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A. Blanc-Potard, S. Pont, F. Nilly, P. Plésiat

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WS06.06**Effect of elxacaftor/tezacaftor/ivacaftor on structural lung damage after one year detected by magnetic resonance imaging**

S. Conci¹, A. Paciaroni², G. Dinnella¹, S. Moser¹, E. Proietti¹, U. Pradal¹. ¹APSS Rovereto Hospital, Pediatric Unit - Cystic Fibrosis Center, Rovereto, Italy; ²APSS Rovereto Hospital, Radiology, Rovereto, Italy

Objectives: The aim of our study was to investigate if treatment with ELX/TEZ/IVA causes a regression of the structural lung damage, after 6 and 12 months, and if it is detectable using a non invasive method such as MRI without contrast.

Methods: We prospectively selected CF subjects eligible for ELX/TEZ/IVA treatment in our CF center in Rovereto. We collected clinical data and performed a thorax MRI before starting treatment in stable clinical condition, then after 6 months and lastly after 12 months of treatment. We evaluated MRI using a semi-quantitative MR score (ranging from 0 to 60, Eichinger 2012). Additionally, we compared the type of bronchiectasies (the most prevalent and the worst grade detectable, grading range from 1 to 3) and the presence of inflammation (using diffusion weighted imaging - DWI) before treatment, and after 6 and 12 months. For the statistical analysis we used the Wilcoxon signed-rank test.

Results: In our center 16 CF subjects started treatment with ELX/TEZ/IVA between February 2020 and December 2021. 4 subjects were lost to follow up and therefore we paired the data of 12 subjects aged 31 ± 10 years (6 females, 4 homozygous for F508del). At 6 months we observed a reduction of the bronchiectasy's grade from a median of 1.5 to 1 (p-value 0.037) for the prevalent grading and from a median of 2.5 to 2 (p-value 0.017) for the worst grading. The MRI showed the presence of inflammation before treatment in 4 subjects. After treatment inflammation was undetectable. The median MRI score decreased from 16.5 to 5.5 (p-value 0.004) at 6 months and to 4.0 (p-value 0.005) at 12 months.

Conclusion: This study shows a significant regression of the structural lung damage and the complete resolution of inflammation after treatment with ELX/TEZ/IVA. These data suggest that a partial but significant recovery of structural lung damage, e.g. bronchiectasies, is possible and that MRI is a reliable, non invasive, radiation free tool to detect and monitor such changes.

WS07 – Pathogenesis and adaptation of microbes in the CF airways

WS07.01**Inter-species interactions alter antimicrobial susceptibility in polymicrobial cultures**

E.B. Benyei¹, P.M. Ho¹, M. Welch¹. ¹University of Cambridge, Department of Biochemistry, Cambridge, United Kingdom

People with cystic fibrosis (p/wCF) often develop chronic lung infections punctuated by periods of acute pulmonary exacerbation. One of the pathogens present in many p/wCF is *Pseudomonas aeruginosa* (PA). In many infection scenarios, PA shares the airway environment with a veritable “zoo” of other microbes, including Gram-negative, Gram-positive and fungal species – yet these are rarely taken into account when testing new anti-pseudomonal agents. The aim of this project is to examine how the presence of other co-habiting species affects the biology of PA during antibiotic challenge.

Due to the technical difficulties of growing PA in polymicrobial cultures *in vitro*, little is currently known about how interspecies interactions influence the biology of PA. However, we have developed an optimized continuous flow system that provides a robust experimental platform for studying CF-associated polymicrobial cultures *in vitro*. Notably, we are able to stably co-culture PA in artificial sputum medium for many days with other CF airway-associated microbes, including *Staphylococcus aureus* (SA) and *Candida albicans* (CA).

Our data show that interactions between PA and its neighbours can significantly enhance resistance against antimicrobial agents. This enhanced resistance has heritable and non-heritable components.

Moreover, we find that a clinically-used combination of antibiotics can lead to a long-term increase in titres of species not explicitly targeted by the treatment.

In order to provide the most effective antibiotic treatment for people with CF, we need to take a more holistic view of the microbial ecology in the CF airways. We are now in a position to do this by investigating the response of *in vitro* polymicrobial cultures to antibiotic challenge. Our data support the notion that interspecies interactions increase antimicrobial resistance, and furthermore, show how antibiotics are potent population remodelling agents.

WS07.02**Zebrafish infection model to assess persistence of *Pseudomonas aeruginosa* clinical isolates and treatment efficiency**

A. Blanc-Potard¹, S. Pont¹, F. Nilly¹, P. Plésiat². ¹Montpellier University, LPHI, Montpellier, France; ²Besançon University, Besançon, France

Objectives: *Pseudomonas aeruginosa* is a main cause of chronic infections in patients with cystic fibrosis (CF). Our aim was to set up *P. aeruginosa* persistent infection in zebrafish (*Danio rerio*), a non-mammalian vertebrate model suitable for real-time imaging of infection and assessment of treatment efficacy.

Methods: Our novel model is based on the infection of clinical *P. aeruginosa* isolates from CF patients in zebrafish embryos (using transgenic fish lines with fluorescent phagocytic cells). Bacteria were counted in infected embryos up to 3 days after infection and visualized by confocal microscopy to address their interaction with innate immune cells. The efficacy of known antibiotics, as well as novel molecules, was evaluated at different times post-infection. Experiments can be conducted in normal context or in CF embryos (using *cfr* morphans or a CFTR-deficient fish line).

Results: Quantification of the bacterial load of infected embryos allowed to identify clinical isolates with a persistent phenotype. We focused on a particular persistent isolate, showing aggregated bacteria at the infection site, which are often located inside macrophages. The persistence of this isolate is associated with a decreased susceptibility to several antibiotics classically used in clinics. The efficiency of anti-biofilm molecules, in combination or not with antibiotics, to eliminate persistent bacteria is currently tested. Studies will be conducted both in normal and CF embryos.

Conclusion: We established for the first time a persistent model of *P. aeruginosa* infection in zebrafish. The persistence of a *P. aeruginosa* clinical isolate from CF patient in the zebrafish embryo is associated with tolerance to antibiotics, which mimics the failure of antibiotic treatment during chronic clinical infections. We thus propose a novel model relevant to study the efficacy of innovative treatments in the context of a *P. aeruginosa* persistent infection.

WS07.03**A long-term study of *Pseudomonas aeruginosa* early patient isolate adaptation to the hypoxic environment**

J. Drabinska¹, A. Mazhova¹, N. Duggan¹, S. McClean¹. ¹University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland

Pseudomonas aeruginosa is an opportunistic bacterial pathogen which causes chronic lung infections in people with cystic fibrosis (CF) and is a major contributor to morbidity and mortality. Despite this, the mechanism driving its adaptation towards chronic colonisation in the CF lung is not yet fully understood. This work focuses on the adaptations of *P. aeruginosa* to hypoxia, one of the important environmental pressures present in the lung. Long term hypoxia has yet to be examined as a driver of adaptation towards the persistence of this bacterium in CF patients. We have studied the effect of long term adaptation to 6% oxygen for up to 28 days. Interestingly, distinctive colony morphotypes developed throughout the experiments, including small colony variants (SCV). One of the observed morphotypes appeared exclusively under low-oxygen pressure. Importantly, the colonies with visibly different morphologies were more common in hypoxia adapted cultures, comprising up to 98% of the population, while they never exceeded 35% in normoxia adapted cultures. Proteomic analysis showed changes in the abundance of over 700 proteins within 5 days of adaptation, including those involved in respiration and carbon metabolism, quorum sensing and phenazine biosynthesis. Interestingly, comparable