function Testing). Forced expiratory volume in 1 s (FEV_1) and functional vital capacity (FVC) were considered the primary outcome parameters to assess differences between groups.[11]

Statistical analysis

Frequency tables (*n*, %) were used for categorical variables and descriptive statistics (mean, median, standard deviation, etc.) were used for numerical variables. Group comparisons of categorical variables were performed with crosstab statistics (Chi-squared test). Assuming that numerical variables were not normally distributed, analyses were performed with nonparametric statistical methods. The Kruskal–Wallis test was used for 3 groups in comparisons of the groups. The relationships between the variables were investigated by Spearman's correlation analysis and the correlation coefficient was expressed as *r*. Analyses were performed using SPSS 21. In all analyses, $P < 0.05$ was taken to indicate statistical significance. IRB approval was obtained from local ethics committe of Bezmialem Vakif University.

Results

Study population

The study population consisted of 54 (52%) female patients and 49 (48%) male patients with a mean age of 11.7 ± 3.4 (min–max: 6–17) years. In terms of etiology, primary ciliary dyskinesia (PCD) was detected in 42 patients (40%), postinfectious BE in 16 (15.5%) patients, bronchiolitis obliterans in 9 (8.7%) patients, asthma in 3 (2.9%) patients, immunodeficiency in 4 (3.9%) patients, and idiopathic BE in 29 (28.2%) patients.

Bronchoscopic findings

In the classification according to the bronchoscopic findings of the bronchi, mucosal inflammation-abnormality (Type 1) was detected in 36 (35%) patients, bronchomalacia (Type 2) in 4 (3.9%) patients, obliteration-like conditions (Type 3) in 9 (8.7%) patients, and normal bronchoscopy findings (Type 5) in 38 (36%) patients. In addition, while both Type 1 and Type 2 bronchoscopy findings were observed in 16 (15.5%) patients, none of our patients had Type 4 (malacia + obliteration) bronchoscopy findings.

When evaluated according to the bronchoscopic secretion score, Grade I was found in 2 (2%) patients, Grade II in 4 (4%) patients, Grade III in 9 (9%) patients, Grade IV in 17 (16%) patients, Grade V in 25 (24%) patients, and Grade VI in 46 (45%) patients.

Radiological findings

According to the Bhalla scoring system, 45 (44%) patients had mild BE, 37 (36%) had moderate BE, and 21 (20%) had severe BE.

Airway microbiology

Microbial growth was detected in BAL fluid from 50 (49%) patients. Isolated aerobic microorganisms were identified as *Streptococcus pneumoniae* ($n = 26$), *Haemophilus influenzae* ($n = 14$), *Moraxella catarrhalis* ($n = 3$), and *Pseudomonas aeruginosa* ($n = 1$), and anaerobic microorganisms were identified as *Bacteroides* spp. ($n = 3$), *Prevotella intermedia* ($n = 3$), *Fusobacterium necrophorum* ($n = 1$), *Clostridium ferments* ($n = 1$), and *Actinomyces meyeri* ($n = 1$). One patient showed 2 microorganisms in the BAL fluid (*H. influenzae* and *M. catarrhalis)*

Statistical analysis

Bronchoscopic secretion score. The relationships between pulmonary function test, Bhalla scoring, microbiological growth in BAL fluid, and bronchoscopic imaging of bronchi were examined based on the bronchoscopic secretion score.

FEV_1 and FVC decreased with increasing bronchoscopic secretion grade, and these trends were statistically significant ($P = 0.048$ and $P = 0.04$, respectively) (Fig. 1). Comparison of the bronchoscopic secretion score and Bhalla score indicated that the degree of radiological severity increased in parallel with the bronchoscopic secretion score, and this relationship was statistically significant ($P = 0.007$) (Table 1). Bronchoscopic secretion grade and bronchoscopic appearance classification were compared. As the bronchoscopic secretion score increased, mucosal inflammation was increased, and most cases of mucosal inflammation were classified as Grade V or VI (Table 1). In the 50 patients with detection of microbial growth in BAL fluid, the results indicated that the rate of BAL fluid microorganism growth increased with increasing bronchoscopic secretion grade (Grade VI: 57.8%; Grade V: 52%; and Grade IV: 35.3%)

