Eur Respir J 2009; 34: 1079–1085
DOI: 10.1183/09031936.00006009

Prevalence and clinical significance of auto-antibodies in adults with cystic fibrosis

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ABSTRACT: The aim of this study was to determine the prevalence of different auto-antibodies in adult, French cystic fibrosis (CF) patients and to look for a correlation between autoimmunity, patient characteristics and survival.

The sera of 144 patients were screened for a wide range of antibodies. Clinical, biological and bacteriological characteristics and the cystic fibrosis transmembrane conductance regulator genotype were recorded and progression of lung disease was examined.

113 (78.5%) patients displayed one or several auto-antibodies, predominantly immunoglobulin (Ig)A anti-*Saccharomyces cerevisiae* antibodies (ASCA; 43.7%) and antineutrophil cytoplasmic antibodies (ANCA; 40%), of which 59% showed bactericidal/permeability-increasing protein (BPI) specificity. The presence of BPI-ANCA was associated with the number of antibiotic courses, low body mass index, *Pseudomonas aeruginosa* colonisation, the presence of resistant *P. aeruginosa*, low forced expiratory volume in 1 s, CF-related liver disease, hypergammaglobulinaemia, male sex and inflammatory syndrome. The presence of ASCA-IgA was correlated with male sex and hypergammaglobulinaemia. 41 patients presented with chronic respiratory failure and/or requested lung transplantation or died during follow-up. These events were more frequent in patients with BPI-ANCA or ASCA-IgA.

These findings confirm the high frequency of auto-antibodies in CF, particularly BPI-ANCA and ASCA-IgA, and the link between BPI-ANCA, severity of lung disease and CF prognosis.

KEYWORDS: Antineutrophil cytoplasmic antibodies, autoimmune diseases, bactericidal/permeability-increasing protein, cystic fibrosis, inflammation, *Pseudomonas aeruginosa*

Cystic fibrosis (CF) is the most common recessively inherited disease in Caucasians. The main cause of morbidity and mortality is progressive deterioration of pulmonary function, resulting from a long sequence of bronchial inflammation and infection, and particularly from chronic infection with *Pseudomonas aeruginosa* [1]. The systemic inflammation resulting from the chronic airway infection may contribute to CF-related diabetes mellitus, CF-related bone disease, CF-related arthropathy and vasculitis [2].

Several studies have underlined the high frequency of antineutrophil cytoplasmic antibodies (ANCA) in CF, especially those with anti-bactericidal/permeability-increasing protein (BPI) specificity (BPI-ANCA), and their link with pulmonary infections and poor respiratory function [3–7]. Nevertheless, the pathological significance of these auto-antibodies remains to be determined: whether they are specifically involved in the pathogenesis of lung disease or whether they only represent a marker of poor pulmonary condition remains unknown.

Other antibodies, such as anti-*Saccharomyces cerevisiae* antibodies (ASCA), rheumatoid factor (RF) and antinuclear antibodies (ANA), known as antibodies with specific clinical expression, have selectively been described [8–11]. The clinical significance of the presence of such antibodies in CF and their link with disease severity is not yet established. These antibodies could be considered as markers of a particular subgroup of patients with a specific differential inflammatory profile and prognosis, but their importance has not been previously studied.

In this single-centre study, we analysed the prevalence of a wide range of auto-antibodies in a large population of adult, French CF patients. We particularly looked for organ-specific auto-antibodies directed against organs involved in CF and for antibodies related to systemic autoimmune diseases. The aim of this study was to determine the prevalence of these different antibodies and to look for a correlation between autoimmunity and patients' phenotype, genotype and survival.

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Received:
Jan 14 2009
Accepted after revision:
April 16 2009
First published online:
May 14 2009

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

PATIENTS AND METHODS

Patients

All patients aged >16 yrs attending the adult CF centre in Lyon, France, between January 2000 and January 2007 were considered for the study. The diagnosis of CF had been established on typical clinical features, increased sweat chloride concentrations and detection of CF-inducing mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [12]. Pregnant females and patients who had previously undergone lung or liver transplantation were excluded. Informed consent was obtained from all patients for the preparation of a sera collection. 144 patients were included, which represents 85% of the total number of patients who attended our centre during this period. A control population of 92 blood donors (mean age 40 ± 19 yrs) was tested for the presence of ASCA. Use of the sera for systematic screening of antibodies was approved by the Ethical Committee of the Hospices Civils de Lyon (Lyon, France).

