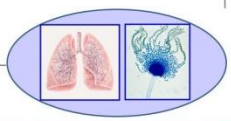
Moulds and cystic fibrosis: what can we learn from studying lung mycobiota?' Recent updates and links with other microbial communities

Linh Nguyen Do Ngoc[1], Laurence Delhaes[1,2]

[1]Pasteur Institute of Lille, Center for Infection and Immunity of Lille, Inserm U1019, UMR CNRS 8204, University Lille Nord de France, Lille, France
[2]Department of Mycology-Lille Hospital, Lille 2 University, Lille Cedex, France



INTRODUCTION & AIM:

✓ Recent studies using culture-independent microbial detection methods (Deep-sequencing) enable exploration of microbial community composition in both healthy and abnormal lungs. These methods reveal that cystic fibrosis (CF) airway bacterial communities are more diverse than previously appreciated and vary in both short- and long-term. **Each community has its own composition and evolution, unique and specific to each patient. The microbiota dynamics might account for CF disease outcome especially during acute exacerbations.** Furthermore, although fungi and viruses are increasingly recognized as important agents in pulmonary exacerbations, only a limited number of small deep-sequencing studies have focused on viruses and phages, and/or fungi.

✓ Given the polymicrobial nature of pulmonary infections in CF patients and the recent evidence that fungi may be of clinical relevance in the decline of CF lung function, **we developed a high-throughput sequencing approach to extensively explore the diversity and dynamics of fungal and prokaryotic populations in CF upper airways.**

➔**Specifically, we explore links between pulmonary acute exacerbation and moulds (such as *Aspergillus fumigatus* and *Scedosporium*), taking into account the context of CF and the polymicrobial nature of airway community.**

- ❖ Pulmonary exacerbation = key event in CF lung alteration
- ❖ What is the role of MBPA (allergic bronchopulmonary mycosis) in such exacerbation?
- ❖ With the idea of deciphering the place of *Aspergillus/Scedosporium*

MATERIAL & METHODS:

• Population and samples:

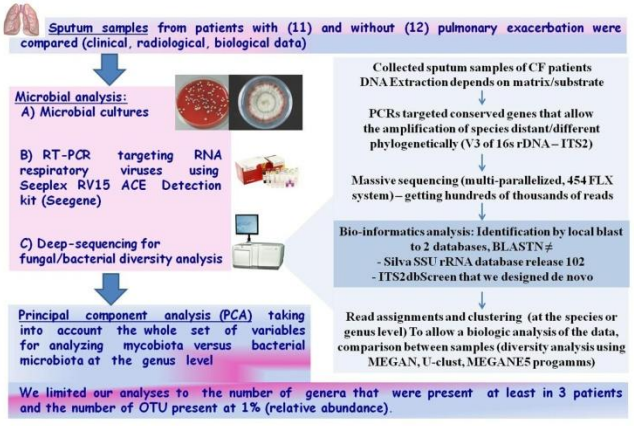
Sputum samples from CF patients with (11 patients) and without (12 patients) pulmonary exacerbation were compared

• Methods:

→ *Microbiological analysis:* Moulds, bacteria and respiratory viruses were identified using conventional methods, RT-PCR and deep-sequencing approach.

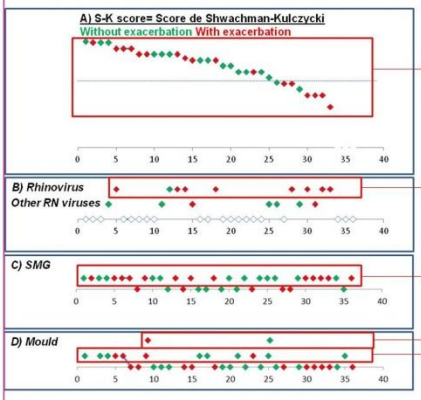
→ *Pyrosequencing (454 FLX) approach* was used to address fungal diversity in lung (i.e. lung mycobiota), as previously described *[Delhaes et al, 2012]*.

