Monitoring Respiratory Disease Severity in Cystic Fibrosis

Jane C Davies MB ChB MRCP MRCPCH MD and Eric WFW Alton MD FRCP FMedSci

Measurements of disease severity provide a guide for the physician to tailor therapies, for the patient and family to gauge progress, and are required for clinical trials. For many respiratory diseases, including cystic fibrosis, sensitive, noninvasive measurements are few, and some of those that are available are applicable only to certain subgroups of patients or lack sufficient sensitivity. We discuss currently available measurements in 4 groups: physiology, infection, inflammation, and radiology. For each group we highlight strengths and weaknesses, ask how we could improve upon these, and provide details of alternative methods. *Key words: cystic fibrosis, monitoring*. [Respir Care 2009;54(5):606–615. © 2009 Daedalus Enterprises]

Jane C Davies MB ChB MRCP MRCPCH MD is affiliated with the Department of Gene Therapy, Imperial College London, and the Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, London, United Kingdom. Eric WFW Alton MD FRCP FMedSci is affiliated with the Department of Gene Therapy, Imperial College London, and the Department of Respiratory Medicine, Royal Brompton Hospital, London, United Kingdom.

The authors have disclosed no conflicts of interest.

Dr Davies presented a version of this paper at the 43rd RESPIRATORY CARE Journal Conference, "Respiratory Care and Cystic Fibrosis," held September 26-28, 2008, in Scottsdale, Arizona.

Correspondence: Jane C Davies MB ChB MRCP MRCPCH MD, Department of Gene Therapy, Imperial College, Emmanuel Kaye Building, Manresa Road, London SW3 6NP, United Kingdom. E-mail: j.c.davies@imperial.ac.uk.

Introduction

Every interaction between a person with cystic fibrosis (CF) and a health professional will involve some degree of assessment of well-being and disease status. Consensus documents provide guidance as to which tools should be employed, both routinely and for more detailed annual assessments.[1,2] So, are the tools we have at our disposal and the methods with which we employ them up to the task? Do they provide sufficient insight into an individual's current status and allow us to gauge the direction and rate of progression of disease? Do they provide us with the optimal pieces of information that allow us to tailor therapeutic interventions, assess their success, and provide prognostic information? In this paper we argue that the answer to these questions is "no". We discuss the investigations in common use, with their limitations, some of which are specific to disease stage or age group. Finally, we discuss newer techniques, many of which are only performed in the research setting, which may offer a more sensitive and detailed insight into lower airway disease in the future.

What Should Be the Goals of Monitoring?

It is our opinion that we should perform measurements that, either alone or in combination, are: sensitive; allow detection of change both long-term and short-term; repeatable and reproducible; minimally invasive or noninvasive and well-tolerated; applicable across age groups and to patients of different illness severity; and complementary in the information they provide. Such an approach would allow individually tailored management and provide long-term prognostic information to patients and professionals.

Current Monitoring

Techniques in current clinical use and listed in management guidelines fall broadly into 4 groups: physiology, infection, inflammation, and radiology. For each of these categories we outline strengths and limitations and make some suggestions as to how we could do better in the future.

Physiology

Current Clinical Practice

Patients old enough to form a seal with their lips and perform prolonged forced expiratory maneuvers routinely undergo spirometry at every clinic visit and at periods of clinical instability or exacerbation. In addition, annual plethysmographic and diffusion-capacity (gas-transfer) measurements are recommended in some guidelines.

Spirometry. Spirometry has long been the accepted standard in disease monitoring. Forced expiratory maneuvers such as forced expiratory volume in the first second (FEV_1) and forced vital capacity are well understood, and almost universally FEV_1 is used to define mild ($> 60\%$ or 70% of predicted), moderate, and severe ($< 40\%$ or 30%) disease. The predicted values have been generated with various models, based on healthy persons and height, sex, and age. The different reference ranges should be borne in mind when comparing or extrapolating data sets, and absolute values should therefore also be obtained and recorded.

What Are the Problems With Spirometry? Although the coefficient of variability for FEV_1 in healthy people is reported to be around 2–3%,[3] it is much higher in patients with CF[4] and for flows at lower lung volumes (eg, the forced expiratory flow during the middle half of the forced expiratory maneuver [$FEF_{25\%-75\%}$]), which may reflect small airways, which is the site of interest. The measurements are highly technique-dependent and effort-dependent. Some patients find such maneuvers difficult, and they are not routinely performed in young children ≤ 5 years old. The measurements also lack sensitivity, particularly in mild, early stages of disease or when looking for small changes in response to an intervention, and there is currently a very slow rate of decline (1–2% per year) in the CF population treated in modern centers.[5] This means that, though patients who are deteriorating rapidly or over a short time period can be easily identified, observing any improvement on this rate of decline in an individual patient will be almost impossible. Finally, although FEV_1 has historically been used in defining severity, there is some evidence to suggest it is not a very useful tool on which to base prognosis; FEV_1 is no longer included in the lung allocation score[6] as part of transplant-waiting-list assessment in the United States.

Plethysmography. Lung volumes and diffusion capacity are listed in some of the current consensus documents for annual assessment, and are performed in the majority of large centers, at least in Europe.

What Are the Problems With Plethysmography? These techniques are expensive, technically challenging, and time-consuming for staff and patient. Similarly to spirometry, they require cooperation and are, in general, not suitable for very young patients. In addition, they are probably less well understood by the clinical team, and in our experience the results may not in fact be paid a great deal of attention. Finally, recent data suggest that lung-volume values add very little to spirometry for the majority of patients.[7]

So we appear to be using expensive resources, in terms of both equipment and skilled manpower, and asking patients to spend substantial time, for a relatively small gain.

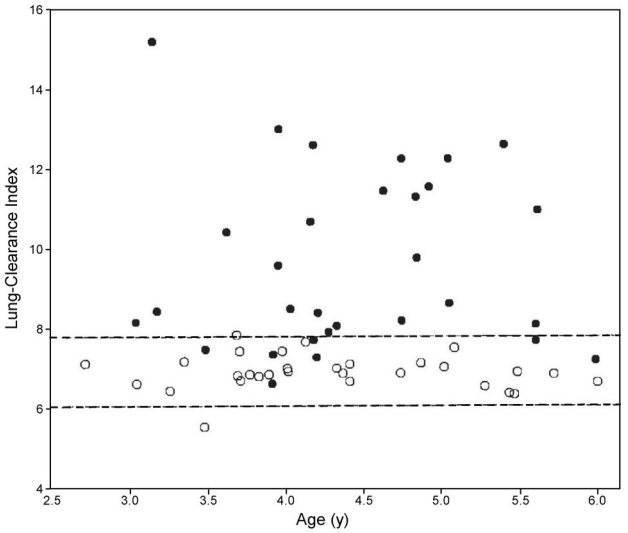


Fig. 1. There is a narrow range of lung-clearance index in normal subjects (circles), compared to patients with cystic fibrosis (dots), and this is similar for older children and adults, which obviates adjustment for age or size. (From Reference 9, with permission.)