Auto-antibodies

Serum samples were obtained from 144 clinically stable patients during a routine visit to the CF centre and stored at -80°C until assayed. All samples were systematically screened for a wide range of antibodies. ANA were detected with an indirect immunofluorescence technique (IIF) using HEp2 cells (Bio-Rad, Mames La Coquette, France). Auto-antibodies against dsDNA were searched in ANA-positive (>1/80) samples using a radioimmunological test (Dade Behring, Paris, France) and extractable nuclear antigen (ENA) auto-antibodies using an ELISA (Bio-Rad). Anti-cyclic citrullinated peptide antibodies (anti-CCP) and immunoglobulin (Ig)M and IgA RF were detected with a commercial CCP2 ELISA (Inova, Menarini, France) and a homemade ELISA, respectively. The titre was considered positive when $\geqslant$25 IU·mL^{-1} for anti-CCP and >1.3 AU·mL^{-1} for RF. Anti-actin, anti-liver-kidney-microsomal (LKM1) and anti-mitochondrial (M2) auto-antibodies were detected using IIF on cryostat mouse stomach and kidney sections (BMD, Mame La Vallée, France). The cut-off level was 1/50. IgA anti-tissue transglutaminase auto-antibodies, IgA and IgG anti-gliadin (AGA) antibodies, and anti-endomysial antibodies were detected by ELISA (Phadia D-tek, Saint-Quentin en Yvelines, France) and IIF on cryostat monkey oesophagus sections (Bio-Rad), respectively. The AGA titre was positive when >50 IU·mL^{-1}. ANCA were first screened by IIF on human neutrophils fixed in ethanol (Inova). The pattern of fluorescence was identified as cytoplasmic (c)-ANCA, perinuclear (p)-ANCA or atypical p-ANCA. The specificity of ANCA, *i.e.* proteinase-3, lactoferrin, myeloperoxidase, elastase, cathepsin G and BPI, was analysed by ELISA (Bio Advance, Bussy Sa, France). The titre was considered positive $\geqslant$1 AU·mL^{-1}. ASCA-IgA and -IgG were measured by ELISA (Inova). The cut-off level was 25 IU·mL^{-1} for both IgA and IgG isotypes. Anti-glutamate decarboxylase (GAD) and anti-islet antigen-2 (IA-2A) auto-antibodies were detected by radioimmunoassay (Cis Biointernational, Bagnols/Céze, France). Anti-thyroperoxidase and anti-thyroglobulin auto-antibodies were investigated by ELISA (Phadia D-tek).

To assess the reproducibility of these immunological tests, analysis was repeated within 3 months for four patients: no variations in terms of presence or absence and concentrations of auto-antibodies were observed. To provide longitudinal data on reproducibility of measurements and to look for prospective changes in the auto-antibody status of the patients, auto-antibody screening was repeated after 1–7 yrs (mean 4.3 yrs) in 20 other patients.

Clinical evaluation

We retrospectively reviewed the medical charts of 144 patients. The following data were collected at the time of blood sampling: sex, age, past medical history of autoimmune or systemic disease, past medical history of drug hypersensitivity, body mass index (BMI), and pulmonary involvement. The CF centre's policy for intravenous courses of antibiotics was to treat patients when suffering from a pulmonary exacerbation (new crackles, increased cough frequency, decrease in forced expiratory volume in 1 s (FEV1) % predicted or haemoptysis) and not routinely. The numbers of *i.v.* courses of antibiotic for pulmonary exacerbation in the previous 12 months were reported. FEV1 % pred and reversibility of airway obstruction with β_2-agonists were recorded. Patients were divided into three groups: FEV1 >75% pred, 75%$\geqslant$FEV1$\geqslant$30% pred and FEV1 <30% pred. Extrapulmonary clinical phenotypes were noted: pancreatic insufficiency, chronic diarrhoea, chronic sinusitis, CF-related diabetes mellitus, arthralgia or arthritis, and liver disease. The criteria used to define diabetes were: fasting plasma glucose level $\geqslant$126 mg·dL^{-1}; post-prandial plasma glucose $\geqslant$200 mg·mL^{-1}; or plasma glucose levels 2 h after glucose load (1.75 g·kg^{-1}) of $\geqslant$200 mg·dL^{-1}, reconfirmed on a subsequent day. Oral glucose tolerance tests (OGTTs) were routinely performed in all patients every 1 or 2 yrs. Patients were considered to have CF-related liver disease when presenting with cirrhosis or portal hypertension, or when disclosing persistent significant elevation of hepatic enzymes.

Laboratory tests

The following parameters were recorded at the time of blood sampling: complete blood count, C-reactive protein, liver enzymes, serum protein electrophoresis with detection of M-component and albuminaemia. All patients were negative for HIV, hepatitis B virus and hepatitis C virus infections.

