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 sensitization. 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 MyxEx™ (Myconostica Ltd, UK). Real time PCR to detect Aspergillus DNA was performed using a CE marked commercial assay, MycAssay™ Aspergillus (Myconostica Ltd).

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

Seraological tests included:
- total IgE
- specific IgE, A. fumigatus
- specific IgG, A. fumigatus
- Phadia ImmunoCAP® assays
- A. fumigatus precipitin performed using counterimmunoelectrophoresis.

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.
- 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.

Results 3 Serology and PCR
Patients on antifungal treatment were excluded.
1) ABPA - 2003 CF consensus conference criteria – minimum diagnostic criteria
2) Sensitisation – Specific IgG ≥ class 2 and IgG ≥ 65mg/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

Contact Details
caroline.baxter@manchester.ac.uk