Aspergillus and *Pseudomonas* lung infections: that's what friends are spore

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***Aspergillus fumigatus* is a significant airway pathogen in chronic suppurative lung diseases. Poore *et al.* demonstrate the impact of mucus composition, and the importance of competition with *Pseudomonas aeruginosa* that reduces fungal growth in co-infection** https://bit.ly/4mdTvYQ

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Aspergillus species are the most prevalent fungal airway pathogens in people with chronic suppurative lung diseases. Sputum culture positivity for *Aspergillus fumigatus* (Af) in the UK Cystic Fibrosis (CF) Registry is reported in 4–8% of adults living with CF [1]. Similarly in non-CF bronchiectasis, the EMBARC registry data suggests approximately 5% of people living with bronchiectasis have positive sputum cultures for Af [2]. The reasons for susceptibility to *Aspergillus* infection in these patient cohorts is not clear.

The availability of highly effective CF transmembrane conductance regulator (CFTR) modulator drugs (*e.g.* elexacaftor/tezacaftor/ivacaftor (ETI)) has been transformational for many aspects of CF care and has significantly reduced the sputum burden experienced by many people living with CF. This does present some challenges for airway microbiological surveillance, particularly with respect to fungal detection, where a switch to a dependence on cough or throat swabs is likely to disproportionately impact the successful culture of fungal organisms compared to classic bacterial CF pathogens. Epidemiological data from ivacaftor monotherapy studies describes reduced prevalence of *Aspergillus* lung infection, however whether this represents a true reduction or simply reduced detection is not known [3, 4]. Data reporting the impact of ETI is relatively scarce but there are some indications that rates of *Aspergillus* spp. detection also decline after the introduction of ETI [5].

In this issue of *ERJ Open Research*, Poore *et al.* [6] report on their experimental findings exploring the impact of mucus on Af growth. A key methodological strength of the study is the sputum media, where rather than using primary expectorated mucus or artificial CF sputum media, this group diligently harvested mucus from human bronchial epithelial (HBE) cell cultures from CF and non-CF tissue donors. They report decreased levels of Af growth as mucus was serially diluted, an effect that was seen in mucus from CF and non-CF cells. Sputum established over longer periods resulted in increased growth, again regardless of CF status. These findings suggest some universal contributors to the prevalence of Af in muco-obstructive diseases but also represent potential therapeutic targets with muco-active drugs or optimised chest clearance. However, the characteristics of sputum alone were not the sole determinant of Af growth. For example, the representation of airway inflammation through IL-4 stimulation of HBE cells increased the % solid content of its mucus, with an associated increase in Af growth. The fact that this increase was observed only in CF derived mucus when adjusted for the rise in % solid content is interesting, particularly when considering previous data supporting a rise in CFTR activity, and therefore airway hydration, with IL-4 stimulation [7].

This study also highlights the potential implications of *Pseudomonas aeruginosa* (PsA) co-infection for Af growth. The inhibition of Af growth in the presence of purified pyocyanin (a PsA virulence factor) as

previously reported by Sass *et al.* [8] was not seen in this study. Interestingly though, pre-exposure of HBE cells to pyocyanin ultimately led to an increase in Af growth in CF and non-CF mucus even when controlling for % solid composition. Furthermore, co-infection of mucus with live PsA 1 h prior to introducing Af conidia caused a marked reduction in Af growth irrespective of CF status, an effect which was lost with a tobramycin-treatment step. It is important that this Af inhibition effect was seen in CF and non-CF mucus, supporting the literature in which PsA anti-*Aspergillus* strategies are widely reported and thought to be largely driven by competition for environmental nutrients *via* the secretion of PsA virulence factors such as pyoverdine [8–10]. The exact mechanisms behind this inhibition remain unclear, but are worthy of future work given that there are implications for patient treatment if the result is a falsely negative Af culture from a sputum sample co-infected with PsA [11].

There are some limitations to consider, for example the overall sample size is low and although the authors took extra steps to ensure validity and replication within the donor samples, larger studies are likely required. Poore and colleagues did not elucidate the mechanism for the changes in Af growth in relation to sputum dilution, which could be due to reduced nutrients, reduced mucins or simply directly as a result of altered rheological structure of the sputum. Similarly, not all clinical isolates of PsA have a pyocyanin production phenotype [12], and given the variable responses between different host-derived inflammatory cytokines seen here, there may also be heterogeneity in responses to different PsA derived stimuli. Further work reproducing the impact of PsA on Af growth with a wider range of PsA clinical isolates would be beneficial, particularly using chronic PsA isolates from people living with CF in order to more accurately recapitulate airway microbial interactions.

A common limitation in studies using pre-ETI derived tissue lines is the generalisability to people living with CF receiving CFTR modulators, however Poore and colleagues were able to explore this using a small number of paired sputum samples from patients in the PROMISE study both pre- and post-ETI initiation [13]. Inoculating Af into these samples revealed less growth in post-ETI samples despite no corresponding changes in % solids. This would suggest that there are direct or indirect CFTR-related changes in sputum composition following the introduction of ETI which alter sputum hospitability for Af growth. Speculatively, these could be changes in competing pathogens within the lung microbiome, changes in pH or indeed ratios of mucins, nutrients or other constituents that result in a deleterious effect on Af growth. Additionally, there are *in vitro* data suggesting a direct impact of ETI on Af biofilm biomass, mediated by cell wall permeability [14]. Samples from this aspect of the study were from a small number of sputum producers but could suggest the reduced detection of Af infection after effective modulator treatment initiation is a true signal rather than a quirk of sampling methods [3–5]. Even with declining prevalence of Af infections in people living with CF receiving ETI, work to establish the underlying mechanisms is warranted given the implications for therapeutic targets in non-CF populations.

In summary, Poore and colleagues improve our understanding as to some of the mechanistic and lung microenvironment factors that may predispose or encourage *Aspergillus* infection in people living with chronic suppurative lung diseases and highlight potential avenues for further research and therapeutic development with a view to combatting fungal lung infections.

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