Cystic fibrosis overview and update on infant care[*]

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ABSTRACT

Cystic fibrosis is now the most common serious, autosomal-recessive disease. It has been described in every population in all parts of the world. In the US, newborn screening is available in all states. Current studies demonstrate that newborn screening, early diagnosis, early nutritional and environmental intervention, and an understanding of the pathophysiology of the disease may increase life expectancy and quality-of-life. Evidence-based guidelines encourage the use of early nutritional intervention and initiation of pulmonary therapies. The average lung function of patients with cystic fibrosis corresponds with genetic makeup, age of intervention, and environmental factors including the socioeconomic status of the patient's family. We reviewed and updated current understanding of the pathophysiology of the disease, diagnosis, and the benefits of early diagnosis via newborn screening in evaluating the nutritional status of our youngest cystic fibrosis patients; the direct correlation of malnutrition to pulmonary- and cognitive-function outcomes are reviewed and updated from literature.

1. BACKGROUND

Cystic fibrosis (CF) is an autosomal-recessive, generalized, multi-organ-system disease caused by a single biochemical abnormality: the defective chloride channel caused by the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The pathophysiology of the disease lies in different gene mutations that encode abnormalities in the structure of this protein. These abnormalities lead to a physiologic defect in ion (chloride and sodium) transport at the apical membrane of epithelial surfaces [1]. This defect affects all the exocrine gland functions: sweat glands, salivary glands, pancreas, hepatobiliary system, lungs, intestines, and reproductive system. It is characterized by the clinical phenotype of recurrent respiratory infections, pancreatic insufficiency, and an elevated sweat chloride. The major morbidity and mortality are related to the involvement of the lungs, which appear free of infection at birth but within a few weeks exhibit small airway mucus plugging and recurrent chronic infection. Approximately three to seven percent of patients may develop hepatobiliary involvement [2] with focal biliary cirrhosis leading to portal hypertension and liver failure needing liver transplant. CF has been described in every population, in every part of the world, and is currently the most common serious, autosomal-recessive inherited disease. The highest incidence of CF is in people of northern European extraction, with an approximate incidence of one in every 3300 [3]. Currently, there are an estimated 30,000 patients in the United States [4] and an estimated 70,000 patients with CF worldwide. More than 40% of these patients are adults. Over 1720 mutations have been reported [5]. More than 12 million (one in 20) Americans carry the gene, and many are unknowing, asymptomatic carriers [6]. Continuance of high gene-frequency has lead geneticists to postulate a heterozygote advantage of 2% to 2.5%. Carriers are resistant to tuberculosis and other infections, such as chloride-secreting diarrhea and infantile gastroenteritis.

Cystic fibrosis is at the forefront of quickly evolving medical discoveries. Currently, every state in the US participates in some form of newborn screening for CF, and it is estimated that over two million newborns are screened annually for the disease. CF has become the paradigm for biomedical research and the study of genetic diseases and, indeed, for the medicine of the future. It is possible to identify a gene whose structure and function are unknown and use that information to develop "designer" therapies. The accomplishments to date have been absolutely outstanding and have provided a foundation for this field that goes much beyond CF.

1.1. CF Gene/Pathophysiology

The CFTR gene, isolated in 1989 by Lap Chi Tsui and

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Francis Collins, is located on chromosome 7q31.2. A large sized gene, it contains 27 exons and 250,000 base pairs. Through the efforts of the Cystic Fibrosis Mutation Database, maintained by the Cystic Fibrosis Foundation, a consortium comprised of approximately 80 laboratories worldwide have identified 1720 mutations in the years since the CF gene isolation. Seventy percent of the mutations are a result of deletion of three base pairs of nucleotides, leading to loss of a single amino acid, phenylalanine (F), in position 508 [7]; this is referred to as delta-F508 mutation. The gene codes for a cyclic AMP-mediated chloride channel referred to as CFTR protein. There are five major classes of CFTR mutation causing CF [8,9]. Class I contains a defective protein production. There is defective processing, regulation and conduction in class II, III and IV, respectively, and there is reduced synthesis in class V. The most common delta-F508 mutation is a block in the processing; this is a class II mutation where the protein is misfolded and the internal quality-control system prevents it from coming to the surface to function as a chloride channel. The milder mutations—classes III, IV, and V—are all potentially more amenable to therapy. Regarding the genotype-phenotype relationship, patients homozygous for delta-F508 have pancreatic insufficiency from birth, higher sweat-chloride content, and a greater chance of being diagnosed at an earlier age due to clinical presentation of symptoms [10]. Regarding pulmonary-function tests, however, there does not seem to be a close correlation between genotype and phenotype; this may be related to multiple factors, such as modifier genes and environmental factors that may work in concordance with socioeconomic status. Primary care providers will be able to better counsel their patients with this background knowledge.

