Treatment of cystic fibrosis following infant screening

Newborn screening for cystic fibrosis has become more widespread over the last 5 years with the recent introduction of screening programs across the USA and UK, as well as many other parts of Europe. Most of Australia and New Zealand have had newborn screening programs for more than 15 years. Newborn screening has been shown to improve both short- and long-term nutritional outcomes and provide an important opportunity for the provision of information and education to families, in addition to the opportunity of early referral to a specialist cystic fibrosis center. There is a growing awareness of early lung disease in infants diagnosed through newborn screening, while evidence for treatment of infants to prevent progression of lung disease following newborn screening remains limited owing to the lack of interventional trials in this young age group.

Newborn screening should provide opportunities to improve clinical outcomes and prevent complications. Care in a cystic fibrosis (CF) specialist center and early referral to a specialist CF center are associated with improved lung function [1–3]. Guidelines have been published on the early management of infants diagnosed through newborn screening [4,5]. There is good evidence from randomized clinical trial data of nutritional benefits from newborn screening [6–8]. The Wisconsin randomized controlled trial of newborn screening reported that infants diagnosed with CF through newborn screening had a more rapid normalization of weight, length and head circumference compared with infants diagnosed clinically. Long-term evaluation to the age of 16 years of children in this study, showed improved long-term weight and height growth in children diagnosed through newborn screening compared with children diagnosed with CF clinically [8]. In addition, infants diagnosed clinically with pancreatic insufficiency and vitamin E deficiency had lower cognitive scores in later childhood (aged 7.3–16.9 years) and smaller head circumference compared with children diagnosed by newborn screening with or without vitamin E deficiency at diagnosis [9]. The evidence therefore suggests that both long-term cognitive and growth outcomes may be optimized through newborn screening and early intervention.

Theoretically there should be opportunities for improvement in long-term pulmonary outcomes from newborn screening. Analyses from national registry studies are supportive of improved pulmonary outcomes following newborn screening [10]. However, the data from the only randomized controlled trial were less compelling. The Wisconsin newborn screening randomized controlled trial found no differences in pulmonary outcomes measured using serial respiratory cultures, pulmonary function with spirometry and chest radiography with quantitative scoring [11]. The authors suggested that over time there may be many confounding issues including early airway infection with *Pseudomonas aeruginosa* or other pathogens. The major concern however is that there are also no recognized specific treatments for prevention of lung disease in infants and preschool children and a lack of clinical trial data in this important group remains a huge gap in clinical management that urgently needs to be addressed.

**Nutritional management**

Nutritional status has long been recognized as an independent predictor of long-term survival for patients with cystic fibrosis [12,13]. Most of the mortality in CF is due to lung disease, and while nutritional status and lung disease are clearly linked, the nature and mechanisms of the link akin to the ‘chicken and egg’ relationship is not fully understood. A full discussion of this important topic is beyond the scope of this review; however, this relationship has been well covered by others [14]. Nutritional status in...
infancy also appears to be associated with long term health outcomes. In the Wisconsin Cystic Fibrosis Neonatal Screening Project, infants with CF and pancreatic insufficiency without meconium ileus who recovered weight within the first 2 years of life had better chest radiograph scores, less cough and higher lung function at 6 years of age compared with those that did not [15]. Children who did not do so well clinically, at age 6 years, had received higher caloric intake and yet these children did not recover weight in the first 2 years of life suggesting that weight recovery in infancy is a marker for more severe clinical status. A more recent longitudinal observational study in young children followed from the time of diagnosis by newborn screen or with meconium ileus to the age of 3 years, reported an association between airway inflammation and BMI standard deviation score [16]. It is unclear whether targeted intervention could impact this group at risk of poorer outcomes but it may require intervention that addresses both inflammatory lung disease and nutritional status.

