NOTE: The Consensus Conference Program was initiated by the Cystic Fibrosis Foundation (CFF) about a decade ago to synthesize the knowledge of experts on a selected topic in cystic fibrosis (CF) patient care together with the current literature. The goal is to establish guidelines for patient care. Whenever the need to examine a topic in patient care is apparent, CFF organizes a conference to review current knowledge. Two chairpersons are recruited on the basis of their level of expertise on that topic; a panel of 20 to 30 CF care givers and other experts on the chosen topic is then selected to share their expertise and ideas throughout the meeting. These 2-day meetings culminate in the consensus panel arriving at recommendations that reflect current trends while also anticipating future areas critical to patient care. The aims of the consensus document are provision of a uniform level of care to all patients with CF, education of CF care givers, and suggestions for future research.—Robert J. Beall, PhD

The diagnosis of cystic fibrosis: A consensus statement

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Cystic fibrosis is the most common life-limiting recessive genetic disorder in Caucasians, with an incidence of 1 in 3200 newborns in the United States.[1] It is less common in African Americans (1 in 15,000) and in Asian Americans (1 in 31,000).[1] The CF gene product, CF transmembrane conductance regulator, functions as a cyclic adenosine monophosphate–regulated chloride channel at the apical surface of epithelial cells.[2] Thus far, more than 500 mutations of the CFTR gene have been identified.[3] In the presence of CFTR mutations, dysfunctional epithelial transport leads to the clinical manifestations seen in patients with CF (Table I). In the United States, the diagnosis of CF is established by 1 year of age in the majority (71%) of patients.[4] However, in 8% of patients the diagnosis is not established until after age 10 years, and the diagnosis is now being made in an increasing number of adults.[4]

See related articles, p. 563 and p. 596.

It is essential to confirm or exclude the diagnosis of CF in a timely fashion and with a high degree of accuracy to avoid unnecessary testing, to provide appropriate therapeutic interventions and prognostic and genetic counseling, and to ensure access to specialized medical services. In the majority of cases, the diagnosis of CF is entertained because of the presence of one or more typical clinical features (Table II) and then confirmed by demonstrating an elevated (>60 mmol/L) sweat chloride concentration. Almost all patients have chronic sinopulmonary disease and, in postpubertal men, obstructive azoospermia.[4] Approximately 85% to 90% of all patients have exocrine pancreatic insufficiency.[5]

CBAVD	Congenital bilateral absence of the vas deferens
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
PD	Potential difference

In recent years the ability to detect CF mutations and to measure transepithelial bioelectric properties has greatly expanded the CF clinical spectrum. In approximately 2% of patients, there is an "atypical" phenotype, which consists of chronic sinopulmonary disease, pancreatic sufficiency, and either borderline (40 to 60 mmol/L) or normal (<40 mmol/L) sweat chloride concentrations.[6-9] In addition, there are patients in whom a single clinical feature (e.g., electrolyte abnormalities,[10] pancreatitis,[11,12] liver disease,[13] sinusitis,[14] or obstructive azoospermia[15-18]) predominates. In such cases, demonstration of CF mutations in each CFTR gene or the in vivo demonstration of abnormal ion transport across nasal epithelium can be used as diagnostic aids.[7-9] Of particular interest are individuals with congenital bilateral absence of the vas deferens and

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Table I. Initial features of 20,096 patients reported to the Cystic Fibrosis Foundation National Patient Registry in 1995

Feature	Number*	Percent*
Acute or persistent respiratory symptoms	10,141	50.5
Failure to thrive/malnutrition	8,628	42.9
Steatorrhea/abnormal stools	7,024	35.0
Meconium ileus/intestinal obstruction	3,788	18.8
Family history	3,368	16.8
Electrolyte imbalance	1,094	5.4
Rectal prolapse	677	3.4
Neonatal screening	459	2.3
Nasal polyps/sinus disease	404	2.0
Genotype	242	1.2
Hepatobiliary disease	175	0.9
Prenatal diagnosis (CVS, amniocentesis)	154	0.8
Other	236	1.2
Unknown	380	1.9

Courtesy of Stacey FitzSimmons, Cystic Fibrosis Foundation, Bethesda, Maryland.
CVS, Chorionic villus sampling.
*Not mutually exclusive.

