Association Between Chronic Colonization or Infection With *Pseudomonas aeruginosa* and Bronchial Hyperreactivity in Patients With Cystic Fibrosis

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OBJECTIVE: In patients with cystic fibrosis, bronchial hyperreactivity is a common finding that has not been conclusively associated with atopy. The objective of the present study was to determine the relationship between chronic colonization or infection with *Pseudomonas aeruginosa* and bronchial hyperreactivity in a group of patients with cystic fibrosis.

PATIENTS AND METHODS: A nonspecific histamine bronchial provocation test was administered to a group of 32 cystic fibrosis patients with a mean (SD) age of 11.25 (3.7) years. The presence of atopy and of chronic colonization or infection with *P aeruginosa* was also studied.

RESULTS: Nine of the 32 patients (28.1%) studied showed bronchial hyperreactivity. The clinical status of these 9 patients was significantly worse and all were colonized or infected with *P aeruginosa*. Atopy was present in 17 of the 32 patients (53.1%) in the study group, but in only 3 of the 9 patients (33.3%) with bronchial hyperreactivity. Bronchial hyperreactivity was significantly associated with colonization or infection with *P aeruginosa* (*P*<.001), but not with atopy (*P*=.12). In the patients without atopy, colonization was significantly associated with bronchial hyperreactivity (*P*=.017). In the group with normal lung function (forced expiratory volume in 1 second ≥80%) this association was also significant (*P*=.044), while the association between bronchial hyperreactivity and atopy was not (*P*=.11).

CONCLUSIONS: The results of the present study suggest that in patients with cystic fibrosis, bronchial hyperreactivity may be associated with colonization or infection with *P aeruginosa*, and that this may be a more important risk factor for bronchial hyperreactivity than atopy.

Key words: *Atopy. Cystic fibrosis. Bronchial hyperreactivity.* Pseudomonas aeruginosa.

Asociación entre colonización-infección crónica por *Pseudomonas aeruginosa* e hiperreactividad bronquial en pacientes con fibrosis quística

OBJETIVO: La hiperreactividad bronquial (HRB) es un hallazgo común en la fibrosis quística (FQ), que no se ha relacionado de forma concluyente con la atopia. El objetivo del estudio ha sido investigar la relación existente entre la colonización-infección crónica por *Pseudomonas aeruginosa* y la HRB en un grupo de pacientes con FQ.

PACIENTES Y MÉTODOS: Se realizó la prueba de broncoprovocación inespecífica con histamina a un grupo de 32 pacientes con FQ cuya edad media ± desviación estándar era de 11,25 ± 3,7 años. Además se investigó en estos pacientes la existencia de atopia y colonización-infección crónica por *P. aeruginosa*.

RESULTADOS: De los 32 pacientes estudiados, se encontró HRB en 9 (28,1%), cuya situación clínica era significativamente peor. Estos 9 pacientes con HRB presentaron todos colonización-infección crónica por *P. aeruginosa*. Tenían atopia 17 pacientes (53,1%) de la muestra estudiada, pero sólo 3 (33,3%) de los 9 con HRB. La HRB se asoció significativamente con la colonización-infección crónica por *P. aeruginosa* (p < 0,001), pero no con la atopia (p = 0,12). Entre los pacientes sin atopia, la colonización se asoció significativamente con HRB (p = 0,017). Además, en el grupo de pacientes con función pulmonar normal (volumen espiratorio forzado en el primer segundo ≥ 80%) esta asociación fue también significativa (p = 0,044), mientras que la asociación entre HRB y atopia no lo fue (p = 0,11).

CONCLUSIONES: Los resultados del presente estudio indican que en pacientes con FQ la HRB podría estar relacionada con la colonización-infección crónica por *P. aeruginosa*, que podría ser un factor de riesgo de HRB más importante que la atopia.

Palabras clave: *Atopia. Fibrosis quística. Hiperreactividad bronquial.* Pseudomonas aeruginosa.

Introduction

Bronchial obstruction plays a fundamental role in the pathophysiology of cystic fibrosis[1] and wheezing is one of the most constant features among the auscultatory findings in patients with this disease.[2] Such patients also frequently show a positive response to bronchodilators.[3]

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Manuscript received November 10, 2006. Accepted for publication October 27, 2007.