Microbiological tests

The results of sputum analysis at the time of blood sampling were recorded. Chronic *P. aeruginosa* and *Staphylococcus aureus* bronchial colonisation was considered when three or more sputum cultures were persistently positive over a period of 6 months. The duration of *P. aeruginosa* and *S. aureus* colonisation was noted. Anti-biograms of these two germs were analysed: sensitive, multidrug-resistant (MDR), pan-resistant *P. aeruginosa* and methicillin-sensitive and methicillin-resistant *S. aureus*. We used the Centers for Disease Control and Prevention criteria to define MDR *P. aeruginosa* infection [13]. An MDR *P. aeruginosa* isolate was defined as an organism that was resistant to at least three of the five anti-pseudomonal classes of antimicrobial agents: carbapenems, quinolones, piperacillin-tazobactam or ticarcillin-clavulanic acid, cefepime or ceftazidime, and aminoglycosides. Pan-resistant *P. aeruginosa* was defined as an organism resistant to all antibiotics excluding colistin. The presence of *Burkholderia cepacia* complex and/or *Stenotrophomonas maltophilia* in sputum cultures at

inclusion was recorded, as well as the presence of *Aspergillus fumigatus* precipitins.

Clinical course

The clinical course of patients after the time of blood sampling was analysed. Progressive events, defined as death from any cause, lung transplantation or chronic respiratory insufficiency, were recorded. Chronic respiratory insufficiency was defined by a partial pressure of arterial oxygen <55 mmHg, in clinically stable patients. The end date for analysis was September 1, 2007.

Statistical analysis

The association between major clinical, laboratory and microbiological findings at inclusion and the presence of BPI-ANCA and ASCA-IgA was individually analysed. Differences in percentages were tested using raw frequencies with Fisher's exact test.

The impact of the presence of BPI-ANCA and ASCA-IgA on the occurrence of progressive events was analysed using the log-rank test. Multivariable logistic regression models (including age, sex, presence and duration of *P. aeruginosa* colonisation and presence of BPI-ANCA) were generated to analyse the influence of these parameters on FEV1. A Cox proportional model was used. Progression-free survival was determined using the life-table algorithm according to the Kaplan–Meier method. It was calculated from the date of blood sampling to the date of occurrence of any progressive event. The significance level was $p<0.05$.

RESULTS

Demographic, clinical, biological and microbiological findings

144 patients (71 females and 73 males, sex ratio 1.03) were included in this study. The mean (range) age at inclusion was 25 (17–43) yrs. Clinical features and *CFTR* genotype are summarised in table 1. The baseline serum chemistry values, blood counts and microbiological findings are summarised in table 2.

Autoimmunity and correlation with patient genotype and phenotype

Three (2.1%) patients had documented autoimmune disease at inclusion: histologically proven Crohn's disease (n=1), systemic lupus (n=1) and rheumatoid arthritis associated with autoimmune adrenal insufficiency (n=1).

113 (78.5%) patients displayed one or several auto-antibodies (table 3). IgA-ASCA were present in 43.7% (associated with ASCA-IgG in 21.8%), and in 1% of blood donors. In total, 40% of patients displayed ANCA *versus* 0% in the control group, and BPI specificity was identified for 59% of these ANCA. ANA were identified in 25% of patients. The only patient with positive anti-dsDNA auto-antibodies presented no clinical or biological symptoms of lupus. Anti-CCP auto-antibodies were observed in 7.6% of patients, in association with RF in two cases. Neither of these two patients had arthritis. Rheumatological manifestations were reported in two patients with anti-CCP alone. IgA-AGA antibodies were present in 12.5% of patients, but they had no anti-transglutaminase or anti-endomysial auto-antibodies or clinical symptoms of coeliac disease. Two (1.5%) patients presented with anti-actin auto-antibodies without associated hepatitis, three patients had anti-thyroperoxidase auto-antibodies with normal thyroid function tests and without clinical signs of thyroiditis. Five patients had anti-GAD and/or anti-IA-2A auto-antibodies without diabetes mellitus (systematic OGTTs revealed impaired glucose tolerance in one out of these five patients).

Factors significantly associated with the presence of BPI-ANCA were the number of *i.v.* antibiotic courses for pulmonary exacerbation in the previous year (p=0.001), low BMI (p=0.006), presence of MDR or pan-resistant *P. aeruginosa* (p=0.012), low FEV1 (p=0.012), presence of CF-related liver disease (p=0.012), hypergammaglobulinaemia (p=0.018), male sex (p=0.03), elevated C-reactive protein (p=0.036) and *P. aeruginosa* colonisation (p=0.039). Multivariate analysis confirmed a significant link between low FEV1 and the presence of

TABLE 1 Initial clinical features and cystic fibrosis (CF) genotype of the study patients

