## 147 Azithromycin long-term therapy in CF patients: microbiological and clinical effects

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Macrolides long-term therapy has demonstrated beneficial effects on pulmonary status in CF patients, but there is a lack of literature on microbiological variations possibly associated with it. We studied lung function (FEV1%), nutritional status (BMI) and sputum culture in 65 CF patients (32 males, mean age 29.11 years), in whom long-term Azithromycin therapy (250–500 mg/die, 3/7 days a week, for at least 1 year) was performed. Moreover we compared, for sputum cultures results, 10 patients (Group A: 4 males, mean age 23.5 years, range 12.5–44, Sputum culture: S.a.oxa S in 7/10, S.a. oxa R in 1/10, S.a.+ P.a. in 2/10) out of these series, with 10 CF patients (Group B) matched for age and sputum culture, in whom long-term Azithromycin therapy was never performed.

In 44/65 patients (68%) mean BMI improved (T0: 19.64; T12: 19.83); in 41/64 patients (63%) mean FEV1% increased (T0: 56.17%; T12: 60.21%); in 20/65 patients sputum bacteria showed new multiresistance antibiotic pattern and in 14/65 (21.5%) sputum cultures exhibited small colony variant (SCV) as new bacterial phenotypes: S.a. in 9 pts, P.a. in 6 pts and mucoid P.a. in 1 pt.

The sputum culture comparison between Group A and Group B showed no difference on new multiresistence antibiotic bacteria developement, but 2 SCV S.a. and 1 SCV P.a. in Group A and no SCV bacteria in Group B were isolated.

In conclusion our data, according to international leterature, demonstrate that long-term Macrolides therapy have a positive impact on respiratory function and nutritional status in CF, but we hypothesize that this therapy could be associated with SCV bacteria development in CF sputa.

## 148* Virulence of mucoid *Pseudomonas aeruginosa* strains in cystic fibrosis: New sequential infection model

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**Background:** Chronic lung infection with *P. aeruginosa* is the dominating cause of death in patients with CF. The chronic lung infection often lasts for decades with just one clone. As a result of inflammation, antibiotic treatment and different niches in the lungs the clone undergoes genetic changes resulting in diversifying geno- and phenotypes. Such adaptation may generate different host responses. However, experimental animal infections with *P. aeruginosa* are limited to 2 weeks.

**Experiments:** To experimentally reflect the year-long lung infection in CF, groups of BALB/c mice were infected with mucoid or non-mucoid isotypic clones isolated during different periods (1980, 1988, 1997, 1999 and 2003) of the lung infection of one CF patient, using the seaweed alginate model.

**Results:** The non-mucoid clones reduced their virulence over time, resulting in faster clearing of the bacteria from the lungs ($p < 0.035$), improved histopathology ($p < 0.05$) and reduced pulmonary MIP-2 and G-CSF ($p < 0.005$). In contrast, the mucoid clones were more virulent. Moreover, their virulence increased with time resulting in impairment of clearing of the latest clone ($p < 0.0001$), severe inflammation in peripheral airways ($p < 0.05$) and increased pulmonary MIP-2 and G-CSF production ($p < 0.04$). Similar results were obtained using comparable sets of isotypic clones from four additional CF patients.

**Conclusion:** Adaptation of *P. aeruginosa* in CF is reflected by changed ability to establish lung infection and results in distinct host responses to mucoid and non-mucoid phenotypes. The present new infection model reflects important characteristics of the year-long *P. aeruginosa* lung infection in CF.

## 149* Respiratory syncytial virus (RSV) enhances *Pseudomonas aeruginosa* lung infection in mice

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**Background:** Cystic fibrosis has a typical course of exacerbations and remission of lung disease, suggesting influence of external factors like respiratory viral infections. We reported clinical evidence suggesting that RSV influences acquisition and chronic infection with *P. aeruginosa*. We recently described that in vitro RSV infection increases infectivity of *P. aeruginosa* in respiratory epithelial cells. We studied whether RSV has an enhancing effect on acute *P. aeruginosa* lung infection in a mouse model.

**Methods:** We used Balb/c mice and a non-mucoid, early *P. aeruginosa* clinical isolate. Mice were inoculated with bacteria alone or together with RSV. Lung function was measured at baseline and at termination of the experiment. Lungs were harvested 24 hours after inoculation and randomly selected for either histopathology or bacteriology.

**Results:** A significantly higher number of bacteria was found in the lungs of co-infected mice, compared to mice infected with bacteria alone (median $1.8 \times 10^5$ and $9.1 \times 10^1$ CFU/mouse respectively, $p < 0.01$). Lung function changes were most outspoken in co-infected mice. Histopathology showed alveolar inflammation in both groups.

**Conclusion:** In mice, co-infection with RSV and *P. aeruginosa* leads to reduced clearance of bacteria and a trend towards more outspoken lung function changes, compared to infection with bacteria alone. This supports clinical and in vitro evidence for an enhancing effect of RSV on *P. aeruginosa* infectivity, which is important in the understanding of the initial mechanisms of *P. aeruginosa* colonization in CF patients.

## 150* The paranasal sinuses are focus for colonisation and chronic biofilm lung infection in CF patients

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Acute and chronic sinusitis is common complications of CF. We have genotyped *P. aeruginosa* (PA) lung isolates from CF children by using PFGE. After antibiotic eradication of PA, new colonising isolates had the same genotype as the initial colonising strain. It has been suggested that the sinuses may serve as a reservoir for bacterial lung infections.

Twenty-five CF patients (median age: 18 yrs, range 6–50) have been treated with FESS to evaluate whether their sinuses serve as a bacterial reservoir. Eleven were chronically infected (7 with PA, 2 with BC, 1 with AX, 1 with Moraxella) and 14 were intermittently colonised (8 with PA and 6 with other CF pathogens) in their lungs.

We found good agreement between lung and sinus bacteriology in 24/25 (95%) of the patients. Seven patients chronically infected with PA had the same genotype in their lungs for >15 years and this genotype was identical to the PA obtained from their paranasal sinuses. Six of eight children colonised with PA had growth of the same genotype of PA in their sinuses and sputum samples collected up to 2 years prior to the FESS operation. In Gram stained smears from the sinuses we found that the bacteria were organised in biofilm structures similar to those seen in the lungs. In sinus smears we detected few inflammatory cells compared to lung smears.

The sinus colonisation is immunological silent in intermittently colonised patients and causes only an insignificant anti-PA antibody response (Th1 response). Because of the weak signs of infection, the sinus colonisation can proceed unnoticed into a permanent infectious focus that cannot be eradicated with antibiotics.