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Original Article

Inflammatory and immunological biomarkers are not related to survival in adults with Cystic Fibrosis

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Abstract

Background: Chronic *Pseudomonas aeruginosa* pulmonary infection is associated with a decline in lung function and reduced survival in people with Cystic Fibrosis (CF). Damaging inflammatory and immunological mediators released in the lungs can be used as markers of chronic infection, inflammation and lung tissue damage.
Methods: Clinical samples were collected from CF patients and healthy controls. Serum IgG and IgA anti-*Pseudomonas* antibodies, sputum IL-8 and TNFα, plasma IL-6 and urine TNFr1 were measured by ELISA. Sputum neutrophil elastase (NE), cathepsin S and cathepsin B were measured by spectrophotometric and fluorogenic assays. The relationship between IgG and IgA, inflammatory mediators and long-term survival was determined.
Results: IgG and IL-6 positively correlated with mortality. However, multivariate analysis demonstrated that after adjusting for FEV_1, IgG was not independently related to mortality. A relationship was observed between IgG and IL-6, TNFα, TNFr1 and between IgA and IL8, cathepsin S and cathepsin B.
Conclusions: These data indicate that biomarkers of inflammation are not independent predictors of survival in people with CF.

Keywords: Antibody titre; Inflammation; Proteases; *Pseudomonas aeruginosa*; Cystic Fibrosis; Mortality

1. Introduction

Chronic *Pseudomonas aeruginosa* pulmonary infection is associated with a decline in lung function, increased exacerbations and reduced survival in people with CF. In the lungs, chronic infection is accompanied by airway inflammation, resulting in the release of damaging inflammatory and immunological mediators from a number of sources including the airway epithelium and circulating neutrophils and macrophages [1]. Such mediators, including neutrophil elastase (NE), IL-8 and TNFα, correlate with disease severity in diverse populations of CF patients [2–4] and can be used as markers of chronic infection, inflammation and lung tissue damage.

The initial humoral response to bacterial challenge in the lungs is the production of secretory IgA (sIgA) by plasma cells in the pulmonary mucosa, followed by the systemic release of IgG. Antibody response to *P. aeruginosa* in CF can be used as a biomarker and lung inflammation and tissue damage are related to antibody titres [5–8]. Specific antibodies against *P. aeruginosa* antigens increase when chronic infection is established and a high number of anti-*Pseudomonas* precipitins have been shown to be related to poor prognosis [5,9]. Despite increased anti-pseudomonal antibodies, the humoral response does not confer protective immunity against this pathogen in the CF lung [10].

In some studies, increased antibody titre against *P. aeruginosa* precedes isolation of the pathogen from the respiratory tract and

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serological studies demonstrate a higher incidence of infection than that of microbial culture [11,12]. However, this pattern has not been consistently demonstrated and this may be due to variation in sample collection, assay methods and microbiological facilities available [13]. Furthermore, study results may be further confounded by not all patients showing an early antibody response [14]. Therefore, serological and molecular techniques may not be particularly helpful in detection of early or intermittent colonisation. To date, there has been little agreement regarding the clinical value of measuring serum antibody response against pathogens such as *P. aeruginosa* [15], particularly in relation to CF children [16]. Anti-pseudomonal IgG titre may have potential diagnostic value and can be used as a prognostic tool to identify CF patients at risk of establishing a chronic infection [17,18]. For many years this method has been used diagnostically in the Danish CF centre in Copenhagen to distinguish between intermittently colonised and chronically infected patients [19].

The primary aim of this study was to determine whether serum IgG and IgA titre against *P. aeruginosa* in clinically stable, chronically infected adult CF patients correlated with mortality and the production of host inflammatory mediators. We hypothesised that IgG antibodies to *P. aeruginosa* would predict long-term survival.

