Aspergillus-Associated Endophenotypes in Bronchiectasis

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Semin Respir Crit Care Med 2021;42:556–566.

Abstract

Bronchiectasis is a chronic condition of global relevance resulting in permanent and irreversible structural airway damage. Bacterial infection in bronchiectasis is well studied; however, recent molecular studies identify fungi as important pathogens, either independently or in association with bacteria. *Aspergillus* species are established fungal pathogens in cystic fibrosis and their role is now increasingly being recognized in noncystic fibrosis bronchiectasis. While the healthy airway is constantly exposed to ubiquitously present *Aspergillus* conidia in the environment, anatomically damaged airways appear more prone to colonization and subsequent infection by this fungal group. *Aspergilli* possess diverse immunopathological mechanistic capabilities and when coupled with innate immune defects in a susceptible host, such as that observed in bronchiectasis, it may promote a range of clinical manifestations including sensitization, allergic bronchopulmonary aspergillosis, *Aspergillus* bronchitis, and/or invasive aspergillosis. How such clinical states influence "endophenotypes" in bronchiectasis is therefore of importance, as each *Aspergillus*-associated disease state has overlapping features with bronchiectasis itself, and can evolve, depending on underlying host immunity from one type into another. Concurrent *Aspergillus* infection complicates the clinical course and exacerbations in bronchiectasis and therefore dedicated research to better understand the *Aspergillus*-host interaction in the bronchiectasis airway is now warranted.

Keywords
- noncystic fibrosis bronchiectasis
- Aspergillus
- endophenotypes
- fungi
- mycobiome

Bronchiectasis is a chronic, progressive, and irreversible airways disease characterized by bronchial dilatation and copious sputum production, and it is often complicated by recurrent chronic pulmonary infections and exacerbations.[1] Globally, the disease is increasing in prevalence despite continued under recognition, and it remains an important cause of respiratory morbidity and poorer quality of life.[2] While approximately 50% of cases are idiopathic, one of the commonest causes of bronchiectasis is postinfection, predominantly after severe pneumonia or tuberculosis. Other important causes include cystic fibrosis (CF), primary ciliary dyskinesia, immunodeficiencies, and allergic bronchopulmonary aspergillosis (ABPA). However, importantly bronchiectasis is often seen in relation to other primary lung diseases including severe asthma and/or chronic obstructive pulmonary disease (COPD). In this article, we use the term "bronchiectasis" in relation to etiologies unrelated to CF.

While bacteria are traditionally recognized as the predominant infection-driving pathogens in bronchiectasis, recent advances in next generation microbiome sequencing approaches reveal an important role for other kingdoms including fungi. Fungi, such as *Aspergillus* may act in isolation

Issue Theme Bronchiectasis: Advances in Diagnosis and Treatment; Guest Editor: James D. Chalmers, MBChB, PhD, FRCPE, FERS

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0041-1730947.
ISSN 1069-3424.

as respiratory pathogens, or alternately in complex interplay with established bronchiectasis pathogens such as *Pseudomonas*.[3,4] Disease progression is facilitated through the vicious cycle, or the more recently described vicious vortex of infection, inflammation, epithelial dysfunction, and impaired mucociliary clearance.[5] Considering this model of pathogenesis in relation to fungal exposure and disease, a healthy immunocompetent host, armed with effective mucociliary clearance mechanisms and robust immunity, can successfully clear inhaled fungal conidia and avoid disease.[6–10] Hosts such as those with established bronchiectasis, however, lack such protective mechanisms, making them inherently susceptible to fungal colonization and/or infection. *Aspergillus fumigatus* is therefore recognized as an important colonizer of the bronchiectasis airway and remains the most widely recognized fungus in relation to bronchiectasis. Despite high frequencies of airway *Aspergillus* in bronchiectasis, much of our current understanding in regard to its pathogenic potential is extrapolated from other respiratory disease states such as CF and COPD. However, this filamentous fungal pathogen has been associated with increased mucus production, purulence, and exacerbation frequency in bronchiectasis.[11]

Aspergillus-associated lung disease states and their related clinical consequences in bronchiectasis remain understudied and warrant attention in view of their increasing recognition, frequency, and clinical importance.[4,12,13] Traditional and challenging methods of fungal detection including culture and/or microscopy are now complemented by more sophisticated and sensitive molecular detection methods including quantitative polymerase chain reaction (qPCR) and, increasingly, next-generation sequencing (NGS) approaches, which have allowed earlier detection and therefore renewed attention to the deleterious consequences of *Aspergillus*-associated pathologies in chronic lung diseases such as bronchiectasis.[3] Here, we review the key *Aspergillus*-associated pathologies as relevant to bronchiectasis, with a focus on NGS approaches in the context of endophenotyping this complex and heterogenous respiratory disease.

The Clinical Significance and Spectrum of *Aspergillus*-Associated Disease in Bronchiectasis

While the role *of Aspergillus* in CF, asthma and COPD is recognized, *Aspergillus*-associated disease in the setting of bronchiectasis remains understudied and significant gaps exist regarding its epidemiology, pathogenesis, diagnosis, and management.[12,14] Fungal cultures are not routinely performed and remain limited by their lack of sensitivity to detect fungi in comparison with molecular approaches including NGS, which thus far have only been employed in research settings.[15,16] Variable recovery rates of *Aspergillus* from sputum culture, ranging from 6.9 to 24.0%, are routinely reported between centers, demonstrating the significant diagnostic challenge this organism presents in routine clinical practice (►**Fig. 1**).[11,17] Chronic and invasive forms of *Aspergillus*-associated disease are more readily detectable in those immunocompromised; however, less consensus on diagnostic approaches in stable immunocompetent bronchiectasis exists. The role and relevance of serum galactomannan, a widely used biomarker in chronic and invasive aspergillosis (IA), is not fully understood in the context of bronchiectasis, particularly in patients with features of early pulmonary aspergillosis. An additional diagnostic challenge in bronchiectasis are the largely indistinguishable radiological features that differentiate underlying bronchiectatic change from ongoing pulmonary aspergillosis, leading to under-recognition of the latter.[14] Dependent on underlying host immunity, *Aspergillus* can act independently to cause

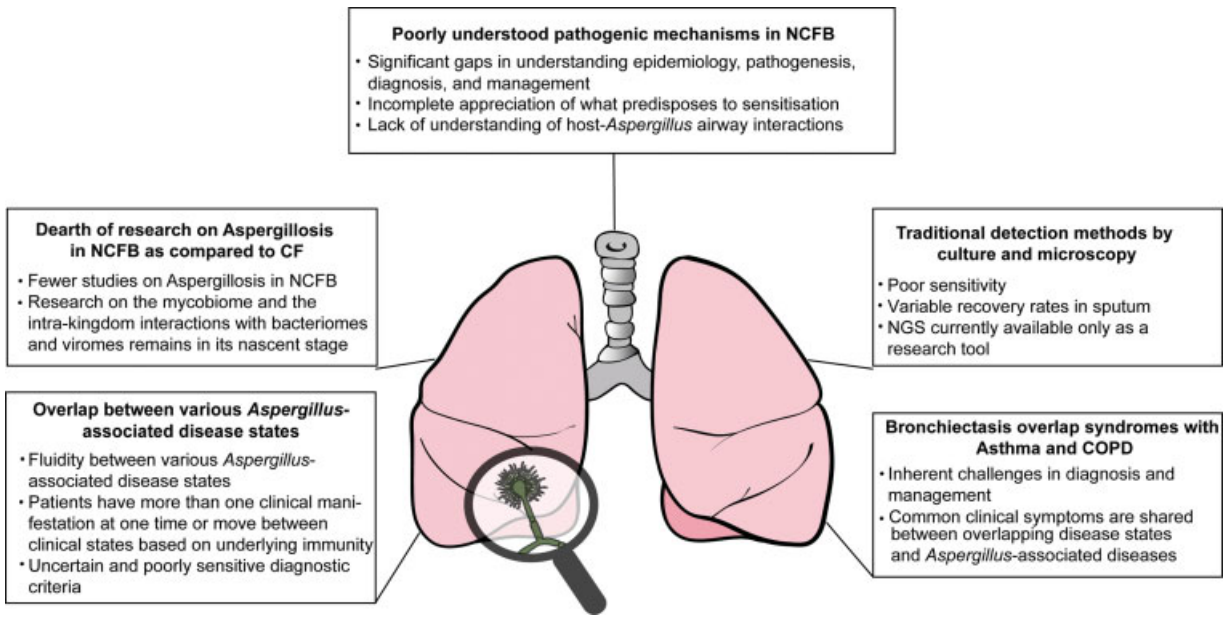

Fig. 1 Diagnostic challenges in *Aspergillus*-associated disease in bronchiectasis. NCFB, noncystic fibrosis bronchiectasis; CF, cystic fibrosis; NGS, next generation sequencing.

