Università di Foggia

PhD Course in Translational medicine and food: innovation, safety and management

(Cycle 33rd)

"Molecular study of the lung microbiome in patients with non-cystic fibrosis bronchiectasis: the contribution of *Pseudomonas aeruginosa* infection to clinical outcomes"

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Academic year: 2019/2020

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Abbreviations

ABPA Allergic bronchopulmonary aspergillosis

AC Airway clearance

AST Antibiotic susceptibility testing

BSI Bronchiectasis Severity Index

BTS British Thoracic Society

CF Cystic fibrosis

COPD Chronic obstructive pulmonary disease

DLCO Diffusing capacity of the lungs for carbon dioxide

EMBARC European Multicentre Bronchiectasis Audit and Research Collaboration

EPS Extracellular polymeric substance

ERN-LUNG European Reference Network for Rare Pulmonary Diseases

ERS European Respiratory Society

FEV1 Forced expiratory volume in 1 s

HRCT Chest high-resolution computed tomography

HNPs Human neutrophil peptides

ICAM-1 Intercellular Adhesion Molecule 1

IG Immunoglobulin

IL-8 Interleukin-8

LTB4 Leukotriene-B4

MAC Mycobacterium avium complex

mPA Mucoid *Pseudomonas aeruginosa*

NE Neutrophil elastase

Non-mPA Non-mucoid *Pseudomonas aeruginosa*

NOR No organism reported

NTM Non-tuberculous mycobacteria

PCD Primary ciliary dyskinesia

PPM Potential pathogenic microorganisms

QoL Quality of Life

ROS Reactive oxygen species

TNF-α Tumor necrosis factor

Abstract

Introduction: Bronchiectasis is a chronic disease characterized by a pathologic dilation of the bronchi and bronchioles, due to a repetitive cycle of inflammation followed by infections causing structural damage and recurrent exacerbations. *Pseudomonas aeruginosa* is the most common bacteria detected in bronchiectasis in Southern Europe and could acquire a mucoid phenotype due to mutations in mucA (mucoid Pseudomonas aeruginosa - mPA) that is a hallmark of poor prognosis. Despite the higher prevalence of *Pseudomonas aeruginosa* in bronchiectasis, how mPA phenotype could affect viscoelastic properties of sputum is unknown. Bronchiectasis exacerbations are often treated with prolonged antibiotic use, even though there is limited evidence for this approach. More severe and frequent exacerbations are associated with worse quality of life and respiratory function, more hospital admissions, higher mortality, and increased economic burden.

Aims: Our aims were: 1) to determine the relationship between *Pseudomonas aeruginosa* phenotypes isolation, the viscoelastic properties of sputum and the clinical outcomes in patients with bronchiectasis; 2) to investigate the baseline clinical and microbiological findings associated with long courses of antibiotic treatment in exacerbated bronchiectasis patients; 3) to evaluate patient characteristics during an exacerbation requiring hospital admission associated with mortality during a one-year period.

Methods: A cross-sectional first study was conducted of sputum samples obtained by spontaneous expectoration and sent for microbiology and rheology analysis. Elasticity and viscosity were measured at two oscillatory frequencies (1 and 100 rad/s). Furthermore, we conducted a second bi-centric prospective observational study of bronchiectasis exacerbated adults. We compared groups receiving short (<14 days) and long (15–21 days) courses of antibiotic treatment. Previous medical history, radiological features, symptoms, and laboratory and microbiological were recorded.

Finally, all patients were re-examined one year after hospital discharge to assess mortality.

Results: Firstly, we analyzed 17 patients with mPA, 14 with non-mPA and 17 with no organism reported (NOR). Compared with the NOR group, the mPA group showed higher elasticity (median 10.30 vs. 5.70, p=0.023), viscosity (2.40 vs. 1.50, p=0.039), and stiffness (10.70 vs. 6.00, p=0.024). Values in the mPA group tended to be higher compared with non-mPA. Clinically, the mPA group showed greater hospitalizations during the previous year and greater affected lobes than the non-mPA and NOR groups. Secondly, we enrolled 191 patients (mean age 72 (63, 79) years; 108 (56.5%) females), of whom 132 (69%) and 59 (31%) received short and long courses of antibiotics, respectively. Multivariable logistic regression of the baseline variables showed that long-term oxygen therapy (LTOT), moderate–severe exacerbations, and microbiological isolation of *Pseudomonas aeruginosa* were associated with long courses of antibiotic therapy. When we excluded patients with a diagnosis of community-acquired pneumonia (n = 49), in the model we found that an etiology of *Pseudomonas aeruginosa* remained as factor associated with longer antibiotic treatment, with a moderate and a severe FACED score and the presence of arrhythmia as comorbidity at baseline. Thirdly, we followed up 185 exacerbated bronchiectasis patients admitted to hospital (94 females, 71.8 (11.8) years, 66.5% BSI stage severe) for one-year. Twenty-three (12.4%) patients died during the one-year follow up. The major causes of death were respiratory related (68%), cardiovascular (18%), and septic shock (14%). LTOT, mechanical ventilation and white blood cell count at day 1 of hospitalization >13.64x 10^9/L are variables associated with an increased risk of one-year mortality in patients hospitalized with moderate or severe bronchiectasis exacerbation. On the other hand, influenza vaccination appears as a protective factor.

Conclusions: The mPA phenotype is associated with increased elasticity, viscosity and stiffness of bronchiectatic sputum. Viscoelastic properties could be used as a marker of poor mucociliary clearance in mPA, with potentially important clinical implications.

Decisions about the duration of antibiotic therapy should be guided by clinical and microbiological assessments of patients with infective exacerbations. A future study addressing the risk of one-year mortality after a hospitalization for moderate to severe bronchiectasis exacerbation is desirable.

1. INTRODUCTION

Bronchiectasis is a structural respiratory disease characterized by permanently dilated bronchi. The most accepted pathogenetic hypothesis consists of chronic bronchial infection, neutrophil-mediated inflammation, structural lung disease, and impaired mucociliary clearance. In the first part of the study, we focused on the damage to the mucociliary system in bronchiectasis patients. Whereas normal bronchial mucus (10–100mL) is continuously produced and easily propelled by expiratory airflow and by the bronchial cilia, mucociliary clearance could be hindered due to excessive production and changes in the viscoelastic properties of mucus in patients with bronchiectasis[1,2]. *Pseudomonas aeruginosa* is among the most common bacteria detected in bronchiectasis in Southern Europe. The wild-type non-mucoid could acquire a mucoid phenotype due to mutations in mucA for the adaptation to the lung environment, that is a hallmark of poor prognosis in patients with bronchiectasis[3]. Despite the higher prevalence of *Pseudomonas aeruginosa* in bronchiectasis, how mucoid *Pseudomonas aeruginosa* (mPA) and non-mucoid *Pseudomonas aeruginosa* (non-mPA) phenotypes could affect viscoelastic properties of sputum is unknown. The effect of changes to the extracellular polymeric substance (EPS) in the mPA biofilm on mucus viscoelasticity is a growth area of biofilm research. Characterization of the viscoelastic properties of mucus focuses on the elasticity (or storage modulus, G') and viscosity (or loss modulus, G"), and together describe the rheology of complex biological fluids[4]. The elasticity measures the tendency for a material to recover its original shape following stress-induced deformation, whereas the viscosity, measures the extent to which the material resists the tendency to flow. A high viscosity allows mucus to remain intact, while low elasticity promotes airflow-mucus interactions by preventing mucus recoil during a burst of high-velocity air[5]. Pulmonary diseases, such as asthma, chronic obstructive pulmonary disease and CF, generally result in mucus hypersecretion and increased viscoelasticity, owing in part to reduced water content and an increased fraction of glycoproteins that impair mucociliary clearance. Therefore, we evaluated the relationship between different *Pseudomonas aeruginosa* phenotypes isolation, the

viscoelastic properties of sputum, and severity outcomes in patients with non-CF bronchiectasis.

This pathology relapses and remits during the course of the disease, leading to increased bacterial load and recurrent exacerbations[6]. As the frequency of exacerbations increases, there is an associated reduction in the forced expiratory volume in 1 s (FEV1), an increased severity of lung disease on computed tomography (CT), and an increase in chronic infection with *Pseudomonas aeruginosa* and *Haemophilus influenzae*[7]. In addition, more severe and frequent exacerbations are associated with a worse quality of life, more hospital admissions, higher mortality, and increased economic burdens[8].

In a recent consensus, experienced clinicians proposed a working definition of an exacerbation of bronchiectasis for use in clinical research[9]. Most therapeutic interventions focus on reducing exacerbations to improve short- and long-term outcomes, so it is recommended practice to initiate oral or intravenous antibiotic treatment based on knowledge of the likely causative organism. Existing guidelines predominantly recommend empirical antibiotic choices based on whether *Pseudomonas aeruginosa* is being targeted[10]. To date, the optimal duration of antibiotic treatment for exacerbations of bronchiectasis has not been systematically studied. The practice of 14 days' therapy for infective exacerbations has been extrapolated from the treatment approach used for cystic fibrosis (CF), without any definitive evidence for the approach in non-CF bronchiectasis populations.

In the guidelines on non-CF bronchiectasis by the European Respiratory Society[11], the authors systematically reviewed the literature comparing short (<14 days) and long (14–21 days) treatment durations. They reported only one small study in which the outcomes were similar at 7 and 14 days[12].

We hypothesized that clinical and microbiological characteristics, as chronic bronchial infection with *Pseudomonas aeruginosa*, exist that can be associated with the optimal duration of antibiotic treatment during an exacerbation. In the second part of this study,

we have identified the main clinical and microbiological factors associated with long-course antibiotic treatment for exacerbations of bronchiectasis.

Finally, we focused on the disease burden in terms of risk of mortality due to exacerbation of bronchiectasis. Previous studies reported that the in-hospital mortality rate is 9%, the one-year mortality rate is 30%, and the median survival is 46.6 months[13]. In the last part of the study, we evaluated clinical and microbiological factors during a hospitalization due to bronchiectasis exacerbation associated with risk of mortality during a one-year period.

2. NON-CYSTIC FIBROSIS BRONCHIECTASIS

2.1 Background

Non-cystic fibrosis (CF) bronchiectasis is the third most common chronic inflammatory respiratory disease after asthma and chronic obstructive pulmonary disease (COPD) and is closely related to both. This condition is characterized radiologically by abnormal and permanent dilatation of the bronchial lumen that can be caused by different etiologies. Clinically, it usually presents with chronic cough and sputum production, as well as recurrent infectious exacerbations. It can cause chronic bronchial infection and a progressive decline in lung function, all of which can lead to a deterioration in quality of life and increased morbidity and mortality.

A diagnosis of bronchiectasis should be suspected when a patient presents with a recurrent or persistent (>8 weeks) cough, with production of purulent or mucopurulent sputum, particularly with the relevant associated risk factors, such as COPD, difficult-to-treat asthma, rheumatoid arthritis, inflammatory bowel disease (IBD), chronic rhinosinusitis or the presence of persistent sputum pathogens, especially *Pseudomonas aeruginosa*. It is a neglected disease, but recent initiatives including the European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC) and the European Union supported European Reference Network for Rare Pulmonary Diseases (ERN-LUNG) are beginning to raise the diseases profile and stimulate new research[14].

2.2 Epidemiology

Although the actual prevalence of bronchiectasis is unknown, it is estimated to be between 42 and 566 cases per 100000 population (higher in women and the elderly), although it is recognized as being significantly underdiagnosed[15].The prevalence and incidence of bronchiectasis in primary care in Italy in 2015 were 163 per 100000 population and 16.3 per 100000 person-years, respectively. Prevalence and incidence increased with age and overall rates e highest in men over 75 years old[16]. The incidence and prevalence of bronchiectasis is rising, possibly due to the growing longevity of the

population, the chronic nature of underlying diseases, the recently observed association between bronchiectasis and other highly prevalent entities (such as asthma or COPD) and, above all, the wide spread use of imaging techniques to confirm diagnosis (chest high-resolution computed tomography [HRCT]). The cost of bronchiectasis is high (the average cost of annual treatment in Spain is estimated to be close to €4700), and is greater the more severe the disease (around €10000 annually in severe cases)[17], if there is coexisting COPD, a higher number of exacerbations, and when there is chronic bronchial *Pseudomonas aeruginosa* infection. Most of the cost is due to exacerbations and inhaled antibiotic treatment in severe bronchiectasis.

2.3 Pathogenesis

Bronchiectasis mechanistically results from chronic inflammatory microenvironments that trigger airway tissue breakdown. The complex interplay between infection and inflammation feeds a pro-inflammatory vicious circle consisting of lesion of the mucociliary system, inflammation, infection and airway repair, which progressively drives the generation of bronchiectasis and the destruction of the pulmonary architecture. Damage to the mucociliary system makes it difficult to eliminate secretions and facilitates bacterial growth and bronchial inflammation, with the latter two being responsible for the bronchial structural damage and perpetuation of the vicious pathogenic circle (*figure 1*). An imbalance between pro- and anti-inflammatory products, and persistent infection and inflammation despite the immune response and treatment, could play an important role in disease progression. Inflammation of the airways has mostly a neutrophilic profile. A high percentage of patients with bronchiectasis also present systemic inflammation in the stable phase of the disease, which has been related with more severe forms[18].

Inflammatory immune cells (mainly activated macrophages and neutrophils) represent the major infiltrating population in disease conditions associated with bronchiectasis and contribute significantly to tissue damage and bronchiectasis generation through the release of their harmful cellular ingredients. Particularly, cell-derived proteases and

reactive oxygen species represent key mediators in the degradation and destruction of extracellular pulmonary tissue components, leading to bronchiectasis formation. The precise early immune-mediated mechanisms that trigger and maintain the formation of bronchiectasis remain yet incompletely understood. Regulated immune homeostasis seems to be essential since both immune deficiencies as well as hyperactive immune responses are associated with bronchiectasis. Particularly, the protease/anti-protease imbalance as found in CF and COPD airways is considered as key pathogenic component in degrading extracellular matrix[19].

2.3.1 Microbiome

Pseudomonas aeruginosa is a common and dominant pathogen found in the airways of both CF and non-CF bronchiectasis patients, but *Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catharralis* and *Staphylococcus aureus,* also make up the core microbiota observed in bronchiectasis. Recent improvements in microbiological methods have led to an increase in the isolation of *enterobacteriae*, Gram-negative non-fermenting bacteria such as *Achromobacter (Alcaligenes) xylosoxidans* and *Stenotrophomonas maltophilia*, *Nocardia spp*., Non-tuberculous mycobacteria (NTM) belonging to the *Mycobacterium avium complex* (MAC) group are also highly prevalent in bronchiectasis with a female preponderance, even if it appear poorly associated with disease severity and exacerbations when compared to *Pseudomonas aeruginosa*[20].Yet, few studies have investigated *methicillin-resistant S. aureus (MRSA)* in bronchiectasis, although its incidence may be rising.

Interestingly, bacterial populations do not drastically change between stable and exacerbation states in bronchiectasis. However, viral load (*coronavirus*, *rhinovirus* and *influenza A/B* virus families) has been positively correlated with exacerbations.

Although fungi (*Aspergillus fumigatus* and *Candida albicans*) are frequently isolated from the same airways, the role of the pulmonary mycobiome in the pathogenesis of these disease states remains largely elusive.

