
Diagnostic Aspects of Cystic Fibrosis

A close-up, slightly blurred photograph of a young man with dark hair, smiling broadly. He is holding a white mobile phone to his ear with his right hand. The background is a soft, out-of-focus light color.

As the most common lethal genetic disorder in the Caucasian population, cystic fibrosis needs to be diagnosed early to allow for appropriate treatment and genetic counselling.

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Cystic Fibrosis (CF) is the most common lethal genetic disorder in the Caucasian population, affecting approximately 1:3,200 live births. Other ethnic groups, such as those of African (1:15,000) or Asian origin (1:31,000) are less affected. In Canada there are approximately 3,200 affected patients. While median survival is now into the fourth decade of life, cohort analysis shows that patients born more recently are benefiting from therapeutic advances and have a much better prognosis.¹ Within this decade, at least half the patients will be of adult age.

CF is a clinical diagnosis, based upon a compatible history, supported by laboratory

results. Most (98%) patients have elevated concentrations of electrolytes in their sweat. Approximately 70% of patients are diagnosed in the first year of life, and 92% by 10 years of age.

CF is an autosomal recessive disorder due to a defect in the gene, located on chromosome 7, coding for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). CFTR is a cyclic adenosine monophosphate (cAMP)-stimulated chloride channel that resides on the apical membrane of epithelial cells. While over 1,000 mutations have been described, approximately 70% of patients carry at least one copy of

the mutation known as F508. This is a 3-base pair deletion coding for phenylalanine, and results in CFTR being trapped in the endoplasmic reticulum, and not translocating to the epithelial surface. Other mutations can result in production of truncated nonfunctional protein; structural changes that result in poor protein trafficking; CFTR with diminished responsiveness to cAMP; or CFTR that has a poorly functioning chloride channel even when stimulated. Diminished CFTR function, whether because of diminished amounts of CFTR at the apical surface, or dysfunctional CFTR, results in defective chloride transport. However, the mechanisms by which altered chloride transport results in progressive lung destruction are unclear at this time.

A diagnosis of CF should be considered when there is an appropriate clinical picture. Prompt confirmation of the diagnosis allows for the institution of appropriate therapy and genetic counselling. Typical clinical features can be found in Table 1. A few clinical aspects deserve particular attention. Approximately 15% of patients will present in the newborn period with meconium ileus. In addition, newborns who have meconium plug also deserve investigation. Nasal polyps in children, or digital clubbing in any patient requires

investigation. Persistent or recurrent sinopulmonary disease is the most common presenting feature, and the principal cause of mortality. Failure to thrive, or malnutrition, is the second most common presenting feature and it should be recognized that about 90% of patients have exocrine pancreatic insufficiency that results in malabsorption of fat, fat soluble vitamins, and protein. Hyponatremic dehydration or persistent metabolic alkalosis also must be evaluated for CF. More recently, it has been recognized that some mutations only result in obstructive azoospermia; however, the long-term pulmonary function of these patients has yet to be evaluated. Similarly, the incidence of CF mutations is increased in patients with chronic pancreatitis, bronchiectasis or sinusitis. Some of these patients will be carriers of a single recognized mutation, while others will be CF variants.

CF must also be considered in siblings of affected individuals. Full siblings have a 25% chance of having CF. If siblings do not have CF, then they have a two out of three chance of being a CF carrier. Compared to the general population, half-siblings of CF patients are at increased risk for CF. Patients can also be diagnosed when they are asymptomatic through newborn screening programs. Antenatally,

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Table 1

Clinical Features of CF

Sinopulmonary Disease

- Persistent or recurrent chest infiltrates
- Difficult to control, or prolonged or recurrent wheezing
- Prolonged sputum production
- Nasal polyps
- Digital clubbing
- Colonization with CF pathogens such as mucoid strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*
- Hemoptysis

Gastrointestinal Disease

- Failure to thrive with poorly formed, malodorous stools
- Meconium ileus or plug
- Rectal prolapse

Electrolyte abnormalities

- Hyponatremic dehydration
- Chronic metabolic alkalosis

Obstructive azoospermia

the diagnosis is occasionally suspected on the basis of a hyperechoic fetal bowel pattern on prenatal ultrasonic examinations.