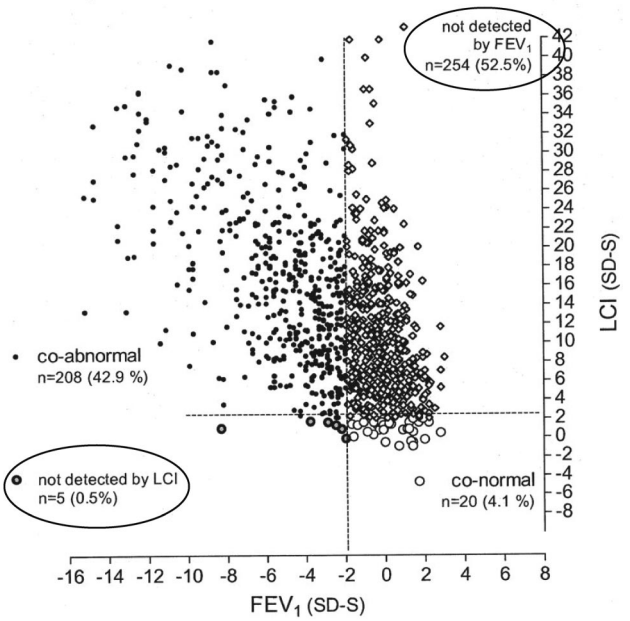


Fig. 2. In the early stages of cystic fibrosis lung disease, the lung-clearance index (LCI) is more sensitive than standard spirometry. Of 274 children with a normal forced expiratory volume in the first second (FEV_1) Z score, 254 (93%) had an abnormality detected via LCI. In contrast, the LCI failed to detect an abnormal FEV_1 in only 2% (5 of 213) of patients. SD-S = standard deviation score. (From Reference 12, with permission.)

How Could We Do Better?

Several new approaches show substantial promise and may be clinically useful in the near future.

Lung-Clearance Index. The lung-clearance index uses multiple-breath wash-out of a nonabsorbable gas (originally nitrogen, but, more commonly now, sulfur hexafluoride) to measure ventilation inhomogeneity caused by airway narrowing from inflammation or partial mucus obstruction. The subject inhales a low concentration of sulfur hexafluoride, via either mask or mouthpiece, until the concentration in the lung is in equilibrium with the concentration administered (wash-in phase). The supply is then switched off and, during continued tidal breathing, wash-out is monitored. Gas analyzers include conventional mass spectrometer and, more recently, photoacoustic and ultrasonic-based technologies. Wash-out is defined, for practical purposes, as the point when the sulfur hexafluoride reaches 1/40th of its original concentration. Patients with more severe disease take longer to wash out, because gas is trapped in narrowed airways; therefore, they have a higher lung-clearance index.

One advantage of the lung-clearance index is that it has a relatively narrow range of normal values that changes very little with age, which obviates the requirement for age/size-adjusted normal values (Fig. 1).[8] The technique is also: harmless; easy to perform (requires only tidal breathing, and no additional coordination, cooperation, or forced maneuvers); can be performed at all ages, including infancy and pre-school ages[8-10]; repeatable, reproducible,[11] and more sensitive at the early stages of disease than is spirometry (Fig. 2).[12] Finally, it is at least as sensitive as forced expiratory maneuvers in infants[10] and correlates better with structural changes on high-resolution computed tomography (CT) than does FEV_1 (Table 1).[13]

An important disadvantage of the lung-clearance index is that completely obstructed lung regions do not contribute to the overall measurement because the inhaled gas does not reach those regions. So in patients who have totally obstructed lung regions, the lung-clearance index could underestimate disease severity. Also, the technique may be more burdensome for the most severely affected patients, who require much longer wash-in and wash-out times. Some of the more portable technologies, such as the Innocor, which relies on photoacoustic analysis of exhaled gas, may currently be less applicable with small children (who have faster respiratory rates), because of the somewhat slower response time than a mass spectrometer. However, mass spectrometers are expensive to set up and may be challenging to maintain.

Lung Function Tests Applicable to Infants and Pre-School Children. The last decade has seen a massive increase in the number of studies that reported lung function in infants and young children, many of which have focused on CF. Consensus guidelines have been published.[14] It is clear from the studies that: sensitive mea-

Table 1. Agreement Between Lung-Clearance Index, FEV_1, and FEF_{75}, and Structural Lung Changes Classified as Abnormal or Normal in a Study of Patients With Cystic Fibrosis ($n = 44$)

	Bronchiectasis		HRCT Score		Air-Trapping	
	Yes	No	> 5%	< 5%	> 30%	< 30%
Lung-Clearance Index (*n*)*						
Abnormal	22	9	25	6	15	16
Normal	4	9	2	11	1	12
	($P = .03$)		($P < .001$)		($P = .03$)	
FEV_1 (*n*)						
Abnormal	5	2	7	0	4	3
Normal	21	16	20	17	12	25
	($P = .76$)		($P = .06$)		($P = .41$	
FEF_{75} (*n*)						
Abnormal	16	3	17	2	12	7
Normal	10	15	10	15	4	21
	($P = .008$)		($P = .003$)		($P = .004$)	

* In a study of patients with cystic fibrosis, there was strong agreement between structural abnormalities identified via high-resolution computed tomography (HRCT) and lung-clearance index or forced expiratory flow at 75% of the forced expiratory maneuver (FEF_{75}). In contrast, agreement with forced expiratory volume in the first second (FEV_1) was poor. (Adapted from Reference 13.)

surements can detect abnormalities in pre-symptomatic babies with CF[10,15]; these changes occur early, although there may be a window of preserved lung function during the first few months of life in babies diagnosed via newborn screening[16]; once physiologic changes are present, these may persist despite the initiation of standard management, so children with CF fail to catch up to their healthy peers (Fig. 3)[15,17]; and it has been difficult to explain these findings on the basis of infection and/or inflammation, from the limited number of studies that have included bronchoalveolar lavage (BAL) fluid.[16] Further research is needed.

Unfortunately, there are few specialized infant and preschool child lung-function-testing laboratories. Most such work is done in the research setting. We hope that in the future some of these measurements will be routinely performed in the clinic on young children with CF, or that easier measurement methods will be established, which would allow less specialized laboratories to participate.

