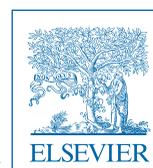# Cystic Fibrosis Foundation Consensus Guidelines for Diagnosis of Cystic Fibrosis

Guest Editors

Philip M. Farrell, MD, PhD
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Introduction to "Cystic Fibrosis Foundation Consensus Guidelines for Diagnosis of Cystic Fibrosis"

Philip M. Farrell, MD, PhD[1], and Terry B. White, PhD[2]

This Supplement focuses on quality improvement in the diagnosis of cystic fibrosis (CF) and provides a comprehensive description of the long-standing diagnostic challenges with an informative historical perspective and in the context of the most recent advances. The featured component is a group of 27 new consensus statement recommendations from the US CF Foundation. Readers are encouraged to begin with the first article,[1] which gives a succinct overview of the statements, and then proceed sequentially to appreciate the historical aspects, genetics, and then the issues and evidence related to diagnosis in screened and nonscreened populations (ie, the elements that led to the consensus statements). A summary of the challenges faced when diagnosing CF and consensus-seeking processes employed follows.

CF, the most common life-threatening autosomal recessive disease among Caucasians in the US, is a multisystem disorder caused by mutations in the gene for the CF transmembrane conductance regulator (CFTR) ion channel with more than 2000 mutations identified to date.[2] A diagnosis of CF has traditionally relied on recognition of characteristic clinical signs and symptoms, as described previously[3,4] and reviewed herein[5] for nonscreened populations; however, with universal CF newborn screening (NBS) and more prenatal testing, an increasing proportion of diagnoses are being made before symptoms occur. Although the majority of infants who screen positive for CF can be readily diagnosed after a confirmatory sweat test showing high sweat chloride concentration, the diagnosis is not clear in some individuals.[4,6] In addition, universal CF NBS was implemented only recently in the US, and many individuals born there before 2010 have not been screened. Lastly, all clinicians agree that diagnoses of CF in unscreened populations can be difficult because the age of onset and severity of signs/symptoms as a result of CFTR dysfunction can be highly variable.

The value in providing an accurate, early CF diagnosis is clear; both for the newborn, who can then receive early therapy and potentially retain optimal health for decades,[7] and for the older individual presenting with symptoms, who can benefit from the application of appropriate therapies, as well as from simply understanding the cause of symptoms that may have required a lengthy "diagnostic odyssey" before diagnosis.[1] The need to re-examine the rules for a CF diagnosis for newborns became increasingly apparent as NBS programs collected more and more data showing a significant number of screen-positive infants with unclear diagnostic results.[4,6] A new look at diagnostic guidelines for the individual presenting symptomatically also is timely, as all clinicians agree that diagnoses of CF in unscreened populations can be difficult because of variability in age of onset and severity of signs/symptoms, and new genetic information and CFTR functionality testing can now be usefully applied to the problem. Since the most recent diagnostic consensus conference,[4] there has been significant growth of phenotypic and genotypic information on CF that can help with interpretation of the disease status in many of these patients. International collection of clinical data from individuals with CF and recent laboratory advances[8-11] have provided new insights into the physiological impact of the most common mutations.[12,13] Because of this new information, and to seek harmony with the diagnostic criteria and terminology[14] of the European CF Society (ECFS), it was decided that the diagnostic guidelines of the CF Foundation[4] published in 2008 should be revised.

Consequently, the CF Foundation under the leadership of Drs Preston Campbell and Bruce Marshall planned an extraordinary, in fact unique, consensus conference with worldwide participation, appointed a steering committee of 3 cochairs (Drs Philip Farrell, Clement Ren, and Patrick Sosnay). Six months later, they convened and supported a committee of 32 experts in the diagnosis of CF representing 9 countries. During the planning phase, a decision was made to engage the ECFS in an effort to harmonize diagnostic criteria and terminology[14] applied to the 2 largest populations of patients with CF.