2.3.2 Inflammation

Neutrophil-dominant inflammation is a key feature of bronchiectasis. Both interleukin-8 (IL-8) and leukotriene-B4 (LTB4) are key chemo-attractants required for migration and infiltration of neutrophils into airways; high systemic IL-8 levels are detectable in individuals withbronchiectasis[21]. Antibacterial neutrophil responses (such as reactive oxygen species [ROS]formation) are activated through the IL-8CXCR1 axis, but proteolytic cleavage mediated by neutrophil elastase (NE), which itself is associated with exacerbations and lung function decline in bronchiectasis, impairs antibacterial neutrophil functions[22]. Uncontrolled NE activity causes further respiratory tissue damage through degradation of extracellular proteins (such as surfactant proteins) and cellular surface receptors (such as complement receptors); high NE levels correlating with disease severity and poorer lung function are described in both CF and non-CF bronchiectasis settings[23]. In this context, CXCR receptor antagonists are hypothesized to inhibit neutrophil airway influx and have been shown to be effective in modulating the inflammatory state in bronchiectasis. Serine proteases are also important neutrophil derived products, released in response to TNF-α signaling. They degrade proteoglycans in the respiratory epithelium subsequently inducing airway damage. In bronchiectasis, activated airway neutrophils secrete an abundance of human neutrophil peptides (HNPs), which have been described to inhibit their phagocytic ability. Poorer clearance of neutrophils by alveolar macrophages further augments the inflammatory state in bronchiectasis.

T-cells constitute another key component of the inflammatory response in bronchiectasis: activation of T-helper 17 antigen-specific pathways have been described in non-CF bronchiectasis[24]. Bacterial load in non-CF bronchiectasis has been correlated with increases in airway (NE, IL-8, IL-1β and TNF-α) and systemic (ICAM-1, E-selectin) derived inflammatory markers, phenomena confirmed in vitro using bronchial epithelial cell lines treated with sputum from bronchiectasis patients[25].

Vitamin-D deficiency observed in CF is associated with increased bacterial infection, exacerbations and poorer lung function. This is corroborated in non-CF bronchiectasis

where it indicates disease severity and associates with more infection, bacterial colonization, airway inflammation and consequently frequent exacerbations[26].

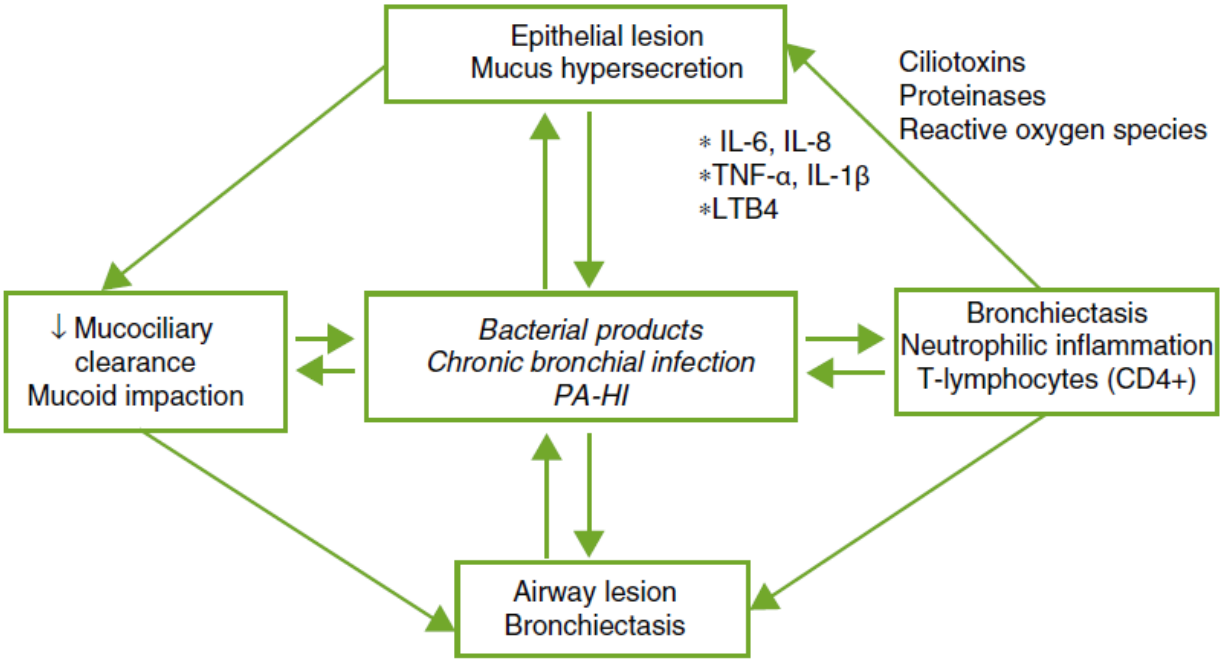


Figure 1. Pathogenesis of bronchiectasis. *Source: M.Á. Martínez-García et al.*[17]

Abbr. HI: Haemophilus influenza; IL: interleukin; LTB4: leukotriene B4; PA: Pseudomonas aeruginosa; TNF: tumor necrosis factor.

2.4 Etiology

Bronchiectasis may be caused by several factors, including previous severe respiratory infections (e.g., bacterial pneumonia or tuberculosis), allergic bronchopulmonary aspergillosis (ABPA), impairment of ciliary clearance (e.g., primary ciliary dyskinesia), and primary or secondary immunodeficiency, and it may be associated with other diseases, such as chronic obstructive pulmonary disease (COPD) and severe asthma. The identification of the underlying causes of bronchiectasis is a complex process related to several variables, such as the geographical origin of data and the diagnostic workup, and remains an important challenge[27]. In patients with a new diagnosis, the European Respiratory Society (ERS) guidelines concerning the management of adult bronchiectasis patients[11]suggest the performance of a standard number of etiological tests including total blood count, serum immunoglobulins (total

IgG, IgA, IgM) and tests for ABPA. However, the recommendation is conditional and has a very low level of evidence. Furthermore, the recent British Thoracic Society (BTS) guidelines for adult patients with bronchiectasis[28] suggest that a panel of investigations should be performed to establish the underlying cause of bronchiectasis (grade of recommendation: B). The BTS guidelines recommend: a) the recording of comorbidities and past medical history to identify possibly causative disease such as rheumatoid arthritis or COPD; b) measurement of full blood count, serum total IgE and assessment of sensitization to Aspergillus fumigatus; c) levels of total IgG, IgA, IgM; d) consideration of baseline antibodies against Streptococcus pneumoniae and response to vaccine; e) evaluation of tests for CF and for primary ciliary dyskinesia (PCD) inpatients with supporting clinical features; f) performance of sputum cultures in all patients for bacterial and mycobacterial culture.

The relative frequency of these etiologies depends on the geographical area in which it is studied, the characteristics of the patient and the clinic attended (general or specialized clinics). The post-infection diagnosis can be assumed when patient reported a history of symptoms due to bronchiectasis with an onset after a severe respiratory infection, such as pneumonia or tuberculosis, according to clinical judgment and regardless of the latency between the event and the occurrence of symptoms; it is the most common in most studies (30%). Bronchiectasis of unknown origin (or idiopathic) are considered to be those in which the cause is unknown despite a comprehensive etiological study, and could account for between 25% and 45% of cases, according to the references[29,30]. It is believed that a significant percentage of these idiopathic bronchiectasis could be due to selective immune deficiencies, gastroesophageal reflux, infections not reported by the patient or other airway diseases such as COPD or asthma not yet diagnosed. A significant percentage (6.3% - 13.7%) of patients with bronchiectasis have associated chronic respiratory diseases (COPD, asthma or alfa-1 antitrypsin deficiency), 5% - 9.4% immune deficiencies, 2.5%–2.9% ciliary dyskinesia, 0.9%–2.6% APBA, and 1.4%–3.8% systemic diseases (rheumatoid arthritis, lupus, Sjogren syndrome, sarcoidosis)[31,32]. Ielpo et al.[33] identified a panel of

variables, including clinical, radiological and laboratory values, with multivariate logistic regression models able to predict the probability to have an etiological diagnosis of bronchiectasis.

The use of a standardized etiological algorithm would improve the ability to diagnose the etiology of bronchiectasis without performing unnecessary tests. An example of etiological algorithm (*figure 2*) was generated in the 2018 Spanish guidelines[17].

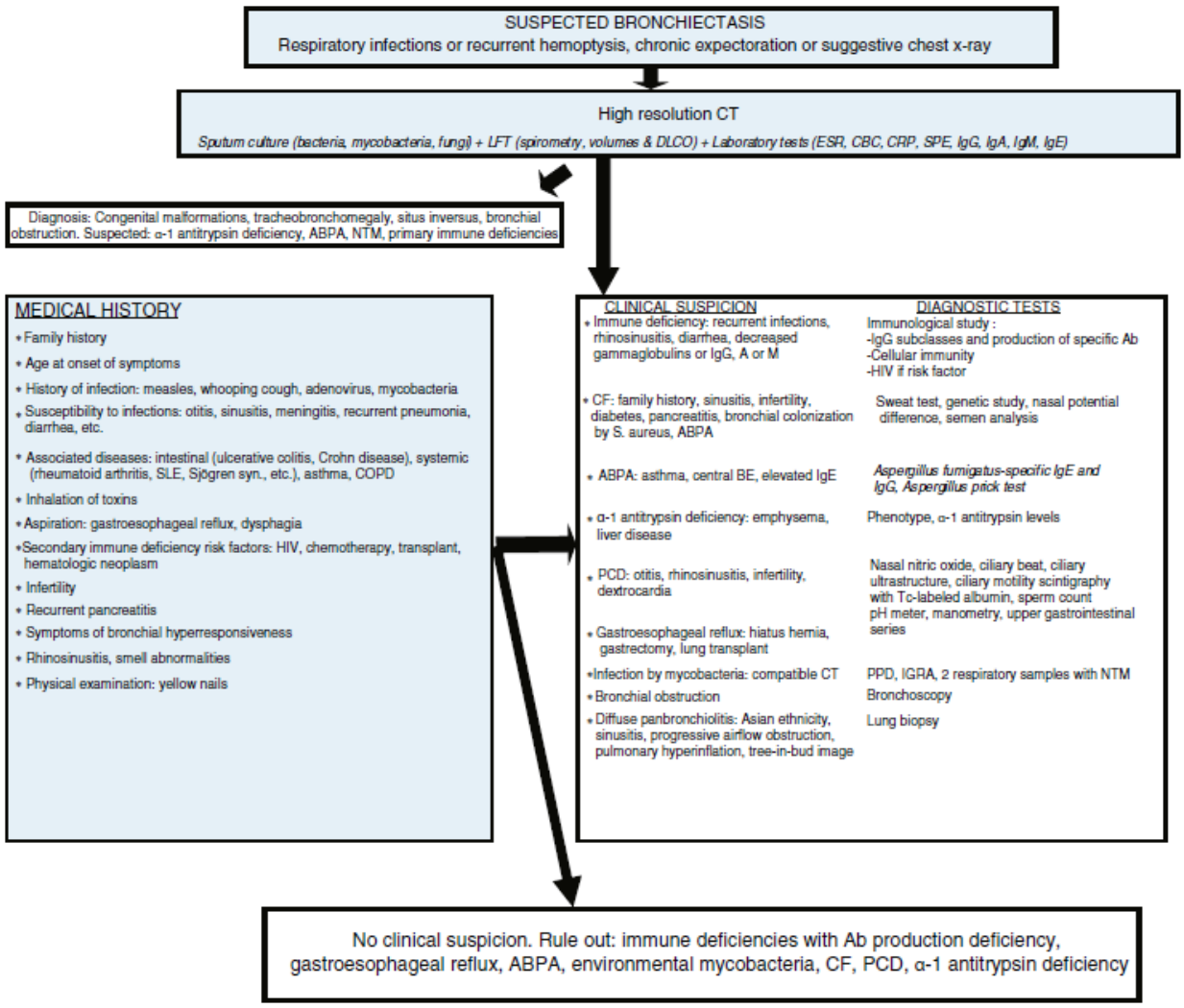


Figure 2. Diagnostic algorithm. *Source: M.Á. Martínez-García et al.*[17]

Abbr. Ab: antibodies; ABPA: allergic bronchopulmonary aspergillosis; BE: bronchiectasis; CBC: complete blood count; CF: cystic fibrosis; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; CT: computed tomography; PCD: primary ciliary dyskinesia; DLCO: diffusing capacity of the lungs for carbon monoxide; ESR: erythrocyte sedimentation rate; FEV1: forced

expiratory volume in the first second; HIV: human immunodeficiency virus; Ig: immunoglobulin; IGRA: interferon gamma release assay; LFT: lung function tests; NTM: non-tuberculous mycobacteria; PPD: purified protein derivative skin test; S: Staphylococcus; SLE: systemic lupus erythematosus; SPE: serum protein electrophoresis; Syn: syndrome; Tc: technetium; UGI: upper gastrointestinal.

2.5 Diagnosis

2.5.1 Clinical aspects

Bronchiectasis patients are clinically characterized by sputum production (upon exercise or spontaneously) leading to productive coughing with mucopurulent masses of yellowish, greenish or brown sputum in the morning or over the day, and repeated respiratory infections, but can remain asymptomatic between these episodes. It is useful to quantify the daily volume (semi-quantitatively, marked by the patient in a graduated container) and color of the sputum (Murray scale – *figure S1*)[34]. Other symptoms that often present are dyspnea, hemoptysis, intermittent chest pain and fatigue. Sinusitis is very common, especially in primary ciliary dyskinesia and in primary immune deficiencies.

The most common functional abnormality in bronchiectasis is chronic persistent airflow obstruction (with an average decline of ~50 ml/year in FEV1 and normal or slightly reduced forced vital capacity), more marked in smokers or COPD patients. Mixed patterns can appear in the post-tuberculous, fibrotic or destructive forms, although a pure restrictive pattern is rare. A slight decrease may be observed in the diffusing capacity of the lungs for carbon dioxide (DLCO). Bronchial hyper-responsiveness has been observed in 30%–69% of cases.

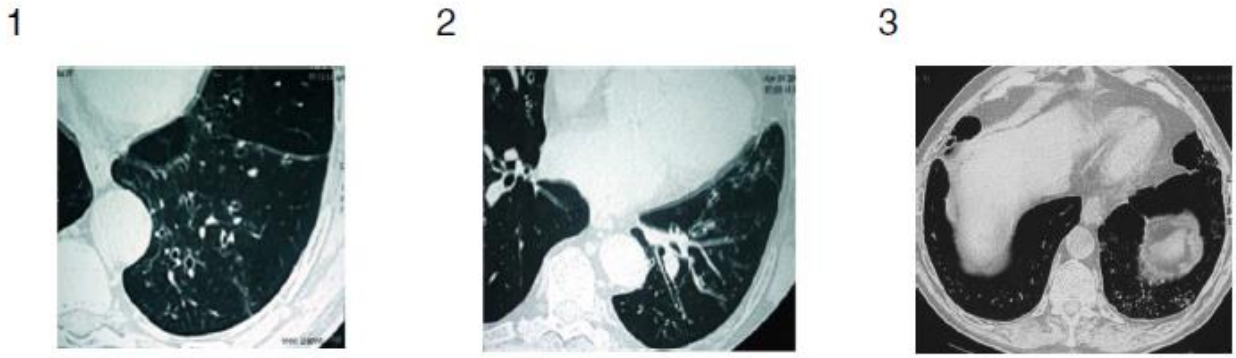
Disease activity is often fluctuating with recurrent exacerbations and increasing bacterial load. As the frequency of exacerbations increases, there is an associated reduction in the FEV1, an increased severity of lung disease on CT, and an increase in chronic infection with *Pseudomonas aeruginosa* and *Haemophilus influenzae*. In addition, more severe and frequent exacerbations are associated with quality of life worsening, more hospital admissions, higher mortality, and increased economic

burden. In a recent consensus[35], experienced clinicians proposed a working definition of an exacerbation of bronchiectasis for use in clinical research: “a person with bronchiectasis with a deterioration in three or more of the following key symptoms for at least 48 h: cough, sputum volume and/or consistency, sputum purulence, breathlessness and/or exercise tolerance, fatigue and/or malaise, hemoptysis and a clinician determines that a change in bronchiectasis treatment is required”.

