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REVIEW

Cystic fibrosis, atopy, asthma and ABPA

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Abstract

The role of atopy on cystic fibrosis (CF) progression remains unclear but evidence suggests that it may influence the appearance of co-morbid conditions such as CF asthma or allergic bronchopulmonary aspergillosis (ABPA).

Recognising asthma in patients with CF is not always easy but the identification of atopic markers favours the diagnosis. Physicians should be aware of this fact in order to achieve a better control of respiratory symptoms in patients with CF.

Bronchial mucosa inflammation and abnormal mucus predispose to mould colonisation. These patients are at higher risk of allergic sensitisation, especially when atopic susceptibility is present. In the particular case of *A. fumigatus*, allergic sensitisation precedes ABPA development, which occurs in up to 10% of CF patients. Progression of lung function deterioration is most strikingly pronounced in patients with ABPA. Therefore, sensitisation with *A. fumigatus* should be regularly tested in patients with CF, especially those at higher risk.

Recombinant allergens constitute an important advance in differentiating *Aspergillus* sensitisation from ABPA itself.

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Background

In the past, several reports have suggested an increased prevalence of atopy in cystic fibrosis (CF) patients compared to the general population. This was sustained by an increase in positive allergy skin prick tests, raised immunoglobulin E (IgE) and IgE antibodies in these patients.¹

The major allergic responses in CF patients are to *Aspergillus fumigatus* and to a lesser extent, other mould allergens such as *Cladosporium herbarum* and *Alternaria alternata*.^{2–4}

In more recent studies however, the sensitisation rate to conventional allergens such as house dust mite and grass pollen matches the common population.^{2,5} According to available data, prevalence of allergy in CF seems to increase progressively with age as well.¹

Historically, Lowe et al. established, in 1949, that 30% of the relatives of CF patients had a clinical history of allergic

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disease.⁶ Others studies reported a prevalence of allergy in patients with CF from 16 to 24%.^{7,8} A Mexican study involving 30 patients with CF showed that 26.6% had respiratory allergy, diagnosed on clinical and laboratory grounds.⁹ About 40% had positive skin prick tests to at least one allergen, and that *Aspergillus fumigatus* (*A. fumigatus*) was the most frequently involved allergen (23% of the cases).⁹

It is well known that a large number of patients with CF report symptoms that suggest an allergic type disorder, even without previous diagnosis of asthma, allergic rhinitis or eczema.⁷

The coexistence of respiratory allergy in the form of bronchial asthma, allergic rhinitis or both is often present in CF. Recognising these diseases will contribute to a more global and proper approach of these patients with obvious improvement in their quality of life.

Although there is great controversy regarding the role atopy plays in CF, a deep knowledge is required on how the hypersensitivity phenomenon begins and how the immunoinflammatory response vicious circle can be controlled. This might lead to the development of new lung protection drugs and, thereafter, prolonged survival in these patients.

Cystic fibrosis

Cystic fibrosis (CF) is a well-characterised monogenic disease; it has an autosomal recessive inheritance pattern and is caused by a single-gene mutation on the CF transmembrane conductance regulator (CFTR).¹⁰

It is relatively uncommon, although prevalence varies considerably depending on ethnicity and geography, and affects preferentially Caucasians of European descent¹¹; prevalence can reach as many as 1:2500 births among certain Caucasian groups.¹²

The molecular mechanisms of the CF mutation have been well described in previous studies. A strong genotype-phenotype correlation can be observed. A significant allelic heterogeneity exists, resulting in a continuum of disease expression varying from very mild to very severe. Different explanations have been proposed for such a wide spectrum of clinical manifestations.¹³ These considerations are, nevertheless, beyond the scope of our review.

Inflammation is a cardinal feature of lung disease in CF. Although the primary genetic defect was detected in CF in 1989¹⁴ and lung inflammation is ubiquitous in CF patients, we still know very little about how mutations in the CFTR gene are related to infection and inflammation. On the one hand, abnormal intracellular chloride retention and increased sodium reabsorption contribute to more viscous secretions which impair the mucociliary clearance. As a consequence, chronic colonisation by opportunistic pathogens, such as *Staphylococcus aureus* or *Pseudomonas aeruginosa* supervenes, leading to persistent inflammation and causing inexorably long-term lung destruction. On the other hand, lung inflammation can also precede lung infection. Even though the lungs are essentially normal at the time of birth, several studies have demonstrated inflammation and infectious changes before clinically evident lung disease is present, even in infancy.¹⁵⁻¹⁷

The presence of high neutrophil counts, neutrophil elastase and inflammatory cytokines in broncho-alveolar

lavage (BAL) specimens, obstructive mucous plugging and epithelial metaplasia has been reported, in young children with CF, even in the absence of positive microbiological cultures.¹⁸ One can still suggest that organisms could be present in a number too small to appear on sputum cultures even though they are present in sufficient quantity to trigger an inflammatory response. Nevertheless, once bacterial clearance is usually reduced when inflammation is enhanced, the consequences of continuous inflammation due to the primary gene defect could well result in facilitated colonisation of the CF airways.^{19,20}

Cystic fibrosis and asthma

Cystic fibrosis and asthma are not always easily distinguished. Wheeze, whether in asthmatic or CF patients, involves airway mucosal oedema, mechanical obstruction by accumulated secretions, airway smooth muscle contraction, and dynamic collapse of airways.