The goals of the consensus-seeking processes included probable revising/updating of diagnostic guidelines and, if possible, achieving global standardization of definitions and terminology. Thus, the mission of this committee was to develop clear and actionable consensus guidelines on diagnosis of CF and other conditions associated with mutations in the *CFTR* gene such as CFTR-related metabolic syndrome (CRMS)[6] or CF screen positive, inconclusive diagnosis (CFSPID),[15] and CFTR-related disorder.[16] The recommendations in this report address individuals with both clear and unclear

CF	Cystic fibrosis
CFSPID	CF screen positive, inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
CRMS	CFTR-related metabolic syndrome
ECFS	European CF Society
NBS	Newborn screening

http://dx.doi.org/10.1016/j.jpeds.2016.09.062

diagnoses, including infants with positive NBS and/or prenatal presumptive diagnosis,[13] and individuals with CF-like symptoms who were either never screened or who had false negative newborn or prenatal screening results.[5]

After studying the most relevant literature, the committee convened to review evidence, illustrative cases, and potential consensus statements in Phoenix, Arizona, on October 6, 2015, before the North American CF Conference. A carefully selected, international subgroup of the participants served as an executive subcommittee ("working/writing group") before and after the conference to consider a variety of issues as consultants. Following the conference, the consensus statements were developed by the executive subcommittee and voted on by the entire consensus committee. An a priori threshold of ≥80% affirmative votes was required for acceptance of each statement. The consensus committee approved 27 of 28 statements, 7 of which needed revisions and another round of voting. The executive subcommittee also reviewed an initial, lengthy proceedings manuscript that served as the precursor to the 6 articles published herein and subsequently gave special attention to the consensus summary article[1] as did all the conference participants.

After the 27 consensus statements were approved by at least 80% of the consensus committee participants, the list was presented for review and discussion at the annual meeting of the ECFS Diagnostic Network Working Group in February 2016 and later presented to the ECFS Board. Next, the consensus summary manuscript was sent for review and comments to the Canadian CF Foundation, the national CF organizations in Australia and New Zealand, a group of parents of children identified with CRMS, all CF center directors in the US, and the CF Foundation centers committee. Although the recommendations herein are those of the US CF Foundation, all of the feedback and suggested revisions were taken into account in completing the overview article[1] and others in the Supplement.

The differences between the 2008 diagnostic criteria and the recommendations herein are summarized in the fourth article[13] focused on screened populations. First and foremost, it is recommended as essential that diagnoses of disorders associated with *CFTR* mutations be established in all individuals from newborn to adult by evaluation of CFTR function with a sweat chloride test or, if this is not possible, another established test of chloride channel functioning as described herein by Farrell et al[13] and Ren et al.[17] Newborn infants with a high level of immunoreactive trypsinogen and inconclusive CFTR functional and genetic testing may be designated either CRMS or CFSPID; these 2 terms are now merged and equivalent, so that CRMS/CFSPID may be used as was done recently by Castellani et al.[18] The committee also recommends that the latest mutation classifications annotated in the CFTR2 project[19] should be used to aid in CF diagnosis. Finally, as described in the Supplement article on genetic aspects by Sosnay et al,[12] all patients with CF should be genotyped, even if the diagnosis was confirmed with sweat test results and/or they had DNA analyses as part of NBS.

Currently, in association with this Supplement, efforts will be put in place to facilitate implementation. Most importantly, all CF clinic/center leaders are urged to read carefully every article in the Supplement because, as emphasized under historical aspects herein[20] and elsewhere,[21] "Every physician's first duty is to diagnose–accurately and promptly. Diagnosis is the first step of treatment." ∎

Author Disclosures

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Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation

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Objective Cystic fibrosis (CF), caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene, continues to present diagnostic challenges. Newborn screening and an evolving understanding of CF genetics have prompted a reconsideration of the diagnosis criteria.

Study design To improve diagnosis and achieve standardized definitions worldwide, the CF Foundation convened a committee of 32 experts in CF diagnosis from 9 countries to develop clear and actionable consensus guidelines on the diagnosis of CF and to clarify diagnostic criteria and terminology for other disorders associated with *CFTR* mutations. An a priori threshold of ≥80% affirmative votes was required for acceptance of each recommendation statement.