Diagnostic Laboratory Investigations

Evaluation of Electrolyte Transport Abnormalities

The standard diagnostic test is the sweat chloride test, which should be interpreted in light of the clinical context. The test

must be carried out in a centre that performs this on a regular basis, with well-defined quality control measures. The only recognized valid method is the pilocarpine iontophoresis sweat test. In this procedure, pilocarpine is introduced under the skin to stimulate sweat production. The sweat is collected over a 30-minute period, and a minimum amount of sweat must be collected to ensure an adequate sweating rate. The most widespread measure used is known as the Gibson-Cooke procedure, and requires a minimum of 75 μL of sweat, while the Wescor Macroduct coil system requires a minimum of 15 μL . Such collections can be difficult to perform on infants under six weeks of age. Inadequate sweat collection in the 30-minute period must be interpreted as an insufficient quantity and not be used to include or exclude a diagnosis of CF.

Sweat tests are standardized as to the chloride concentration. Typically, CF patients have sweat chloride concentrations $> 60\text{mmol/L}$. A value of 40-60 mmol/L could represent a CF variant, a patient who carries a single mutation, or some other process unrelated to CF. For children less than three months of age, a value >30 mmol is suspicious, and > 40 mmol is highly suggestive of a CF diagnosis. Sweat tests always require a second confirmatory test. Most causes for a falsely positive sweat test are clinically distinct enough to allow for differentiation from CF; however, malnutrition can also cause falsely elevated sweat chloride results. Paradoxically, significant protein-calorie malnutrition, which can occur in untreated

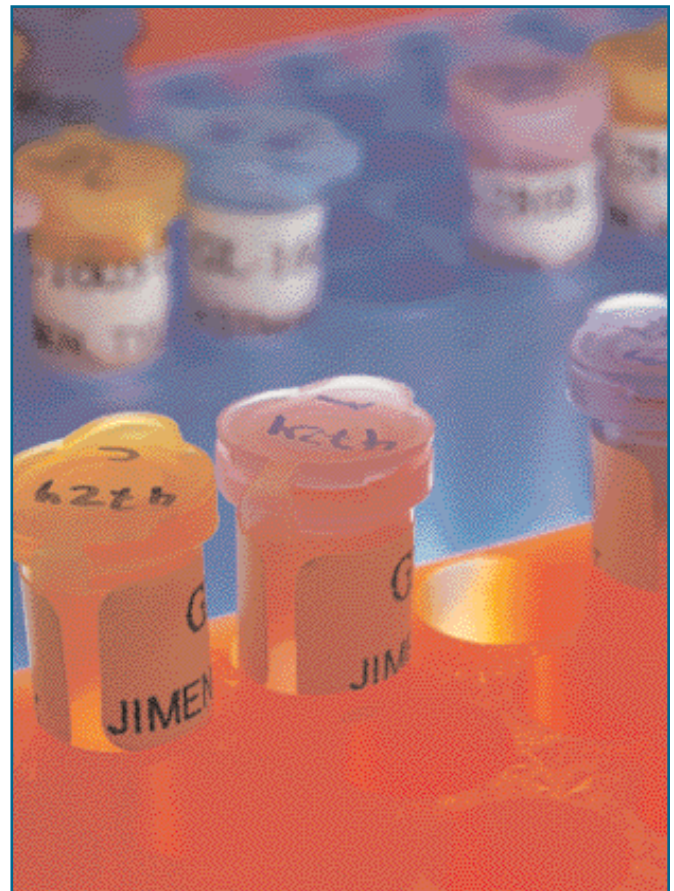
CF patients, can cause a false-negative result.

In highly specialized centres, nasal potential differences can be measured. Due to alterations in ion transport seen in CF, there are alterations in the membrane potential across the respiratory epithelial. This can be assessed across the nasal epithelium by examining the voltage difference between the epithelial surface in the nose and the subdermal epithelium on a limb. The technical expertise for such testing is very demanding, but such testing can aid when the sweat-test result is indeterminate. However, false-negative results can occur in the context of nasal inflammation, a common occurrence in CF patients.

Genetic Mutation Analysis

The diagnosis of CF is typically suggested by its characteristic clinical and laboratory features and confirmed by a sweat test. In a patient who exhibits one or more phenotypic features consistent with CF, or has a history of CF in a sibling, the diagnosis of CF can be confirmed by the identification of two known CF mutations using genetic mutation analysis. This test is used for prenatal diagnosis, provided both parents are shown to be carriers, and carrier testing takes place in families with a previously affected child. Mutation analysis also can be used for carrier detection in the general population, but it is not yet recommended for population-based screening in Canada.

