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DISTINCT 'IMMUNO-ALLERTYPES' OF DISEASE AND HIGH FREQUENCIES OF SENSITISATION IN NON-CYSTIC-FIBROSIS BRONCHIECTASIS

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AT A GLANCE COMMENTARY

Scientific knowledge on the subject: Atopy and sensitization are established prognostic indicators in chronic respiratory diseases including asthma and chronic obstructive pulmonary disease (COPD) where they represent 'treatable traits'. Their role in bronchiectasis not due to cystic fibrosis is unclear, and few studies have assessed the frequency of atopy in adult bronchiectasis. Reports to date are limited to single centre studies, relatively small cohorts and have demonstrated conflicting results.

What this study adds to the field: We report, to our knowledge, the largest multi-centre study of atopy in bronchiectasis including patients from geographically distinct Asian (Singapore and Malaysia) and European (Scotland) cohorts. Our results illustrate high rates of allergic sensitization against a broad panel of allergens including house dust mite and fungi that correlate with worse clinical outcome. The airway inflammatory profile of 'sensitized bronchiectasis' reveals novel endo-phenotypes of disease in our 'matched' and geographically distinct populations. This work demonstrates the importance of identifying sensitization and atopy in bronchiectasis, its clinical relevance and geographic variability. We further demonstrate two clinically relevant endo-phenotypes, each driven by a specific allergen response profile and airway immune signature: fungal-driven pro-inflammatory and house-dust mite driven chemokine dominant. These 'immuno-allertypes' are clinically relevant and identify 'high risk' subgroups of bronchiectasis where appropriate therapeutic intervention may be offered.

Online data supplement statement: This article has an online data supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

ABSTRACT: Rationale: Allergic sensitization is associated with poor clinical outcomes in asthma, chronic obstructive pulmonary disease and cystic fibrosis however its presence, frequency and clinical significance in non-CF bronchiectasis remain unclear. **Objective:** To determine the frequency and geographic variability that exists in sensitization pattern to common and specific allergens including house dust mite and fungi and, to correlate such patterns to airway immune-inflammatory status and clinical outcomes in bronchiectasis. **Methods:** Patients with bronchiectasis were recruited in Asia (Singapore & Malaysia) and the United Kingdom (Scotland) (n=238) forming the Cohort of Asian and Matched European Bronchiectasis (CAMEB) which matched recruited patients on age, gender and bronchiectasis severity. Specific-IgE response against a range of common allergens was determined, combined with airway immune-inflammatory status and correlated to clinical outcomes. Clinically relevant patient clusters based on sensitization pattern and airway immune profiles ("immuno-allertypes") were determined. **Measurements and Main Results:** A high frequency of sensitization to multiple allergens was detected in bronchiectasis, exceeding that in a comparator cohort with allergic rhinitis (n=149). Sensitization associated with poor clinical outcomes including decreased pulmonary function and more severe disease. 'Sensitized-bronchiectasis' was classified into two 'immuno-allertypes': one fungal-driven and pro-inflammatory versus house dust mite-driven, chemokine-dominant with the former demonstrating poorer clinical outcome. **Conclusion:** Allergic sensitization occurs at high frequency in bronchiectasis patients recruited from different global centres. Improving endo-phenotyping of 'sensitized-bronchiectasis', a clinically significant state, and 'treatable trait' permits therapeutic intervention in appropriate patients and may allow improved stratification in future bronchiectasis research and clinical trials.

Key words: Bronchiectasis; Sensitization; Allergy; House dust-mite; Aspergillus

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INTRODUCTION

Bronchiectasis not due to cystic fibrosis is a disease experiencing a clinical and research renaissance.(1) Characterized by persistent cough, mucopurulent secretions and recurrent infection, permanent and irreversible bronchial dilatation ensues.(2) The failure of therapies used in cystic fibrosis (CF) to translate to bronchiectasis is exemplified by trials of recombinant DNAse therapy, which in contrast to CF, increases exacerbations in bronchiectasis.(3) Such failures suggest fundamental differences in disease associated mechanisms necessitating data-driven, endo-phenotyping approaches toward improved patient stratification in this heterogeneous disease.(2) While disease-associated effects on host immunity, infection and inflammation are recognized, the role of allergic sensitization in the setting of bronchiectasis lacks dedicated study.

Atopy is a known risk factor for the development and/or progression of chronic respiratory disease including asthma, chronic obstructive pulmonary disease (COPD) and CF.(4, 5) While allergic bronchopulmonary aspergillosis (ABPA) is an identified cause for bronchiectasis, the specific role of atopy and sensitization as a consequence of disease remains to be established. This is particularly important as the presence of atopy; fungal-sensitization and ABPA in asthma, COPD and CF are all recognized associations of poorer clinical outcome including decreased pulmonary function, more frequent exacerbations and even the development of bronchiectasis.(4, 6)

Prior reports of atopy in bronchiectasis are small, conflicting and limited to single centres. In addition, none have thus far addressed the potential for geographic variation across countries with differing allergen exposures and climates.

Here, we describe the largest study of atopy in bronchiectasis to date including patients from clinically 'matched' cohorts of Asian and European origin. Sensitization to a range of allergens was assessed with their clinical associations and accompanying airway inflammatory signatures. This allows patient stratification into clinically relevant groups defined as 'immuno-allertypes'; specific endo-phenotypes of 'sensitized bronchiectasis' with therapeutic implications. Some of the results of these studies have been previously reported in the form of abstracts (7, 8).

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METHODS

Study population(s)

Patients with stable bronchiectasis, defined by British Thoracic Society (BTS) guidelines, were recruited across three countries as part of the CAMEB study (a cross-sectional Cohort of Asian and Matched European Bronchiectasis).(9, 10) Recruitment included three sites in Singapore (Singapore General Hospital, Changi General Hospital and Tan Tock Seng Hospital; n=124), one Malaysian site (UKM Medical Centre, Kuala Lumpur, Malaysia; n=14) and an age-, sex- and disease-severity matched group from a single European site (Ninewells Hospital, Dundee, UK; n=100). The study was conducted between March 2016 and July 2017 (total CAMEB study population, n=238). All patients had radiologically confirmed bronchiectasis by high-resolution computed tomography (HRCT) scanning of the thorax interpreted in accordance with clinical practice guidelines of the BTS.(9) Patients were recruited during routine visits to the outpatient clinic and were clinically stable at recruitment. Clinical stability was defined as the absence of new symptoms and where no change had occurred to their bronchiectasis therapy in the preceding six-week period. Patients were excluded if they had a primary diagnosis of any other major respiratory diagnosis (asthma or COPD) (as defined by clinical symptoms and established spirometric criteria),(11, 12) were pregnant or breastfeeding, had active mycobacterial disease (identified through symptoms, chest radiograph, and sputum microbiology),(13), or were on chemotherapy for malignancy. Patients with any active infection requiring use of antibiotics or systemic corticosteroids in the four weeks preceding recruitment were also excluded. From the Singapore-Kuala Lumpur (SG-KL) cohort a total of n=100 patients were matched individually by age, sex and total bronchiectasis severity index (BSI) score (assigned at time of sample acquisition) to patients in the Dundee (DD) cohort (14). Patients with any prior history of or active ABPA (defined as meeting established ISHAM criteria including either

Type 1 *Aspergillus* skin test positivity or elevated IgE levels against *A. fumigatus* and elevated total IgE>1000IU/mL plus at least two of the following three criteria: precipitating or serum IgG antibodies against *A. fumigatus*, radiographic change consistent with ABPA or total eosinophil count >500 cells/μl in steroid naïve patients) at enrolment were excluded.(15) A separate cohort of 149 allergic rhinitis (AR) patients, among which bronchiectasis was excluded by radiological examination, were recruited from the Otolaryngology outpatient clinic at National University Hospital, Singapore, to serve as a comparator group with high allergic sensitization. Allergic rhinitis was confirmed by evidence of sensitization to at least one allergen (by skin prick or serum IgE test) using established clinical criteria.(16) The institutional review board of all the participating hospitals approved the study and written informed consent was obtained from each patient.

Full details on clinical data and specimen collection, immuno-dot blot assay for specific IgE measurement, multiplex analysis for cytokine/chemokine quantification as well as statistics and data analysis are provided in the supplementary material.

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RESULTS

Clinical characteristics and patient demographics are shown in Table 1.

High frequencies of sensitization to a range of allergens are detected in bronchiectasis: Based on prior findings from our group and others, we sought to comprehensively assess sensitization levels to multiple specific allergens in the CAMEB cohort (10, 17-20). Measured specific-IgE (sIgE) titres against house dust mite (HDM), *Alternaria alternata* (Alt a) and recombinant allergens of *Aspergillus fumigatus* (rAsp) revealed high frequencies of sensitization in patients with bronchiectasis (Figure 1). Sensitization of class 3 or above (to at least one allergen) was observed in 57·6% (n=137) of bronchiectasis patients compared to 26·9% (n=40) in the AR cohort, that served as an unmatched comparator group ($p < 0·0001$). Of all allergens tested, extracts of HDM (Der p and Blo t) elicited the highest median sIgE titres in bronchiectasis (Figure 1, Table E1). Less than 3% (n=4) of AR patients exhibited sIgE titres in the 'very high' (class 4 and above) range versus 33·2% (n=79) in the bronchiectasis cohort. Only patients in the bronchiectasis group registered sIgE titres in the 'very high' (class 6) range (n=35; 14·7%).