Results After reviewing relevant literature, the committee convened to review evidence and cases. Following the conference, consensus statements were developed by an executive subcommittee. The entire consensus committee voted and approved 27 of 28 statements, 7 of which needed revisions and a second round of voting.

Conclusions It is recommended that diagnoses associated with *CFTR* mutations in all individuals, from newborn to adult, be established by evaluation of CFTR function with a sweat chloride test. The latest mutation classifications annotated in the Clinical and Functional Translation of CFTR project (http://www.cftr2.org/index.php) should be used to aid in diagnosis. Newborns with a high immunoreactive trypsinogen level and inconclusive CFTR functional and genetic testing may be designated CFTR-related metabolic syndrome or CF screen positive, inconclusive diagnosis; these terms are now merged and equivalent, and CFTR-related metabolic syndrome/CF screen positive, inconclusive diagnosis may be used. *International Statistical Classification of Diseases and Related Health Problems, 10th Revision* codes for use in diagnoses associated with *CFTR* mutations are included. *(J Pediatr 2017;181S:S4-15).*

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disease in the US, affecting approximately 1 in 4000 newborns in the US,[1-3] and occurring at higher frequencies in some European countries.[4,5] CF is a multisystem disorder caused by mutations in the gene for the CF transmembrane conductance regulator (*CFTR*), which encodes an ion channel protein,[6] with more than 2000 mutations identified to date (http://www.genet.sickkids.on.ca/cftr/app[7]).

A diagnosis of CF initially relied on phenotype, with clinical recognition of characteristic signs and symptoms.[8,9] However, because of widespread CF newborn screening (NBS), at least 64% of new CF diagnoses in the US now occur in

CF	Cystic fibrosis
CFSPID	CF screen positive, inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
CFTR2	Clinical and Functional Translation of CFTR
CRMS	CFTR-related metabolic syndrome
ECFS	European CF Society
ICD-10	*International Statistical Classification of Diseases and Related Health Problems, 10th Revision*
ICM	Intestinal current measurement
IRT	Immunoreactive trypsinogen
NBS	Newborn screening
NPD	Nasal potential difference

asymptomatic or minimally symptomatic infants following a positive NBS result.[10] Although the majority of infants who screen positive can be readily diagnosed with CF after a confirmatory test showing high sweat chloride concentration, the diagnosis is not clear in some individuals,[11,12] leading to persistent challenges[13] and stresses, including a potentially disturbed parent/child relationship.[14-16] Furthermore, universal NBS was implemented only recently in the US, and many individuals born prior to 2010 have not been screened. Diagnosis of CF in the nonscreened population can be challenging because the age of onset and severity of symptoms can differ greatly as a result of highly variable levels of CFTR dysfunction. Presenting manifestations can include pancreatitis, respiratory symptoms, chronic sinusitis, and male infertility.[9,17-19]

The last few years have seen significant growth of phenotypic and genotypic information on CF that can help with interpretation of the disease status in many patients. International collection of clinical data from individuals with CF[20] and laboratory advances[21] provide insight into the functional and physiological impact of the most common mutations.[22] Because of this new information, and to seek harmony with the diagnostic criteria and terminology[23] of the European CF Society (ECFS), it was decided that the 2008 diagnostic guidelines[24] of the CF Foundation should be revised.

