The biology of pulmonary exacerbations in bronchiectasis

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"The hardest thing of all is to find a black cat in a dark room, especially if there is no cat." There is a lack of knowledge about exacerbation of bronchiectasis. Future efforts are required to better define the biology of exacerbations. http://bit.ly/2XgjaHG

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ABSTRACT Bronchiectasis is a heterogeneous chronic disease. Heterogeneity characterises bronchiectasis not only in the stable state but also during exacerbations, despite evidence on clinical and biological aspects of bronchiectasis, exacerbations still remain poorly understood. Although the scientific community recognises that bacterial infection is a cornerstone in the development of bronchiectasis, there is a lack of data regarding other trigger factors for exacerbations. In addition, a huge amount of data suggest a primary role of neutrophils in the stable state and exacerbation of bronchiectasis, but the inflammatory reaction involves many other additional pathways. Cole's vicious cycle hypothesis illustrates how airway dysfunction, airway inflammation, infection and structural damage are linked. The introduction of the concept of a "vicious vortex" stresses the complexity of the relationships between the components of the cycle. In this model of disease, exacerbations work as a catalyst, accelerating the progression of disease. The roles of microbiology and inflammation need to be considered as closely linked and will need to be investigated in different ways to collect samples. Clinical and translational research is of paramount importance to achieve a better comprehension of the pathophysiology of bronchiectasis, microbiology and inflammation both in the stable state and during exacerbations.

Heterogeneity is the major challenge of bronchiectasis

Interest and awareness of bronchiectasis are increasing across both scientific and patient communities [1]. Bronchiectasis is recognised as a chronic respiratory disease characterised by an anatomic alteration (abnormal and permanent dilatation of the bronchi) associated with specific clinical characteristics (cough, sputum production and recurrent respiratory infections) that represents the final common pathway of different disease processes [2, 3]. The identification of the underlying aetiology of bronchiectasis is one of the main challenges, along with the heterogeneity, which also applies to other aspects of the disease [4]. From a radiological point of view, the extent of bronchiectasis can range from focal to diffuse disease, and only recently a dedicated score to evaluate radiological severity has been developed and validated in Europe [5].

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From a functional point of view, patients with bronchiectasis might show a variety of patterns ranging from normal lung function to pathophysiological abnormalities, including obstructive, restrictive, isolated air trapping or mixed patterns [6, 7]. From a clinical point of view, some patients might be paucisymptomatic. In other patients, bronchiectasis may be detected unexpectedly through haemoptysis or pneumonia, whereas others again may have daily symptoms of cough and sputum production with periodic exacerbations [8]. From a microbiological point of view, *Pseudomonas aeruginosa* and *Haemophilus influenzae* are the most common bacteria detected in bronchiectatic airways but other pathogens including fungi, mycobacteria and viruses can colonise and/or infect patients with bronchiectasis [9–11]. Detected microorganisms and the balance between different microorganisms may vary among different continents and nations as pointed out in a recent paper by Chandrasekaran *et al.* [12]. Geographic variations in the airway microbiology of bronchiectasis contribute to the observed differences in the epidemiology of the disease [12].

In light of the huge heterogeneity of the disease, international efforts have been made to identify clinical phenotypes in patients with bronchiectasis [13, 14]. A clinical phenotype identifies a cluster of bronchiectasis patients sharing similar clinical characteristics and biological pathways, and who might respond to the same treatment [13]. Data from five European bronchiectasis registries identified four clinical phenotypes mainly based on microbiological isolations and respiratory symptoms: *Pseudomonas* (16%), other chronic infection (24%), daily sputum (33%) and dry bronchiectasis (27%) [13]. None of these phenotypes have been replicated in independent cohorts. Furthermore, these studies did not consider the presence of specific endotypes. An endotype is defined as a specific biological trait which characterises a group of patients with bronchiectasis and that could also act as a target for a specific intervention [15]. Expanding work on phenotypes and endotypes is of paramount importance to better understand the pathophysiologic development of bronchiectasis and to select possible treatable traits of the disease [16]. Heterogeneity characterises bronchiectasis not only in the stable state but also during exacerbations, although evidence on clinical and biological aspects of bronchiectasis exacerbations still remain poorly understood.