Patients with bronchiectasis have poorer quality of life (QoL) scores than the general population. This deterioration has been related largely with age, chronic bronchial *Pseudomonas aeruginosa* infection, grade of dyspnea, number of exacerbations, poorer lung function, presence of respiratory failure and symptoms of depression and anxiety. The only QoL questionnaires designed specifically for use in bronchiectasis are the Quality of Life-Bronchiectasis questionnaire[36] and the recently published Bronchiectasis Health Questionnaire[37] (*figure S2*). Other validated questionnaires are St. George’s Respiratory Questionnaire[38]and the Leicester Cough Questionnaire[39] (*figure S3*), the latter for the specific assessment of the impact of cough.

2.5.2 Radiological aspects

Imaging is of crucial importance in diagnosing and monitoring bronchiectasis. Chest radiograph shows low sensitivity and specificity for the diagnosis of bronchiectasis. HRCT is currently the gold standard for both diagnosis and to assess disease morphology, extent and progression. It also helps in therapeutic decision-making and the diagnosis of concomitant findings. Bronchiectasis are diagnosed according to the criteria described by Naidich[40] (*figure 3*). Depending on its appearance, bronchiectasis is classified as cylindrical (uniform dilatation with non-tapering walls), varicose (undulating dilatation) or saccular/cystic (progressing dilatation towards the periphery)[41].



DIRECT SIGNS
-1.Bronchial dilatation: bronchoarterial ratio greater than 1
- Abnormal bronchial contour
- Signet ring sign (transverse slice)
- Tram-track sign (horizontal slice)
- String of pearls sign (horizontal slice)
- Bunch of grapes sign

-2.Lack of bronchial tapering
-3.Visualization of peripheral bronchi
- Within 1 cm of the costal pleura
- In contact with the mediastinal pleura

INDIRECT SIGNS
-Peribronchial thickening
-Mucoid impaction
- Tubular or Y-shaped structures
- Rounded or branching opacities (transverse slice
- Air-fluid levels

-Mosaic pattern
-Centrilobular nodules, tree-in-bud nodules
-Focal areas of air trapping
-Atelectasis/consolidation

*Figure 3.*Radiological signs of bronchiectasis (images above the table of the 3 principle criteria or direct signs of Naidich et al.[40]). *Source: M.Á. Martínez-García et al.*[17]

In addition to its appearance, the distribution of bronchiectasis can also help to characterize the disease; there are several scores that had been implemented for evaluation of bronchiectasis such as the modified score of Reiff et al[42]. Therefore, each lobe (with the lingula considered as a separate lobe) is scored for the extent of involvement (0=none, 1=one or partial segment, 2=two or more segments); severity of bronchial dilatation (0=normal, 1=less than twice the diameter, 2=2±3 times the

diameter, and 3=more than 3x the diameter of the adjacent pulmonary artery); severity of the bronchial wall thickening (0=normal, 1=half the diameter, 2=0.5-1x diameter, and 3=more than 1x the diameter of the adjacent pulmonary artery); type of bronchiectasis (1=cylindrical, 2=varicose, or 3 = cystic). Later, the lobar distribution of bronchiectasis (0=widespread, 1=predominantly upper lobe, 2=predominantly middle lobe, 3=predominantly lower lobe, 4=middle and lower lobes equally involved, or 5 = unclassifiable) is registered. In case of situs inversus or heterotaxy, right-sided changes are assigned to the left site according to the architecture of the lobes. The modified Bhalla[43] score system is recommended if more extensive or detailed radiological information is needed. The correlation between both scores is very high.
In some cases, HRCT can reveal the etiology. Diffuse bronchiectasis suggests an underlying systemic problem, those due to tuberculosis predominate in upper fields and those secondary to ABPA are usually central. The presence of associated multiple small nodules, predominantly in the lingual and middle lobe, suggests NTM infection.

2.5.3 Microbiological aspects

Pathogenic colonization, which is usually expressed as “bronchial infection”, is a “passive pathogenesis” model caused by the growth of microorganisms on the surface of the respiratory mucosa without invading the adjacent tissues and which causes a local inflammatory effect. Different stages in the infection can be distinguished in bronchiectasis, which are important in clinical management and antimicrobial treatment: initial infection (first culture positive for a potentially pathogenic microorganism not isolated in previous periodic cultures), intermittent infection (positive and negative cultures for the same potentially pathogenic microorganism in consecutive samples taken at least one month after the initial infection), and chronic infection (3 or more consecutive cultures positive for the same pathogenic microorganism within a period of at least 6 months in samples taken at least 1 month apart).

Eradication of a certain pathogenic microorganism is usually considered the absence of cultures positive for the microorganism in at least three sputum samples taken at least 1 month apart over a period of 6 months. Colony count in the culture can help assess treatment efficacy.

The microscopic examination of sputum should exclude contamination from the upper respiratory tract, so >25 leukocytes and <10 epithelial cells should be observed. Samples must be collected, transported and processed within 6 h. If this is not possible, they should be kept for no longer than 24 h at room temperature, prefer-ably stored at 4◦C rather than −20◦C. For longer periods, they should be kept at −80◦C. General differential and selective media should be included in the culture to increase yield. Different morpho-types of the same microorganism can appear in cultures and should be detected using specific antibiotic susceptibility testing (AST) in each case. Although AST results are the gold standard in antimicrobial treatment, correlation between conventional in vitro sensitivity and in vivo response can be poor, especially with microorganisms that grow in biofilms, or while using inhaled antibiotics that reach very high concentrations in the bronchial mucosa.

2.6 Prognosis

Bronchiectasis is an irreversible, chronic disease with variable progression. As the disease progresses, a greater number of exacerbations and hospital admissions, progressive airflow obstruction, chronic bronchial infection caused by *Pseudomonas aeruginosa* and other multi-resistant microorganisms, progressive dyspnoea, respiratory failure, and death (especially due to respiratory exacerbations) usually appear. The presence of systemic inflammation, chronic bronchial *Pseudomonas aeruginosa* infection and severe exacerbations has been associated with more rapid progression of bronchiectasis.

Two multidimensional scores are used to assess the prognosis and initial severity of bronchiectasis: the FACED[44] (*figure S4a*) and the Bronchiectasis Severity Index (BSI)[8] (*figure S5*), as well as a modification of the former (E-FACED)[45] (*figure S4b*), which

also includes the number and severity of exacerbations in the previous year. For the initial clinical management and assessment of the patient, the E-FACED score is recommended for its simplicity. The variables should be collected as close as possible to the time of diagnosis. Both the FACED and E-FACED have shown good prognostic capacity for mortality. E-FACED also presents a good prognostic capacity for the number and severity of exacerbations. The E-FACED should be obtained annually to assess clinical progression of the disease. Although the BSI is more complex, it has also shown good prognostic capacity for quality of life and lung function decline[46].

2.7 Treatment

Treatment is primarily based on the principles of preventing or suppressing acute and chronic bronchial infection, improving mucociliary clearance and reducing the impact of structural lung disease (*figure 4*).

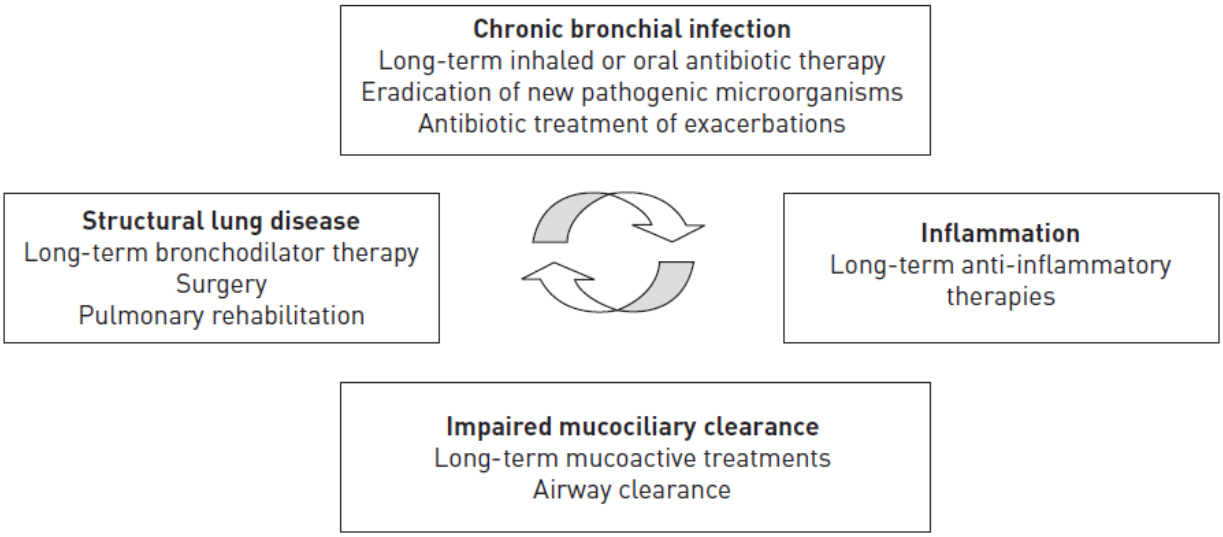


Figure 4. Treatments for bronchiectasis considered in this guideline according to the vicious cycle concept of bronchiectasis. *Source: Polverino E. et al*[47].

2.7.1 Treatment of bronchial infection

Firstly, it is very important to identify bronchiectasis etiologies that have specific treatment, in order to start therapy as soon as possible to control symptoms and prevent

progression of lung damage (intravenous or subcutaneous immunoglobulins for defective antibody production, oral corticosteroids and anti-fungal agents for ABPA, intravenous alfa-1 antitrypsin for alfa-1 antitrypsin deficiency, treatments for asthma or COPD according to clinical guidelines, mucociliary clearance techniques for primary ciliary dyskinesia, etc.). Treatment of the underlying disease should be reviewed at each clinical assessment.

For the primary infection, the decision to apply an eradication treatment for potentially pathogenic microorganisms should be made on an individual basis according to the patient's symptoms and the microorganism in question, since there is no strong evidence on its contribution to the pathogenesis of bronchiectasis. Based mainly on the benefit of *Pseudomonas aeruginosa* eradication in CF, eradication in non-CF bronchiectasis patients should be attempted[48], even if no *Pseudomonas aeruginosa* eradication protocol has demonstrated superiority over another. After each step, it is recommended to repeat sputum sampling for *Pseudomonas aeruginosa* and to progress to the next step if the culture remains positive. If three treatment strategies fail, the chronic infection protocol should be applied (*figure 5*). There is no evidence to support eradication of organisms other than *Pseudomonas aeruginosa* and in organisms that are not so clearly associated with poorer outcomes, the risk-benefit ratio is less in favor of eradication treatment.

The main aim of antibiotic treatment of chronic bronchial infection is to lower bacterial density to break the vicious pathogenic circle of airway infection-inflammation, reducing both as far as possible and thereby slowing clinical–functional decline. Prolonged antibiotic treatment is recommended in the following situations: (a) in all patients who present with chronic bronchial *Pseudomonas aeruginosa* infection; (b) in patients with chronic bronchial infection due to other potentially pathogenic microorganisms, who also present at least 2 exacerbations or 1 hospitalization for exacerbation during the previous year, marked decline in lung function or deterioration in quality of life evidenced by an increase in sputum volume or purulence, dyspnoea or cough[49] (*figure 6*).

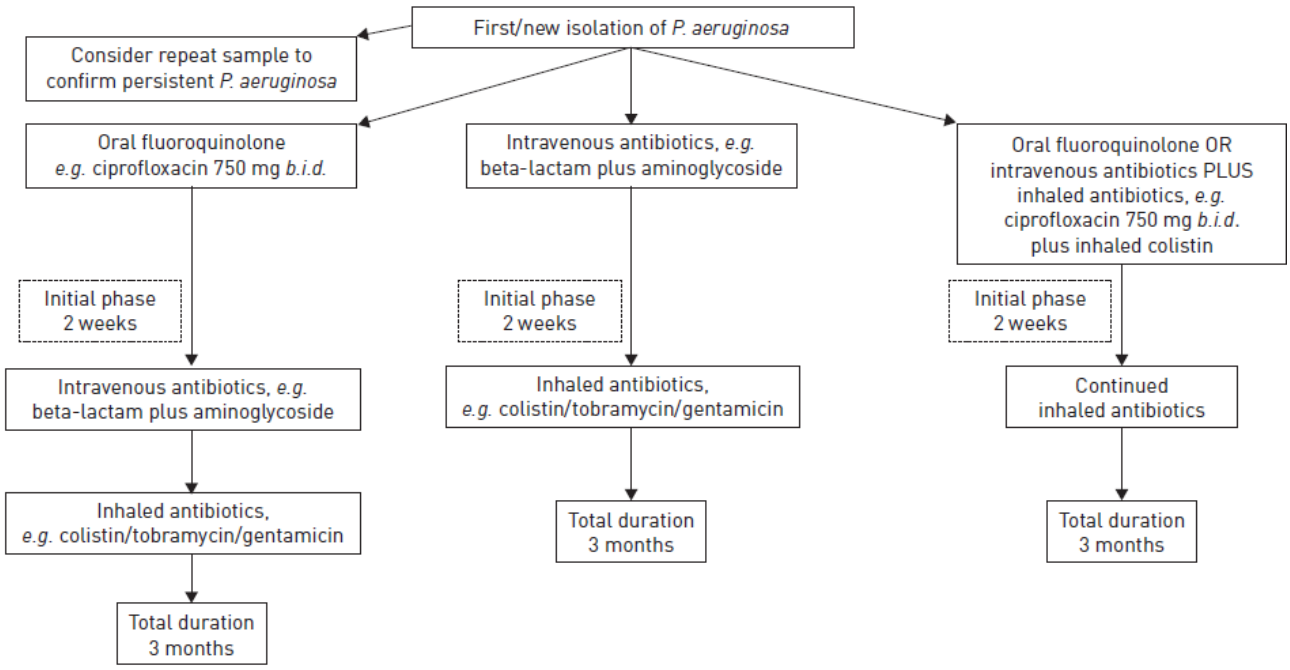


Figure 5. Treatment of the Primary Infection. *Source: Polverino E. et al*[47].

Inhaled rather than systemic antibiotics are recommended due to their high effectiveness (significant reduction of bacterial load, decrease in local inflammation, improved quality of life and reduction in the number of exacerbations) and good safety profile (high antibiotic concentrations at the infection site with minimal systemic side effects and lower rate of resistance)[50].

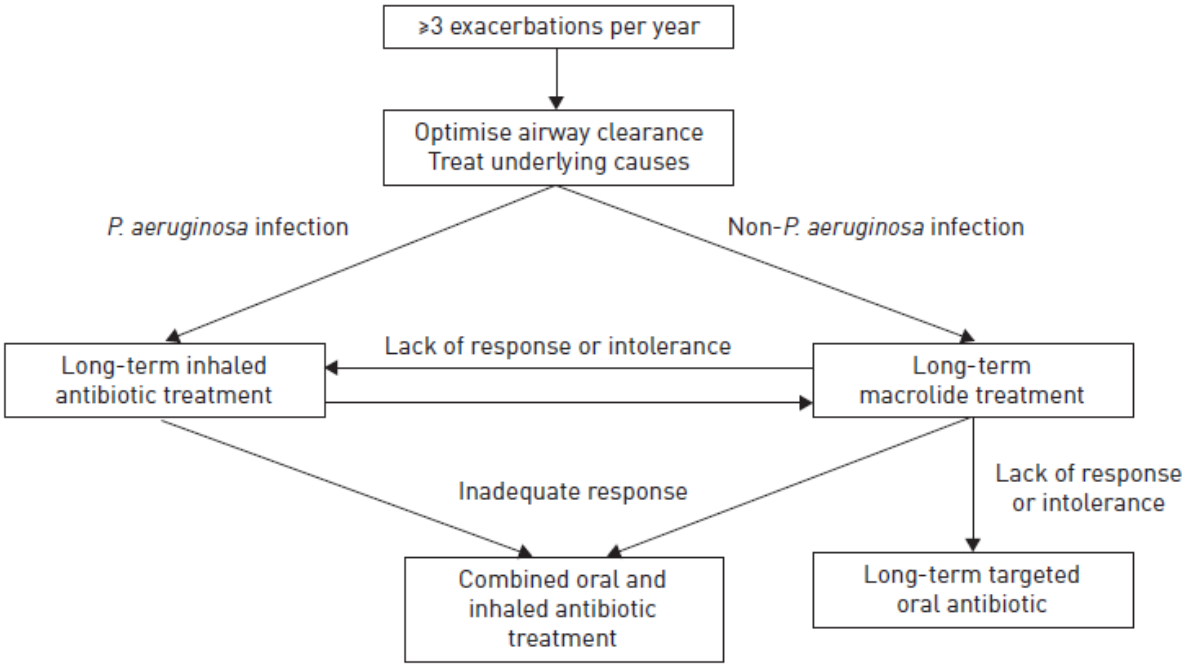


Figure 6. Treatment of Chronic Infection. *Source: Polverino E. et al*[47].