Pancreatic insufficiency

The cystic fibrosis transmembrane conductance regulator (CFTR) gene codes for the CFTR protein that is localized in the apical surface of exocrine glands including the pancreas. The CFTR protein functions as a cyclic AMP-dependent chloride channel providing chloride ions into the pancreatic ducts. Luminal chloride ions in the pancreatic duct are exchanged for bicarbonate providing an alkaline environment. Alteration in bicarbonate secretion leads to a more acidic environment and precipitation of secretions with consequent obstruction and organ damage. Pancreatic status is strongly linked to the CF genotype [17]. Patients who carry two or more severe CF mutations from classes I, II or III develop pancreatic insufficiency [18]. While many will have pancreatic insufficiency at the time of diagnosis by newborn screening, some may be pancreatic sufficient initially and develop pancreatic insufficiency over time. Estimates of the prevalence of pancreatic insufficiency vary, although it is thought that around 82–90% of patients will develop pancreatic insufficiency [19,20]. Those who carry at least one mild mutation are more likely to be pancreatic sufficient and are at risk of developing pancreatitis [17,19].

Pancreatic insufficiency is characterized by steatorrhea, which may be detected prior to the development clinical symptoms by measuring pancreatic elastase-1 in stool samples making the test particularly suitable for monitoring pancreatic sufficient patients longitudinally [21]. Healthy infants and premature infants may have low levels in the first 2 weeks of life and the test is therefore unsuitable for diagnosis of pancreatic insufficiency in newborns until at least 2 weeks of age [22]. In newborns the coefficient of fat absorption may be used as an alternative measure although the test is not easy to do for families and in a child who is symptomatic with appropriate genotype it may be reasonable to treat and measure fecal elastase-1 after 2 weeks as the test is unaffected by the use of pancreatic enzyme supplements. Pancreatic insufficiency should be diagnosed promptly and treated with appropriate pancreatic enzyme replacement therapy.

■ Feeding

The benefits of breast feeding are well recognized. However, there has been little data in the literature on the benefits (or otherwise) of breast feeding for infants with CF. An observational study in 103 infants diagnosed with CF through newborn screening in Wisconsin compared outcomes in infants using breast and formula feeding and categorized infants by duration of exclusive breast feeding [23]. There was some suggestion that the previously recognized benefits of reduced respiratory infections seen in breast fed infants in the general population may also be applicable to infants with CF. Breast feeding was associated with a reduced frequency of acquisition of P. aeruginosa regardless of the length of exclusive breast feeding [23]. Exclusive breast feeding for less than 2 months was not associated with compromised growth, although prolonged exclusive breast feeding in pancreatic insufficient infants was associated with a decline in weight standard deviation scores over the first 6 months of life [23]. Benefits with regards to higher lung function in children who were breast fed were also reported from a retrospective study in 146 children with CF in Milan, Italy [24].

■ Vitamin supplementation

A combination of pancreatic insufficiency, which leads to reduced absorption of fat soluble vitamins even with pancreatic enzyme supplementation, and what is thought to be increased utilization through higher levels of oxidative stress render patients with CF at risk of fat soluble vitamin deficiency [25]. Vitamin supplementation is routine in most CF centers, however there are a
lack of data available to guide optimal levels and optimal dosing for maximizing health outcomes in CF at different ages. A full discussion of this increasingly complex topic is beyond the scope of this review and has been covered by others [26]. Infants diagnosed with CF through newborn screening, may have vitamin deficiency at the time of diagnosis [27]. Vitamin D deficiency has been reported in infants diagnosed through newborn screening however, vitamin D levels were unrelated to pancreatic status or other fat soluble vitamin levels and improved with time [28]. The long-term consequences of this vitamin D deficiency in early infancy are unclear. There has been a growing interest in the role of vitamin D across a range of disorders from osteopenia and increased risk of falls, through to infectious diseases, cardiovascular disease and common cancers [29]. Vitamin D deficiency is common in CF and the role of vitamin D is likely to be important in muscle strength, bone metabolism, respiratory infection and pulmonary inflammation [30]. Strategies to optimize vitamin D levels across the age ranges for patients with CF will need to be developed.

Salt replacement
Acute dehydration with hyponatremia is a recognized complication of CF and is related to high sweat rates and increased loss of salt in the sweat as well as suppressed thirst sensation [31,32]. Infants, particularly during hot weather, are also at risk of more gradual lowering of serum electrolytes associated with failure to thrive and metabolic alkalosis [33]. Salt replacement is now standard practice particularly, in warmer climates [4]. However, there are no available data on optimal dosing or types of salt replacement in infancy or in children and adults.

