First Aspergillus fumigatus IgG seroconversion is associated with more severe disease in CF children

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Abstract

Background: Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasian population. Allergic bronchopulmonary aspergillosis (ABPA) is one of the severe complications of CF, on which diagnosis is based on symptoms and blood IgE levels. Many techniques of specific IgG levels measures are used, which signification is still unclear. We evaluated evolution of patients who presented a first aspergillosis IgG seroconversion. Methods: Monocentric pediatric case-control study led in Rouen, France. Every patient with a first aspergillosis IgG seroconversion was paired with a seronegative patient. Clinical data, functional respiratory investigations, CT-scan and biologic data were collected a year before (Y_{-1}), a year after (Y_{+1}) and at the moment of the first aspergillosis seroconversion. Results: 36 cases, paired with 36 controls. Median age was 8. Forced expiratory volume in 1 second was significantly lower at Y_{+1} (p=0.025) and Vital Capacity was significantly lower at Y_{0} (p=0.027) in the case-population. More respiratory exacerbations were observed in the case-population (p=0.047). Higher specific IgE against A. fumigatus levels were observed at Y_{0} (p=0.014), Y_{-1} (p=0.001) and Y_{+1} (p=0.04) in the case-population. Total IgG were significantly higher at Y_{0} in the case-population. On the CT-scan, bronchiectasis and pulmonary infiltrates were more important in the case-population (p=0.01 and p=0.003 respectively). Conclusion: Aspergillosis seroconversion is associated with changes of clinical, respiratory functional, biologic and radiologic parameters in CF population. Aspergillosis seroconversion is a milestone in the evolution of CF. A systematic research is needed, to evaluate actions to be taken.

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SUMMARY

Background: Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasian population. Allergic bronchopulmonary aspergillosis (ABPA) is one of the severe complications of CF, on which diagnosis is based on symptoms and blood IgE levels. Many techniques of specific IgG levels measures are used, which signification is still unclear. We evaluated evolution of patients who presented a first aspergillosis IgG seroconversion.
Methods: Monocentric pediatric case-control study led in Rouen, France. Every patient with a first aspergillosis IgG seroconversion was paired with a seronegative patient. Clinical data, functional respiratory investigations, CT-scan and biologic data were collected a year before (Y₁⁻), a year after (Y₁⁺) and at the moment of the first aspergillosis seroconversion.

Results: 36 cases, paired with 36 controls. Median age was 8. Forced expiratory volume in 1 second was significantly lower at Y₁⁺ (p=0.025) and Vital Capacity was significantly lower at Y₀ (p= 0.027) in the case-population. More respiratory exacerbations were observed in the case-population (p=0.047). Higher specific IgE against A. fumigatus levels were observed at Y₀ (p=0.014), Y₁⁻ (p=0.001) and Y₁⁺ (p=0.04) in the case-population. Total IgG were significantly higher at Y₀ in the case-population. On the CT-scan, bronchiectasis and pulmonary infiltrates were more important in the case-population (p=0.01 and p=0.003 respectively).

Conclusion: Aspergillosis seroconversion is associated with changes of clinical, respiratory functional, biologic and radiologic parameters in CF population. Aspergillosis seroconversion is a milestone in the evolution of CF. A systematic research is needed, to evaluate actions to be taken.

Key words: cystic fibrosis- Allergic bronchopulmonary aspergillosis- aspergillosis seroconversion

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BACKGROUND

Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasian population, with 6600 patients actually registered in France, and 200 new cases each year. Pulmonary aspergillosis is the most common complication associated with Aspergillus fumigatus (Af), which can aggravate prognosis (1). Several forms can be described: aspergillosis colonization, which frequency in the expectorations varies between 10% et 60% according to several studies (2), aspergillosis infection, less frequent in CF, and allergic bronchopulmonary aspergillosis (ABPA). ABPA is defined by American and British diagnostic recommendations. ABPA’s prevalence varies from one study to another, between 2.1% and 13.6% in CF population (3) (4). ABPA can be diagnosed in CF-patients older than 6, and is associated with early colonization to Pseudomonas aeruginosa in expectorations (5) (6). In patients with Pseudomonas aeruginosa chronic infection, decline of respiratory functions is more important if patients have ABPA too (6). Thus, early detection is needed and 5 stages are defined by Patterson (7) to guide therapeutic measures.