2. Methods

2.1. Patients and controls

Two separate cohorts of clinically stable CF patients were recruited at the Manchester and Belfast Adult CF centres [3,20]. Manchester patients were recruited over a 6-month period between July and December 2001, and Belfast patients during the year 2000. Data and clinical samples were collected from 63 CF patients attending the Belfast Centre (mean age 27.7 ± 8.9 years, 37M, 26F) and from 118 age-matched healthy controls [19]. Patients from Manchester (n = 40, mean age 27.7 ± 8.3 years, 20M, 20F) were subdivided into two groups based on the *P. aeruginosa* strain type causing infection: those infected with sporadic strains and those infected with a transmissible (*P. aeruginosa* strain, MA) strain. Of these 103 CF patients, 36 had no *P. aeruginosa* infection and 67 were chronically infected with *P. aeruginosa* for at least 12 months prior to recruitment. Both studies had ethical approval and patients and controls gave written informed consent. Demographic and clinical data was recorded for all CF patient cohorts at baseline including age, sex, CF genotype, %FEV_1, %FVC, white cell count (WCC), C-reactive protein (CRP) and body mass index (BMI) (Table 1). Mortality data was retrospectively obtained in 2013 and the time in months until death or lung transplantation used for subsequent analysis.

2.2. Biochemical measurements

Biochemical analysis of samples (sputum, urine, serum, plasma) from these patients was performed as part of two previously reported studies [3,4]. Sputum IL-8 and TNF-α, plasma IL-6 and urine TNFr1 were measured by ELISA. Sputum NE, cathepsin S and cathepsin B were measured by spectrophotometric and fluorogenic assays.

Table 1
Demographics of Cystic Fibrosis groups.

	Manchester	Belfast	Combined
Sex, male (female)	20 (20)	37 (26)	57 (46)
CF genotype, no. (%)			
F508/F508	23 (57.5)	20(31.7)	43 (41.7)
F508/other	14 (35)	24 (38.1)	38 (36.9)
Other/other	3 (7.5)	19 (30.2)	22 (21.4)
Age, y, mean (SD)	27.9 (8.5)	27.7 (8.9)	27.8 (8.7)
% FEV_1, mean (SD)	44.1 (18.69)	68.0 (24.99)	58.77 (25.5)
BMI, mean (SD)	20.42 (2.71)	22.47 (4.34)	21.74 (3.94)

2.3. Comparison of anti-pseudomonal immunological response with survival

Serum samples which had been stored at −80 °C since collection were assayed by ELISA for IgG and IgA antibodies against *P. aeruginosa* antigens. Micro-titre plates (96-well) were coated overnight with antigenic preparation from St-Ag: 1–17 (Statens Serum Institute). This antigenic preparation has been shown to have a high sensitivity of 97% [21]. Following blocking, diluted serum was added to each well and incubated for 2 h at room temperature. Horseradish peroxidase conjugated anti-human IgG or IgA (DAKO Co. Golstrup, Denmark) was added to each well. Optical Density was measured at 450 nM and the titre was arbitrarily defined as ELISA units (EU) from a standard curve developed using *P. aeruginosa* positive serum (Statens Serum Institute). Samples were assayed in duplicate and data presented as mean ± SD. The relationship between IgG and IgA and both inflammatory mediators and survival was then determined.

2.3. Statistical analysis

Where necessary, results were log transformed to achieve approximate normal distribution of data. A Pearson's correlation coefficient was used to measure the strength of the linear association between antibody titres, demographic/clinical parameters and sputum inflammatory biomarkers. Immunological response in control subjects, CF patients chronically infected with *P. aeruginosa* and *P. aeruginosa* negative CF patients were analysed using one-way ANOVA with post-hoc testing. The relationship between IgG and long-term survival was determined using Cox proportional hazard modelling to calculate hazard ratios (and 95% confidence intervals) and allowed adjustment for individual level confounders (age, gender, lung function and BMI). Hazard ratios (and 95% confidence intervals) were also calculated for the association between sputum inflammatory biomarkers and survival. Statistical analysis was performed with the SPSS (SPSS Version 19, Chicago, Illinois, USA) software package.