As the data of the European Epidemiologic Registry of Cystic Fibrosis show, a large proportion of patients have asthma-like symptoms, and this proportion is higher in patients with poorer lung function.[2,4] The difficulty lies in determining whether such symptoms are due to the coexistence of asthma or whether, on the contrary, they are caused by the cystic fibrosis itself.[5]

Bronchial hyperreactivity related to chronic airway inflammation and the presence of allergy is a typical finding in asthma. Both factors are also found in patients with CF. In the pathophysiology of cystic fibrosis the combination of inflammation and infection plays a considerable role.[1,6] Recent studies have shown that the interaction between infection and inflammation occurs in the early stages of life; the 2 are related and may be present in many patients as early as the first year of life.[7,8] There is a direct association between the degree of infection and the severity of the inflammatory process.[9] Despite the similarities between the inflammation found in cystic fibrosis and that found in asthma, there are also certain differences—exhaled nitric oxide is elevated in asthma, but not in cystic fibrosis.[10] Furthermore, the cell profiles obtained by bronchoalveolar lavage are different in the 2 diseases: lymphocyte and eosinophil levels are elevated in asthma, while neutrophils and macrophages are found more typically in cystic fibrosis.[11] However, macrophage levels are also elevated in severe asthma.[12] A predominance of T helper 2 cells has also been reported in the blood of patients colonized with *Pseudomonas aeruginosa.*[13]

The presence of bronchial hyperreactivity in cystic fibrosis has been widely studied. Its prevalence ranges from 24% to 100% depending on the method and positivity criterion used in the test.[14] However, the exact relation between bacterial colonization or infection and bronchial hyperreactivity has not yet been determined. The aim of the present study was to analyze how bronchial hyperreactivity and bacterial colonization or infection are related in a population of patients with cystic fibrosis.

Patients and Methods

Our study group included 32 patients (19 boys) between the ages of 6 and 19 years (mean [SD] age, 11.25 [3.7] years) diagnosed with cystic fibrosis (sweat test >60 mmol/L) and with a forced expiratory volume in 1 second (FEV_1) more than 25% of predicted. All were able to perform spirometry correctly and none had presented any clinical exacerbation in the previous 3 months or any upper respiratory infection in the previous 4 months. None had allergic bronchopulmonary aspergillosis and none were taking any drugs or substances that could affect lung function tests. All patients underwent a nonspecific bronchial challenge test with increasing concentrations of histamine (according to the method described by Cockcroft et al[15]) using the Mefar-B3 dosimeter (Mefar SRL, Bovezzo, Italy). Lung function was measured with the Datospir-92 spirometer (Sibel SA, Barcelona, Spain) with specifications that meet the requirements of the Spanish Society of Pulmonology and Thoracic Medicine (SEPAR). The bronchial hyperreactivity test was considered positive if there was a decrease in FEV_1 of at least 20% with a histamine concentration of 8 mg/dL or less.

In addition to spirometric variables—forced vital capacity (FVC), FEV_1, FEV_1/FVC, forced midexpiratory flow rate, and peak expiratory flow—the evaluation of each patient included determining Chrispin-Norman and Shwachman scores, presence of δF508 mutation, and body mass index z-score, calculated in comparison with standard values for the Spanish population. Atopy was determined by a skin prick test in which the following allergens were used: *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, dander, fungi (*Alternaria* and *Aspergillus*), tree pollens (*Betula, Alnus, Corylus,* and olive), grass pollens (*Dactylis*, *Lolium*, *Festuca*, *Poa*, *Phleum*, and oat grass), and *Parietaria*. The test was considered positive when a maximum wheal diameter at least 3 mm was found after subtracting that of the negative control. A patient was considered to be chronically colonized or infected with *P aeruginosa* when the bacterium was detected in of at least 3 sputum cultures, spaced 1 month apart, in the previous 6 months.[16] Colonization or infection was not quantified, however.

Statistical Analysis

Once normal distribution had been confirmed, the parametric *t* test was used to compare means in children with and without bronchial hyperreactivity for each of the continuous variables. To compare the association between dichotomous variables and bronchial hyperreactivity the Fischer exact test was used. The χ^2 test could not be used because the condition that 80% of the cells have an expected cell count of more than 5 was not met. A logistic regression model could not be constructed because all the patients with bronchial hyperreactivity were colonized or infected with *P aeruginos*a and thus a positive test for bronchial hyperreactivity predicted colonization perfectly. In order to rule out the effects of a longer disease progression and consequently of poorer lung function, the association between colonization or infection and bronchial hyperreactivity was also analyzed in the subgroup of patients with normal lung function (FEV_1 ≥80%). The association was also investigated in those without atopy. All analyses were performed using the SPSS statistical package version 13.0 (SPSS Inc, Chicago, Illinois, USA).