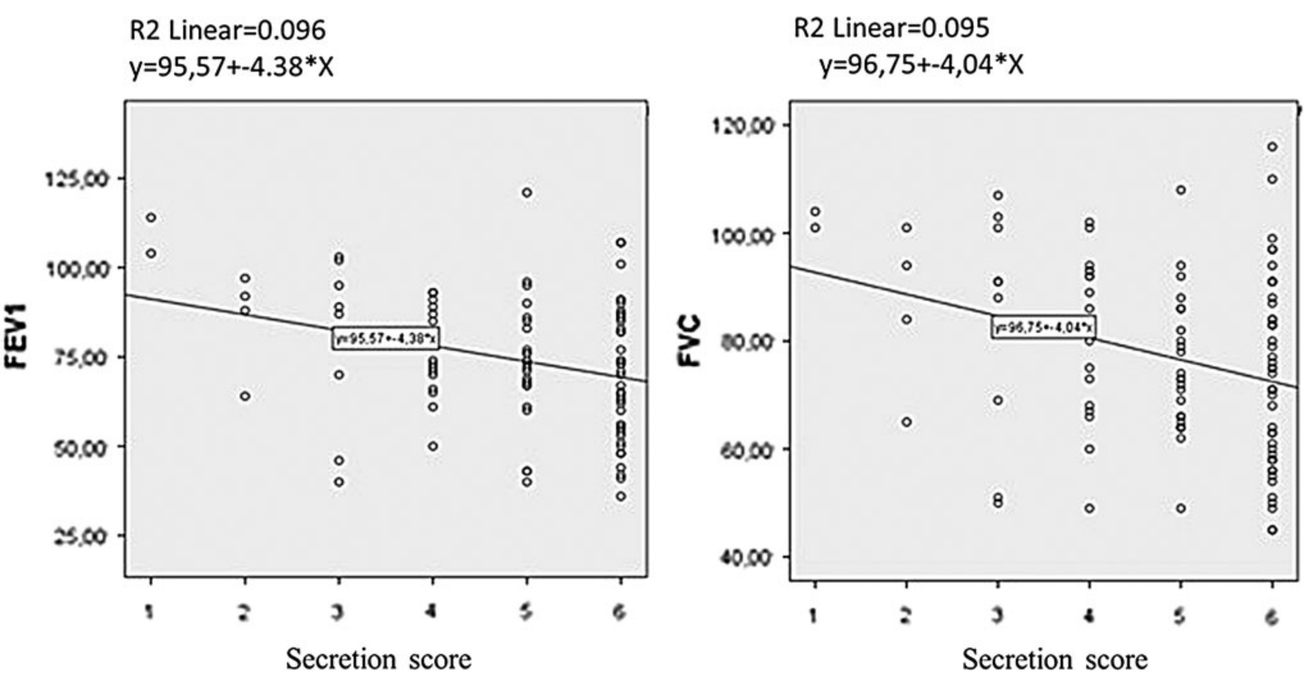

FIG. 1. The relationship between pulmonary function test and bronchoscopic secretion score: FEV_1 and FVC decreased with increasing bronchoscopic secretion grade The English in this document has been checked by at least 2 professional editors, both native speakers of English. FEV_1, forced expiratory volume in 1 s; FVC, functional vital capacity.

Table 1. The Relationships Between Bronchoscopic Secretion Score, Bronchoscopic Imaging Score, Bhalla Score, and Bronchoalveolar Lavage Culture