Defining exacerbations in bronchiectasis: a clinical challenge

The identification of specific thresholds for the variation of each patient's daily symptoms and the minimum time required to start an exacerbation represent important challenges in the clinical management of bronchiectasis. Finding the specific parameters necessary to define an exacerbation is equally tricky. For this purpose, different definitions of exacerbations have been proposed over the past decade. Kapur *et al.* [17] found that changes in cough frequency or character, fever and increase in purulent sputum are the most common features of bronchiectasis exacerbations. In the EMBRACE trial, an exacerbation was defined as an increase or the new onset of more than one of the following pulmonary symptoms: sputum volume, sputum purulence and dyspnoea [18]. The Spanish guidelines [19] and the British Thoracic Society (BTS) guidelines [20] proposed different clinical definitions of exacerbations of bronchiectasis and, despite the definitions seeming to be similar, there are some peculiarities that differentiate them considerably (table 1). The updated version of the Spanish guidelines [21] also introduced the concept of "very severe exacerbations" which includes haemodynamic instability, altered mental status or the need for admission to an intensive or intermediate care unit.

In 2016, an international group of investigators developed a consensus definition of exacerbations for clinical trials based on a systematic review of papers published over the past 15 years [22]. An exacerbation was defined as a change in bronchiectasis treatment associated with 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; or haemoptysis. We note that this definition still awaits validation in different settings [22, 23].

Clinical impact of bronchiectasis exacerbations

Exacerbations have a cornerstone role in bronchiectasis in terms of healthcare costs and negative impact on patient prognosis [24, 25]. A large number of studies have shown that an increased frequency of exacerbations results in increased airway and systemic inflammation and are associated with progressive lung damage, worse quality of life, accelerated lung function decline and increased mortality [13, 25–27]. Reduction of the frequency of exacerbations and/or shortening time to the first exacerbation represent two of the most reported outcomes in clinical trials in bronchiectasis [23, 28]; however, the pathobiology of exacerbations is poorly understood and even their clinical manifestations are widely heterogeneous. Exacerbations are generally considered as infectious events triggered by bacterial agents, and current guidelines suggest antibiotic treatment to manage exacerbations [23, 28]. Despite these recommendations, other potential causes of exacerbations in bronchiectasis have not thoroughly been investigated and comorbidities could also play a relevant role, as is the case in other respiratory diseases [29, 30]. For example, the association between high levels of environmental pollution and exacerbations suggests a role for pollutants in increasing airway inflammation, suppressing host immunity and disturbing the biofilm,

TABLE 1 Definition of exacerbation according to different guidelines

Spanish guidelines 2008 [19]	British guidelines 2010 [20]	Consensus definition for clinical research 2016 [22]	Spanish guidelines 2017 [21]
At least one of the following: 1. Changes in sputum characteristics (increased volume, thicker consistency, greater purulence, or haemoptysis) 2. Increased breathlessness unrelated to other causes	Worsening of one or more of the following: 1. Increasing sputum volume or purulence 2. Worsening dyspnoea 3. Increased cough 4. Decline in lung function 5. Increased fatigue/ malaise	Deterioration in three or more of the following key symptoms for at least 48 h: 1. Cough 2. Sputum volume and/or consistency 3. Sputum purulence 4. Breathlessness and/or exercise tolerance 5. Fatigue and/or malaise 6. Haemoptysis	Increasing cough AND changes in sputum characteristics (increased volume, thicker consistency, greater purulence)
May be accompanied by: 1. Worsening of cough 2. Fever 3. Asthenia 4. General discomfort 5. Anorexia 6. Weight loss 7. Pleuritic chest pain 8. Physical changes in the lungs found during examination 9. Chest radiograph findings suggestive of infection 10. Declining lung function 11. Elevated markers of systemic inflammation	AND new appearance of one or more of the following: 1. Fever 2. Pleurisy 3. Haemoptysis 4. Requirement of antibiotic treatment	AND a clinician determines that a change in bronchiectasis treatment is required	May be accompanied by: 1. Worsening dyspnoea 2. Fever 3. Asthenia 4. General discomfort 5. Anorexia 6. Weight loss 7. Chest pain 8. Haemoptysis 9. Changes in thoracic objective exam 10. Requirement of changes in bronchiectasis treatment 11. Declining lung function

