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HIGH FREQUENCY OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS IN BRONCHIECTASIS-COPD OVERLAP

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Running head: ABPA in bronchiectasis-COPD overlap

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Abbreviation List

BCO (Bronchiectasis-COPD overlap), BMI (body mass index), BSI (bronchiectasis severity index), CF (cystic fibrosis), COPD (chronic obstructive pulmonary disease), FEV_1 (forced expiratory volume in the first second), IQR (interquartile range), ITS (internal transcribed spacer), OR (odds ratio), rAsp f (recombinant *Aspergillus fumigatus*), ABPA (allergic bronchopulmonary aspergillosis), sIgE (specific-IgE)

Abstract

Background: Allergic bronchopulmonary aspergillosis (ABPA) is associated with frequent exacerbations and poor outcomes in chronic respiratory disease but remains underdiagnosed. The role of fungal sensitization in bronchiectasis-COPD overlap (BCO) is unknown. **Research question:** What is the occurrence and clinical relevance of *Aspergillus* sensitization and ABPA in BCO when compared to individuals with COPD or bronchiectasis without overlap? **Study Design:** Prospective, observational and cross-sectional. **Methods:** We prospectively recruited n=280 patients during periods of clinical stability with bronchiectasis (n=183), COPD (n=50) and BCO (n=47) from six hospitals across three countries (Singapore, Malaysia, and Scotland). We assessed sensitization responses (as specific IgE) to a panel of recombinant *Aspergillus fumigatus* (rAsp f) allergens and the occurrence of ABPA (ABPA) in relation to clinical outcomes. **Results:** Individuals with BCO illustrate an increased frequency and clinical severity of ABPA compared to COPD and bronchiectasis without overlap. BCO-associated ABPA demonstrates more severe disease, higher exacerbation rates and lower lung function when compared to ABPA occurring in the absence of overlap. BCO with a severe bronchiectasis severity index (BSI) ($\geq$9) significantly associates with the occurrence of ABPA that is unrelated to underlying COPD severity. **Conclusions:** BCO demonstrates a high frequency of ABPA that associates with a severe BSI (>9) and poor clinical outcomes. Clinicians should maintain a high index of suspicion for the potential development of ABPA in BCO patients with high BSI.

Keywords: Bronchiectasis-COPD overlap, *Aspergillus*, ABPA, sensitization, BCO

Adverse clinical consequence associates with fungi in severe asthma, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and bronchiectasis [1-13]. In bronchiectasis, persistent fungal colonization leads to more purulent sputum and frequent exacerbations, whereas the clinical consequences in COPD remain largely unclear with no clear differences noted either in disease severity and/or exacerbation frequency [5,14-16]. Severe asthma with fungal sensitization (SAFS) is an established asthma phenotype associated with poor disease control, while in COPD, the importance of fungal sensitization including a high-risk mycobiome is linked to symptomatic disease, exacerbations, and mortality[17-21]. While sensitization and allergy, including to fungi, are not traditionally associated with bronchiectasis, a large multicenter study of Asian and European patients demonstrates the importance and clinical relevance of the fungal microbiome and its role in endophenotypes of bronchiectasis associated to fungal sensitization and poorer disease outcomes[2,22-24]. The overall poor clinical outcomes observed in association with fungal, and specifically *Aspergillus*-associated sensitization across a range of respiratory diseases has led to considering their potential role in overlap respiratory syndromes[7,25]. Matthes and colleagues report asthma-like features including sensitization in patients with severe COPD, and in such asthma-COPD overlap, the exacerbations experienced appear more clinically severe[26]. Increased risks of developing bronchiectasis-COPD overlap (BCO) are observed in COPD sensitized to *Aspergillus fumigatus*, however, rates of allergic bronchopulmonary aspergillosis (ABPA) in this setting and its clinical relevance remain uncertain[27]. ABPA remains an important clinical consequence related to a range of lung diseases and is associated to increased exacerbations, poorer lung function and the development of bronchiectasis[17,28]. Despite the adverse clinical outcomes reported for *Aspergillus* sensitization (and ABPA) in COPD and bronchiectasis, no clear recommendations currently exist for the routine assessment of *Aspergillus* in patients demonstrating sensitization. While oral corticosteroids and anti-fungals remain the treatment of choice for ABPA, their role, if any, in *Aspergillus* sensitization remains unclear [29]. Reported prevalence rates of BCO are increasing, and this clinical state associates with higher exacerbation risk, poorer lung function and greater mortality when compared to bronchiectasis and/or COPD as individual entities[30]. Whether

bronchiectasis and COPD co-exist by chance, share common pathophysiology or have an interrelated relationship resulting in overlap remains uncertain. Rates of *Aspergillus* sensitization are increased in BCO, however the role of ABPA remains unclear and prior BCO studies have predominantly focused on comparisons with COPD rather than bronchiectasis[27]. Moreover, crude *Aspergillus* allergens are routinely used to establish sensitization responses despite their lack of reproducibility and specificity when compared to recombinant *Aspergillus* allergens. Further assessment including crude and recombinant allergens in the context of sensitization and ABPA in COPD, bronchiectasis and BCO will provide an improved appreciation of their individual clinical utility[31].

Here, we assess the occurrence and clinical relevance of *Aspergillus* sensitization and ABPA in BCO in comparison to individuals without overlap.

Methods

Ethical approval: Institutional review boards (IRBs) of all participating hospitals approved the study and written informed consent was obtained from all participants. IRB reference numbers were as follows: CIRB 2016/2715, CIRB 2017/2933, CIRB 2017/2109, CIRB 2016/2073 mutually recognized by DSRB; NTU IRB-2016-01-031, UKMMC FF-2016-440, UMMC 2018725-6524 and NHD 12/ES/0059.