Infection

Infection of the lower respiratory tract occurs early in CF, with what we had believed, until recently, to be a relatively well defined group of bacteria.[18] In addition, viral infections are thought to play an important role, for example, in infective exacerbations, although this role has been less well studied and is not completely clear. Fungi, in particular *Aspergillus fumigatus*, cause problems with allergic sensitization, and there is also a substantial disease burden from nontuberculous mycobacteria, most notably, *Mycobacterium abscessus*.

Current Clinical Practice

The importance of bacterial infection has long been recognized, and modern treatment is based on attempts to identify organisms early, eradicate them if possible, and suppress their numbers in the chronically infected state. To this end, guidelines recommend: culture and sensitivity at every out-patient consultation and at the start and during admissions for intravenous antibiotic treatment; that nonexpectorating patients (the majority of children) undergo oropharyngeal, cough swab, or cough plate cultures; and that CF subjects with their first isolation of *Pseudomonas aeruginosa* should undergo eradication therapy,[19] which is usually a combination of nebulized and systemic (oral or intravenous) antibiotics. Cross-infection between patients should be limited by strict infection-control protocols that prevent patient contact and emphasize the importance of simple measures such as staff handwashing.[20] Patients with particularly worrisome bacteria such as organisms of the *Burkholderia cepacia* complex, should attend separate clinics.

What Are the Problems?

It is our opinion that there are many opportunities for improvement regarding both the ways we obtain samples and the detection/sensitivity of the methods in conventional laboratories. It is clear from the literature that the methods of sampling from nonexpectorating patients lack sensitivity and specificity.[21,22] Some patients have repeatedly negative cultures despite substantial disease; it is unknown whether this truly reflects a sterile lower airway,

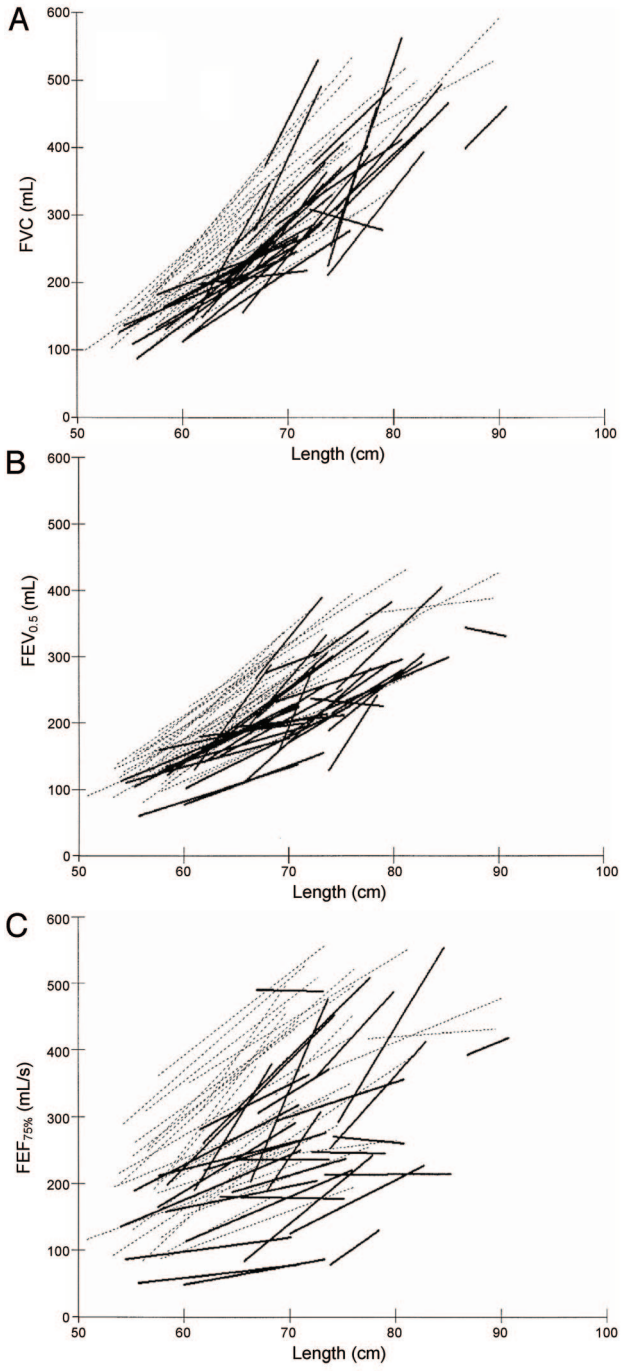


Fig. 3. Infants with cystic fibrosis (solid lines) have significantly poorer lung physiology, based on forced expiratory volume in the first 0.5 second ($FEV_{0.5}$), forced vital capacity (FVC), and forced expiratory flow at 75% of the forced expiratory maneuver (FEF_{75}) than do their healthy counterparts (dashed lines) at diagnosis, even in the absence of any previous respiratory symptoms. And once treatment is initiated, they do not catch up: the cystic fibrosis cohort continues to have lower values than the controls. (From Reference 15, with permission.)

but recent molecular tools suggest that is unlikely (see below). In vitro sensitivity testing has important limitations in terms of translation into clinical practice; commonly, patients respond to antibiotics when they have organisms that are believed to be resistant, or fail to respond to theoretically useful combinations. This most likely reflects the different growth conditions of bacteria on culture plates compared to within biofilms in the airway. Finally, we have rather blunt tools with which to assess microbiological response to interventions. Quantification of bacterial load is via serial dilution and culture of sputum or BAL; however, different numbers, and even different pathogens, have been obtained from different areas of individual patients' lungs,[23] which makes this measurement prone to noise. It is not clear whether in fact a clinical response requires such a decrease, or, rather, therapies in some way alter bacterial phenotype (eg, expression of virulence factors). Furthermore, most clinical laboratories focus on bacterial (and fungal) detection systems. Virological detection methods are rarely employed, and the role of viruses in CF pathophysiology is rather poorly understood.

How Could We Do Better?

This topic is covered in greater detail in another paper from this Journal Conference.[18] There are several opportunities for improvement, some of which could be made available now, whereas others require further research, development, and validation.