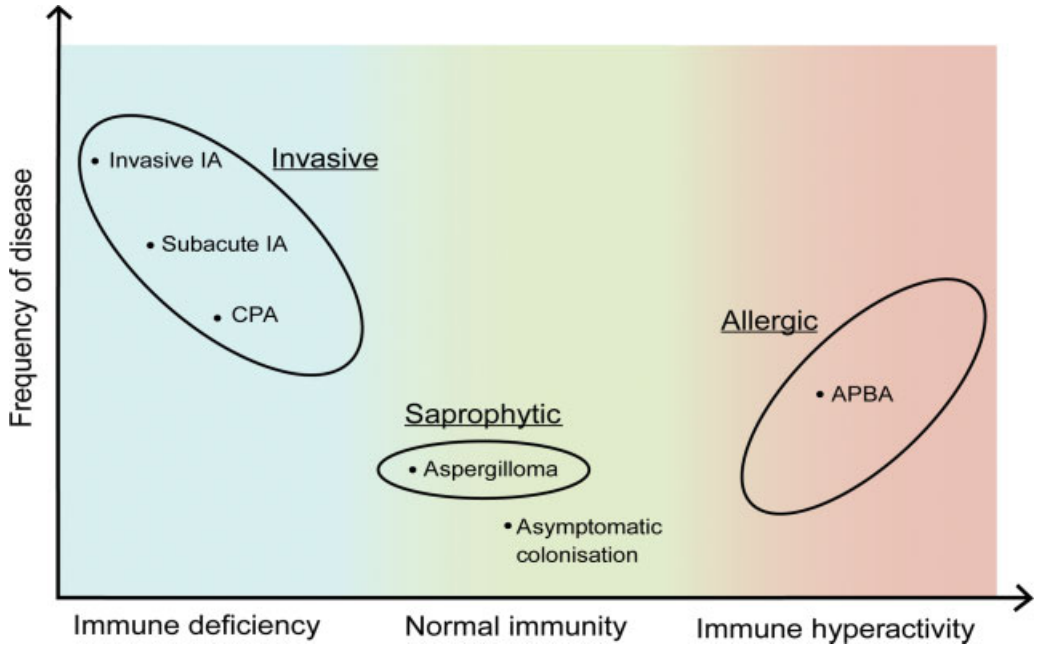

Fig. 2 The clinical spectrum of pulmonary *Aspergillus*-associated disease. ABPA, allergic bronchopulmonary aspergillosis; CPA, chronic pulmonary aspergillosis; IA, invasive aspergillosis.

direct pulmonary damage leading to bronchiectasis or alternately trigger a spectrum of syndromes that complicate preexisting bronchiectasis[13,18–20](►**Fig. 2**). The ubiquitous nature of *Aspergillus* spores in the surrounding environment coupled to their small size (2–5 µm) favor dispersal to the most distal airways, and the inhaled fungal burden may be especially high in hot and humid environments potentially explaining the geographic variation in *Aspergillus*-associated disease.[16] The clinical gamut of *Aspergillus* lung disease primarily depends on fungal–host interaction, a key area of ongoing research.[7,12,21,22] The balance between host defenses and *Aspergillus* proliferation broadly determines the clinical outcome, which can range from asymptomatic colonization to sensitization and ABPA in the immunocompetent and allergic to chronic and invasive disease in the immunocompromised[7,10,18,23] (►**Fig. 2**). The type and severity of *Aspergillus* disease therefore directly relates to the host immune response and underlying anatomical abnormalities, including bronchiectasis, that demonstrate immunodeficiency states and high frequencies of sensitization in a significant number of patients.[24] It is also common to observe *Aspergillus*-related pathology evolve from one defined clinical state into another, in the same patient and over time, under the influence of the immune system and/or treatment the patient may be receiving such as steroids. Such dynamic ongoing change to host immunity, particularly in the setting of bronchiectasis, increase the difficulties in diagnosing and managing such infections.[14]

Broadly, clinical syndromes related to pulmonary aspergillosis in the setting of bronchiectasis may be classified as allergic disease, saprophytic infection, or invasive disease[20] (►**Fig. 2**). ABPA represents one of the commonest *Aspergillus*-related endophenotypes in bronchiectasis and represents a hypersensitivity response to *Aspergillus* antigens, located in the airway in a sensitized host. Involving an unrestrained immune-inflammatory airway response by macrophages, neutrophils, and fungal proteases, excessive damage to the airways can result.[13] A complex association between ABPA and bronchiectasis exists, and its underlying pathogenic mechanisms are of increasing research interest. ABPA is an established cause of bronchiectasis but more commonly is a consequence of preexisting disease. An ABPA prevalence of up to 10% is observed in idiopathic bronchiectasis, while causal links between ABPA and bronchiectasis are described due to the persistent and exaggerated immune response characteristic of ABPA.[25,26] Current data suggest that ABPA leads to poorer clinical outcomes and a higher risk of exacerbations in bronchiectasis.[27–29] Disproportionate immune responses and variable *Aspergillus* virulence can predispose to fungal bronchitis and chronic pulmonary aspergillosis (CPA) in bronchiectasis, comparable to that observed in other chronic lung diseases such as COPD.[12,30] Interestingly, tuberculous and nontuberculous mycobacterial (NTM) infection established bronchiectasis etiologies closely associated with CPA occurrence.[19,31] Direct correlations between NTM and *A. fumigatus* sensitization are observed, and both *Aspergillus* lung disease and NTM remain important independent predictors of mortality in patients with bronchiectasis.[19,31] Cavitary lesions in individuals with post-tuberculosis (TB) bronchiectasis can result in saprophytic noninvasive Aspergillomas, while coexisting bronchiectasis in patients with asthma or COPD increase the risk of *Aspergillus*-associated disease while conversely, chronic *Aspergillus* infection in any setting can increase the chance of developing subsequent bronchiectasis.[32–34] Invasive aspergillosis represents the most serious and lethal form of *Aspergillus*-associated disease, most commonly seen in the immunocompromised, but importantly described in

association with structural airway damage such as that seen in bronchiectasis which increases risk.[13]

Aspergillus-Associated Disease and Its Related Endophenotypes in Bronchiectasis

Increasing molecular evidence including NGS studies reveal important *Aspergillus*-associated endophenotypes in bronchiectasis which merit recognition owing to their potential increased risk of exacerbations and poorer clinical outcomes coupled to the inherent complexity in their diagnosis and management.[12] The etiopathogenesis of aspergillosis in bronchiectasis is wide ranging and leads to both the development of further bronchiectasis and decompensation of existing disease.[13] Studies in patients with bronchiectasis and aspergillosis illustrate that they experience a higher frequency of hospitalization, exacerbation, and overall poorer clinical outcomes, especially in older individuals and those receiving chronic antibiotic therapy.[13,14]

