187 Nitric oxide production by polymorphonuclear leukocytes in sputum from cystic fibrosis patients with chronic lung infection

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Objective: Chronic *Pseudomonas aeruginosa* lung infection in CF patients is characterized by persisting mucoid biofilms in hypoxic endobronchial mucus. These biofilms are surrounded by numerous polymorphonuclear leukocytes (PMNs), which are the major consumers of O_2 due to production of O_2^-. In this study, we hypothesized that nitric oxide (NO) is also produced by the NO synthases (NOS) in the O_2-consuming PMNs in the endobronchial mucus.
Methods and Results: This hypothesis was tested on fresh expectorated sputum from chronically infected CF patients by quantifying and visualizing the NO production. A positive correlation was observed between the concentration of PMNs and stable endproducts of NO production in CF sputum; NO_3^- ($p < 0.04$, r: 0.66, $n = 10$) and NO_2^- ($p < 0.006$, r: 0.78, $n = 11$) using the Griess Reagent System, indicating a production of NO by PMNs. PMNs stained with the NO-indicator, DAF-FM, could be identified by fluorescence microscopy. In addition, microsensor analysis revealed the presence of discrete occurrences of NO. To further verify NO production by active NOS in the PMNs, we are presently estimating O_2 consumption and DAF-FM fluorescence during inhibition of NOS in CF sputum.
Conclusions: The present study suggests that besides consumption of O_2 for production of reactive oxygen species, the PMNs in CF sputum can also consume O_2 for reactive nitrogen species, i.e. NO.

188 A comparison of the precipitation technique and ImmunoCAP® FIEA for measurement of IgG antibodies to *Aspergillus*

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Purpose: *Aspergillus* precipitins aid diagnosis and monitor treatment of aspergillosis. Phadia's ImmunoCAP® fluorescent immunoenzyme assay (FIEA) is a new fast, automated method to detect IgG antibodies to *Aspergillus fumigatus*. This assay has not been validated in individual patient groups and the clinical significance of levels is not known. This study aimed to compare the ImmunoCAP® IgG with *Aspergillus* precipitins in patients with cystic fibrosis (CF).
Method: Serum from 100 adult CF patients was analyzed by the ImmunoCAP® assay for specific IgG *A. fumigatus*, and by counterimmunoelectrophoresis for *A. fumigatus* precipitating antibodies. Phadia recommends an ImmunoCAP® result >40 mg/L as positive. The precipitin titre was recorded. For clinical analysis, patients were grouped into those with ABPA (2003 consensus criteria), sensitisation ($\geqslant$ class 2 specific IgE to *A. fumigatus*) and controls (no evidence of sensitisation).
Results: There was 50% concordance between a positive IgG result and a positive precipitin result. Non concordance in 38% was due to a positive IgG but negative precipitin. A ROC curve, with an area under the curve of 0.593, demonstrated that raising the cut-off level for a positive IgG result did not improve concordance. There was no correlation between IgG level and precipitin titre ($r = 0.199$). Neither precipitin titre nor IgG level correlated with disease group. There was no significant difference in the mean IgG between controls and ABPA patients ($p = 0.154$).
Conclusion: In CF there is little correlation between the ImmunoCAP® assay and precipitating antibodies. Neither the precipitin test nor the ImmunoCAP® IgG is a good predictor of disease in CF.

189 Should immunoglobulins (Igs) be measured in the CF Annual Review?

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The UK CF Trust recommends the routine measurement of serum Igs as part of the Annual Review. We assessed the usefulness of these tests in our clinic population. The Annual Screen results from the preceding 12 months of our adult CF patients ($n = 253$) were reviewed. All patients who had a full Ig panel (IgG, IgA, IgM and serum electrophoresis) with contemporaneous white cell count were included. 189 patients (75%) had a complete Ig panel available for analysis. The results are shown in Table 1.

Table 1

	IgG	IgA	IgM
Normal	159 (84%)	106 (56%)	134 (71%)
Reduced	4 (2%)	8 (4%)	33 (17%)
reduced >10%	1 (0.5%)	7 (3.7%)	23 (12%)
Raised	26 (14%)	75 (40%)	22 (12%)
raised >10%	9 (5%)	66 (35%)	20 (11%)

20 patients had a polyclonal increase on electrophoresis, but no monoclonal bands were seen. A raised IgA correlated well with a raised white cell count (WCC) ($r = 0.175$, $p = 0.02$), but there was no significant correlation between raised WCC and IgG or IgM.Only one patient had a reduction in more than one Ig class, and this did not have a clinical effect.
Abnormal Ig results are often seen in the CF Annual Review. Many of these are likely to be due to an acute phase response in patients who are exacerbating. Significant immunodeficiency has not been identified by the Annual Screen process, and no significant management alterations have been made as a result of routinely measuring Ig levels. An Ig panel plus electrophoresis costs £18.19 per test.
Routine measurement of Igs as part of the CF Annual Review is not indicated. By discontinuing this, we can save up to £4600 (€5155) per year.

190 The effect of systemic antibiotics, leukotriene receptor antagonist montelukast and azithromycin on Th1/Th2 cytokine levels in cystic fibrosis patients

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Objectives: Recurrent respiratory infections trigger the chronic inflammatory process in cystic fibrosis (CF). The immune response to chronic *Pseudomonas aeruginosa* lung infection in CF is predominantly of the Th2 type. The aim of this study is to determine the long-term (6 months) effects of montelukast and azithromycin and the effect of systemic antibiotics for 3 weeks during acute pulmonary exacerbations on Th1/Th2 cytokine levels in children with CF prospectively.
Material and Methods: CF patients ($n = 29$) (8.66 ± 3.55 years) were chosen as study group and patients with non-CF bronchiechtasis ($n = 10$) (12.71 ± 2.92 years) as control group. Study group received montelukast ($n = 12$), azithromycin ($n = 9$) for 6 months as prophylaxis and systemic antibiotics for acute respiratory exacerbations ($n = 8$) for 21 days. The Th1/Th2 cytokines (IL-2, IL-4, IL-5, TNF-α, IFN-γ, IL-10) were measured by Cytometric Bead Array (BD Biosciences, USA) on flow cytometry in serum of all patients and in sputum of patients with acute respiratory exacerbations.
Results: The montelukast and azithromycin prophylaxis during 6 months period prevented the acute respiratory exacerbations, increased the Shwachman Clinical Scores without adverse effect were observed. There were no statistically significant differences in pre- and post-treatment cytokine levels in serum samples of CF patients who received montelukast or azithromycin for prophylaxis and in sputum samples of CF patients with exacerbations.
Conclusion: In spite of no statistically significant differences in serum and sputum Th1/Th2 cytokine levels, montelukast and azithromycin for prophylaxis and sytemic antibiotics for treatment cause clinical improvement in CF children.