TABLE 1
Characteristics of the Patients Included in the Study (n=32)[a]

Mean age, y	11.25 (3.7)
Sex, male/female	19/13
BMI, z score	–0.21 (1.1)
Chrispin-Norman score	9.31 (1.31)
Shwachman score	82.34 (15.8)
Mutations (n=31)	
δF508/Other	17/31
δF508/δF508	4/31
Other/Other	10/31
Atopy	17/32
Colonization or infection with *P aeruginosa*	17/32
Lung function, % of predicted	
FVC	88.69 (21.7)
FEV_1	79.13 (26.6)
FEV_1/FVC	88.44 (11.9)
$FEF_{25\%-75\%}$	55.88 (32.9)
PEF	91.94 (31.5)
Positive for BHR	9/32

Abbreviations: BHR, bronchial hyperreactivity; BMI, body mass index; $FEF_{25\%-75\%}$, forced midexpiratory flow rate; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow.
[a] Quantitative variables are expressed as means (SD).

TABLE 2
Patient Characteristics According to BHR[a]

	BHR		*P*
	Positive (n=9)	Negative (n=23)	
Mean age, y	13.4 (4.4)	10.4 (3.2)	<.05
Sex, male	5/9	14/23	.78
BMI, z score	–0.66 (0.8)	–0.10 (1.1)	.25
Chrispin-Norman score	14.7 (7.8)	7.2 (6.2)	<.01
Shwachman score	67.7 (18.4)	88.1(10.4)	<.001
Mutations (n=31)			
δF508/Other	6/8	11/23	.18
δF508/δF508	1/8	3/23	.97
Other/Other	1/8	9/23	.16
Colonization or infection with *P aeruginosa*	9/9	8/23	.001
Atopy	3/9	14/23	.12
Lung function, % of predicted			
FVC	74.0 (25.2)	94.4 (17.5)	.013
FEV_1	54.9 (26.6)	88.6 (20.0)	.001
(FEV_1/FVC)	76.7 (9.4)	93.0 (9.4)	.001
$FEF_{25\%-75\%}$	26.4 (18.4)	67.4 (30.2)	.001
PEF	65.9 (26.0)	102.1 (27.7)	.002

Abbreviations: BHR, bronchial hyperreactivity; BMI, body mass index; $FEF_{25\%-75\%}$, forced midexpiratory flow rate; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow.
[a] Quantitative variables are expressed as mean (SD).

Results

Patient characteristics are shown in Table 1. The bronchial hyperreactivity test was positive in only 9 (28.1%) of the 32 patients studied. Patients with bronchial hyperreactivity scored significantly worse on clinical and radiological scales and had poorer lung function (Table 2).

TABLE 3
Association Between Chronic Colonization or Infection With *Pseudomonas aeruginosa* and BHR in Patients With Normal Lung Function

	BHR		*P*
	Positive	Negative	
Atopy	2/2	6/15	.11
Colonization or infection with *P aeruginosa*	2/2	2/15	.044

Abbreviation: BHR, bronchial hyperreactivity.

TABLE 4
Association Between Chronic Colonization or Infection With *Pseudomonas aeruginosa* and BHR in Patients Without Atopy

	BHR		*P*
	Positive	Negative	
Normal lung function	1/5	9/10	.018
Colonization or infection with *P aeruginosa*	4/5	1/10	.017

Abbreviation: BHR, bronchial hyperreactivity.

All patients with bronchial hyperreactivity showed chronic colonization or infection with *P aeruginosa*. However, the prevalence of atopy among patients with bronchial hyperreactivity was only 33%. We found a significant association between bronchial hyperreactivity and chronic colonization or infection (*P*<.001), while the association between bronchial hyperreactivity and atopy was not statistically significant (Table 2).

Among the patients with normal lung function (n=17), that is, an FEV_1 of 80% or more of predicted, bronchial hyperreactivity was also associated with chronic colonization or infection (*P*=.044), but not with atopy (*P*=.11). The association between bronchial hyperreactivity and chronic colonization or infection was also found in patients without atopy (*P*=.017). In these patients an association between normal lung function and a negative test for bronchial hyperreactivity was also found (Tables 3 and 4).