Bronchiectasis patients are typically given prolonged courses of antibiotics of 14 days' duration for infective exacerbations based on the patient's prior microbiology testing and the severity of the exacerbation. Exacerbations are classified as mild or moderate when they can be controlled with oral antibiotic treatment and are considered severe when they require intravenous antibiotic treatment or hospitalization, and/or when they present with at least one of the following conditions: exacerbated acute or chronic respiratory failure, significant deterioration in oxygen saturation, high temperature or other criteria for sepsis, frank hemoptysis or significant deterioration in lung function. Shorter or longer courses of antibiotics may be appropriate in some cases, depending on specific clinical conditions (such as exacerbation severity, patient response to treatment, or microbiology) (*figure 7*).

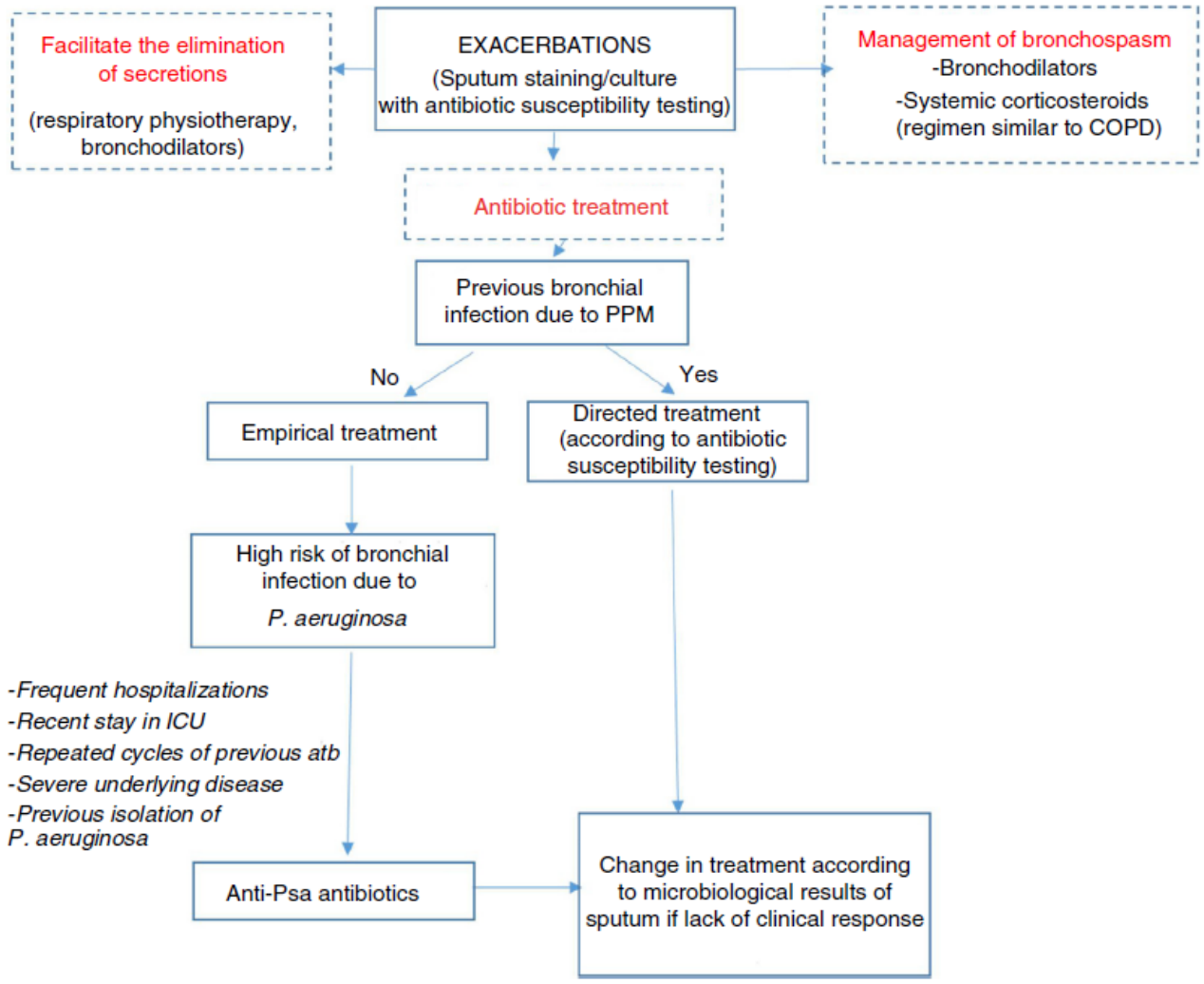


Figure 7. Algorithm for the management of exacerbations. *Source: M.Á. Martínez-García et al.*[17] *Abbr. antiPsa: anti-pseudomonas; Atb: antibiotic; COPD: chronic obstructive pulmonary disease; ICU: Intensive Care Unit; PPM: potentially pathogenic microorganisms.*

2.7.2 Treatment of inflammation

In addition to their antibiotic action, macrolides can modulate bronchial inflammation and interfere in biofilm formation. They can reduce the number of exacerbations, as well as the amount of sputum, improve quality of life and attenuate lung function decline. They are recommended in patients with clinically stable BE but with at least two annual exacerbations despite appropriate background treatment[51].Their clinical efficacy should be re-evaluated every 6 months based on the decrease in the number of exacerbations.

There is scant evidence of the clinical efficacy of other anti-inflammatory treatments, such as neutrophil elastase inhibitors, anti-leukotrienes, phosphodiesterase-4 inhibitors or statins, inhaled corticosteroids. Therefore, routine use of these is not recommended, except in the case of concomitant COPD, asthma or other comorbidities. Care should be taken with inhaled corticosteroid treatment in patients with chronic bronchial infection caused by pathogenic microorganisms, as these drugs can increase susceptibility to infection[52].

2.7.3 Treatment of impaired mucociliary clearance

Airway clearance (AC) techniques by a trained respiratory physiotherapist, adjuncts such as mucolytics and hyperosmolar agents, alter mucus viscosity and/or enhance mucociliary clearance. The use of short-acting beta-agonists (salbutamol or terbutaline) is recommended as premedication before respiratory physiotherapy and/or mucolytics to facilitate AC and the use of inhaled antibiotics or hypertonic saline solution.

AC techniques to perform once or twice daily are safe and recommended in adult patients with clinically stable bronchiectasis with productive cough, because they significantly improve quality of life, especially hyper-secretory patients or those with frequent exacerbations[53].The choice of technique should be based on the patient's preference, their ability, comorbidity and interference in daily life; AC techniques can be either manual (autogenic drainage, slow expiration with glottis opened and active cycle of breathing techniques) or instrumental (positive expiratory pressure, oscillating positive expiratory pressure and high frequency chest wall oscillation). All reduce the symptoms of dyspnea, cough, and facilitate expectoration. AC is contraindicated in unstable disease, hemoptysis, bronchospasm, intracranial hypertension, pneumothorax and recent eye surgery.

Long-term (≥3 months) treatment with hypertonic substances is recommended for in bronchiectasis patients with expectoration greater than 10 mL per day or ≥2

exacerbations per year despite correct background treatment[54], while there is insufficient evidence to recommend the routine use of mucolytics in bronchiectasis.
The indication and type of treatment given should be tailored to each individual patient according to their baseline symptom profile (frequency and severity of exacerbations, quality of life, bronchial hyper-reactivity, and sputum viscosity), baseline lung function and patient preferences.

2.7.4 Treatment of structural lung disease

The use of long-acting beta-agonists is recommended in patients who present with symptomatic and/or functional airflow obstruction, with significant breathlessness on an individual basis, provided the advantages outweigh the adverse effects, and to step down inhaled steroid doses[55]. There is no information on the use of anti-cholinergics, so they should only be used in cases of concomitant asthma or COPD, or in particular, cases in which other treatments have not produced the desired effect. Furthermore, the use of bronchodilators is recommended before physiotherapy, inhaled mucoactive drugs, as well as before inhaled antibiotics, to increase tolerability and optimize pulmonary deposition in diseased areas of the lungs (good practice point, indirect evidence).
Surgery (lobectomies or segmentectomies) should be considered a therapeutic option only in curative treatment in localized BEs that are refractory to clinical management, provided that underlying diseases that contribute to onset have been ruled out. Surgery with palliative intent is also indicated in cases of severe hemoptysis with ineffective embolization, or of abscessed areas that cannot be cured with antibiotic treatment. Factors such as the existence of residual bronchiectasis, *Pseudomonas aeruginosa* or non-tuberculous mycobacteria infection and immunosuppression can result in a poorer clinical response following surgery[56].
Physical training, within pulmonary rehabilitation programs, is recommended in stable patients with dyspnea and impaired exercise capacity. All interventions should be

tailored to the patient's symptoms, physical capability and disease characteristics and aim to improve exercise tolerance and quality of life.

2.8 Follow up

In general, patients who are monitored in specialized bronchiectasis clinics or units should been seen at least once every 6–12 months. More severe or unstable patients are advised to attend once every 1–3 months, with clinical and microbiological study performed at all visits. After an acute exacerbation, clinical assessment should be performed within the first month.

3. THE MICROBIOME IN BRONCHIECTASIS: *PSEUDOMONAS AERUGINOSA*

The human respiratory tract represents the major portal of entry for numerous microorganisms, primarily those occurring as airborne particles such as viral and bacterial entities, or fungal spores. Microorganism characteristics coupled with the local host immune response will determine whether they will be cleared or adhere and colonize the airways leading to acute or chronic pulmonary disease.

The "microbiome" is defined as the combined genetic material of all microorganisms in an environment. This is distinct from the microbiota, which is defined as the ecological community of commensal, symbiotic and pathogenic microorganisms found in a specific niche. In practice, these terms are often used interchangeably[57]. Common phyla identified by 16S rRNA sequencing in the healthy lung include Proteobacteria, Firmicutes and Bacteroidetes, while at the genus level *Streptococcus*, *Prevotella* and *Veillonella* predominate, with lesser contributions from *Haemophilus* and *Neisseria*[58]. Like bacteria, fungi can cause severe lung diseases, but their infection rates are much lower[59].

The healthy lung microbiome is believed to be transient and depends on three factors, as follows: 1) microbes moving into the airways from inhalation, micro-aspiration and direct mucosal dispersion; 2) the speed at which the mucociliary system and innate immune system remove microbes; and 3) whether conditions in certain regions of the lung are able to favor microbial growth[60].

Lung disease alters the structure of the lung and changes regional growth conditions. In bronchiectasis the airways become widened, leading to a failure of mucociliary clearance allowing bacterial adherence, increased bacterial loads and the development of chronic infection.

The field of microbiome research in bronchiectasis is less advanced than the field of other respiratory diseases such as cystic fibrosis and COPD, with fewer published studies, but existing data show lung bacterial communities dominated by

Pseudomonas, *Haemophilus* and *Streptococcus*, while exhibiting intra-individual stability and large inter-individual variability. *Pseudomonas*- and *Haemophilus*-dominated microbiomes have been shown to be linked to severe disease and frequent exacerbations.

Pseudomonas aeruginosa is a ubiquitous and opportunistic gram-negative bacterium and is considered one of the most significant pathogens that produce chronic bronchial infection and infection of the lower respiratory tract, especially in people with chronic inflammatory airway diseases such as asthma, COPD, CF, and bronchiectasis. From a microbiological viewpoint, the presence and persistence of *Pseudomonas aeruginosa* over time are characterized by adaptation within the host, followed by a reduced susceptibility to bacterial invasion (albeit with a consolidation of the bacterial presence) that precludes any rapid, devastating injury to the host[61].

Longitudinal studies on CF have shown that initial *Pseudomonas aeruginosa* colonization occurs by wild-type non-mucoid *Pseudomonas aeruginosa* (non-mPA), but that as the disease progresses, there is adaptation to the lung environment. Thus, the non-mPA phenotype may acquire a mucoid *Pseudomonas aeruginosa* (mPA) phenotype due to mutations in mucA, beginning to overproduce the exopolysaccharide alginate. This conversion to the mPA phenotype is a hallmark of chronic infection and predicts a poor prognosis because of its recalcitrance to clearance by antibiotics and the immune response through the extracellular polymeric substance (EPS) of the biofilm[62]. This biofilm has been linked to the persistence of *Pseudomonas aeruginosa* and the difficulties involved in its eradication, even under antimicrobial treatment with either intravenous or inhaled antibiotics.

The effect of changes to the EPS in the mPA biofilm on mucus viscoelasticity is a growth area of biofilm research. Characterization of the viscoelastic properties of mucus focuses on the elasticity (or storage modulus, G') and viscosity (or loss modulus, G"), and together describe the rheology of complex biological fluids[4]. The “elasticity” measures the tendency for a material to recover its original shape following stress-induced deformation, whereas the “viscosity”, measures the extent to which the

material resists the tendency to flow. A high viscosity allows mucus to remain intact, while low elasticity promotes airflow-mucus interactions by preventing mucus recoil during a burst of high-velocity air[5]. Pulmonary diseases, such as asthma, chronic obstructive pulmonary disease and CF, generally result in mucus hypersecretion and increased viscoelasticity, owing in part to reduced water content and an increased fraction of glycoproteins that impair mucociliary clearance. However, how mPA and non-mPA phenotypes may affect viscoelastic properties in patients with bronchiectasis is still unknown.

Pseudomonas aeruginosa is the potentially pathogenic microorganism that has been most widely studied in bronchiectasis but there remains much to learn about its natural history and the epidemiology of bronchial infection in this disease. Many of the notions about the behavior of *Pseudomonas aeruginosa* in the airways of people with bronchiectasis have been extrapolated from CF, but there are certain differences between the two diseases. For example, while bronchial infections in adults with CF tend to be caused by the same strain of *Pseudomonas aeruginosa*, this is not true of non-CF bronchiectasis[63].

The presence of *Pseudomonas aeruginosa* in the airways of these people has been linked to a more marked decline in lung function, worsening of symptoms, more frequent exacerbations or flares, reduced quality of life, increased morbidity and mortality, greater local and systemic inflammation, higher medical costs, and a higher mortality rate[64].

Preventing the chronic bronchial infection of *Pseudomonas aeruginosa* is very significant in avoiding associated lung function and quality of life decline and development of resistance. Once established, *Pseudomonas aeruginosa* chronic infection becomes almost impossible to eradicate although “seasonal” airway presence can take place with periods of re-infection and colonization.

4. AIMS

In the first part of the study, we focused on the damage to the mucociliary system in bronchiectasis patients. The primary aims were to evaluate the sputum viscoelastic properties in these patients and to determine the relationship between different *Pseudomonas aeruginosa* phenotypes (mPA or non-mPA) isolation, the viscoelastic properties of sputum, and severity outcomes. The secondary aim was to analyze the possible association between *Pseudomonas aeruginosa* chronic bronchial infection with viscoelastic properties and clinical outcomes.