→ *Data were analysis according to the following workflow* in collaboration with Pegase consortium



RESULTS:

• Conventional microbial analysis:



No correlation between SK-score and pulmonary exacerbation

We confirmed the significant association between HRV and pulmonary exacerbation *[Goffard et al. J Clin Virol. 2014 Feb 25]*

No association between Milleri group Streptococcus and pulmonary exacerbation

Only 2 patients colonized with *Scedosporium apiospermum*

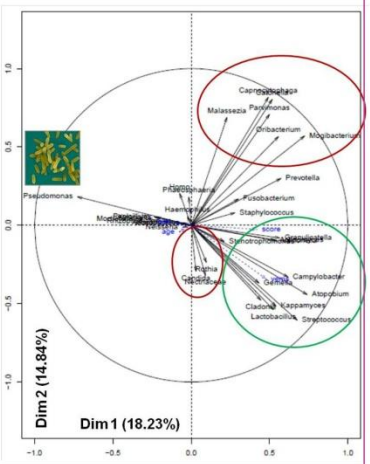
No association between *A. fumigatus* and exacerbation

• Deep-sequencing results:

- ✓ 953 999 reads size from 315 to 468 pb - 2/3 16s rDNA + 1/3 ITS2
- ✓ Optimal rarefaction curves
- ✓ CPA and modelization under process

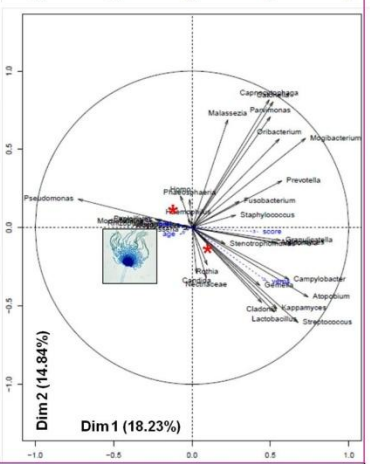
✓ **Pseudomonas**

- is alone in agreement with published data *[Zemanick et al. 2013]*
- not correlated with "Malassezia plus Prevotella group" *[Zemanick et al 2013]*
- neither with the "Candida plus Rothia group" (which is not well explained by our axes since the arrows are short)
- but is negatively correlated with the "group of oral flora including streptococcus plus some environmental fungi", as well as FEV1 – SK-score *[Zemanick et al. 2013]*



✓ **Aspergillus**

- Unfortunately, our PCA model explained poorly this mold (short arrows, anti-correlated to SK-score, FEV1,),
- Neither exacerbation status: There was no differentiation between the group of patients with and without pulmonary exacerbation (according to PCA-barycenter (*) of each patient group)



DISCUSSION & CONCLUSION:

• Using deep-sequencing and PCA approaches, we confirmed the relation between *Pseudomonas aeruginosa* and alteration of respiratory function as well as an anti-correlation between *P. aeruginosa* and bacteria of the mouth community as previously described *[Zemanick et al. 2013]*. Most of current studies have focused on bacterial microbiota in CF patients while viruses and/or moulds have been reported as pathogens *[Alouat et al 2014]*.

• While Rhinoviruses (recombined HRV-Ca) were significantly associated to acute pulmonary exacerbation ($p = 0.027$) *[Goffard et al 2014]*, we didn't observe any correlation between *Aspergillus fumigatus* and exacerbation in our population.

This might reflect the fact that ***Aspergillus* spp. especially *A. fumigatus* isolated from respiratory secretions is often a dilemma for the CF clinician in terms of clinical relevance and treatment** *[Liu et al. 2013]*.

• We currently continue the statistical analysis by focusing on *Streptococcus* species and less abundant (but more diverse) components of the mycobiota (rare biosphere - <0.1%). Larger studies are also required to deeply analyze the role of each microorganisms (bacteria, viruses and fungi), in combination with a more ecological analysis *[Whiteson et al. 2014]*.

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