It occurs frequently in both pathologies and for this reason it is difficult to determine which patients have concomitant asthma and which wheeze as a result of their underlying CF lung disease. However, it is important to readily recognise "CF asthma" once it can contribute to a better control of respiratory symptoms in patients with CF. It is clear from the North American Epidemiologic Study of Cystic Fibrosis (ESCF) that the diagnosis of asthma in CF does influence prescribing practice, in particular increasing the use of inhaled corticosteroids.^{21,22}

There is, however, no consensus on how to define CF asthma. Acute airway obstruction reversed by bronchodilators, especially if seasonal or allergen induced, can be a valuable but fallible indicator, and laboratory data such as eosinophilia or high IgE levels, are also of limited value, as explained in later sections.

A personal history (eczema or allergic rhinitis) and a family history of atopy (including asthma) in first degree relatives are probably the most useful predictors of asthma.²³

Standard spirometric tests, although useful in assessing lung disease severity, are not reliable tools to diagnose asthma in CF patients. The reason for this apparent ineffectiveness of lung function testing in CF patients suspected of having asthma is the degree of variability in lung function measures. Forced expiratory volume in one second and forced vital capacity can vary as much as 15–20%, even when testing on the same day.^{24,25}

Likewise, the role of bronchodilator responsiveness in patients with CF is not well defined and several studies report important limitations.^{26,27} CF patients often show a degree of bronchodilator responsiveness whether they have CF asthma or not.^{28,29}

Bronchial hyperresponsiveness (BHR) is a typical finding in, but not exclusive to, asthma and even though it is also found in many CF patients, especially those with poorer lung function, the underlying mechanism is still debatable.^{29,30}

Even the hypothesised role of atopy in "CF asthma" needs to be clarified. According to some studies, a strong association exists between atopy and BHR.^{31,32} Other authors, on the contrary, believe it might not be a significant risk factor for BHR development in patients with CF.³³⁻³⁵

In a study with 20 children with CF, BHR was found to correlate with coexistent asthma, particularly for positive skin testing patients.³¹

In a study by Eggleston et al., with CF patients with (20) and without (16) methacholine hyperreactivity, a significantly higher proportion of atopic patients was found in the first group, but no discriminative power between both groups was found for allergy history.³²

On the other hand, several other authors found BHR not conclusively associated with atopy, even though it is a common finding in CF patients.^{33–35} Mitchell et al. performed methacholine challenges in 113 CF patients and found a positive response in 51% of the cases and asthma in 98% of those. However, there was no relationship between allergic rhinitis or positive allergen skin tests and positive methacholine response.³³ These findings are concordant with other studies, by Sanchez or Valverde-Molina with 22 and 32 patients, respectively, keeping this chapter still unfinished.^{34,35}

According to Valverde-Molina et al., BHR may be associated with colonisation or infection with *P. aeruginosa* in patients with CF, and this may be a more important risk factor than atopy.³⁵

Once other causes of serum total IgE elevation are excluded, particularly allergic bronchopulmonary aspergillosis (ABPA), it can also constitute a guide to atopic status, strengthening the diagnosis of CF asthma in opposition to “CF wheezers” without asthma.³⁶ Atopy can also be confirmed through positive skin prick testing or serum specific IgE tests when common aeroallergens such as house dust mite, cat, dog, grass and tree pollens, are involved. On the other hand, as stated by Balfour-Lynn, a reaction to *Aspergillus* does not necessarily denote atopy, but this will be discussed later.²³ Nevertheless, a positive result would still support the diagnosis of CF asthma.³⁷

Asthma and CF differ not only clinically but in terms of immunopathology as well. In CF, it is mainly neutrophil driven, whilst in asthma it depends mostly on eosinophils and lymphocytes, with elevated exhaled nitric oxide levels; more severe forms of asthma, however, do tend to be associated with neutrophils.^{38,39} A Th2 balanced immune response is strongly characteristic of atopic asthma but, in general, CF does not quite fit into the Th1/Th2 pattern.³⁹ Patients with chronic *Pseudomonas aeruginosa* infection and ABPA also develop predominantly a Th2 type response.^{40,41}

Chronic airway inflammation constitutes a determinant factor for BHR and, despite the similarities between the inflammation found in CF and asthma, immunopathological mechanisms are considerably different.