In the second part of the study, we hypothesized that clinical and microbiological characteristics, as chronic infection with *Pseudomonas aeruginosa*, exist that can be associated with the optimal duration of antibiotic treatment during an exacerbation. The aim was therefore to identify the main clinical and microbiological factors associated with long-course antibiotic treatment for exacerbations of bronchiectasis.

Finally, a small preliminary study was carried out, with the aim to evaluate clinical and microbiological factors during an exacerbation of bronchiectasis associated with risk of mortality during a one-year period.

5. MATERIALS AND METHODS

5.1 Study design and patients

The first part of the study was conducted, with a cross-sectional design, in the pulmonology service of Hospital Clínic of Barcelona - University of Barcelona (Spain). Patients were enrolled consequently between October 2018 and July 2019. The inclusion criteria were as follows: (1) age ≥18 years; (2) a bronchiectasis diagnosis confirmed by HRCT and symptoms of the disease; (3) clinically stable disease (no exacerbation and no significant change in symptoms or therapy in the last four weeks); (4) good quality sputum sample provided for microbiology culture; and (5) ability to perform all clinical tests and to understand the process and purpose of the study. The following exclusion criteria were also applied: (1) positive microbiological result of any pathogenic bacteria other than *Pseudomonas aeruginosa*; (2) any physical and psychological disorder that might interfere with protocol compliance; (3) diagnosis of CF, sarcoidosis, ciliary dyskinesia, pulmonary fibrosis, active tuberculosis, or non-tuberculosis mycobacterial infection; (4) exacerbation of any comorbidity; (5) participation in any clinical trial that included changes in pharmacological treatment in the preceding 6 months; and (6) respiratory insufficiency and/or oxygen therapy.

In the second part of the study, conducted with a prospective observational design, consecutive adult patients (>18 years) with bronchiectasis exacerbations treated by the pulmonology services of two tertiary care university hospitals in Spain (Hospital Clínic, Barcelona and Hospital Universitario y Politécnico La Fe, Valencia) between October 2018 and July 2019 were enrolled. The main inclusion criterion was a clinical history compatible with exacerbation, with diagnosis of bronchiectasis confirmed by HRCT before recruitment. Disease etiology was established according to Spanish guidelines[17]. The following exclusion criteria were applied: (a) diagnosis of CF, ciliary dyskinesia, pulmonary interstitial disease, active tuberculosis, or NTM infection during treatment; (b) exacerbation of any comorbidity; and (c) participation in any clinical trial that included changes in pharmacological treatment in the preceding 6 months.

For comparison, two groups were formed according to the treatment duration chosen according to Spanish guidelines[65]: a short-course group (≤14 days) and a long-course group (15–21 days). We did not follow a standardized algorithm for the treatment decision-making. The two groups were formed according to the treatment duration chosen after an independent clinical decision made by the attending physician of the emergency department who were not involved in the study and determined the different therapeutic options and the course lengths at the onset depending on the etiologic microbiological diagnosis and severity of the exacerbation. Treatment was stopped once clinical success was achieved, which was the complete resolution or a reduction of signs and symptoms associated with the exacerbation without new signs and symptoms developing at the end of the treatment. Poor clinical response was defined as the persistence of >2 symptoms for >5 days at the end of the treatment.

5.2 Clinical measurements

We collected socio-demographic and clinical data, such as the etiology of bronchiectasis, the current treatment, presence of comorbidities[66] as well as bronchiectasis severity score[8,44], number of exacerbations and hospitalizations due bronchiectasis in the previous year, presence of dyspnoea[67], and the lobes affected on HRCT. Lung function was tested with an EaseOneTM WorldSpirometer (ndd Medical Technologies, Zurich, Switzerland) and was classified according to the guidelines of the American Thoracic Society/European Respiratory Society (ERS)[68]. Current exacerbations that presented with a new infiltrate on chest X-ray were included. The decision to admit to hospital was made by the attending physician in the emergency department based on the presence or absence of acute findings consistent with a moderate to severe exacerbation. Blood and microbiological extractions were performed within 24 h of initial assessment.

All surviving patients were re-examined 30 days and 1 year after hospital discharge or at the end of treatment in an outpatient clinic to assess their clinical response.

5.3 Microbiology

Sputum samples were collected from spontaneous expectorations into 50mL sterile containers. Samples were divided, with one part used for microbiological culture and the other stored at −80°C for rheological analysis. Microbiological cultures were analyzed at the microbiology laboratory, using only good quality sputum samples. They were immersed in Sputolysin-Dithiothreitol (1:1) and sonicated in ultrasonic cleaning equipment (Branson 3510E-MT, Bransonic, Danbury, USA) for 5min at 40kHz[69]. All samples were plated on blood, chocolate, MacConkey and Ziehl–Neelsen staining and Lowenstein and Mycobacteria Growth Indicator Tube (MGIT) liquid culture incubated in automatized BDBACTEC™ system were performed. The cultures were evaluated for growth after 48h and Lowenstein cultures (for Mycobacterium spp.) after 6 weeks. Susceptibility testing was performed using disc diffusion, E-test when needed, and samples were classified as sensitive, intermediate or resistant according to the criteria published by the EUCAST[70]. Microorganisms were identified by MALDI-TOF[71] and were classified as potential pathogenic microorganisms (PPMs), which included *Haemophilus influenza*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, Gram-negative bacilli, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and non-potential pathogenic microorganisms (non-PPMs), which included normal flora such as *Streptococcus viridans, Neisseria spp, Candida spp, Corynebacterium spp, Haemophilus parainfluenzae and Staphylococcus epidermidis*. *Pseudomonas aeruginosa* were classified primarily as mPA and non-mPA phenotypes, with "no organism reported" (NOR) used when normal saprophytic flora grew. Chronic infection was defined according to the ERS guidelines as two or more isolates of the same organism at least 3 months apart in 1 year[11]. Sputum color was classified using the Murray scale as mucoid, muco-purulent, and purulent[34].

5.4 Rheology

Rheological analysis was done at the Chemical Engineering department of the Barcelona University. Each sample was processed using a rheometer (HAAKE MARS III, Thermo Scientific, Germany), with a plate-plate spindle diameter of 35mm and gap

of 0.4mm, under a temperature of 37°C. Two oscillation frequencies were used: 1 and 100 rad/s (simulating ciliary movement and cough, respectively). At 1rad/s 10 repetitions were measured and at 100 rad/s 40. The mean of the storage modulus (elasticity, G′) and loss modulus (viscosity, G″) were measured and the magnitude of the complex modulus (stiffness, |G*|) and phase angle (δ) were calculated. The phase angle (G″/G′) characterized mucus as $\delta = 0°$ for a Hookean solid, $\delta = 90°$ for a pure viscous liquid; $\delta < 45°$ for a viscoelastic solid, and $\delta > 45°$ for a viscoelastic liquid[4] ($|G^*| = \sqrt{G'^2 + G''^2}$ - $\delta^o = \tan - 1(G''/G') \cdot 180/\pi$).

In order to reduce potential sources of bias, two independent analysts did the rheological and microbiological analysis.

5.5 Statistical analysis

Categorical variables were reported as numbers and percentages, whereas continuous variables were reported as medians and first to third quartiles. Group comparisons for categorical variables were conducted with the chi-square test or Fisher exact test, and comparisons for continuous variables were conducted using the non-parametric Mann–Whitney test. Unless stated otherwise, the significance level was set at 0.05 (two-tailed). All analyses were performed using IBM SPSS version 25.0 (IBM Corp., Armonk, USA).

Logistic regression analyses were performed to examine the risks factors for long-course antibiotic treatment. Each risk factor was first tested individually before we added all risk factors that showed a univariate association ($p < 0.10$) to the multivariable model. Next, backward stepwise selection ($pin < 0.05$, $pout > 0.10$) was used to determine the factors associated with long antibiotic treatment courses. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated.

A multiple imputation method was used to handle missing data in the multivariable analyses. Multicollinearity was checked by calculating the variance inflation factor. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate the adequacy of the model. In addition, the area under the receiver operating characteristic curve (AUC)

was calculated for the ability of the final model to predict patients requiring long-course antibiotic treatment. Finally, to measure possible overfitting and instability of the selection variables in the final model, we performed internal validation using ordinary non-parametric bootstrapping with 1000 bootstrap samples and bias-corrected accelerated 95% Cis.

6. RESULTS

6.1 Pseudomonas aeruginosa and sputum viscoelastic properties.

Of the 57 stable patients with bronchiectasis enrolled, 9 (16%) were not considered because these patients had sputum positive for other pathogens but *Pseudomonas aeruginosa*. The study population therefore comprised 48 patients, which were classified in three groups according to the sputum culture: 31 (65%) *Pseudomonas aeruginosa* (17 (35%) mPA, 14 (29%) non-mPA) and 17 (35%) NOR.

No differences were found in baseline characteristics between study groups but in severity variables. Compared with the non-mPA group, the mPA group had more hospitalizations in the previous year and more lobes affected on HRCT. Additionally, the mPA group had greater exacerbations and greater hospitalizations in the last year, more affected lobes, and more purulent sputum than the NOR group; by contrast, the non-mPA and NOR groups did not differ (*table 1*). In terms of the viscoelastic properties of the sputum, at 1 rad/s, the mPA group showed higher median G′ (10.30 [8.30–17.46] vs. 5.70 [4.63–8.84]), G″ (2.40 [1.95–3.40] vs. 1.50 [1.20–2.30]), and |G*| (10.70 [8.56–17.58] vs. 6.00 [4.83–9.11]), compared with the NOR group (p values of 0.023, 0.039, and 0.024, respectively; *figure 8A*). There were no differences in rheology between the non-mPA vs. NOR groups and mPA vs. non-mPA groups although there was a trend to higher viscosity, elasticity, and stiffness in the mPA group compared to the non-mPA group. There were no differences between any group at 100 rad/s (*figure 8B*), and there were no differences in delta values between any group at either 1 or 100 rad/s.

Table 1. Population baseline characteristics.

	All patients	PA		NOR	p value
		mPA	Non-mPA		
	N=48	N=17 (35%)	N=14 (29%)	N=17 (35%)	
Baseline characteristics					
Female sex, n (%)	31 (64)	13 (76)	7 (50)	11 (65)	0.389
Age, mean (SD), years	68.5 (16)	61.7 (18.4)	71.8 (11.4)	72.5 (15.6)	0.096
Chronic colonization, n (%)	36 (75)	15 (88)	13 (93)	8 (47)	0.003
Pseudomonas aeruginosa	34 (95)	15 (88)	13 (93)	6 (86)	<0.001
Haemophilus influenza	2 (5)	0 (0)	0 (0)	2 (12)	0.419
Dyspnea (MRC Scale, 1-5), median [Q1; Q3]	2 [2; 3]	2 [2; 3]	3 [2; 3]	2 [2; 2.5]	0.118
Etiology, n (%)					0.114
Post-infectious	19 (40)	6 (35)	6 (43)	7 (41)	0.905
Idiopathic	13 (27)	8 (47)	2 (14)	3 (18)	0.081
Others	16 (33)	3 (17)	6 (43)	7 (41)	0.083
Variables of severity					
Exacerbations last year, median [Q1; Q3]	2 [2; 3]	2 [2; 4]	2 [1; 3]	2[1.5; 2]	0.031[b]
Hospitalizations last year, median [Q1; Q3]	0 [0; 1]	1 [0; 1]	0 [0; 0]	0 [0; 0]	0.021[bc]
Lobes affected (HRCT), median [Q1; Q3]	3 [3; 5]	5 [3.5; 5.5]	3 [2.75; 4]	3 [2; 4]	0.015[bc]
BE severity (BSI stages), n (%)					0.527
Mild: 0-4	4 (8)	0 (0)	1 (7)	3 (17)	0.172
Moderate: 5-8	11 (23)	5 (29)	3 (21)	3 (17)	0.691
Severe: ≥9	31 (65)	11 (65)	10 (71)	10 (59)	0.867
Sputum color, n (%)					
Mucoid	1 (1)	0 (0)	0 (0)	1 (5)	0.402
Mucopurulent	19 (39)	4 (23)	5 (35)	10 (59)	0.108
Purulent	28 (58)	13 (76)	9 (64)	6 (35)	0.048[b]

Abbr. mPA: mucoid Pseudomonas aeruginosa; Non-mPA: Non-mucoid Pseudomonas aeruginosa; NOR: no organism reported; SD: standard deviation; MRC: medical research council; Q1: first quartile; Q3: third quartile; HRCT: high-resolution computed tomography; BE: bronchiectasis; BSI: bronchiectasis severity index. Percentages calculated on non-missing data.

[a]Could have more than 1 medication. [b]$p < 0.05$ for comparison between mPA and NOR. [c]$p < 0.05$ for comparison between mPA and non-mPA.

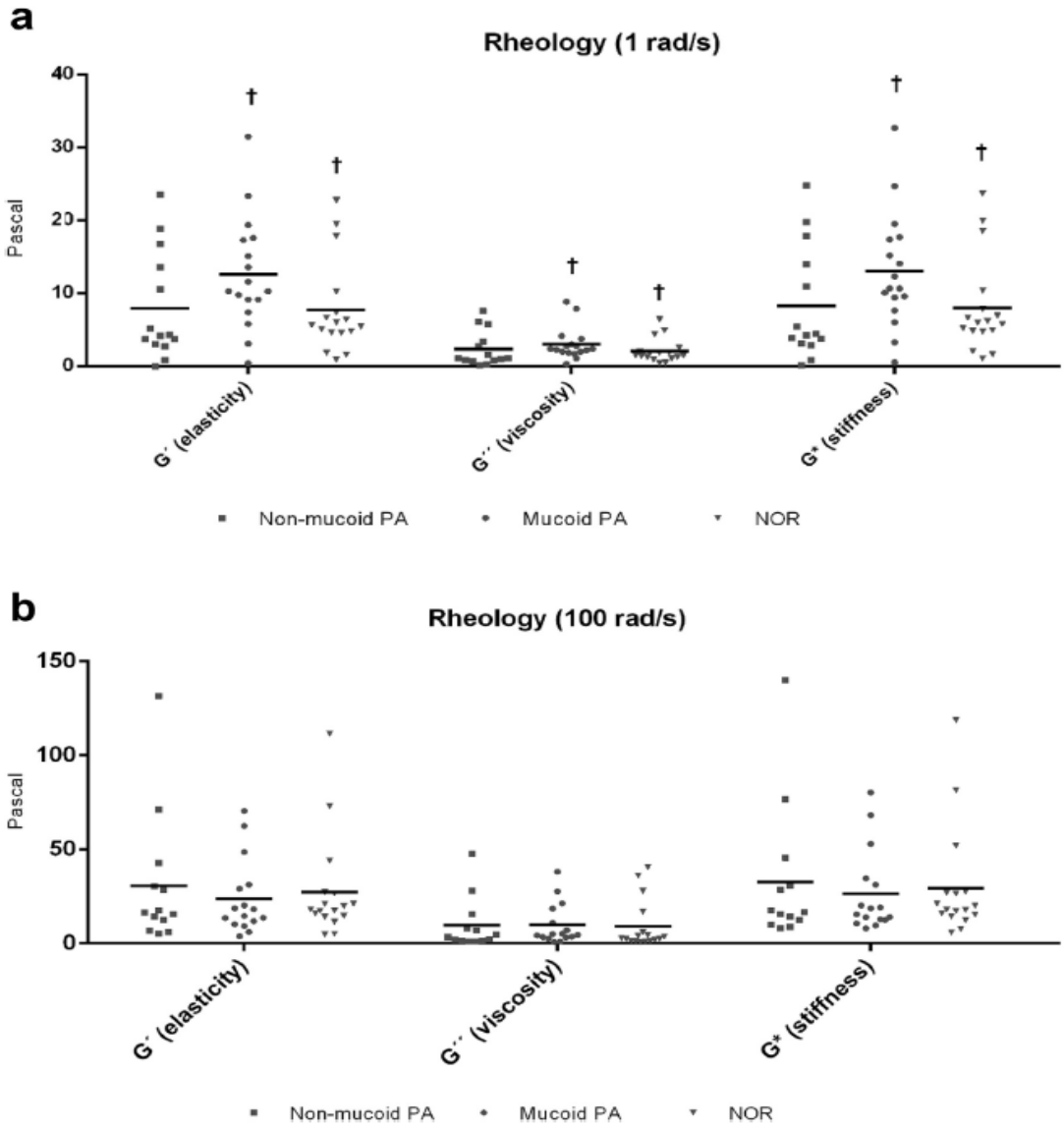


Figure 8. (A) Viscoelastic results at 1 rad/s. (B) Viscoelastic results at 100 rad/s. *Source: Alcaraz-Serrano V. et al*[72].