The gold standard for diagnosing CF is defined by two positive sweat-tests obtained on two separate sites, performed on different days, and performed in a laboratory accredited by the Cystic Fibrosis Foundation with continual levels of high quality-control. Sweat chloride over 60 is considered diagnostic of CF, 40 to 60 is considered the indeterminate range, and less than 40 is normal [11]. The notable exception is infants under the age of approximately six months; in this population, the lower limit of abnormal sweat chloride is 30 [12,13]; this is an important new information of which primary care pediatricians need to be aware. The consensus statement from Cystic Fibrosis Foundation [14] reports that the CF diagnosis is suggested by the presence of one or more characteristic clinical features, a history of CF in a sibling, or a positive newborn screening test. This will then be confirmed by laboratory evidence for CFTR dysfunction, namely, two positive sweat tests or identification of two CF-causing mutations. For patients in whom sweat chloride concentrations are normal or borderline and in whom two CF mutations are not identified, abnormal nasal PD measurements recorded on two separate days can be used as evidence for CFTR dysfunction [14]. Furthermore, infants screened with immunoreactive trypsin (IRT) who have an elevated IRT (> 95th percentile), a borderline sweat chloride, and only one disease-causing mutation may have what is now referred to as CF-related metabolic syndrome and need to be followed closely for manifestations of classic CF [15].

The sodium chloride at the bottom of the sweat duct has the same concentration of salt in normal patients and in CF patients. With the presence of the CFTR protein in the normal lining membrane, as the sweat moves up the duct, sodium and chloride are reabsorbed and a low-salt-containing sweat is seen on the surface. In CF patients, because of the lack of CFTR protein in the sweat-duct lining, there is no reabsorption of sodium and chloride; thus, high salt-levels are seen in the sweat.

1.2. Hypothesis for CF Airway Disease

Current theories provide two hypotheses for the genesis of CF airway disease: the high-salt hypothesis and the depleted- (or low-) volume theory [16]. Based on the high-salt hypothesis, β-defensins on the epithelial surfaces of the lungs and trachea are salt-sensitive and do not function properly in the relatively hypertonic airway-surface liquid in CF airways. Alternately, the low-volume hypothesis theorizes that ciliary clearance is affected by dehydration of airway-surface liquid n the periciliary liquid layer in CF individuals [17]. Normal airway-surface liquid contains sol and gel layers. For the cilia to stand straight up and beat fast forward with a slow, backwards motion, the height of the sol layer is important in mucociliary defense. In CF patients, secondary to the abnormal chloride channel with excessive sodium reabsorption, the periciliary liquid is dehydrated. The cilia cannot function normally. The CFTR dysfunction leads to decreased chloride secretion, increased amiloride-sensitive sodium absorption, and a diagnostically significant raised potential difference across the CF airway [17]. The defective transepithelial fluid transport in CF airways points directly to the conclusion that a mutation in CFTR causes CF disease.

1.3. CFTR

In the mid-1990s, CFTR was initially described as a chloride channel with two membrane-spanning domains, two nucleotide-binding domains, pyruvate kinase and ATP-binding domains, leading to the chloride channel. However, more research into CFTR uncovered that intermolecular interactions between the C-terminus of

CFTR and regulatory networks are vital for CFTR function. Through a number of mechanisms, CFTR controls the epithelial sodium channel as well.[1] In normal individuals, 75% of the CFTR protein is degraded, and 25% functions as a chloride channel. In CF patients, 99% of the CFTR protein is degraded, and only 1% functions as a chloride channel. Current research postulates that if at least 4% of the CFTR protein can be made to function as a chloride channel, one can reverse the changes of CF. However, increased sulfation of mucins may enhance adherence of *Pseudomonas aeruginosa* [18]. Within the Golgi apparatus, the CFTR protein derangement leads to an abnormally high pH; such a change will affect the activity of the most pH-sensitive enzymes, sialyl-transferases, which covalently modify newly synthesized molecules. Less-impaired enzymes and less-pH-sensitive sulfur transferases take over. As a result of this modulation of enzyme activity, abnormally sulphated membrane constituents may be transported to the cell surface and cause more avid adherence of *P. aeruginosa* to the surface of the cell [19].