In the past, some authors also suggested a common genetic background linking asthma and CF, suggesting that atopy in CF patients could result from the same genetic defect responsible for CF.⁴² Nevertheless, studies that tried to assess the association between asthma and CFTR gene mutations heterozygosity have led to conflicting results. In a study carried out in a Norway, no association was found between asthma and heterozygous CF.⁴³

A study from 2008 found that, although the relative risk of asthma did not differ between heterozygous CF and no heterozygous CF, the values of FEV1, and FEV1/FVC ratio were lower in carriers, suggesting that, according to pulmonary function testing, heterozygosity may be related with a silent obstructive pulmonary profile.⁴⁴

Using 15-year follow-up data from the Copenhagen City Heart Study, Dahl et al. found that CF $\Delta F508$ heterozygotes may be overrepresented among individuals with asthma and may have poorer lung function than non-carriers. Furthermore, $\Delta F508$ heterozygosity in context with familial predisposition to asthma may be associated with a greater annual FEV1 decline.⁴⁵

Pathophysiology insights

In this section, the possible relationships between mould sensitisation, atopy and cystic fibrosis will be revised in a few words.

Cystic fibrosis patients show several risk factors which might influence atopic progression, such as increased permeability of the bronchial mucosa,⁴⁶ defective secretory IgA system⁴⁷ and entrapment of antigens in infected areas of the lungs⁴⁷ in association with ciliar dysfunction.⁴⁸ This would result in increased antigenic access through the tracheobronchial tree, favouring a constant stimulation of IgE production.

Under normal circumstances, despite high concentrations of exposure to mould spores, no sensitisation would occur.⁴⁹ This low sensitisation potential contrasts, however, with hyphal elements, significantly more allergenic. It has been known that exposure to high concentrations of hyphal elements, rather than spores, will have a chance of producing sensitisation. Spores are inhaled into the bronchial airway, becoming trapped in the luminal mucus of patients with progressively more severe airway inflammation.

In patients with CF, there will be enough time for spores to germinate, forming mycelia, with consequent release of allergenic proteins and sensitisation.^{50,51} Indeed, it is quite common to be able to culture *Aspergillus fumigatus* and observe hyphal networks in the sputum of CF patients, with up to 30% having positive cultures.⁵²

The sensitisation process occurs in genetically susceptible individuals and requires allergen processing by antigen presenting cells, which then stimulate T cells within the bronchoalveolar lymphoid tissue (BALT) in a Th2 balanced way.

Different theories have been proposed concerning the role of chronic *P. aeruginosa* colonisation in mould sensitisation and atopy. The highest IgE levels are associated with chronic *Pseudomonas* colonisation and frequent exacerbation of infection. These data have been used to suggest either that allergy contributes to the lung pathology or, alternatively, that serious lung infection facilitates allergen sensitisation.^{2,53}

Aspergillus fumigatus

Special attention should be given to *A. fumigatus*, the most prevalent mould allergen identified in CF patients.² Colonisation of the lower respiratory tract with *A. fumigatus* is particularly frequent in patients with CF, with a reported incidence of 57%,⁵⁴ and a prevalence of 40%.⁵⁵

Aspergillus fumigatus, a widely distributed spore-bearing fungus, causes multiple diseases in humans,^{56–58} such as invasive pulmonary aspergillosis, aspergilloma, and different

forms of hypersensitivity diseases. These include allergic asthma, characterised by a Th2 response^{59,60}; hypersensitivity pneumonitis, with Th1 response dominance,⁶¹ and ABPA, which will be presented in later sections.

The immune response to *A. fumigatus* differs in atopic and non-atopic patients. In the first group a Th2 CD4+ T-cell response and IgE and IgG antibody responses prevail with a clinical spectrum of allergic sensitivity to ABPA and in non-atopic patients predominates a Th1 response.⁶⁰

The most common allergens include *A. fumigatus* and *Aspergillus clavatus*, but other species exist, causing mainly invasive disease.

Aspergillus has a very small spore size (3–5 µm), which enables it to penetrate deeply into the lung. Spores are capable of withstanding extraordinary atmospheric conditions (and suboptimal host defences).⁶²

As mycelia grow, it releases allergens that are processed by antigen-presenting cells bearing HLA-DR2 or -DR5. Proteases of *A. fumigatus*, with elastolytic and collagenolytic activities, may play a role facilitating antigen transport across the epithelial cell layer, by damaging the epithelial integrity and through direct interaction with epithelial cell surface receptors, resulting in production of proinflammatory cytokines and corresponding inflammatory responses. As previously explained, in susceptible individuals, the immune response to *Aspergillus* allergens becomes skewed toward a Th2 CD4+ cell response.⁶³

ABPA

ABPA is a disease primarily occurring in patients with asthma (1%–2% of asthma patients) or with cystic fibrosis (1%–7.8% of CF patients).^{60,64–71}

ABPA is a lung disease caused by immediate hypersensitivity reaction against *Aspergillus fumigatus*. The most clinically relevant characteristics are impaired mucociliary clearance, mucoid impactions, wheezing, pulmonary infiltrates, bronchiectasis and fibrosis.⁵⁶ Some immunological manifestations include recurrent episodes of eosinophilic airways inflammation, peripheral blood eosinophilia, increased serum concentrations of IL-2 receptor, high total serum IgE levels, and increased circulating specific IgE and IgG against *A. fumigatus* antigen.^{56,72,73}

The pathophysiology of ABPA still remains largely speculative, nonetheless several predisposing factors have been proposed for ABPA development.