Abbr. non-mPA, non-mucoid Pseudomonas aeruginosa; mPA, mucoid Pseudomonas aeruginosa; NOR, no organism reported. (A): the mPA storage modulus (elasticity; G'), loss modulus (viscosity; G''), and magnitude of the complex modulus (stiffness; |G|) were 10.30 (8.30–17.46), 2.40 (1.95–3.40), and 10.70 (8.56–17.58), respectively. The NOR G', G'', and |G*| were 5.70 (4.63–8.84), 1.50 (1.20–2.30), and 6.00 (4.83–9.11).*

Of all the patients, 36 (75%) had chronic *Pseudomonas aeruginosa* colonization and 12 (25%) did not (*table 2*). The chronic *Pseudomonas aeruginosa* group presented poorer lung function, higher dyspnea scores, and increased BSI severity scores compared with the non-chronic *Pseudomonas aeruginosa* (BSI severe stage: 81% vs.

36%, p < 0.001), but no differences were found in sputum viscoelasticity between these two groups.

Table 2. Clinical characteristics of chronic *Pseudomonas aeruginosa* vs non-chronic colonization subjects.

	CHRONIC PA	**NON-CHRONIC**	**p value**
	N=36 (75%)	N=12 (25%)	
Microbiologic result, n (%)			0.004
Mucoid PA	15 (41.6)	2 (16.7)	
Non-mucoid PA	13 (36.1)	1 (8.3)	
No organism reported	8 (22.2)	9 (75)	
Clinical Characteristics, median [Q1; Q3]			
Exacerbations last year	2 [2; 3]	2 [2; 2]	0.462
Hospitalizations last year	0 [0; 1]	0 [0; 0]	0.254
Lobes affected (HRCT)	4 [3; 5]	3 [2.25; 3.75]	0.227
Dyspnea (MRC Scale, 1-5)	2 [2; 3]	2 [1.25; 2]	0.006
Pulmonary Function mean (SD)			
FEV_1, % predicted	59.09 (18.6)	85.29 (23.55)	<0.001
FEV_1, L	1.52 (0.6)	2.02 (0.9)	0.029
FVC, % predicted	73.06 (16.5)	84.79 (17.24)	0.033
FVC, L	2.59 (0.83)	2.69 (0.92)	0.704
FEV_1/FVC, %	66.45 (19)	80.57 (15)	0.017

Abbr. PA: Pseudomonas aeruginosa: Q1: first quartile; Q3: third quartile; HRCT: high-resolution computed tomography; MRC: medical research council; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; SD: standard deviation. Percentages calculated on non-missing data.

6.2 Pseudomonas aeruginosa and duration of antibiotic treatment for exacerbations.

Of the 283 exacerbated patients attended by the two participating hospitals with exacerbations of bronchiectasis during the study period, 92 (33%) were excluded from the analysis because they did not receive antibiotic treatment, or the duration of the antibiotic treatment was not recorded. The population therefore comprised 191 patients with bronchiectasis exacerbations, of which most were women (57%; n = 108) and elderly (median age, 72 (63, 79) years). Participants were divided in the short-course

group (69%; n = 132) and the long-course group (31%; n = 59) based on their antibiotic treatment duration, as decided by the attending physician (*figure 9*).

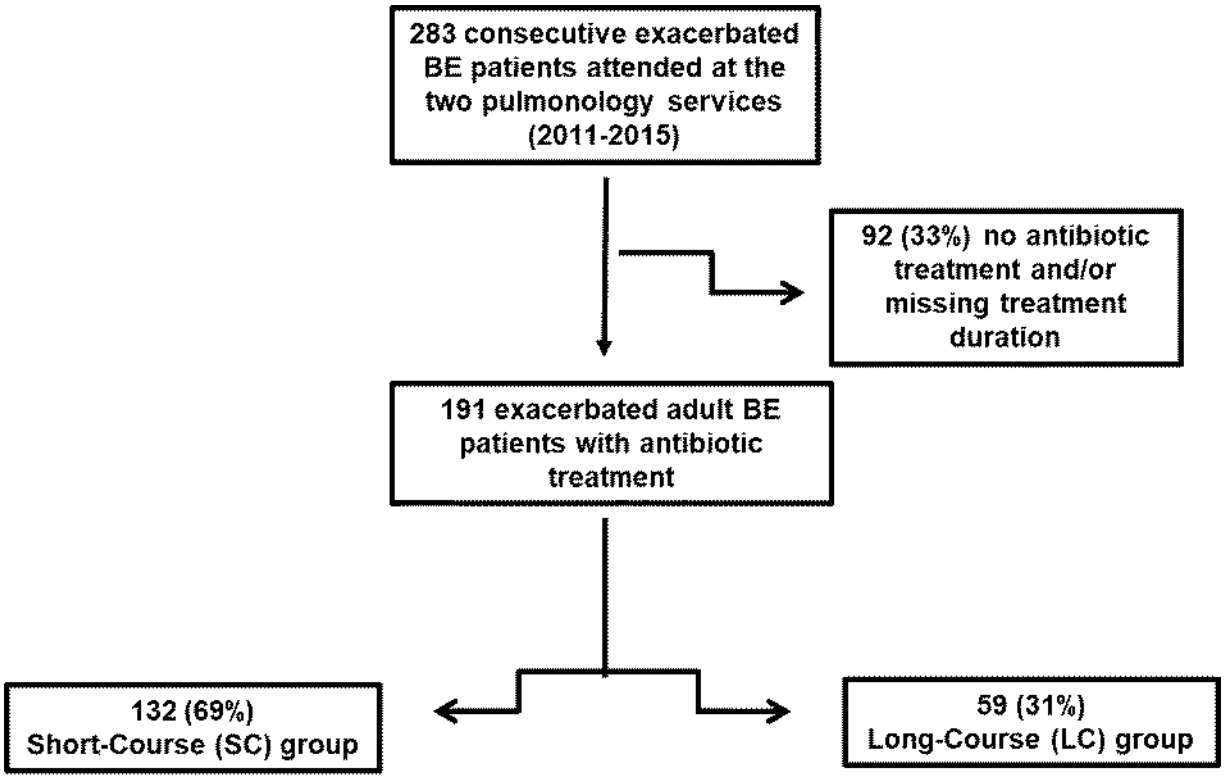


Figure 9. Flow chart for study enrollment. *Sources: Scioscia G. et al*[73].

Patients with longer durations of antibiotic therapy received LTOT, had cylindrical or cystic bronchiectasis on high-resolution CT, had a higher incidence of chronic colonization with *Pseudomonas aeruginosa*, had more exacerbations and hospitalizations due to bronchiectasis in the last year, and more often had a severe FACED score. Patients with short-term therapy more frequently had a mild FACED score. Twenty-six patients (13.6%) received LTOT at baseline. All of them had a severe BSI stage, 15 (57.7%) had a severe FACED score, while 4 (15.4%) presented a mild FACED score and 7 (26.9%) a moderate one ($p < 0.001$) (*table 3*).

Mild exacerbations were more frequent in patients receiving short-course therapy, whereas moderate to severe exacerbations were associated with longer courses of antibiotic therapy. The mean exacerbation and hospitalization lengths were also significantly higher in patients with longer courses of antibiotic treatment. Overall, an

etiologic microbiological diagnosis was obtained at baseline in 141 patients (74%). Patients with longer courses of antibiotic treatment more frequently had *Pseudomonas aeruginosa* isolated and initially received three or more antibiotics compared with patients who received short-term therapy. We detected a change in the initial decision in 60 (31.4%) patients that corresponded to the cases in which the treatment had been started empirically according to previous cultures and then improved based on the current susceptibility testing (*table 4*).

Univariate logistic regression analyses were performed for the main characteristics of patients at baseline and during an acute exacerbation. Several variables were significantly associated with longer courses of antibiotic treatment (*table 5*). However, when all significant variables were put into the multivariable model, only three factors remained as independent associated with longer antibiotic treatment. These were the use of LTOT (OR 2.51 (95% CI 1.02 to 6.22)), the presence of a moderate to severe exacerbation (OR 3.31 (95% CI 1.26 to 8.65)), and an etiology of *Pseudomonas aeruginosa* confirmed by microbiological isolation (OR 2.89 (95% CI 1.43 to 5.82)). The AUC was 0.73 (95% CI 0.65 to 0.80) for this final model predicting longer antibiotic treatment duration.

We repeated all regression analyses after excluding patients with a diagnosis of community-acquired pneumonia (n = 49). In the multivariable model, we found that an etiology of *Pseudomonas aeruginosa* confirmed by microbiological isolation (OR 3.07 (95% CI 1.31 to 7.18)) remained as independent factor associated with longer antibiotic treatment, with a moderate FACED score (OR 2.97 (95% CI 2.09 to 8.14)), a severe FACED score (OR 4.31 (95% CI 1.49 to 12.42)), and the presence of arrhythmia as comorbidity at baseline (OR 0.19 (95% CI 0.05 to 0.73)). The AUC was 0.77 (95% CI 0.68 to 0.85) for this final model predicting longer antibiotic treatment duration.

However, no statistically significant differences were found between the two groups in terms of the history of exacerbations or hospitalizations during the 1-year follow-up. Similarly, the mortality rates during the exacerbation and at 30 days and 1 year of follow-up did not differ between the two groups.

6.3 Pseudomonas aeruginosa and risk of one-year mortality due to exacerbations.

We follow up 185 exacerbated bronchiectasis patients admitted to hospital (94 females, 71.8 (11.8) years, 66.5% BSI stage severe) for one-year. Twenty-three (12.4%) patients died during the one-year follow up. In our preliminary results, we found no significant differences between the two groups (survivors and non-survivors) in terms of chronic colonization by *Pseudomonas aeruginosa* or microbiological isolation with *Pseudomonas aeruginosa* during the hospitalization (*table 6*). The major causes of death were respiratory related (68%), cardiovascular (18%), and septic shock (14%).

In a preliminary multivariate Cox regression analysis, we found that LTOT, mechanical ventilation and white blood cell count >13.64x10^9/L at day 1 of hospitalization are variables associated with an increased risk of one-year mortality in patients hospitalized with moderate or severe bronchiectasis exacerbation. On the other hand, influenza vaccination appears as a protective factor. The AUC was 0.51 (0.33 to 0.68) for this final model predicting one-year mortality.

Table 3. Patient baseline characteristics.

	All patients	SC ≤14 days	LC 15–21 days	P value[a]
Patients	N=191	N=132	N=59	
Clinical				
Female sex, n (%)	108 (56.5)	76 (57.6)	32 (54.2)	0.667
Age, median [Q1; Q3], years	72 [63; 79]	72 [62.5; 79]	73 [64; 78]	0.881
Etiology, n (%)				0.535
Post-infectious	65 (34)	49 (37.1)	16 (27.1)	
Idiopathic	61 (32)	38 (28.8)	23 (39)	
Asthma	13 (6.8)	8 (6.1)	5 (8.5)	
COPD	36 (18.8)	25 (18.9)	11 (18.6)	
Others	16 (8.4)	12 (0.08)	4 (6.8)	
Therapy, n (%) [b]				
Mucolytics	76 (39.8)	47 (35.6)	29 (49.2)	0.077
Inhaled Antibiotic	43 (22.5)	27 (20.5)	16 (27.1)	0.308
Bronchodilators	263 (68.8)	179 (67.8)	84 (71.18)	0.268
Inhaled steroid	154 (80.6)	108 (81.8)	13 (22)	0.534
Oral corticosteroids (<28 days)	16 (8.4)	9 (6.8)	7 (11.9)	0.418
LTOT	26 (13.6)	11 (8.3)	15 (25.4)	0.001
Severity				
Lobes affected (HRCT), n (%)				0.312
< 3	77 (41)	56 (43.4)	21 (35.6)	
≥ 3	111 (59)	73 (56.6)	38 (64.4)	
Type of BE (HRCT), n (%)				<0.001
Cylindrical	58 (30.7)	33 (25.4)	25 (42.4)	0.019
Varicose	12 (6.3)	7 (5.4)	5 (8.5)	0.521
Cystic	13 (6.9)	5 (3.8)	8 (13.6)	0.026
Chronic colonization, n (%)				
Pseudomonas aeruginosa	89 (46.6)	52 (39.4)	37 (62.7)	0.003
Others	46 (24.3)	31 (23.8)	15 (25.4)	0.815
History of exacerbations due to BE (1 year), n (%)	143 (75.3)	94 (71.8)	49 (83)	0.095
History of pneumonia (1 year), n (%)	32 (16.8)	25 (19.1)	7 (11.9)	0.218
History of hospitalizations due to BE (1 year), n (%)	97 (50.8)	60 (45.5)	37 (62.7)	0.028

		All patients	SC ≤14 days	LC 15–21 days	P value[a]
Patients		N=191	N=132	N=59	
BSI stage, n (%)					0.315
	Mild 0–4	19 (9.9)	16 (12.1)	3 (5.1)	
	Moderate 5–8	45 (23.6)	31 (23.5)	14 (23.7)	
	Severe ≥ 9	127 (66.5)	85 (64.4)	42 (71.2)	
FACED score, n (%)					0.029
	Mild 0–2	94 (49.2)	72 (54.5)	22 (37.3)	0.027
	Moderate 3–4	61 (31.9)	41 (31.1)	20 (33.9)	0.697
	Severe 5–7	36 (18.8)	19 (14.4)	17 (28.8)	0.019

Abbr: BD, bronchodilator; BE, bronchiectasis; BMI, body mass index; BSI, Bronchiectasis Severity Index; COPD, chronic obstructive pulmonary disease; HRCT, high-resolution computed tomography; LC, long-course; LTOT, Long-Term Oxygen Therapy; SC, short-course. Percentages calculated on non-missing data. [a] The p values correspond to differences between the two groups (short-course group (<14 days) vs. long-course group (15–21 days)). [b] Could have more than one medication.

Table 4. Patient characteristics during exacerbation.

