Role of Real time PCR and Galactomannan in the classification of Aspergillus disease in CF

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Introduction
Adults with cystic fibrosis (CF) demonstrate a wide spectrum of immunological responses to Aspergillus ranging from simple sensitisation (65%) to allergic bronchopulmonary aspergillosis (ABPA) (15%). Standard sputum culture identifies 12-57% colonised with Aspergillus. Consensus diagnostic criteria have been developed to identify those with ABPA and antifungal treatment is routine. However, there are no criteria to classify those who do not have ABPA and it is unknown whether these patients would similarly benefit from antifungal treatment. More reliable methods to detect and monitor Aspergillus in respiratory secretions and criteria to classify those without ABPA are needed.

Aim
This study aimed to validate two new methods to detect Aspergillus in CF sputum (real time PCR and galactomannan (GM) antigen) and then interpret these tests with standard serological analysis to identify patients groups that may benefit from antifungal therapy.

Materials
146 adult CF patients were recruited from the Manchester Adult CF Unit. Each patient provided:
- a fresh sputum sample (30 patients provided 2 samples over 9 months)
- a blood sample for Aspergillus serology
- clinical details were collected from case notes

Sputum samples were homogenized with Sputasol (Oxoid Ltd, UK) and sonication (Sonic 2000C) at 120 seconds at amplitude of 295µm.

- 10µL of homogenized sputum was used for real-time PCR.
- A sample for Aspergillus serology was collected.

Results 1: Real time PCR
- 39 of the 146 (37%) sputum samples grew A. fumigatus on SAB agar. 2 samples additionally grew A. flavus.
- 108 of the 146 (74%) sputum samples were PCR positive for Aspergillus spp.
- 16 patients were on azole(s), 7 of whom were PCR positive.
- Reproducibility of PCR
  - Within a sample (n = 30):
    - 15 negative remained negative (Ct >38).
    - 15 positive remained positive (<1 Ct cycle variability).
  - Over 9 months (n = 30):
    - 18 positive remained positive. 15 of which had a reduction in Ct value (rise in DNA) over the 9 months (mean 3.6 cycles).

Results 2: Galactomannan
- 68 of the 146 sputum samples were galactomannan positive.
- 66 of the 108 positive PCR samples were GM positive.

Results 3: Latent Class Analysis
- Prior to latent class analysis patients on azoles (16) were excluded: n = 130

Results 4: Clinical Characteristics
- Lung function:
  - Class 1 had a significantly slower decline in FEV1 over 2 years compared to other classes (ANOVA: F (1, 126) = 7.64, p = 0.007).

Conclusions
- Real time PCR is more sensitive than standard culture for identifying CF patients with Aspergillus in their sputum.
- Both real time PCR and GM demonstrate good reproducibility in CF sputum and may be useful to monitor treatment response.
- Latent class statistical analysis suggests the presence of 4 distinct patient groups based on PCR, GM and serological data.
- A clinical trial of antifungal treatment is needed to determine clinical response of classes and the value of PCR/GM in monitoring disease activity.

References