ABPA diagnosis is still complicated (8): diagnostic criteria proposed to associate clinical symptoms or/and radiologic abnormalities with biological markers. In 2002, UK Cystic Fibrosis Trust proposed a total IgE threshold > 500 KU/L or a strong increase of the basal level, positive specific IgE or positive prick tests and presence of Af in secretions. American recommendations (US Cystic Fibrosis Foundation (CFF), 2003), the same year, describe total IgE > 1000 KU/L, and positive specific IgG as biological criteria (9). However these criteria are not very sensitive and specific (9). Specific IgE are though interesting as a marker of ABPA (10) and recombinant IgE could improve diagnosis sensitivity and specificity (11). Af-IgG4, TARC (Thymus and Activation Regulated Chemokine) and basophils stimulation tests have also been described as diagnostic markers (12) (13).

Although Aspergillus fumigatus IgG are detected by research of precipitins, reference method, and several others ELISA methods for a few years (14), and are quoted in the American CFF recommendations, their diagnostic value is often studied with the other ABPA markers. Lately, anti-aspergillus IgG presence have been considered as a diagnostic criteria of chronic bronchopulmonary aspergillosis (1). Anti-aspergillus IgG appearance has rarely been associated with a severe aspergillosis.

We propose here a retrospective study of cystic fibrosis patients, which anti-Af IgG serology became positive. Clinical data, functional respiratory investigations, chest CT scans and biologic data were collected a year.
before, a year after and at the moment of a first anti-Af IgG seroconversion.

**METHODS**

We conducted a monocentric, retrospective, pediatric, case-control study, directed within CFRC (CF reference and competence center) of Rouen, Normandy, France. Inclusion criteria were: patients < 18 years, followed in the pediatric department of Rouen Hospital and who benefited from systematic annual aspergillosis IgG serology since CF diagnosis. Seroconversion was defined by appearance of serum anti-Af IgG in patients whom had ever been negative. Each case was paired with a control (CF seronegative patient) on age and sex (Figure 1).

Demographic criteria, genetic criteria and sweat test values were collected. Clinical status was determined 12 months before seroconversion (Y<sub>-1</sub>), at the moment of seroconversion (Y<sub>0</sub>), and 12 months after (Y<sub>+1</sub>). CF patients were followed every 3 months at the hospital: they underwent a specialist medical consultation, sputum examination, and lung function tests (respiratory flow volumes and rates) if they were able to. Patient inclusion was made between 2007 and 2013 (Y<sub>0</sub>). The primary study endpoint was the forced expiratory volume in 1 second (FEV), in expected percentage of the age group, given by respiratory flow volumes and rates according to ATS/ERS recommendations (Figure 2).

Secondary endpoints were: Body Mass Index (BMI), sweat test values at the diagnosis (mmol/L), Vital Capacity (VC, in expected percentage of the age group), total IgE levels (KUI/L), specific IgE levels (KUI/L), total IgG (g/L), serum eosinophils (G/L), Pseudomonas aeruginosa and Staphylococcus aureus quantitative colonization (CFU/mL), number of respiratory exacerbations, antifungals prescription and presence of bronchiectasis and pulmonary infiltrates on chest CT scans.

The number of respiratory exacerbations was defined by the number of respiratory deteriorations which needed oral or intravenous antibiotics, leading or not to hospitalization. We also studied the frequency of antifungal administrations after positive serology, and duration of treatments.

Aspergillosis serologies were performed at the parasitology laboratory of the Rouen’s Hospital, Normandy, France. Several serological techniques exist: precipitins technic or indirect hemagglutination, or ELISA, confirmed by immune-electrophoresis. At each change, serum was analysed with the precedent removal for continuity of interpretation.

Sputum bacteriologic colonization was systematically looked for after a physiotherapy session. We collected the sputum analyses at each annual evaluation.