Table II. Phenotypic features consistent with a diagnosis of CF

1. Chronic sinopulmonary disease manifested by
   a. Persistent colonization/infection with typical CF pathogens including *Staphylococcus aureus,* nontypeable *Haemophilus influenzae,* mucoid and nonmucoid *Pseudomonas aeruginosa,* and *Burkholderia cepacia*
   b. Chronic cough and sputum production
   c. Persistent chest radiograph abnormalities (e.g., bronchiectasis, atelectasis, infiltrates, hyperinflation)
   d. Airway obstruction manifested by wheezing and air trapping
   e. Nasal polyps; radiographic or computed tomographic abnormalities of the paranasal sinuses
   f. Digital clubbing
2. Gastrointestinal and nutritional abnormalities including
   a. Intestinal: meconium ileus, distal intestinal obstruction syndrome, rectal prolapse
   b. Pancreatic: pancreatic insufficiency, recurrent pancreatitis
   c. Hepatic: chronic hepatic disease manifested by clinical or histologic evidence of focal biliary cirrhosis or multilobular cirrhosis
   d. Nutritional: failure to thrive (protein-calorie malnutrition), hypoproteinemia and edema, complications secondary to fat-soluble vitamin deficiency
3. Salt loss syndromes: acute salt depletion, chronic metabolic alkalosis
4. Male urogenital abnormalities resulting in obstructive azoospermia (CBAVD)

other forms of obstructive azoospermia, many of whom have CF mutations on one or both CFTR genes or an incompletely penetrant mutation (5T) in a noncoding region (intron 8) of CFTR.[15-18]

A CF diagnosis can also be considered in the absence of clinical features. An individual with an affected sibling has a 1 in 4 or 25% chance of having the disease. Half-siblings are also at increased risk compared with the general population (Caucasians, 1 in 112; African Americans, 1 in 244; and Asian Americans, 1 in 352). These high risks justify careful clinical monitoring and, in appropriate situations, sweat testing of full and half-siblings. In many parts of the world, a diagnosis of CF is being established in the neonatal period with increased frequency because of the inclusion of tests for CF in newborn screening programs.[19,20] In these programs the diagnosis is suggested by an elevated level of immunoreactive trypsinogen in the blood and then confirmed by mutation analysis or sweat testing. Although some of these infants may be free of symptoms at the time of initial testing, it can be predicted that virtually all such patients will experience clinical manifestations of CF. Finally, because of the availability of accurate prenatal testing, an in utero diagnosis of CF, based on the detection of two CF mutations in the fetus, is being made with an appreciable frequency (Table I). Such testing is usually carried out in a family that has had a previously affected child or because of the detection of fetal echogenic bowel on routine ultrasonography.[21] Additional cases may be diagnosed as a result of the expansion of CF carrier screening in the general population.[22]

The goal of the consensus panel was to more precisely define the criteria for a diagnosis of CF in the context of our evolving understanding of the diverse clinical features seen in patients with CF and the availability of new diagnostic procedures.

CRITERIA FOR THE DIAGNOSIS OF CF

It is the consensus of the panel that the diagnosis of CF should be based on the presence of one or more characteristic phenotypic features (Table II), a history of CF in a sibling, or a positive newborn screening test result plus laboratory evidence of a CFTR abnormality as documented by elevated sweat chloride concentration, or identification of mutations in each CFTR gene known to cause CF or in vivo demonstration of characteristic abnormalities in ion transport across the nasal epithelium.

EVIDENCE OF CFTR ABNORMALITY

Sweat Test

In most cases the diagnosis of CF will be confirmed by measurement of chloride

concentration in sweat after iontophoresis of pilocarpine. Sweat testing should be carried out in accordance with the guidelines of the National Committee for Clinical Laboratory Standards.[23] It is crucial that testing be carried out by experienced personnel using standardized methods in facilities that perform adequate numbers of tests to maintain laboratory proficiency and quality control. The only acceptable procedure is the quantitative pilocarpine iontophoresis sweat test. A minimum acceptable volume (15 μl for the Wescor Macroduct coil system) or weight (75 mg for the Gibson-Cooke procedure) of sweat must be collected during a 30-minute period to ensure an average sweat rate of more than 1 gm/m^2/min.[23] Alternative sweat test procedures, such as direct-reading conductivity measurements[24] or a paper-patch indicator system,[25] are associated with an increased incidence of false-positive and false-negative results and should never be used as the basis of a definitive CF diagnosis.[23,26,27] When a physician orders a sweat test, it is mandatory that he or she know the method being used, as well as reference laboratory values.[27]