Sensitization to multiple allergens associates with more severe disease and poorer lung function in bronchiectasis: Having identified high levels of sensitization in bronchiectasis, we next investigated if this was associated with disease severity and poorer clinical progression focusing on the primary clinical outcomes of BSI, lung function (FEV_1 % predicted) and exacerbation rate in the prior year. Patients were categorized by the number of allergens to which each individual was sensitized (defined as sensitization ≥ class 3). Higher sIgE titres were observed in those sensitized to ≥ 2 allergens. These individuals more

frequently had titres in the 'very high' (class 4-6) range (Figure 2, $p < 0{\cdot}00001$). Patients with sensitization to ≥3 allergens had greatest disease severity and poorest lung function (Figures 2A and 2B) but no association was found with exacerbation frequency (Figure 2C).

Sensitization patterns in bronchiectasis demonstrate geographic variation: In patients from both cohorts: SG-KL and DD, the association between sensitization and poorer lung function (Figure 2b) was statistically significant ($p < 0{\cdot}01$), whereas the effect on BSI (Figure 2a) was more pronounced in the SG-KL cohort ($p < 0{\cdot}01$) compared to the DD cohort ($p = 0{\cdot}06$). Interestingly, we found distinct geographic allergen response profiles based on patient origin. Patients from the SG-KL cohort exhibited higher responses to HDM allergens compared to the DD cohort, of which the response to *D. pteronyssinus* (Der p) was significant (Figure 3). In contrast, the DD cohort had greater responses to fungal allergens including *A. alternata* and *A. fumigatus* (rAsp f 6, f 8, f 15, f 17) with one exception, the *A. fumigatus* major allergen rAsp f 1, whose response was significantly elevated in the SG-KL cohort (Figure 3). In specific matched patient analyses, the significant responses to Der p and rAsp f 1 remained in the SG-KL cohort while the response to rAsp f 17 was the single relationship maintained in the DD cohort (Table E2 and E3). The clinical consequence of varied geographic sensitization pattern did not necessarily correlate with their observed frequency: sensitization to HDM allergens and rAsp f 1 were associated with poorer lung function in SG-KL and DD cohorts respectively, while sensitization to rAsp f 17 was only linked to a higher exacerbation frequency in patients from the SG-KL cohort despite its predominance in matched patients from the DD cohort (Table E4).

Sensitization to the *A. fumigatus* minor allergen rAsp f 17 is enriched in bronchiectasis-associated serological-ABPA (s-ABPA): All recruited bronchiectasis patients were

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managed in accordance with established ERS guidelines that advise screening for ABPA at diagnosis: we excluded all patients that screened positive in accordance with our study criteria but in some cases this screening would have been several years prior.(2) Despite this strict exclusion criteria, when all *Aspergillus*-sensitized patients in our bronchiectasis cohort were considered (n=224; 95·8%), 43 (18·1%) met the criteria for s-ABPA based on the published classification used in CF-related bronchiectasis.(21)

In these patients, we next assessed the relationship between the presence or absence of s-ABPA, and sensitization to specific *A. fumigatus* allergens. This revealed a significant association between s-ABPA and a rAsp f 17 specific response, a relationship statistically driven by differences in the DD cohort (Figure 4A and 4B). Importantly, however, a trend toward enrichment of a sensitization response to rAsp f 17 was also observed in the SG-KL cohort (Figure 4B). When all s-ABPA patients are considered (from both SG-KL and DD cohorts), a significantly higher proportion have sensitization responses to rAsp f 17 in the class 3 or above range (Table E4). Those with rAsp f17 sensitivity also had higher rates of qPCR positivity for *A. fumigatus* (69% vs 45%, p = 0.017) while their sputum galactomannan levels were comparable (65% vs 69%, p = 0.801), measures determined in prior reported analysis of the CAMEB cohort.(10) Interestingly, whilst the highest observed responses to rAsp f 17 were in Scottish patients with s-ABPA, Singaporean and Malaysians who had significant responses to rAsp f 17 also had significantly more exacerbations (Median = 1·5 versus 0; p<0·05) suggestive that responses to this allergen are clinically relevant in both populations (Figure 4, Table E4).

Clinically relevant 'immuno-allertypes' are defined by sensitization pattern and airway immune profiling in bronchiectasis: We next investigated whether clinically relevant

patient clusters based on sensitization pattern and airway immune profiles ("immuno-allertypes") occur in bronchiectasis. To address this, we used multiplex sputum cytokine and chemokine profiling approach, previously validated in COPD, to assess immunological signals and linked this to sensitization patterns and accompanying clinical phenotypes (22). Hierarchical cluster analysis of resultant data revealed two distinct patient immuno-allertypes characterised by distinct sensitization patterns and immune profile (Figure 5A). Assignment into immuno-allertypes was not driven by geographic origin or the presence of s-ABPA as, within each group, we found equal proportions of patients from both SG-KL and DD cohorts as well as an equal distribution of s-ABPA ($p>0{\cdot}5$) (Figure 5A). Each immuno-allertype was instead defined by a unique sensitization pattern and immune profile: patients in the fungal-driven pro-inflammatory (FDPI) group exhibited marked responses to the fungal allergens Alt a and rAsp coupled to a pro-inflammatory profile characterised by elevated airway TNFα, IL-1α and IL-1β. Asthma-like symptoms, long-term antibiotics ($p=0.0620$) or increased ICS ($p=0.932$) use was not significantly apparent in either immuno-allertype (Table E5), nor was ICS use significantly different in those exhibiting sensitization (sIgE class ≥ 3, $p=0.580$). However, higher levels of sputum galactomannan, trending toward significance, were observed in FDPI bronchiectasis patients ($p = 0{\cdot}065$, Table E5). In contrast, patients in the HDM-driven chemokine-dominant (HDCD) group exhibited lower sensitization to fungal allergens but significant responses to HDM allergens, that was accompanied by a chemokine-dominant airway profile characterised by high GRO (CXCL1), MCP-1 (CCL2) and eotaxin-1 (CCL11). This HDCD immuno-allertype also exhibited anti-inflammatory signatures including elevated IL-1RA, IL-10 and G-CSF (Figure 5A). Following patient stratification by underlying immuno-allertype, a significant association was observed of the FDPI pattern with bronchiectasis severity. The FDPI immuno-allertype conferred significantly worse disease (Figure 5B, Table E5) and poorer lung function (Figure 5C, Table E5) while frequent

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exacerbators were equally observed in both groups (Figure 5D). Post-infective bronchiectasis and greater long-term antibiotic use was also apparent in the FDPI group (Table E5).

Geographic patient origin drives intra-immuno-allertype variation in bronchiectasis: Given our observed geographic differences in sensitization pattern in bronchiectasis (Figure 3, Table E2 and E3), we next assessed if there was intra-immuno-allertype variation, based on patients' geographic origin. Our previously defined immuno-allertypes were based on unsupervised hierarchical clustering and categorized patients into two groups, with equal frequencies of patients from each of our geographic cohorts (SG-KL and DD) (Figure 5A-ii). To investigate intra-immuno-allertype patient variation based on geographic origin, we used a supervised Markov blanket approach to identify features within each immuno-allertype associated with SG-KL and DD respectively. As such, intra-immuno-allertype variability was assessed in terms of information shared between sensitization pattern, immune cytokine/chemokine profile and geographic patient origin (Figure 6). In the FDPI group, Asian origin (SG-KL cohort) was associated with a predictive set of features including IFNα2, TNFβ and the PDGF isoforms AA and AB/BB while European origin (DD cohort) correlated with greater levels of sCD40L, IL-1RA and IL-17A (Figure 6A). In the HDCD group, patients from the SG-KL cohort exhibited an increased MCP-3, IL-1β, PDGF-AB/BB and a significant HDM allergen response, while IL-9, IL-10, TGFα, MDC, Eotaxin and FGF-2 were more predictive of DD cohort membership (Figure 6B). This suggests the existence of sub-groups based on geographic origin within each of the defined immuno-allertypes and indicates the potential clinical importance of geographic heterogeneity in bronchiectasis. The strongly associated HDM response identified in the SG-KL cohort (Figure 6B) is consistent with our earlier analyses (Figure 3) which lends further credence that immuno-allertypes are clinically relevant. Independent of immuno-allertype, the SG-KL and DD cohorts further

illustrate significant differences in airway inflammatory signature, for example elevated PDGF-AB/BB ($p<0{\cdot}0001$) and MCP-3 ($p<0{\cdot}05$) in Singaporeans and Malaysians patients, substantiating regional differences in immune response. Eotaxin is significantly elevated in 'sensitized bronchiectasis' ($p < 0{\cdot}05$) but no differences in inflammatory patterns are observed between bronchiectasis patients with different underlying aetiologies (e.g. idiopathic, post-infectious or other causes).

DISCUSSION

Atopy and sensitization are important 'treatable traits' in asthma, COPD and their associated overlap syndromes, but their role in bronchiectasis remains uncertain (5, 17, 23). The present study is the most comprehensive to date addressing this and detected high frequencies of sensitization to a range of specific allergens, exceeding that found in a comparator AR cohort, which although unmatched, served as an important positive control group of heightened sensitization. Sensitization has clinical implications in bronchiectasis and demonstrates geographic variation in allergen response pattern. Individual allergen responses correlate with specific clinical outcomes in bronchiectasis including FEV_1 (HDM and rAsp f 1), exacerbations (rAsp f 17) and the presence of s-ABPA (rAsp f 17). Two clinically relevant 'immuno-allertypes' are described: fungal-driven, pro-inflammatory (FDPI) and HDM-driven, chemokine dominant (HDCD), with the FDPI pattern linked to poorer clinical outcome.