Severe exacerbation: a severe exacerbation is one in which there is tachypnoea, acute respiratory failure, exacerbated chronic respiratory failure, a significant decline in oxygen saturation or respiratory function, hypercapnia, fever >38°C, haemoptysis, haemodynamic instability, and/or impaired cognitive function. Very severe exacerbation: haemodynamic instability, altered mental status or the need of admission to an intensive or intermediate care unit.

thus leading to an exacerbation [31]. Gastro-oesophageal reflux disease is one of the most important comorbidities in bronchiectasis and has been associated with an increased number of exacerbations and hospitalisations [30, 32, 33].

The frequent exacerbator phenotype

In recent years, some attempts have been made to identify the "frequent exacerbator" phenotype in bronchiectasis to identify future targets for preventive therapies [34, 35]. To be useful in clinical practice the phenotype needs to be stable over time and linked to relevant outcomes. Chalmers *et al.* [34] demonstrated a positive correlation between the median number of exacerbations in the previous year and the risk of future exacerbations. These data were particularly strong in patients with more than three exacerbations per year, reflecting on outcomes such as a worse quality of life measured with the St George's Respiratory Questionnaire and an increased rate of hospitalisation and mortality over 5 years [34]. Furthermore, these data were supported by the findings of Martinez-Garcia *et al.* [35] in a South American cohort showing higher mortality rates in patients with a history of two exacerbations or hospitalisation due to bronchiectasis in the previous year.

Despite these data, there is no consensus concerning the minimum number of exacerbations that defines a "frequent exacerbator patient" although the 2017 European Respiratory Society guidelines identified three or more exacerbations as the threshold to start chronic antibiotic therapy to prevent future exacerbations [23]. Moreover, little is known regarding the causative factors that lead to an increased rate of exacerbations. Chronic infection seems to be a relevant microbiological factor. Indeed, bronchiectasis patients with *P. aeruginosa* chronic infection have worse outcomes, such as an increased risk of hospitalisation and an

increased frequency of exacerbations [36–39]. Other chronic infections, as highlighted in a study by Chalmers *et al.* [34], are associated with an increased frequency of exacerbations, despite the lack of a clear association with mortality. Araújo *et al.* [40] showed that the combination of *P. aeruginosa* infection and a higher number of exacerbations and hospitalisations in the previous year led to an increased risk of death in bronchiectasis. However, the same study found that there was a small group of patients with *P. aeruginosa* chronic infection without an increased rate of exacerbations. It is difficult to determine the reason why these patients do not exacerbate frequently but the possibility that this group is at increased risk of death cannot be excluded. From this perspective, research priorities include the need to better understand the role of *P. aeruginosa* and identify the risk factors leading to disease progression, increased exacerbation rate and poor outcomes in patients with bronchiectasis [41].

Exacerbations sustain the "vicious vortex" of bronchiectasis

Our understanding of bronchiectasis pathophysiology is limited, even though Cole's "vicious cycle hypothesis" is widely accepted as the paradigm of bronchiectasis development and progression [42]. The hypothesis emphasises the role of four different components: impaired lung defences, inflammatory response, airway damage and infection. The "entry point" which triggers the cycle is variable depending on the underlying aetiology of bronchiectasis [43]. For instance, primary ciliary dyskinesia and immunodeficiency impair host defences leading to an increased susceptibility to infection [44, 45]. Inflammatory bowel disease and rheumatoid arthritis stimulate an exaggerated lung inflammatory response promoting lung structural damage [46, 47]. Aspiration causes direct tissue damage [48]. Moreover, infections due to nontuberculous mycobacteria (NTM) encourage structural damage and lung inflammation [11]. Some comorbidities act on the vicious cycle through several entry points. In chronic rhinosinusitis, an inflammatory response begins in the upper airway and subsequently involves the lower airway; chronic infection from the upper airway often extends to the lower airway through the migration of secretions [49]. In addition, migration of secretions from the upper airway may also impair mucus clearance in the lower airways [49].