Patient recruitment: Patients with stable COPD, bronchiectasis and BCO overlap were recruited into a prospective observational cross-sectional study between 2016 and 2018 across six tertiary centres in three countries: Singapore General Hospital, Changi General Hospital and Tan Tock Seng Hospital (Singapore), University Malaya Medical Centre and UKM Medical Centre (Kuala Lumpur, Malaysia), and Ninewells Hospital (Dundee, United Kingdom). COPD was defined according to the global initiative for chronic obstructive lung disease (GOLD) criteria 2018 [32]. Bronchiectasis was defined as the presence of cough, shortness of breath, chronic sputum production, and/or recurrent respiratory infection with high-resolution computed tomography (HRCT) confirmation of the presence of

bronchiectasis indicated by a broncho-arterial ratio > 1, lack of tapering, and/or airway visibility within 1cm of the pleural surface [33,34]. BCO was defined as the presence of respiratory symptoms such as cough, sputum production, shortness of breath, and/or wheezing with a >10 pack year smoking history, an FEV_1/FVC ratio of <0.7 and HRCT confirmation of bronchiectasis without definitive aetiology such as immunodeficiency, primary ciliary dysfunction, rheumatoid arthritis, connective tissue disease, inflammatory bowel disease, aspiration, Kartagener's syndrome, Young's syndrome, α-1 antitrypsin deficiency, human immunodeficiency virus infection, Williams-Campbell, Marfan or Mounier-Kuhn syndrome [32-34]. Patients with a documented diagnosis of asthma, defined by GINA guidelines (variable respiratory symptoms and airflow limitation), individuals on long-term oral corticosteroids and/or immunosuppressive agents, active mycobacterial disease, malignancy on chemotherapy and recent active infection and/or exacerbations requiring antibiotics or systemic corticosteroids in the 4 weeks preceding study recruitment were excluded [35]. ABPA was defined as an elevated total immunoglobulin-E (IgE) (>500 IU/ml) with specific immunoglobulin-E (sIgE) against *Aspergillus fumigatus* with any two of the following criteria: elevated serum anti-*Aspergillus* IgG antibodies, peripheral eosinophilia and radiological features consistent with ABPA as previously described [29,36]. Sensitization occurs at values > 0.35 kU/L, and polysensitization refers to sensitization to 2 or more allergens. *Aspergillus* sensitization (AS) was defined as sensitization to crude *Aspergillus fumigatus* and/or *A. terreus* recombinant allergen and *Aspergillus* colonization (AC) refers to the detection of *Aspergillus fumigatus* and/or *A. terreus* by sputum culture and/or quantitative polymerase chain reaction (qPCR). Exacerbations was defined as an acute worsening of respiratory symptoms requiring additional therapy (i.e. antibiotics and/or corticosteroids) [32]. Frequent exacerbators were defined as two or more exacerbations in the preceding year for COPD and BCO and three or more exacerbations in the preceding year for bronchiectasis in line with published criteria [37,38]. Clinical data was collated from all patients and includes demographics, smoking history, pulmonary function, modified Medical Research Council dyspnoea (mMRC) score, Bronchiectasis Severity Index (BSI), sputum microbiology, number of exacerbations and/or hospitalizations requiring antibiotics and/or corticosteroid treatment in the year preceding recruitment. All recruited patients had a prior HRCT thorax that was assessed for the presence of bronchiectasis as described above. All patients included into the COPD group had no evidence of

radiological bronchiectasis. Bronchiectasis Severity Index (BSI) ranges from 0-26 and is categorized as mild (0-4), moderate (5-8) or severe (>9) [39]. COPD severity was defined as GOLD ABCD based on exacerbation frequency and symptoms (as CAT score) and GOLD grouped according to FEV_1 % predicted; group 1 (>80%) , group 2 (50-79%), group 3 (30-49%) and group 4 (<29%) [32].

Venous blood and sputum processing, immunological assays and microbiome assessment: Venous blood was collected from all patients at recruitment and serum isolated by centrifuging at 1300g for 10 minutes at 18°C followed by storage in aliquots at -80°C until further processing. Spontaneous expectorated sputum where accessible, were collected from a subset of patients (n=156) and subjected to microbiome sequencing[40]. Full details of all immunological assays and microbiome sequencing is provided in the online supplement.

Statistical analysis: Statistical analysis was performed using R (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria) and normality testing using the Shapiro-Wilk test. All continuous variables were non-normally distributed and therefore presented as medians with interquartile ranges (IQRs). The Kruskal-Wallis test with Benjamin-Hochberg correction for false discovery was used in group comparisons (for continuous variables) and the chi-square or fisher exact test used as appropriate (for categorical variables). Multivariate logistic regression was performed using the *'glm'* function in R and variables with a p-value<0.05 on univariate analysis and which were clinically relevant were used in the presented multivariate regression model. Alpha diversity (Shannon and Simpson index) was calculated with the "*diversity*" function R package "vegan". Beta diversity was visualized using the principal coordinate analysis (PCoA) plot with Bray-Curtis dissimilarities, and permutational multivariate analysis of variance using distance matrices (PERMANOVA) was computed with 10,000 permutations using the "*adonis"* function of R package "vegan". Comparisons of taxa between patient groups was performed using Metastats [41]. A p-value<0.05 was considered significant for all analyses.

Results

BCO demonstrates an increased frequency of ABPA in comparison to bronchiectasis and/or COPD without overlap. A total of 280 stable patients with BCO (n=47), bronchiectasis (n=183) and COPD (n=50) were recruited from Singapore, Malaysia and Scotland. Their median age was 65 (IQR 61-74) with a male predominance in the COPD (98%) and BCO (66%) groups. ABPA was present in 37 (13.2%) of the recruited cohort with the highest proportion in patients with BCO (n=16, 34.0%) compared with bronchiectasis (n=19, 10.4%) and COPD (n=2, 4.0%). Patient demographics and associated clinical characteristics are illustrated in Table 1. Clinical associations and outcomes related to BCO were assessed by multivariate logistic regression analyses against bronchiectasis or COPD. BCO significantly associates with male gender (OR 2.25; 95% CI: 1.04 - 4.86; p=0.040), greater symptoms (mMRC dyspnea score) (OR 3.37; 95% CI: 1.41 - 8.07; p=0.006), a higher frequency of ABPA (OR 3.85; 95% CI: 1.58 - 9.4; p=0.003) and a lower number of involved lobes (OR 0.46; 95% CI: 0.21 - 0.99; p=0.047) compared to bronchiectasis (Figure 1a). When BCO was assessed in comparison to COPD, it demonstrates significantly higher odds of exacerbations (ORs 5.22; 95% CI: 1.77 - 15.33; p=0.003), higher ABPA frequencies (OR 10.68; 95% CI: 1.07 - 107.1; p=0.044) but a lower smoking pack year history (OR 0.96; 95% CI: 0.92 - 1.00; p=0.038) (Figure 1b). Taken together, these data suggest that BCO is an important clinical entity compared to bronchiectasis and/or COPD without overlap. BCO demonstrates greater symptoms, exacerbations, and is enriched for the occurrence of ABPA despite a lower number of involved lobes (for bronchiectasis) and shorter smoking history (for COPD).