Laboratories that perform direct CFTR mutation analysis require expertise and



experience in the use of sophisticated tools in molecular biology, and must possess expertise in human genetics for accurate interpretation of the results. Testing of symptomatic patients or carrier status typically requires a source of whole blood, however buccal swabs, dried blood spots, saliva and other human tissue samples containing DNA are also acceptable. Testing for prenatal diagnosis requires fetal or placental cellular sampling from chorionic villus samples (CVS) at about 10 weeks gestation, or from amniocentesis (direct or cultured) between 15 to 18 weeks gestation.

The incidence of single mutations is increased in populations with chronic sinopulmonary and pancreatic inflammation.

For direct mutation analysis, a number of methodologies are used, the most common being hybridization of patient DNA with mutation-specific oligonucleotide probes. Technical accuracy of testing for detecting a specific genetic mutation is more than 99%.² The overall sensitivity depends on the number of CF gene mutations included in the core CFTR mutation test panel by the performing laboratory. With over 1,000 reported mutations in the CF gene, it is not practical or cost-effective to screen for more than a small percentage of

the total number of mutations. Furthermore, not all mutations in CFTR cause CF. Most laboratory test panels screen for five to 80 CFTR mutations that appear in the population at more than 1%. Test panels typically contain the most common mutation, F508; as well, test panels may also contain mutations found at high frequency in other ethnic groups (e.g., W1282X found in Ashkenazi Jews).^{3,4}

Overall test sensitivity will vary according to the specific ethnic, demographic, or racial group of the patient being tested.

Results for this test are reported as negative (no mutation identified); heterozygous positive (one mutant allele identified); homozygous positive (two copies of the same mutant allele identified); or com-

ound heterozygous positive (one copy of each of two different mutations identified). It is important to note that a negative result indicates that the individual is not a carrier of any CF mutation **included in the DNA test panel**; thus a negative test only reduces the risk of being a carrier and does not completely eliminate it. Heterozygous-positive individuals are carriers of one of the CF mutations included in the DNA test panel, and usually have no biochemical or physiological alterations by which they could otherwise readily be identified. However, as previously mentioned, the incidence of single mutations is increased in populations with chronic sinopulmonary and pancreatic inflammation. Homozygous and compound heterozygous-positive individuals are patients affected by cystic fibrosis; however, clinical severity of CF can be quite variable. Depending on which mutations are included in the test panel, this method may potentially identify patients with atypical or asymptomatic CF who have normal sweat tests.

Prior to any genetic testing, it is highly recommended that detailed education or genetic counselling be offered to patients and their families, and that the person, or their legal guardian, signs a consent form and submits it with the sample. It is strongly recommended that the caregiver explain the nature of the residual risk of a negative test and the clinical implications of a positive test.

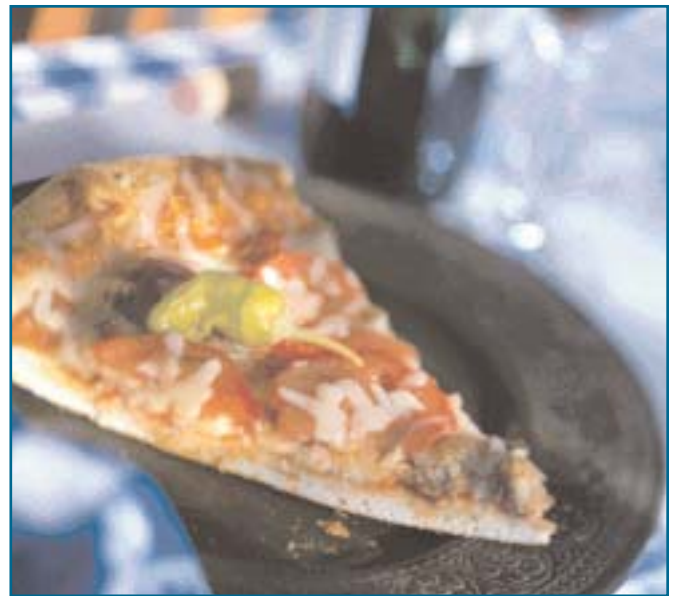
Neonatal Screening

Several centres, particularly in the United

States and Australia, have begun population screening in the neonatal period. Such testing involves analysis of the blood spot collected as part of neonatal screening for the concentration of immunoreactive trypsinogen. In the face of pancreatic duct obstruction, immunoreactive trypsinogen will be elevated in the blood. In order for such screening to be sufficiently sensitive (no false-negatives at the expense of some false-positives), further evaluation is carried out on patients with levels above the 99th percentile for the particular assay used. Such screening will miss the 10% to 15% of CF patients with adequate pancreatic function in the newborn period. A positive test is then followed by genetic mutation analysis and sweat testing. There is still significant controversy concerning whether such population-based screening should be done. Data from some centres suggest that presymptomatic detection improves long-term outcome, although this has not been found universally. However, with the growing knowledge of pathogenesis, the realization that interventions must occur early, and the potential of new therapeutic strategies, early identification of patients before they are symptomatic will become part of the routine therapeutic strategy for CF care in the near future.