A sweat chloride concentration of more than 60 mmol/L is consistent with the diagnosis of CF, but the result must be interpreted in the context of the patient's age and clinical picture by a physician knowledgeable about CF. Some data suggest that in infants younger than 3 months of age, a sweat chloride concentration of more than 40 mmol/L is highly suggestive of a diagnosis of CF.[28] The diagnosis of CF should be made only if there is an elevated sweat chloride concentration (>60 mmol/L) on two separate occasions in a patient with one or more clinical features consistent with the CF phenotype or a history of CF in a sibling. Because sweat sodium concentrations of 60 to 80 mmol/L can be seen in individuals with diseases other than CF,[29-31] measurement of sodium alone is not recommended. However, in some cases, especially those with borderline sweat test results, measurement of both sodium and chloride concentrations can be helpful. In patients with CF, both analytes should be proportionately elevated (within 15 mmol/L), and the chloride/sodium ratio is almost always greater than 1.0.[31] A sweat chloride concentration of more than 160 mmol/L is physiologically impossible[32] and suggests an error in collection or analysis. Tests with such results should be repeated.

Table III. Mutations that cause cystic fibrosis

Mutation	Frequency*	Evidence†
G85E	0.2	4
R117H‡	0.3	1
621+1G→T	0.7	1,3
711+1G→T	0.1	1,3
1078delT	0.1	2
R334W	0.1	1
R347P	0.2	1
A455E‡	0.1	1
ΔI507	0.2	4
ΔF508	66.0	1,4
1717-1G→T	0.6	2
G542X	2.4	3
S549N	0.1	4
G551D	1.6	1,4
R533X	0.7	2
R560T	0.1	4
1898+1G→T	0.1	3
2184delA	0.1	2
2789+5G→A‡	0.1	1,4
R1162X	0.3	2
3659delC	0.1	3
3849+10kbC→T‡	0.2	1,4
W1282X	1.2	2
N1303K	1.3	1,4

*Caucasian population [4].
†(1) Causes a change in the amino acid sequence that severely affects CFTR synthesis and/or function, (2) introduces a premature termination signal, (3) alters the "invariant" nucleotides of spliced sites, (4) causes a change in the amino acid sequence that does not occur in the normal genes from at least 100 carriers of CF mutations from the same ethnic group.
‡Mutations that may be associated with normal or borderline sweat electrolyte levels.

Mutation Analysis

Cloning of the gene responsible for CF and identification of disease-producing mutations have raised the possibility that DNA testing may be substituted for the sweat test in certain circumstances. Considerable evidence for the deleterious consequences of a number of CF mutations has accumulated.[33] Thus the presence of mutations known to cause CF in each CFTR gene predicts with a high degree of certainty that an individual has CF. To date, more than 500 putative CF mutations have been described,[3] and it is not surprising that different defects in the CFTR gene may give rise to cases with overlapping phenotypic features. Alterations in the CFTR gene, designated as *CF-causing mutations*, should fulfill at least one of the following criteria. The mutation has been shown to: (1) cause a change in the amino acid sequence that severely affects CFTR synthesis and/or function, (2) introduce a premature termination signal (insertion, deletion, or nonsense mutations), (3) alter the "invariant" nucleotides of intron splice sites (the first two or last two nucleotides), or (4) cause a novel amino acid sequence that does not occur in the normal CFTR genes from at least 100 carriers of CF mutations from the patient's ethnic group. A list of mutations that are included in currently available CF