BCO-associated ABPA illustrates greatest disease severity, highest exacerbation frequency and poorest lung function. Having identified a significantly higher frequency of ABPA in BCO, we next evaluated for key clinical associations of ABPA in patients with or without BCO. BCO-associated ABPA demonstrates significantly greater disease severity (median BSI 12; IQR: 10 - 14; p=0.001) (Figure 2a), highest exacerbation frequency (median 4; IQR: 2 - 6, p=0.002) (Figure 2b) and poorest lung function (median 49.3% FEV_1 % predicted; IQR: 36.9 - 65.1; p=0.001) (Figure 2c) with a trend

toward greater symptoms (as mMRC score) (median 2; IQR 1 - 2; p=0.061) (Figure 2d) in comparison to individuals without ABPA or non-BCO-associated ABPA. When the patients were grouped as non-diseased (i.e. non-sensitized or colonized), *Aspergillus* colonized, *Aspergillus* sensitized or ABPA, ABPA demonstrates the highest disease severity (median BSI 12; IQR 10-14, p=0.004), exacerbation frequency (median 3; IQR 1-5, p=0.002) and poorest lung function (median FEV_1% predicted 58; IQR: 40-75; p=0.01) however no differences in symptomatology (e-Figure 1).

BCO and bronchiectasis exhibit comparable *Aspergillus fumigatus* sensitization patterns. As BCO demonstrates higher frequencies of ABPA compared to bronchiectasis and COPD, we next assessed whether sensitization patterns including polysensitization, with specific focus on *Aspergillus*, varied between disease states as sensitization is an important ABPA precursor. No differences in total IgE or the sensitization response to house dust mite or crude *Aspergillus* allergens were detected between the three groups (Figure 3a, e-Figure 2a and 2b), however, sIgE responses against the major and minor recombinant *Aspergillus* allergens rAsp f 1 (p<0.001), f 2 (p<0.001), f 6 (p=0.005), f 15 (p<0.001) and f 17 (p=0.009) were significantly higher in BCO and bronchiectasis (Figure 3b) suggesting that bronchiectasis, regardless of overlap, portends toward a higher occurrence of sensitization. To further interrogate this, we next evaluated for any relationship to the underlying airway microbiome. Only patients demonstrating sensitization to the major rAsp f allergens (rAsp f1 and/or f2) illustrate lower α- and β- mycobiome diversity with an increased relative abundance of *Tricosporon* (p=0.003) (e-Figure 3, e-Table 1). No significant difference was observed in bacterial microbiomes (e-Figure 4, e-Table 1). We next considered if polysensitization could explain the higher occurrence of ABPA in patients with BCO. Patients with BCO and bronchiectasis demonstrate higher rates of polysensitization to rAsp f allergens compared to COPD (p<0.001) (Figure 4a). Polysensitization associates with higher symptomatic burden (mMRC score) (p=0.022) (Figure 4b) across all three groups which interestingly remains significant in non-frequent exacerbators (<2 exacerbations/year for COPD and BCO and <3 exacerbations/year for bronchiectasis) (e-Figure 5a). Of note, we did not detect any association between polysensitization and lung function or exacerbation frequency in any group (Figure 4c-d, e-Figure 5b).

Taken together, the higher observed frequency and severity of BCO-associated ABPA is not related to differences in sensitization response patterns, including polysensitization or the underlying airway microbiome. BCO and bronchiectasis (without overlap) therefore appear comparable in terms of sensitization responses, airway microbiomes and polysensitization.

Sensitization to rAsp f 2 and 17 is enriched in ABPA. Having demonstrated the higher frequency and clinical severity of BCO-associated ABPA, that has comparable sensitization to bronchiectasis, we next sought to determine if specific rAsp f allergens were enriched in BCO-associated ABPA to assist in its early identification. Sensitization to rAsp f 2 ($p=0.003$), and f 17 ($p=0.002$) although enriched in ABPA, were no different between BCO and bronchiectasis (Figure 5a). Irrespective of overlap however, ABPA patients sensitized to rAsp f 2 have significantly more severe bronchiectasis but no difference in exacerbations, lung function or symptoms (Figure 5b, d, f and e-Figure 6a), while ABPA patients sensitized to rAsp f 17 demonstrate increased exacerbations and reduced lung function but no difference in bronchiectasis severity and/or symptoms (Figure 5c, e, g and e-Figure 6b). Therefore, while a detectable response to rAsp f 2 or rAsp f 17 may have clinical relevance in the setting of ABPA, importantly, they do not help in the identification or risk stratification of individuals with BCO-associated ABPA.

BCO-associated ABPA is observed at highest frequency in individuals with a severe BSI and associates with poor clinical outcomes. As no differences in overall sensitization pattern or in the response to specific rAsp f allergens were found in association with BCO-associated ABPA despite its high frequency and clinical severity, we next assessed which disease component of the overlap (i.e. bronchiectasis or COPD severity) portended toward greater risk for ABPA. Such evaluation allows for clinical risk stratification in BCO. Bronchiectasis severity (as BSI) and COPD severity (based on GOLD grade or ABCD group) were assessed in relation to the presence or absence of ABPA in the BCO group (Figure 6a-b). BCO with a severe BSI (>9), unrelated to COPD severity, significantly associated with

the occurrence of ABPA (p=0.017) (Figure 6a-b). When BCO-associated ABPA was compared with bronchiectasis-associated ABPA (without overlap), the former importantly associated with significantly more exacerbations and symptoms but less lobar involvement indicative of the clinical importance in identifying and stratifying this patient group (Figure 6c-e).

Discussion

We report an increased frequency of ABPA in BCO compared to COPD and/or bronchiectasis. Individuals with BCO-associated ABPA also demonstrate poorest clinical outcomes despite comparable sensitization patterns to bronchiectasis. While sensitization to rAsp f 2 and f 17 was enriched in ABPA, this was not restricted to BCO. BCO-associated ABPA occurred at highest frequencies in individuals with a severe BSI (>9) and no association with COPD GOLD ABCD grade and/or stage was identified. BCO is known to be associated with worse clinical outcomes than either disease alone independent of radiological severity or lung function. Our data suggests that a higher frequency of aspergillus sensitization may contribute to the increased morbidity in this group.