Exacerbations are acute events that may accelerate the vicious cycle irrespective of the cause that is responsible for the exacerbation, generating a hypothetical vicious vortex [50]. Targeting one aspect in isolation, such as antibiotic use during an exacerbation to eliminate or reduce bacterial infection, only blocks a single pathway of the disease and is likely to exert a modest impact on the overall progression of the condition and on clinical outcomes. A multimodal approach would be optimal in order to better target all the aspects of the disease that may be interdependent when the vortex is generated [16, 50]. Therefore, identifying the aetiologies of the exacerbation and finding biomarkers capable of predicting future exacerbation risk and/or early detection of exacerbation development and response to therapy are of paramount importance [41].

Microbiology during the stable state and exacerbations

Infection is one of the key points in both the stable state and during exacerbations of bronchiectasis. Current guidelines suggest obtaining sputum cultures at least every year in stable bronchiectasis [23, 51]. Collecting lower airway specimens for microbiological examination is also recommended at the beginning of an exacerbation to address targeted antibiotic therapy [23, 51].

Through traditional sputum cultures, the most common bacteria detected in patients with stable bronchiectasis are *P. aeruginosa* and *H. influenzae*. Other bacteria isolated in sputum are NTM, *Moraxella catarrhalis, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella* spp. and *Escherichia* spp. [9, 10, 25]. The relative frequencies of these bacteria in sputum samples vary among different populations (table 2). In European studies, *H. influenzae* and *P. aeruginosa* are the most prevalent organisms isolated during the stable state as shown by the EMBARC registry [52, 53]. In Northern Europe the most common bacteria isolated in bronchiectasis patients is *H. influenzae*, while *P. aeruginosa* is the predominant bacteria in Southern Europe [53]. In particular, the proportion of bronchiectasis patients with *P. aeruginosa* infection in Italy, Greece and Turkey is >50% [53].

In Asia, as well as in Europe, data provided from different cohorts indicates that *P. aeruginosa* and *H. influenzae* are the most common bacteria isolated with a low detection rate for NTM [54–56].

The US Bronchiectasis Research Registry provides different data compared with European and Asian cohorts. The prevalent microorganisms detected in the airways of bronchiectasis patients are NTM with an isolation rate of ~50% [57]. The high rate of NTM isolation could be explained by the abundance of NTM lung disease referral centres involved in the Bronchiectasis Research Registry [57–59]. Among NTM, the most common species isolated are *Mycobacterium avium* complex, *M. abscessus* and *M. chelonae* [57]. In over 1800 patients evaluated by the Bronchiectasis Research Registry, other bacteria isolated less frequently

TABLE 2 Differences in microbiological prevalence among different continents

	USA[#]	Europe[¶]	Asia[+]
Haemophilus influenzae	8	57	
Streptococcus pneumoniae	3	33	
Staphylococcus aureus	12	23	1
Pseudomonas aeruginosa	33	49	20
Stenotrophomonas maltophilia	5	16	1
Klebsiella pneumoniae	2	14	4
Moraxella catarrhalis	1	24	
Achromobacter	1	7	
Alcaligenes	1		
Serratia marcescens	2	10	
***Burkholderia* species**	0		
Escherichia coli		13	1
Acinetobacter		6	1
Enterobacter cloacae		5	1
Proteus mirabilis		5	
Mycobacterium tuberculosis			4
NTM	50	3	2
***Mycobacterium avium* complex**	37		
Mycobacterium abscessus/chelonae	10		
Mycobacterium kansasii	1		
Mycobacterium gordonae	3		
Other mycobacterial species	3		
Nocardia	1		
***Aspergillus* species**	19	10	6
Scedosporium apiospermum	3		
Other fungal species	36		
***Candida* species**		11	6

Data are presented as %. NTM: nontuberculous mycobacteria. [#]: n=60; [¶]: n=56; [+]: n=59.

than NTM are *P. aeruginosa* (33%), *S. aureus* (12%), *H. influenzae* (8%) and *Stenotrophomonas maltophilia* (5%) (table 2) [57].