Respiratory
Lung disease is responsible for most of the morbidity and mortality in CF. The spectrum of structural lung damage in end-stage lung disease appears to vary between patients and includes a mixture of air trapping and chronic suppurative damage [34]. Exactly how these lesions arise is not yet fully understood although recent data suggest that structural lung disease with air trapping and bronchial dilatation may be detected even in very young children [35,36] and is associated with airway inflammation and early infection with P. aeruginosa [35]. The role of chest computed tomography (CT) scanning in routine surveillance in infants and young children diagnosed through newborn screening is unclear. Case reports suggest potential clinical benefits, however, the optimal frequency and potential disadvantages of radiation exposure and need for sedation or anesthesia need to be carefully considered [37].

The underlying defect in CF of epithelial-cell-transmembrane electrolyte transport with impaired chloride transport and hyper-absorption of sodium, leads to a lung microenvironment characterized by depletion of the periciliary liquid layer of the airway epithelium, abnormal mucus and submucosal gland hypertrophy [38,39]. These changes result in impaired mucociliary transport with retention of viscid mucus and the formation of microaerophilic or anaerobic-mucous plugs [39]. This leaves the airway epithelial surface susceptible to characteristic pathogens and exuberant airway inflammation. Anti-inflammatory therapies have not yet been tested in infants although a placebo controlled randomized clinical trial of azithromycin to prevent bronchiectasis in infants diagnosed through newborn screening (COMBAT CF) will be undertaken across Australasia in the near future [101].

Microbiology & antibiotic therapies
In the first 2 years of life Staphylococcus aureus and Haemophilus influenzae are the most commonly cultured pathogens [40]. The importance of these organisms in the development of structural lung disease is unclear, however, infection of the lower respiratory tract with S. aureus is associated with increased airflow inflammation and has an additive effect on inflammation associated with lower respiratory tract infection with P. aeruginosa [41]. Treatment of respiratory symptoms in young children is usually aimed at these organisms although optimal regimens have not been identified. The role of anti-staphylococcal prophylaxis in the first few years of life is also unclear and practices vary considerably. CF centers in the UK and some European countries commonly use prophylaxis, often with flucloxacillin, while CF centers in the USA tend not to use prophylaxis. This may be due to the results of the largest randomized clinical trial from the USA, comparing cephalaxin with placebo, which resulted in no apparent clinical benefit with the suggestion of a possible increased risk of the acquisition of P. aeruginosa associated with treatment [42]. Drug availability is also a factor that is likely to contribute to differences in practice between countries and flucloxacillin is not available for anti-staphylococcal prophylaxis in the USA. Systematic review of the available
data provides evidence that anti-staphylococcal prophylaxis, regardless of type of treatment, is associated with reduced *S. aureus* in respiratory cultures although clinical benefits have not been consistently reported and concerns about the potential for an increased risk of acquisition of *P. aeruginosa* remain [43]. Further good quality clinical trial data are required.

Acquisition of *P. aeruginosa* in the respiratory tract is a sentinel event and if infection is persistent the organism adapts to the CF lung environment with the development of a biofilm, which protects the organism from host defenses. Adaptation commonly involves production of alginate and the development of a mucoid phenotype, which is associated with increasing antibiotic resistance and an increase in both morbidity and mortality. With time, the organism becomes impossible to eradicate using current therapies. Eradication of early infection has now become a widespread clinical practice that is thought to be associated with a delay in the development of chronic infection and is associated with both clinical and health–economic benefits [44–46].

The optimal treatment strategy for eradication or prevention of acquisition of *P. aeruginosa* infection has not previously been established. A number of randomized controlled trials have however provided important insights. The ELITE study demonstrated a similar benefit in using 56 days rather than 28 days of inhaled tobramycin inhalation solution (300 mg/5 ml) with 93% children being free of infection 1 month after 28 days of treatment compared with 92% after 56 days, and 66% remaining free of infection after 27 months of study compared with 69%, respectively [47]. Cycled antibiotic therapy using inhaled colistin and oral ciprofloxacin for 3 weeks every 3 months did not reduce the risk of initial infection or chronic infection with *P. aeruginosa* in children with CF [48]. Similarly, data from the Early Pseudomonas Infection Control (EPIC) trial suggest that cycled therapy using inhaled tobramycin for inhalation solution was no better than culture-based treatment in reducing time to use of intravenous antibiotics or for the microbiological end points and the use of oral ciprofloxacin did not appear to add benefit above the use of inhaled tobramycin solution for inhalation [49]. There are a range of eradication regimens in the literature and most seem to provide similar success rates [46].