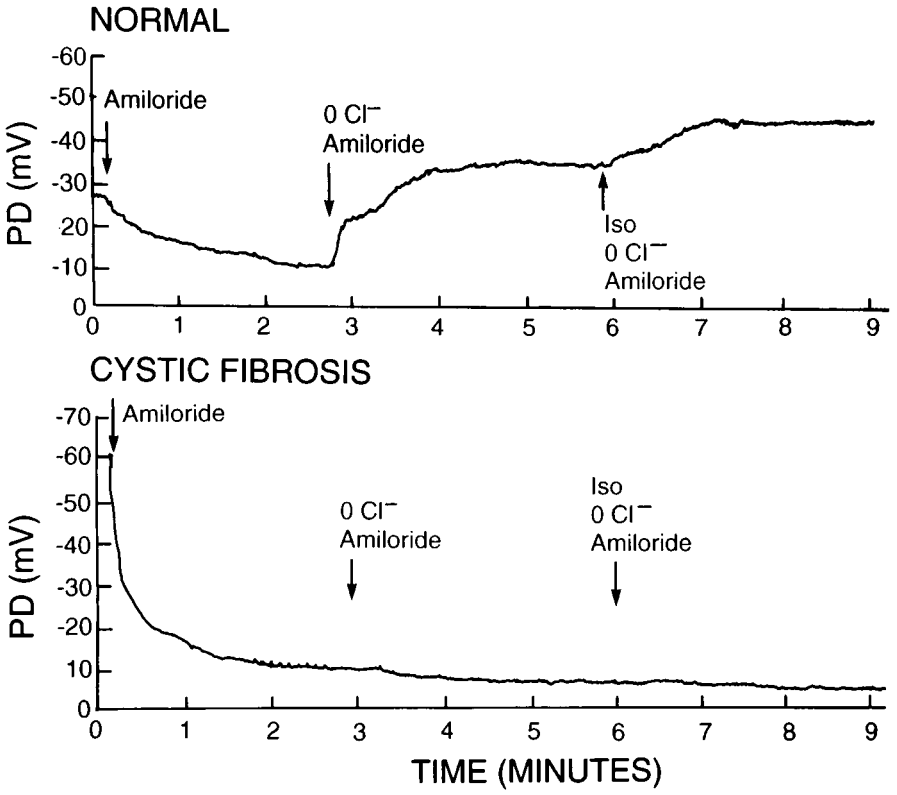

Figure. Nasal PD tracing in a normal subject **(top panel)** and a patient with CF **(bottom panel)**. Tracings illustrate response of PD to perfusion with amiloride (10^{-4} mol/L), addition of a Cl^--free solution (gluconate buffer) to amiloride, and addition of isoproterenol (10^{-5} mol/L) to the Cl^--free solution containing amiloride (see text).

mutation tests and that meet one or more of these criteria is shown in Table III; improvement in DNA technology indicates that CF mutation tests in the future will include a larger panel of mutant alleles. Each additional mutation should meet one or more of the four criteria listed above to provide a reasonable degree of certainty that it is disease-producing. A more complicated situation is presented by the R117H and 5T mutations. Presence of both mutations in the same gene (R117H-5T) is associated with CF.[34] However, neither the R117H mutation alone (i.e., R117H with the common splice variant 7T) nor the 5T mutation alone meets the criteria for a CF mutation. Although these mutations have been associated with male infertility caused by CBAVD, diagnosis of CF in patients carrying R117H-7T or 5T will require demonstration of a CFTR abnormality by sweat testing or nasal potential difference testing.

Confirming the diagnosis of CF on the basis of the presence of two CF-producing mutations is highly specific but not very sensitive. The sensitivity of mutation analysis is decreased because of the large number of CF alleles. Current commercially available mutation screening panels detect at most only 80% to 85% of CF alleles. In the United States, variability in the mutation detection rate reflects the ethnic origin of individuals in various regions of the country.[35] However, some CF mutations occur with increased frequency, or even uniquely, in specific population groups, such as Ashkenazi Jews,[33] African Americans,[1] and patients with specific clinical features (e.g., pancreatic sufficiency[36] or normal or borderline sweat electrolyte concentrations).[7-9] By customizing mutation panels to match the patient's ethnic background and phenotype, the sensitivity of DNA testing could be enhanced, although this is not routine clinical practice.

Increasing test sensitivity dramatically increases the fraction of patients with CF with two mutations identified. However, certain patients with CF will carry an unidentified mutation, even when test sensitivity approaches 95%. These patients will have to be diagnosed by using other measures of CFTR dysfunction (sweat test or nasal potential difference testing). Perhaps the most difficult diagnostic situation facing the clinician is the patient with clinical features consistent with CF but a nondiagnostic sweat test result and only one identified CF mutation. In such cases, evaluation involves weighing the possibility that the individual is a carrier of a CF mutation against the possibility that the patient has atypical CF. Nasal PD testing and ancillary laboratory tests (see below) may be particularly helpful for this group of patients. If CFTR dysfunction cannot be demonstrated by any method (sweat test, mutation analysis, or nasal PD), a definitive diagnosis cannot be made, and the decision to monitor or treat the patient rests on the strength of that individual's clinical presentation.