Sensitization induced by HDM is common and may associate with poor clinical outcomes in allergic respiratory disease.(4, 24) The effect of HDM exposure in asthma, for instance, relates to a Th2-mediated, chemokine-associated allergic response of deleterious clinical consequence.(24, 25) In COPD, increased sensitization is implicated in disease pathogenesis

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and correlates to exacerbations.(5) In our work, significant numbers of apparently stable bronchiectasis patients show HDM-sensitization which presumably reflects either co-existing subclinical allergic airways disease or a predisposition to atopy following airway damage caused by bronchiectasis. Given the dearth of bronchiectasis therapy currently available and the known implications of allergic sensitization on clinical outcomes in other respiratory diseases, addressing HDM sensitization in bronchiectasis offers an important therapeutic avenue. The role of fungal allergy in respiratory disease is well recognized and fungal sensitization is an established prognostic indicator in severe asthma with fungal sensitization (SAFS) and ABPA, and has also been reported in COPD where specific fungal responses are implicated as a risk factor for progression to bronchiectasis.(4, 17, 23) This latter work supports our findings of high rates of atopy and sensitization in bronchiectasis highlighting a potential role for *A. fumigatus* even in non-ABPA respiratory disease.(6, 17) Our comprehensive immune-allergy assessment of stable bronchiectasis patients importantly identified patients with s-ABPA; a potentially treatable condition, which would have been missed by guideline-recommended screening alone particularly as the s-ABPA developed after the initial diagnosis of bronchiectasis.

Allergen-based sensitization patterns exhibit regional and global variability and marked differences have been observed across Asian and European populations.(4, 24, 26) Our group has previously reported a high prevalence of HDM sensitization in the Asian setting; a feature confirmed in bronchiectasis.(23, 26) Allergen exposure varies by country and with different ethnic backgrounds and, as such, a key strength of our study is in its multicentre design. Geographically distinct allergen profiles were observed across our matched cohorts, not just to HDM but also to *A. alternaria* and specific *A. fumigatus* allergens. To our knowledge, this is the first study to compare such populations in bronchiectasis. Singapore

and Malaysia (where our Asian patients were recruited) have tropical climates and distinct allergen profiles, which, when coupled to genetic and other environmental differences including air quality and use of air-conditioning, may potentially explain the observed differences compared to temperate oceanic climates seen in Scotland (where our European patients were recruited).(4, 24) Differences in sensitization between populations (for instance, to the same *A. fumigatus* specific allergen) may be due to host genetic and immunological variation, but allergen cross-reactivity with other fungi may also be important, given the potential contrasting environmental exposures. Further, *B. tropicalis* (Blo t) sIgE responses were observed amongst the European population despite the absence of this tropical dust mite species in Dundee. We suspect this is due to the recognition of cross reactive epitopes of *D. pteronyssinus* or other HDM species rather than those of *B. tropicalis*. Critically, our observed allergen-associated patterns relate differently to clinical outcomes in the two regions: HDM and rAsp f 1 responses relate to FEV_1 in Asians and Europeans respectively while increased exacerbations were linked to the rAsp f 17 response in Asians and to s-ABPA in both populations. An improved understanding of the role of environmental exposures and its geographic variation will ultimately require a more direct environmental measurement of patient exposure, preferably at the point of sampling, to fully explicate the precise influence a patient's environment may have on sensitisation and disease progression in bronchiectasis.

A response to rAsp f 17 is of particular importance in bronchiectasis. This allergen, encoded by the Afu4g03240 gene of the *A. fumigatus* AF293 genome, was originally characterised by Yuen *et al*. as the first species-specific antigenic cell wall galactomannoprotein,(27) but its clinical relevance outside invasive aspergillosis is unclear. Our study, therefore, also represents the first clinical evidence of a potential role for this allergen in chronic airways disease, and specifically in bronchiectasis where it is linked to exacerbations and s-ABPA.

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Interestingly, Gibbons and colleagues demonstrate that rAsp f 17 exhibits significant up-regulation (>200-fold) in *A. fumigatus* biofilms, much more than any other specific allergen.(28) Taken together; this links rAsp f 17 sensitization with *A. fumigatus* biofilm production and matrix galactomannan concentration. Therefore, further work is now warranted to better understand its role in immuno-pathogenesis and potential clinical use in *Aspergillus*-associated allergic disease.

When sensitization pattern and airway immune response are considered together in unsupervised analyses, two distinct immuno-allergic signatures of clinical relevance emerge. The first of these groups - FDPI - is fungal-driven, characterised by specific responses to rAsp allergens and an airway rich in the pro-inflammatory cytokines IL-1α, IL-1β and TNF-α. These cytokines trigger release of intercellular-adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) from the endothelium leading to neutrophil and eosinophil airway recruitment, and their expression in the FDPI group is notable given the central importance of neutrophils in bronchiectasis (29-32). Following cell recruitment, increased airway smooth muscle contractility and hyper-responsiveness may result, potentially explaining the poorer lung function and increased bronchiectasis severity in this group. This contrasts the HDM-associated (HDCD) immuno-allertype identified by specific responses to Der p and Blo t and an airway rich in chemokines (eotaxin, IP-10), lymphokines (IL-9, IL-2), growth factors (TGF-α) and elevated concentrations of Th2-related cytokines (IL-5, IL-10 and IL-13) consistent with Th2-dominant airway inflammation, suggestive of eosinophilic involvement. Taken together, these identified endo-phenotypes of bronchiectasis may be amenable to targeted treatments including anti-inflammatories, corticosteroids, anti-Th2-cytokine or antifungal therapy; a strategy which has shown positive outcomes for CF patients colonized by *A. fumigatus* (33). Frequent exacerbators were seen in both immuno-

allertypes with equal frequency suggesting that this important phenotypic endpoint used in clinical trials of bronchiectasis therapies may be underpinned by alternate mechanisms necessitating more targeted approaches. Though the idea that different immunological pathways could drive sensitization and exacerbations in bronchiectasis is intriguing, we recognise that our study is limited by its size. Smaller sub-groups associated with exacerbations may exist which our study lacked power to detect. Notwithstanding this, the fact that exacerbation rates were equivalent among the different immuno-allertypes serves to highlight that potential 'exacerbation types' may exist in bronchiectasis, analogous to observations in COPD and asthma, where potentially 'treatable traits' include specific endotype-driven exacerbations.(1, 34-36). Moreover, while exacerbation remains an key bronchiectasis phenotype, its use as an endpoint in clinical trials for bronchiectasis has proven challenging (37, 38). 'Treatable traits' like 'sensitised bronchiectasis' do not themselves represent clinical endpoints, however, identifying such endo-phenotypes, amenable to focused therapy addresses other important clinical symptoms in bronchiectasis.(1, 39-41) Our distinct immunoallertypes, when assessed together with bronchiectasis symptomology, may allow better patient stratification, which in turn permits tailored focused and effective interventions for this highly heterogeneous disease.

A limitation of this work is its cross-sectional nature, precluding assessment of the temporal dynamics of the identified immuno-allertypes, their risk factors and underlying causal mechanisms. Our ability to assess cause-effect relationships between sensitisation and clinical outcome was largely constrained by our decision to focus on matching our patient cohorts on age, sex and disease severity, which by nature creates logistical challenges for longitudinal sampling. Longitudinal studies in bronchiectasis are nonetheless an important and an on-going research focus of our group and others, as we seek to explicate further the

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clinical correlates of immuno-allertype profiles as well the as therapeutic factors, such as long-term macrolide therapy, which may influence the immuno-allertype itself through immunomodulation. Such effects were likely undetected in this study due to its cross-sectional design. Further, we did not perform analyses in other comparator pulmonary cohorts such as asthma or assess sputum immune cells, which would have further substantiated the observed immuno-allertypes, and while we identified an increasing trend in galactomannan sputum positivity in FDPI patients, we did not investigate corresponding airway markers in the HDCD group such as dust mite protease. Though our allergen panel was broad, it can be further extended to include cockroach, animal dander or geographically prominent pollens – all of which are areas of future work.

Bayesian network analysis revealed specific immune-profiles among our geographically distinct cohorts, suggesting regional variability within immuno-allertypes with implications for endo-phenotyping in bronchiectasis. In the SG-KL cohort, the FDPI immuno-allertype had associated features linked to an airflow-limiting airway remodelling process, notably PDGF and TNF-β expression. In contrast, patients from the DD cohort exhibiting the FDPI immuno-allertype had distinctive immune signatures comparable to those observed in allergic asthma implicating IL-RA and IL-17A.(42-44) Both geographic groups classed as HDCD demonstrated a chemokine-dominant, pro-inflammatory milieu with increased growth factor expression reflective of a fibrotic airway remodelling process. The implication of PDGF-AB/BB is of particular interest given the recently described haematopoietic potential of the lung and reported links between exacerbation and platelets in COPD.(45, 46) Though interesting, replication of these geographic differences in larger cohorts is required before true clinical importance can be established. Perhaps more intriguing was the degree of

similarity seen across such geographically distinct populations, serving to broadly validate the observed immuno-allertypes and their association with clinical outcome in bronchiectasis. The interplay between allergens, sensitization and the immune-inflammatory response in bronchiectasis is complex with apparent geographical variation. Our work reveals, for the first time, high frequencies and distinct patterns of sensitization in bronchiectasis. While therapies employed in other allergic respiratory diseases may now be considered in patients with bronchiectasis complicated by allergic sensitization, we must address the significant and inherent heterogeneity in this disease. The immuno-allertypes presented here represent an important starting point of future work focusing on improved patient endo-phenotyping in bronchiectasis, which in turn will allow stratified therapeutic approaches to 'sensitized bronchiectasis': a key treatable trait.