1. We need a clearer picture of which bacteria, viruses, and fungi are infecting an individual's airway at any one time, and data to help us understand which of these are most likely to be detrimental to respiratory health. More sensitive measurements of microbial response to treatment would also be of benefit. Ways to achieve these aims might include:

a. Molecular tools to identify the pathogens probably present in most patients' airways, without the limitations of conventional culture[24]

b. Longitudinal studies with those molecular tools to distinguish between detrimental and nonharmful (or even beneficial?) organisms

c. Qualitative bacterial assays (such as measurement of virulence factors) and an understanding of how (if at all) these impact clinical status, which would allow us to move beyond relying solely on quantitative data

d. Noninvasive techniques to identify and quantify surrogate markers of infection,[25,26] such as volatile bacterial products in breath or condensate ("electronic nose" technology)

2. We need better, more relevant systems for determining microbial sensitivity and resistance patterns. The existing systems for growing bacteria in biofilms in the laboratory are complex and cumbersome, but might provide more useful information on which to base treatment decisions.[27,28]

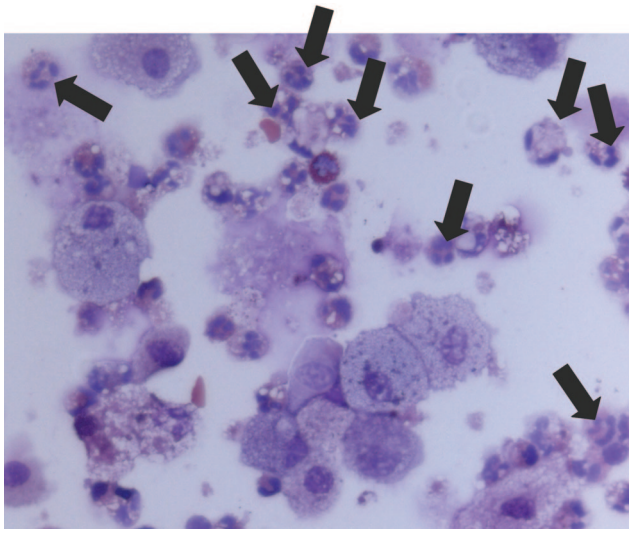


Fig. 4. In contrast to bronchoalveolar lavage fluid from healthy patients, which is largely macrophage-dominated, there is a massive excess of neutrophils (arrows) in both sputum and bronchoalveolar lavage fluid from patients with cystic fibrosis. The number of neutrophils increases as disease severity progresses, and the levels of soluble mediators (eg, elastase) released by those cells inversely correlate with lung function. (Courtesy of Thomas N Hilliard MD, Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, London, United Kingdom.)

Inflammation

Severe neutrophil-mediated inflammation is characteristic of CF (Fig. 4). It occurs early in life, is exaggerated compared to non-CF subjects, and is prolonged. It is also incompletely effective and commonly fails to eradicate the microbes to which it is directed. Some data suggest that the CF airway may be inherently pro-inflammatory, although the CF world is divided on this issue.

Current Clinical Practice

Broadly speaking, although measurements of inflammation are often performed in clinical trials, they do not play a part in routine clinical monitoring.

What Are the Problems?

We accept inflammation as key in the irreversible airway damage in CF. Indeed, much research attention focuses on therapeutic anti-inflammatory strategies. However, we have no tools with which we routinely assess inflammation in clinical practice. If we consider inflammation to be so important, shouldn't we be developing these tools?

How Could We Do Better?

For the majority of chronic lung diseases, measurements of airway inflammation have been extensively reported in the literature, but with the exception perhaps of exhaled nitric oxide (NO) in asthma, the majority of these have been in the research setting. Techniques include: direct sputum analysis (sometimes after induction with hypertonic saline); BAL; exhaled breath; exhaled-breath condensate; and airway mucosal blood flow measurements. Indirect evidence of airway inflammation may also come from measurements in blood or urine.

Sputum. CF sputum contains high levels of inflammatory cells, pro-inflammatory cytokines, and proteolytic enzymes; certain of these appear to correlate with other measurements of pulmonary severity, such as spirometry, although whether they cause the pulmonary damage is less clear. There is also a well-described relative deficiency of anti-inflammatory cytokines (eg, interleukin 10) and antiproteases. Cytokines can be measured reproducibly, even in sputum from young children,[29] and several of these are reduced by treatment with conventional intravenous antibiotics[30,31] and other therapies. A study that found changes in sputum cytokines after nebulized heparin found no corresponding changes in spirometry,[32] which may suggest either that these measurements are more sensitive to detect change than conventional lung function, or may simply reflect the fact that changes in lung function may only occur later. Encouragingly, results suggest that spontaneously expectorated sputum and samples obtained via induction methods based on nebulization of hypertonic saline are not significantly different with respect to inflammatory markers. This technique has been confirmed as safe in children with CF, which is the group in which it is most likely to be required.[33] One limitation of the interpretation of these data is the varied and often unvalidated methods that have been used in the processing stage. For example, mucolytics such as dithiothreitol are often used. Dithiothreitol, by cleaving disulphide bonds, affects the levels of many proteins and may adversely affect reagents in the assay system.[34] A second concern relates to the lack of standardization of the nature and concentration of protease inhibitors used. Both of these issues may be specific to the type of assay used and the substance measured. There is an urgent need to address these methodological issues, specifically for CF sputum, before this test can be routinely used in the clinical context.

Bronchoalveolar Lavage. BAL is often considered the accepted standard technique for airway sampling. Similar patterns of inflammation have been described as in sputum, although there is a paucity of data to compare in-

flammation in those 2 types of sample. However, BAL, whether bronchoscopic or nonbronchoscopic, is highly invasive and not easily repeated in a short time period. Adverse effects such as fever have been reported, although this is rarely important in our experience. Extensive safety data in young children were recently reported by the Australasian study that addressed the utility of regular versus symptomatic BAL, further results of which are eagerly awaited.[35] Further limitations include the large and unknown dilution factor (markers for dilution are of limited use, and recent guidelines suggest they are not helpful[36]) and the fact that the technique samples only a small part of the airway, which may be problematic in a disease known to be inhomogeneous.

Exhaled Breath. Both exhaled breath and breath condensate are easy and noninvasive to obtain, and can be used reproducibly, even in young children, as long as attention is paid to methodological detail. Much interest has focused on the observation that the level of exhaled NO is reduced in CF. Given the anti-inflammatory and anti-infective properties of NO, some think it may play an important primary role in CF pathophysiology—a hypothesis supported by the low level of NO synthase messenger ribonucleic acid in relatively undamaged airways.[37] An alternative view is that NO production is itself adversely affected by inflammation and that the low NO level is *secondary* to CF lung disease. The NO level is extremely low in primary ciliary dyskinesia, a disease with a generally much better outlook than CF. A recent clinical trial of orally administered L-arginine (an NO donor) with CF subjects used exhaled NO as an outcome and reported a sustained increase in NO production, although this was not mirrored by any significant effect on lung function.[38] However, no studies have addressed longitudinal change or correlations with other clinical variables, which would support this measure as a useful monitoring tool in the clinic.