Discussion

The results of the present study suggest that colonization or infection with *P aeruginosa* is itself a risk factor independent of atopy (and probably of pulmonary deterioration as well) for bronchial hyperreactivity as measured by a nonspecific bronchial challenge test with histamine. The prevalence of bronchial hyperreactivity in the patients included in the study was relatively low (28.1%) and comparable to those found by Mellis and Levison[17] (24%) and Tobin et al[18] (35%), which are among the lowest published. This low prevalence could be due to the fact that in cystic fibrosis the response to histamine is lower than the response to other substances such as methacholine.[19]

In the present study patients with bronchial hyperreactivity had poorer clinical and radiological scores and poorer lung function than those without bronchial hyperreactivity.[19-22] Probably due to the effect of disease duration, patients with bronchial hyperreactivity were significantly older. This is consistent with the findings of Mellis and Levison.[17]

Bronchial hyperreactivity is a typical finding in, but not exclusive to, asthma. Due to the high prevalence of this disease, it is quite likely that it may coexist with cystic fibrosis.[5] However, nonspecific bronchial hyperreactivity in cystic fibrosis patients has been shown to differ from bronchial hyperreactivity in asthmatic patients. Firstly, the dose of histamine needed to cause a 20% decrease in FEV_1 is larger in cystic fibrosis than in asthma and asthmatic patients respond better to bronchodilators than do cystic fibrosis patients. Secondly, exercise and hypertonic saline solution can provoke a bronchodilator response in patients with cystic fibrosis due to mobilization of mucus and instability of the bronchial wall.[23-25]

Atopy is the most important risk factor for the development of asthma in childhood and is also frequent in the general population. We found atopy in 53.1% of patients, a higher prevalence than in the general population of the same age (37%).[26] Other studies have reported a greater prevalence of atopy among cystic fibrosis patients than in the general population.[19,27] In our study we did not

find atopy to be a significant risk factor for bronchial hyperreactivity in line with some previous studies.[18,19,21] Other studies, however, have found a strong association between atopy and bronchial hyperreactivity in patients with cystic fibrosis.[20,22]

Apart from the coexistence of atopy and asthma, the presence of bronchial hyperreactivity in patients with cystic fibrosis could be associated with the type of genetic mutation responsible for the disease. This association has not been studied in depth, but some studies suggest the possible association of asthma in patients with δF508 heterozygosity[28] as well as in individuals with amino acid (missense) mutations in the cystic fibrosis gene.[29] We classified our patients into 3 groups: those without δF508 mutation, those with δF508 homozygosity, and those with δF508 heterozygosity. No association, however, was found between these groups and the presence of bronchial hyperreactivity.

In the present study, all patients with bronchial hyperreactivity were colonized or infected with *P aeruginosa*, including those with normal lung function (Table 3). It is not known whether inflammation precedes infection or vice versa in cystic fibrosis patients, although we do know that inflammation is already present in the first year of life.[7] For reasons that are not yet well understood, *P aeruginosa* chronically infects the lungs of patients with cystic fibrosis and causes permanent neutrophilic inflammation in the airways.[30] This type of inflammation, which does not require the presence of concomitant infection, is found in young children with wheezing.[31] In adults, neutrophilic asthma is a well-defined type of asthma that is associated with greater disease severity.[32] The mechanism by which neutrophils contribute to asthma symptoms has not been clearly established, but it has been shown that these cells are capable of producing significant concentrations of leukotriene (LT) C_4, transferring unstable LTA_4 to cells containing LTC_4 synthetase (such as the endothelial cells) by means of a process known as transcellular synthesis of cysteinyl LT.[33] The process of chronic airway inflammation produces remodeling characterized by hyperplasia without hypertrophy of the smooth bronchial muscle.[34] The increase in the number of smooth muscle cells may provide an additional explanation for the presence of bronchial hyperreactivity in cystic fibrosis. An area that should be studied is how colonization or infection with *P aeruginosa* can affect the correct functioning of muscarinic receptors, especially M_2 receptors, which are important in controlling bronchial hyperreactivity.[35]

The present study has 2 main limitations, both of them related to the impossibility of using a logistic regression model to analyze the data. Firstly, the number of patients was too small to construct a sufficiently powerful model in which bronchial hyperreactivity would be the dependent variable and atopy, colonization or infection, age, and pulmonary function, the independent variables. Secondly, all the children with bronchial hyperreactivity were colonized or infected, and this made it impossible for us to use this factor as an independent variable. However, there was a significant association between colonization or infection and bronchial hyperreactivity in the subgroup of patients without atopy and in that of patients with normal lung function. This would indicate that atopy and probably lung function do not affect the association between the development of bronchial hyperreactivity and colonization or infection, although we do not know which occurs first.

In summary, the results of the present study suggest that, in patients with cystic fibrosis, bronchial hyperreactivity may be associated with colonization or infection with *P aeruginosa*, a more important risk factor than atopy.

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