The pulmonary mycobiome in stable bronchiectasis

Chandrasekaran R[1], Mac Aogáin M[1], Lim AY[2], Low TB[3], Tan GL[4], Yii AC[4], Choo XN[3,4], Chai HZ[3], Jones LA[5], Narayanan S[5], Au V[5], Ng AH[6], Poh TY[1], Niranjan N[6], Connolly JE[5], Abisheganaden J[2], Koh MS[4], Chotirmall SH[1]

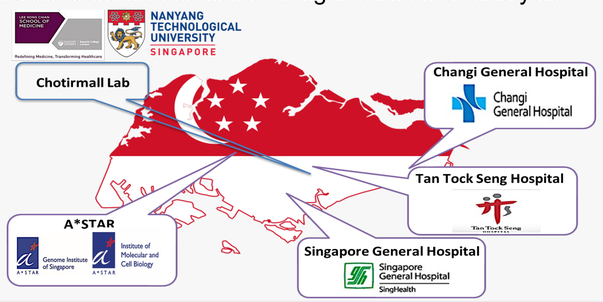
[1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, [2]Department of Respiratory and Critical Care Medicine, Tan Tock Seng Hospital, Singapore, [3]Department of Respiratory and Critical Care Medicine, Changi General Hospital, Singapore, [4]Department of Respiratory and Critical Care Medicine, Singapore General Hospital, Singapore, [5]Institute of Molecular and Cell Biology, A*STAR, Singapore. [6]Genome Institute of Singapore, A*STAR, Singapore. Corresponding author: Chotirmall SH, email: schotirmall@ntu.edu.sg

Background

Aspergillus species, described in cystic fibrosis, where it influences disease, has yet to be investigated in non-cystic fibrosis bronchiectasis. The recent statement on research priorities for bronchiectasis clearly illustrate that defining the mycobiome is a high priority area for investigation[1]. We aim to determine the frequency of *Aspergillus spp.* in sputum and to characterise its associated inflammatory consequences in the airways of patients with non-CF bronchiectasis. Additionally, we determined in a subset, the associated mycobiome to investigate if other fungal species have a role in bronchiectasis.

Methods

We prospectively recruited n=57 patients with stable bronchiectasis at three separate clinical sites across Singapore. Our previously published probe-based quantitative PCR (qPCR) method was used to identify the presence of *Aspergillus* in sputum and if detectable its associated conidial burden[2]. We concurrently assessed the airway inflammatory state by assessing a panel of 41 different cytokines and chemokines related to fungal pathogenesis. In a subset of patients (n=12), we performed 18s ITS targeted amplicon sequencing to determine the mycobiome at the species level. A p-value<0.05 was considered significant for all analysis.

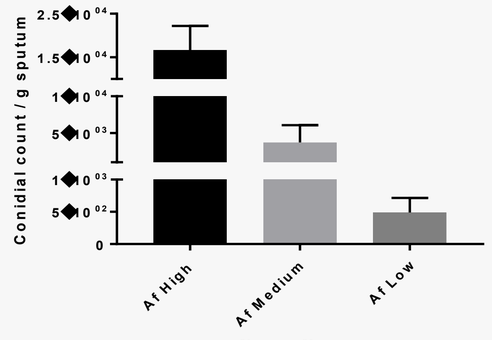


Results

Mean age (+/-SD) and forced expiratory volume (FEV_1) of the patient cohort was 65±12 years and 66±23% predicted respectively with an equal gender distribution. Aetiology of the bronchiectasis was as follows: idiopathic n=31 (54.5%); post-tuberculosis n=16 (28.0%) and others n=10 (17.5%). None of the patients had *Aspergillus* growth on routine culture however n=26 (45.6%) were positive on qPCR.