The CF Foundation convened an international committee of experts in the diagnosis of CF to update diagnostic guidance and achieve standardization in definitions worldwide. The mission of this committee was to develop clear and actionable consensus guidelines on diagnosis of CF and other conditions associated with mutations in the *CFTR* gene such as CFTR-related metabolic syndrome (CRMS)[25] or CF screen positive, inconclusive diagnosis (CFSPID),[26] and CFTR-related disorders.[27] The recommendations in this article address individuals with both clear and unclear diagnoses, including infants with positive NBS (defined as any result other than normal) and/or prenatal diagnosis,[28] and individuals with CF-like symptoms who were either never screened or who had false negative newborn or prenatal screening results.[9] Case studies, designed to show how the recommendations should be applied in challenging clinical scenarios, can be found in additional articles published throughout this Supplement.[9,28,29]

Methods

An international consensus committee was selected and tasked with the development of guidelines on the diagnosis of CF; 32 experts made up this committee. Committee selection was designed to include participants representative of worldwide CF care communities, particularly pediatric CF providers with NBS experience, and other relevant specialists, including adult CF providers. Before the consensus conference, the committee reviewed the existing CF Foundation diagnosis guidelines[24] and a list of publications on CF diagnosis published since the 2008 CF Foundation Diagnosis Guidelines, including 10 key articles selected by conference cochairs. The conference was held immediately prior to the North American CF Conference in October 2015.

At the consensus conference, committee members presented and discussed new studies and data on CF diagnosis. An executive subcommittee, consisting of 10 representatives from 4 countries, developed the consensus statements at subsequent meetings. These statements were reviewed by the entire consensus committee and voted on by the members using an electronic survey tool (SurveyMonkey, Palo Alto, California).[30] An a priori threshold of ≥80% affirmative votes was required for acceptance. Individuals voting against a statement were asked to provide a revised statement and/or explanation for their vote. Feedback on the statements that did not reach 80% agreement was reviewed by the committee cochairs, and those statements were revised with input from the rest of the executive subcommittee. The revised statements were then resubmitted for voting.

After the recommendation statements were agreed upon, they were presented to the ECFS at the Diagnostic Network Working Group annual meeting in February 2016 to help engage all parties in the discussion. The draft manuscript was distributed for feedback from the executive subcommittee, conference committee, the CF Foundation's CF Center Committee, all CF centers in the US, parents of screened infants, and a variety of international organizations and their members during a public comment period.

Results

In the survey, participants were able to vote in agreement, disagreement, or to abstain. However, in each of the 2 surveys distributed for reviewing the consensus statements and voting, 1 committee member (a different person each time) did not respond. Thus, the 1 committee member who did not participate in the first voting exercise did not constitute an abstention. A vote was taken on 28 statements initially; 8 did not reach at least 80% agreement. The 8 statements that did not pass were reviewed and revised, and reduced to 7 statements by the chairs and the executive committee and sent out for a second round of voting. All but 1 member of the 32 committee members participated in this vote (ie, 1 was nonresponsive). All 7 of the revised statements passed the 80% threshold in the second round of voting.

The committee approved 27 consensus statements (**Table I**) in 4 overlapping categories that apply to: (1) both screened and nonscreened populations; (2) newborn screened populations and fetuses undergoing prenatal testing; (3) infants with uncertain diagnosis and designated either CRMS or CFSPID (now considered to be the same); and (4) patients presenting clinically who represent nonscreened populations, including children born at home or in regions before NBS implementation, those with false negative screening tests, and older nonscreened individuals.

The **Figure** provides a simplified algorithm for how these consensus statements should be applied to individuals suspected of having CF because of a positive NBS result, the appearance of signs or symptoms, or recognition of immediate family history of CF (most often sibling, but may also include