Principles of Management

Current management largely aims to overcome the symptoms of CF. The central factor that has had the largest impact on survival of CF patients is the organization



Current gastrointestinal management encourages a high-caloric diet relatively rich in fat (approximately 35%), which is actually quite similar to the standard North American diet.

of multi-disciplinary clinics dedicated to CF care. Working in conjunction with their family physician, almost all affected Canadians are followed at CF clinics.

It is now recognized that there is an overly exuberant neutrophilic inflammation in the lungs, especially in response to bacterial colonization. This results in bronchial wall damage, bronchiectasis, and parenchymal destruction, such that lung function is the primary determinant of mortality. Based on such a paradigm, a variety of anti-inflammatory strategies are currently under investigation, including high-dose ibuprofen,

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anti-proteases, and novel antimicrobial peptides. As well, a variety of novel therapies in development aim to improve the production of CFTR, which can traffic through the endoplasmic reticulum, then through the Golgi apparatus, to reach the apical membrane and be responsive to cAMP. Other approaches include stimulating the function of chloride channels other than CFTR which already exist on the apical surface. Taking a much longer view is gene replacement therapy which is also under vigorous investigation; however, patients will need to have good preservation of lung function to benefit from these approaches.


Current gastrointestinal management encourages a high-caloric diet relatively rich in fat (approximately 35%), which is actually quite similar to the standard North American diet. The aim of nutritional therapy is normal growth and development. Patients with pancreatic insufficiency require enzyme replacement with all meals and snacks containing protein and lipids. Water miscible forms of the fat soluble vitamins A, D, and E are routinely provided. Replacement vitamin K is not provided routinely, however recent evidence suggests that CF patients may have subclinical vitamin K deficiencies that could be contributing to the osteoporosis that is frequently found in adolescents and adults with CF. Patients are advised to liberally salt their food.

Respiratory therapy currently aims to diminish the amount of pulmonary secretions and reduce bacterial load. Patients are encouraged to carry out daily chest physical therapy. A variety of techniques exist,

but the mainstay is percussion and drainage. Such therapy usually requires the help of someone to administer such treatment. Preadolescent and older patients can often utilize an expiratory resistive device that allows secretions to move more centrally where they can be expectorated. This technique alleviates the need for someone to administer the treatment. All these techniques take about 20 minutes and patients are encouraged to do physiotherapy once or twice a day. Vigorous physical exercise is strongly encouraged, but is not a replacement for well-recognized forms of chest physical therapy. Some patients utilize nebulized deoxyribonuclease (DNase), as a mucolytic. The major cause for the viscous secretions seen in CF is the DNA resulting from the abundant dead neutrophils in the respiratory secretions. DNase will break up these DNA clumps and make the secretions more fluid. At least 20% of patients also have a bronchospastic component to their lung disease and require asthma medications including inhaled corticosteroids and beta-agonists.

In an attempt to reduce the exaggerated inflammatory response to resident bacteria, antibiotics are used frequently to diminish the bacterial load. The most commonly encountered organisms are *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa*, especially mucoid strains. In fact, culture of mucoid *Pseudomonas aeruginosa* in respiratory secretions should raise a suspicion of CF. Many patients take antibiotics on a continuous basis, either using oral or inhaled antibiotics.

Conclusion

Significant strides have been made in the care and management of CF patients, so that they are living longer and healthier lives. It is important that CF be diagnosed early, so that treatment can be implemented to prevent and delay permanent damage. Newer therapeutic interventions hold significant promise for even further gains in morbidity and mortality. Prompt recognition of CF and referral to a CF clinic will allow patients to benefit from therapeutic advances. 

*The Canadian Cystic Fibrosis Foundation (CCFF) is a world leader in the fight against CF. Canadian researchers discovered the gene responsible for cystic fibrosis in 1989, and continue to play a leading role in the worldwide race to develop new treatments for the disease. With developments in research and treatment in Canada, the median age of survival has increased from four years in 1960, to over 30 years today. For more information about cystic fibrosis and the CCFF please visit the following Web site:
www.cysticfibrosis.ca.*

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Recommended Reading

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