Familial occurrence of ABPA has been reported, suggesting a possible genetic contribution to the disease in CF.^{74,75} Firstly, Chauhan et al. suggested that Th2 reactivity to a major *Aspergillus fumigatus* antigen was restricted by HLA-DR2 or HLA-DR5 alleles. Later, the same authors concluded that HLA-DR molecules DR2, DR5, and possibly DR4 or DR7 contribute to susceptibility to ABPA, while HLA-DQ2 appears to be a protective factor.⁷⁶ In addition, there may be increased sensitivity of T cells, B cells, NK cells, and eosinophils to IL-4 stimulation due to mutations of IL-4R alpha and/or the Jak/STAT pathway genes.⁷⁷ The presence of IL-4R alpha single nucleotide polymorphisms, principally ile75val, appears to be a genetic risk for the development of ABPA.⁷⁸ This suggests a strong immunogenetic susceptibility in ABPA progression.

Likewise, CFTR mutation has also been implicated in the aetiology of ABPA⁷⁴ but no significant association with any particular CFTR genotype has been documented.^{71,79} Miller et al. found a higher incidence of ABPA among atopic patients with CF and an increased frequency of heterozygous mutations of the CFTR gene was identified in this group.⁸⁰ Patients with ABPA, but without CF, have been demonstrated to have higher frequencies of CFTR mutations as well.⁸⁰ This could probably be due to abnormal mucus production with spores trapping in the respiratory mucosa.

Atopy appears to be another important risk factor for ABPA. ABPA was diagnosed in 22% of atopic individuals with CF but in only 2% of non-atopic patients.⁶⁹ In another study, the majority of patients with ABPA were atopic, as defined by positive skin prick test to at least one common aeroallergen, other than *A. fumigatus*.⁶⁷ Total IgE concentration was found to be increased in *A. fumigatus* sensitised patients with CF but with no ABPA.⁶⁸ All these data therefore suggest that atopy, especially in CF patients, may facilitate ABPA development.

Fungal colonisation and mycelia growth in CF has also been related to increased use of antibiotics for recurrent bacterial infections, facilitating *A. fumigatus* sensitisation and ABPA progression.⁸¹

Although previous works detected no association between ABPA and *P. aeruginosa* colonisation,⁸² Nikolaizik et al. reported a positive correlation between the two entities, suggesting that *P. aeruginosa* colonisation favours the process of *A. fumigatus* sensitisation.⁸³

The causal relationship between *Aspergillus* sensitisation and *Pseudomonas* colonisation has motivated great discussion over the years as it is difficult to disentangle the influences of the *Pseudomonas* from that of *Aspergillus*.

In vitro studies report a possible link between *Aspergillus* and *Pseudomonas*, as one of the major allergens of *A. fumigatus* is selectively released when *Aspergillus* is co-cultured with *Pseudomonas*. This could explain the in vivo relationship between colonisation with these two organisms and the subsequent development of allergy.

Allergic sensitisation and clinical relevance

Sensitisation to *A. fumigatus* can be assessed by skin prick testing, a cheap, rapid, easy and highly sensitive screening test with high negative predictive value, or by a serological battery of assays with high specificity, but more expensive and less sensitive.⁸⁴

Sensitisation to *A. fumigatus* in CF has been reported to increase with age and to be as high as 56%.⁸⁵ When considering persistent asthma in west European countries, it is identified in 20%–25% of patients.^{66,86,87} In other studies involving CF, 31–59% of patients were sensitised to *A. fumigatus*.^{67,88}

When considering CF and ABPA, although sensitisation to *A. fumigatus* is crucial to ABPA diagnosis, it is important to differentiate lung colonisation by *Aspergillus* (40%),⁶⁹ allergic sensitisation (20–60%),^{66,85} and clinically proven ABPA, a sizeable minority ranging from 1 to 11%.^{71,89}

The evolution from simple sensitisation to ABPA constitutes a crucial phase which urges to be correctly diagnosed in order to prevent disease progression.

It has been proved that CF patients with *A. fumigatus* sensitisation and “true” ABPA differ in terms of specific IgE responses to recombinant allergens (rAspf1, rAspf2, rAspf3, rAspf4 and rAspf6).⁹⁰

A. fumigatus sensitised asthmatic patients react with 100% specificity and 88% sensitivity to rAspf1 and rAspf3, achieving positive and negative predictive values of 100% and 63% respectively, in terms of *A. fumigatus* sensitisation.⁸⁶ On the other hand, patients with ABPA present almost exclusively specific IgE to rAspf4 and rAspf6, suggesting that specific IgE to recombinant allergens of *A. fumigatus* could help in the early detection of sensitisation and ABPA itself.^{86,88} In terms of diagnostic specificity, recombinant allergens have proven superior to allergen extracts.⁹¹

However, the relationship between *A. fumigatus* sensitisation and pulmonary function is still to be clarified. This issue has been studied by different groups, with conflicting results,^{68,71,89,92} thus reinforcing the need for adequate longitudinal studies aimed at establishing the effect of *A. fumigatus* sensitisation in the decline of pulmonary function, particularly in CF patients. Strong evidence exists that ABPA and bacterial infection or chronic colonisation are independently linked to a more rapid decline in lung function. Some authors even suggest that each entity may influence differently specific parameters of lung function testing but more research is required before any conclusions are stated.⁷⁹

Conclusion

In conclusion, strong evidence exists that the atopic status may influence the appearance of co-morbid conditions in patients with CF, such as CF asthma, mould sensitisation and ABPA.