	All patients	SC ≤14 days	LC 15–21 days	P value
Patients	N=191	N=132	N=59	
Severity				
Severity of exacerbation				0.005
Mild	48 (25.1)	42 (31.8)	6 (10.2)	0.001
Moderate to severe	143 (74.9)	90 (61.2)	53 (89.8)	0.001
Duration of exacerbation, median [Q1; Q3], days	11 [8; 15]	10 [7; 14]	15 [11; 20]	<0.001
Duration of hospitalization, median [Q1; Q3], days	8 [6; 11]	8 [6; 10]	9.5 [7;17]	0.002
Diagnosis of CAP, n (%)	49 (25.7)	31 (23.5)	18 (30.5)	0.304
Complications, n (%) [a]				
Intubation / MV	4 (2.1)	2 (1.5)	2 (3.4)	0.589
NIV	7 (3.7)	3 (2.3)	4 (6.8)	0.205
Sepsis	7 (3.7)	3 (2.3)	4 (6.8)	0.205
Septic Shock	4 (2.1)	1 (0.8)	3 (5.1)	0.088
Acute myocardial infarction	2 (1)	0	2 (3.4)	0.094
Arrhythmia	8 (4.2)	6 (4.5)	2 (3.4)	>0.999
Microbiology, n (%)				
Patients with defined etiology	141 (73.8)	90 (68.2)	51 (86.4)	0.008
Haemophilus influenza[b]	12 (8.5)	11 (12.2)	1 (2)	0.056
Escherichia coli[b]	5 (3.5)	4 (4.4)	1 (2)	0.654
Klebsiella pneumonia[b]	1 (0.7)	1 (1.1)	0	>0.999
Moraxella catarrhalis[b]	3 (2.1)	3 (3.3)	0	0.553
Pseudomonas aeruginosa[b]	28 (19.9)	12 (13.39)	16 (31.4)	0.010
Staphylococcus aureus[b]	2 (1.4)	0	2 (3.9)	0.129
Mycoplasma pneumonia[b]	2 (1.4)	2 (2.2)	0	0.535
Streptococcus pneumoniae[b]	11 (7.8)	6 (6.7)	5 (9.8)	0.527
Virus[b]	6 (4.3)	5 (5.69)	1 (2)	0.418
Other[b]	17 (12.1)	13 (14.4)	4 (7.8)	0.247
Polymicrobial[b]	52 (36.9)	32 (35.69)	20 (39.2)	0.665
Antibiotic treatment				
Number of initial antibiotics, n (%)				<0.001
1	94 (49.2)	75 (56.8)	19 (32.2)	0.002

		All patients	SC ≤14 days	LC 15–21 days	P value
Patients		N=191	N=132	N=59	
	2	74 (38.7)	49 (37.1)	25 (42.4)	0.491
	≥3	23 (12)	8 (6.1)	15 (25.4)	<0.001

Abbr. CAP, Community-acquired pneumonia; i.v., intravenous; LC, long course; MV, Mechanical ventilation; NIV, Non-invasive ventilation; PaO2, partial pressure of oxygen; SC, short-course. Percentages calculated on non-missing data. +[a]Could have more than one complication. [b]The percentages of pathogens are related to the number of patients with etiologic diagnosis in each group.

Table 5. Univariate and multivariable logistic regression analyses in predicting long-course antibiotic treatment.

Variable	Univariate			Multivariable[a, b, c]		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
LTOT	3.75	1.60–8.78	0.002	2.51	1.02–6.22	0.046
FACED score			0.033	–	–	–
Mild 0–2	1.00	–	–	–	–	–
Moderate 3–4	1.60	0.78–3.27	0.20	–	–	–
Severe 5–7	2.93	1.30–6.58	0.009	–	–	–
Site of treatment: Hospital ward / Intensive Care Unit / Intermediate Care Unit	2.68	1.21–5.96	0.015	–	–	–
Moderate to severe exacerbation	4.12	1.64–10.35	0.003	3.31	1.26–8.65	0.015
Pseudomonas aeruginosa	3.76	1.94–7.29	<0.001	2.89	1.43–5.80	0.003
MRSA	6.02	1.13–31.98	0.035	–	–	–

Abbr. CI, confidence interval; i.v., intravenous; LC, long-course; LTOT, Long-Term Oxygen Therapy; MRSA, Methicillin-resistant Staphylococcus aureus; MV, mechanical ventilation; OR, odds ratio.

Data are shown as estimated ORs (95% CIs) of the explanatory variables in the LC group. The OR represents the odds that LC antibiotic treatment will occur, given exposure to the explanatory variable, compared to the odds of the outcome occurring in the absence of that exposure. P-values are based on the null hypothesis that all ORs relating to an explanatory variable equal unity (i.e., no effect).

[a]Adjusted for center. [b]Hosmer–Lemeshow goodness-of-fit test, p = 0.35. [c]Probability of LC antibiotic treatment = Exp (β) / (1 + Exp (β)), where β = -2.543 + 0.528 (for Valencia center) + 0.922 (for LTOT) + 1.196 (for moderate to severe exacerbation) + 1.061 (for Pseudomonas aeruginosa microbiology). In the presence of all risks factors, the probability of LC antibiotic treatment was 76.2%, while in the absence of all these risk factors, the probability of LC antibiotic treatment was 7.3%.

Table 6. Population baseline characteristics during hospitalization.

	All patients	Survivors	Non-survivors	p value
	N=185	N=162 (87.6)	N=23 (12.4)	
Demographics				
Female	94 (50.8)	87 (53.7)	7 (30.4)	0.037
Age, years	71.8 (11.8)	70.8 (12.0)	78.8 (7.3)	0.002
BMI, Kg/m^2	25.9 (4.8)	26.0 (4.9)	24.9 (4.0)	0.425
Former smokers	78 (42.2)	65 (40.1)	13 (56.5)	0.413
Ex-alcoholic	6 (3.2)	3 (1.8)	3 (13)	<0.001
Influenza vaccination	132 (71.4)	119 (73.5)	13 (56.5)	0.093
Pneumococcal vaccination	79 (48.2)	71 (48.6)	8 (44.4)	0.737
Chronic colonization by *Pseudomonas aeruginosa*	98 (53)	85 (52.5)	13 (56.5)	0.716
Number exacerbations/previous year	2 [0-3]	2 [0-2]	2 [1-3]	0.391
Number hospitalizations/ previous year	0 [0-1]	0 [0-1]	1 [0-3]	0.003
Number of lobes affected in the HRCT	3.3 (1.8)	3.4 (1.8)	3.0 (2.0)	0.233
Long term oxygen therapy	31 (16.8)	23 (14.2)	8 (34.8)	0.013
Etiology				0.583
Post-infectious	59 (31.9)	50 (30.9)	9 (39.1)	
Idiopathic	54 (29.2)	50 (30.9)	4 (17.4)	
COPD	48 (25.9)	39 (24.1)	9 (39.1)	
Others	24 (13)	23 (14.2)	1 (4.3)	
Severity				
Charlson Comorbidities Index	5 [3-6]	4 [3-6]	6 [5-8]	0.004
BSI Stages				0.025
Mild	11 (5.9)	11 (6.8)	0 (0)	
Moderate	51 (27.6)	49 (30.2)	2 (8.7)	
Severe	123 (66.5)	102 (63)	21 (91.3)	
Pulmonary Function				
FEV_1, % predicted	57.8 (22.2)	58.1 (20.9)	54.7 (32.5)	0.597
FVC, % predicted	81.6 (23.7)	81.2 (23.5)	84.8 (26.4)	0.606
FEV_1/FVC, %	54.7 (11.8)	55.4 (11.1)	48.0 (16.4)	0.031

	All patients	Survivors	Non-survivors	p value
	N=185	N=162 (87.6)	N=23 (12.4)	
Hospitalization episode				
Duration, days	8 [6-11]	8 [6-10]	8 [6-16]	0.446
Diagnosis of CAP	69 (37.3)	58 (35.8)	11 (47.8)	0.265
Pneumonia Severity Index	4 [3-4]	3.5 [2.5-4]	4 [4-4]	0.021
Microbiological diagnosis				0.515
Pseudomonas aeruginosa	30 (16.2)	25 (15.4)	5 (21.7)	
Streptococcus pneumoniae	13 (9)	12 (9.6)	1 (5.3)	
Haemophilus influenza	11 (7.6)	9 (7.2)	2 (10.5)	
Complications				
IMV	2 (1.1)	1 (0.6)	1 (4.3)	0.105
NIMV	5 (2.7)	1 (0.6)	4 (17.4)	<0.001
Sepsis	8 (4.3)	7 (4.3)	1 (4.3)	0.995

Abbr. BMI, body mass index; BSI, Bronchiectasis Severity Index; CAP, Community-acquired pneumonia; COPD, chronic obstructive pulmonary disease; FEV1, Forced expiratory volume in 1 second; FVC, Forced Volume Vital Capacity; HRCT, high-resolution computed tomography; IMV, invasive mechanical ventilation; LTOT, Long-Term Oxygen Therapy; NIMV, non-invasive mechanical ventilation.

Table 7. Factors independently associated to increased one-year mortality in patients hospitalized with moderate to severe bronchiectasis exacerbation.

	Univariate		Multivariable	
Variables	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Influenza vaccination	0.33 (0.14 to 0.76)	0.010	0.36 (0.15 to 0.90)	0.028
LTOT	3.03 (1.27 to 7.23)	0.012	4.76 (1.91 to 11.91)	0.001
MV	14.25 (4.13 to 49.24)	<0.001	12.08 (2.98 to 49.04)	<0.001
WBC count (day 1) >13.64 x 10^9/L	3.09 (1.35 to 7.04)	0.007	3.35 (1.42 to 7.89)	0.006

Abbr. CI, confidence interval; LTOT, Long-Term Oxygen Therapy; MV, mechanical ventilation; OR, odds ratio; WBC, white blood cell.

7. DISCUSSION

This is a large study of non-CF bronchiectasis patients with the aim to assess the burden of *Pseudomonas aeruginosa* colonization. The main findings are as follows. a) Bronchiectasis patients with mPA phenotype infection have worse severity outcomes compared with the non-mPA and NOR ones (e.g., increased number of hospitalizations in the previous year and a greater number of affected lobes). b) mPA phenotype cause poor viscoelastic properties of sputum (e.g. elasticity, viscosity and stiffness) implying a reduced mucociliary clearance. c) Chronic colonized patients have poorer lung function, greater dyspnea, and an increased disease severity (BSI) compared with non-chronic ones, but there are no differences in terms of viscoelastic properties. d) Longer courses of antibiotic treatment are provided to patients treated with LTOT and who have a more severe disease at baseline. e) Moderate to severe bronchiectasis exacerbations lasting more days, and for which *Pseudomonas aeruginosa* is isolated also tend to receive longer antibiotic courses. f) LTOT, moderate to severe exacerbations and microbiological isolation of *Pseudomonas aeruginosa* are associated with the need for longer courses of antibiotic treatment. g) An association between one-year mortality and *Pseudomonas aeruginosa* colonization is not demonstrate in patients hospitalized with moderate or severe bronchiectasis exacerbation. h) LTOT at baseline, mechanical ventilation and white blood cell count $>13.64x10^9$/L at day 1 of hospitalization for bronchiectasis exacerbation are factors associated with an increased risk of one-year mortality.

In healthy subjects, the viscoelasticity of mucus maintains a balance that promotes clearance by cough and mucociliary mechanisms[74]. In lung disease such as CF, hypersecretion and a change in the viscoelastic properties of mucus alter this balance. Our findings provide evidence that the mPA phenotype is not only a marker of disease severity, determined by more advanced disease on HRCT and higher number of hospitalizations, but it is also associated with a trend of poorer viscoelastic properties than non-mPA phenotype. We assume that the lack of significance in rheology measures between mPA and non-mPA phenotypes is due to the lower number of

samples. Moreover, compared to NOR group, mPA had poor elasticity, viscosity and stiffness at ciliary velocity (1 rad/s) but not at cough velocity (100 rad/s). We could relate this last finding to the mutation of the mPA phenotype that causes the over-production of exopolysaccharide alginate that is probably responsible for the poor mucociliary clearance, but it not affects the cough mechanisms. Finally, the non-mPA phenotype did not differ by the NOR patients in terms of viscoelastic properties and severity outcomes. Our results are consistent with those of previous studies in patients with CF[75]. One study in a pediatric population indicated that the presence of mPA was associated with a decline in lung function, and that it was a marker of progressive transition to more severe disease stages[76]. In a dynamic assessment, Gloag et al[5] demonstrated how the viscoelastic properties of the exopolysaccharide *Pseudomonas aeruginosa* matrix change from early to mature mucoid biofilms, suggesting that this contributes to decreases in mucociliary and cough clearance as CF progresses.

Previous studies have shown that patients colonized by *Pseudomonas aeruginosa* had poor quality of life[77], worse lung function, higher inflammatory responses[78], more exacerbations[79] and increased mortality. In our study, chronic colonization with mPA or non-mPA was associated with poor lung function, greater dyspnoea, and more severe BSI score, despite a similar number of exacerbations between the groups. Similarly, Lee et al[80] correlated the colonization status of *Pseudomonas aeruginosa* to disease severity using the FACED scoring system. They found that, despite *Haemophilus influenzae* being the most commonly isolated genus, it was significantly more abundant in patients with mild bronchiectasis, whereas *Pseudomonas aeruginosa* was more prevalent in the moderate/severe group.

In patients with CF, Locke et al[81] reported that those with chronic *Pseudomonas aeruginosa* colonization had a reduced mucociliary clearance compared with those who had non chronic colonization. In contrast to this finding, we identified no correlations between chronic *Pseudomonas aeruginosa* colonization and the viscoelastic parameters. However, our data did strongly indicate that viscoelasticity was increased in the presence of the mPA phenotype. The fact that we found similar

frequencies of colonization by mPA (41.6%) and non-mPA (36.1%) phenotypes might have been responsible for this lack of correlation between viscoelasticity and *Pseudomonas aeruginosa* colonization. We suggest that it is recommendable to identify the *Pseudomonas aeruginosa* phenotype responsible for the colonization because it represents significant information about the severity of the disease. In the colonized group, we also found patients with sputum cultures labelled as NOR (22.2%). Therefore, although sputum should provide a representative sample of the content of the lower respiratory tract, it is possible that these samples were obtained from the upper airways.

The first results of this study may justify the application of rheological analysis in clinical practice to analyze sputum viscoelasticity of bronchiectasis patients. However, rheological analysis needs specific equipment and technical formation, which may difficult its implementation. We believe that the evaluation of the sputum viscoelasticity as an outcome could be useful in clinical practice to personalize the therapeutic approach to the patient with bronchiectasis. Our findings could suggest that patients infected by mPA phenotype may benefit from physiotherapy strategies and/or use of hyperosmolar-nebulized agents in order to fluidize sputum, but further longitudinal investigations and randomized clinical trials are needed to confirm this suggestion.

The main limitation of this first part of research was the low number of samples included in the study. Secondly, the samples were stored at −80 °C before performing the rheological analyses. Previous research has shown that different storage durations (2, 10, 30, and 90 days) and two temperatures (−20 °C and −80 °C) did not affect the viscoelastic properties[82]. Further investigation is needed to compare viscoelastic properties between mPA vs other pathogens in order to confirm if mPA phenotype is the only organism causing poor viscoelastic properties of sputum.

The optimal duration of either oral or intravenous antibiotic therapy has not been studied for non-CF bronchiectasis, with current practice extrapolated from that used for CF. In the absence of any direct data comparing long and short courses, 14 days of

treatment continues to be recommended in guidelines[7,83,84]. Some authors have demonstrated a favorable impact with 14 days of intravenous antibiotic therapy for exacerbations, with improvements shown in sputum bacterial volume, markers of inflammation, the incremental walk test, and the St. George's Respiratory Questionnaire; however, spirometry results remained unchanged[78,85]. Collaco et al[86] also observed that patients with CF who received longer courses of intravenous antibiotics tended to have worse lung function and they demonstrated that the greatest improvement in lung function may occur within the first week of therapy, indicating that courses lasting 14–21 days may provide no additional benefit.

Consistent with the recent guideline of the British Thoracic Society[28], we observed that patients treated with longer courses of antibiotics presented with more markers of severe disease than patients treated with shorter-term therapy. When we assessed the two main severity scores, the FACED score was milder in the short-course group and more severe in the long-course group. By contrast, no differences were found for the BSI score. Rosales et al[46] demonstrated that severity stratified by FACED and BSI scores can show relevant differences in patient distribution.