BCO remains an understudied clinical entity, suffering from a lack of consensus definition and therefore complicated by wide ranging reported prevalence [30]. BCO however is importantly associated to increased airway inflammation, greater symptoms, higher exacerbations, and all-cause mortality when compared to COPD[42-44]. These findings are broadly consistent with our work which incrementally identifies a higher ABPA frequency in BCO. Critically, BCO-associated ABPA associates with more severe disease, higher exacerbation rates and poorest lung function when compared to ABPA occurring in the absence of overlap. This suggests the importance of identifying BCO early to stratify patients optimally for their risk of developing ABPA. Furthermore, almost all prior work on BCO uses COPD as the sole reference comparator with little work assessing bronchiectasis[27,42,45]. Our study overcomes this by employing both COPD and bronchiectasis as comparators to BCO to provide important additional clinical insight. For instance, despite the increased symptoms and greater frequency of ABPA

in BCO, a significantly smaller number of lobes were found to be affected by bronchiectasis in BCO when compared to bronchiectasis without overlap, suggestive of mechanisms going beyond structural change to explain the higher occurrence and poorer clinical outcomes associated with ABPA in BCO.

Fungal sensitization represents an important 'treatable' trait in patients with chronic airways disease including asthma, COPD, and bronchiectasis and is considered a precursor to the later occurrence of ABPA. For example, fungal sensitization is associated to exacerbations, symptoms, and poor lung function in COPD. Fungi, particularly *Aspergillus* sensitization is prevalent in patients with post-tuberculosis-related bronchiectasis and fungal driven pro-inflammatory immuno-allertypes in bronchiectasis associate with airway inflammation and adverse clinical outcomes[18,22,46]. The role of fungal sensitization and ABPA in BCO however has lacked study with only a single previous report indicating some association between BCO and recombinant *Aspergillus fumigatus* sensitization in patients with COPD[27]. Further, current COPD (GOLD) guidelines do not provide any specific recommendations for the screening of *Aspergillus* sensitization and/or ABPA despite increasing evidence supporting an association with poorer clinical outcome[5,18]. Our study extends this by identifying and comparing specific sensitization patterns to a variety of crude and recombinant allergens including *Aspergillus fumigatus* and explores polysensitization between BCO, COPD and bronchiectasis. Although BCO demonstrates differences in sensitization pattern to COPD, we found remarkably similar sensitization profiles with bronchiectasis, unidentified in prior work, and indicative of the important influence of structural lung damage on measurable sensitization.

ABPA represents a significant allergic response to airway *Aspergillus* with sensitization considered antecedent to its occurrence. ABPA is described in relation to asthma, COPD and CF and is considered both a cause and consequence of bronchiectasis[17,47,48]. Its early diagnosis and treatment have important prognostic implications and may prevent irreversible lung damage however, little is known about its occurrence and/or clinical relevance in BCO[17]. Here, we identify high frequencies of ABPA in BCO,

importantly higher than COPD or bronchiectasis. Its clinical relevance in BCO is important, associating with greater disease severity, significant symptoms and higher exacerbations compared to individuals without overlap. Whether BCO predisposes to ABPA or ABPA occurring in the setting of COPD or bronchiectasis itself incites the development of BCO cannot be established from this work and necessitates prospective longitudinal studies to elucidate. What is clear however is that these relationships are complex, for instance we found comparable patterns of *Aspergillus fumigatus* sensitization between BCO and bronchiectasis, however, the occurrence of ABPA remained significantly higher in the setting of BCO, even where a lesser number of lobes were affected by bronchiectasis. This suggests that host rather than structural factors may be most important in determining the risks and clinical consequences associated with the development of ABPA.

As clinical BCO represents the concurrent presence of COPD and bronchiectasis respectively, albeit to differing extents in different patients, we assessed for the potential contribution of COPD and/or bronchiectasis respectively to the development of BCO-associated ABPA. While no relationship between COPD severity and ABPA occurrence was identified, either by GOLD ABCD group or stage, we did find that a severe bronchiectasis severity index (i.e. >9) conferred the strongest link to BCO-associated ABPA suggesting that clinicians maintain a high index of suspicion for the development of ABPA in BCO patients with high BSI.

A further strength of this work is the use of specific recombinant *Aspergillus* allergens to assess for the IgE response and classify ABPA. Crude allergens have been most used in prior work to assess such responses however, they can elicit variable outcomes owing to batch-to-batch variation, inter-strain differences and an altered allergenicity based on fungal growth and culture conditions employed during synthesis[49]. In addition, crude allergens cannot adequately differentiate cross-reactivity from co-sensitivity and the use of recombinant allergens, at least partially, overcomes such collective limitations[50]. Recombinant allergens, produced by DNA cloning and protein purification offer a more

standardized and reproducible approach where large scale production is feasible[50]. Importantly, although we did not find any significant differences in sensitization pattern between bronchiectasis, COPD and BCO to crude *Aspergillus* allergens, key differences were observed when recombinant *Aspergillus fumigatus* allergens were assessed, attesting to their importance and greater specificity. Interestingly, polysensitization to rAsp f allergens associated with greater symptoms in non-frequent exacerbators, an important clinical finding likely to have been missed if only crude allergens were evaluated. Certain specific recombinant *Aspergillus fumigatus* allergens are reported to correlate with ABPA: increased specific-IgE responses to rAsp f 2, 4, and 6 are enriched in ABPA as compared to allergic asthma and healthy individuals while rAsp f 17 occurs in bronchiectasis-associated ABPA[22,51]. Here, we report increased sensitization rates to rAsp f 2 and f 17 with occurrences of ABPA, a finding consistent with prior studies. These responses importantly associate with poorer clinical outcomes and are not limited to BCO-associated ABPA. Interestingly, we also detect a reduced α- and β-airway mycobiome diversity in association with sensitization to the major recombinant *Aspergillus fumigatus* allergen rAsp f 2 suggesting a potential role of this sensitization response in the development of ABPA through an altered mycobiome.