Exhaled-Breath Condensate. Condensate can be collected simply by asking a subject, even a quite small child, to exhale into a cold tube during tidal breathing. The condensate contains a small (but variable and undetermined) volume of airway-lining fluid, the pH of which is abnormally low in CF (Fig. 5)[39] and other inflammatory airway diseases.[40] Thus, the condensate provides an "inflammamometer" to assess interventions. However, this technique appears to lack sufficient sensitivity for use with an individual patient. Attempts to measure other substances from patients with CF have met with variable success, which might be partly related to methodological issues.[41-43]

Airway Mucosal Blood Flow. A universal downstream effect of inflammation at any site in the body is an increase in blood flow. Measurements to detect this, based on the rate of disappearance of an inhaled, absorbable gas from the airway, have shown some promise in asthma,[44] in which studies have found a raised level, which is reduced by anti-inflammatory agents. There are, as yet, no published data available from patients with CF, and current techniques require multiple, accurately-timed breath-hold maneuvers, which would probably restrict the technique to older children and adults. However, this type of technique, unlike measurements of specific inflammatory markers, is not dependent on a complete understanding of the complex inflammatory milieu within the airway, and may therefore be more applicable as a generalized marker of inflammation.

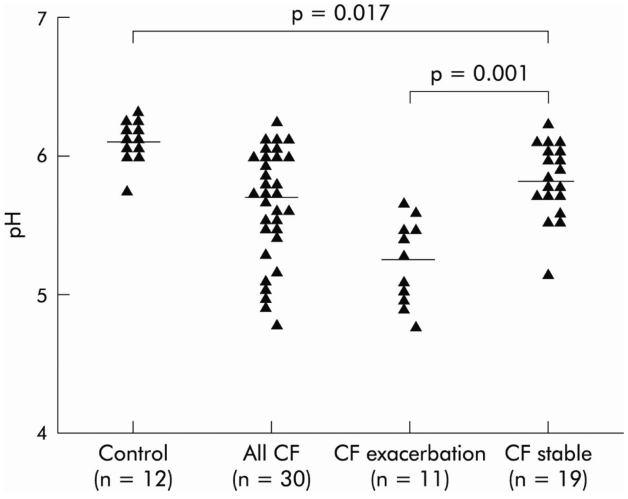


Fig. 5. Exhaled-breath condensate from patients with cystic fibrosis (CF) is significantly more acidic than that from healthy controls. Patients with and without CF pulmonary exacerbation also had significant pH differences. This is unlikely to be directly related to the cystic fibrosis transmembrane regulator defect, as it has also been shown to occur in other inflammatory airway diseases, such as asthma. (From Reference 39, with permission.)

Blood. Blood and serum markers, including inflammatory (total white blood cells and differential) cell counts and acute-phase reactants such as C-reactive protein and immunoglobulin G, are recommended in many current clinical guidelines. Together with circulating cytokine levels, they have been used both as efficacy and safety outcome measurements in CF clinical trials. In many studies in both those contexts they have proved useful. However, although there are not the same methodological issues as exist with the airway-sampling techniques described above, blood/serum would probably be considered by most to be an adjunct to, rather than a substitute for, such direct measurements. No studies have found a tight correlation between such measurements and direct airway inflammation.

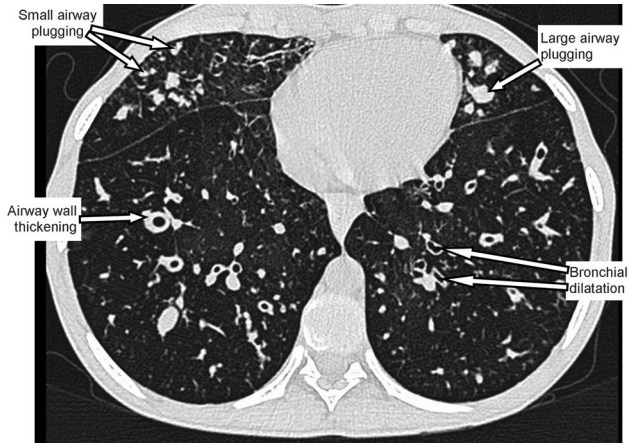


Fig. 6. Typical changes on a computed tomogram of a patient with moderately severe cystic fibrosis lung disease.

Urine. Several groups have reported increased tissue-degradation products, such as desmosine and isodesmosine, in the urine of patients with chronic lung diseases, including CF,[45] but the levels fluctuate rapidly, which might limit the applicability of these measurements in the clinical (or trial) context.

Radiology

Current Clinical Practice

Consensus guidelines recommend plain chest radiographs as part of the annual detailed assessment and at other periods of clinical concern.

What Are the Problems?

Plain chest radiographs lack sensitivity, particularly in the early stages of disease. The scoring systems in clinical use also differ markedly, and although some researchers have reported confidence in the utility of these, others view them as highly subjective and lacking in consistency. However, they carry a low radiation burden and are relatively cheap.

How Could We Do Better?

Computed Tomography. A very small number of centers around the world advocate regular (every 1–2 years) CT, on the basis that CT is more sensitive than radiograph, and this was the topic of recent good reviews.[46,47] The changes at various disease stages include air-trapping and bronchial wall thickening (Fig. 6), which appear to be at least partially reversible, which renders them useful markers of clinical progression and response to therapy. Several scoring systems, of various complexities, have been devised, and some authors strongly favor composite scores with, for example, spirometric indices.[48] The routine use of CT in clinical practice is not proposed in the majority of consensus guidelines and has not been widely accepted by the CF community, in particular by pediatricians, probably because the risk from radiation is thought to outweigh the benefits of the knowledge gained from the CT. However, the radiation is substantially less with some of the more modern CT scanners[49] and could be further reduced with a CT protocol that takes fewer CT slices. The requirement that the patient lie still and (as advocated by some) perform respiratory maneuvers[50] may limit the use of CT in some age groups.

Magnetic Resonance Imaging. Magnetic resonance imaging was, until recently, widely regarded as lacking sufficient resolution for lung imaging, but in a small clinical trial the addition of hyperpolarized helium 3 improved the sensitivity so that significant differences were visible after bronchodilator treatment.[51] If further progress is made, this is potentially an attractive, radiation-free technique.

Positron Emission Tomography. This technique is relatively new in the context of lung disease. Labeled glucose uptake indicates areas of inflammation. A recent study found greater uptake in patients with CF than in healthy controls, and the difference was particularly marked in subjects with more impaired spirometry, and correlated with BAL neutrophilia.[52] Research is ongoing on positron emission tomography in lung disease.

Summary

Patients with CF in developed countries are surviving longer than ever before. In the United Kingdom, adults with CF now outnumber children with CF, and almost 50% of United States patients with CF are over 18 years old. Milder disease and a slower rate of decline make monitoring more difficult, whereas the increasing number of interventions available, and the fact that response to treatment is often unpredictable, make the requirement for good monitoring interventions even more pressing. This is particularly problematic in certain patient groups, such as infants and young children. There are several opportunities for improved monitoring with currently available techniques, and many new techniques are under investigation, fuelled largely by the requirement of more sensitive measurements for clinical trials. As an example, the United Kingdom CF Gene Therapy Consortium[53] is assessing newer techniques in both interventional and longitudinal clinical studies, to help determine which outcome measurements to use in our forthcoming multi-dose gene-therapy trial. We hope that certain of these techniques, and perhaps others as yet undeveloped, will show sufficient

promise for routine clinical use and give us more insight into our patients' respiratory health.