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Table 1. Demographic table illustrating patient cohorts of allergic rhinitis (n=149) compared to the CAMEB cohort of non-CF bronchiectasis (n=238).

Characteristic	Allergic rhinitis (n=149)	Bronchiectasis patients (n=238)	Bronchiectasis patients SG-KL (n=138)	Matched cohorts		
				SG-KL (n=100)	DD (n=100)	p-value
Age : median (IQR)	28 (23-35)	68 (64-71)	65 (58-73)	65 (58-74)	69 (64-76)	0.021
Gender : n (%)						0.392
Female	60 (40%)	130 (55%)	77 (55%)	59 (59%)	53 (53%)	
Male	89 (60%)	108 (45%)	61 (45%)	41 (41%)	47 (47%)	
Aetiology n (%)						0.040
Idiopathic	-	145 (61%)	85 (62%)	63 (63%)	60 (60%)	
Post-infection (non-mycobacterial)	-	51 (21%)	25 (18%)	18 (18%)	26 (26%)	
Post-infection (mycobacterial)	-	19 (8%)	18 (13%)	9 (9%)	1 (1%)	
Other	-	23 (10%)	10 (7%)	10 (10%)	13 (13%)	
Smoking status n (%)						0.013
Never	-	170 (70%)	108 (78%)	80 (80%)	62 (62%)	
Current	-	11 (5%)	7 (5%)	4 (4%)	4 (4%)	
Past	-	57 (25%)	23 (17%)	16 (16%)	34 (34%)	
BSI status : n (%)						0.355
Severe	-	147 (62%)	84 (61%)	63 (63%)	63 (63%)	
Moderate	-	71 (30%)	45 (33%)	26 (26%)	26 (26%)	
Mild	-	20 (8%)	9 (6%)	11 (11%)	11 (11%)	
BSI score : median (IQR)	-	9 (6-13)	10 (7-14)	10 (7-14)	9 (6-12)	0.054
BMI (kg/m2) : median (IQR)	-	21 (18-27)	19 (17-22)	19 (17-22)	27 (22-31)	<0.001
MRC dyspnea score : n (%)						
1-3	-	200 (84%)	121 (88%)	90 (90%)	79 (79%)	0.001
4	-	26 (11%)	10 (7%)	6 (6%)	16 (16%)	
5	-	12 (5%)	7 (5%)	4 (4%)	5 (5%)	
FEV_1 % predicted: : median (IQR)	-	74 (54-87)	69 (51-84)	69 (52-84)	76 (57-96)	0.067
Radiological severity : n (%)						0.123
1-2 lobes involved	-	106 (45%)	62 (45%)	43 (43%)	44 (44%)	
3 or more lobes involved	-	132 (55%)	76 (55%)	57 (57%)	56 (56%)	
No. of exacerbations in previous year : n (%)						<0.001
0	-	84 (35%)	69 (50%)	44 (44%)	15 (15%)	
1-2	-	82 (35%)	51 (37%)	41 (41%)	31 (31%)	
3 or more	-	72 (30%)	18 (13%)	15 (15%)	54 (54%)	
Hospital admissions before study : n (%)						0.004
Yes	-	88 (37%)	63 (46%)	43 (43%)	25 (25%)	
No	-	150 (63%)	75 (54%)	57 (57%)	75 (75%)	
Colonization with other organisms : n (%)						0.002
Yes	-	127 (53%)	60 (43%)	44 (44%)	67 (67%)	
No	-	111 (47%)	78 (57%)	56 (56%)	33 (33%)	
Pseudomonas colonisation : n (%)						0.032
Yes	-	23 (10%)	18 (13%)	15 (15%)	5 (5%)	
No	-	215 (90%)	120 (87%)	85 (85%)	95 (95%)	
Bronchodilator use n (%)						0.158
Yes	-	107 (45%)	58 (42%)	39 (39%)	49 (49%)	
No	-	131 (55%)	80 (58%)	61 (61%)	51 (51%)	
Inhaled corticosteroids n (%)						<0.001
Yes	-	80 (34%)	21 (15%)	14 (14%)	59 (59%)	
No	-	158 (66%)	117 (85%)	86 (86%)	41 (41%)	
Mucolytics n (%)						<0.001
Yes	-	118 (50%)	60 (44%)	45 (45%)	13 (13%)	
No	-	120 (50%)	78 (56%)	55 (55%)	87 (87%)	
Long-term antibiotics n (%)						0.032
Yes	-	48 (20%)	22 (16%)	14 (14%)	26 (26%)	
No	-	190 (80%)	116 (84%)	86 (86%)	74 (74%)	

Table 1: Demographic table illustrating the patient cohorts with allergic rhinitis (n=149) and non-CF bronchiectasis (n=238). The non-CF bronchiectasis cohort includes a matched cohort of patients from Singapore-Kuala Lumpur (SG-KL) and Dundee (DD). Matching was performed based on age, gender and disease severity (total score on the bronchiectasis severity index; BSI). The variables defining the composite BSI score include: Body Mass Index (BMI), shortness of breath (MRC) dyspnea score, forced expiratory volume in the 1st second (FEV_1) % predicted values, Radiological severity by number of involved lobes, number of exacerbations and hospitalizations in the preceding year, microbial colonization with other organisms and colonization by *P. aeruginosa* are illustrated. Relevant therapy is documented including bronchodilator, inhaled corticosteroid, mucolytic or long-term prophylactic antibiotic use. Data are presented as median (interquartile range; IQR) or number of patients (n) (percentage; %) and p-values for differences observed between matched cohorts indicated in the rightmost column.

FIGURE LEGENDS

Figure 1: High frequencies of sensitization to house dust mite (HDM), *Alternaria alternata* (Alt a) and recombinant allergens of *Aspergillus fumigatus* (rAsp) are detected in stable non-cystic fibrosis (CF) bronchiectasis. Studied allergens are denoted as follows; HDM (*Dermatophagoides pteronyssinus* [Der p], *Blomia tropicalis* [Blo t]), *Alternaria alternata* (Alt a), and rAsp (f 1, f 2 [Major allergens] and f 6, f 8, f 15, f 17 [minor allergens]). Specific immunoglobulin-E (sIgE) titres (as kU/L) against each allergen are indicated: Bronchiectasis (B; black) and Allergic Rhinitis (AR; grey). Median values for all groups are illustrated (red lines).

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Figure 2: Sensitization to multiple (≥3) allergens in non-CF bronchiectasis is associated with more severe disease and poorer pulmonary function but does not effect exacerbations. The number of allergens to which an individual is sensitized (defined as sIgE class ≥3) was examined in relation to (A) disease severity (as bronchiectasis severity index; BSI), (B) pulmonary function (as percent predicted forced expiratory volume in the first second; FEV_1) and (C) exacerbation frequency in the preceding year. Dot coloration indicates specific sIgE class range as: non-diseased to moderate sensitization (Class 0-2; grey); high to very high (Class 3-4; purple) and very high (Class 5-6; pink). For patients exhibiting sensitization to more than a single allergen, the colouration corresponding with the highest sIgE class range is illustrated. Median values for all groups are shown (black lines). ns : non-significant, * $p \leq 0{\cdot}05$.

Figure 3: Geographic variation in sensitization profiles to specific recombinant allergens are observed in non-CF bronchiectasis. Patients from the SG-KL cohort (red dots) exhibit significantly higher sIgE titres to *D. pteronyssinus* (Der p) and the major *A. fumigatus* allergen rAsp f 1 while patients from the DD cohort (blue dots) have higher responses to *A. alternata* (Alt a) and all minor *Aspergillus* allergens (rAsp f 6, f 8, f 15 and f 17). Median values for all groups are shown (black lines). * $p \leq 0{\cdot}05$, ** $p \leq 0{\cdot}01$.

Figure 4: Sensitization to rAsp f 17 is enriched in serologic allergic bronchopulmonary aspergillosis (s-ABPA). (A) Specific-IgE (sIgE) titres against recombinant *A. fumigatus* allergens (major: f 1 and f 2 and minor: f 6, f 8, f 15 and f 17) in the absence or presence of s-ABPA (N and A respectively) illustrates enrichment for significant responses to rAsp f 17 in s-ABPA and (B) significant sensitization to rAsp f 17 is observed in the DD (blue dots) but not SG-KL (red dots) cohorts with s-ABPA. Median values for all groups are shown (black lines). rAsp f: recombinant *A. fumigatus* allergen, ns: non-significant ,*p ≤ 0·05, **p ≤ 0·01.