While our study importantly advances our understanding of sensitization and ABPA in the setting of BCO, it does have limitations. All included patients were recruited from major tertiary referral hospitals which may enrich for patients with a high disease burden and therefore a higher frequency of sensitization. Due to the cross-sectional nature of our work, longitudinal outcomes, including the stability of the measured sensitization response was not assessed. Critically, a lack of consensus diagnostic criteria for BCO exists, and therefore our definitions may have over or under-diagnosed individuals with BCO. Nevertheless, in view of our strict criteria for a diagnosis of BCO in this study, the number of included BCO patients was relatively small and future studies with larger cohorts will be important to validate our findings. As patients were recruited from six different centers, CT thorax was reported at each respective center. While the diagnosis of bronchiectasis is based on defined radiological criteria, other radiological findings that may be associated with ABPA such as mucus impaction or

hyper attenuated mucus were not systemically assessed and therefore cannot be reported in this study. In addition, we did not quantify degree of radiological emphysema that may have been beneficial in combination with the bronchiectasis severity radiological CT score (BRICS) in more clearly definng the BCO endotype [52]. Further, we recognize that our COPD cohort, comprising a smaller number of patients in comparison to bronchiectasis, had milder disease (i.e. GOLD A-B) which may not be representative of a wider more general COPD population.

Interpretation

BCO is an important clinical entity compared to COPD or bronchiectasis. It demonstrates high frequencies of ABPA that associate with poor clinical outcomes. Clinicians should maintain a high index of suspicion for the potential development of ABPA in BCO particularly those with high BSI.

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Authors' contributions: TPY and SHC take full responsibility for the content of the manuscript, including the data analysis. TPY: Study design, patient recruitment and performance of experimental work, data collection, interpretation and analysis including writing of the final manuscript. AYHL, HRK, AJD, MMA, SLP, LTB, TMH, MEP, HX, THO, MSK, JAA, AT, FTC and JDC: patient recruitment, clinical data, specimen collection, experimental work and data analysis and SHC: Study design and conception of experiments, obtained study funding, interpretation of results, data analysis and writing of the final manuscript. All authors reviewed and approved the final draft of the manuscript.

Take Home Point:

Study Question: What is the role of fungal sensitization in bronchiectasis-COPD overlap (BCO)?

Results: BCO demonstrates a high frequency of ABPA that associates with a severe BSI (>9) and poor clinical outcomes.

Interpretation: Clinicians should maintain a high index of suspicion for the potential development of sABPA in BCO patients with high BSI.

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Table 1: Summary of patient demographics and clinical characteristics of the study population including bronchiectasis-COPD overlap (BCO), bronchiectasis (BE) and chronic obstructive pulmonary disease (COPD).

Characteristic	Total	BCO	BE	COPD	p-value
n (%)	280	47	183	50	
Age, Median (IQR)	68.0 (61.0-74.0)	70 (63-76)	67 (59-73)	69 (64-75)	0.014
Gender, n (%)					<0.001
Male	164 (58.6)	31 (66)	84 (45.9)	49 (98.0)	
Female	116 (41.4)	16 (34)	99 (54.1)	1 (2.0)	
BMI kg/m^2, Median (IQR)	21.3 (18.7-26.1)	24.2 (20.2-27.7)	20.8 (18.7-25.7)	21.6 (17.7-23.5)	0.028
Smoking status, n (%)					<0.001
Never	158 (56.4)	0 (0.0)	158 (86.3)	0	
Ex-smoker	89 (31.8)	39 (83.0)	19 (10.4)	31 (62.0)	
Current smoker	33 (11.8)	8 (17.0)	6 (3.3)	19 (38.0)	
BSI, Median (IQR)		10 (9-13)	9 (6-12)		0.013
Mild, n (%)	31 (13.5)	1 (2.1)	30 (16.4)		0.007
Moderate, n (%)	60 (26.1)	9 (19.2)	51 (27.9)		
Severe, n (%)	139 (60.4)	37 (78.7)	102 (55.7)		
mMRC, Median (IQR)	1 (1-2)	2 (1-4)	1 (1-2)	2 (1-3)	<0.001
FEV_1 % predicted, Median (IQR)	70.1 (53.0-85.0)	58.0 (41.6-75.5)	76.0 (61.0-89.0)	58.0 (41.0-75.0)	<0.001
FEV_1 >80%	96 (34.3)	8 (17.0)	79 (43.2)	9 (18.0)	<0.001
FEV_1 50-80%	124 (44.3)	22 (46.8)	81 (44.3)	21 (42.0)	
FEV_1 30-50%	51 (18.2)	14 (29.8)	18 (9.8)	19 (38.0)	
FEV_1<30%	9 (3.2)	3 (6.4)	5 (2.7)	1 (2.0)	
FEV_1/FVC % predicted, Median (IQR)	70.0 (56.0-81.3)	55.7(45.0-565.0)	77.0 (70.0-87.0)	54.6 (44.3-63.8)	<0.001
Bronchiectasis aetiology, n (%)					0.102
Idiopathic	156 (55.7)	32 (68.1)	124 (67.7)		
Post-infection (mycobacteria)	27 (9.6)	6 (12.8)	21 (11.5)		
Post infection (non-mycobacterial)	24 (8.5)	9 (19.1)	15 (8.2)		
Others	23 (8.2)	0 (0.0)	23 (12.6)		
Radiological severity, n (%)					0.021
1 to 2 lobes	82 (35.7)	24 (51.0)	58 (31.7)		
>2 lobes	148 (64.3)	23 (49.0)	125 (63.3)		
Pseudomonas colonization, n (%)					0.006
Yes	27 (9.6)	2 (4.3)	25 (13.7)	0 (0.0)	
No	253 (90.4)	45 (95.7)	158 (86.3)	50 (100)	
Colonization: other bacteria organisms, n (%)					0.024
Yes	111 (39.6)	15 (31.9)	96 (52.5)	0 (0.0)	
No	169 (60.4)	32 (68.1)	87 (47.5)	50 (100)	
Aspergillus colonization, n (%)					
Yes	173 (61.8)	39 (83.0)	121 (66.1)	13 (26.0)	<0.001
No	107 (38.2)	8 (17.0)	62 (33.9)	37 (74.0)	
No. of hospitalized exacerbations in the preceding year					ns
Yes	108 (38.6)	21 (44.7)	69 (37.7)	18 (36.0)	
No	172 (61.4)	26 (55.3)	114 (62.3)	32 (64.0)	
No. of exacerbations in the preceding year					<0.001
0	116 (41.4)	10 (21.3)	63 (34.4)	43 (86.0)	