Allergic Bronchopulmonary Aspergillosis

Most studies on aspergillosis in bronchiectasis have focused on the development of bronchiectasis because of ABPA, and the accompanying clinical consequences of coinfection. Bronchiectasis develops over time following acute ABPA, and pathogenesis directly relates to the host immune response including type I, III, and IV hypersensitivity reactions to *Aspergillus* antigens, immunoglobulin mediation, and the degranulation of mast cells and eosinophils in airways during ABPA exacerbations. Taken together, like the vicious cycle of bronchiectasis itself, these events lead to a self-perpetuating cycle of inflammation and airway dilatation.[35,36] This causes mucus impaction, atelectasis, and eventually permanent structural damage to the airway resulting in bronchiectasis.[37] Mucus plugging itself, through selective induction of MUC5AC by *A. fumigatus* is described as a possible mechanism toward developing ABPA.[38] A recent multicenter study demonstrated trends toward higher ABPA occurrence in patients with higher Bronchiectasis Severity Index, suggesting a potential link between existing disease severity, occurrence of ABPA, and further disease progression.[2] A major challenge however remains the difficultly in determining true ABPA incidence in bronchiectasis, due to overlapping symptoms with infective exacerbations and variability in the applied diagnostic criteria across geographic regions.[39–41] Central bronchiectasis was traditionally viewed as an important feature characteristic of ABPA-related bronchiectasis. However, there are other causes of central bronchiectasis and some ABPA patients display solely peripheral bronchiectasis.[34,42] The characterization of ABPA in bronchiectasis is also further complicated as the presence of bronchiectasis itself is considered an independent criteria to establish a diagnosis of ABPA.[43] Immunodeficiency remains another important etiology of bronchiectasis and primary immunodeficiency disorders including defects in the nicotinamide adenine dinucleotide phosphate oxidase (NADPH) oxidase complex in chronic granulomatous disease (CGD) or *STAT3* mutations can predispose to *Aspergillus* colonization and *Aspergillus*-associated disease. Notably, secondary immunosuppression through necessary steroid use in ABPA treatment confers the risk of transitioning to other *Aspergillus*-associated disease states including CPA.[41,44,45]

Chronic Pulmonary Aspergillosis

CPA, also referred to as semi-invasive or subacute aspergillosis, is most commonly observed as a complication of established and severe bronchiectasis.[13,34,46] Unlike IA, that remains characterized by vascular invasion, CPA is limited to slowly progressing cavitary lesions of the lung parenchyma that follows *Aspergillus* infection (►**Table 1**).[47] Denning et al propose a CPA classification into three distinct categories based on radiological pattern as clinical features overlap.[48] Chronic cavitary pulmonary aspergillosis (CCPA) is characterized by multiple progressive cavitary lung lesions while the presence of fibrosis, as sequelae of these expanding cavities lead to chronic fibrosing pulmonary aspergillosis (CFPA). The final category, chronic necrotizing pulmonary fibrosis (CNPA), or subacute invasive pulmonary aspergillosis (subacute IPA) is distinguished by its slowly progressive and invasive disease due to the enlargement of a single cavity (►**Table 1**). This latter condition occurs most frequently in individuals with significant immune compromise including diabetes and those receiving long-term corticosteroid therapy. Long-term follow-up of clinical progression in CPA demonstrates that all affected individuals had some preexisting lung disease, while approximately just over one quarter demonstrate bronchiectasis-related change on either radiology and/or histology.[48] A South Korean-based evaluation of pulmonary aspergillosis observed similar proportions of patients with underlying bronchiectasis and further documented strong associations with NTM infection.[49] Of note, coinfection with NTM in bronchiectasis also independently associates with higher mortality.[31]

Invasive Pulmonary Aspergillosis

Tissue invasion, either by angioinvasion or invasion of the airway, by septate fungal hyphae indicates IA (►**Table 1**). This

Table 1 Summary of degree of tissue invasion based on type of *Aspergillus*-associated disease

Noninvasive no tissue invasion by hyphae	Invasive hyphae invade tissue		
	Superficial		Deep
Colonization/ Sensitization	CCPA	CPA	IPA
ABPA	CFPA		IA
Aspergilloma	CNPA/Subacute IPA		
	ATB		

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; ATB, *Aspergillus* tracheo-bronchitis; CCPA, chronic cavitary pulmonary aspergillosis; CFPA, chronic fibrosing pulmonary aspergillosis; CNPA, chronic necrotizing pulmonary aspergillosis; CPA, chronic pulmonary aspergillosis; IA, invasive aspergillosis; IPA, invasive pulmonary aspergillosis.

is most prevalent in the severely immunosuppressed or those with preexisting chronic respiratory pathology such as bronchiectasis.[18,34] This is a serious fungal consequence that associates with high mortality. *A. fumigatus* is the most reported species causing IPA although infections with others such as *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus terreus* are reported.[13,34] Prolonged neutropenia is an important risk factor; however, IPA is documented in immunocompetent hosts including advanced COPD and/or bronchiectasis.[34,50] It can be particularly challenging to detect IPA, especially in non-neutropenic states such as that observed in bronchiectasis, leaving such patients undiagnosed for long periods as the disease progresses. Chronic airway damage such as that characteristic of bronchiectasis predisposes to *Aspergillus* colonization, and it remains highly probable that such structural change, and its accompanying immune-related effects are an under-recognized risk factor for IPA. Immunodeficiency syndromes including CGD occur with coexisting bronchiectasis and represent additional risks for the development of IPA.[51–53] Whether routine screening for IPA is warranted in bronchiectasis care remains to be determined; however, it is advised, especially in high-risk patients with bronchiectasis and a significant immunodeficiency.

Aspergillus Tracheo-Bronchitis

ATB is considered a subgroup of IPA, but unlike IPA, tissue invasion is confined to the superficial mucosal layers of the tracheo-bronchial tree (►**Table 1**).[18,34] This condition has lacked overall study but is identified in small groups of patients (~3%) among large cohorts, where aspergillosis was assessed.[54] In the context of bronchiectasis, it predominantly occurs in patients with minor immune deficits and is characterized by mucoid impaction of the airways and bronchial plugging, with or without accompanying ulceration.[54,55] Other forms of ATB are described and include obstructive, pseudomembranous and ulcerative subtypes.[34,56] Most interestingly, unlike IA, obstructive ATB may present without evidence of mucosal invasion and is characterized by an absence of airway inflammation; however, this form is yet to be described explicitly in association to bronchiectasis and warrants further study.[56]

Aspergilloma

Aspergillomas are a saprophytic manifestation of pulmonary aspergillosis. They represent a localized mass of hyphae and cellular debris, often developing in areas of lung with preexisting structural damage, including lung cavities in patients with post-tuberculosis bronchiectasis.[47,57] Most remain asymptomatic and are detected incidentally; however, life-threatening hemoptysis is a dreaded complication if fungal hyphae invade the bronchial vasculature. In this setting, surgical resection of the mass or embolization of the involved vasculature may be necessary, especially with a large or otherwise intermittent frequent bleeds. The precise occurrence of aspergillomas in bronchiectasis remains unknown and is likely underestimated.

Bronchiectasis Overlap Syndromes and *Aspergillus*-Associated Disease

Bronchiectasis may overlap with the presence of other chronic respiratory diseases including asthma and COPD. These present inherent diagnostic challenges due to common symptoms and the relative lack of clinical guidelines for identification and management.[58–60] Patients with severe asthma and concurrent fungal sensitization are more prone to colonization with *A. fumigatus*, and studies have shown an approximate twofold increase in the risk for developing bronchiectasis, which is then complicated by poor lung function.[61,62] It remains unclear what specifically predisposes these patients to *Aspergillus* sensitization in the first place, but what is clearly evident is that once this occurs, it represents an important risk for the subsequent development and progression of bronchiectasis, likely due to the chronically inflamed airways and the consequent remodeling process. Bronchiectasis COPD overlap is diagnosed when patients fulfill physiological and structural diagnostic criteria for both COPD and bronchiectasis.[63] Bronchiectasis has been commonly associated with COPD and when present is identified as an independent risk for mortality.[64–68] In a study of COPD patients, bronchiectasis was interestingly most frequently observed in patients with demonstrable sensitization to *Aspergillus* antigens. Furthermore, these "sensitized" patients exhibited a higher frequency of coinfection with bacterial pathogens such as *P. aeruginosa*, which in itself complicates bronchiectasis.[69] Such work further emphasizes the existence of *Aspergillus* endophenotypes in bronchiectasis, which first must be recognized before we can consider the various approaches necessary for accurate diagnosis, an understanding of their clinical course and required therapeutic interventions. While *Aspergillus*-associated disease endophenotypes in relation to bronchiectasis are clearly influenced by the presence of an overlap syndrome, the spectrum of *Aspergillus*-associated disease itself means that some disease states can coexist in the same patient or evolve from one entity to another dependent on the underlying host immune system. General risks for the evolution of disease include multiple respiratory pathologies, prolonged corticosteroid therapy, high fungal load, and/or host genetic susceptibility.[34] Aspergillomas and ABPA are frequently codiagnosed and likely due to an expanding bronchiectasis developing cavitation and subsequent *Aspergillus* colonization.[70,71] Alternately, hyperimmune responses cause the evolution of an aspergilloma into ABPA or ABPA treated with prolonged steroids can result in CPA.[72] ABPA with an element of invasion is rare but is linked to prolonged corticosteroid use where immune suppression promotes a displacement of hyphae from the airways into the lung parenchyma.[73] Concurrent *Aspergillus* pathologies while presenting an even more significant diagnostic challenge need to be recognized as they can lead to more severe bronchiectasis and a higher exacerbation risk.