Figure 5: Pattern of allergen sensitization and associated airway inflammatory state defines clinically relevant immunological clusters of allergy in non-CF bronchiectasis ("immunoallertypes"). (A) Hierarchical cluster analysis based on systemic specific-IgE (sIgE) titre against a range of allergens and airway cytokine/chemokine expression reveals two distinct clusters ("immunoallertypes") in bronchiectasis: FDPI (cluster 1) and HDCD (cluster 2). A dendrogram illustrating hierarchical clustering of bronchiectasis patients' and their associated immune profiles (by heat-map) are illustrated. Coloured bars (below dendrogram) denote (i) cluster membership (cluster 1: purple, cluster 2: turquoise); (ii) geographic patient origin (SG-KL: red; DD: blue) and (iii) serologic allergic bronchopulmonary aspergillosis (s-ABPA) status (non-ABPA: orange; s-ABPA: green). Membership of Cluster 1 is associated with (B) increased disease severity (based on bronchiectasis severity index; BSI) and (C) poorer pulmonary function (as percent predicted forced expiratory volume in the 1st second; % predicted FEV_1) however (D) no difference in exacerbation frequency was detected between clusters. ns: non-significant; *p ≤ 0·05, **p ≤ 0·01.

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Figure 6: Geographic origin associates with intra-cluster variation in immune profiles that distinguish bronchiectasis patients of Asian and European origin. Mutual information networks illustrating associations between immune profile and geographic patient origin are shown for (A) cluster 1: FDPI and (B) cluster 2: HDCD. A central circle represents the target node of geographic patient origin (SG-KL: red or DD: blue) and positively correlated immune profiles (by Pearson correlation) are represented by outer circles (sized to reflect the mutual information shared with the target node). Line thickness illustrates the strength of the correlation and line colouration indicates geographic origin: SG-KL (red) and DD (blue) respectively. Cytokines and chemokines (white outer circles) and allergens (grey outer circles) are shown. Immune analytes common to both networks are further highlighted by a thickened border.