Table I. Consensus recommendations for diagnosis of CF*

Statement numbers	Consensus statements	Vote	Abstain (n)
1	Sweat chloride testing should be performed according to approved procedural guidelines published in established, international protocols such as the CLSI 2009 Guidelines.	100%	0
2	Newborns with a positive CF newborn screen, to increase the likelihood of collecting an adequate sweat specimen, should have the test performed bilaterally and when the infant weighs >2 kg, and is at least 36 wk of corrected gestational age.	87%	0
3	Newborns greater than 36 wk gestation and >2 kg body weight with a positive CF newborn screen, or positive prenatal genetic test, should have sweat chloride testing performed as soon as possible after 10 d of age, ideally by the end of the neonatal period (4 wk of age).	93%	1
4	In infants with presumptive CF identified through NBS, CF treatment should not be delayed while efforts to establish a diagnosis of CF are initiated.	83%	1
5	Sweat chloride analysis should be performed within a few hours of sweat collection and the results and interpretations should be reported to clinicians and parents or patients, as soon as possible and certainly on the same day.	90%	0
6	In individuals presenting with a positive newborn screen, clinical features consistent with CF, or a positive family history, a diagnosis of CF can be made if the sweat chloride value is ≥60 mmol/L.	93%	0
7	Individuals who are screen-positive and meet sweat chloride criteria for CF diagnosis should undergo *CFTR* genetic testing if the *CFTR* genotype was not available through the screening process or is incomplete.	100%	0
8	In individuals with a positive newborn screen, a sweat chloride <30 mmol/L indicates that CF is unlikely.	82%	2
9	Individuals with clinical features that may be consistent with CF who have a sweat chloride <30 mmol/L indicates that CF is less likely. It may, however, be considered if evolving clinical criteria and/or *CFTR* genotyping support CF and not an alternative diagnosis.	80%	0
10	Individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range (30-59 mmol/L) on two separate occasions may have CF. They should be considered for extended *CFTR* gene analysis and/or CFTR functional analysis.	90%	0
11	The latest classifications identified in the CFTR2 project (http://www.cftr2.org/index.php) should be used to aid with CF diagnosis: • CF-causing mutation: individuals with 2 copies on separate alleles will likely have CF (clinical sweat confirmation needed) • Mutation of varying clinical consequence (MVCC): a mutation that in combination with a CF-causing mutation or another MVCC mutation may result in CF • Uncharacterized mutation/mutation of UNK: mutation that has not been evaluated by CFTR2 and may be disease causing or of variable clinical consequence or benign • Non-CF-causing mutation: individuals with 1 or more are unlikely to have CF (as a result of that allele)	100%	0
12	In individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, the identification of 2 CF-causing mutations (defined by CFTR2) is consistent with a diagnosis of CF. Sweat chloride testing is necessary, though, to confirm the diagnosis.	87%	0
13	The absence of detection of 2 CF-causing *CFTR* mutations does not exclude a diagnosis of CF.	93%	1
14	If further CF functional testing is needed (NPD and ICM), it should be performed in a validated reference center with trained staff certified by the CF Foundation TDN or ECFS Clinical Trial Network.	100%	0
15	In individuals with a positive newborn screen but variable or uncharacterized *CFTR* mutations (<2 CF-causing mutations), the diagnosis of CF can be made by demonstrating CFTR dysfunction (a sweat chloride ≥ 60 mmol/L or CF-typical NPD or ICM).	93%	0
16	The term CRMS is used in the US for healthcare delivery purposes and CFSPID is used in other countries, but these both describe an inconclusive diagnosis following NBS.	96%	2
17	The term CRMS/CFSPID is reserved for individuals who screen positive without clinical features consistent with a diagnosis of CF.	83%	1
18	The definition of CRMS/CFSPID is an infant with a positive NBS test for CF and either: • A sweat chloride value <30 mmol/L and 2 *CFTR* mutations, at least 1 of which has unclear phenotypic consequences *OR* • An intermediate sweat chloride value (30-59 mmol/L) and 1 or 0 CF-causing mutations	86%	1
19	Children designated as CRMS/CFSPID should undergo at least one repeat sweat chloride test at CF centers with suitable expertise, such as an accredited CF center.	86%	1
20	Children designated as CRMS/CFSPID should have clinical evaluation performed by CF providers to identify the minority that may develop clinical symptoms.	83%	1
21	Children designated as CRMS/CFSPID can be considered for extended *CFTR* gene analysis (sequencing and or deletion duplication testing), as well as CFTR functional analysis (NPD/ICM) testing to further define their likelihood of developing CF.	80%	0
22	The decision to reclassify children designated as CRMS/CFSPID as CF is an integrated decision that should take into account functional assessment of CFTR (sweat chloride, and possibly NPD/ICM), *CFTR* genetic analysis, and clinical assessment by the CF clinicians caring for the patient.	90%	0
23	Genetic counseling should be offered to families of individuals followed for CRMS/CFSPID, including a discussion of the risk in future pregnancies.	100%	1
24	Research Recommendation: Infants with a designation of CRMS/CFSPID (by definition) do not have clinical features consistent with a diagnosis of CF and further research is needed to determine the prognosis and best practices for frequency and duration of follow-up.	96%	0
25	For individuals presenting with CF symptoms, the same diagnostic criteria recommended for the screened population for sweat chloride testing, *CFTR* genetic analysis, and CFTR functional testing should be used to confirm a CF diagnosis.	93%	0
26	The diagnosis of CFTR-related disorder has been defined as a monosymptomatic clinical entity (CBAVD/pancreatitis/bronchiectasis) associated with CFTR dysfunction that does not fulfill the diagnostic criteria for CF.	86%	2
27	Clinicians should avoid the use of terms like classic/nonclassic CF, typical/atypical CF, delayed CF, because these terms have no harmonized definition and could be confusing for families or caregivers.	83%	1