Subjects n	144
Age yrs	25±5.9 (17–43)
Sex ratio	1.03
Male	73 (50.7)
Female	71 (49.3)
Past medical history of autoimmune disease	4 (2.8)
Past medical history of drug hypersensitivity	50 (34.7)
***CFTR* gene mutation**	
F508del/F508del	57 (39.6)
Others	87 (60.4)
Pulmonary involvement	143 (99.3)
Chronic exocrine pancreatic insufficiency	134 (93.1)
Chronic sinusitis	78 (54.2)
BMI ⩽19 kg·m^{-2}	67 (46.5)
BMI kg·m^{-2}	19.57±2.8
Chronic diarrhoea	51 (35.4)
CF-related liver disease	46 (31.9)
Diabetes mellitus	34 (23.6)
Arthralgia	13 (9)
Associated with polyarthritis	2 (1.3)
Associated with oligo/monarthritis	3 (2.1)
FEV1 % pred	52.2±21.9
FEV1 >75% pred	23 (16)
75%⩾FEV1⩾30% pred	90 (65.2)
FEV1 <30% pred	31 (21.5)
Reversible airway obstruction with β_2-agonists	37 (25.7)
Antibiotic courses for pulmonary exacerbation in past 12 months#	117 (81.3)
0	27 (18.7)
1	35 (24.3)
2	26 (18.1)
3	36 (25)
⩾4	20 (13.9)

Data are presented as mean±SD (range), n (%) or mean±SD, unless otherwise stated. *CFTR*: CF transmembrane conductance regulator gene; BMI: body mass index; FEV1: forced expiratory volume in 1 s; % pred: % predicted. #: mean number of courses=1.6.

TABLE 2 Initial laboratory findings in the 144 cystic fibrosis patients

Microbiology	
P. aeruginosa colonisation	113/144 (78.5)
Multidrug-resistant Pseudomonas	66/136 (48.5)
Pan-resistant Pseudomonas	8/136 (5.9)
S. aureus colonisation	117/144 (82.5)
Methicillin-resistant *S. aureus*	22/109 (20.2)
Presence of *Burkholderia cepacia* complex and others[#]	20/142 (14.1)
Duration of *P. aeruginosa* colonisation yrs	10.2±5.6
Duration of *S. aureus* colonisation yrs	11.8±6.1
Presence of *Aspergillus fumigatus* precipitins	38/116 (32.8)
Haematology	
Neutrophilia $>8\times10^9$ mm^{-3}	56/138 (40.6)
Thrombocytosis $>400\times10^9$ mm^{-3}	28/138 (20.3)
Hypereosinophilia $>0.5\times10^9$ mm^{-3}	18/138 (13)
Lymphopenia $<1\times10^9$ mm^{-3}	8/138 (5.8)
Anaemia[¶]	6/138 (4.4)
Lymphocytosis $>4\times10^9$ mm^{-3}	5/138 (3.6)
Thrombocytopenia $<150\times10^9$ mm^{-3}	4/138 (2.9)
Biochemistry	
Elevated CRP	96/137 (70)
5<CRP<30	63/137 (46)
30<CRP	33/137 (24)
Hepatic cholestasis	34/140 (24.3)
Hypoalbuminaemia <35 g·L^{-1}	28/124 (22.6)
Hepatic cytolysis	6/140 (4.3)
Hypergammaglobulinaemia >14 g·L^{-1}	75/143 (52.4)
Circulating monoclonal Ig[+]	29/142 (20.4)

Data are presented as n/N available (%) or mean± SD. *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; CRP: C-reactive protein; Ig: immunoglobulins. [#]: or heterogeneity restriction; [¶]: haemoglobin <11.5 g·dL^{-1}; [+]: *Stenotrophomonas maltophilia*.

TABLE 3 Prevalence of antibodies in the cystic fibrosis patients

Subjects n	144
ASCA-IgA >25 IU·mL^{-1}	43.7
ASCA-IgA associated with ASCA-IgG	21.8
ANCA >1/20	40
Anti-BPI ⩾1 AU·mL^{-1}	23.6
Anti-PR3, anti-MPO	0
ANA >1/80	25
Anti-dsDNA antibodies ⩾5 IU·mL^{-1}	0.7
ENA	0
IgM rheumatoid factor >1.3 AU·mL^{-1}	2.7
Anti-CCP antibodies >25 IU·mL^{-1}	7.6
Anti-CCP associated with IgM rheumatoid factor	1.5
Anti-gliadin IgA >50 IU·mL^{-1}	12.5
Anti-GAD >1 IU·mL^{-1}	2
Anti-IA-2 >0.75 IU·mL^{-1}	1.5
Anti-actin >1/50	1.5
Anti-thyroperoxidase >60 IU·mL^{-1}	2

Data are presented as %, unless otherwise stated. ASCA: anti-*Saccharomyces cerevisiae* antibodies; Ig: immunoglobulins; ANCA: antineutrophil cytoplasmic antibodies; BPI: bactericidal/permeability-increasing protein; PR3: proteinase-3; MPO: myeloperoxidase; ANA: antinuclear antibodies; ENA: extractable nuclear antigens; CCP: cyclic citrullinated peptide; GAD: glutamic acid decarboxylase; IA-2: Islet antigen-2.