Despite initial success in clearing early infection with antibiotics, some children are unable to clear the infection and with increasing age *P. aeruginosa* becomes more common, with around 80% of adults being infected [39]. Establishment within the lower airway of *P. aeruginosa* strains, particularly those bearing the mucoid phenotype, is associated with increased morbidity and mortality [50–52]. If chronic infection is established in young children aggressive management following guidelines for treatment of chronic infection as well as preventative strategies to maximize airway clearance and nutritional outcomes should be followed [53,54].

Diagnosing *P. aeruginosa* infection accurately in nonexpectorating CF patients can be difficult. Although oropharyngeal cultures are used widely, they have limited sensitivity and variable specificity for predicting the presence of lower airway pathogens [55]. Bronchoalveolar lavage (BAL) has been used as an alternative diagnostic tool when young CF children cannot provide sputum [56–59]. A recent randomized controlled trial compared prevalence of *P. aeruginosa* on BAL cultures and total CF CT score on chest high-resolution CT scan outcomes at the age of 5 years, in children and who were diagnosed through newborn screening who were randomized in the first 6 months of life to BAL-directed or standard therapy using oropharyngeal cultures. The BAL group underwent BAL at enrollment, when hospitalized for pulmonary exacerbations, if *P. aeruginosa* was detected in oropharyngeal specimens, and after *P. aeruginosa* eradication therapy. BAL-directed therapy did not provide any clinical, microbiologic or radiographic advantage and led to an increased risk of predominantly mild adverse events as a direct result of bronchoscopy [36,60]. This study resulted in a low prevalence of *P. aeruginosa* cultured from BAL at the age of 5 years (10–12%) and only one child out of 157 children who completed the trial met the criteria for chronic infection with *P. aeruginosa* at the age of 5 years. These results were achieved using an average of three to four oropharyngeal cultures collected per year per child. The authors concluded that “BAL remains a useful tool and should perhaps be reserved for young children who are deteriorating despite parental antibiotic therapy, when unusual or antibiotic-resistant pathogens including clonal *P. aeruginosa* strains are suspected [61,62] and to diagnose those with chronic *P. aeruginosa* infection” [36].

There are some concerns about the potential for increasing antibiotic resistance and the emergence of antibiotic-resistant organisms as
intensive antibiotic usage becomes widespread especially in younger patients. The microbiology of respiratory samples from patients with CF in the USA aged 6 years and older infected with *P. aeruginosa* who met the same eligibility criteria for the original Phase III trials of tobramycin for inhalation, were compared with the microbiology from the original studies between 1995 and 2008. Greater aminoglycoside resistance was seen for *P. aeruginosa* strains over time as well as an increasing prevalence of *S. aureus* and multiresistant organisms such as methicillin-resistant *S. aureus*, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* [63]. There is also an emerging awareness of the importance of fungal infections. Persistent isolation from respiratory samples of *Aspergillus fumigatus*, which is the most commonly isolated fungus, is associated with an increased risk for hospitalization [64]. The role of particular antibiotic regimens in the emergence of these and other organisms in young children is currently unclear and requires careful study. In addition, different methodologies for the collection of respiratory samples may lead to quite different reporting of, in particular, *A. fumigatus* with bronchoalveolar lavage providing superior detection compared with either sputum or oropharyngeal samples and this will need to be carefully considered in the future [65]. Understanding the changes in CF airway microbiology with time and the implications for therapeutic intervention will be areas of key importance for future research.