FIGURE 1

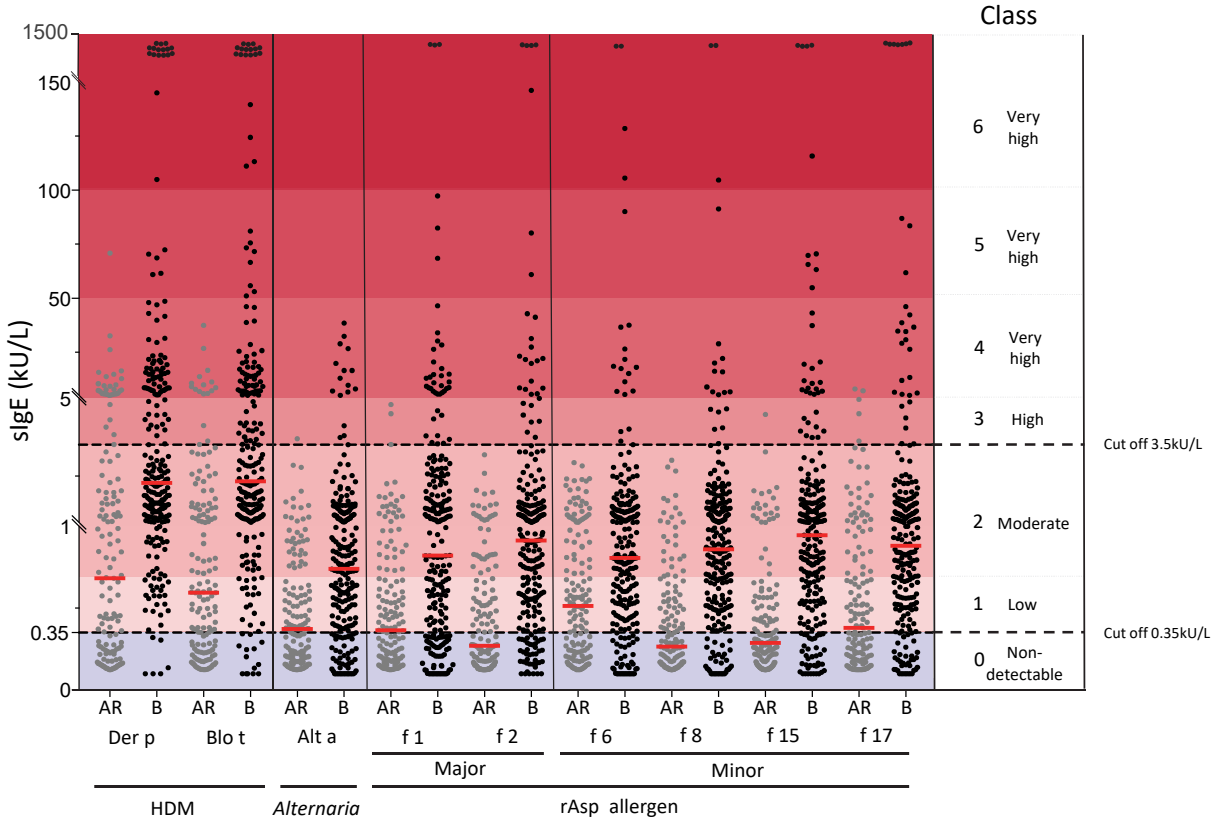

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FIGURE 2

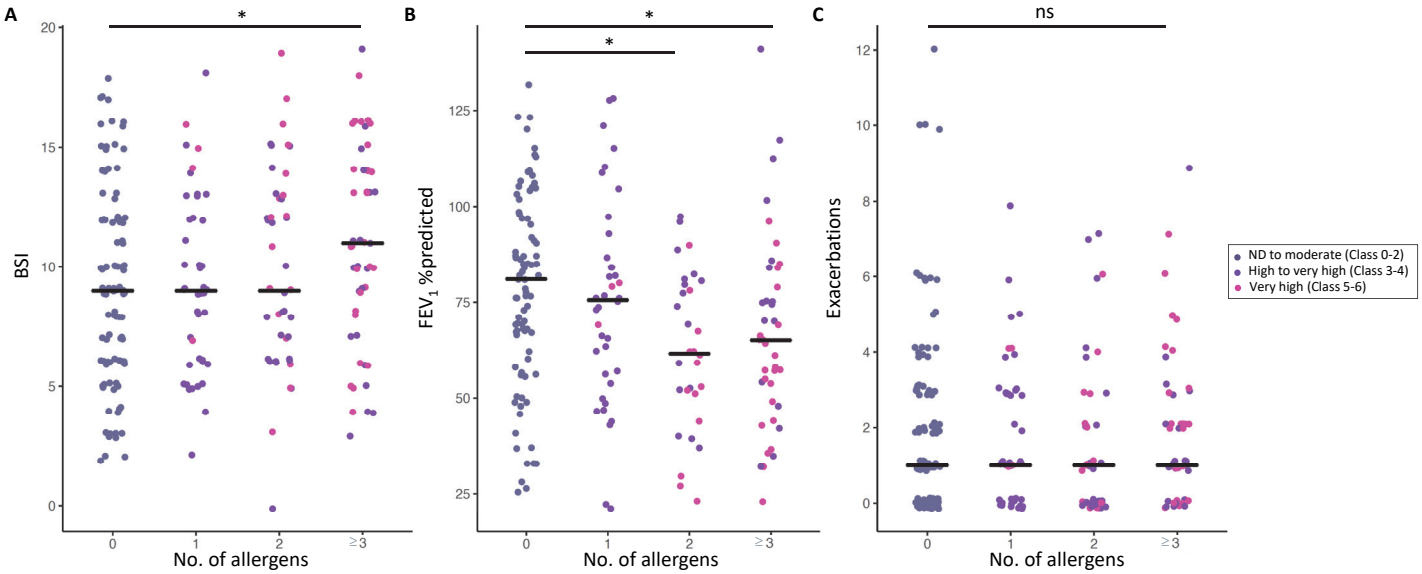

FIGURE 3

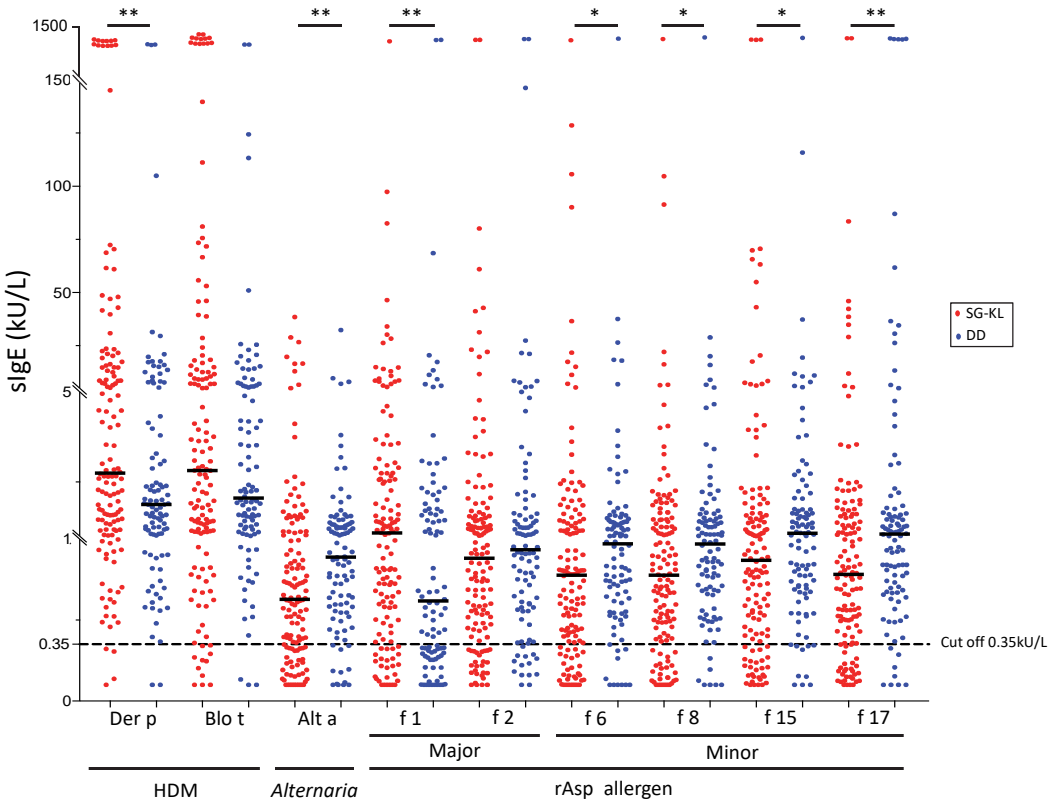

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FIGURE 4

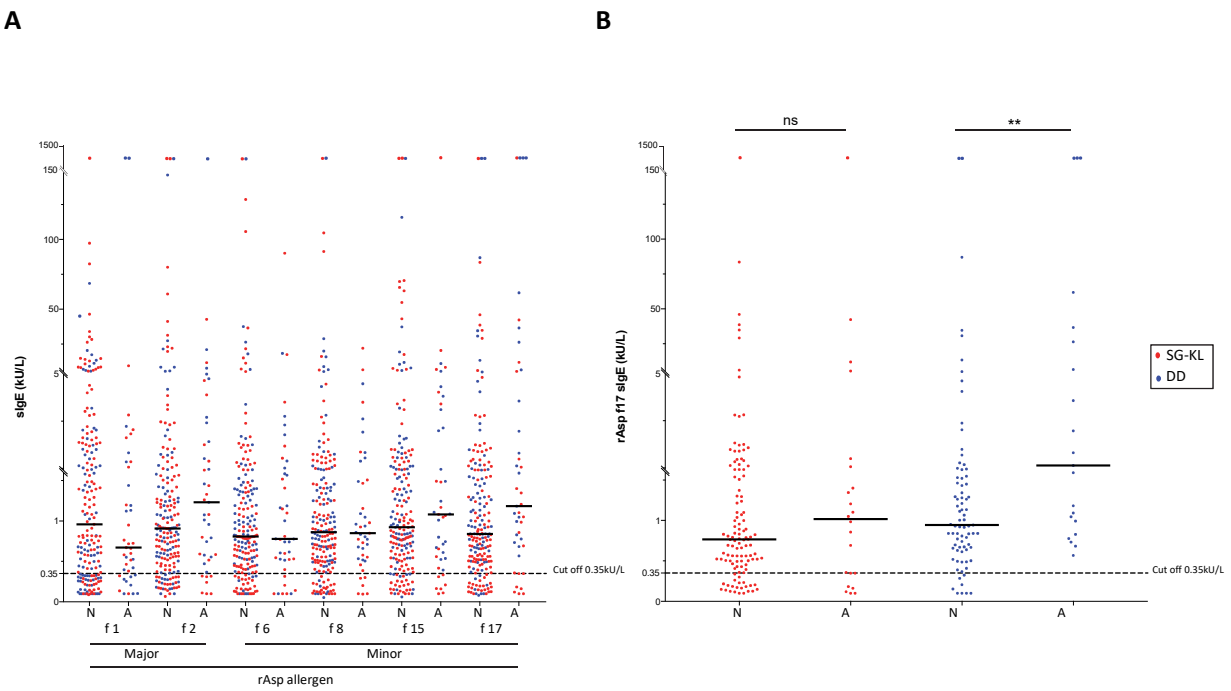

FIGURE 5

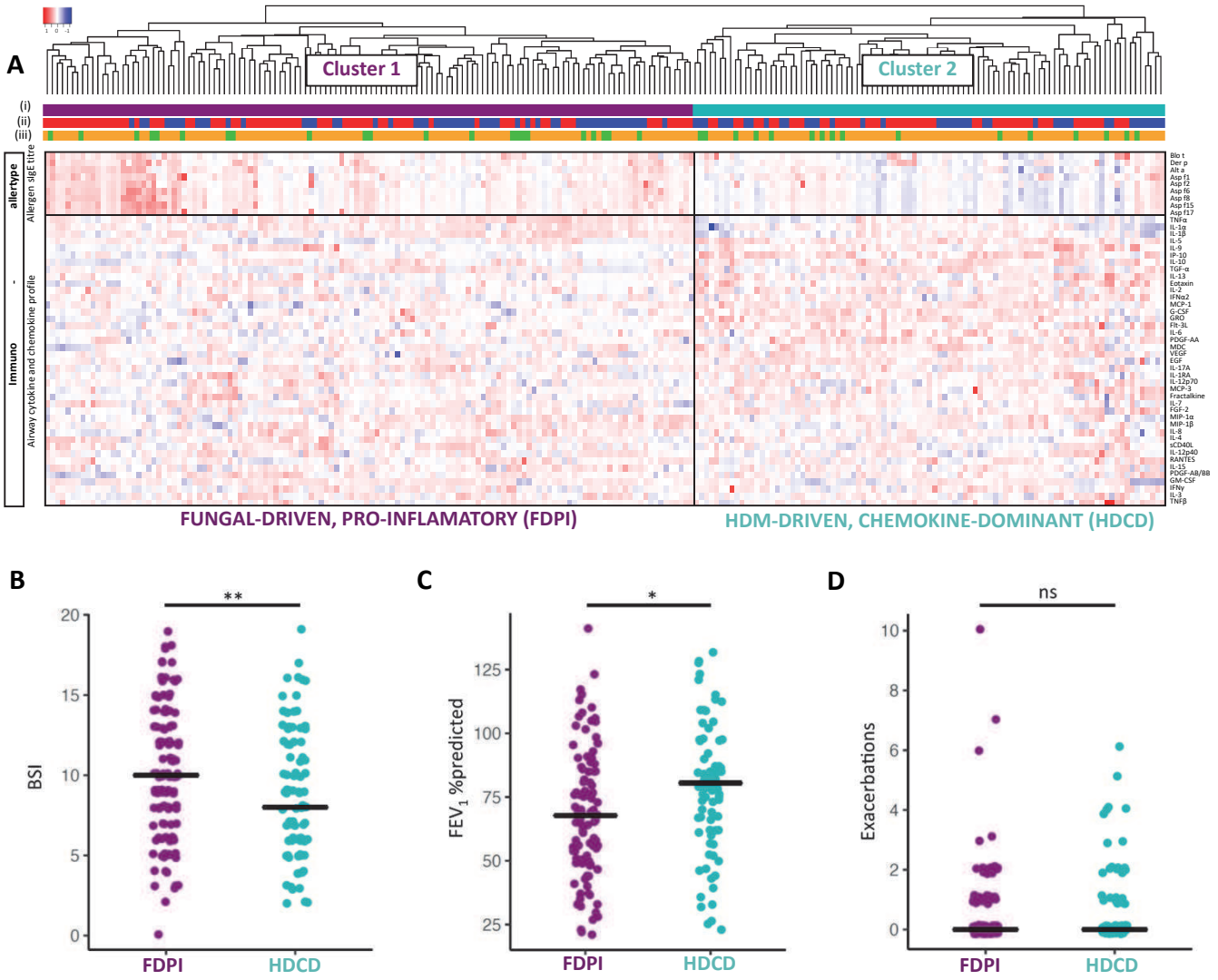

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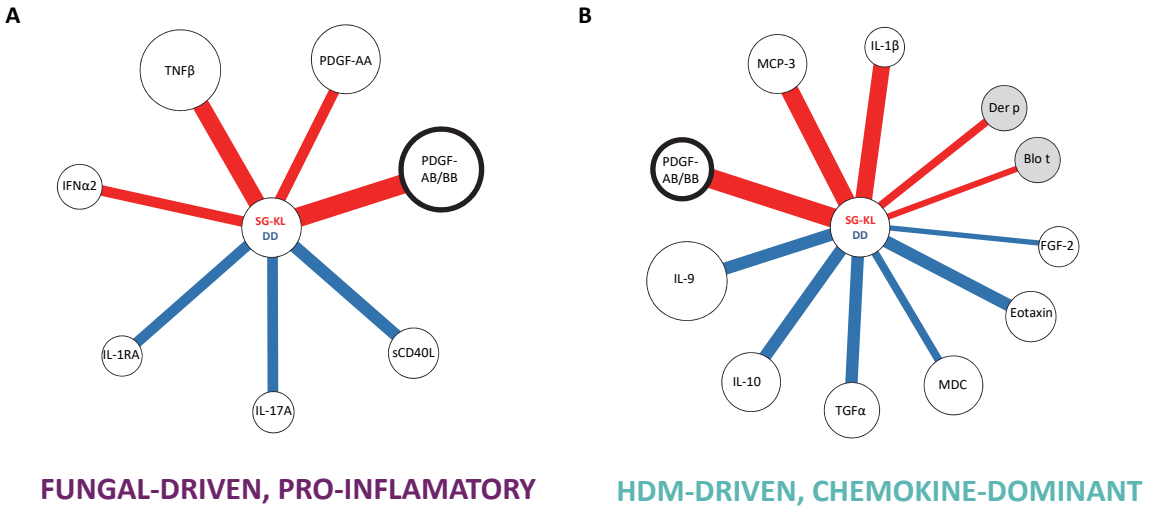
FIGURE 6



Online Data Supplement

DISTINCT 'IMMUNO-ALLERTYPES' OF DISEASE AND HIGH FREQUENCIES OF SENSITISATION IN NON-CYSTIC-FIBROSIS BRONCHIECTASIS

SUPPLEMENTARY MATERIALS AND METHODS

Ethical approval: This study was approved by the institutional review boards of all participating institutes as follows: CIRB 2016/2073 mutually recognized by DSRB; NTU IRB-2016-01-031; UKMMC FF-2016-440; NHD 12/ES/0059; NHG DSRB B/04/055 and NUS IRB 07-023.