Cystic fibrosis patients appear to be more prone to mould sensitisation, in particular to *A. fumigatus*.

Age, bacterial lung colonisation, genetics and immunology appear to be important factors for mould sensitisation. The relationship between atopy and CF remains under scope but the immunoallergic evaluation assumes a great interest for a correct approach of these patients.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Mogayzel Jr PJ, Flume PA. Update in cystic fibrosis 2009. *Am J Respir Crit Care Med*. 2010;181:539–44.
2. Warner JO, Taylor BW, Norman AP, Soothill JF. The association of cystic fibrosis with allergy. *Arch Dis Child*. 1976;51:507–11.
3. Warren CP, Tai E, Batten JC, Hutchcroft BJ, Pepys J. Cystic fibrosis immunological reactions to *A. fumigatus* and common allergens. *Clin Allergy*. 1975;5:1–12.
4. Allan JD, Moss AD, Wallwork JC, McFarlane H. Immediate hypersensitivity in patients with cystic fibrosis. *Clin Allergy*. 1975;5:255–61.
5. Warner JO. The variability of skin test hypersensitivity reactions in cystic fibrosis and asthma. *Clin Allergy*. 1977;7:385–9.
6. Lowe CU, May CD, Reed SC. Fibrosis of pancreas in infants and children: a statistical study of clinical and hereditary features. *Am J Dis Child*. 1949;78:349–74.
7. Kulczycki LL, Mueller H, Shwachman H. Respiratory allergy in patients with cystic fibrosis. *JAMA*. 1961;175:358–64.
8. Rachelefsky GS, Osher A, Dooley RE, Ank B, Stiehm ER. Coexistent respiratory allergy and cystic fibrosis. *Am J Dis Child*. 1974;128:355–9.
9. Torres-Lozano C, Núñez-Núñez ME, Pedroza-Meléndez A, Lezana JL, Huerta-López JG. Respiratory allergy in Mexican patients with cystic fibrosis. *Allergol Immunopathol*. 1999;27:294–7.
10. Barnes KC. Genetic studies of cystic fibrosis and atopic asthma: current approaches and future aspects. *Pediatr Allergy Immunol*. 1996;7:74–81.
11. Welsh MJ, Tsui LC, Boat FT, Beaudet AL. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic and molecular bases of inherited disease*, vol. 3. New York: McGraw-Hill; 1995. p. 3799–876.
12. Zielenski J, Tsui LC. Cystic fibrosis: genotypic and phenotypic variations. *Annu Rev Genet*. 1995;29:777–807.
13. Rozmahel R, Wilschanski M, Matin A, Plyte S, Oliver M, Auerbach W, et al. Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. *Nature Genet*. 1996;12:280–7.
14. Tizzano EF, Buchwald M. Cystic fibrosis: beyond the gene to therapy. *J Pediatr*. 1992;120:337–49.
15. Wilmott RW, Kassab JT, Kilian PL, Benjamin WR, Douglas D, Wood RE. Increased levels of interleukin-1 in bronchoalveolar washings from children with bacterial pulmonary infections. *Am Rev Respir Dis*. 1990;142:365–8.
16. Konstan MW, Hilliard KA, Norvell TM, Berger M. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. *Am J Respir Crit Care Med*. 1994;150:448–54.
17. Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med*. 1995;151:1075–82.
18. Bedrossian CW, Greenberg SD, Singer DB, Hansen JJ, Rosenberg HS. The lung in cystic fibrosis. A quantitative study including prevalence of pathologic findings among different age groups. *Hum Pathol*. 1976;7:195–204.
19. Doring G, Dauner HM. Clearance of *Pseudomonas aeruginosa* in different rat lung infection models. *Am Rev Respir Dis*. 1988;138:1249–53.
20. Dal Nogare AR, Toews GB, Pierce AK. Increased salivary elastase precedes gram-negative bacillary colonization in postoperative patients. *Am Rev Respir Dis*. 1987;135:671–5.
21. Brand PLP. Bronchodilators in cystic fibrosis. *J R Soc Med*. 2000;93:37–9.
22. van Haren EH, Lammers JW, Festen J, van Herwaarden CL. Bronchial vagal tone and responsiveness to histamine, exercise and bronchodilators in adult patients with cystic fibrosis. *Eur Respir J*. 1992;5:1083–8.
23. Balfour-Lynn IM, Elborn JS. “CF asthma”: what is it and what do we do about it? *Thorax*. 2002;57:742–8.
24. Cooper PJ, Robertson CF, Hudson IL, Phelan PD. Variability of pulmonary function tests in cystic fibrosis. *Pediatr Pulmonol*. 1990;8:16–22.
25. Sanders DB, Rosenfeld M, Mayer-Hamblett N, Stamey D, Redding GJ. Reproducibility of spirometry during cystic fibrosis pulmonary exacerbations. *Pediatr Pulmonol*. 2008;43:1142–6.
26. Dahl M, Tybjaerg-Hansen A, Lange P, Nordestgaard BG. DeltaF508 heterozygosity in cystic fibrosis and susceptibility to asthma. *Lancet*. 1998;351:1911–3.
27. Heijerman H, Westerman E, Conway S, Touw D, Döring G; consensus working group. Inhaled medication and inhalation devices for lung disease in patients with cystic fibrosis: A European consensus. *J Cyst Fibros*. 2009;8:295–315.

28. Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S; International Study of Asthma and Allergies in Childhood Phase Three Study Group. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax*. 2009;64:476–83.
29. Weinberger M. Airways reactivity in patients with CF. *Clin Rev Allergy Immunol*. 2002;23:77–85.
30. Schroeder SA, Gaughan DM, Swift M. Protection against bronchial asthma by CFTR deltaF508 mutation: a heterozygote advantage in cystic fibrosis. *Nature Med*. 1995;1:703–5.
31. van Asperen P, Mellis CM, South RT, Simpson SJ. Bronchial reactivity in cystic fibrosis with normal pulmonary function. *Am J Dis Child*. 1981;135:815–9.
32. Eggleston PA, Rosenstein BJ, Stackhouse CM, Alexander MF. Airway hyperreactivity in cystic fibrosis. Clinical correlates and possible effects on the course of the disease. *Chest*. 1988;94:360–5.
33. Mitchell I, Corey M, Woenne R, Krastins IRB, Levison H. Bronchial hyperreactivity in Cystic Fibrosis and Asthma. *J Pediatr*. 1978;93:744–8.
34. Sánchez I, Powell RE, Chernick V. Response to inhaled bronchodilators and nonspecific airway hyperreactivity in children with cystic fibrosis. *Pediatr Pulmonol*. 1992;14:52–7.
35. Valverde-Molina J, Sánchez-Solis M, Pastor-Vivero MD, García-Marcos L. Association between chronic colonization or infection with *Pseudomonas aeruginosa* and bronchial hyperreactivity in patients with cystic fibrosis. *Arch Bronconeumol*. 2008;44:180–4.
36. Sanchez I, Powell RE, Pasterkamp H. Wheezing and airflow obstruction during methacholine challenge in children with cystic fibrosis and in normal children. *Am Rev Respir Dis*. 1993;147:705–9.
37. Holzer FJ, Olinsky A, Phelan PD. Variability of airways hyperreactivity and allergy in cystic fibrosis. *Arch Dis Child*. 1981;56:455–9.
38. Darga LL, Eason LA, Zach DM, Polgar G. Cold air provocation of airway hyperreactivity in patients with cystic fibrosis. *Pediatr Pulmonol*. 1986;2:82–8.
39. Balfour-Lynn IM. Cardiopulmonary function testing. In: Pryor JA, Prasad SA, editors. *Physiotherapy for respiratory and cardiac problems. Children and adults*, 3rd ed.. Edinburgh: Harcourt Sciences; 2002. p. 102–26.
40. Yamashita Y, Okano M, Yoshino T, Hattori H, Yamamoto T, Watanabe T, et al. Carbohydrates expressed on *Aspergillus fumigatus* induce in vivo allergic Th2-type response. *Clin Exp Allergy*. 2002;32:776–82.
41. Allard JB, Poynter ME, Marr KA, Cohn L, Rincon M, Whittaker LA. *Aspergillus fumigatus* generates an enhanced Th2-biased immune response in mice with defective cystic fibrosis transmembrane conductance regulator. *J Immunol*. 2006;177:5186–94.
42. Warner JO, Norman AP, Soothill JF. Cystic fibrosis heterozygosity in the pathogenesis of allergy. *Lancet*. 1976;1:1990–1991.
43. Munthe-Kaas MC, Lødrup Carlsen KC, Carlsen KH, Skinningsrud B, Håland G, Devulapalli CS, et al. CFTR gene mutations and asthma in the Norwegian Environment and Childhood Asthma study. *Respir Med*. 2006;100:2121–8.
44. Douros K, Loukou I, Doudounakis S, Tzetis M, Priftis KN, Kanavakis E. Asthma and pulmonary function abnormalities in heterozygotes for cystic fibrosis transmembrane regulator gene mutations. *Int J Clin Exp Med*. 2008;1:345–9.
45. Dahl M, Nordestgaard BG, Lange P, Tybjaerg-Hansen A. Fifteen-year follow-up of pulmonary function in individuals heterozygous for the cystic fibrosis phenylalanine-508 deletion. *J Allergy Clin Immunol*. 2001;107:818–23.
46. Leskowitz S, Salvaggio JE, Schwartz HE. An hypothesis for the development of atopic allergy in man. *Clin Allergy*. 1972;2:237–46.