Chest CT scans were performed more rarely; so we collected the results of the scan performed at the closest date after seroconversion. Chest CT scans were analysed by pediatric radiologists at Rouen’s hospital. We collected the following criteria: bronchiectasis and pulmonary infiltrates according to the pediatric radiologist.

**Statistics**

Statistic data were included in a Windows excel table. Initial descriptive analysis was performed. Qualitative values were expressed in percentages or/and their effective in concerned population (%; n/N). Quantitative values were expressed with medians and interquartile ranges [1-3]. Data univariate analysis was made with statistics software (TGV STAT). Significance threshold was p < 0.05. Mann-Witney non-parametric tests were used for quantitative comparisons. Chi<sup>2</sup> and Student’s tests were used for qualitative comparisons. Data bivariate analysis was made with statistics software (TGV STAT). Significance threshold was p < 0.05.

**RESULTS**

We included 36 patients in each group, with a median age at 8 years old [5-11]. BMI, sweat test values and F508del homozygotes mutations were not significantly different in each group (Table 1).
We analysed lung function tests of 25 children paired, who were old enough to perform them. No significant difference was observed of FEV1 at Y_{-1} and Y_0 (Table 2). On the other hand, FEV1 was significantly lower at Y_{+1} in the case population compared to the controls (p= 0.025). VC was significantly lower at Y_0 in the case population compared to controls (p= 0.027). No significant difference of VC at Y_{-1} and Y_{+1} was noted.

Concerning total IgE (Figure 2), no significant difference between cases and controls was observed over time. Significant increases of specific IgE at Y_{-1} (p= 0.001), at Y_0 (p= 0.014) and at Y_{+1} (p= 0.029) were observed in the case-group compared with the controls. Total IgG were significantly higher at Y_0 (p= 0.04) in the case-group compared with the controls, and not at Y_{-1} and Y_{+1}.

No significant difference was shown in serum eosinophils (p= 0.87), chronic colonizations in secretions by Pseudomonas aeruginosa (p= 0.72) and Staphylococcus aureus (p=1) between cases and controls.

Every chest CT scan was performed in the systematic follow-up of patients, never in an emergency situation. More bronchectasis (p= 0.014) and more infiltrates (p<0.001) are significantly described by the radiologists in the case-group compared to controls.

Respiratory exacerbations between Y_{-1} and Y_{+1} are significantly more frequent in the case-group (p= 0.047) (Figure 3), with more than 3 exacerbations during the period, versus 2 in the control group.

Number of patients treated with antifungals and duration of treatment are noted in the table 3. Among treatments, itraconazole was prescribed in 71% cases, voriconazole in 27% cases.

**DISCUSSION**

ABPA is the expression of aspergillosis in cystic fibrosis with various clinical presentations (15) associated with variable markers and biological thresholds according to the different recommendations. We performed a retrospective case-control study reporting aspergillosis seroconversion forms in pediatric patients with cystic fibrosis. The main results showed a more altered FEV1, a larger number of exacerbations and higher specific IgE levels in the case-population.

FEV1, as the primary endpoint, was analysed in 50 patients paired on age and sex. Our 2 populations were comparable, and FEV was significantly decreased a year after aspergillosis seroconversion, and CV was significantly decreased at the moment of seroconversion in the case-population compared to controls. On the other hand, there was no significant decrease between Y_{-1} and Y_{+1} with a median variation equal to 1.5% [-16; 15] and -0.5 [-8; 15]. In the European study (3), no relation between FEV1 decrease and ABPA was observed, highlighting heterogeneous evolution within a big cohort.

Interestingly, patients with seroconversion have significantly more respiratory exacerbations. We noted exacerbations with antibiotherapy; this cannot be explained by more frequent infections by Pseudomonas aeruginosa or Staphylococcus aureus, because colonizations and chronic infections was similar in the two populations. A higher frequency of chronic infections by Pseudomonas aeruginosa would be expected (5). But this result goes in the same direction as FEV1 decrease observed in the seroconversion group: exacerbations frequency is associated with a higher FEV1 decrease. Concerning possible role of Aspergillus fumigatus sensitization, in previous studies, a FEV1 decrease is noted in patients sensitized at Aspergillus fumigatus in cystic fibrosis (11) and also asthma (12).