1 to 2	78 (27.9)	15 (31.9)	56 (30.6)	7 (14.0)	
>2	86 (30.7)	22 (46.8)	64 (35.0)	0 (0.0)	
ABPA					<0.001
Yes	37 (13.2)	16 (34.0)	19 (10.4)	2 (4.0)	
No	243 (86.8)	31 (66.0)	164 (89.6)	48 (96.0)	
Presence of upper lobe bronchiectasis, n (%)	14 (38)	4 (8.5)	10 (5.5)	0 (0)	0.662
GOLD ABCD					<0.001
A	22 (22.7)	7 (14.9)		15 (30.0)	
B	25 (25.8)	8 (17.0)		17(34.0)	
C	18 (18.5)	14 (29.8)		4 (8.0)	
D	32 (33.0)	18 (38.3)		14 (28.0)	

Data is presented as number of patients (n) with respective percentages (%) or median with interquartile range (IQR). BMI: body mass index, BSI: bronchiectasis severity index, mMRC: modified MRC dyspnea score, FEV_1: forced expiratory volume in the 1st second, FVC: forced vital capacity, ABPA: allergic bronchopulmonary aspergillosis, GOLD: global initiative of chronic obstructive lung disease, ns: non-significant.

Figure 1: Patients with bronchiectasis-COPD overlap (BCO) demonstrate a high frequency of allergic bronchopulmonary aspergillosis (ABPA) and varied clinical characteristics compared to bronchiectasis (BE) and COPD respectively. Forest plot with odds ratio (OR) and 95% confidence interval (CI) illustrating the clinical differences between (a) bronchiectasis (BE) and BCO and (b) COPD and BCO. Dots represent the odds ratio and colouration indicates level of significance: red ($p<0.05$) and grey (ns). Error bars correspond to the 95% CI.

Figure 2: Bronchiectasis-COPD overlap (BCO)-associated allergic bronchopulmonary aspergillosis (ABPA) exhibits poor clinical outcomes. Scattered box plots illustrating (a) disease severity (as bronchiectasis severity index (BSI)), (b) exacerbation frequency (in the year preceding study recruitment) (c) lung function (as FEV_1% predicted) and (d) patient symptoms (as mMRC score) in individuals without ABPA (i.e. non-ABPA), ABPA and BCO-associated ABPA (i.e. BCO ABPA). Dot colouration corresponds to the respective patient group: BCO (red), bronchiectasis (green) and COPD (blue). Box and whisker plots illustrating the median and interquartile range (IQR) and the largest and smallest values (within 1.5 times the IQR) above or below the 75th and 25th percentile respectively. **$p<0.01$, ns: non-significant.

Figure 3: Bronchiectasis (BE) and bronchiectasis-COPD overlap (BCO) exhibit similar sensitization patterns to crude *Aspergillus* and recombinant *Aspergillus fumigatus* allergens compared to COPD. Scattered box plots illustrating systemic specific-IgE binding (log_{10}) to (a) crude *Aspergillus fumigatus* (Asp f) and *Aspergillus terreus* (Asp t) allergen and (b) recombinant major (rAsp f1 and 2) and minor (rAsp f6, 8, 15 and 17) *Aspergillus fumigatus* allergens. Horizontal dotted lines correspond to >0.35 kU/L (international cut-off indicating a positive response). Dot colouration corresponds to the respective patient group: BCO (red), bronchiectasis (green) and COPD (blue). Box and whisker plots illustrating the median and interquartile range (IQR) and the largest and smallest values (within 1.5 times the IQR) above or below the 75^{th} and 25^{th} percentile respectively. *p<0.05, **p<0.01, ***p<0.001, ns: non-significant.

Figure 4: Polysensitization to recombinant *Aspergillus* allergens demonstrates greater symptomatic burden but no effect on lung function and/or exacerbation frequency. Scattered boxplots illustrating (a) the total number of recombinant *Aspergillus* allergens to which each individual is sensitised to (by disease group) and its association to (b) symptoms (as mMRC score), (c) lung function (as FEV_1 % predicted) and (d) exacerbation frequency (in the year preceding study recruitment). Dot colouration corresponds to each patient group: BCO (red), bronchiectasis (green) and COPD (blue). Box and whisker plots illustrating the median and interquartile range (IQR) and the largest and smallest values (within 1.5 times the IQR) above or below the 75^{th} and 25^{th} percentile respectively. *p<0.05, ***p<0.01, ns: non-significant.

Figure 5: Sensitization responses to the recombinant *Aspergillus fumigatus* allergens (rAsp) f2 and f17 are enriched in allergic bronchopulmonary aspergillosis (ABPA) and associate with disease severity and exacerbations in bronchiectasis-COPD overlap (BCO) and bronchiectasis (BE) (a) Scattered boxplots illustrating the systemic specific-IgE binding (log_{10}) to the various recombinant *Aspergillus fumigatus* allergens (rAsp f) compared between individuals with and without ABPA (indicated as A

and N respectively). Scattered boxplots illustrating (b-c) disease severity (as bronchiectasis severity index (BSI)) in non-ABPA and ABPA patients sensitised to (b) rAsp f 2 and (c) rAsp f 17 respectively. (d-e) exacerbation frequency (in the year preceding study recruitment) and (f-g) lung function (as FEV_1 % predicted) in non-ABPA and ABPA patients sensitised to (d and f) rAsp f 2, and (e and g) rAsp f 17 respectively. Dot colouration corresponds to the respective patient group: BCO (red), bronchiectasis (green) and COPD (blue). Box and whisker plots illustrating the median and interquartile range (IQR) and the largest and smallest values (within 1.5 times the IQR) above or below the 75^{th} and 25^{th} percentile respectively. *p<0.05, **p<0.01, ns: non-significant.

Figure 6: Bronchiectasis-COPD overlap (BCO)-associated allergic bronchopulmonary aspergillosis (ABPA) exhibits higher bronchiectasis severity index (BSI) but is unrelated to COPD GOLD grade and/or ABCD group. Scatterplot illustrating (a) GOLD grade and BSI and (b) GOLD ABCD group and BSI in BCO patients with (purple) and without ABPA (grey). Scattered boxplots illustrating (c) exacerbation frequency (in the year preceding study recruitment) (d) symptoms (as mMRC) score and (e) number of lobes affected in ABPA associated with bronchiectasis (green) and BCO (red) respectively. Dotted lines differentiate mild-moderate (BSI ≤9) versus severe BSI (BSI >9) scores. Box and whisker plots illustrating the median and interquartile range (IQR) and the largest and smallest values (within 1.5 times the IQR) above or below the 75^{th} and 25^{th} percentile respectively. *p≤0.05.