REFERENCES

1. Cystic Fibrosis Trust. Standards for the clinical care of children and adults with cystic fibrosis in the UK 2001: a revised, expanded and referenced version of the Cystic Fibrosis Trust's 1996 guidelines. May 2001. http://www.cftrust.org.uk/aboutcf/publications/consensusdoc/c_3000standards_of_care.pdf. *Accessed March 18, 2009.*
2. Kerem E, Conway S, Elborn S, Heijerman H; Consensus Committee. Standards of care for patients with cystic fibrosis: a European consensus. J Cyst Fibros 2005;4(1):7-26.
3. Cotes JE, Leathart GL Lung function, physiology, measurement and application in medicine, 6th edition. Blackwell Science; 1993.
4. Cooper PJ, Robertson CF, Hudson IL, Phelan PD. Variability of pulmonary function tests in cystic fibrosis. Pediatr Pulmonol 1990; 8(1):16-22.
5. Que C, Cullinan P, Geddes D. Improving rate of decline of FEV_1 in young adults with cystic fibrosis. Thorax 2006;61(2):155-157.
6. Davis SQ, Garrity ER Jr. Organ allocation in lung transplant. Chest 2007;132(5):1646-1651.
7. Rosenthal M. Annual assessment spirometry, plethysmography, and gas transfer in cystic fibrosis: do they predict death or transplantation. Pediatr Pulmonol 2008;43(10):945-952.
8. Aurora P, Gustafsson P, Bush A, Lindblad A, Oliver C, Wallis CE, Stocks J. Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. Thorax 2004; 59(12):1068-1073.
9. Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, et al; London Cystic Fibrosis Collaboration. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2005;171(3):249-256.
10. Lum S, Gustafsson P, Ljungberg H, Hülskamp G, Bush A, Carr SB, et al; London Cystic Fibrosis Collaboration. Early detection of cystic fibrosis lung disease: multiple-breath washout versus raised volume tests. Thorax 2007;62(4):341-347.
11. Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, et al. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. Thorax 2008;63(2):135-140.
12. Kraemer R, Blum A, Schibler A, Ammann RA, Gallati S. Ventilation inhomogeneities in relation to standard lung function in patients with cystic fibrosis. Am J Respir Crit Care Med 2005;171(4):371-378.
13. Gustafsson PM, De Jong PA, Tiddens HA, Lindblad A. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. Thorax 2008;63(2):129-134.
14. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med 2007;175(12):1304-1345.
15. Ranganathan SC, Stocks J, Dezateux C, Bush A, Wade A, Carr S, et al. The evolution of airway function in early childhood following clinical diagnosis of cystic fibrosis. Am J Respir Crit Care Med 2004;169(8):928-933.
16. Linnane BM, Hall GL, Nolan G, Brennan S, Stick SM, Sly PD, et al.; on behalf of the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF). Lung function in infants with cystic fibrosis diagnosed by newborn screening. Am J Respir Crit Care Med 2008;178(12):1238-1244.
17. Kozlowska WJ, Bush A, Wade A, Aurora P, Carr SB, Castle RA, et al.; London Cystic Fibrosis Collaboration. Lung function from infancy to the preschool years after clinical diagnosis of cystic fibrosis. Am J Respir Crit Care Med 2008;178(1):42-49.
18. Davies JC, Bilton D. Bugs, biofilms, and resistance in cystic fibrosis. Respir Care 2009;54(5):628-638; discussion 638-640.
19. Wood DM, Smyth AR. Antibiotic strategies for eradicating *Pseudomonas aeruginosa* in people with cystic fibrosis. Cochrane Database Syst Rev 2006;(1):CD004197.
20. Festini F, Buzzetti R, Bassi C, Braggion C, Salvatore D, Taccetti G, Mastella G. Isolation measures for prevention of infection with respiratory pathogens in cystic fibrosis: a systematic review. J Hosp Infect 2006;64(1):1-6.
21. Equi AC, Pike SE, Davies J, Bush A. Use of cough swabs in a cystic fibrosis clinic. Arch Dis Child 2001;85(5):438-439.
22. Rosenfeld M, Emerson J, Accurso F, Armstrong D, Castile R, Grimwood K, et al. Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis. Pediatr Pulmonol 1999;28(5):321-328.
23. Gutierrez JP, Grimwood K, Armstrong DS, Carlin JB, Carzino R, Olinsky A, et al. Interlobar differences in bronchoalveolar lavage fluid from children with cystic fibrosis. Eur Respir J 2001;17(2): 281-286.
24. Rogers GB, Carroll MP, Serisier DJ, Hockey PM, Jones G, Kehagia V, et al. Use of 16S rRNA gene profiling by terminal restriction fragment length polymorphism analysis to compare bacterial communities in sputum and mouthwash samples from patients with cystic fibrosis. J Clin Microbiol 2006;44(7):2601-2604.
25. Carroll W, Lenney W, Wang T, Spanel P, Alcock A, Smith D. Detection of volatile compounds emitted by *Pseudomonas aeruginosa* using selected ion flow tube mass spectrometry. Pediatr Pulmonol 2005;39(5):452-456.
26. Ryall B, Davies JC, Wilson R, Shoemark A, Williams HD. *Pseudomonas aeruginosa*, cyanide accumulation and lung function in CF and non-CF bronchiectasis patients. Eur Respir J 2008;32(3):740-747.
27. Moskowitz SM, Foster JM, Emerson JC, Gibson RL, Burns JL. Use of *Pseudomonas* biofilm susceptibilities to assign simulated antibiotic regimens for cystic fibrosis airway infection. J Antimicrob Chemother 2005;56(5):879-886.
28. Caraher E, Reynolds G, Murphy P, McClean S, Callaghan M. Comparison of antibiotic susceptibility of *Burkholderia cepacia* complex organisms when grown planktonically or as biofilm in vitro. Eur J Clin Microbiol Infect Dis 2007;26(3):213-216.
29. Ordonez CL, Kartashov AI, Wohl ME. Variability of markers of inflammation and infection in induced sputum in children with cystic fibrosis. J Pediatr 2004;145(5):689-692.
30. Colombo C, Costantini D, Rocchi A, Cariani L, Garlaschi ML, Tirelli S, et al. Cytokine levels in sputum of cystic fibrosis patients before and after antibiotic therapy. Pediatr Pulmonol 2005;40(1):15-21.
31. Ordonez CL, Henig NR, Mayer-Hamblett N, Accurso FJ, Burns JL, Chmiel JF, et al. Inflammatory and microbiologic markers in induced sputum after intravenous antibiotics in cystic fibrosis. Am J Respir Crit Care Med 2003;168(12):1471-1475.
32. Ledson M, Gallagher M, Hart CA, Walshaw M. Nebulized heparin in *Burkholderia cepacia* colonized adult cystic fibrosis patients. Eur Respir J 2001;17(1):36-38.
33. Suri R, Marshall LJ, Wallis C, Metcalfe C, Shute JK, Bush A. Safety and use of sputum induction in children with cystic fibrosis. Pediatr Pulmonol 2003;35(4):309-313.
34. Kim JS, Hackley GH, Okamoto K, Rubin BK. Sputum processing for evaluation of inflammatory mediators. Pediatr Pulmonol 2001; 32(2):152-158.
35. Wainwright CE, Grimwood K, Carlin JB, Vidmar S, Cooper PJ, Francis PW, et al. Safety of bronchoalveolar lavage in young children with cystic fibrosis. Pediatr Pulmonol 2008;43(10):965-972.
36. Haslam PL, Baughman RP. Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL. Eur Respir J 1999;14(2):245-248.