CBAVD, congenital bilateral absence of the vas deferens; *CLSI*, Clinical and Laboratory Standards Institute; *CTN*, Clinical Trial Network; *ICM*, intestinal current measurement; *MVCC*, mutation of varying clinical consequence; *NPD*, nasal potential difference; *TDN*, Therapeutics Development Network; *UNK*, unknown clinical consequence.
*In each of the 2 surveys distributed for reviewing the consensus statements drafted and voting, 1 committee member, a different person each time, did not respond.
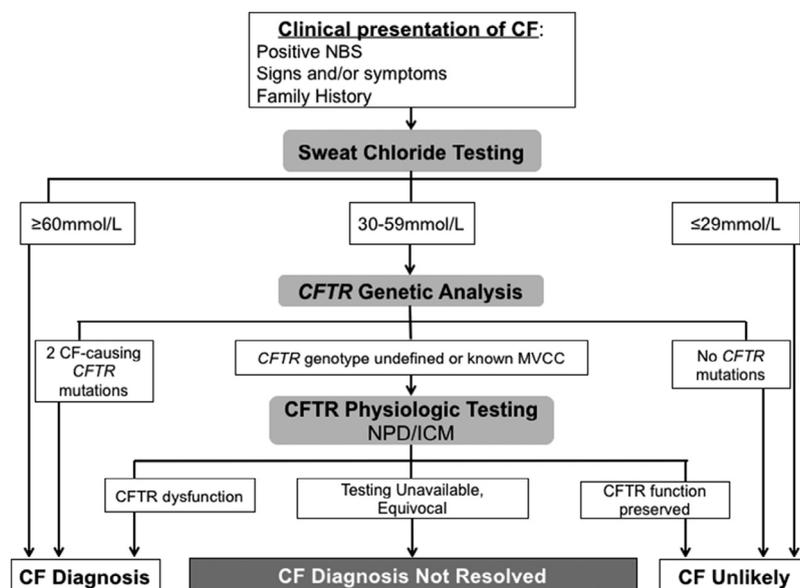
Figure. CF is diagnosed when an individual has both a clinical presentation of the disease and evidence of CFTR dysfunction. The tests of CFTR function are not always done in this order, but hierarchically to establish the diagnosis of CF, sweat chloride should be considered first, then *CFTR* genetic analysis, and then CFTR physiologic tests. All individuals diagnosed with CF should have a sweat test and a *CFTR* genetic analysis performed. Rare individuals with a sweat chloride <30 mmol/L may be considered to have CF if alternatives are excluded and the other confirmatory tests (genetic, physiologic testing) support CF. If only 1 *CFTR* variant is identified on limited analysis, further ("extended") *CFTR* testing should be performed.[22] CF is possible if both alleles possess CF-causing, undefined, or mutation of varying clinical consequence (MVCC) mutations; CF is unlikely if only non-CF-causing mutations are found. If a CF diagnosis is not resolved, CRMS/CFSPID (following NBS) or CFTR-related disorder should be considered.[9,29] Rarely, no distinct label may be appropriate but further follow-up may be warranted. In these cases, the use of "CF carrier" or the specific clinical problem should be used for characterization/labeling purposes.

parent or child). It should be noted that a positive NBS result does not mean the infant has CF; the probability of a CF diagnosis following a positive result varies greatly depending on the NBS method used.