Limited data suggest that during exacerbations, patients with bronchiectasis frequently isolate the same bacterial species that they typically grow in sputum when stable [60, 61]. The species isolated during an exacerbation could be the result of an increase in bacterial load of a pre-existing bacterial strain or may be associated with acquisition of new strains as has been observed in COPD [62]. Polverino *et al.* [63] considered two different groups of bronchiectasis patients during exacerbation. The first group included patients with exacerbation leading to community-acquired pneumonia (CAP), whereas the second group included exacerbated patients without CAP. Exacerbation was defined according to the Spanish guidelines and the authors differentiated the presence of CAP in the context of bronchiectasis *versus* an exacerbation without CAP when a new radiological infiltrate was detected on the chest radiograph by the attending physician and confirmed by an external radiologist [19]. If the radiologist did not agree on the diagnosis of pneumonia, the exacerbation was considered as a case of an exacerbation without CAP. The microbiology samples show that *S. pneumoniae* was the most frequent bacteria isolated in the CAP group, whereas *P. aeruginosa* was the most represented in the non-CAP group [63].

Among the infective causes of an exacerbation, bacteria play a leading role but, as stated in the 2019 BTS guidelines, we lack data regarding the impact of viruses and fungi [51]. There are only a few studies investigating the prevalence of respiratory viruses in adults and children with bronchiectasis during the stable state and exacerbations [64–66]; however, the role of viruses is well defined in other respiratory diseases as trigger factors for exacerbations, particularly in asthma and COPD [67–70]. Data from a Chinese cohort show that virus prevalence is higher during exacerbations than in stable state bronchiectasis [64]. The previously mentioned study by Polverino *et al.* [63] confirms a high prevalence of respiratory viruses during exacerbations in Europe.

The role of fungi in bronchiectasis and particularly their role as a trigger factor for exacerbation is still unclear and there is a great paucity of data on this argument [41]. Most microbiological studies in bronchiectasis involve bacteria, while fungi are often considered as an incidental detection [12, 41]. *Aspergillus* spp. are the most common fungi isolated in the sputum of patients with bronchiectasis. *A. fumigatus* can be both a

pathogen or an allergen but its role in provoking exacerbations is still unclear [71, 72]. Other fungi less commonly isolated are *Penicillium, Scedosporium* and *Fusarium* spp. Interestingly, in a Spanish study, antibiotic therapy was associated with a higher prevalence of fungal colonisation [73]. In two studies from the UK, *A. fumigatus* colonisation or sensitisation was related to NTM infection and associated with a higher mortality rate in bronchiectasis [74, 75].

In recent years, interest concerning NTM has grown considerably. The estimated prevalence of these ubiquitous environmental organisms ranges from 0% to 60% in patients with bronchiectasis [57, 76–78]. It is as yet unclear whether NTM actively cause bronchiectasis development or whether these agents colonise and infect patients with a pre-existing predisposing condition (a chicken and egg situation). NTM lung disease seems to be increasing in recent years, partly as a result of newer and more sensitive laboratory detection methods [79]. Although there are a number of published guidelines regarding these pathogens, more studies are needed to understand their role in exacerbations [79, 80].

From sputum culture to DNA analysis: the role of microbiome and mycobiome

DNA-sequencing technologies have changed our understanding of pulmonary microbiology in bronchiectasis [81]. In recent decades, huge interest has grown around the role of the pulmonary microbiome and its clinical implications. 16S rRNA gene sequencing is the most common method targeting housekeeping genes to study bacterial phylogeny and taxonomy. The 16S rRNA gene contains several regions that are present in almost all bacteria and it may be used to recognise and classify different taxa and to estimate the richness and evenness of the pulmonary microbiota [81–83]. In the past, the lung of healthy subjects was considered a sterile site because of negative results on standard sputum cultures. Nowadays, through the use of DNA-sequencing technologies, an increasing body of evidence has shown that viruses, bacteria and fungi coexist in the lungs of both healthy and ill people [84, 85]. Furthermore, it is important to consider potential interactions that may increase virulence and pathogenicity as described in nonrespiratory contexts [86]. The development of the microbiome depends on several factors such as translocation from the upper airways, micro-aspiration and the efficacy of host defences [87, 88].