37. Moeller A, Horak F Jr, Lane C, Knight D, Kicic A, Brennan S, et al. Inducible NO synthase expression is low in airway epithelium from young children with cystic fibrosis. Thorax 2006;61(6):514-520.
38. Grasemann H, Grasemann C, Kurtz F, Tietze-Schillings G, Vester U, Ratjen F. Oral L-arginine supplementation in cystic fibrosis patients: a placebo-controlled study. Eur Respir J 2005;25(1):62-68.
39. Tate S, MacGregor G, Davis M, Innes JA, Greening AP. Airways in cystic fibrosis are acidified: detection by exhaled breath condensate. Thorax 2002;57(11):926-929.
40. Carpagnano GE, Barnes PJ, Francis J, Wilson N, Bush A, Kharitonov SA. Breath condensate pH in children with cystic fibrosis and asthma: a new noninvasive marker of airway inflammation? Chest 2004;125(6):2005-2010.
41. Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. Thorax 2005;60(1):22-26.
42. Carpagnano GE, Barnes PJ, Geddes DM, Hodson ME, Kharitonov SA. Increased leukotriene B4 and interleukin-6 in exhaled breath condensate in cystic fibrosis. Am J Respir Crit Care Med 2003; 167(8):1109-1112.
43. Rosias PP, Dompeling E, Hendriks HJ, Heijnens JW, Donckerwolcke RA, Jobsis Q. Exhaled breath condensate in children: pearls and pitfalls. Pediatr Allergy Immunol 2004;15(1):4-19.
44. Wanner A, Mendes ES, Atkins ND. A simplified noninvasive method to measure airway blood flow in humans. J Appl Physiol 2006; 100(5):1674-1678.
45. Bode DC, Pagani ED, Cumiskey WR, von Roemeling R, Hamel L, Silver PJ. Comparison of urinary desmosine excretion in patients with chronic obstructive pulmonary disease or cystic fibrosis. Pulm Pharmacol Ther 2000;13(4):175-180.
46. Robinson TE. High-resolution CT scanning: potential outcome measure. Curr Opin Pulm Med 2004;10(6):537-541.
47. Brody AS. Scoring systems for CT in cystic fibrosis: who cares? Radiology 2004;231(2):296-298.
48. Robinson TE, Leung AN, Northway WH, Blankenberg FG, Chan FP, Bloch DA, et al. Composite spirometric-computed tomography outcome measure in early cystic fibrosis lung disease. Am J Respir Crit Care Med 2003;168(5):588-593.
49. Huda W. Radiation doses and risks in chest computed tomography examinations. Proc Am Thorac Soc 2007;4(4):316-320.
50. Long FR. High-resolution computed tomography of the lung in children with cystic fibrosis: technical factors. Proc Am Thorac Soc 2007;4(4):306-309.
51. Mentore K, Froh DK, de Lange EE, Brookeman JR, Paget-Brown AO, Altes TA. Hyperpolarized HHe 3 MRI of the lung in cystic fibrosis: assessment at baseline and after bronchodilator and airway clearance treatment. Acad Radiol 2005;12(11):1423-1429.
52. Chen DL, Ferkol TW, Mintun MA, Pittman JE, Rosenbluth DB, Schuster DP. Quantifying pulmonary inflammation in cystic fibrosis with positron emission tomography. Am J Respir Crit Care Med 2006;173(12):1363-1369.
53. UK Cystic Fibrosis Gene Therapy Consortium. Current research. http://www.cfgenetherapy.org.uk/consortiumresearch.htm. *Accessed March 18, 2009*.

Discussion

Geller: The lung-clearance index seems like a nice, noninvasive, sensitive way of evaluating early disease, and we're struggling with outcome measures in clinical trials where we're dealing with healthier and healthier kids with CF, in whom we don't see large changes in variables such as FEV_1, which is the standard outcome measure. I would think the lung-clearance index would be high on that list of new variables to consider, but in the United States it's not receiving a lot of interest yet. How difficult and/or expensive is it to set up for and collect the data to calculate the lung-clearance index? Has it been commercialized or standardized?

Davies: It's extremely easy, for the patient and the technician, after a quite short training. It relies on tidal breathing and requires either a mask or that the patient be able to keep a lip-seal on a mouthpiece. It requires patient cooperation, and we've found that providing a cartoon or something to watch usually achieves that cooperation. The setup required used to be much more difficult, because all the techniques were based on mass spectrometry, and the machinery was constantly breaking down, unreliable, or difficult or expensive to maintain, but the machines are now commercially available, although they're not being heavily marketed.

We're using an Innocor photoacoustic machine, which is very much easier to use. I think it costs about $30,000 or $40,000. We usually do 3 tests, which takes up to a half an hour. In very severely affected patients, wash-in and wash-out take much longer. Of the patient outcome variables we have to choose from and are considering, I think the airway-clearance index currently tops the list. We'd never use FEV_1.

Ratjen: Though the Innocor lung-clearance-index technique may be fine for older individuals, it may not be ideal for infants, with whom there have been some issues with this technology. For infants you probably still have to use a mass-spectrometry-based system, which costs about $100,000 in North America, and those can be a bit difficult to set up.

What we still don't fully understand is how responsive the various measurements, such as the lung-clearance index, are in patients who have very mild disease, and we are very interested in those patients because they have normal FEV_1. We're targeting that population in interventional studies with the lung-clearance index, hypertonic saline, and DNAse [recombinant human deoxyribonoclease], to see whether we can pick up a signal in this group. But using these data that we have from pulmonary exacerbations, where we use a population that is not stable at baseline, may not be the

ideal comparative group to the kind of intervention we're ultimately looking at, because if we do interventional trials we usually do the interventions in stable patients.