Figure 1

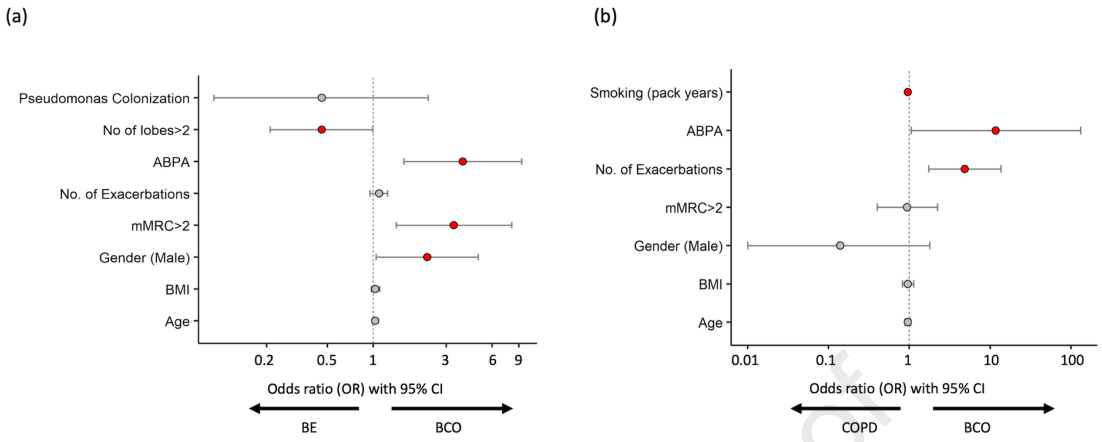


Figure 2

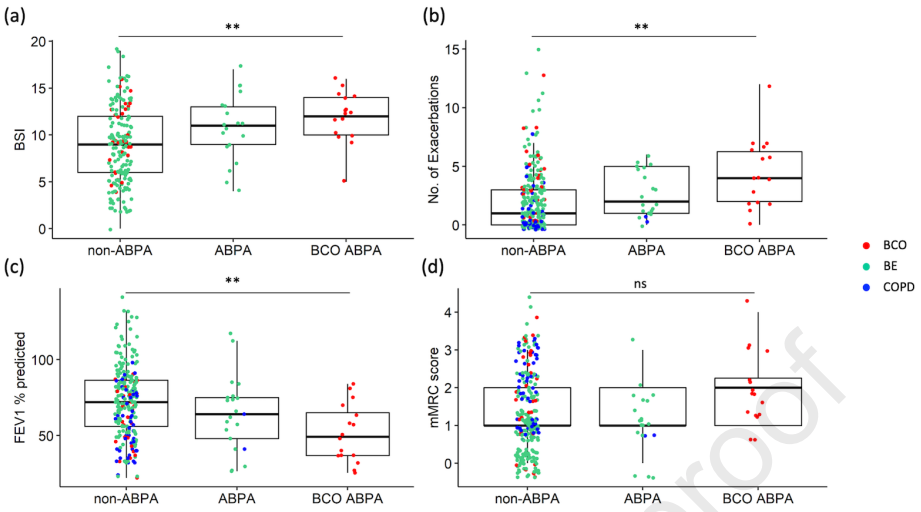


Figure 3

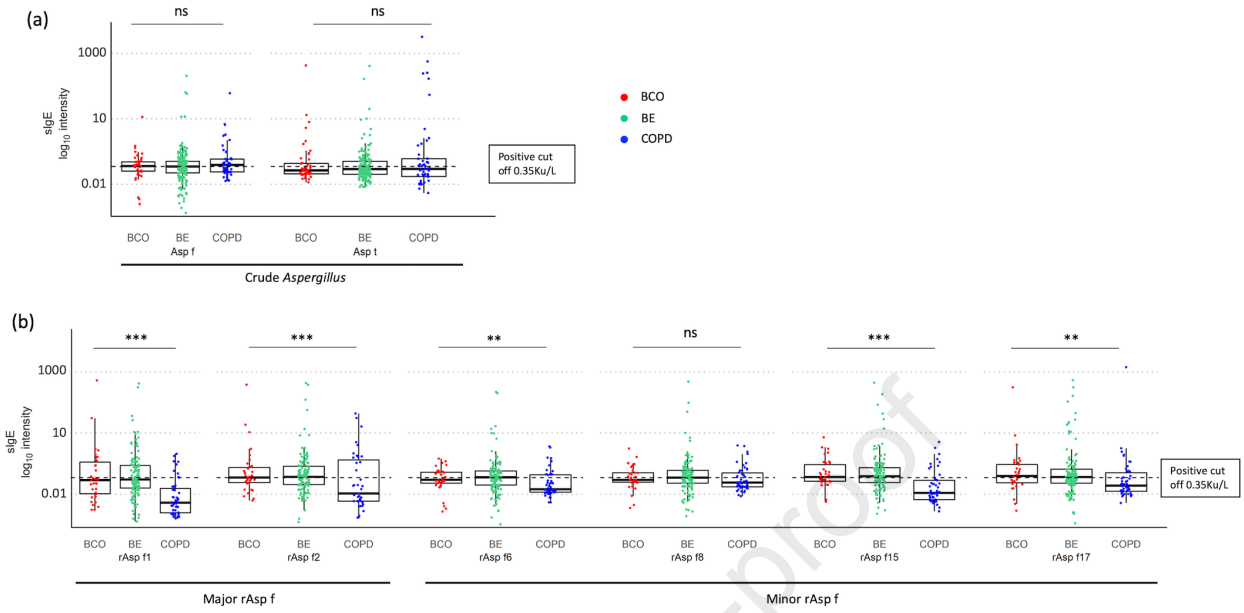


Figure 4

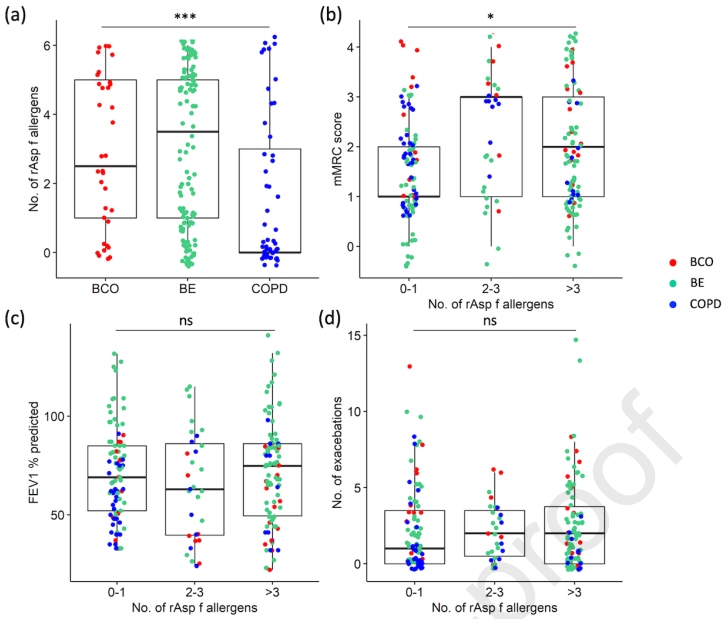


Figure 5

(a)

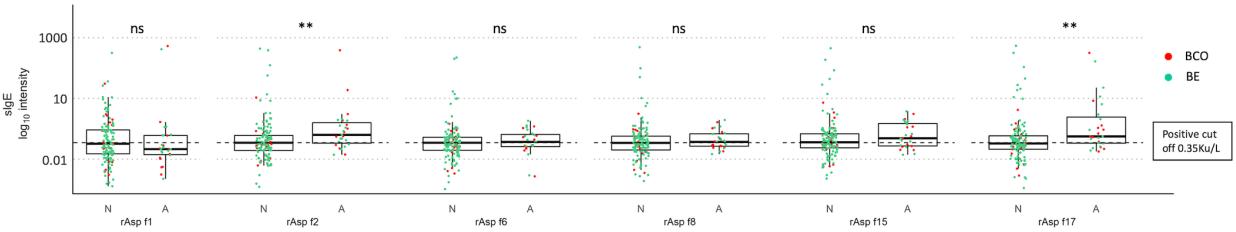


Figure 5

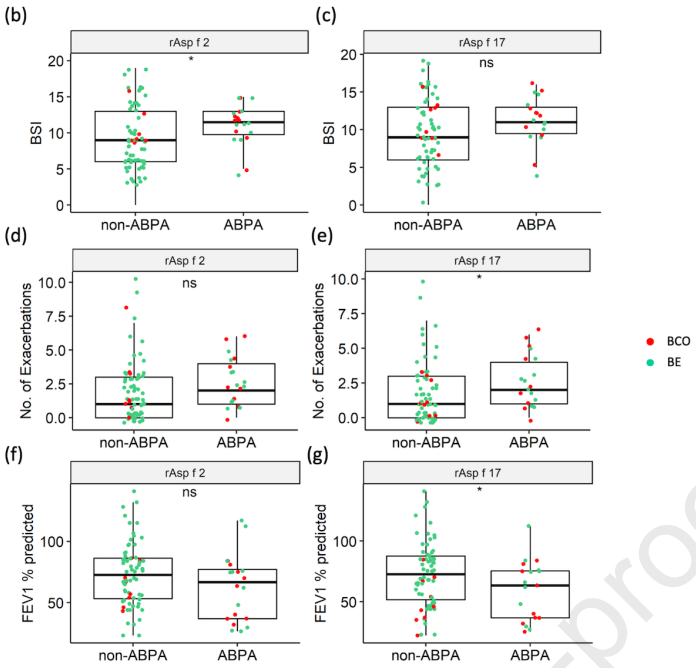


Figure 6

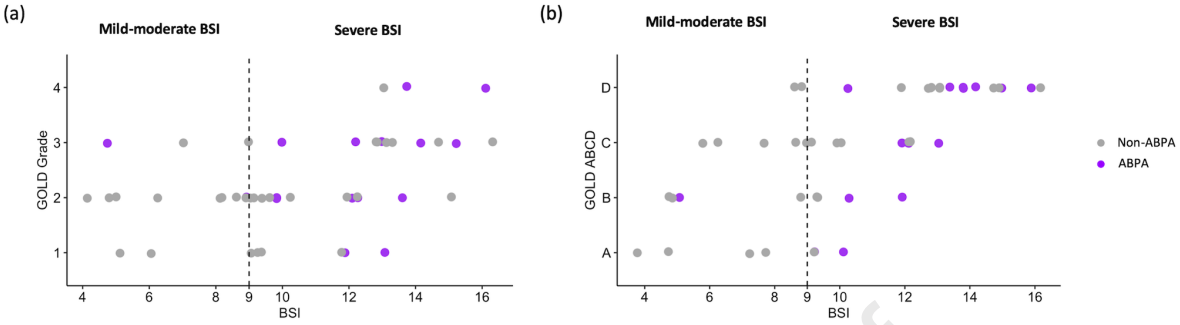
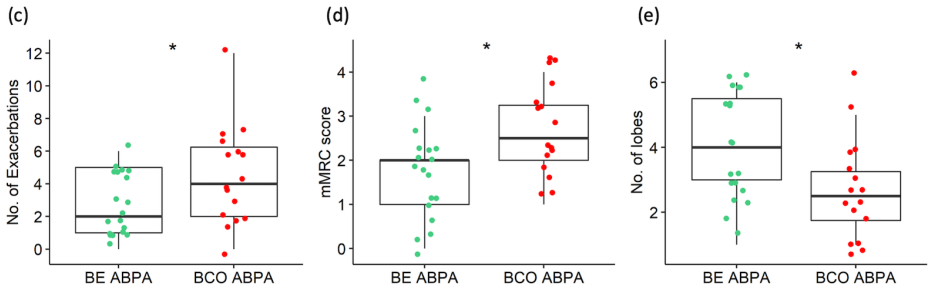


Figure 6



Abbreviation List

BCO (Bronchiectasis-COPD overlap), BMI (body mass index), BSI (bronchiectasis severity index), CF (cystic fibrosis), COPD (chronic obstructive pulmonary disease), FEV_1 (forced expiratory volume in the first second), IQR (interquartile range), ITS (internal transcribed spacer), OR (odds ratio), rAsp f (recombinant *Aspergillus fumigatus*), sABPA (serological allergic bronchopulmonary aspergillosis), sIgE (specific-IgE)