Clinical data and specimen collection: Disease severity of each bronchiectasis patient was assigned according to the BSI and further divided into 'Mild' (BSI; 0-4), 'Moderate' (BSI; 5-8) or 'Severe' (BSI; 9 and above) (1). These disease categories served as the basis for disease severity matching of cohorts of Asian and European origin in the CAMEB study as described.(2) Target samples sizes of matched cohorts were estimated at 100 patients per group based on detection of modest effect sizes of 0.5 for sensitization with >90% power. All clinical data comprising the BSI including age, Body Mass Index (BMI), Medical Research Council (MRC) dyspnea score, FEV_1 percentage predicted values, radiological severity, number of exacerbations (defined by BTS consensus criteria) in the preceding year, hospitalizations in the preceding year, microbial colonization with other organisms and colonization by *P. aeruginosa* was recorded for each patient, as was data on gender, disease etiology and smoking status (1, 3). Data on use of bronchodilators, inhaled corticosteroids, mucolytics and long-term prophylactic antibiotics was also recorded, together with a range of molecular assays including sIgE response to crude Aspergillus extract, Anti-Aspergillus IgG

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response, qPCR-based assessment of Aspregillus presence and sputum galactomannan assays as described elsewhere.(2) Spontaneously expectorated 'representative' sputum from a deep cough with the assistance of a chest physiotherapist (where appropriate) was collected in sterile containers and transported (on ice) for evaluation (4). All CAMEB specimens from clinical sites were transported promptly, appropriately and processed centrally in Singapore to ensure consistency and standardization of all experimental work. To ensure quality control of materials transported from sites outside Singapore, specimens were temperature controlled and their integrity checked on arrival to Singapore before experimental use.

Immuno-dot blot assay for specific IgE (sIgE) measurement: The specific IgE (sIgE) response to crude protein extract from *Dermatophagoides pteronyssinus* (Der p), *Blomia tropicalis* (Blo t), *Alternaria alternata* (Alt a) and recombinant protein of *A. fumigatus* were assessed using an immuno-dot blot assay previously described.(5-9) The recombinant proteins used were rAsp f 1 (M83781), rAsp f 2 (U56938), rAsp f 6 (U53561), rAsp f 8 (AJ224333), rAsp f 15 (AJ002026) and rAsp f 17 (AJ224865). Briefly, 1µg/ml of each allergen was blotted in duplicate on nitrocellulose membranes, with serially diluted IgE concentrations (1000 IU/mL to 0·195 IU/mL; National Institute for Biological standards) as a standard. One microgram of Bovine Serum Albumin (BSA) and elution buffers were used as negative controls. Membranes were air-dried prior to blocking with 1X PBS 0·1% Tween-20 for an hour and incubated overnight with diluted serum (1:10 in PBS) at 4°C. Subsequently, the membranes were washed with 1X PBS 0·05% Tween-20 and incubated for two hours with anti-human IgE antibodies conjugated with alkaline phosphatase. Alkaline phosphatase activity was detected by adding nitroblue tetrazolium (NBT)/5-bromo-4-chloro-3'-indolyphosphate (BCIP) solution (Thermo Fisher Scientific) for 10 minutes. The intensities of the spots were recorded and measured using Syngene imaging software. Spot intensities

were calculated after removing background scores and inter- and intra assay concordances of 90% and 95% respectively were observed demonstrating strong assay reproducibility. The levels of sIgE were categorized into class 0 to 6; class 0: ≤0·35 kU/L, class 1: 0·36-0·69 kU/L, class 2: 0·70-3·49 kU/L, class 3: 3·50-17·40 kU/L, class 4: 17·50-49·0 kU/L, class 5: 50-99 kU/L and class 6: ≥100 kU/L. A value of >0·35 kU/L is considered positive while ≥3·5 kU/L considered high.

***Anti-Aspergillus* IgG:** Serum anti-*Aspergillus* specific Immunoglobulin-G (IgG) antibodies were measured using the Platelia Anti-*Aspergillus* IgG kit (Bio-rad) according to the manufacturer's instructions. The assay detection range was 0-80 AU/mL and all samples were run in duplicate with a set of *Aspergillus* IgG calibrating standards run on each microplate. Observed readings were assessed in combination with those of sIgE titres against *Aspergillus* recombinant allergen in the definition of serological Allergic Bronchopulmonary Aspergillosis (s-ABPA) based on immunological breakpoints for *Aspergillus* sIgE of >0·35 kU/L and *Aspergillus* specific IgG of >5 AU/ml.

Sputum galactomannan assay: *Aspergillus*-associated sputum GM antigen was measured using the Platelia *Aspergillus* Ag kit (Biorad) according to the manufacturer's instructions and as previously described with samples run in duplicate and values ≥ 0·5 considered positive.(10)

Multiplex analysis for cytokine/chemokine quantification in sputum: Sputum expression levels of 41 different cytokines and chemokines were determined using the MILLIPLEX Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore) following previously described methodology (11). For analysis, 25µL of sample supernatant from sputum pre-

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treated with sputasol and protease inhibitor (Thermo Scientific) was used. Samples were incubated for 30 minutes with streptavidin-R-phycoerythrin conjugate, as per manufactures instruction, and analyzed with a Flexmap 3D instrument using Bio-Plex Manager software, v4·1 (Bio-Rad). The concentrations of each individual analyte were normalized to the total protein concentration in sputum which was determined by Bradford assays (Bio-rad).

Statistics and data analysis: Data were analyzed using Prism (version 7, GraphPad Software), R (version 3·2·4, R Foundation for Statistical Computing) and BayesiaLab software (BayesiaLab 7, Bayesia S.A.S). The Shapiro-Wilk normality test was used to examine data distributions. All continuous variables assessed deviated significantly from normality and hence median values with interquartile range (IQR) are reported. Continuous variables were analyzed using the Mann-Whitney U and Kruskal-Wallis tests as appropriate. Categorical variables were compared using Fisher's exact test or Chi-square test as appropriate. P-values of $<0{\cdot}05$ were considered significant with Benjamini-Hochberg correction for multiple comparisons applied as appropriate. Data from the MILLIPLEX Human Cytokine/Chemokine Magnetic Bead Panel was adjusted for batch variation using the R package 'MdimNormn'.(12) Normalized data was combined with sIgE allergen titre values generating a single dataset. Log-transformed values for each immunological analyte were scaled and visualized as hierarchically clustered heat maps using the R package 'heatmap.plus', while the optimal number of clusters present in the dataset was assessed using the 'NbClust' R package. To determine potentially complex associations between immunological analytes and target nodes of interest (i.e. geographic origin) within identified clusters, Bayesian network analysis based on information theory rather than classical statistical modelling was employed. Discretization of variables by supervised multivariate analysis was performed after which a Markov-blanket-based model was implemented using BayesiaLab 7.(13) A Bayesian network based on all immunological measures was

constructed, keeping the features that made the target node conditionally independent of all other nodes in the model. Immune measures ('features'), which were predictive of the target node (geographic origin) in the network, were thus identified.

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SUPPLEMENTARY TABLES AND LEGENDS

Table E1

Allergen			Median sIgE (IQR) kU/L	Class n (%)						
				0	1	2	3	4	5	6
House dust mite		Der p	2·28 (1·16-9·32)	6 (2·5)	23 (9·7)	118 (49·6)	49 (20·6)	20 (8·4)	5 (2·1)	17 (7·1)
		Blo t	2·33 (1·12-9·27)	14 (5·9)	17 (7·1)	109 (45·8)	56 (23·5)	15 (6·3)	8 (3·4)	19 (8)
Alternaria		Alt a	0·74 (0·39-1·30)	51 (21·4)	58 (24·4)	112 (47·1)	12 (5)	5 (2·1)	0	0
Aspergillus	Major	rAsp f 1	0·82 (0·33-2·51)	62 (26)	40 (16·8)	95 (39·9)	29 (12·2)	6 (2·5)	3 (1·3)	3 (1·3)
		rAsp f 2	0·91 (0·52-1·59)	41 (17·2)	47 (19·8)	120 (50·4)	14 (5·9)	9 (3·8)	2 (0·8)	5 (2·1)
	Minor	rAsp f 6	0·81 (0·46-1·42)	42 (17·6)	52(21·9)	123 (51·7)	10 (4·2)	6 (2·5)	1 (0·4)	4 (1·7)
		rAsp f 8	0·86 (0·51-1·49)	35 (14·7)	55 (23·1)	128 (53·8)	13 (5·4)	3 (1·3)	1 (0·4)	3 (1·3)
		rAsp f 15	0·95 (0·54-1·76)	38 (16)	40 (16·8)	125 (52·5)	20 (8·4)	5 (2·1)	5 (2·1)	5 (2·1)
		rAsp f 17	0·88 (0·52-1·63)	37 (15·5)	52 (21·9)	118 (49·6)	12 (5)	9 (3·8)	3 (1·3)	7 (2·9)