Bronchoscopic secretion score	Bronchoscopic imaging score						Bhalla score			Bronchoalveolar lavage culture		
	Type 1: Mucosal inflammation	Type 2: Bronchomalacia	Type 3: Obliterative-like lesion	Type 5: Normal structure	Type 1+2	Total	Mild	Moderate	Severe	Positive	Negative	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Grade I	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)
Grade II	0 (0.0)	0 (0.0)	1 (25.0)	2 (50.0)	1 (25.0)	4 (100.0)	3 (75.0)	0 (0.0)	1 (25.0)	1 (25.0)	3 (75.0)	4 (100.0)
Grade III	0 (0.0)	1 (11.1)	1 (11.1)	7 (77.8)	0 (0.0)	9 (100.0)	5 (55.6)	3 (33.3)	1 (11.1)	4 (44.4)	5 (55.6)	9 (100.0)
Grade IV	6 (35.3)	0 (0.0)	1 (5.9)	6 (35.3)	4 (23.5)	17 (100.0)	11 (64.7)	4 (23.5)	2 (11.8)	6 (35.3)	11 (64.7)	17 (100.0)
Grade V	10 (40.0)	0 (0.0)	4 (16.0)	6 (24.0)	5 (20.0)	25 (100.0)	8 (32.0)	13 (52.0)	4 (16.0)	13 (52.0)	12 (48.0)	25 (100.0)
Grade VI	20 (43.5)	3 (6.5)	2 (4.3)	15 (32.6)	6 (13.0)	46 (100.0)	16 (34.8)	16 (34.8)	14 (30.4)	26 (57.8)	19 (42.2)	45 (100.0)
Total	36 (35.0)	4 (3.9)	9 (8.7)	38 (36.9)	16 (15.5)	103 (100.0)	45 (43.7)	36 (35.0)	22 (21.40)	50 (49.0)	52 (51.0)	102 (100.0)
Mantel-Haenzsel test			$P=0.012$					$P=0.007$			$P=0.031$	

and this relationship was statistically significant ($P=0.03$) (Table 1). There were no significant correlations between the anaerobic or aerobic nature of the growing microorganisms and bronchoscopic secretion grade ($P=0.15$ and $P=0.43$, respectively),

Bronchoscopic appearance score. There was no significant relationship between the bronchoscopic appearance score and Bhalla score ($P=0.52$) (Table 2). Similarly, there were no statistical correlation between the bronchoscopic appearance score, pulmonary function test results, and microbiological growth in BAL fluid ($P>0.05$) (Table 2).

Bhalla score. The relationships between Bhalla score, pulmonary function test results, and microorganism growth in BAL fluid were investigated. The mean FEV_1 and FVC values were 84% and 85.3% in patients with a mild Bhalla score, 68.5% and 72.3% in those with a moderate Bhalla score, and 62.9% and 67.1% in those with a severe Bhalla score, respectively. The mean values of FEV_1 and FVC were lower in patients with a severe Bhalla score, and these relationships were statistically significant ($P<0.001$ and $P<0.001$, respectively). There was no statistically significant relationship between the severity of Bhalla score and the growth rate of microorganisms in BAL fluid ($P=0.403$) (Table 2).

Discussion

This study was performed to examine relationships between bronchoscopic findings and radiological, microbiological, and pulmonary function tests in patients with non-CF BE. As the bronchoscopic secretion score increased, pulmonary function decreased and the radiological severity and microbiological growth rate increased. Mucosal inflammation increased in parallel with the BE secretion score. The radiological findings, pulmonary function test results, and microbiological growth rates did not change according to the subtype of bronchoscopic appearance. As expected, patients with severe radiological scores were found to have low respiratory function. However, no relationship was detected between microbiological growth rate and radiological findings.

Flexible bronchoscopy is a commonly used method for the diagnosis and treatment of chest diseases. However, there have been few reports regarding bronchoscopic findings in non-CF-related chronic lung diseases that cause BE. In a previous study, Chang et al.[4] classified the bronchoscopic findings of 28 patients with non-CF BE into 5 different groups. They reported that the most common anomalies in these patients were mucosal inflammation and edema (Type 1: 58%) followed by bronchomalacia (Type 2: 18.8%). Other findings were obliteration-like lesions (Type 3: 16.7%), combination of malacia and obliterative lesions (Type 4: 4.2%), and normal structure (Type 5: 2.1%). In this study, the most common structural anomalies in the airway were mucosal edema and inflammation. There have been no previous report regarding the associations of bronchoscopic appearance with radiological findings, pulmonary function test results, and microbiological growth in patients with non-CF BE. The results of pulmonary function tests, microbiological growth rates, and radiological severity did not change in accordance with the subtypes of bronchoscopic appearance.