BPI-ANCA (p=0.012). The presence of ASCA-IgA was significantly correlated with male sex (p=0.008) and hypergammaglobulinaemia (p=0.042), while the association between ASCA-IgA and *P. aeruginosa* colonisation, low FEV1 and low BMI was of borderline significance (p=0.054, p=0.055 and p=0.064, respectively). No correlation between autoimmunity and genotype was identified.

No significant changes in auto-antibody status and concentrations were observed over time for anti-CCP, anti-thyroperoxidase, ENA, anti-dsDNA, anti-actin, anti-GAD and anti-IA-2A auto-antibodies and RF in the 20 patients analysed. ANA appeared in one patient within 3 yrs, with no evident clinical manifestation and no association with anti-dsDNA auto-antibodies. No variability over time was observed for BPI-ANCA in 19 out of the 20 patients. For one patient, BPI-ANCA was found to be negative 4 yrs after a positive test; no evident change in clinical status was observed between the two measurements. For ASCA-IgA, no variability was described in 18 out of 20 patients; positive ASCA-IgA became negative in one patient and negative ASCA-IgA turned to positive in one patient within 4 yrs, without any modification of respiratory or digestive status.

Clinical follow-up and correlation with the presence of BPI-ANCA and ASCA-IgA auto-antibodies

Six (4.2%) patients developed an autoimmune or systemic disease during follow-up: three patients had rheumatoid-like inflammatory arthritis, two patients had bilateral ankle arthritis associated with cutaneous vasculitis (palpable purpura on lower limbs), and one patient inflammatory colitis. The diagnosis of infectious or drug-related vasculitis was excluded. Except for one patient with rheumatoid-type arthritis in whom anti-CCP auto-antibodies had been identified a few years earlier, at inclusion none of these patients had auto-antibodies that could have been associated to the clinical symptoms observed.

With a median follow-up of 3.2 yrs, chronic respiratory insufficiency occurred in 26 (18%) patients and death in 18 (12.5%) patients, while lung transplantation was performed in 16 (11%) patients. Overall, 41 (28.5%) patients presented one or several progressive events.

Progressive events occurred in 44% of patients with positive BPI-ANCA at inclusion *versus* 22.7% of patients without BPI-ANCA at inclusion (p=0.027). Of patients with positive ASCA-IgA, 36.6% presented with one or several progressive events during follow-up *versus* 21% of patients without ASCA-IgA (p=0.06, nonsignificant).

As shown in figure 1, progression-free survival was better for patients without ASCA-IgA than for those with ASCA-IgA (p=0.045). The negative influence of the presence of BPI-ANCA on progression-free survival was of borderline significance (p=0.05).

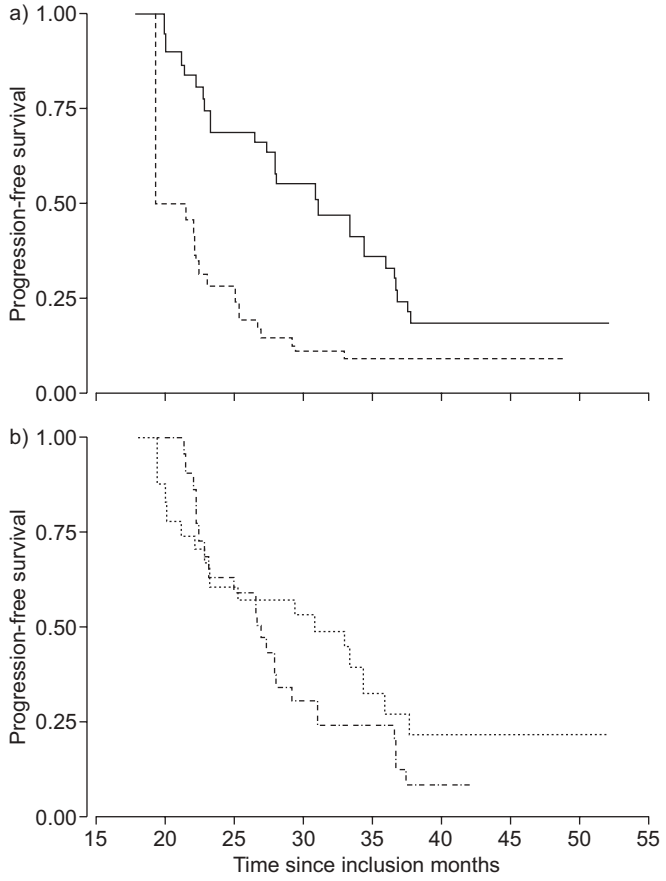

FIGURE 1. Influence of the presence of a) bactericidal/permeability-increasing protein-antineutrophil cytoplasmic antibodies (BPI-ANCA) and b) immunoglobulin A-anti-*Saccharomyces cerevisiae* antibodies (IgA-ASCA) on progression-free survival in the 144 cystic fibrosis patients. ——: BPI-ANCA not present; - - - - -: BPI-ANCA present; ·····: IgA-ASCA not present; – – · – – ·: IgA-ASCA present. a) p=0.05, b) p=0.045.

