

Correspondence

Reply

To the Editor:

We thank our colleagues Fu et al¹ for their interest in our work.² Our pilot study reported several associations between the indoor microbial exposure, human sputum microbiota and mycobiota, and the occurrence of severe asthma among patients.

Especially, we established that fungal taxa (ie, fungal Operon Taxon Unit [OTU] proportions, Fig 3, *F*) shared between the indoor environment electrostatic dust collector (EDC) samples and the corresponding sputa was significantly higher when sputa were collected during asthma exacerbation, compared with pairs of sputum and EDC samples collected during stable states.

To describe this finding, we estimated the proportions of common bacterial and fungal OTUs (Fig 3, *E* and *F*, respectively) in sputum expressed in % between sputum and EDC samples, taking into account the clinical status of patients (stable state or exacerbation).

It refers to the number of reads of each common OTU calculated as follows:

$$\text{Common OTUs in sputa (in \%)} = \sum \left(\frac{\text{Number of reads of all the OTUs shared between a pair of sputum and EDC sample}}{\text{Number of total reads of the sputum sample}} \times 100 \right)$$

This approach is congruent with Venn diagrams findings (Fig 4) that showed a fungal core (Fig 4, *B*) larger than the bacterial core (Fig 4, *A*) and composed of 9 OTUs associated with sputa collected during an exacerbation and 17 OTUs belonging to the fungal core independently from the clinical state, but only 1 (*Alternaria brassicae*) associated with sputa collected at stable state. Of note, Venn diagram data were not fully comparable to those of Fig 3, *E* and *F*, because they took into account only those OTUs that were present in at least 20% of each group of sputa (clustered according to the presence or absence of an exacerbation when sputa were collected) and in at least 90% of the corresponding EDC samples.

Regarding Principal Coordinate Analysis (PCoA), bacterial composition of the indoor and respiratory microbiome was clearly different between the 2 type of samples (Fig 3, *C*). Conversely, the fungal composition indicated high fungal compositional variations between indoor and respiratory mycobiomes but with 2 clear clusters within sputum samples (Fig 3, *D*), 1 of these 2 clusters being more close to the EDC sample cluster. Despite high proportions in sputum of common bacterial and fungal OTUs between sputum and EDC samples (Fig 3, *E* and *F*, respectively), the greater diversity found in EDC samples, compared with sputum (Fig 3, *A*), explains the dissimilarity found between EDC samples and sputum (Fig 3, *E* and *F*). On the whole, these results were congruent with Figs 3 and 4, and renewed interest in deciphering the interactions between the indoor environment, fungi, and the host response to facilitate the development of novel therapeutic managements in asthma.³

Comparing our finding observed on a severe asthma population of adults with data recently published by Gupta et al⁴ is difficult, because they focused on environmental analysis of a

pediatric population (without clinical links such as presence or absence of asthma) and demonstrated that bed dust flora appeared to be influenced by multiple environmental exposures (especially type of living environment and pets as previously reported^{5,6}).

The authors compared endogenous (airways) and exogenous (bed dust) microbiotas, by evaluating whether associations existed between the microbiotas of bed dust collected when children were 6 months old and the microbiotas of infant airways sampled at 3 months. Using Spearman correlation (based on $P < .05$ after false-discovery rate adjustment), they identified several positive correlations between airway bacterial and bed dust bacterial plus fungal microbiomes, for which the biological relevance was tempered by the authors especially regarding the difference in sampling time between the airways and bed dust samples.

A co-occurrence network was also produced without highly relevant findings.⁵ In fact, robust co-occurrence networks are difficult to set up given the compositional nature of the microbiota and mycobiota data. Several approaches have been proposed to address co-occurrence networks including the Spearman correla-

tion analysis used by Gupta et al^{4,5} and suggested by ours colleagues Fu et al.¹ These methods usually deal with a number of taxa (OTUs) large relative to the sample size and assume sparsity (an abundance of 0 entries) to achieve stable precision matrix estimation.⁷ However, inadequate (small) sample sizes remain a major drawback, resulting in unstable estimates of precision matrices for which applying a penalization scheme is recommended as we and other authors previously reported.⁷ Even when using Lasso as an efficient solution in the domain of sparse precision matrix estimation, having a limited sample size (<30 samples) tends to produce unstable co-occurrence data and networks to our knowledge.

To conclude, our pilot study pointed out several microbial species (especially fungal species) from the indoor flora that appear related to the endotypes of severe asthma. Further larger studies are now warranted to understand better how these species interact with the asthmatic epithelium and with the other microbial species copresent in the respiratory tract.

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interests.

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<https://doi.org/10.1016/j.jaci.2020.10.021>