1.4. Role of CFTR Protein

The physiologic properties of CFTR are numerous: it acts as a chloride channel, regulates the epithelial sodium channel and endosomal pH, is an interaction partner of many cellular proteins, and may be responsible for promoting *P. aeruginosa* adherence [20,21]. A mutant CFTR affects ion and fluid transport across the epithelial membrane, which may impair mucociliary clearance and encourage bacterial colonization of the airways. Mutant CFTR may act as a receptor for *P. aeruginosa* [21]. In addition to mutant CFTR, environmental factors and genetic modifiers also affect outcomes and phenotypic presentation. This helps the pediatrician to understand and explain a differing phenol-type despite having the same CF gene mutation. Comprehending this basic pathophysiology, clinicians will appreciate the manifestations of the disease and understand the rationale for prescribed treatment modalities, such as mucolytics, and hydrating agents, such as hypertonic saline inhalations.

1.5. Mucus in CF/Structural Changes in Lung

Bronchoscopic findings reveal the very thick, viscous and extremely elastic mucus adhering to the airways in CF patients. The pulmonary abnormalities start as mucous plugging and infection in the peripheral airways, and as the disease progresses, more central airways become involved, leading to bronchiolitis, bronchitis, and bronchiectasis. The histopathological evidence of the

disease reveals airways filled with mucopurulent material and inflammatory exudates, peribronchial inflammation, and some arterial changes of medial-wall hypertrophy.

In 1976, Bedrossian *et al.* [22] reported a qualitative study of lower respiratory tracts in *Human Pathology*. The 82 patients were divided into five groups based on the age as follows: group one, 0 to 4 months, 16 patients; group two, 4 to 24 months, eight patients; group three, 2 to 6 years, 28 patients; group four, 6 to 10 years, 14 patients; and group five, 2 to 24 years, 17 patients. Autopsies were reviewed with respect to pathologic changes in the lung and their prevalence among different age groups. Bronchitis, mucopurulent plugging, bronchopneumonia, loss of cilia, and epithelial metaplasia with stratified squamous epithelium were seen in more than 50% of patients aged less than four months. Goblet-cell hyperplasia and hyperplastic bronchial glands were seen as a result of chronic airway infection, along with increased mucous; epithelial metaplasia, crippling of mucociliary defense, and retention of mucopurulent exudates; bronchiectasis; and bronchopneumonia. They also noted intimal thickening of small muscular pulmonary arteries. The complex vascular structure suggesting anastomosis was seen in bronchial circulation—this is what frequently causes hemoptysis. Increased pulmonary artery pressure explains the right ventricular hypertrophy in CF patients. Microscopic sections of subsegmental bronchi revealed findings of squamous metaplasia of the epithetlium. A mucopurulent plug fills the bronchial lumen, and the wall is surrounded by extensive inflammation. The occurrence of bronchiectasis in CF patients aged less than four months was 20%, in patients four months to two years was 75%, and in patients aged more than two years was 100%. Bronchiectasis was seen early in the disease in conjunction with *P. aeruginosa* infection. This information helps pediatricians to understand the vital need to advocate early intervention in their newly diagnosed infants. The disease clearly starts early in life, even without any overt respiratory symptoms, with mucus plugging and early asymptomatic infection/inflammation.

1.6. CF Lung Infections

About 30% of CF patients may be colonized for *P. aeruginosa* by one year of age; as such, a young infant with CF seen in a primary care doctor's office with an incidental increase in cough will have to be evaluated for early infections. *Staphylococcus aureus* and *Haemophilus influenzae* colonization remain constant through the age groups [4]. As CF patients continue to achieve longer life spans, different kinds of infections are emerging in this population, such as *Stenotrophomonas maltophilia*, *Burkolderia cepacia*, xylosoxidans, asper-

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[1]Welsh, M.J. "Bactericidal Activity of Airway Surface Fluid." Presented at the Tenth Annual North American Cystic Fibrosis Conference; October 24-27, 1996, Orlando, FL.