Concerning the microbiological results, we showed that prior chronic colonization with *Pseudomonas aeruginosa* or etiologic isolation during a bronchiectasis exacerbation influenced the need for prolonged antibiotic treatment. Recently, Crisafulli et al[87] also demonstrated that *Pseudomonas aeruginosa* affected the length of hospitalization during exacerbations of COPD. We could explain the longer exacerbation and hospitalization durations of our patients in the long-course group by *Pseudomonas aeruginosa* isolation in 31.4% of cases. It was also possible to explain the initial use of three or more antibiotics in 15 patients from the long-course group by the isolation of multidrug resistant *Pseudomonas aeruginosa* in 15.2%. Univariate analysis of the main characteristics at baseline and during exacerbations revealed several variables that were significantly associated with longer courses of antibiotic treatment. However, the multivariate analysis only confirmed that LTOT, the presence of a moderate to severe exacerbation, and the microbiological isolation of *Pseudomonas aeruginosa* during an

exacerbation were significant associated with longer treatment durations. In recent study, Sundh et al[88] concluded that LTOT prescribed for 24h each day did not decrease hospital admissions compared therapy for just 15–16 patients with oxygen-dependent COPD. Together with our finding, this could indicate that LTOT usage is associated with considerable healthcare costs. On the other hand, we can say that LTOT is a clinical factor that reflects disease severity. Previous studies have shown that *Pseudomonas aeruginosa* isolation in bronchiectasis patients was associated with accelerated loss of lung function and increased daily sputum production[89], as well as a poorer quality of life, regardless of whether it is a cause or consequence of functional deterioration[77]. The findings of our study also suggest that longer antibiotic treatment during exacerbations is appropriate for patients with *Pseudomonas aeruginosa* infection (with and without diagnosis of community- acquired pneumonia), consistent with the latest recommendations of the British Thoracic Society[28]. Finally, when we repeated all regression analyses after excluding patients with a diagnosis of community-acquired pneumonia, we found that the presence of arrhythmia as comorbidity at baseline appear a factor negatively associated with long-term therapy. A potential explanation is that attending physicians tended to shorten the course of antibiotic therapies to avoid cardiac complications in patients with a baseline arrhythmic comorbidity, or it could simply be a random response without any clinical significance.

The strengths of this part of the study are the prospective inclusion of consecutive patients in two centers, the inclusion of clinically relevant variables, and the long-term follow-up. The results provide important preliminary evidence on which recommendations for decisions about antibiotic treatment durations for exacerbations of bronchiectasis can be based. However, there are some main limitations. First, there is a significant imbalance between patients treated with longer courses as compared to shorter courses. Second, the microbiological study was performed during an exacerbation and we did not establish whether the identified microorganisms were responsible for prior chronic bronchial infection. Third, we could not perform a

complete microbiological study in all cases, despite almost all patients providing at least one respiratory sample.

The last part of the study shows preliminary results on the disease burden in terms of risk of mortality due to exacerbation of bronchiectasis. Several studies have identified *Pseudomonas aeruginosa* chronic infection as a risk factor for mortality in bronchiectasis patients[90,91]. In terms of mortality, the relationship between *Pseudomonas aeruginosa* and an increased risk of death, described previously in the literature, seems to be closely related with the fact that these patients have more exacerbations and more hospitalizations, rather than just the simple fact of being chronically infected by this pathogen. Our preliminary results cannot fully account for the question of which characteristic comes first in disease. It is not known whether patients with *Pseudomonas aeruginosa* have more exacerbations and therefore have worse outcomes, or whether frequently exacerbating patients have a poor prognosis with frequent antibiotic courses leading to *Pseudomonas aeruginosa* infection and poor outcomes. The present study was not powered to detect differences in mortality rates because this was not a study goal. Nevertheless, based on the one-year follow-up results, we speculate that an association between *Pseudomonas aeruginosa* chronic colonization or microbiological isolation and one-year mortality is not demonstrate in patients hospitalized with moderate or severe bronchiectasis exacerbation. On the other hand, the preliminary multivariate analysis only evidenced that LTOT at baseline, mechanical ventilation and white blood cell count $>13.64x10^9$/L at day 1 of hospitalization for bronchiectasis exacerbation are factors associated with an increased risk of one-year mortality. The most surprising finding in the present analysis was the independent association between regular vaccination and preventive influence on the mortality of bronchiectasis. Although, numbers of respiratory infections were associated with an inflammatory response and the release of proteolytic enzymes, which results in damage to the airway wall, the relationship between immunization and survival has not been researched. Besides, the prognostic value of regular vaccination in patients who were treated with conventional treatment is more difficult to explain

because there is not enough study available to compare with the present study in directly addressing immunization and the long-term survival of bronchiectasis patients. A future study addressing the risk of one-year mortality for patients with bronchiectasis exacerbations and the identification of clinical and microbiological factors associated with the risk of death after a hospitalization for moderate to severe bronchiectasis exacerbation is desirable.

8. CONCLUSIONS

The first conclusion is that the mPA phenotype is associated with worse severity outcomes and increased sputum purulence, elasticity, viscosity, and stiffness in patients with bronchiectasis. Our results suggest that viscoelasticity assessment with rheology could be a new clinical tool in order to optimize and personalize bronchiectasis management, but further investigation is needed to confirm our hypothesis. Secondly, we conclude that LTOT use, the presence of a moderate to severe exacerbation, and microbiological isolation of *Pseudomonas aeruginosa* each are associated with the need for a longer course of antibiotic therapy during bronchiectasis exacerbations. Patients receiving longer courses of treatment also tend to have more severe disease, colonization with *Pseudomonas aeruginosa*, and a history of worse outcomes in terms of previous hospitalizations. Accordingly, decisions about the duration of antibiotic therapy should be guided by complete clinical and microbiological assessments of patients when they present with an acute exacerbation. Our study is really the reflection of clinical practice and if on the one hand it has a potential bias in the process of treatment decision-making, on the other it provides insights into a subset of exacerbated patients who could benefit from longer antibiotic treatment. However, more evidence is still needed to support clinic decision-making. Studies are currently underway to determine the optimal length of treatment in CF, and similar studies are needed for patients with non-CF bronchiectasis. Finally, we can conclude that LTOT, mechanical ventilation and white blood cell count at day 1 of hospitalization for bronchiectasis exacerbation are independent risk factors for one-year mortality, all three are simple to evaluate and requires no special equipment or time. In addition to this, vaccination has preventive effects on the mortality, but we do not know whether this would be useful indicator of the outcome in clinical trials.

Supplementary materials

Figure S1. Table to assess the color of sputum from least to most purulent (Murray scale[34]). *Abbr. M: mucous; MP: mucopurulent; P: purulent.*

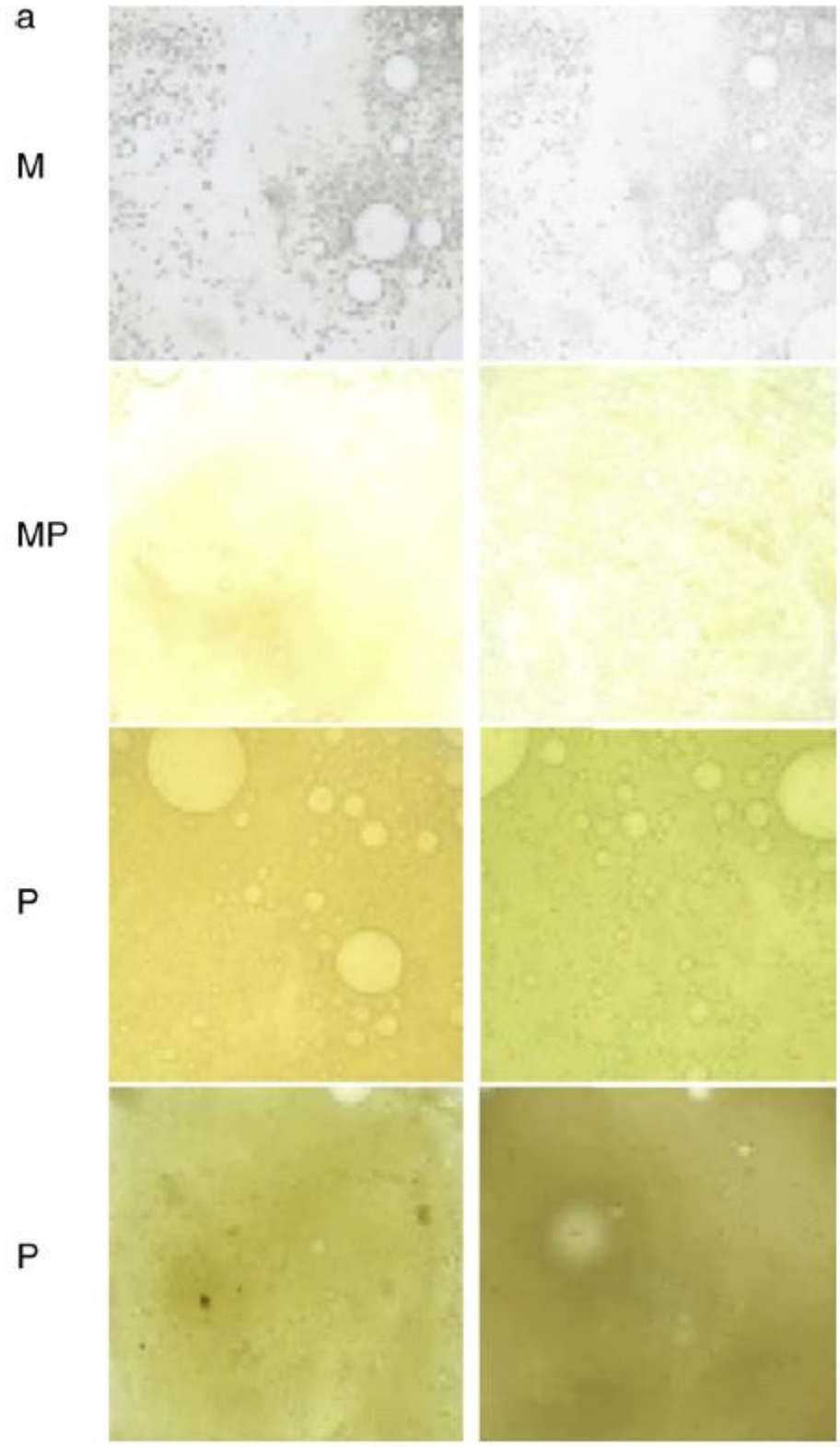


Figure S2. Bronchiectasis Health Questionnaire[37].

The Bronchiectasis Health Questionnaire (BHQ)© 2014

This questionnaire is designed to assess how bronchiectasis affects your life. Please read each question carefully and answer by SELECTING the response that best applies to you. It is important that you answer all questions as honestly as you can.

1. In the last 14 days, I have been tired.

1. All of the time 2. Most of the time 3. A good bit of the time 4. Some of the time 5. A little of the time 6. Hardly any of the time 7. None of the time

2. In the last 14 days, I have been much slower at doing things than other people of my age.

1. All of the time 2. Most of the time 3. A good bit of the time 4. Some of the time 5. A little of the time 6. Hardly any of the time 7. None of the time

3. In the last 14 days, I have felt anxious.

1. All of the time 2. Most of the time 3. A good bit of the time 4. Some of the time 5. A little of the time 6. Hardly any of the time 7. None of the time

4. In the last 14 days, my chest has felt clear.

1. All of the time 2. Most of the time 3. A good bit of the time 4. Some of the time 5. A little of the time 6. Hardly any of the time 7. None of the time

5. In the last 14 days, I have been embarassed because of my phlegm (sputum).

1. All of the time 2. Most of the time 3. A good bit of the time 4. Some of the time 5. A little of the time 6. Hardly any of the time 7. None of the time

6. In the last 14 days, I have felt short of breath.

1. All of the time 2. Most of the time 3. A good bit of the time 4. Some of the time 5. A little of the time 6. Hardly any of the time 7. None of the time

7. In the last 14 days, my sleep has been disrupted because of my bronchiectasis.

1. Every night 2. Most nights 3. Several nights 4. Some nights 5. Occasionally 6. Rarely 7. Never

8. In the last 14 days, I have had coughing fits.

1. Every day 2. Most days 3. Several days 4. Some days 5. Occasionally 6. Rarely 7. Never

9. In the last 14 days, my phlegm (sputum) contained blood.

1. Every time 2. Most times 3. Several times 4. Sometimes 5. Occasionally 6. Rarely 7. Never

10. In the last 12 months, I have taken antibiotic treatments for a chest infection.

1. More than five times 2. Five times 3. Four times 4. Three times 5. Twice 6. Once 7. None

Thank you for completing this questionnaire!

Figure S3. Leicester Cough Questionnaire[39].

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by CIRCLING the response that best applies to you. Please answer ALL questions, as honestly as you can.

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

1	2	3	4	5	6	7
Every time	Most times	Several times	Some times	Occasionally	Rarely	Never

3. In the last 2 weeks, have you been tired because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

4. In the last 2 weeks, have you felt in control of your cough?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

6. In the last 2 weeks, my cough has made me feel anxious

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

7. In the last 2 weeks, my cough has interfered with my job, or other daily tasks

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

8. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

9. In the last 2 weeks, exposure to paints or fumes has made me cough

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

10. In the last 2 weeks, has your cough disturbed your sleep?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

11. In the last 2 weeks, how many times a day have you had coughing bouts?

1	2	3	4	5	6	7
All of the time (continuously)	Most times during the day	Several times during the day	Some times during the day	Occasionally through the day	Rarely	None

12. In the last 2 weeks, my cough has made me feel frustrated

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

13. In the last 2 weeks, my cough has made me feel fed up

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

14. In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

15. In the last 2 weeks, have you had a lot of energy?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

16. In the last 2 weeks, have you worried that your cough may indicate serious illness?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

17. In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

18. In the last 2 weeks, my cough has interrupted conversation or telephone calls

1	2	3	4	5	6	7
Every time	Most times	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

19. In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends

1	2	3	4	5	6	7
Every time I cough	Most times when I cough	Several times when I cough	Some times when I cough	Occasionally when I cough	Rarely	Never

Thank you for completing this questionnaire.

Figure S4a. FACED score[44]. http://bronchiectasis.com.au/assessment/medical/faced-score

> F – FEV1 (≥ 50% = 0 points, < 50% = 2 points)
>
> A – Age (< 70 years = 0 points, ≥ 70 years = 2 points)
>
> C – Chronic colonisation (no Pseudomonas = 0 points, presence of Pseudomonas = 1 point)
>
> E – Extension (1 – 2 lobes affected = 0 points, > 2 lobes affected= 1 point)
>
> D – Dyspnoea – modified Medical Research Council scale – mMRC (0 – 2 = 0 points, 3 – 4 = 1 point)

Abbr. FEV1: forced expiratory volume in the first second; MRC: Medical Research Council.

FACED classification of severity:

0–2 points: mild bronchiectasis.

3-4 points: moderate bronchiectasis.

5-7 points: severe bronchiectasis.

Figure S4b. E-FACED score. *Adapted from: Martínez-García et al*[45].

Variable	Values	Score
Exacerbaciones with hospital admission (previous year)	No	0
	At least 1	2
FEV$_1$ (% predicted)	At least 50%	0
	Less than 50%	2
Age	Less than 70 years	0
	At least 70 years	2
Chronic bronchial infection (***C***olonization) by *P. aeruginosa*	No	0
	Yes	1
Radiological ***E***xtension (no. of lobes)[a]	1–2 lobes	0
	More than 2 lobes	1
Dyspnea (modified MRC scale)	0–II	0
	III–IV	1

[a] *Middle lobe and lingula considered as independent lobes.*

Abbr. FEV1: forced expiratory volume in the first second; MRC: Medical Research Council.

E-FACED classification of severity:

0–3 points: mild bronchiectasis.

4–6 points: moderate bronchiectasis.

7–9 points: severe bronchiectasis.

Figure S5. Bronchiectasis Severity Index (BSI)[8].
https://www.bronchiectasis.eu/severity-assessment

Severity criteria	0 points	1 point	2 points	3 points	4 points	5 points	6 points
Age	<50		50-69	-	70-79	-	80+
BMI kg/m2	≥18.5		<18.5	-	-	-	-
FEV1 % predicted	>80%	50-80%	30-49%	<30%	-	-	-
Hospital admissions in the past 2 years	No					Yes	
Exacerbation frequency in last 12 months	0-2		3 or more				
MRC dyspnoea score	1-3		4	5			
Colonisation status	Not colonised	Chronic colonisation		*P. aeruginosa* colonisation			
Radiological severity	<3 lobes involved	3 or more lobes or cystic changes					

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