In bronchiectasis, several studies have shown that heterogeneity is the hallmark characteristic in microbiome composition. Indeed, the heterogeneity in microbiome composition has implications on lung function, severity of disease and inflammatory patterns [89, 90].

As previously observed in other respiratory diseases, a loss of bacterial diversity or the predominance of a small group of taxa can be observed in bronchiectasis [91–95]. A loss of bacterial diversity is associated with disease progression, worse lung function and an increased number of exacerbations [60, 89, 90].

The most commonly found genera are *Haemophilus*, *Pseudomonas*, *Moraxella*, *Streptococcus*, *Veilonella, Prevotella, Rothia* and *Klebsiella*. There are differences between standard sputum culture and DNA sequencing [90]. The presence of *H. influenzae* and *M. catarrhalis* seems to be under-recognised with traditional techniques, while *P. aeruginosa* and *S. aureus* may be overestimated on standard culture, probably due to their capacity to outgrow other bacteria [90]. Moreover, the prevalence of anaerobic bacteria varies among sputum culture and DNA-sequencing techniques, and despite the role for anaerobic bacteria in the onset of exacerbations being hypothesised, more studies are needed to clarify the possibility [60].

Changes in richness and evenness of the microbiome, measurable through different indexes, such as the Shannon–Wiener index, are related to worsening lung function in bronchiectasis [96].

The relationship between antibiotic therapy and changes in the microbiome is still unclear. A secondary analysis of the BLESS study (a randomised controlled trial analysing the effect of long-term administration of erythromycin on exacerbation frequency) found that patients without *Pseudomonas* before the start of treatment had changed their microbiome composition at the end of the trial with a great emergence of *Pseudomonas* spp. [97]. These data have important clinical impact, in fact dominance of *Pseudomonas* spp. is associated with more exacerbations and higher levels of inflammatory markers, such as metalloproteinases [97, 98]. A recent study found that the microbiome is similar in the stable state, during an exacerbation managed with antibiotics, and after full recovery from an exacerbation [90]. Indeed, the use of antibiotics to treat an exacerbation only seems to exert a temporary effect on microbiome composition. A few weeks after treatment it is no longer possible to highlight differences in microbiome composition [60]. Byun *et al.* [99] investigated differences in the microbiome during the stable state and during exacerbations in patients with bronchiectasis but failed to find differences in microbial community composition. This suggests that exacerbations may be triggered by a shift in microbiota behaviour rather than a shift in its composition [99].

Standard bacterial studies on 16S rRNA sequencing have failed to clarify the role of fungi and viruses in chronic respiratory diseases [100]. There are some data regarding mycobiome composition conducted on

the internal transcribed spacer region of fungi suggesting that in respiratory disease there is a correlation between reduction in fungal diversity and decline of lung function [101, 102]. In cystic fibrosis (CF), mycobiome composition stability was reported after the completion of antifungal therapy during an exacerbation [103]. *Aspergillus* is significantly more represented in the mycobiome of individuals with bronchiectasis compared with healthy people and its presence correlates with exacerbations [104].

Little is known about the virome in bronchiectasis, and molecular methods to identify viruses present in the respiratory tract without *a priori* knowledge of the organism are expensive. Moreover, the lack of a universal viral molecular marker, such as 16S rRNA, or internal transcribed spacer region represents an important challenge in the understanding of the virome [105]. A study conducted on patients with respiratory tract infections showed that the most represented viruses are Paramyxoviruses followed by Picornaviruses and Orthomyxoviruses, whereas in a small sample of patients with CF reticuloendotheliosis virus, Epstein–Barr virus, Human Herpesvirus 6B and Human Herpesvirus 8 were found [106, 107]. More studies are needed to assess the role of the virome in bronchiectasis.