DISCUSSION

To the best of our knowledge, this is the first study to investigate clinical autoimmunity and to screen for a wide range of antibodies in a single-centre cohort of adult patients with CF. It provides a useful contribution to the knowledge of autoimmunity in CF. Our results draw attention to the high prevalence (78.5%) of antibodies, mainly BPI-ANCA and ASCA-IgA, in adult CF patients and to the frequency of autoimmune or systemic disease in this population (6.3%).

BPI protein is a membrane-associated protein found in the azurophilic granules of neutrophil granulocytes which has a potent antimicrobial activity against Gram-negative bacteria, for example *P. aeruginosa*, by neutralising the endotoxin and bactericidal effect and by playing a part in opsonisation of the bacteria [14]. ANCA with BPI specificity have been identified in various diseases associated with Gram-negative bacterial colonisation, such as inflammatory bowel diseases and bronchiectasis, and are frequently present in CF patients [3, 4, 6, 15, 16]. We confirmed, in accordance with previous reports [3–7, 15, 17, 18], the high prevalence of BPI-ANCA in CF patients and the significant link between these auto-antibodies, poor respiratory function and *P. aeruginosa* colonisation. We also established a link between the presence of BPI-ANCA, inflammatory syndrome and hypergammaglobulinaemia. These findings support the hypothesis that BPI-ANCA are generated following chronic exposure to *P. aeruginosa*, but also suggest that the generation of these auto-antibodies may be promoted by chronic inflammation and nonspecific immune activation. We also confirmed, in a larger population, the correlation previously established by CARLSSON *et al.* [4] between the presence of BPI-ANCA and poor prognosis of CF disease. This correlation could either be due to a pathogenic role of these auto-antibodies or to the fact that they are present in more severe patients. Further investigations should elucidate the potential role of BPI-ANCA in the disease process and in the development of lung damage. Longitudinal studies of BPI-ANCA in children and young CF patients, and correlating these findings with changes in respiratory function could help us to understand this role. We believe that such findings may have clinical relevance and that screening for BPI-ANCA in CF patients could lead to more appropriate treatment.

Circulating ASCA has been identified in 60–70% of patients with Crohn's disease [19]. ASCA-IgA seemed more specific to Crohn's disease than ASCA-IgG, which has also been described in other clinical conditions, such as ulcerative colitis or coeliac disease. The frequency of ASCA seropositivity in CF patients has only been analysed in one paediatric study [8]. In this study, ASCA were identified in 21% of patients, with ASCA-IgA being identified in 10%. The prevalence of ASCA-IgA in our population was higher (43.7%) and the specificity of our technique was confirmed in a control population of blood donors. The clinical significance of the presence of ASCA-IgA remains to be determined. LLOYD-STILL [20] has already suggested an association between Crohn's disease and CF. We did not find any clinical features of inflammatory bowel disease in our positive patients. However, as no digestive endoscopies were performed we could not be certain that they did not have Crohn's disease. We found a significant correlation between the presence of ASCA-IgA and hypergammaglobulinaemia, as well as a probable link between ASCA-IgA seropositivity and *P. aeruginosa* colonisation. These results suggest that the high prevalence of ASCA-IgA could be nonspecific and explained by chronic immune stimulation secondary to exposure to infectious agents or to systemic inflammation. Owing to the high prevalence of ASCA-IgA in CF patients, we believe that this marker should not be used in evaluating these patients for inflammatory bowel diseases.

Many musculoskeletal manifestations have been described in association with CF [21]. The most common is episodic arthritis, often associated with bronchial exacerbation and correlated with a poor respiratory prognosis [22]. Some cases of rheumatoid arthritis have been described [23]. RF, especially IgM type, was found in CF patients in two previous reports (21% and 54%, respectively) and, thus, could be useful for the diagnosis of rheumatoid arthritis in these patients [9, 10]. We did not observe this high prevalence, but we found another biological marker of rheumatoid arthritis, *i.e.* anti-CCP auto-antibodies. To our knowledge, our study is the first to test a large series of patients for the presence of such auto-antibodies, and we found a prevalence of 7.6%, which was associated with joint symptoms in two cases. Furthermore, one patient with anti-CCP auto-antibodies developed confirmed rheumatoid arthritis after 2 yrs of follow-up. These results suggest that patients with anti-CCP auto-antibodies should be clinically

followed and investigated for rheumatoid arthritis when presenting with arthritis.