The Contribution of Nonfumigatus and Other Fungi in Bronchiectasis

While *A. fumigatus* remains the most common fungi implicated in bronchiectasis-related aspergillosis, other *Aspergillus* species including *A. niger*, *A. versicolor*, and *A. flavus* have

been identified in relation to bronchiectasis.[30] *A. niger*, *A. flavus*, and *A. terreus* are reported to induce ABPA while the less frequent *A. nidulans* does exhibit an association with CGD and an aggressive course of disease.[30,52,74–79] Importantly, coinfection by more than a single *Aspergillus* species has also been reported.[80,81] Geographic variation in non-fumigatus species have been described: *A. niger* and *A. terreus* are more prevalent in Japan while *A. flavus* predominates in India and China.[30,77,82,83] A key study in bronchiectasis, the Cohort of Asian and Matched European Bronchiectasis (CAMEB) study, demonstrates that *A. fumigatus* profiles dominate patients of Asian origin while in an age- and sex-matched cohort of European origin, *A. terreus* was more frequent. Correlation with airway conidial burden reiterated this regional variation, even when both species coexisted, and interestingly, higher conidial burdens were associated with a greater number of exacerbations.[84] Other fungi that commonly associate with bronchiectasis include *Fusarium*, *Mucor*, *Rhizopus*, and *Scedosporium*.[14,36] Yeasts, including *Candida albicans* and *Exophiala dermatitidis* have also been isolated in bronchiectasis.[11,36] *C. albicans*, for instance, was isolated in over 40% of patients with bronchiectasis in a Spanish study while *E. dermatitidis* albeit rarely does cause a significant deterioration in pulmonary function.[11] A global review on allergic bronchopulmonary mycosis caused by fungi other than *Aspergillus* reports that up to 60% of the identified cases can be caused by *C. albicans*, an important consideration in bronchiectasis.[85]

The Pulmonary Mycobiome in Bronchiectasis

Many fungi have been proposed as contributors to airway infection in chronic lung diseases including bronchiectasis.[36] With increasing urbanization, climate change, and the ubiquitous nature of fungal presence in the surrounding environment, the relationship between fungi and human lung disease has received renewed attention.[86,87] Early culture-based assessment coupled to deep metagenomic sequencing now illustrates the complexity of fungal consortia that exist in outdoor air and demonstrate cyclical variability in abundance, whose consequences for human respiratory health remain to be fully appreciated.[88–90] Fungal conidia, owing to their small size, can reach the smallest airways, but are then removed by innate immune mechanisms including mucociliary clearance and macrophage engulfment.[91] Daily fungal exposure, although plentiful, is of little consequence in healthy immunocompetent individuals due to their successful elimination by the tightly regulated host mucosal defenses.[91,92] In bronchiectasis, however, chronic infection and associated immunopathogenic dysfunction lead to dysregulated host responses and fungal colonization.[11,93] Inflammatory cytokines, elastases, and matrix metalloproteinases (MMPs) then damage the structural integrity of the airway and lead to anatomic distortion and subsequent fungal sensitization, an increasingly recognized contributor to the pathology of bronchiectasis.[94–96] Several studies now underscore the importance of fungi in bronchiectasis, in particular the increased colonization by *Aspergillus* and *Candida* species, the increased expression of antifungal chitinase enzymes, and the heightened sensitization response to fungal antigens.[11,24,84,97] Given these developments, the role of the mycobiome, a collective assessment of fungal consortia present in the lung has become a key the subject of focused for NGS-based analyses in bronchiectasis.[98,99]

Bronchiectasis is a markedly heterogenous disease which differs further based on geographic boundaries, ethnicities, etiologies, and response to therapy.[100] Most clinical intervention to date focuses on prevention of exacerbations and airway clearance although targeting microbes such as *P. aeruginosa* in the airway confers clinical benefit. Recent NGS sequencing studies uncover a milieu of complex multikingdom organisms including bacteria, viruses, and fungi that potentially interact within the bronchiectasis airway, which explains the inherent heterogeneity and vastly contrasting clinical course observed between patients.[3,99] The European Multicenter Bronchiectasis Audit and Research Collaboration consensus statement for bronchiectasis attempts to address these important gaps in our understanding of this disease and identified exploration of the pulmonary mycobiome as a key research priority.[101] The recent CAMEB study is notable for its first report on the pulmonary mycobiome in bronchiectasis across continents and in age- and sex-matched populations from distinct geographical regions. It provided key insights into *Aspergillus*-associated disease in bronchiectasis.[84] By using high throughput 18S-28S ITS sequencing, this work first reaffirms the importance of *Aspergillus* and *Aspergillus*-associated disease in bronchiectasis and identifies distinct mycobiome profiles dominated by *A. terreus* in patients from Dundee, Scotland and *A. fumigatus* in patients from Singapore and Kuala Lumpur, Malaysia. These findings exemplify the existence and relevance of considering geographic differences in the clinical assessment of the bronchiectasis mycobiome. Quantification of conidial burden by quantitative polymerase chain reaction (q-PCR) reveals significant associations between higher conidial burden and occurrence of exacerbations. Further clinical correlations were elucidated by grouping patients based on meeting criteria for the various *Aspergillus*-associated disease states. Patients with serological ABPA (sABPA) had more severe disease, greater exacerbations, and poorer lung function when compared with those *Aspergillus* colonized and/or sensitized. This serves to demonstrate the clinical significance of *Aspergillus* in the etiopathogenesis and progression of bronchiectasis and, screening for this fungus, even in clinically stable states may offer important clinical insight.

While the *Aspergilli* remain the best characterized fungi in the bronchiectasis airway, *Candida* species are the most widely detected.[4,14,98] Other important fungi to emerge from culture-based studies include *Saccharomyces cerevisiae*, *Trichosporon spp*., *Scedosporium*, and *Penicillium*.[14] Given the inherent challenges and lack of standardization in fungal culture protocols, it is possible that these data are skewed and underestimate the true in vivo composition of

the bronchiectasis mycobiome.[102,103] Emerging NGS-based mycobiome research in bronchiectasis performed by our group, and others offers fresh insight into the mycobiome in this setting and remain less susceptible to the biases of culture-based assessments.[84,99] The CAMEB study, as previously described also highlighted an overrepresentation of fungal taxa that go beyond *Aspergillus* and include *Penicillium* and *Cryptococcus* while concurrently identifying, in view of its study design, several taxa that vary geographically. This latter group includes *Simplicillium* and *Trichosporon* which predominate in Asians, and *Wickerhamomyces*, *Clavispora,* and *Cryptococcus* that demonstrate a higher abundance in Europeans. High *Basidiomycota* loads within a bronchiectasis mycobiome was generally associated with a more favorable prognosis; however, some key *Basidiomycota* fungi including *Trichosporon*, *Cryptococcus*, *Clavispora*, *Alternaria*, *Botrytis*, *Wickerhamomyces*, and *Cladosporium* were relevant in *Aspergillus* sensitization and sABPA demonstrating the complexity of the bronchiectasis mycobiome and need for future ongoing research to better understand its clinical correlates and usefulness in patient stratification.[16,84]

Immunoallertypes and the Role of *Aspergillus* Sensitization in Endophenotyping Bronchiectasis

