

Use of Real time PCR and Specific IgG in Identifying CF Patients with *Aspergillus* Colonisation

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Introduction

Aspergillus fumigatus causes significant morbidity in cystic fibrosis (CF). Adults with CF demonstrate a wide spectrum of immunopathological responses to *A. fumigatus* ranging from simple atopy (60%) to allergic bronchopulmonary aspergillosis (ABPA) (15%). Accurate diagnosis of ABPA allows effective antifungal therapy. It is not known whether antifungal therapy would also benefit patients colonized with *Aspergillus*. No link has yet been found between *A. fumigatus* colonization and development of ABPA or sensitisation. However, sputum culture can be insensitive due to sampling error and culture conditions as demonstrated by the variability in prevalence reporting (12-57%).

Aim

To accurately identify patients with *Aspergillus* colonisation, using Real time PCR, and examine the relationship to markers of *A. fumigatus* sensitisation and infection.

Method

104 patients were recruited from the Manchester Adult CF Unit. Each patient provided:

- a fresh sputum sample
- a blood sample for *Aspergillus* serology
- and underwent fungal skin prick tests (SPT).

Sputum samples were homogenized with Sputasol (Oxoid Ltd, UK). 10µL of homogenized sputum was plated onto Sabouraud Dextrose (SAB) agar (Oxoid, UK) and cultured at 30, 37 and 45 C for 72 hours. Positive cultures were identified by microscopy. The remainder of the sample was used for DNA extraction. DNA was extracted using a commercial DNA extraction kit MycXtra™ (Myconostica Ltd, UK). Real time PCR to detect *Aspergillus* DNA was performed using a CE marked commercial assay, MycAssay™ *Aspergillus* (Myconostica Ltd).

Figure 1 MycAssay™ *Aspergillus*



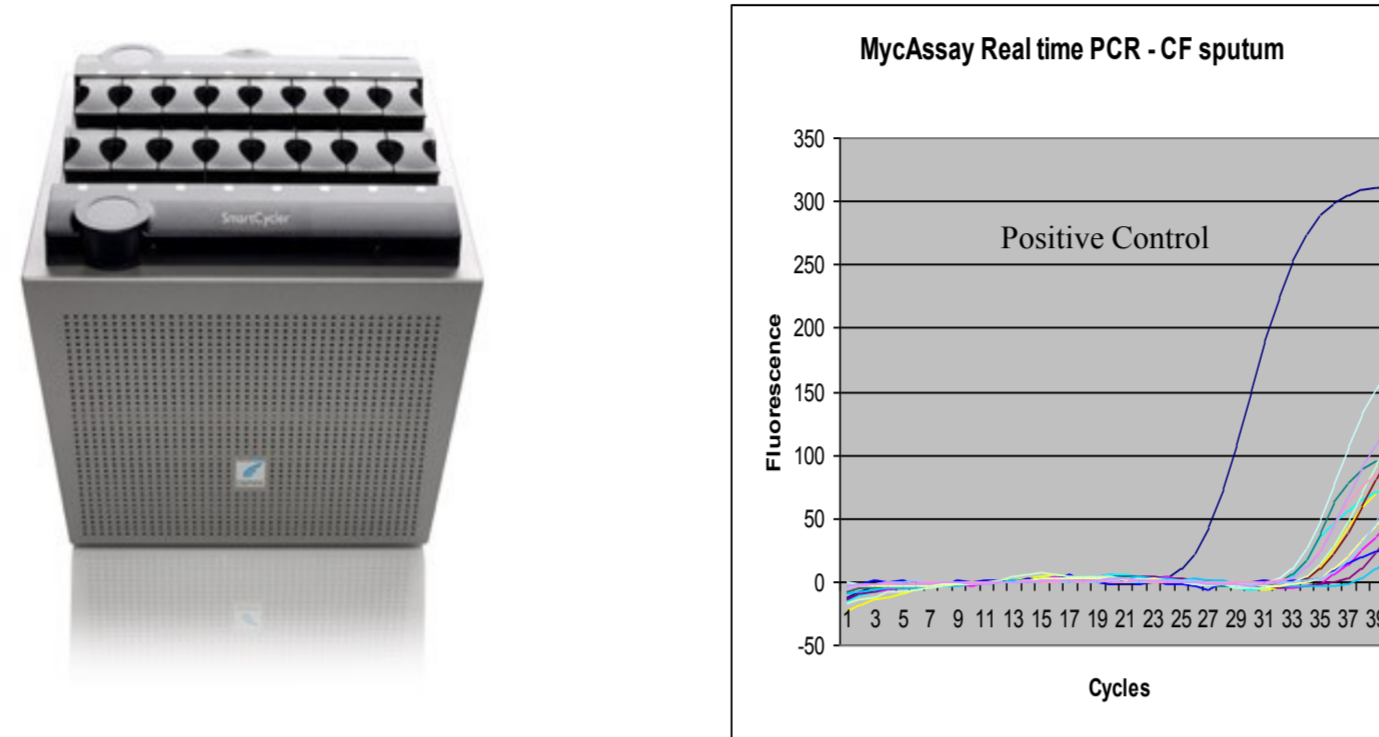
This assay utilizes molecular beacons targeting the 18S ribosomal unit of *Aspergillus* spp. Real time PCR was performed on a SmartCycler® (Cepheid, USA) platform.