In summary, in individuals with clinical features consistent with CF, identification of two known CF mutations by a Clinical Laboratory Improvement Amendment–accredited DNA diagnostic laboratory confirms the diagnosis. Individuals with one CF mutation should be diagnosed on the basis of clinical features together with other measures of CFTR dysfunction. Inability to detect CF mutations does not rule out a diagnosis of CF. It should be emphasized that in the majority of cases, the diagnosis will be confirmed by a positive sweat test result and not by the identification of two CF mutations. However, it is the consensus of the panel that in such cases mutation analysis is desirable because it can be used for: (1) confirmation of the diagnosis, (2) provision of genetic information for interested family members, (3) prediction of certain phenotypic features such as pancreatic status, and (4) categorization of patients for research protocols. It should be offered to the patient or family after appropriate genetic counseling has been provided.

Nasal PD Measurements

Respiratory epithelia, including nasal epithelia, regulate the composition of fluids that bathe airway surfaces by transport of ions such as sodium (Na^+) and

chloride (Cl^-). This active transport of ions generates a transepithelial electrical PD, which can be measured in vivo.[37] Abnormalities of ion transport in respiratory epithelia of patients with CF are associated with a different pattern of nasal PD compared with normal epithelia (Figure).[38,39] This provides a rationale for the use of nasal PD as a diagnostic aid.[40,41] Specifically, three features distinguish CF: (1) higher (raised) basal PD, which reflects enhanced Na^+ transport across a relatively Cl^--impermeable barrier; (2) greater inhibition of PD after nasal perfusion with the Na^+ channel inhibitor, amiloride, which reflects inhibition of accelerated Na^+ transport; and (3) little or no change in PD in response to perfusion of the nasal epithelial surface with a Cl^--free solution in conjunction with isoproterenol, which reflects an absence of CFTR-mediated Cl^- secretion.[38] Although the measurement of nasal PD may assist in the diagnosis or exclusion of CF, there are important variables that need to be rigorously addressed to ensure the safety and accuracy of testing. The technique is safe, provided that the PD equipment (high-impedance voltmeter) meets appropriate clinical electrical engineering standards and subcutaneous skin bridges are prepared in an aseptic manner. Technical considerations mandate that nasal anatomy be clearly understood, because the sites of PD measurements are critical to the accuracy of the measurement. The equipment that is used must be rigorously validated, and the protocol should be well-defined and standardized. These technical considerations have been described in great detail.[38] Nasal PD can be measured in patients as young as a few hours of life[42]; older children (age 2 to 5 years) rarely may require light sedation. The presence of nasal polyps or inflamed mucosa alters bioelectric properties and may yield a false-negative result.[38]

Interpretation of PD measurements requires a clear understanding of the ion-transport characteristics of the nasal epithelium and the PD responses to perfusion with different probes of ion transport. For example, a raised basal nasal PD is strong evidence for the diagnosis of CF. However, the absence of a raised PD does not rule out CF because a false-negative result may occur in the presence of inflamed epithelium. As with any laboratory test that is used to confirm a diagnosis, a raised PD must be duplicated on more than one occasion to be valid as a diagnostic adjunct. It should be emphasized that the absence of a large CFTR-mediated Cl^- conductance (voltage change) in response to perfusion with a low (or zero) Cl^- solution and a β-agonist does not establish the diagnosis of CF, because there are nonspecific effects that inhibit the CFTR-mediated Cl^- conductance. However, the presence of a large response to Cl^--free perfusion is strong evidence against CF. Any laboratory planning to establish nasal PD as a clinical diagnostic tool must carry out a sufficient number of studies in patients with CF and defined mutations, normal subjects, and disease control subjects to establish reference values and to ensure adequate rigor of the technique.

Table IV. Clinical evaluation of atypical cases

1. Respiratory tract microbiology
2. Assessment for bronchiectasis
   a. Plain radiography
   b. Computed tomography
3. Evaluation of paranasal sinuses
   a. Plain radiography
   b. Computed tomography
4. Quantitative assessment of pancreatic function
5. Male genital tract evaluation
   a. Semen analysis
   b. Urologic examination
   c. Ultrasonography
   d. Scrotal exploration
6. Exclusion of other diagnoses
   a. Ciliary structure and function
   b. Immunologic status
   c. Allergy
   d. Infection

ANCILLARY TESTS TO ASSESS THE PATIENT'S PHENOTYPE

In patients who initially have an "atypical" phenotype, it is important to carry out a comprehensive clinical, radiographic, and laboratory evaluation (Table IV) for features known to be consistent with the CF phenotype or for alternative diagnoses.