gillus and other fungal infections, typical and atypical mycobacterium, methicillin-resistant *S. aureus*, etc. The primary-care physicians involved in the care of children with CF need an increased index of suspicion for a wider variety of pathogens. The neutrophils respond to infection in the lung, engulf the bacteria with pseudopodia, and form phagosomes; they fuse with lysosome-containing digestive enzymes and destroy the bacteria. As the white cells degrade, they release elastase and oxidants, which cause epithelial and cell-wall damage. As there are adequate antielastase and antioxidants, the epithelial damage can be repaired with the resolution of pneumonia in non-CF patients. However, in CF patients, because of the excessive number of white cells, the naturally occurring antioxidants and antielastase systems are overwhelmed, and destruction of the lungs occurs with resultant bronchiectasis. In CF patients, the bronchoalveolar lavage, compared with that of normal infants, reveals an increased number of white cells even without an infection. Khan *et al.* [23] reported on bronchoalveolar lavage fluid from 16 infants with CF, with a mean age of six months, and 11 disease control infants examined for neutrophil count, activity of free neutrophil elastase and levels of interleukin (IL)-8, and the expression of IL-8 mRNA transcripts by airway macrophages. The numbers of neutrophils and the expression of IL-8 were increased in infants with CF who had negative cultures. Airway inflammation may be present in infants with CF as young as four weeks. The source of IL-8 and inflammation of the airways appears to be alveolar macrophages. The airway inflammation in CF appears to be neutrophil dominated. The neutrophils can secrete oxygen radicals, and the cellular contents DNA and F-actin make the mucous very thick and viscous. The neutrophils also produce chemoattractants, eicosanoids, leukotriene B4 (LTB$_4$), IL-8, and proteases including elastase. The neutrophil-mediated inflammation in CF lung disease and elastase down-regulates the immune system; cleaves the complements and the antibodies; and causes elastin degradation and structural damage, bronchectasis, and an increase in macromolecular secretion with plugging of the airways. Elastase has been shown to cause opsonin and receptor mismatch. Elastase can cleave the IgG antibodies to *P. aeruginosa* [24]. It can cause hypertrophy of mucus-secreting glands as seen in a mouse model [25].

1.7. Airway Inflammation in CF

The CFTR mutations may promote and perpetuate airway inflammation by disrupting autocrine control of cytokine secretion of epithelial cells. In the healthy controls, IL-10 is in excess of IL-8, and inflammation is restricted. However, in CF patients, there is neutrophil accumulation and increased amounts of IL-8, and not enough IL-10 at the epithelial surfaces, promoting infection in immune hyper-responsive CF airways. This environment promotes bacteria to attach easily via flagella-adhesion molecules being activated, and microcolonies form. The colonization and infection noted with *P. aeruginosa* is an example of such invasions that take place in the CF airways. Furthermore, the biofilm community is seen with extracellular matrix surrounding them, turned on by quorum-sensing genes that have been described [26]. The lack of oxygen in thickened CF mucus simultaneously promotes and perpetuates infection. At birth, there is no noted infection; however, shortly thereafter, one can have an *S. aureus* or *H. influenzae* positive culture and a negative *P. aeruginosa* culture. Subsequent *P. aeruginosa* culture with intermittent infection can lead to mucoid transformation and chronic infection, promoting inflammation and leading to bronchiectasis.

It is estimated that approximately 30% of CF patients will be colonized with *P. aeruginosa* by one year of age. As part of the ongoing Wisconsin newborn screening project, Kosorok *et al.* [27] examined 56 patients diagnosed with CF on newborn screening. With a retrospective review of chest radiographs and lung function that were compared pre and post *P. aeruginosa* colonization, there is an expected worsening of both chest radiograph scores and lung function. Both forced expiratory volume in one second (FEV$_1$) and forced vital capacity (FVC) decline by 1.29% per year prior to acquisition of *P. aeruginosa*, and they decline by 1.8% per year post acquisition, with a *P* value of 0.001. Newborns have no bacteria in the lungs; however, with a host-defense defect related to CFTR mutation, there is promotion of intermittent infection and then bacterial adaptation with biofilm formation leading to permanent colonization with *P. aeruginosa*.

Abdominal complications, especially involvement of intestinal mucous glands, predispose patients with CF to intestinal obstruction referred to as distal intestinal obstruction syndrome. The general pediatrician evaluating a child with CF needs to be aware of the non-pulmonary manifestations of CF. The bile is inspissated and causes biliary cirrhosis. With repeated inflammation, the pancreas can form cystic changes and become fibrotic, leading to endocrine pancreatic dysfunction and necessitating the use of insulin.

1.8. Sinus Disease in CF

Acute and chronic sinusitis is a common complication of CF. The true incidence of sinusitis is not known, but a great majority of patients with CF develop sinus symptoms, usually between the ages of 5 and 14 years [28]. The sinus disease associated with CF has unique features that suggest the diagnosis, such as nasal polyps. Nasal

polyps in CF have fewer eosinophils (<4%), and mucus glands contain acid rather than neutral mucins [29]. The prevalence of nasal polyps on CT has been reported as high as 48% and appears to be proportional to age [30]. Agenesis or lack of proper formation of frontal sinuses is common in CF seen on plain radiographs because of obstruction of sinuses early in life. On CT and MRI, more than 75% opacification on maxillary and ethmoid sinus is considered a hallmark of CF. Bulging lateral nasal wall near middle meatus and resorption of uncinate process is considered distinctive of CF. Sinus disease can cause significant symptoms and, in some cases, may contribute to the worsening of lung disease [31].