Despite the high frequency of diabetes mellitus in adult CF patients (24% in our study), we did not identify anti-GAD or anti-IA-2 auto-antibodies in diabetic patients. These results are in accordance with previous findings [24] and may reinforce the hypothesis that CF-related diabetes mellitus is not an auto-immune disease, but may be secondary to both insulin deficiency induced by pancreatic disease and insulin resistance induced by chronic infection [25]. Nevertheless, other studies have suggested that autoimmunity may play a role in the pathogenesis of CF diabetes, in the wake of bacterial hyper-immunisation [11]. Further investigations should increase our understanding of the pathophysiology of CF-related diabetes.

A wide variety of hepatobiliary disorders are described in adult CF patients, ranging from common moderate elevation of aminotransferase to cirrhosis to intrahepatic biliary changes resembling primary sclerosing cholangitis [26, 27]. Despite the frequency of CF-related disease in our population (32%), we did not establish a link between this disease and the presence of anti-actin, anti-LKM1 or anti-M2 auto-antibodies.

Knowledge of the mechanism of autoimmunity in CF and the clinical significance of this autoimmunity is incomplete. Thus, it may be a nonspecific consequence of chronic infection by means of inflammatory response and hypergammaglobulinaemia. Autoimmunity may also be a marker of a pro-inflammatory status that could promote severe infections and lung damage. Therapies attempting to directly treat the excessive inflammatory response, such as corticosteroids, ibuprofen, montelukast, fish oil, low-dose immunosuppressive drugs or azithromycin, have been used in previous studies with varying degrees of success [28, 29]. The balance of benefit to harm is still uncertain. For example, the clinical benefits of prednisone have been well demonstrated but this treatment was accompanied by unacceptable adverse events [30]. The search for auto-antibodies, as well as other markers of systemic inflammation, could perhaps help us to select patients, possibly paediatric patients, who could benefit from early anti-inflammatory treatments. The study of some modifier genes, such as tumour necrosis factor-α, transforming growth factor-β1 and mannose-binding lectin 2, could also be useful in selecting such patients with exacerbated immune response [31, 32].

In conclusion, our study confirmed the high frequency of some auto-antibodies in CF, especially ANCA with BPI specificity and ASCA-IgA, and the link between the presence of BPI-ANCA, the severity of lung disease and CF prognosis. These findings suggest that BPI-ANCA may be an important tool in the care of patients with CF. The clinical significance of the presence of ASCA-IgA remains to be determined.

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

We would like to thank M. Rabilloud and A. Bissery (Dept of Biostatistics, Hospices Civils de Lyon, France) who contributed to the statistical analysis, P. Damaso (Immunology Dept, CHLS, Pierre Bénite, France) for secretarial assistance and N. Crowte (Alibi, France) for revision of the English manuscript.