Heightened Th2 responses are associated with fungal sensitization and allergy and remain a component of several chronic respiratory disease states.[36] Early suggestions of a role in bronchiectasis came from studies in CF, where sensitization and allergy correlate with the development of CF-ABPA and lung function declines.[104–106] Further work revealed that even outside the CF setting, that a significantly elevated atopic response was detectable in bronchiectasis and, that the association between sensitization and lung function decline, as identified in CF, remained consistent.[107] Interestingly, sensitization to *A. fumigatus* was identified as a risk factor for the development of bronchiectasis in COPD cohorts, with most of the heightened risk attributed to the recombinant *A. fumigatus* allergens rAsp f1 or f3.[69] Building on these existing works, and employing the CAMEB cohort, our group next assessed the frequency and clinical relevance of fungal sensitization in stable bronchiectasis. We identified a high prevalence of both the *Aspergillus* fungi and related sensitization response including significant (and largely clinically undetected) proportions of sABPA.[12,24,36,108,109] Significant levels of polysensitization, going beyond fungi and including common environmental allergens were identified independent of patient origin. These include crude allergens of the house dust mites *Dermatophagoides pteronyssinus* and *Blomia tropicalis* and the fungi *Alternaria alternata*. Importantly, a comprehensive panel of *A. fumigatus* recombinant allergens was explored and includes rAsp f 1, f 2, f 6, f 8, f 15, and f 17. Comprehensive immune-inflammatory profiling was concurrently performed and when assessed in combination to airway sensitization responses in bronchiectasis revealed two distinct "immuno-allertypes" including a predominantly house dust mite sensitized patient group, characterized by a chemokine-dominant airway profile including growth-regulated oncogene (CXCL1), monocyte chemoattractant protein-1 (CCL2), and eotaxin-1 (CCL11) in addition to the anti-inflammatory cytokines interleukin 1RA, interleukin 10, and granulocyte colony–stimulating factor. In contrast, a second group of patients with a predominantly fungal-driven sensitization response, and poorer clinical outcomes was also identified. This group, marked by a proinflammatory airway cytokine signature including tumor necrosis factor α (TNF-α), IL-1α, and IL-1β demonstrated significant associations with poorer lung function and increased disease severity.[24,110] A marked geographic variation in allergic profiles was also evident, suggestive of perhaps distinct endophenotypes that warrant further study. Asians with bronchiectasis in the CAMEB cohort exhibit a higher sensitization to house dust mite allergens and the *A. fumigatus* major allergen, rAsp 1, in contrast to Europeans who had higher levels of sensitization to *Alternaria* and the *A. fumigatus* allergens rAsp f 6, f 8, f 15, and f 17. A further dissection of these patterns within each "immuno-allertype" revealed specific endophenotypic subgroups relating to a patient's country of origin, reflective of the clinical heterogeneity in sensitization responses that likely exist. While the overall picture is complex, the observed increases in sensitization responses to the major *Aspergillus* allergen rAsp1 in Asian patients remains consistent with the higher detected *A. fumigatus* conidial burden in this region suggesting that a combination of geographic origin, host response, and fungal exposure levels all have importance. Endophenotypic variability, based on sensitization pattern and other features, is therefore an important consideration for patient stratification and the design of clinical trials in bronchiectasis, particularly when multiple centers across wide ranging geographic regions are included. Careful consideration is required to the presence of underlying sensitization in bronchiectasis, and while complex, it likely contributes to disease heterogeneity.[111,112] This complexity extends further to the underlying etiology of bronchiectasis at the individual level, and recent work substantiates this, illustrating the critical importance of fungal sensitization in bronchiectasis while identifying TB-related bronchiectasis as an independent risk factor for *Aspergillus* sensitization.[113] Taken together, collective data does suggest that sensitization in bronchiectasis, particularly to fungi is significant and clinically relevant. This provides scope for improved patient stratification and potentially the development of targeted and personalized interventions based on geographic origin.

Going Beyond Fungi: Interkingdom Interaction in the Bronchiectasis Airway

While clearly relevant, the mycobiome cannot be considered in isolation, given its existence within an integrated microbial ecosystem in the airway that encompasses fungi, bacteria, and viruses, all of which contribute to bronchiectasis.[16] While bacteriomes have been investigated, the role of viruses and the "virome" including bacteriophages remains poorly

understood in bronchiectasis and necessitates research.[114–116] As studies continue to emerge that improve our understanding of the airway microbiome and its relevance to human health and disease, it is apparent that holistic "multibiome" analyses, which encompasses complex microbial interaction networks, represents a logical progression in bronchiectasis microbiome research efforts. Efforts to model host microbiomes as an integrated microbial network have already been advanced in CF to understand exacerbations.[117] Such models seek to assess the microbiome as a network, integrating coexisting, commensal, and/or 'pathobiont' microbes in addition to classical "culprit" organisms. Network-based analysis can therefore account more accurately for observed clinical differences seen in patient cohorts and represent a promising platform for further explication of microbiome-driven endophenotypes of respiratory disease including bronchiectasis.[118] The plausibility of potentially variable interactions is evident in existing clinical and co-culture analyses of *P. aeruginosa* and *A. fumigatus*, the most well-studied interkingdom interaction of relevance to respiratory disease.[119–121] Interkingdom communication between fungi and bacteria remains an active research area with relevance to bronchiectasis, where the application of "multibiome; approaches may yield insight into complex and currently poorly understood endophenotypes.[3,16,84,122] While each individual microbial kingdom has its own relevance and is examined independently, an integrated and holistic interkingdom approach remains an important and likely rich avenue for future microbiome studies in bronchiectasis.

Conclusion

The emerging role of fungi and in particular *Aspergillus* in bronchiectasis has been the subject of numerous studies and remains a key research priority.[101] Although significant progress has already been made, further studies focusing on epidemiology, strain variation, and clinical relevance in relation to bronchiectasis endophenotypes are required. Our current understanding is largely shaped by the increasing applied systems-level analysis that employs data-rich microbial profiling of host airways and their associated systemic response. Recent exploratory work underscores the potential of data-driven molecular approaches to identify and stratify patients according to their underlying fungal endophenotypes. While current work on the bronchiectasis mycobiome provides a platform for future research, it also highlights several challenges for the field including the development and optimization of ITS protocols to ensure adequate fungal coverage as compared with those robustly established for the bacterial microbiome.[99,123] Metagenomics offers an alternate approach, but here challenges also exist, most notably in the development and availability of public reference databases that lag significantly behind those available for bacteria, which potentially leads to classification errors.[124,125] Notwithstanding this, the exploratory microbiome studies have been steadily increasing in bronchiectasis, which provides scope for improved patient stratification and resolving the inherent disease heterogeneity that exists.[3,117] Applying intrakingdom analytical approaches may be the next logical step to address key knowledge gaps in bronchiectasis mycology. Longitudinal measures of *Aspergillus* sensitization and its associated immune response may allow the identification of "at risk" groups at an early stage and prior to the onset of overt fungal disease, permitting the identification of fungal endophenotypes at the molecular level that can be individually targeted for appropriate intervention. An improved understanding of the role for antifungal therapy in bronchiectasis is also required, therefore addressing fungal endophenotypes and their accompanying clinical outcomes remain a priority. The role of bacterial taxa such as *P. aeruginosa* and members of the NTM family, both of which can interact with *Aspergillus* may contribute to fungal endophenotypes and disease progression in bronchiectasis and warrant dedicated investigation. In addition to leveraging key technologies, an increased international effort to address the geographic variation through the establishment and maintenance of large bronchiectasis cohorts will be important to drive this field forward and recognize the true relevance of *Aspergillus* and other fungi in bronchiectasis.

Funding
This research is supported by the Singapore Ministry of Health's National Medical Research Council under its Clinician-Scientist Individual Research Grant (MOH-000141) (S.H.C) and the NTU Integrated Medical, Biological and Environmental Life Sciences (NIMBELS) [NIM/03/2018] (S.H.C).

Conflict of Interest
None declared.