Chang et al. evaluated bronchoscopic secretion scores in 6 groups in a previous study performed in a cohort of 106 patients with chronic wet cough. An increasing bronchoscopic

Table 2. The Relationships Between Bhalla Score, Bronchoscopic Imaging Score, and Bronchoalveolar Lavage Culture

Bhalla score	Bronchoscopic imaging score: Type 1: Mucosal inflammation	Type 2: Bronchomalacia	Type 3: Obliterative-like lesion	Type 5: Normal structure	Type 1+2	Total	Bronchoalveolar lavage culture: Positive	Negative	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)			
Mild	14 (31.1)	2 (4.4)	3 (6.7)	19 (42.2)	7 (15.6)	45 (100.0)	20 (44.4)	25 (55.6)	45 (100.0)
Moderate	13 (36.1)	2 (5.6)	5 (13.9)	9 (25.0)	7 (19.4)	36 (100.0)	17 (47.2)	19 (52.8)	36 (100.0)
Severe	9 (40.9)	0 (0.0)	1 (4.5)	10 (45.5)	2 (9.1)	22 (100.0)	13 (61.9)	8 (38.1)	21 (100.0)
Total	36 (35.0)	4 (3.9)	9 (8.7)	38 (36.9)	16 (15.5)	102 (100.0)	50 (49.0)	52 (51.0)	102 (100.0)
	Mantel-Haenzsel test ($P=0.520$)						Chi-squared test ($P=0.403$)		

secretion score was shown to be associated with wet cough, as described by family members and the attending physician. As the bronchoscopic secretion grade increased, BAL fluid microorganism cell count also increased, and it was shown to be associated with bronchial inflammation. Moreover, patients with bronchoscopic secretion Grade IV and above were reported to have greater microbiological growth in BAL fluid.[7,12] There have been no previous reports regarding the relationship between bronchoscopic secretion score and microbiological growth in non-CF BE. In this study, the incidence of microorganisms in BAL fluid detected in culture increased in patients with bronchoscopic secretion Grade IV and above, similar to the results in the literature. There was no significant relationship between anaerobic-aerobic microorganism count and bronchoscopic secretion grade.

There have been no previous studies regarding the relationships between bronchoscopic secretion score, pulmonary function test results, Bhalla score, and bronchoscopic appearance classification in patients with non-CF BE. In our patients, the mean FEV_1 and FVC values gradually decreased and the Bhalla score and bronchial mucosal inflammation in the bronchi increased as the bronchoscopic secretion score increased.

Bhalla scoring is the one of the most common system used for evaluation of chest radiology results in patients with BE. Although there have been many reports regarding the relationship between Bhalla score and spirometry in CF patients, few data are available in patients with non-CF BE in children.[13,14] Edwards et al.,[15] reported that as the HRCT score increased in non-CF BE patients, pulmonary function tests (PFT) also decreased. In the same study, as in our study, there was no correlation between radiological severity and sputum growth.[15] In the other 2 studies, it was reported that the deterioration in PFT was correlated with radiological weight scoring.[16,17]

Relationships between CT and PFT in BE patients have also been studied in adults.[18] An adult study reported that severity of bronchial wall thickening and extent of decreased attenuation on the expiratory CT scan correlated strongly with the severity of airflow obstruction.[10] In the same study, it was reported that the closest relationship was seen between decreased FEV_1.

In another adult study, it was reported that the severity of airflow obstruction was primarily related to the extent of mosaic attenuation, but serial changes in pulmonary function indices were only related to serial changes in mucus plugging scores. In addition, it was also reported that changes in mucus plugging on serial CT were related to severity of BE and bronchial wall thickness on serial CT.[19]

Recent studies showed that plethysmography and lung clearance index (LCI) were more sensitive than spirometry in detecting HRCT abnormalities in PCD patients.[20] Further studies are needed to prove the superiority of plethysmography and LCI in showing HRCT abnormalities in non-CF BE children.