Davies: I completely agree; that's a good point. A lot of the data are skewed by picking a population that will regress to the mean anyway. One thing we're doing is a run in a study in which 200 patients are being seen approximately every 4 months, but only at times of complete stability. We're making a basket of measurements, including lung-clearance index, at those time points, and we're going to look at the coefficient of variability, the reproducibility, et cetera, to see whether this would only be useful in the patients who start out abnormal or whether mild degrees of abnormality are detectable.

It's important, what you said about young children; the Innocor does have a slower detection time than the mass-spectrometry techniques, so with a child with a rapid respiratory rate these may not be completely applicable. We've been testing younger children with both the Innocor and the mass spectrometer and should have some comparison data soon.

Ratjen: Oh good. If you do that, then we don't have to.

Davies: Well, you could do it too, and then we could make sure we're all getting the same thing.

Rubin: Jane, that was great. I have 2 queries. The first easy one is about research, and the second (harder) one is practical. Have you any information on the use of hyperpolarized gas imaging to get a better idea of small airways disease in CF? And for the practical one, respiratory therapists at bedside are often asked to evaluate whether a treatment that they're providing to somebody with CF, acutely or chronically, is being beneficial or being harmful. They're the ones at the bedside; they're the ones who get to observe that patient. How would you recommend that they determine if what they're doing is helping the patient?

Davies: I have very limited knowledge about hyperpolarized helium and magnetic resonance imaging. Groups in the United States[1] and the United Kingdom[2] are working on this, and we spoke to one of the United Kingdom groups about whether it should become part of the armamentarium, but we haven't taken it forward because we haven't yet been convinced of the degree of sensitivity it would give us on top of the noninvasive measurements we're already making. It's a developing field, and worth keeping an eye on, but they haven't yet convinced me that it's worth overcoming the technical issues.

With regard to patient assessment and whether a treatment is doing harm or good, that's a very difficult question. At our multidisciplinary meetings we quite often have such issues. For example, a physiotherapist (we call them physiotherapists, and their job description is not quite the same as that of respiratory therapists) might say, "So-and-so really loves being on hypertonic saline, but we think it's not doing anything. They're already nonadherent to the rest of their medication. What should we do?" That's a difficult situation.

It's similar to a study Andy Bush and I did many years ago,[3] in which we asked people how they felt after taking DNAse for several months. And nearly everyone felt better, even patients who'd had a 15% drop in FEV_1, so I think there's a mismatch between the sorts of things we're measuring and the patient-reported outcomes, which I deliberately didn't stray into, because it's a whole new minefield.

But, in general, I do think that the person by the bedside is the best to judge that acute response. And in general those clinicians will say, for instance, "This patient is feeling better; they are expectorating sputum better with hypertonic saline than with DNAse." And we don't know whether that translates into medium or even long-term benefit. I don't have the answer to that question, and I would love to know if anyone else has. [Silence.] Obviously not.

REFERENCES

1. Altes TA, Eichinger M, Puderbach M. Magnetic resonance imaging of the lung in cystic fibrosis. Proc Am Thorac Soc 2007;4(4):321-327.
2. van Beek EJ, Hill C, Woodhouse N, Fichele S, Fleming S, Howe B, et al. Assessment of lung disease in children with cystic fibrosis using hyperpolarized 3-helium MRI: comparison with Shwachman score, Chrispin-Norman score and spirometry. Eur Radiol 2007;17(4):1018-1024.
3. Davies J, Trindade MT, Wallis C, Rosenthal M, Crawford O, Bush A. Retrospective review of the effects of rhDNase in children with cystic fibrosis. Pediatr Pulmonol 1997;23(4):243-248.

Marshall: What is the youngest patient group with whom we can obtain the lung-clearance index?

Davies: Janet Stocks's group[1] and others[2] have done it with newborns. It's similar to getting the child to sleep and then putting on a mask with a good seal, and it's no more difficult—in fact, possibly easier—than some of the raised-volume compression techniques.

The group where I work doesn't do infant lung-function studies. We've done children down as low as age 7 with the Innocor, although we are not completely certain how good those data are. But there's probably a window, somewhere between the ages of 3 and 5 years, where they're neither infants (who will go to sleep nicely) nor cooperative children, depending on how compliant your pediatric cohort is, but in general I would say it's pretty much across the whole age range.

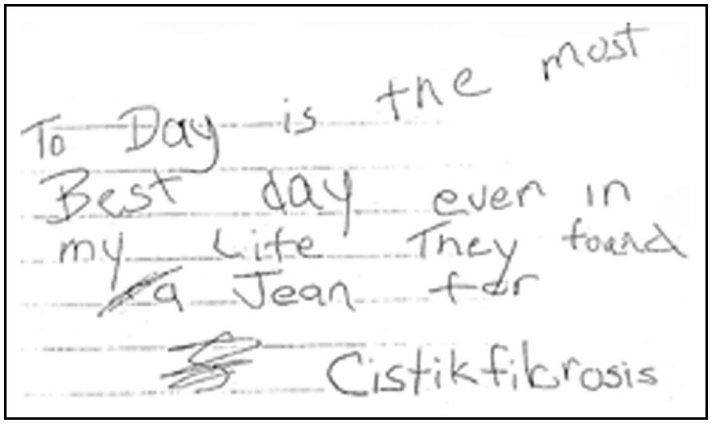
REFERENCES

1. Lum S, Gustafsson P, Ljungberg H, Hülskamp G, Bush A, Carr SB, et al. Early detection of cystic fibrosis lung disease: mul-

tiple-breath wash-out versus raised volume tests. Thorax 2007;62(4):341-347.
2. Horsley A. Lung clearance index in the assessment of airways disease. Respir Med 2009 Feb 24. [Epub ahead of print]

Marshall: You mentioned that lung-clearance index tops your list of possible outcome measurements you're considering at the gene-therapy consortium. What other measurements are you considering?

Davies: I don't want to imply that the consortium has chosen the lung-clearance index as the primary end point. We haven't looked at any of the data from the run-in, but I think it's among the top contenders. Other measurements we're looking at include: mucociliary clearance, for which patients from our center have to travel to Southampton, which is a couple of hours away, and which requires a long 24-hour scan that's quite complicated; computed tomography; various sputum assays; exhaled breath assays; serum assays; normal spirometry; and quality-of-life questionnaires. That's everything you'd probably expect.



Letter to Dr Francis Collins, one of the researchers
who located the gene causing cystic fibrosis,
from J.H., an 8-year-old cystic fibrosis patient, August 25, 1989.
Courtesy National Human Genome Research Institute,
National Institutes of Health