47. Wallwork JC, McFarlane H. The IgA system and hypersensitivity in patients with cystic fibrosis. *Clin Allergy*. 1976;6:349–58.
48. Tobin MJ, Maguire O, Reen D, Tempany E, Fitzgerald MX. Atopy and bronchial reactivity in older patients with cystic fibrosis. *Thorax*. 1980;35:807–13.
49. Warner JA, Little SA, Pollock I, Longbottom JL, Warner JO. The influences of exposure to house dust mite, cat, pollen and fungal allergens in the home on primary sensitization in asthma. *Pediatr Allergy Immunol*. 1990;1:79–86.
50. Little SA, Longbottom JL, Warner JO. Optimised preparations of *Aspergillus fumigatus* extracts for allergy diagnosis. *Clin Exp Allergy*. 1993;23:835–42.
51. Warner JO, Kilburn SA. Cystic fibrosis and allergy. *Pediatr Allergy Immunol*. 1996;7:67–9.
52. Nikolaizik WH, Brueton MJ, Warner JO. *Aspergillus* allergy and allergic bronchopulmonary aspergillosis in cystic fibrosis. *Pediatr Allergy Immunol*. 1991;2:83–6.
53. Pitcher-Wilmott RW, Levinsky RJ, Gordon I, Turner MW, Matthew DJ. *Pseudomonas* infection, allergy and cystic fibrosis. *Arch Dis Child*. 1982;57:582–6.
54. Nelson LA, Callera ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. *Am Rev Respir Dis*. 1979;120:863–73.
55. Schonheyder H, Jensen T, Hoiby N, Andersen P, Koch C. Frequency of *Aspergillus fumigatus* isolates and antibodies to *Aspergillus* antigens in cystic fibrosis. *Acta Pathol Microbiol Immunol Scand*. 1985;93:105–12.
56. Kurup VP, Apter AJ. Allergic bronchopulmonary aspergillosis. *Immunol Allergy Clin North Am*. 1998;18:471–715.
57. Summerbell RC. Taxonomy and ecology of *Aspergillus* species associated with colonizing infections of the respiratory tract. *Immunol Allergy Clin North Am*. 1998;18:549–73.
58. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. *Crit Rev Microbiol*. 2010;36:1–53.
59. Knutsen AP, Mueller KR, Hutcheson PS, Slavin RG. T- and B-cell dysregulation in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Clin Immunol Immunopathol*. 1990;55:129–38.
60. Knutsen AP, Chauhan B, Slavin RG. Cell-mediated immunity in allergic bronchopulmonary aspergillosis. *Immunol Allergy Clin North Am*. 1998;18:575–99.
61. Kurup VP, Banerjee B. Allergic aspergillosis: antigens and immunodiagnosis. *Adv Med Mycol*. 1996;2:133–54.
62. Parta M, Chang Y, Rulong S, Pinto-Da Silva P, Kwon-Chung KJ. HYP1, a hydrophobin gene from *Aspergillus fumigatus*, complements the rodletless phenotype in *Aspergillus nidulans*. *Infect Immun*. 1994;62:4389–95.
63. Knutsen AP, Slavin RG. In vitro T cell responses in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J Lab Clin Med*. 1989;113:428–35.
64. Patterson R, Greenberger PA, Halwig JM, Liotta JL, Roberts M. Allergic bronchopulmonary aspergillosis: natural history and classification of early disease by serologic and roentgenographic studies. *Arch Intern Med*. 1986;146:916–8.
65. Chotirmall SH, Branagan P, Gunaratnam C, McElvaney NG. *Aspergillus*/allergic bronchopulmonary aspergillosis in an Irish cystic fibrosis population: a diagnostically challenging entity. *Respir Care*. 2008;53:1035–41.
66. de Almeida MB, Bussamra MH, Rodrigues JC. Allergic bronchopulmonary aspergillosis in paediatric cystic fibrosis patients. *Paediatr Respir Rev*. 2006;7:67–72.
67. Valletta EA, Braggion C, Mastella G. Sensitization to *Aspergillus* and allergic bronchopulmonary aspergillosis in a cystic fibrosis population. *Pediatr Asthma Allergy Immunol*. 1993;7:43–9.
68. Wojnarowski C, Eichler I, Gartner C, Götz M, Renner S, Koller DY, et al. Sensitization to *Aspergillus fumigatus* and lung function in children with cystic fibrosis. *Am J Respir Crit Care Med*. 1997;155:1902–7.

69. Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis: role of atopy and response to itraconazole. *Chest*. 1999;115:364–70.
70. Hafen GM, Hartl D, Regamey N, Casaulta C, Latzin P. Allergic bronchopulmonary aspergillosis: the hunt for a diagnostic serological marker in cystic fibrosis patients. *Expert Rev Mol Diagn*. 2009;9:157–64.
71. Mastella G, Rainisio M, Harms HK, Hodson ME, Koch C, Navarro J, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis: a European epidemiological study. *Epidemiologic Registry of Cystic Fibrosis*. *Eur Respir J*. 2000;16:464–71.
72. Brown JE, Greenberger PA, Yarnold PR. Soluble serum interleukin 2 receptors in patients with asthma and allergic bronchopulmonary aspergillosis. *Ann Allergy Asthma Immunol*. 1995;74:484–8.
73. Latzin P, Hartl D, Regamey N, Frey U, Schoeni MH, Casaulta C. Comparison of serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur Respir J*. 2008;31:36–42.
74. Marchand E, Verellen-Dumoulin C, Mairesse M, Delaunois L, Brancaleone P, Rahier JF, et al. Frequency of cystic fibrosis transmembrane conductance regulator gene mutations and 5T allele in patients with allergic bronchopulmonary aspergillosis. *Chest*. 2001;119:762–7.
75. Graves TS, Fink JN, Patterson R, Kurup VP, Scanlon GT. A familial occurrence of allergic bronchopulmonary aspergillosis. *Ann Intern Med*. 1979;91:378–82.
76. Chauhan B, Santiago L, Hutcheson PS, Schwartz HJ, Spitznagel E, Castro M, et al. Evidence for the involvement of two different MHC class II regions in susceptibility or protection in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol*. 2000;106:723–9.
77. Stevens DA, Moss RB, Kurup VP, Knutsen AP, Greenberger P, Judson MA, et al. Participants in the Cystic Fibrosis Foundation Consensus Conference. Allergic Bronchopulmonary Aspergillosis in Cystic Fibrosis - State of the Art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis*. 2003;37:225–64.
78. Knutsen AP, Kariuki B, Consolino JD, Warriar MR. IL-4 alpha chain receptor (IL-4Ralpha) polymorphisms in allergic bronchopulmonary aspergillosis. *Clin Mol Allergy*. 2006;17: 4–3.
79. Kraemer R, Deloséa N, Ballinari P, Gallati S, Cramer R. Effect of Allergic Bronchopulmonary Aspergillosis on Lung Function in Children with Cystic Fibrosis. *Am J Respir Crit Care Med*. 2006;174:1211–20.
80. Miller PW, Hamosh A, Macek Jr M, Greenberger PA, MacLean J, Walden SM, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet*. 1996;59:45–51.
81. Burns JL, Van Dalen JM, Shawar RM, Otto KL, Garber RL, Quan JM, et al. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. *J Infect Dis*. 1999;179:1190–6.
82. Skov M, Pressler T, Jensen HE, Hoiby N, Koch C. Specific IgG subclass antibody pattern to *Aspergillus fumigatus* in patients with cystic fibrosis with allergic bronchopulmonary aspergillosis (ABPA). *Thorax*. 1999;54:44–50.
83. Nikolaizik WH, Moser M, Cramer R, Little S, Warner JO, Blaser K, et al. Identification of allergic bronchopulmonary aspergillosis in cystic fibrosis patients by recombinant *Aspergillus fumigatus* IgA-specific serology. *Am J Respir Crit Care Med*. 1995;152:634–9.
84. Greenberger PA, Patterson R. Allergic bronchopulmonary aspergillosis and the evaluation of the patient with asthma. *J Allergy Clin Immunol*. 1988;81:646–50.
85. Hutcheson PS, Knutsen AP, Regent AJ, Slavin RG. A twelve year longitudinal study of *Aspergillus* sensitivity in patients with cystic fibrosis. *Chest*. 1996;110:363–6.
86. Cramer R, Hemmann S, Ismail C, Menz G, Blaser K. Disease-specific recombinant allergens for the diagnosis of allergic bronchopulmonary aspergillosis. *Int Immunol*. 1998;10:1211–6.
87. Schwartz HJ, Citron KM, Chester EH, Kaimal J, Barlow PB, Baum GL, et al. A comparison of the prevalence of sensitization to *Aspergillus* antigens among asthmatics in Cleveland and London. *J Allergy Clin Immunol*. 1978;62:9–14.
88. Hemmann S, Nikolaizik WH, Schoni MH, Blaser K, Cramer R. Differential IgE recognition of recombinant *Aspergillus fumigatus* allergens by cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Eur J Immunol*. 1998;28: 1155–60.
89. Geller DE, Kaplowitz H, Light MJ, Colin AA. Allergic bronchopulmonary aspergillosis in cystic fibrosis: reported prevalence, regional distribution, and patient characteristics. Scientific Advisory Group, Investigators, and Coordinators of the Epidemiologic Study of Cystic Fibrosis. *Chest*. 1999;116: 639–46.
90. Slavin RG, Hutcheson PS, Chauhan B, Bellone CJ. An overview of allergic bronchopulmonary aspergillosis with some new insights. *Allergy Asthma Proc*. 2004;25:395–9.
91. Hemmann S, Menz G, Ismail C, Blaser K, Cramer R. Skin test reactivity to 2 recombinant *Aspergillus fumigatus* allergens in A fumigatus-sensitized asthmatic subjects allows diagnostic separation of allergic bronchopulmonary aspergillosis from fungal sensitization. *J Allergy Clin Immunol*. 1999;104:601–7.
92. Hogaboam CM, Carpenter KJ, Schuh JM, Buckland KF. *Aspergillus* and asthma: any link? *Med Mycol* 2005;43:197–202.