The sinuses are lined by a layer of epithelium similar to lower respiratory tract; the inflammation and infection that causes harm leads to pansinusitis. Infection in CF sinusitis is easy to predict and unlike non-CF sinusitis, as it is a reflection of lung pathogens that are unique to CF, namely *P. aeruginosa*, Staphylococcus, *H. influenzae*, Burkholderia, Achromobacter, and Stenotrophomonas.

Treatments take on many forms: antibiotics, nasal steroids, mechanical clearance with saline antihistamines, decongestants, and surgery. Surgical treatment of sinus disease is generally considered in patients with persistent nasal symptoms, persistent headaches, and infection and in those awaiting lung transplants [32].

1.9. Potential Drug Therapies for CF

There are potential drug therapies for CF in development every step of the way. The CF Foundation hopes the drug development and approval process, which currently takes 17 years on average with a cost of $400 million, eventually will be limited to seven years with a cost of less than $100 million in the safety dosage stage, with phase one, two, and three trials shortened considerably as well. The CF foundation's website, www.CFF.org, lists all the medications in their research pipeline for CF.

Lung transplant remains the only viable option for treatment of end-stage lung-disease with diffuse bronchiectasis and moderate-to-severe lung-disease with repeated exacerbation and oxygen dependency. St. Louis Children's Hospital, the most active pediatric lung transplant center in the world, experiences a five-year survival rate of 55% [33]. However, even with transplantation, other systemic sequelae of CF are not cured.

Gene therapy is held as a possible cure for cystic fibrosis, and current research entails the use of adenovirus, adeno-associated virus, and liposomes as vectors, which, at the present time, do not work well for therapy. Researchers hope for personalized CF care, genomics, and proteomics for individualized treatment of patients with CF. The motto of the CF Foundation is, "spanning the globe and working together".

1.10. Socioeconomic Factors and CF

In 2001, Schechter *et al*. studied the association of socioeconomic status and the outcomes depending on each status in CF patients in the United States [34]. In this historical cohort study of CF registry data between the years 1986 to 1994, with Medicaid status as a proxy for lower socioeconomic status, they examined 20,390 CF registry patients aged under 20 years. The median follow-up was 8 years. Of the 20,390 patients in the eligible group, 1894 (9.3%) received Medicaid during every year they were listed in the registry. The adjusted risk of death was 3.6 times higher in Medicaid patients than in those not receiving Medicaid. Medicaid patients had 1.9% lower FEV_1, and were 2.1 times more likely to be below the fifth percentile for weight, 2.2 times more likely to be below the fifth percentile for height, and 1.6 times more likely to require treatment for pulmonary exacerbation. There was no difference in the number of outpatient clinic visits for Medicaid versus non-Medicaid patients. Schechter *et al*. concluded that low socioeconomic status may be associated with significantly lower outcomes. This finding, however, was not due to access barriers to specialty healthcare, as all patients had an equal number of clinic visits. Therefore, one has to look further to determine what adverse environmental factors might cause worse outcomes in CF patients of low socioeconomic status. Clinicians providing primary care to young infants and children in this scenario need to be aware of the lower outcomes and explore avenues to attempt to modify end-results.

Environmental factors that influence CF lung-disease are cigarette smoke and other pollutants; allergens; viral pathogens; respiratory syncytial virus (RSV) in infancy (clinicians should be aware of the need for palivizumab for the first winter); pathogen acquisition type and timing (namely *P. aeruginosa* acquisition [20,21]); and, as previously mentioned, socioeconomic status. A study by Collaco *et al*. [35] examined interactions between secondhand smoke and genes that affect CF lung disease. Their research showed substantial disease variation in conditions with single gene etiology, such as CF, by simultaneously studying the effects of genes and environment. The study looked at secondhand smoke exposure and lung function in CF, and whether socioeconomic status and secondhand smoke, along with genetic predispositions, combined to create negative outcomes in lung function. This retrospective assessment of lung function looked at 812 patients, stratified by environmental and genetic factors, using data collected from the United States CF Twin and Sibling Study and the CF Registry. Secondhand smoking was found in 23.2% of the subjects, and active maternal smoking during gestation was reported in 16.5%. Secondhand smoke exposure led to a significantly lower cross-sectional lung function