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Table E2

Allergen			Median sIgE (IQR) kU/L					
						Matched cohorts		
			Allergic rhinitis n=149	Bronchiectasis n=238	Bronchiectasis (SG-KL) n=138	Bronchiectasis (SG-KL) n=100	Bronchiectasis (DD) n=100	p-value
House dust mite		Der p	0·68 (0·22-2·75)	2·28 (1·16-9·32)	2·75 (1·29-15·83)	2·70 (1·33-17·19)	1·87(1·05-4·27)	**0·007**
		Blo t	0·59 (0·24-1·54)	2·33 (1·12-9·27)	2·82 (1·12-14·05)	2·91 (1·15-17·78)	2·06 (1·19-5·79)	0·21
Alternaria		Alt a	0·37 (0·18-0·70)	0·74 (0·39-1·30)	0·63 (0·33-1·12)	0·65 (0·33-1·13)	0·89 (0·58-1·34)	**0·015**
Aspergillus	Major	rAsp f 1	0·37 (0·28-0·73)	0·82 (0·33-2·51)	1·09(0·50-2·78)	1·18(0·50-3·01)	0·62 (0·29-1·86)	**0·009**
		rAsp f 2	0·37 (0·25-0·66)	0·91 (0·52-1·59)	0·88 (0·47-1·56)	0·92 (0·52-1·76)	0·93 (0·58-1·63)	0·771
	Minor	rAsp f 6	0·52 (0·33-0·96)	0·81 (0·46-1·42)	0·78 (0·37-1·42)	0·79 (0·43-1·43)	0·97 (0·62-1·43)	0·112
		rAsp f 8	0·26 (0·15-0·56)	0·86 (0·51-1·49)	0·78 (0·41-1·47)	0·81 (0·49-1·50)	0·97 (0·68-1·53)	0·105
		rAsp f 15	0·29 (0·16-0·56)	0·95 (0·54-1·76)	0·87 (0·43-1·67)	0·89 (0·55-1·63)	1·08 (0·70-1·97)	0·124
		rAsp f 17	0·38 (0·18-0·84)	0·88 (0·52-1·63)	0·78 (0·42-1·61)	0·80 (0·47-1·69)	1·06 (0·69-1·77)	**0·021**

Table E3

Allergen			Patients with sIgE titres of class 3 or above (≥3·5 kU/L); n (%)				
						Matched cohorts	
			Allergic rhinitis	Bronchiectasis	Bronchiectasis (SG-KL)	Bronchiectasis (SG-KL)	Bronchiectasis (DD)
			n=149	n=238	n=138	n=100	n=100
House dust mite		Der p	33 (22%)	91 (38%)	63 (46%)	46 (46%)	28 (28%)
		Blo t	17 (11%)	98 (41%)	60 (44%)	46 (46%)	38 (38%)
Alternaria		Alt a	1 (1%)	17 (7%)	11 (8%)	9 (9%)	6 (6%)
Aspergillus	Major	rAsp f 1	3 (2%)	41 (17%)	29 (21%)	23 (23%)	12 (12%)
		rAsp f 2	0	30 (13%)	16 (12%)	13 (13%)	14 (14%)
	Minor	rAsp f 6	0	21 (9%)	13 (9%)	9 (9%)	8 (8%)
		rAsp f 8	0	20 (8%)	10 (7%)	7 (7%)	10 (10%)
		rAsp f 15	1 (1%)	35 (15%)	21 (15%)	15 (15%)	14 (14%)
		rAsp f 17	5 (3%)	31 (13%)	14 (10%)	10 (10%)	17 (17%)

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Table E4

Allergen		SG-KL	DD	Median FEV_1						Median Exacerbations						sABPA n (%)						Effect on clinical outcome
				SG-KL			DD			SG-KL			DD			SG-KL			DD			
				sIgE class		p-value	sIgE class		p-value	sIgE class		p-value	sIgE class		p-value	sIgE class		p-value	sIgE class		p-value	
				< 3	≥ 3		< 3	≥ 3		< 3	≥ 3		< 3	≥ 3		< 3	≥ 3		< 3	≥ 3		
House dust mite	Der p	↑↑	↑	**76**	**61**	**0·039***	77	72	0·47	1	1	0·925	3	3	0·47	10 (13%)	12 (19%)	0·484	15 (21%)	6 (21%)	1	Decreased FEV_1 in SG-KL
	Blo t			**75**	**61**	**0·04***	78	75	0·40	0	1	0·908	3	3	0·86	8 (12%)	14 (26%)	0·059	11 (18%)	10 (26%)	0·323	
Alternaria	Alt a	↑	↑↑	68	84	0·31	76	65	0·47	1	1	0·777	3	5	0·14	19 (15%)	3 (33%)	0·382	19 (20%)	2 (33%)	0·603	-
Aspergillus	Major rAsp f 1	↑↑	↑	69	62	0·16	**77**	**55**	**0·028***	1	0	0·407	3	1·5	0·39	17 (16%)	5 (17%)	0·782	19 (22%)	2 (17%)	1	Decreased FEV_1 in DD
	Major rAsp f 2	↑	↑	69	66	0·48	77	67	0·17	0	1	0·617	3	3	0·46	17 (14%)	5 (31%)	0·137	16 (19%)	5 (36%)	0·164	-
	Minor rAsp f 6	↑	↑↑	70	57	0·49	76	67	0·65	0	1	0·144	3	3·5	0·46	18 (14%)	4 (31%)	0·223	19 (21%)	2 (25%)	0·673	-
	Minor rAsp f 8	↑	↑↑	70	56	0·18	76	75	0·93	0	1·5	0·308	3	1	0·42	18 (14%)	4 (40%)	0·054	20 (22%)	1 (10%)	0·684	-
	Minor rAsp f 15	↑	↑↑	70	57	0·12	76	75	0·53	0	1	0·205	3	2	0·73	**15 (13%)**	**7 (33%)**	**0·045***	16 (19%)	5 (36%)	0·164	Increased exacerbations in SG-KL
	Minor rAsp f 17	↑	↑↑	69	65	0·83	78	69	0·16	**0**	**1·5**	**0·035***	3	3	0·80	**16 (13%)**	**6 (43%)**	**0·011***	**16 (19%)**	**8 (47%)**	**0·008****	Increased in sABPA

Table E5

Characteristic	Immuno-allertype		
	FDPI	**HDCD**	**p-value**
BSI score : median (IQR)	10 (7-13)	8 (6-12)	**0.0098**
BSI status : (%)			**0.0245**
Severe	65	47	
Moderate	29	40	
Mild	6	13	
FEV_1 % predicted: median (IQR)	68 (50-86)	80 (62-90)	**0.0114**
Aetiology (%)			**0.0213**
Idiopathic	54	71	
Post-infection	36	19	
Other	10	10	
Long-term antibiotics (%)			0.0620
All	25	14	
Macrolide	19	13	0.2729
Sputum galactomannan (OD_{450})	0.299 (0.07-1.08)	0.115 (0.05-0.94)	0.0645

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Supplementary Table E1: Median specific-IgE (sIgE) titre (by class) against a range of recombinant allergens (house dust mite, *Alternaria* and *Aspergillus*) in non-CF bronchiectasis. Data are presented as median (interquartile range; IQR) and number of patients (n) (percentage; %).

Supplementary Table E2: Specific-IgE (sIgE) titres against house dust mite, *Alternaria alternata* and *Aspergillus fumigatus* recombinant allergens in patients with allergic rhinitis (n=149) and bronchiectasis (n=238). The bronchiectasis cohorts SG-KL and DD (n=100 each) were matched for age, gender and disease severity. Data are presented as median (interquartile range; IQR) and p-values indicated relate to the differences observed between matched cohorts.

Supplementary Table E3: Specific-IgE (sIgE) titres against house dust mite, *Alternaria alternata* and *Aspergillus fumigatus* recombinant allergens in patients with allergic rhinitis (n=149) and bronchiectasis (n=238) expressed as number and percentages of patients exhibiting specific-IgE (sIgE) titres of class 3 and above. As for Supplementary table E2, The bronchiectasis cohort includes SG-KL and DD cohorts (n=100 each) matched for age, gender and disease severity.

Supplementary Table E4: Relationships between observed sensitization patterns against a variety of allergens (house dust mite: Der p and Blo t; *A. alternata*: Alt a and *A. fumigatus*: rAsp f 1, f 2, f 6, f 8, f 15, f 17) and clinical outcomes in bronchiectasis. ↑: elevated sIgE titre, ↑↑: markedly elevated sIgE titre. Comparison of the effect of elevated sIgE titres on FEV_1, exacerbations and sABPA presence is indicated for patients from both the SG-KL and DD

cohorts (n = 238) with p-values for each comparison indicated; *p<0·05, **p<0·01. The final column summarises the main effects seen for each allergen.

Supplementary Table E5: Clinical characteristics of immuno-allertypes observed in NCFB. A comparison of clinically relevant outcomes among patients from the FDPI and HDCD immuno-allertype clusters is summarized. Only variables found to exhibit significant values of <0.1 are illustrated Significant differences in primary outcomes of interest were observed including BSI score/status and lung function (FEV_1 % predicted). Immuno-allertypes exhibited differences in aetiology, while use of long-term antibiotics and observed sputum galactomannan levels were greater in the FDPI cluster.