The third point of our study is the observation of a most important bronchial disease, with bronchiectasis which are twice important in the case population as the controls. We noted a parenchymal disease in 1 child of the control, and in 33% cases. Bronchiectasis are one of lesional damages caused by cystic fibrosis, and infiltrates belong to ABPA criteria. So, our results show that children with seroconversion must be aimed at a more severe illness.

In cystic fibrosis, anti-Af IgG signification remains unclear. First, as it is noted in our study, ELISA techniques are developed with techniques specific to laboratories, and their presence is correlated with precipitins with a better sensibility. In our study, every patient with positive precipitins had high IgE levels by ELISA methods. On the other hand, diagnostic value was every time studied in relation with ABPA,
clinical situation where Aspergillus sensitization type I sensitization is constant. Thus, Barton and al showed a strong IgG level increase in ABPA (16). In contrast, IgG are reported as chronic aspergillosis markers out of ABPA. This forms are not reported in cystic fibrosis, while there are compatible radiologic modifications (infiltrates) (1).

Recently, immunologic classification was proposed (17), with total and specific IgG and IgE, galactoman dosage, and Aspergillus fumigatus detection performed by RT-PCR. Four groups were defined: the first without aspergillosis but which can be healthy carrier; the second, positive for the whole markers corresponding to real ABPA; the third which is a group of patients sensitibilized with low IgG rate; and the fourth corresponding to patients with high IgG levels and no IgE, and a high galactoman level, defined as aspergillosis bronchitis. Patients with ABPA and sensitized were more allergic. So, analysis of our patients with seroconversion shows that 25 patients (70%) are sensitized with specific IgE > 0,1 UI/L, 9 with total IgE > 100 KU/L, and 1 with ABPA. However, among 11 with seroconversion, only 1 became sensitized a year after, showing a possible dissociation between IgG and IgE production, compatible for an aspergillosis bronchitis.

These points mean therapeutic implication. Two important treatments are used in ABPA: oral corticosteroids and antifungals. 67% cases have been treated by antifungals, for a median period of 11,4 months. Itraconazole was the first prescribed. Voriconazole was prescribed more rarely, if serology stayed positive with Itraconazole. No patient received corticosteroids. So these positive serologies were not considered as real ABPA, but like a potential ABPA beginning.

Our study has limits: small groups, retrospective. It was monocentric, so it can assure the longitudinal follow up. Indeed, seroconversion is given with several methods over the years, using precipitins techniques associated to ELISA techniques, with different sensibilities. However, when there was a method change, samples were taken again, so we could test all samples 12 months before and after seroconversion.

CONCLUSION

Results of our case-control study show clinical, functional and injury modifications by patients with IgG-Af seroconversion. These are in favour of early effect of aspergillosis colonizations, and of the interest of following children regularly. Early treatment is also discussed.

ABBREVIATIONS

ABPA: allergic bronchopulmonary aspergillosis
Af: Aspergillus fumigatus
BMI: body mass index
CF: cystic fibrosis
CFF: cystic fibrosis foundation
FEV1: forced expiratory volume in 1 second
VC: vital capacity

REFERENCES


Figure 1- Flow chart of the population

Rouen’s CFRCC  
n= 198  
\[ \rightarrow \]  
102 adults  

Paediatric CFRCC  
n=96  
\[ \rightarrow \]  
CASES: aspergillosis IgG seroconversion  
n=36  

Matching on age and sex  
\[ \rightarrow \]  
INCLUSION  

CONTROLS: negative serologies since birth  
n=36

CFRCC: Cystic Fibrosis Reference and Competence Center
Figure 2- Evolution of total IgE, specific IgE and total IgG the year before (Y-1), at the moment (Y0) and the year after (Y1) IgG seroconversion.
Figure 3- Number of exacerbations between Y₁ and Y₊₁

![Bar chart showing number of exacerbations](https://authorea.com/users/656963/articles/662005-first-aspergillus-fumigatus-igg-seroconversion-is-associated-with-more-severe-disease-in-cf-children)

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