Inflammatory markers and cytokines in stable bronchiectasis and during exacerbations

Neutrophilic inflammation in the stable state and during exacerbations

Bronchiectasis is considered a chronic inflammatory disorder mainly driven by neutrophilic airway inflammation [108]. The presence of an elevated number of neutrophils in the airway of patients with bronchiectasis was found both in both the stable state and during exacerbations [109–111]. Moreover, previous studies found that patients with bronchiectasis with chronic infection, in particular with *P. aeruginosa*, are characterised by higher levels of neutrophils in sputum compared with patients with bronchiectasis without chronic infection [27, 109, 110]. Indeed, bacterial colonisation promotes neutrophil recruitment through upregulation of adhesion markers that are elevated in patients with bronchiectasis [27]. Despite the abundance of neutrophils in the airways of patients with bronchiectasis, the functional capacity of neutrophils is blunted, generating the concept of a neutrophil paradox [112, 113]. A study conducted by Bedi *et al.* [113] found that neutrophils show delayed apoptosis and increased activation in stable state bronchiectasis, irrespective of disease severity. Interestingly, neutrophils isolated in sputum reveal defective phagocytosis, favouring inefficient bacterial killing. Impaired bacterial killing capacity is also evident at the onset of an exacerbation and persists beyond the clinical resolution of the exacerbation [113]. In the same study, Bedi *et al.* [113] illustrated that patients with CAP also show defects in neutrophil killing capacity at the onset of pneumonia, but neutrophil function recovers after a successful course of antibiotics. This study exclusively involved patients with idiopathic bronchiectasis, and its results are in contrast with previously published literature which did not report blood neutrophil defects in bronchiectasis [76, 114]. Further studies are also needed in order to better understand whether the neutrophil paradox is suitable for patients with known aetiologies of bronchiectasis.

Increased degranulation of neutrophil azurophilic granules releases proteases responsible for increased lung damage [108]. One of the best studied proteases is neutrophil elastase (NE), a proteolytic serine-proteinase [108]. NE is involved in several aspects, including ciliary beating rate inhibition, phagocytosis and bacterial killing, extracellular matrix destruction, mucus gland hyperplasia induction, increased mucus production in lung airways and direct airway damage [115–118]. Patients with stable bronchiectasis exhibit higher sputum concentrations of NE than healthy subjects, even in the absence of bacterial colonisation [119]. Moreover, NE progressively increases with increasing bacterial load in the sputum, especially in patients with *P. aeruginosa* chronic infection [27, 109, 120]. As a marker of airway inflammation, NE was also found to increase during exacerbations and decrease after antibiotic treatment [27, 111, 120]. In a study performed by Chalmers *et al.* [27], antibiotic treatment administered for a bronchiectasis exacerbation results in significant reduction of inflammatory markers, such as NE, tumour necrosis factor-α and interleukin-8. Finally, previous evidence showed that higher levels of NE are associated with relevant clinical outcomes in bronchiectasis, such as lung function decline, risk of future exacerbation and all-cause mortality [111, 121]. NE in sputum is easy to measure and seems to be a promising biomarker to categorise disease severity, predict exacerbations and define long-term clinical outcomes in bronchiectasis, but future clinical trials are needed to validate cut-off values for NE activity and implement NE as a useful biomarker in the clinical management of patients with bronchiectasis, both in the stable state and during exacerbations [108].

Beyond neutrophilic inflammation in bronchiectasis

The presence of neutrophilic inflammation in bronchiectasis is widely described in both the stable state and during exacerbations [109–111]. Data concerning the possible role of inflammatory pathways other than neutrophilic during an exacerbation are lacking. Up to 35% of patients in the study by Chalmers *et al.* [111] had levels of NE activity $<0.016\ \mu g \cdot mL^{-1}$ during the stable state and were considered as not

having neutrophilic inflammation. Thus, it seems that inflammation in bronchiectasis is complex and heterogeneous, and the variety in bronchiectasis aetiologies, as well as the lack of an animal model, limit our understanding of the disease pathogenesis. Indeed, up to one-quarter of patients with bronchiectasis have eosinophil-dominant airway inflammation [110, 122]. Macrophages in bronchiectasis airways could be increased [123, 124]. Efferocytosis, the process by which apoptotic or necrotic cells are removed by macrophages (the burying of dead cells), is impaired in bronchiectasis [125]. Failure to clear necrotic cells results in increased inflammatory damage through unopposed granule product release and secondary necrosis of apoptotic cells [126]. Future research should address the role of macrophages and eosinophils not only during the stable state but also during an exacerbation of bronchiectasis.