References

1 Maselli DJ, Amalakuhan B, Keyt H, Diaz AA. Suspecting non-cystic fibrosis bronchiectasis: what the busy primary care clinician needs to know. Int J Clin Pract 2017;71(02): e12924
2 Lonni S, Chalmers JD, Goeminne PC, et al. Etiology of non-cystic fibrosis bronchiectasis in adults and its correlation to disease severity. Ann Am Thorac Soc 2015;12(12):1764–1770
3 Richardson H, Dicker AJ, Barclay H, Chalmers JD. The microbiome in bronchiectasis. Eur Respir Rev 2019;28(153):190048
4 Richardson M, Bowyer P, Sabino R. The human lung and Aspergillus: you are what you breathe in? Med Mycol 2019;57 (Suppl 2):S145–S154
5 Flume PA, Chalmers JD, Olivier KN. Advances in bronchiectasis: endotyping, genetics, microbiome, and disease heterogeneity. Lancet 2018;392(10150):880–890
6 Bhatia S, Fei M, Yarlagadda M, et al. Rapid host defense against Aspergillus fumigatus involves alveolar macrophages with a predominance of alternatively activated phenotype. PLoS One 2011;6(01):e15943–e15943
7 Chotirmall SH, Mirkovic B, Lavelle GM, McElvaney NG. Immunoevasive Aspergillus virulence factors. Mycopathologia 2014; 178(5-6):363–370
8 McCormick A, Loeffler J, Ebel F. Aspergillus fumigatus: contours of an opportunistic human pathogen. Cell Microbiol 2010;12 (11):1535–1543

9 Park SJ, Mehrad B. Innate immunity to Aspergillus species. Clin Microbiol Rev 2009;22(04):535–551
10 Chotirmall SH, Al-Alawi M, Mirkovic B, et al. Aspergillus-associated airway disease, inflammation, and the innate immune response. BioMed Res Int 2013;2013:723129
11 Máiz L, Vendrell M, Olveira C, Girón R, Nieto R, Martínez-García MÁ Prevalence and factors associated with isolation of Aspergillus and Candida from sputum in patients with non-cystic fibrosis bronchiectasis. Respiration 2015;89(05):396–403
12 Chotirmall SH, Martin-Gomez MT. Aspergillus species in bronchiectasis: challenges in the cystic fibrosis and non-cystic fibrosis airways. Mycopathologia 2018;183(01):45–59
13 De Soyza A, Aliberti S. Bronchiectasis and Aspergillus: how are they linked? Med Mycol 2017;55(01):69–81
14 Máiz L, Nieto R, Cantón R, Gómez G de la Pedrosa E, Martinez-García MÁ Fungi in Bronchiectasis: a concise review. Int J Mol Sci 2018;19(01):142
15 Kim ST, Choi JH, Jeon HG, Cha HE, Hwang YJ, Chung YS. Comparison between polymerase chain reaction and fungal culture for the detection of fungi in patients with chronic sinusitis and normal controls. Acta Otolaryngol 2005;125(01):72–75
16 Chandrasekaran R, Mac Aogáin M, Chalmers JD, Elborn SJ, Chotirmall SH. Geographic variation in the aetiology, epidemiology and microbiology of bronchiectasis. BMC Pulm Med 2018;18(01):83
17 Qi Q, Wang W, Li T, Zhang Y, Li Y. Aetiology and clinical characteristics of patients with bronchiectasis in a Chinese Han population: a prospective study. Respirology 2015;20(06):917–924
18 Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. Thorax 2015;70(03):270–277
19 Kunst H, Wickremasinghe M, Wells A, Wilson R. Nontuberculous mycobacterial disease and Aspergillus-related lung disease in bronchiectasis. Eur Respir J 2006;28(02):352–357
20 Warris A. The biology of pulmonary aspergillus infections. J Infect 2014;69(Suppl 1):S36–S41
21 Cramer RA, Rivera A, Hohl TM. Immune responses against Aspergillus fumigatus: what have we learned? Curr Opin Infect Dis 2011;24(04):315–322
22 Hohl TM. Immune responses to invasive aspergillosis: new understanding and therapeutic opportunities. Curr Opin Infect Dis 2017;30(04):364–371
23 Murray MA, Chotirmall SH. The impact of immunosenescence on pulmonary disease. Mediators Inflamm 2015;2015:692546
24 Mac Aogáin M, Tiew PY, Lim AYH, et al. Distinct "immunoallertypes" of disease and high frequencies of sensitization in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med 2019;199(07):842–853
25 Bahous J, Malo JL, Paquin R, Cartier A, Vyas P, Longbottom JL. Allergic bronchopulmonary aspergillosis and sensitization to Aspergillus fumigatus in chronic bronchiectasis in adults. Clin Allergy 1985;15(06):571–579
26 Becker KL, Gresnigt MS, Smeekens SP, et al. Pattern recognition pathways leading to a Th2 cytokine bias in allergic bronchopulmonary aspergillosis patients. Clin Exp Allergy 2015;45(02):423–437
27 Kumar R, Chopra D. Evaluation of allergic bronchopulmonary aspergillosis in patients with and without central bronchiectasis. J Asthma 2002;39(06):473–477
28 Greenberger PA, Miller TP, Roberts M, Smith LL. Allergic bronchopulmonary aspergillosis in patients with and without evidence of bronchiectasis. Ann Allergy 1993;70(04):333–338
29 Wark PA, Saltos N, Simpson J, Slater S, Hensley MJ, Gibson PG. Induced sputum eosinophils and neutrophils and bronchiectasis severity in allergic bronchopulmonary aspergillosis. Eur Respir J 2000;16(06):1095–1101
30 Tashiro T, Izumikawa K, Tashiro M, et al. Diagnostic significance of Aspergillus species isolated from respiratory samples in an adult pneumology ward. Med Mycol 2011;49(06):581–587
31 Zoumot Z, Boutou AK, Gill SS, et al. Mycobacterium avium complex infection in non-cystic fibrosis bronchiectasis. Respirology 2014;19(05):714–722
32 Chabi ML, Goracci A, Roche N, Paugam A, Lupo A, Revel MP. Pulmonary aspergillosis. Diagn Interv Imaging 2015;96(05):435–442
33 Everaerts S, Lagrou K, Vermeersch K, Dupont LJ, Vanaudenaerde BM, Janssens W. Aspergillus fumigatus detection and risk factors in patients with copd-bronchiectasis overlap. Int J Mol Sci 2018;19(02):523
34 Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. Eur Respir Rev 2011;20(121):156–174
35 Shah A, Panjabi C. Allergic aspergillosis of the respiratory tract. Eur Respir Rev 2014;23(131):8–29
36 Knutsen AP, Bush RK, Demain JG, et al. Fungi and allergic lower respiratory tract diseases. J Allergy Clin Immunol 2012;129(02):280–291, quiz 292–293
37 Spiro SG, Silvestri GA, Agustí A Clinical Respiratory Medicine. Philadelphia, PA: Elsevier/Saunders; 2012
38 Oguma T, Asano K, Tomomatsu K, et al. Induction of mucin and MUC5AC expression by the protease activity of Aspergillus fumigatus in airway epithelial cells. J Immunol 2011;187(02):999–1005
39 Page ID, Richardson MD, Denning DW. Comparison of six Aspergillus-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). J Infect 2016;72(02):240–249
40 Agarwal R, Aggarwal AN, Garg M, Saikia B, Chakrabarti A. Cut-off values of serum IgE (total and A. fumigatus -specific) and eosinophil count in differentiating allergic bronchopulmonary aspergillosis from asthma. Mycoses 2014;57(11):659–663
41 Suarez-Cuartin G, Chalmers JD, Sibila O. Diagnostic challenges of bronchiectasis. Respir Med 2016;116:70–77
42 Kumar R. Mild, moderate, and severe forms of allergic bronchopulmonary aspergillosis: a clinical and serologic evaluation. Chest 2003;124(03):890–892
43 Patel AR, Patel AR, Singh S, Singh S, Khawaja I. Diagnosing allergic bronchopulmonary Aspergillosis: a review. Cureus 2019;11(04):e4550
44 Goussault H, Salvator H, Catherinot E, et al. Primary immunodeficiency-related bronchiectasis in adults: comparison with bronchiectasis of other etiologies in a French reference center. Respir Res 2019;20(01):275
45 Coulter TI, Devlin L, Downey D, Elborn JS, Edgar JDM. Immunodeficiency in Bronchiectasis. In: Chalmers J, Polverino E, Aliberti S, eds. Bronchiectasis: The EMBARC Manual. Cham: Springer International Publishing; 2018:77–100
46 Denning DW, Cadranel J, Beigelman-Aubry C, et al; European Society for Clinical Microbiology and Infectious Diseases and European Respiratory Society. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. Eur Respir J 2016;47(01):45–68
47 Tunnicliffe G, Schomberg L, Walsh S, Tinwell B, Harrison T, Chua F. Airway and parenchymal manifestations of pulmonary aspergillosis. Respir Med 2013;107(08):1113–1123
48 Denning DW, Riniotis K, Dobrashian R, Sambatakou H. Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature change, and review. Clin Infect Dis 2003;37(Suppl 3):S265–S280
49 Jhun BW, Jeon K, Eom JS, et al. Clinical characteristics and treatment outcomes of chronic pulmonary aspergillosis. Med Mycol 2013;51(08):811–817
50 Abers MS, Ghebremichael MS, Timmons AK, Warren HS, Poznansky MC, Vyas JM. A critical reappraisal of prolonged neutropenia as a risk factor for invasive pulmonary Aspergillosis. Open Forum Infect Dis 2016;3(01):ofw036–ofw036
51 Farmand S, Sundin M. Hyper-IgE syndromes: recent advances in pathogenesis, diagnostics and clinical care. Curr Opin Hematol 2015;22(01):12–22