and a 9.8% lower FEV_1 with a P value of <0.001. The longitudinal lung function in patients exposed to secondhand smoke was 6.1% lower ($P = 0.007$). Analysis determined that socioeconomic status did not confound the adverse effects of secondhand smoke on lung function: exposure to secondhand smoke will have detrimental effect on lung health regardless of socioeconomic status. Additionally, the interaction between gene variants and secondhand smoke exposure resulted in a significant percentile-point decrease in lung function. Variation of the gene that causes CF (CFTR) and CF modifier gene (TGF beta1) amplify the negative effects of secondhand smoke exposure. Namely, CFTR non-delta-F508 homozygotes had a 12.8% decrease in lung function ($P = 0.001$) and the presence of transforming growth factor gene as a modifier gene led to a 22.7 to 20.3 percentile-point decrease in lung function ($P = 0.005$). Simultaneous study of gene variations and environment will explain the variation of disease expression even with single gene etiology such as CF. General pediatricians need to be aware of the interactions between the gene pool, environment [smoking], and socioeconomic factors to counsel parents and/or care takers appropriately.

1.11. Modifier Genes in CF

Research into mapping the CF-modified genes in the mouse model and in humans is ongoing. Modifier genes in CF, even in individuals with the same CF genotype, will influence different clinical courses. Known modifier genes are tumor necrosis factor (TNF)-alpha, alpha-1 antitrypsin, and alpha-1 antichymotrypsin. The 2006 cross-sectional study by McKone et al. [36] tested the association between CF lung-disease and functional polymorphism in the glutamate-cysetine-ligase gene. Participants included 440 subjects, recruited from CF clinics in Seattle, WA, and centers in Canada, with a mean age of 26 ± 11 years and mild lung disease, with a mean FEV_1 of 62%. The authors concluded that in patients with a milder CFTR genotype, there is a strong association between functional polymorphisms of the glutamate-cysteine-ligase gene and severity of CF lung disease. Clearly, other genes are affecting the phenotypic presentation; this explains the difference in disease expression even among related individuals with CF. Clinicians will be better equipped to counsel their patients with this background information.

1.12. Survival

In 2009, the median age of survival was 35.9 years [4] hoping to increase to 39 to 40 years. In previous years, increases in median survival correlate with the discovery of newer anti-*Pseudomonas aeruginosa* antibiotics. Cur-

rently, at specialized care centers, a team approach to CF-patient care, improvement in nutrition, control of infection, and improvement in early preventive care have advanced survival. The specialist, working hand in hand with the primary care pediatrician, enhances the care given to these patients.

2. BENEFITS OF EARLY SCREENING AND DIAGNOSIS

Because of the availability of newborn screening in all 50 states as of 2010 [37], and the recommendation from the American College of Obstetricians and Gynecologists for carrier screening, CF diagnosis is now occurring at an earlier age than before [3]. There is strong evidence for early onset of malnutrition [38], airway inflammation, and structural changes in infants with CF. Although new CF therapies have been introduced over the past 10 years, none have been studied in infants, due in part to the variability of this patient subgroup. Limited data are available on the utility of available therapies in infants; there are no controlled trials on newer CF therapies, and no all-inclusive, age-specific guidelines specifically relating to the respiratory system. The nutritional benefits of neonatal screening for CF have been evaluated by many authors. Farrell et al. [39] compared nutritional status of patients with CF identified by neonatal screening versus those identified by clinical presentation after birth. The early diagnosis group (12 weeks vs. 72 weeks) had significantly greater height and weight percentiles and a larger head circumference (52nd vs. 32nd percentile; $P = 0.003$). Higher antropometric indexes were found during follow-up despite pancreatic insufficiency and delta-F508 mutation. When there is a delayed diagnosis, severe malnutrition exists throughout infancy, and catch-up growth is more difficult to attain.

An example of a study that can attest to this is the following. Konstan et al. [38] reviewed growth and nutritional indices in early life as predictors of pulmonary function in CF. Using the Epidemiologic Study of Cystic Fibrosis (ESCF) database, they compared 931 patients for weight-for-age and height-for-age percentiles, ideal body weight, and signs of lung disease at three years of age; the patients were retested for lung function at six years. Height-for-age and ideal body weight were poorly associated with lung disease at three years, but strongly associated with lung function at six years. If the weight-for-age was below the fifth percentile at age three, then lung function, or FEV_1, at age six was 86 ± 20. If the weight-for-age at age three was at the 75th percentile, the FEV_1 at age six was 102 ± 18. Patients with signs and symptoms of lung disease at age three had lower lung function at age six years. This work leads to the conclusion that aggressive intervention early in life aimed at growth, nutrition, and lung disease will positively influ-

ence lung function.