Assessment of Exocrine Pancreatic Function

The vast majority of patients with CF, including those without obvious steatorrhea, have abnormal pancreatic acinar and ductular function.[43,44] The exocrine pancreas has a large functional capacity; more than 98% of the pancreatic capacity to secrete enzyme must be lost before signs and symptoms of maldigestion are evident.[43,45] A number of direct and indirect tests (including blood tests) are available to evaluate exocrine pancreatic function[43]; however, all currently available tests have drawbacks. Measurement of serum trypsinogen is only useful as a screening test after the age of 7 to 8 years, and bentiromide is not commercially available in North America. Among the indirect tests, fecal fat analysis with a timed pooled stool collection (minimum of 72 hours) is the most widely used and is probably the most informative. However, it does not measure pancreatic reserve. Thus there is no "ideal" test. Direct tests are highly specific and capable of evaluating the entire range of pancreatic function. They are of great value for identifying aspects of pancreatic fluid and anion secretion in patients with a questionable diagnosis of CF. However, these tests require special skill to perform and interpret, and their invasive nature precludes their use for routine clinical purposes.

Respiratory Tract Microbiology

Characterization of the respiratory microbial flora can be diagnostically helpful in the evaluation of patients with atypical features of CF. The predilection of *Pseudomonas aeruginosa* to colonize the respiratory tract in CF is well known.[46,47] The presence of the mucoid phenotype of *P. aeruginosa* in the respiratory tract (bronchoalveolar lavage fluid, sputum, oropharyngeal swab, sinus aspirate), especially if persistent, is highly suggestive of CF. Persistent colonization with other organisms such as *Staphylococcus aureus*, *Haemophilus influenzae*, and *Burkholderia*

Table V. Criteria for the diagnosis of CF

One or more characteristic phenotypic features —or a history of CF in a sibling —or a positive newborn screening test result
AND an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions —or identification of two CF mutations —or demonstration of abnormal nasal epithelial ion transport

cepacia may support a diagnosis of CF,[46,47] although many of these pathogens are also found in other conditions.

Urogenital Evaluation

One of the most consistent features of the CF phenotype in postpubertal male subjects is obstructive azoospermia, a finding present in 98% to 99% of affected individuals.[5,48,49] Functional sperm and fertility have been reported in male subjects hemizygous or homozygous for the 3849+10kb C→T mutation.[9,50] In the majority of patients with CF, azoospermia occurs as a result of absent or rudimentary vasa deferentia. The evaluation of postpubertal male subjects with atypical presentations should include a careful evaluation of their urogenital status by urologic examination, semen analysis, ultrasonographic study of the urogenital structures, and in rare cases, scrotal exploration.

Individuals who are first seen with CBAVD and other forms of obstructive azoospermia usually have no evidence of respiratory tract or pancreatic abnormalities and may have normal, intermediate, or elevated sweat chloride concentrations.[15,16,51] It is the consensus of the panel that individuals who are first seen with obstructive azoospermia be assigned a diagnosis of CF only if there is evidence of CFTR dysfunction as documented by elevated sweat chloride concentrations, identification of two CF mutations, or the in vivo demonstration of abnormal ion transport across the nasal epithelium. The prognosis for such patients assigned a diagnosis of CF appears to be excellent, but it is recommended that they be closely monitored for the development of other CF-related complications.[51]

SUMMARY

The diagnostic criteria proposed here are not likely to cover every possible clinical scenario, and there will be clinical dilemmas. For the vast majority of patients with CF, the diagnosis will be suggested by the presence of one or more characteristic clinical features, a history of CF in a sibling, or a positive newborn screening test result and will then be confirmed by laboratory evidence of CFTR dysfunction (Table V). Abnormal CFTR function will usually be documented by two elevated sweat chloride concentrations obtained on separate days or identification of two CF mutations. For patients in whom sweat chloride concentrations are normal or borderline and in whom two CF mutations are not identified, an abnormal nasal PD measurement recorded on 2 separate days can be used as evidence of CFTR dysfunction. Clinical judgment will continue to be essential in patients who have typical or "atypical" clinical features but who lack conclusive evidence of CFTR dysfunction. Such patients will require close clinical follow-up along with laboratory reevaluation as appropriate.

FUTURE DIRECTIONS

This consensus statement should be viewed as a work in progress that reflects our current state of knowledge. It will need to be updated and refined as we: (1) develop new insights concerning the CF phenotype; (2) more precisely define the normal, borderline, and abnormal range for the sweat test results; (3) further define the role for nasal PD and other diagnostic methods such as measurement of intestinal currents[52]; and (4) identify additional CF-causing mutations.

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