52 Henriet S, Verweij PE, Holland SM, Warris A. Invasive fungal infections in patients with chronic granulomatous disease. In: Curtis N, Finn A, Pollard AJ, eds. Hot Topics in Infection and Immunity in Children IX. New York, NY: Springer New York; 2013:27–55

53 Bassiri-Jahromi S, Doostkam A. Fungal infection and increased mortality in patients with chronic granulomatous disease. J Mycol Med 2012;22(01):52–57

54 Chrdle A, Mustakim S, Bright-Thomas RJ, Baxter CG, Felton T, Denning DW. Aspergillus bronchitis without significant immunocompromise. Ann N Y Acad Sci 2012;1272(01):73–85

55 Cho BH, Oh Y, Kang ES, et al. Aspergillus tracheobronchitis in a mild immunocompromised host. Tuberc Respir Dis (Seoul) 2014; 77(05):223–226

56 Panchabhai TS, Bandyopadhyay D, Alraiyes AH, Mehta AC, Almeida FAA. A 60-year-old woman with cough, dyspnea, and atelectasis 19 years after liver transplant. Chest 2015;148(04): e122–e125

57 Bongomin F. Post-tuberculosis chronic pulmonary aspergillosis: an emerging public health concern. PLoS Pathog 2020;16(08): e1008742

58 Hurst JR, Elborn JS, De Soyza ABRONCH-UK Consortium. COPD-bronchiectasis overlap syndrome. Eur Respir J 2015;45(02): 310–313

59 Poh TY, Mac Aogáin M, Chan AK, et al. Understanding COPD-overlap syndromes. Expert Rev Respir Med 2017;11(04):285–298

60 Porsbjerg C, Menzies-Gow A. Co-morbidities in severe asthma: clinical impact and management. Respirology 2017;22(04): 651–661

61 Fairs A, Agbetile J, Hargadon B, et al. IgE sensitization to Aspergillus fumigatus is associated with reduced lung function in asthma. Am J Respir Crit Care Med 2010;182(11):1362–1368

62 Menzies D, Holmes L, McCumesky G, Prys-Picard C, Niven R. Aspergillus sensitization is associated with airflow limitation and bronchiectasis in severe asthma. Allergy 2011;66(05): 679–685

63 Pasteur MC, Bilton D, Hill ATBritish Thoracic Society Bronchiectasis non-CF Guideline Group. British Thoracic Society guideline for non-CF bronchiectasis. Thorax 2010;65(Suppl 1):i1–i58

64 Quint JK, Millett ER, Joshi M, et al. Changes in the incidence, prevalence and mortality of bronchiectasis in the UK from 2004 to 2013: a population-based cohort study. Eur Respir J 2016;47 (01):186–193

65 Patel IS, Vlahos I, Wilkinson TM, et al. Bronchiectasis, exacerbation indices, and inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004;170(04):400–407

66 Du Q, Jin J, Liu X, Sun Y. Bronchiectasis as a comorbidity of chronic obstructive pulmonary disease: a systematic review and meta-analysis. PLoS One 2016;11(03):e0150532

67 Martínez-García MA, de la Rosa Carrillo D, Soler-Cataluña JJ, et al. Prognostic value of bronchiectasis in patients with moderate-to-severe chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013;187(08):823–831

68 McDonnell MJ, Aliberti S, Goeminne PC, et al. Comorbidities and the risk of mortality in patients with bronchiectasis: an international multicentre cohort study. Lancet Respir Med 2016;4(12): 969–979

69 Everaerts S, Lagrou K, Dubbeldam A, et al. Sensitization to *Aspergillus fumigatus* as a risk factor for bronchiectasis in COPD. Int J Chron Obstruct Pulmon Dis 2017;12:2629–2638

70 Safirstein BH, D'Souza MF, Simon G, Tai EH, Pepys J. Five-year follow-up of allergic bronchopulmonary aspergillosis. Am Rev Respir Dis 1973;108(03):450–459

71 Israel RH, Poe RH, Bomba PA, Gross RA. The rapid development of an aspergilloma secondary to allergic bronchopulmonary aspergillosis. Am J Med Sci 1980;280(01):41–44

72 Agarwal R, Aggarwal AN, Garg M, Saikia B, Gupta D, Chakrabarti A. Allergic bronchopulmonary aspergillosis with aspergilloma: an immunologically severe disease with poor outcome. Mycopathologia 2012;174(03):193–201

73 Maturu VN, Agarwal R. Acute invasive pulmonary aspergillosis complicating allergic bronchopulmonary aspergillosis: case report and systematic review. Mycopathologia 2015;180(3-4):209–215

74 Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. Pediatrics 2008;121(05):e1286–e1294

75 Henriet SS, Verweij PE, Warris A. Aspergillus nidulans and chronic granulomatous disease: a unique host-pathogen interaction. J Infect Dis 2012;206(07):1128–1137

76 Hoshino H, Tagaki S, Kon H, et al. Allergic bronchopulmonary aspergillosis due to Aspergillus niger without bronchial asthma. Respiration 1999;66(04):369–372

77 Ishiguro T, Takayanagi N, Kagiyama N, Shimizu Y, Yanagisawa T, Sugita Y. Clinical characteristics of biopsy-proven allergic bronchopulmonary mycosis: variety in causative fungi and laboratory findings. Intern Med 2014;53(13):1407–1411

78 Oshima M, Soda H, Oda H, Watanabe A. [A case of allergic bronchopulmonary aspergillosis caused by Aspergillus terreus]. Nihon Kyobu Shikkan Gakkai Zasshi 1997;35(12):1418–1424

79 Tillie-Leblond I, Tonnel AB. Allergic bronchopulmonary aspergillosis. Allergy 2005;60(08):1004–1013

80 Yamamoto K, Abe M, Inoue Y, Yokoyama A, Kohno N, Hiwada K. [Development of infection with Aspergillus flavus in woman being treated for allergic pulmonary Aspergillosis caused by Aspergillus fumigatus]. Nihon Kyobu Shikkan Gakkai Zasshi 1995;33(10):1099–1104

81 Gautier M, Normand AC, L'Ollivier C, et al. Aspergillus tubingensis: a major filamentous fungus found in the airways of patients with lung disease. Med Mycol 2016;54(05):459–470

82 Mortensen KL, Johansen HK, Fuursted K, et al. A prospective survey of Aspergillus spp. in respiratory tract samples: prevalence, clinical impact and antifungal susceptibility. Eur J Clin Microbiol Infect Dis 2011;30(11):1355–1363

83 Chakrabarti A, Sethi S, Raman DS, Behera D. Eight-year study of allergic bronchopulmonary aspergillosis in an Indian teaching hospital. Mycoses 2002;45(08):295–299

84 Mac Aogáin M, Chandrasekaran R, Lim AYH, et al. Immunological corollary of the pulmonary mycobiome in bronchiectasis: the CAMEB study. Eur Respir J 2018;52(01):1800766