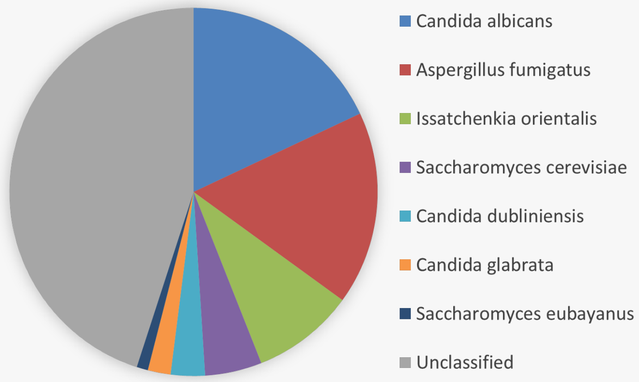
Patient No.	Aspergillus fumigatus status		A. fumigatus conidial load
	Culture	Molecular (qPCR) assessment	
1	Negative	Positive	High
2	Negative	Positive	High
3	Negative	Positive	High
4	Negative	Positive	High
5	Negative	Positive	High
6	Negative	Positive	High
7	Negative	Positive	High
8	Negative	Positive	High
9	Negative	Positive	Intermediate
10	Negative	Positive	Intermediate
11	Negative	Positive	Intermediate
12	Negative	Positive	Intermediate
13	Negative	Positive	Intermediate
14	Negative	Positive	Intermediate
15	Negative	Positive	Intermediate
16	Negative	Positive	Intermediate
17	Negative	Positive	Intermediate
18	Negative	Positive	Low
19	Negative	Positive	Low
20	Negative	Positive	Low
21	Negative	Positive	Low
22	Negative	Positive	Low
23	Negative	Positive	Low
24	Negative	Positive	Low
25	Negative	Positive	Low
26	Negative	Positive	Low

Median conidial burden was 3,402 conidia per gram of sputum (IQR: 806 – 8,660 conidia/g sputum).

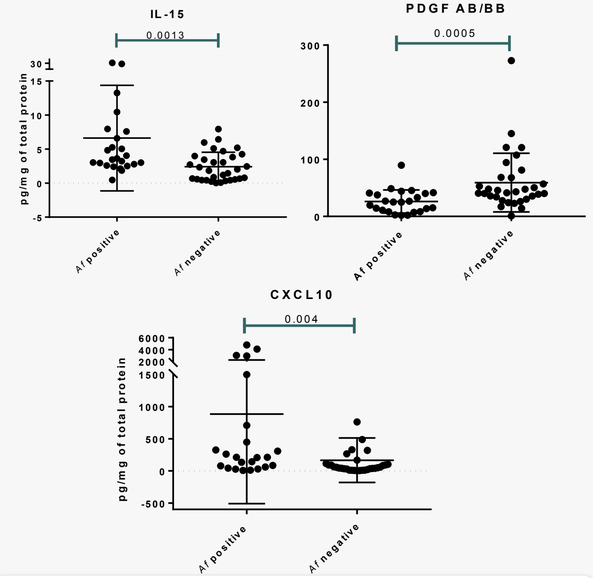


Af high: 1x 10^{04}-2.5x10^{04} conidia
Af Medium: 1x 10^{03} – 1x 10^{04} conidia
Af low: 1x10^{02} – 1x10^{03} conidia

The abundance of *Aspergillus* sequences from 18S ITS sequencing correlated well with conidial burden obtained from qPCR. Other fungal species identified by mycobiome analysis included *Candida, Issatchenkia and Saccharomyces spp.* Abundance of Aspergillus sequences however was significantly associated with increased clinical exacerbations (rate ratio: 1.026, 95% confidence: 1.006-1.045, p=0.009).



Aspergillus positive patients also demonstrated greater airway inflammation by differences in sputum IL15, CXCL10 and PDGF AB/BB (all p<0.05).



Conclusion

We report a high prevalence of *Aspergillus* in the airway of bronchiectasis patients that was associated with increased inflammation and exacerbations. The clinical implication of our findings and other species within the mycobiome require further study and corroboration in non-Asian cohorts.

References

1. Aliberti S et al. *The European respiratory journal* 2016.
2. Coughlan CA et al. *American journal of respiratory and critical care medicine* 2012

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