In this study, Bhalla score and pulmonary function test results were compared in patients with non-CF BE, and the mean FEV_1 and FVC values of the patients were shown to deteriorate in parallel with radiological findings. There was no relationship between the severity of radiological findings and the incidence of microorganism growth in BAL fluid. With these results, our study has made a new contribution to the literature in the patient group with non-CF BE.

In addition, the place of radiological abnormalities in chronic respiratory problems and their relationship with inflammation were compared. Studies in asthmatic patients showed that BE is associated with bronchial wall thickness, severity of asthma, and inflammatory response in asthmatics.[21]

The most common microorganisms found in non-CF BE are *S. pneumoniae*, *H. influenza*, and *M. catarrhalis*. However, no data are available regarding anaerobic microorganisms in such cases. Kapur et al.[22] reported growth in BAL in 68% of 113 newly diagnosed BE patients, and the most common microorganisms isolated were *H. influenza* (32%), *S. pneumoniae* (14%), and *M. catarrhalis* (8%). In another study in pediatric patients, microorganism growth in BAL fluid was detected in 41% of the patients, and the most common microorganisms isolated were also *H. influenzae* ($n=26$), *S. pneumoniae* ($n=26$), and *M. catarrhalis* ($n=7$).[7] In this study, 50% of the patients showed microorganism growth in BAL fluid, and *S. pneumoniae*, *H. influenza*, and *M. catarrhalis* were the most common organisms isolated. Also, an anaerobic growth was determined at a rate of 8.7%. In patients who do not improve with routine treatment, anaerobic factors should be kept in mind and treatment should be added.

In children, bronchoscopy is indicated when BE affects a single lobe to exclude a foreign body. In some acutely ill patients, it may achieve a useful microbiological result. In adults with localized disease, bronchoscopy may be indicated to exclude proximal obstruction. For patients in whom serial testing of sputum does not yield microbiological information and who are not responding well to treatment, bronchoscopic sampling of lower respiratory tract secretions may be indicated. Bronchoscopy is indicated if high-resolution CT (HRCT) suggests atypical mycobacterial infection and sputum culture is negative.[23] In a hospital setting, there is excellent agreement between the description of a wet cough by parents and doctors and the finding of increased endobronchial secretions at bronchoscopy.[24] In addition, bronchoscopy has been shown to be valuable in distinguishing mucosal inflammation from bronchomalacia in children.[4,25] In adults with stable symptoms, BAL and protected specimen brush are both sensitive in detecting lower respiratory tract organisms. Similarly, in our study, it was observed that secretion scoring in bronchoscopy was related to radiological weight level, PFT, and microbiology. We think that bronchoscopy can be included in initial evaluation in selected cases.

Study limitation

This is a cross-sectional retrospective study. The results of BE patients who underwent bronchoscopy were examined and this study did not cover all BE patients. This should be noted as a limitation. In addition, only spirometer was used to show lung functions in this study. Studies showing changes in bronchoscopic, radiological and PFT involving Plethysmography and LCI measurements are needed. Prospective studies are also needed to determine the importance of bronchoscopy in patients with BE.

Conclusions

This study showed that bronchoscopic evaluation and especially scoring of secretions correlate with severe clinical condition, decrease in pulmonary function test, worsening in radiology scores, and increase in microbiological bacterial load in patients. Flexible endoscopic bronchoscopy should be kept in mind in the initial evaluation of non-CF BE patients.

Statement of Ethics

Written informed consent was approved from the parents. Ethical approval was obtained from the Bezmialem Vakif University local committee.

Author Contributions

Study concept and design: E.C., A.A.K., F.B.C., L.M.A.S., F.K.A., F.U.K., M.A.N., and H.D. Analysis and interpretation of data: E.C., S.S.B., and B.S. Drafting of the article: F.B.C. and E.C. Critical revision of the article for important intellectual content: E.C., A.A.K., and F.B.C. Statistical analysis: F.B.C. and E.C.

Author Disclosure Statement

No competing financial interests exist.

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