Immune role of vitamin D

The main role of vitamin D in the regulation of calcium homeostasis and bone health has been known since the beginning of the 20th century [127]. However, it was later discovered that the vitamin D receptor is functional in tissues that are not involved in calcium metabolism [128, 129]. In the past decade, an increasing number of publications focused on the extra skeletal effects of vitamin D, including the effect on the human immune system and both chronic and infectious diseases [130–134]. Vitamin D reduces the production of proinflammatory cytokines and regulates the secretion of antimicrobial peptides such as cathelicidin (LL-37) which has potent antimicrobial activity against *P. aeruginosa* [130–132]. Furthermore, a mouse model study showed that vitamin D deficiency directly caused alterations in the lung structure and reduced lung function [133]. A recent study conducted by Chalmers *et al.* [134] highlighted that 93% of patients with bronchiectasis were either vitamin D deficient or insufficient, a percentage that was significantly higher in comparison with the control group. Patients with vitamin D deficiency are more commonly colonised, have lower forced expiratory volume in 1 s and a more rapid decline of lung function over a 3-year follow-up period, have more frequent pulmonary exacerbations and have higher sputum levels of inflammatory markers (myeloperoxidase, NE and interleukin-8). Given this evidence, it is intriguing to speculate that vitamin D supplementation could be a reasonable therapeutic approach for patients with bronchiectasis. Indeed, in patients with CF, vitamin D has been part of the standard treatment for many decades [135]. The issue of the desirable target serum level (currently set at >30 ng·mL^{-1}) could be thorny if we consider administering vitamin D not just for preserving bone health but with the intent of preventing lung diseases. Based on data currently available for patients with CF, the dose needed in order to obtain a maximum immunomodulatory function is probably higher than the dose required for optimal bone health [136]. A pilot study recently conducted in New Zealand found that serum vitamin D levels in adults with bronchiectasis significantly increased after vitamin D oral supplementation [137]. Designed randomised controlled trials are needed to demonstrate whether vitamin D supplementation is associated with measurable disease outcomes, such as exacerbation rate and lung function decline, to define which patients would benefit most from treatment, together with the serum levels that have to be attained and the optimal doses.

Conclusions

Bronchiectasis is a very heterogeneous disease, both in the stable state and during exacerbations. Although the scientific community recognises that exacerbations play a key role in the progression of the disease, there is a lack of data regarding trigger factors for exacerbations. Currently, infections are considered as the only cause of exacerbations with a leading role reserved for bacteria. We lack data regarding NTM, viruses and fungi in bronchiectasis and their role as trigger factors for exacerbations is still unclear. The increasing use of DNA-sequencing techniques in the study of the microbiota will provide a more complete perspective on the role of microbiology, both in the stable state and during exacerbations of bronchiectasis. If our knowledge on the microbiota is so far scant, even less is known about inflammatory markers and cytokines in bronchiectasis. Data suggest a primary role for neutrophils, but the inflammatory reaction in bronchiectasis involves many other additional factors. Cole's vicious cycle hypothesis, revised by Flume *et al.* [50], illustrates how airway dysfunction, airway inflammation, infection and structural damage are linked. The introduction of the concept of a vicious vortex stresses the complexity of the relationship between the components of the cycle. In this model of disease, exacerbations work as a catalyst, accelerating the progression of disease. The roles of microbiology and inflammation need to be considered as closely linked and will need to be investigated by different ways to collect samples. Translational research is of paramount importance to achieve reliable results. A better comprehension of the pathophysiology of bronchiectasis, microbiology and inflammation, could allow the development of more characterised phenotypes and endotypes in order to develop new clinical trials and find individualised treatment targets.

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