69 Clinical situations and mucoid transition of *Pseudomonas aeruginosa* in Cystic Fibrosis patients

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Background: Mucoid form of *Pseudomonas aeruginosa* (Pa) in CF patients is thought to be responsible for the main part of progression of lung disease. As molecular mechanisms underlying mucoid transition in Pa are progressively elucidated, clinical situations associated with mucoidy acquisition are as yet poorly investigated.
Method: We retrospectively evaluated 52 CF patients colonized by Pa followed at the CF centre of Dijon, France. End-point of follow-up was defined by first mucoid Pa isolation (n=26), or last sputum examination for children remaining free of mucoid Pa (n=26). Any clinical, pathological or therapeutic event occuring during the follow-up period was recorded. A Cox model with time-dependant covariables was used to determine which parameters were linked to mucoid transition of Pa.
Results: Mean follow-up duration was 4.7 years ±4.3, with no difference between both groups. After adjustment on age, three independent parameters were associated with a higher risk of mucoid transition of Pa: persistence of Pa in sputum (OR 7.89; IC95[1.78–34.99]; $p < 0.01$), inhaled bronchodilators (OR 3.40; IC95[1.04–11.12]; $p = 0.04$), and inhaled colimycin (OR 4.04; IC95[1.27–12.9]; $p = 0.02$), while isolation of either *S. aureus*, *H. influenzae* or *S. pneumoniae* in sputum was associated with a lower risk (OR 0.24; IC95[0.09–0.67]; $p < 0.01$). Use of parenteral antibiotics was not an independent risk factor for mucoid transition.
Conclusion: Four bacteriological and therapeutic parameters, reflecting both severity of lung disease and importance of Pa colonization, were associated with mucoid transition of Pa in our study. Though these results are not directly accessible to prophylactic measures, they corroborate the beneficial impact of early eradication of Pa.

70 *Pseudomonas aeruginosa* in the oral cavity: implications for lung infection in patients with Cystic Fibrosis

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Pseudomonas aeruginosa may be transmitted into the lungs of CF patients via oral uptake of contaminated food. Thus, we investigated the contamination rate of common vegetables and the adhesion of *P. aeruginosa* in the oral cavity. 308 vegetable samples, collected from three supermarkets, were screened for *P. aeruginosa*, other *Pseudomonas spp.*, *Burkholderia cepacia* complex, and *Staphylococcus aureus*. For quantification of the bacteria, vegetables were homogenized, diluted, and plated on appropriate agar plates. For quantification of *P. aeruginosa* adherence, one CF patient and two healthy individuals chewed green salad, incubated with 1.4×10^6 cfu, for 5 min followed by bacterial quantification in the expectorated salad. The mouth of the probands was rinsed 0, 30, and 60 min after chewing, followed by quantitation of *P. aeruginosa* in the oral lavage fluid specimens. In 15 vegetable samples (4.9%), *P. aeruginosa* was detected. Other *Pseudomonas ssp.* were present in 73.1% of the samples. In green salad (7.4%), tomato (10.0%) and cucumber (13.8%) samples, a mean value of 5×10^2 *P. aeruginosa*/ml was found. All other vegetable samples were *P. aeruginosa* negative, neither *S. aureus* nor *B. cepacia* complex were found. On the expectorated salad, 4.0×10^5 cfu (35% of the inoculum) were found. Only 3.1×10^3 cfu (0.2%) were detectable in oral lavage fluid specimens 60 min after consumption. Drinking of 200 ml water immediately after chewing reduced bacterial numbers in the lavage fluid to 1.7×10^0 cfu (0.0001%). The study suggests that oral uptake of vegetables contaminated with bacteria does not pose a major risk for *P. aeruginosa* lung infections in CF patients.

71 Phenotypic and genotypic characteristics of a Cystic Fibrosis epidemic strain of *Pseudomonas aeruginosa*

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The Liverpool epidemic strain (LES) of *Pseudomonas aeruginosa* is a transmissible aggressive pathogen of cystic fibrosis (CF) patients. Indeed, a recent survey indicated that the LES is the most common *P. aeruginosa* strain amongst the CF population of England and Wales. The aims of this study were to identify genotypic or phenotypic characteristics that might contribute to the additional properties of this epidemic clone.
Using a combination of suppression subtractive hybridisation (SSH) and data from the ongoing genome sequence project, we have identified three clusters of genes either unique to the LES or found only rarely in other *P. aeruginosa* strains. Transcriptome profiling has indicated that the virulence-related quorum sensing (QS) regulon is considerably up-regulated and prematurely activated in some LES isolates (Hypervirulence [HV] phenotype). The HV phenotype can be identified using assays for pyocyanin production. Isolates from the same CF patient, often taken at the same time, exhibit different phenotypes with respect to pyocyanin production, suggesting that there is a heterogeneous population of the LES during infection of CF patients. LES isolates lacking the HV phenotype often contain different frame-shift mutations in *lasR*. However, the HV phenotype can persist for many years during infection of a CF patient (>7 years).
Genomic islands and/or enhanced virulence characteristics may have contributed to the successful spread of the LES throughout the CF population of the UK.
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72 Diagnosis of *Pseudomonas aeruginosa* infection using microbial, molecular-biology and serology techniques

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Aims: Early detection of *P. aeruginosa* (Pa) essentially influences morbidity and mortality of CF patients. This work resumes and compares results of three different Pa detection techniques.
Methods: Sputum samples of 209 CF patients were tested using microbial techniques. In 192 PCR positivity for Pa was tested. Specific anti-Pa antibodies were investigated in 180 patients using the ELISA method. Phi coefficient and McNemar test were used for comparison of different techniques.
Results: Microbial cultivation positivity for Pa strongly correlated with PCR positivity ($Phi = 0.65$, $P < 0.001$). Positive result was more often seen using PCR technique ($p = 0.029$). The most different results were found in patients Pa intermittently infected (41.4%) and those infected with *B. cepacia* (19.1%). Antibody production against Pa correlates with both microbiology ($Phi = 0.497$) and PCR ($Phi = 0.52$) methods. Patients without any previous reported history of Pa infection were both PCR and microbial Pa negative, and had negative or borderline titres of antibodies production. Only patients with positive Pa history had also positive antibody titres.
Conclusions: Neither microbial cultivation, nor molecular-genetic (PCR) techniques are 100% reliable. Only combination of these techniques and addition of serology could facilitate therapeutic decision making. We always have to consider *P. aeruginosa* infection when patients produce antibodies. However, negative antibody titres (especially in young cases) do not exclude infection.
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