Cross-sectional studies using culture independent analysis of respiratory samples examining bacterial diversity of sputum and BAL specimens from patients with CF have shown complex microbial communities even from young children, which include an abundance and diversity of anaerobes and previously unappreciated species, such as the *Streptococcus milleri* group [66–69]. Oral bacterial species may also be detected in lower respiratory tract secretions and molecular techniques have found that oral bacterial species in CF sputum specimens were unlikely to be the result of contamination by oral flora [70]. In addition, oropharyngeal bacteria can induce quorum sensing of virulence genes in *P. aeruginosa* resulting in enhanced lung damage in a rat lung model [71]. Thus oropharyngeal flora may play a more active role in CF lung disease than previously suspected. A study of 45 children with CF aged 2–16 years examining bacterial communities using molecular techniques from oropharyngeal swab samples has shown an inverse relationship between bacterial community complexity and age, presence of *P. aeruginosa* and antibiotic exposure [72]. In addition, there was a suggestion that CF genotype may have an effect on bacterial communities in the upper airway. It is possible that the airway microbiome plays a role in the development of, or protection from, infections, inflammation and the development of CF lung disease, although as yet little is known about these relationships. Little is also known about the microbiome in the healthy human airways in early life or about the changes over time in infants and young children with CF and further studies particularly longitudinal studies are awaited.

### Infection control

Avoiding cross infection in infants and young children is clearly of key importance and there should be clear infection control guidelines in all CF centers and in all healthcare facilities that manage patients with CF [73]. For young children, avoiding cross infection with viral infections is also of key importance as early viral infections (especially with respiratory syncytial virus) are often associated with an increased risk of pulmonary exacerbation [74]. Some CF centers particularly in the USA, use respiratory syncytial virus immunoprophylaxis with the humanized monoclonal antibody palivizumab [75]. However, there is no current evidence base to support benefit or cost-effectiveness in infants with CF [76].

### Airway clearance, mucolytics & therapies aimed at improving mucociliary clearance

The use of airway clearance techniques in the management of children and adults with CF is based on the logic that airway clearance techniques assist in the removal of the thick tenacious secretions from the airway lumen with concomitant improvement in airway function and health outcomes. Use of airway clearance strategies are universal across CF centers, however, even in older children and adults the optimal strategies for managing airway clearance are unknown [77]. The benefits and best approach to airway clearance in infants diagnosed through newborn screening is also unknown. However, a European consensus of senior physiotherapists achieved good consensus on most aspects of airway clearance management in infants except for the issue of treating ‘asymptomatic’ infants [78]. Most CF centers introduce airway clearance techniques at the time of diagnosis.
it is recognized that structural changes may be apparent on chest CT scans with air trapping and even bronchial dilatation in the first few months of life [45] and that lower respiratory tract infection may also be present in ‘asymptomatic’ infants at a few months of age [40].

In CF, leukocytes accumulate in the airways and release extracellular DNA that contributes to the viscous properties of airway secretions. Even in infants, elevated DNA content is recognized [79]. Dornase alfa is a highly purified solution of recombinant human deoxyribonuclease 1, an enzyme that selectively cleaves DNA and is essentially identical to endogenous human DNase 1. Dornase alfa is administered by nebulization into the airways where it is thought to act as a mucolytic agent. Inhaled dornase alfa has clearly been shown to improve pulmonary function in children aged 5 years and older and also in adults with CF [80,81]. Inhaled dornase alfa has been shown to reduce the risk of pulmonary exacerbations and maintain lung function in children with mild lung disease [81]. It has also been shown to reduce quantitative air trapping in children with mild lung disease. Regional air trapping is one of the earliest radiological abnormalities detected in young children with CF [82]. A randomized trial of inhaled dornase alfa therapy found a reduction in the number of positive bacterial cultures in patients with CF [83] and the use of inhaled dornase alfa has also been associated with a reduction in the increase in neutrophil inflammation that is found over time even in patients with normal lung function [57]. There are some safety data available in infants and young children and inhaled dornase alfa would seem to be an attractive option for prevention of progression of lung disease in infants and young children [84,85]. However there are no randomized controlled trials available to assess potential clinical benefits in newborn screened infants and young children.

Inhaled hypertonic saline is a recognized therapy that improves mucociliary clearance [86] and has been shown to reduce pulmonary exacerbations in children aged 6 years and older and also in adults with CF [87]. Hypertonic saline appears to be well tolerated in infants [88] and the results from that ongoing randomized controlled trial in infants (ISIS study) are eagerly awaited.

Genetics
Newborn screening provides opportunities for families to make informed decisions in family planning and to take advantage of cascade screening. These services should be offered to all families. Some centers have reported a modest decline in the prevalence of CF since the introduction of newborn screening [89] while others have reported limited take up of cascade screening [90]. The optimal model for genetic counseling of families with a child diagnosed with CF through newborn screening is not yet established, although the ‘Wisconsin model’ (a new model aimed at reducing family distress and improving understanding of information given as well as empowering decision making) is currently being assessed and shows promise [91].