In 2004, Kosick *et al.*, also a part of the Wisconsin newborn screening group, evaluated screened newborns versus those given a clinical symptomatic diagnosis as to cognitive function and the possible influence of both early diagnosis through neonatal screening and the potential effect of malnutrition [40]. Using a cognitive-skills index and cognitive-factor scores (verbal, non-verbal, and memory), outcomes of significantly lower cognitive scores correlated with low plasma alpha-tocopherol level. Prevention of prolonged malnutrition by early diagnosis and traditional therapy minimizing the duration of vitamin E deficiency is associated with better cognitive function in children with CF. So clearly, early diagnosis improves nutrition, even as long as 13 years later.

It has also been noted that children have evidence of changes secondary to chronic inflammation in their lungs. The 2004 study by Long *et al.* [41] reviewed full-inflation, controlled-ventilation, high-resolution computed tomography (CT) scan images of the lungs in 34 CF infants with a mean age of 2.4 years. In control infants, the airway-wall thickness, airway-lumen diameter, and vessel diameter were measured. The infants with CF had an airway wall that was thicker, an airway-lumen diameter that was larger, and a vessel diameter that was smaller. The airway-lumen diameter to vessel diameter ratio increased with age in patients with CF compared with those in the control group. Further evidence of this chronic inflammation leading to subsequent development of bacterial infection has also been studied. In 2009, Sly *et al.* (Australian Respiratory Early Surveillance Team for Cystic Fibrosis [AREST-CF]) published a study [42] in which 57 infants with median age of 3.6 months underwent bronchoalveolar lavage and chest CT in Australia. Despite the absence of respiratory symptoms in 48 patients, a substantial proportion of lung disease with bacterial infection was seen. Inflammation was increased with infection; however, most were asymptomatic. There was radiological evidence of abnormal CT scan in 80%, bronchial dilation in 18.6%, bronchial-wall thickening in 45%, and air trapping in 66.7%. Most children with structural lung-disease were asymptomatic and diagnosed by newborn screening at a median age of 30 days; a CT and bronchoalveolar lavage were performed under anesthesia at 4 kg weight. Prevalence of bronchiectasis was 22% and increased with age. Factors associated with bronchiectasis include absolute neutrophil count elevation, neutrophil elastase concentration, and *P. aeruginosa* infection.

In 2007, Padman *et al.* [43] collected and reviewed ESCF data to assess whether the patterns of care for infants with CF at sites with superior average lung-functions at six to 12 years of age showed any difference from those at the lowest outcome sites. A total of 755 infants in 12 upper-quartile sites and 743 infants from 12 lower-quartile sites were reviewed. Upper-quartile sites had more infants whose disease was diagnosed by family history or newborn screening, fewer infants with symptoms at diagnosis, higher weight-for-age at enrollment, more Caucasian patients, and more delta-F508 homozygotes. Medical conditions and respiratory tract microbeology differed between sites. Infants at upper-quartile sites had more office and sick visits, more respiratory tract cultures, and frequent use of antibiotics, oral steroids, mast-cell stabilizers, and mucolytics. The authors concluded that both enrollment characteristics and infant care patterns are associated with lung function outcomes in later childhood. Analysis also suggested that pulmonary function of older children may be improved through specific interventions during the first three years of life. More antibiotics, specifically more oral antibiotics, inhaled steroids, mucolytics, and cromolyn sodium, were used in the top quartile. There was an increase in aggressive therapy for infants with CF and increasing detection of *S. aureus* and other organisms. Specific practices, including more frequent visits, more cultures, and more therapies can lead to a better outcome in terms of lung function as long as six to 12 years later.

All of the preceding studies collectively suggest early intervention in nutrition and initiation of pulmonary therapies as a means of prolonging lung remodeling and addressing failure to thrive. In a retrospective cohort study of CF registry data (1993-2004) from three CF centers, Padman *et al.* [44] compared initial management with respiratory, antimicrobial, and nutritional agents in infants. They examined the association between dornase alfa use prior to two years of age and body mass index (BMI) percentile over time, controlling for possible factors including genes, gender, race, CF center, presentation, age at diagnosis, sweat value, delta-F508 status, first *P. aeruginosa* infection, second-year weight percentile, supplemental feeding use, and pancreatic enzyme use. Patient characteristics and prescribed therapies were similar at all sites for 165 patients. One CF center prescribed dornase alfa more frequently, 82% vs. 10%, and supplemental feeding significantly less frequently, 56% vs. 78% ($P = 0.04$). Dornase alfa prior to two years of age is associated with a 10% increase in BMI through six years as compared with infants treated with dornase alfa. Treating infants aged less than two years with dornase alfa may improve nutritional outcomes up to six years of age.