Serological tests included:

- total IgE
- specific IgE *A. fumigatus*
- specific IgG *A. fumigatus* (Phadia ImmunoCAP® assays)

- *A. fumigatus* precipitins performed using counterimmunoelectrophoresis.

Figure 2 Smartcycler® Real time PCR machine



PCR results were compared to serology in order to identify any correlations.

Results 1 Real time PCR

- 33 of the 104 (32%) sputum samples grew *A. fumigatus* on SAB agar. 2 samples also grew *A. flavus* species and 2 grew *Penicillium* species.
- 75 of the 104 (72%) sputum samples were PCR positive for *Aspergillus* spp.

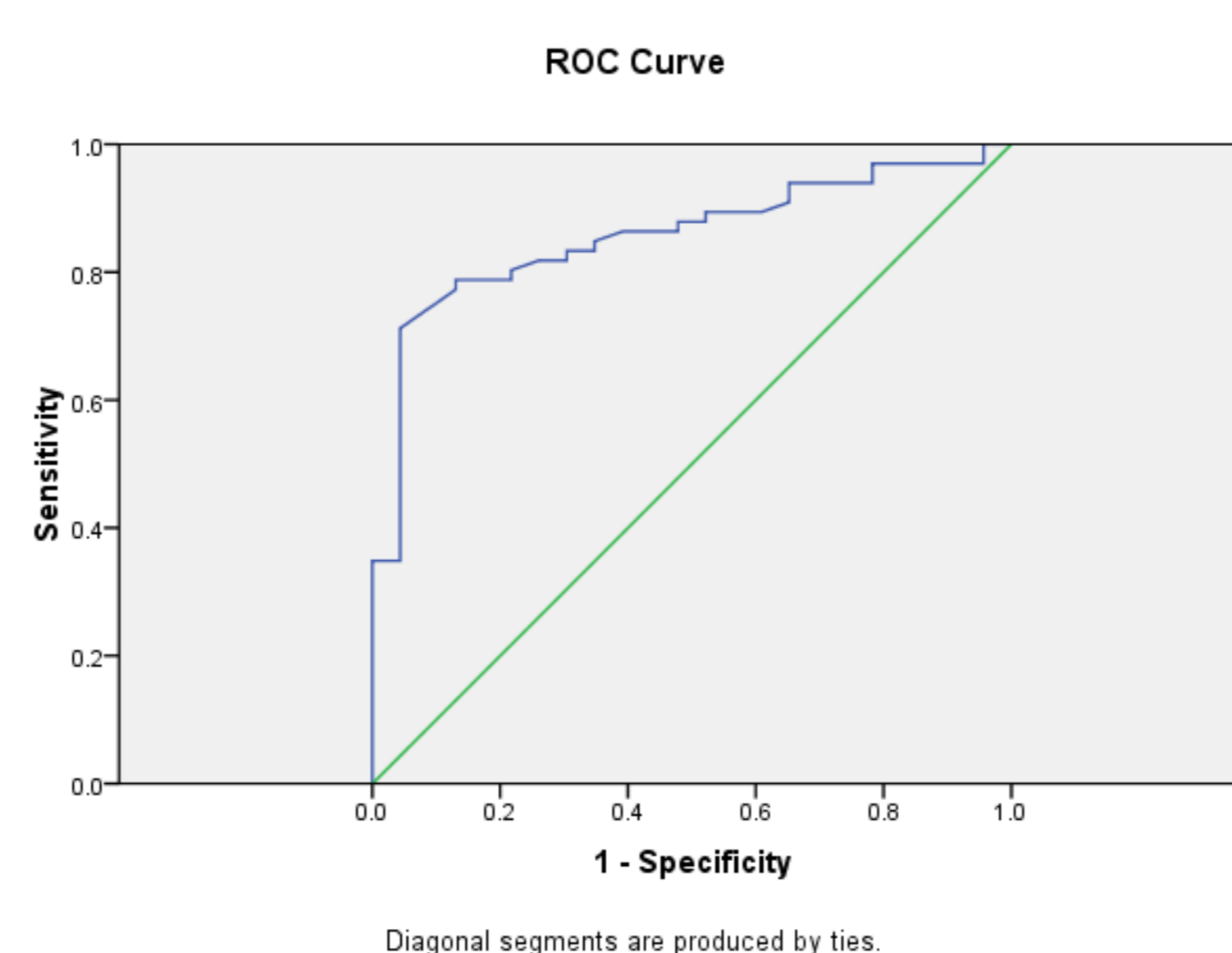
	PCR Positive	PCR Negative
Culture Positive	33	0
Culture Negative	42	29

