Volatile organic compound analysis in the early detection of Pseudomonas aeruginosa in cystic fibrosis

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Background: Infection with Pseudomonas aeruginosa (PA) is a major cause of morbidity and mortality in patients with cystic fibrosis (CF). We have investigated emission of volatile organic compounds (VOCs) from sputum, saliva and bacterial cultures in order to identify VOCs associated with PA.

Methods: Fifteen samples of sputum and saliva from stable patients with CF were studied; 11 had PA. Sputum and saliva samples, 0.2–1 mL, were decanted in to septum topped headspace vials and analysed on the day of collection. VOC content was measured using solid phase micro-extraction fibres (SPME) and gas chromatography mass spectrometry (GCMS), following incubation for 30 mins at 60°C and SPME headspace extraction for 20 mins. Sputum samples were also cultured on blood agar for 3 days. Following incubation, samples were allowed to equilibrate at 22°C for 1 h prior to headspace SPME extraction for 1 h. VOCs were assigned an identification using Perkin Elmer Turbomass proprietary software and NIST 2005 mass spectral database.

Results: VOCs from sputum of patients with PA were similar to those of patients without, except for 2-Butanone which was present in 9/11 PA patients and 1/4 patients without PA. VOCs emitted from culture and saliva samples were similar, irrespective of the presence of PA.

Discussion: The point of care recognition of PA in the sputum of patients with CF has the potential to influence clinical care. Our initial results suggest that 2-butanone would yield a sensitivity of 82% and a specificity of 75% for PA. This marker may be identifiable from fresh samples using a sensor device. This small study needs further work.

Supported by: A grant from the David Telling Charitable Trust.

Early detection of Pseudomonas aeruginosa (PA) in patients with cystic fibrosis (CF) by real-time PCR (RT-PCR): original method developed for a preliminary study

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Background: Early detection of PA, the most threatening pathogen in CF patients, is crucial. To date, there is no agreement about the usefulness of PCR in routine probably due to the lack of cohort studies.

Objective: To establish an optimised protocol for the accurate molecular detection of PA directly from CF sputum.

Methods: The reliability of four widely used DNA targets (oprl, oprl, ecfX and rrl) was evaluated: 1) On a set of 30 PA isolates, and on 14 different Pseudomonaceae strains (except PA) commonly found in CF patients 2) On 154 sputum samples from 95 CF patients.

Results: The oprl PCR showed the best specificity (100%) and sensitivity (100%). RT-PCR produced a linear quantitative detection range of 8 logs (r = 0.99857), with a lower detection limit of 10 colony-forming units (CFU)/mL (Ct=38.6±1.33). The standardized Ct and fluorescence of the DICO Extra r-gene detected inhibitors for 18.5% of clinical samples.

Discussion: It is the first time that DICO Extra r-gene has been used as an internal control for the PA molecular detection in CF sputum. This universal control prevents defaults in extraction process and provides accurate detection of inhibitors. With a lower detection limit of 10 CFU/mL as opposed to 100 CFU/mL which is usually found with culture, this study encourages the use of molecular screening for PA in CF patients.

Prospects: The development of this original protocol was the prerequisite of a multicenter prospective study, which began in September 2008 and is running for 3 years in 3 centres (CRCM Roscoff, CRCM Nantes, and CHU Brest) specialized in the management of CF patients (n = 310).

Supported by: Vaincre la Mucoviscidose, DIRC Grand Ouest.