85 Chowdhary A, Agarwal K, Kathuria S, Gaur SN, Randhawa HS, Meis JF. Allergic bronchopulmonary mycosis due to fungi other than Aspergillus: a global overview. Crit Rev Microbiol 2014;40 (01):30–48

86 Nicolaou N, Siddique N, Custovic A. Allergic disease in urban and rural populations: increasing prevalence with increasing urbanization. Allergy 2005;60(11):1357–1360

87 Eguiluz-Gracia I, Mathioudakis AG, Bartel S, et al. The need for clean air: The way air pollution and climate change affect allergic rhinitis and asthma. Allergy 2020;75(09): 2170–2184

88 Gusareva ES, Acerbi E, Lau KJX, et al. Microbial communities in the tropical air ecosystem follow a precise diel cycle. Proceedings of the National Academy of Sciences of the United States of America. Accessed 2019 at: https://www.pnas.org/content/116/46/23299

89 Tiew PY, Ko FWS, Pang SL, et al. Environmental fungal sensitisation associates with poorer clinical outcomes in COPD. Eur Respir J 2020;56(02):2000418

90 Lim SH, Chew FT, Binti Mohd Dali SD, Wah Tan HT, Lee BW, Tan TK. Outdoor airborne fungal spores in Singapore. Grana 1998;37 (04):246–252

91 de Vrankrijker AM, van der Ent CK, van Berkhout FT, et al. Aspergillus fumigatus colonization in cystic fibrosis: implications for lung function? Clin Microbiol Infect 2011;17(09): 1381–1386

92 Romani L. Immunity to fungal infections. Nat Rev Immunol 2011; 11(04):275–288
93 Chalmers JD, Chang AB, Chotirmall SH, Dhar R, McShane PJ. Bronchiectasis. Nat Rev Dis Primers 2018;4(01):45
94 Chalmers JD, Moffitt KL, Suarez-Cuartin G, et al. Neutrophil elastase activity is associated with exacerbations and lung function decline in bronchiectasis. Am J Respir Crit Care Med 2017;195(10):1384–1393
95 Angrill J, Agustí C, De Celis R, et al. Bronchial inflammation and colonization in patients with clinically stable bronchiectasis. Am J Respir Crit Care Med 2001;164(09):1628–1632
96 Taylor SL, Rogers GB, Chen AC, Burr LD, McGuckin MA, Serisier DJ. Matrix metalloproteinases vary with airway microbiota composition and lung function in non-cystic fibrosis bronchiectasis. Ann Am Thorac Soc 2015;12(05):701–707
97 Poh TY, Tiew PY, Lim AYH, et al. Increased chitotriosidase is associated with aspergillus and frequent exacerbations in southeast asian patients with bronchiectasis. Chest 2020;158(02): 512–522
98 Weaver D, Gago S, Bromley M, Bowyer P. The human lung mycobiome in chronic respiratory disease: limitations of methods and our current understanding. Curr Fungal Infect Rep 2019; 13(03):109–119
99 Tiew PY, Mac Aogain M, Ali NABM, et al. The mycobiome in health and disease: emerging concepts, methodologies and challenges. Mycopathologia 2020;185(02):207–231
100 Chotirmall SH, Chalmers JD. Bronchiectasis: an emerging global epidemic. BMC Pulm Med 2018;18(01):76
101 Aliberti S, Masefield S, Polverino E, et al; EMBARC Study Group. Research priorities in bronchiectasis: a consensus statement from the EMBARC Clinical Research Collaboration. Eur Respir J 2016;48(03):632–647
102 Coron N, Pihet M, Fréalle E, et al. Toward the standardization of mycological examination of sputum samples in cystic fibrosis: results from a French multicenter prospective study. Mycopathologia 2018;183(01):101–117
103 Chen SC-A, Meyer W, Pashley CH. Challenges in laboratory detection of fungal pathogens in the airways of cystic fibrosis patients. Mycopathologia 2018;183(01):89–100
104 Baxter CG, Moore CB, Jones AM, Webb AK, Denning DW. IgE-mediated immune responses and airway detection of Aspergillus and Candida in adult cystic fibrosis. Chest 2013;143(05): 1351–1357
105 Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with Aspergillus fumigatus on lung function and hospitalization in patients with cystic fibrosis. Chest 2010;137(01):171–176
106 Nelson LA, Callerame ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. Am Rev Respir Dis 1979;120(04):863–873
107 Murphy MB, Reen DJ, Fitzgerald MX. Atopy, immunological changes, and respiratory function in bronchiectasis. Thorax 1984;39(03):179–184
108 Nguyen LD, Viscogliosi E, Delhaes L. The lung mycobiome: an emerging field of the human respiratory microbiome. Front Microbiol 2015;6:89
109 Mac Aogáin M, Vidaillac C, Chotirmall SH. Fungal infections and ABPA. In: Cystic Fibrosis. Springer; 2020:93–126
110 McShane PJ. A new bronchiectasis endophenotype: immunoallertypes. Am J Respir Crit Care Med 2019;199(07): 811–812
111 Metersky M, Chalmers J. Bronchiectasis insanity: doing the same thing over and over again and expecting different results? F1000 Res 2019;8:8
112 Chalmers JD, Chotirmall SH. Bronchiectasis: new therapies and new perspectives. Lancet Respir Med 2018;6(09): 715–726
113 Sehgal IS, Dhooria S, Prasad KT, et al. Sensitization to a fumigatus in subjects with non-cystic fibrosis bronchiectasis. Mycoses 2020
114 Gao YH, Guan WJ, Xu G, et al. The role of viral infection in pulmonary exacerbations of bronchiectasis in adults: a prospective study. Chest 2015;147(06):1635–1643
115 Mitchell AB, Mourad B, Buddle L, Peters MJ, Oliver BGG, Morgan LC. Viruses in bronchiectasis: a pilot study to explore the presence of community acquired respiratory viruses in stable patients and during acute exacerbations. BMC Pulm Med 2018; 18(01):84
116 Chen CL, Huang Y, Yuan JJ, et al. The roles of bacteria and viruses in bronchiectasis exacerbation: a prospective study. Arch Bronconeumol 2020;56(10):621–629
117 Layeghifard M, Li H, Wang PW, et al. Microbiome networks and change-point analysis reveal key community changes associated with cystic fibrosis pulmonary exacerbations. NPJ Biofilms Microbiomes 2019;5(01):4
118 Layeghifard M, Hwang DM, Guttman DS. Disentangling Interactions in the Microbiome: a network perspective. Trends Microbiol 2017;25(03):217–228
119 Sass G, Ansari SR, Dietl AM, Déziel E, Haas H, Stevens DA. Intermicrobial interaction: Aspergillus fumigatus siderophores protect against competition by Pseudomonas aeruginosa. PLoS One 2019;14(05):e0216085
120 Mowat E, Rajendran R, Williams C, et al. Pseudomonas aeruginosa and their small diffusible extracellular molecules inhibit Aspergillus fumigatus biofilm formation. FEMS Microbiol Lett 2010;313(02):96–102
121 Briard B, Heddergott C, Latgé JP. Volatile compounds emitted by pseudomonas aeruginosa stimulate growth of the fungal pathogen aspergillus fumigatus. MBio 2016;7(02): e00219
122 Tipton L, Müller CL, Kurtz ZD, et al. Fungi stabilize connectivity in the lung and skin microbial ecosystems. Microbiome 2018;6 (01):12
123 Ali NABM, Mac Aogáin M, Morales RF, Tiew PY, Chotirmall SH. Optimisation and benchmarking of targeted amplicon sequencing for mycobiome analysis of respiratory specimens. Int J Mol Sci 2019;20(20):E4991
124 Mac Aogáin M, Chaturvedi V, Chotirmall SH. Mycopathologia-GENOMES: the new 'home' for the publication of fungal genomes. Mycopathologia 2019;184(05):551–554
125 Abdelaziz MT, Hassan M, Damasy DAE, Aziz RK. Why we missed it? Computational analysis reveals distribution patterns of Malassezia furfur, the etiological agent of Pityriasis versicolor, in skin metagenomes. In: Research Square; 2021