Parental education/support
The time of diagnosis of CF is highly stressful for families and coincides with the time that large amounts of information and education are given to families. Individualized assessment and educational programs should be provided where possible to help support families and ensure optimal health and psychological outcomes. Environmental tobacco smoke adversely affects pulmonary outcomes in patients with CF [92]. Newborn screening provides an opportunity to educate families to avoid exposure with environmental tobacco smoke for infants and young children. For infants with CF adherence with standard immunization protocols to avoid infection with B. pertussis are of key importance. The role of immunization for influenza in newborn screened infants with CF is not established although many CF centers suggest families are immunized to protect young infants.

Clinical outcome measures in young children
Measuring outcomes for clinical trials, and also for clinical benchmarking purposes, is of enormous importance although complex and difficult in infants and preschool children. In older children and adults, lung function remains the measurement of choice although there is growing interest in imaging techniques as more patients now have normal lung function. As mentioned earlier, chest CT scanning is being used to detect early lung disease although radiation exposure limits the potential frequency of testing. Alternative imaging modalities such as MRI are being explored [93]. Infant lung function tests as clinical trial end points continue to pose a number of challenges including the local experience with techniques, acceptability rates, variability and potentially large sample size requirements, which have been investigated and described by Davis et al. [94].
Aurora et al. demonstrated that the abnormal lung clearance index, a measure of ventilation inhomogeneity, measured in preschool children aged between 3 and 5 years predicts lung function abnormalities in children aged 6–10 years [95]. In addition, the London Cystic Fibrosis Collaboration have reported similar sensitivity of lung clearance index and chest high resolution CT scanning to detect lung disease in children with a mean age of 7.8 years [96]. However, for individual patients both measures provided different information, suggesting that for monitoring individual patients the one test does not exclude or describe the extent of lung disease. Measurements of inflammatory status have included BAL inflammatory parameters and urine and blood and breath tests. To date, no noninvasive, reliable biomarker of lung disease has emerged. Pulmonary exacerbations remain a risk for progressive decline in lung function in older patients. Sanders et al. report data from the CF Foundation Patient Registry with a worrying 25% of exacerbations treated with intravenous antibiotics resulting in failure to recover baseline lung function [97]. Pulmonary exacerbations are now being considered as outcomes for clinical trials in young children although an agreed definition of a pulmonary exacerbation in young children and the long-term consequences of pulmonary exacerbations in infancy are still unclear. For infants and young children, nutritional parameters and growth remain the most widely used and reliable outcomes for general benchmarking purposes.

**Financial & competing interests disclosure**

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

**Executive summary**

**To capitalize on the benefits from newborn screening programs we need to:**

- Recognize and monitor the development of pancreatic insufficiency in newborn screened infants and institute appropriate measures with pancreatic enzyme replacement therapy.
- Prevent complications including salt dehydration and fat soluble vitamin deficiency.
- Identify early pulmonary infection with *Pseudomonas aeruginosa* using oropharyngeal cultures (average three to four per child per year) and use eradication protocols to clear infection and delay acquisition of chronic infection.
- Institute airway clearance strategies on an individualized and age appropriate basis.
- Follow infection control guidelines and prevent cross infection with *P. aeruginosa, Burkholderia cepacia*-complex and viral infections.
- Provide genetic counseling to enable informed decisions and choices for families.
- Provide education for families and provide preventative strategies including optimizing immunization and preventing exposure of infants and children to environmental tobacco smoke.

**Future perspective**

- Reliable and noninvasive biomarkers or measures of lung disease are needed to monitor individual lung disease progression and provide outcome measures for clinical trials in infants and young children.
- Intervention trials in newborn screened infants are urgently required to provide evidence for prevention of lung disease progression.

**Bibliography**


42. Trenggara MM, Retsch-Bogart G, Mayer-Hamblett N et al. Comparative...


77 Main E, Prasad A, Schans C. Conventional chest physiotherapy compared with other airway clearance techniques for cystic fibrosis. Cochrane Database Syst. Rev. 1, CD002011 (2005).


**Website**