This is also evident radiologically. Nasr *et al.* [45] studied 12 patients, all under 5 years of age, at the University of Michigan CF center. Mean changes in high-resolution CT-scan scores between dornase alfa and placebo groups were found to be significant at the 95% percentile level. The difference in chest radiographic

scores was not significant between the two groups. Administration of dornase alfa was associated with improvement in high-resolution CT scans in CF patients aged less than five years.

Ratjen *et al.* [46] looked at the role of matrix metalloproteases in the remodeling and degradation of extracellular matrix and their possible role in pulmonary tissue destruction. They examined bronchoalveolar lavage on two occasions within 18 months in 23 children with mild CF, of whom 13 were treated with dornase alfa. For comparison, they also studied 11 children without CF or other pulmonary disease who were undergoing elective surgery for nonpulmonary illnesses. Matrix metallopeptidase MMP-8, MMP-9, and the molar ratio were significantly higher in children with CF than in the comparison group. A correlation was found between bronchoalveolar lavage fluid concentrations of MMPs and alpha (2)-macroglobulin, a marker of alveolocapillary leakage. After 18 months, MMPs were increased in dornase-alfa-untreated CF patients and were decreased in patients with CF treated with dornase alfa. This indicates that uninhibited MMPs contributed to pulmonary tissue damage even in patients with CF who have mild lung disease. Treatment with dornase alfa may have a beneficial effect even in CF patients with mild lung disease.

In a 2009 retrospective cohort study, Van der Doef *et al.* [47] looked at 218 patients with CF and examined longitudinal lung function (FVC, FEV1, small airway flow rates) and bacterial colonization rates, and compared the patients that were and were not treated with gastric acid inhibition for fat malabsorption or gasrtoesophageal reflux. Patients with CF who were on gastric-acid inhibition had similar yearly decline in lung function when compared with control CF patients who were not on gastric-acid inhibition. *P. aeruginosa* and *S. aureus* colonization were not different between the two groups. Gastroesophageal reflux disease (GERD) patients had an association with a reduced pulmonary function and an earlier acquisition of *P. aeruginosa* and *S. aureus*, indicating GERD should be aggressively pursued in patients with CF. Gastric-acid inhibition may have beneficial effect on lung function with time as the decline in flow rates were less pronounced with GERD and gastric-acid inhibition in CF patients.

Guidelines for Management of Infants with CF

"The Cystic Fibrosis Foundation Evidence-Based Guidelines for Management of Infants with Cystic Fibrosis" was published as a supplement to the 2009 *Journal of Pediatrics* [2]. The guidelines promote an initial visit to an accredited CF center within 24 to 72 hours of diagnosis and monthly visits to a CF center for the first six months following diagnosis. In the realm of nutrition, the CF Foundation lists the need for supplemental salt (1/8

of a teaspoon per day for the first six months, 1/4 teaspoon per day for the next six months), breast milk feeding or standard infant formula, initiation of caloric-dense feeding for inadequate weight gain, maintenance of weight-for-length greater than or equal to 50th percentile, and prescription of pancreatic enzymes and fat soluble vitamins with monitoring of stooling pattern and vitamin blood levels. Strong infection-control measures are to be exercised, the environment must be smoke-free, airway clearance must be started within the first few months, chest physical therapy should not be performed in a head-down position, and the use of albuterol is encouraged. The care plan should include influenza vaccination, and palivizumab may be considered. Continued surveillance for bacterial infection, discouraging the use of antibiotics as prophylaxis, and treatment of the initial acquisition and persistent infection are necessary. Chest imaging and chronic therapies are recommended, but mucolytics, including dornase alfa, and hypertonic saline are not specified for the respiratory system, as there are no large-scale, double-blind, randomized studies to practice evidence-based medicine at this point in time.

The newborn screen has allowed the early detection of a number of disease entities, CF being a recent addition to the screen. For this screen to be effective in delaying onset of the numerous detrimental aspects of this disease, early, aggressive nutritional intervention and pulmonary therapies are needed. Current pulmonary therapy does not address the neutrophilic inflammation that starts shortly after birth. Clinicians involved in the care of many infants diagnosed by newborn screening need to be aware of these recommendations. They must follow, along with the specialist, both the typical and atypical infants and children with this underlying diagnosis. They must ensure that the routine immunizations are administered, including annual influenza vaccine. Palivizumab is a consideration for the first winter. They must monitor growth parameters closely and maintain year-round surveillance for lung infections. Through close work with CF specialists, clinicians can improve and enhance the lives of young patients with CF.

3. ACKNOWLEDGEMENTS

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