- 15 patients were noted to be on azole antifungals, 9 of these were PCR positive.
- 20 patients met the minimal diagnostic criteria for ABPA (consensus conference 2003). 16 were PCR positive. 8 of the 16 were on azole antifungals. 4 were PCR negative but all 4 were taking azole antifungals.

Results 2 Real time PCR and Specific IgG

- For the purposes of specific Asp IgG evaluation patients on antifungal treatment (n = 15) were excluded from the data analysis.
- 88% of PCR positive samples had a positive specific IgG titre (>40mg/L).
- 48% of PCR negative samples had a positive IgG titre.
- PCR positive patients had a significantly greater mean specific IgG (PCR positive 84mg/L versus PCR negative 40mg/L p= <0.01).
- Using a Receiver Operator Curve (ROC), the area under the curve was 0.857. An IgG level of above 65mg/L gives 74% sensitivity and 91% specificity for positive PCR.

Figure 4 ROC curve - IgG as a predictor of positive PCR



Results 3 Serology and PCR

Patients on antifungal treatment were excluded. Patients were categorized as having:

- 1) ABPA - 2003 CF consensus conference criteria – minimum diagnostic criteria
- 2) Sensitisation – Specific IgE ≥ class 2 and IgG ≥ 65 mg/L but not reaching ABPA criteria
- 3) Atopic – ≥ Class 2 specific IgE *A. fumigatus* or positive SPT, IgG <65mg/L
- 4) IgG rise > 65mg/L, IgE < class 2
- 5) Control - no evidence of sensitisation or infection

• Positive PCR patients (n=67):

- 8 ABPA
- 9 Sensitised
- 10 Atopic
- 31 IgG rise (infection)
- 9 Controls

27 (40%) of PCR positive patients have serological sensitisation.

31 (46%) of PCR positive patients have serological infection without sensitisation

• Negative PCR patients (n= 23):

- 0 ABPA
- 0 Sensitised
- 6 Atopic
- 0 IgG rise (infection)
- 17 controls

74% of PCR negative patients have no evidence of serological sensitisation.

Conclusions

- Real time PCR can be used to identify patients colonised or infected with *Aspergillus*, including those in whom antifungal therapy is inadequate.
- A specific IgG above 65 mg/L gives the optimal sensitivity and specificity to determine positive *Aspergillus* PCR.
- All patients who are not on azole therapy, with ABPA and *Aspergillus* sensitisation or infection (IgE and/or IgG positive) are PCR positive.
- A large number of PCR positive patients have a lone rise in IgG. These patients may represent infected patients, and ‘*Aspergillus* bronchitis’ is the most likely diagnosis.

References

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