REFERENCES

1 Kerem E, Reisman J, Corey M, *et al.* Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; 326: 1187–1191.
2 Elborn JS. How can we prevent multisystem complications of cystic fibrosis? *Semin Respir Crit Care Med* 2007; 28: 303–311.
3 Dorlochter L, Carlsson M, Olafsdottir EJ, *et al.* Anti-neutrophil cytoplasmatic antibodies and lung disease in cystic fibrosis. *J Cyst Fibros* 2004; 3: 179–183.
4 Carlsson M, Eriksson L, Pressler T, *et al.* Autoantibody response to BPI predict disease severity and outcome in cystic fibrosis. *J Cyst Fibros* 2007; 6: 228–233.
5 Berlie I, Leboucher B, Leblanc M, *et al.* Anticorps anticytoplame des polynucléaires neutrophiles au cours de la mucoviscidose. Fréquence et signification [Antineutrophil cytoplasmic antibodies in cystic fibrosis. Frequency and significance]. *Arch Pediatr* 2005; 12: 140–143.
6 Schultz H. From infection to autoimmunity: a new model for induction of ANCA against the bactericidal/permeability increasing protein (BPI). *Autoimmun Rev* 2007; 6: 223–227.
7 Carmona E, Perez-Aguilar F, Lopez JA, *et al.* Anti-neutrophil-cytoplasmic auto-antibodies (ANCA) in 105 patients (56 adults and 49 children) suffering from cystic fibrosis, attending a Spanish hospital. *J Intern Med* 2002; 252: 281–282.
8 Condino AA, Hoffenberg EJ, Accurso F, *et al.* Frequency of ASCA seropositivity in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2005; 41: 23–26.
9 Coffey M, Hassan J, Feighery C, *et al.* Rheumatoid factors in cystic fibrosis: associations with disease manifestations and recurrent bacterial infections. *Clin Exp Immunol* 1989; 77: 52–57.
10 Hassan J, Feighery C, Bresnihan B, *et al.* Prevalence of anti-Fab antibodies in patients with autoimmune and infectious diseases. *Clin Exp Immunol* 1992; 89: 423–426.
11 Stechova K, Kolouskova S, Sumnik Z, *et al.* Anti-GAD65 reactive peripheral blood mononuclear cells in the pathogenesis of cystic fibrosis related diabetes mellitus. *Autoimmunity* 2005; 38: 319–323.
12 Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998; 132: 589–595.
13 Garner JS, Jarvis WR, Emori TG, *et al.* CDC definition for nosocomial infections. *In:* Olmstead RN, ed. Infection Control and Applied Epidemiology: Principles and Practice. Mosby, St. Louis, 1996.
14 Elsbach P, Weiss J. Role of the bactericidal/permeability-increasing protein in host defence. *Curr Opin Immunol* 1998; 10: 45–49.
15 Zhao MH, Jayne DR, Ardiles LG, *et al.* Autoantibodies against bactericidal/permeability-increasing protein in patients with cystic fibrosis. *QJM* 1996; 89: 259–265.
16 Schinke S, Fellermann K, Herlyn K, *et al.* Autoantibodies against the bactericidal/permeability-increasing protein from inflammatory bowel disease patients can impair the antibiotic activity of bactericidal/permeability-increasing protein. *Inflamm Bowel Dis* 2004; 10: 763–770.
17 Aichele D, Schnare M, Saake M, *et al.* Expression and antimicrobial function of bactericidal permeability-increasing protein in cystic fibrosis patients. *Infect Immun* 2006; 74: 4708–4714.
18 Mahadeva R, Dunn AC, Westerbeek RC, *et al.* Anti-neutrophil cytoplasmic antibodies (ANCA) against bactericidal/permeability-increasing protein (BPI) and cystic fibrosis lung disease. *Clin Exp Immunol* 1999; 117: 561–567.
19 Hoffenberg EJ, Fidanza S, Sauaia A. Serologic testing for inflammatory bowel disease. *J Pediatr* 1999; 134: 447–452.
20 Lloyd-Still JD. Crohn's disease and cystic fibrosis. *Dig Dis Sci* 1994; 39: 880–885.
21 Botton E, Saraux A, Laselve H, *et al.* Musculoskeletal manifestations in cystic fibrosis. *Joint Bone Spine* 2003; 70: 327–335.
22 Rush PJ, Shore A, Coblentz C, *et al.* The musculoskeletal manifestations of cystic fibrosis. *Semin Arthritis Rheum* 1986; 15: 213–225.

23 Doyen V, Fournier C, Bautin N, *et al.* Polyarthrite rhumatoïde et mucoviscidose [Rheumatoid arthritis and cystic fibrosis]. *Rev Mal Respir* 2005; 22: 667–671.
24 Minicucci L, Cotellessa M, Pittaluga L, *et al.* Beta-cell autoantibodies and diabetes mellitus family history in cystic fibrosis. *J Pediatr Endocrinol Metab* 2005; 18: 755–760.
25 Costa M, Potvin S, Berthiaume Y, *et al.* Diabetes: a major comorbidity of cystic fibrosis. *Diabetes Metab* 2005; 31: 221–232.
26 Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. *Gut* 2007; 56: 1153–1163.
27 Durieu I, Pellet O, Simonot L, *et al.* Sclerosing cholangitis in adults with cystic fibrosis: a magnetic resonance cholangiographic prospective study. *J Hepatol* 1999; 30: 1052–1056.
28 Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. *Chest* 2008; 133: 489–495.
29 Balfour-Lynn IM. Anti-inflammatory approaches to cystic fibrosis airways disease. *Curr Opin Pulm Med* 2007; 13: 522–528.
30 Eigen H, Rosenstein BJ, Fitzsimmons S, *et al.* A multicenter study of alternate-day prednisone therapy in patients with cystic fibrosis. Cystic Fibrosis Foundation Prednisone Trial Group. *J Pediatr* 1995; 126: 515–523.
31 Dorfman R, Sandford A, Taylor C, *et al.* Complex two-gene modulation of lung disease severity in children with cystic fibrosis. *J Clin Invest* 2008; 118: 1040–1049.
32 Buranawuti K, Boyle MP, Cheng S, *et al.* Variants in mannose-binding lectin and tumour necrosis factor-α affect survival in cystic fibrosis. *J Med Genet* 2007; 44: 209–214.