Even though many individuals enter this algorithm through a positive newborn screen in which *CFTR* genetic testing was done, the diagnosis of CF is primarily based on the direct demonstration of abnormal CFTR function by measurement of chloride concentration in the sweat.[24] Although obtaining an adequate sweat specimen for chloride measurements can be challenging, particularly in very young infants, experience and studies have shown that this is feasible in full-term infants during the first postnatal month (ie, during the neonatal period).[31-34] Following the committee's recommendations will improve reliability of the sweat test result.

Sweat Chloride Testing and Presumptive Diagnosis

(1) All populations: *Sweat chloride testing should be performed according to approved procedural guidelines published in established, international protocols such as the Clinical and Laboratory Standards Institute 2009 Guidelines.* Following appropriate protocols for performing the sweat test[32] is important for achieving accurate results and minimizing collection of inadequate amounts of sweat (quantity not sufficient).[28,33-37]

(2) For newborns: *Newborns with a positive CF newborn screen, to increase the likelihood of collecting an adequate sweat specimen, should have the test performed bilaterally and when the infant weighs >2 kg and is at least 36 weeks' corrected gestational age.* Sweat samples collected bilaterally must not be combined; instead, they should be analyzed separately, providing a useful quality control measure.[33]

(3) For newborns: *Newborns greater than 36 weeks' gestation and >2 kg body weight with a positive CF newborn screen, or positive prenatal genetic test, should have sweat chloride testing performed as soon as possible after 10 days of age, ideally by the end of the neonatal period (4 weeks of age).* For a variety of reasons related to efficient, effective follow-up and optimizing care, sweat chloride testing should occur as soon as possible when positive screening results are reported and can be as early as 48 hours after birth.[33] The committee recognizes that many NBS programs do not report results by this time and, therefore, recommends that sweat chloride testing proceed as soon as possible after results are available; generally, this is no later than 10 days of age. Although gestational age and weight must be considered,[38] testing should occur if at all possible before the end of the neonatal period because malnutrition and other risks such as potentially fatal hyponatremic dehydration may occur even in the first few weeks of life.[39-42]

(4) <u>For newborns:</u> *In infants with presumptive CF identified through NBS, CF treatment should not be delayed while efforts to establish a diagnosis of CF are initiated.* Optimal outcomes depend on early intervention. Efforts to obtain adequate quantities of sweat and accurate sweat chloride values should not delay start of salt supplementation or other appropriate therapies.[43] The CF Foundation recommends that infants with CF have an initial visit at an accredited CF care center within 24-72 hours of diagnosis,[43] and timing of the initial visit for infants with a presumptive diagnosis should aim to meet this timeframe. A presumptive diagnosis of CF for purposes of treatment initiation can include the following clinical circumstances: (1) positive CF newborn screen showing 2 CF-causing *CFTR* mutations (see below); (2) positive CF newborn screen and clinical signs and symptoms of CF; and (3) meconium ileus, with or without a positive newborn screen.

However, definitive diagnosis requires demonstration of CFTR dysfunction. A date of presumptive diagnosis should be recorded to permit evaluation of timeliness of diagnosis and treatment within NBS programs. However, for purposes of providing standardized data to the CF Foundation Patient Registry, the date of the first positive sweat chloride test should be reported as the date of diagnosis.

(5) <u>All populations:</u> *Sweat chloride analysis should be performed within a few hours of sweat collection, and the results and interpretations should be reported to clinicians and parents or patients, as soon as possible and certainly on the same day.* Prompt reporting should be made regardless of sweat test results to reduce family or patient stress.[44-47] A second, confirmatory, sweat test following an initial positive result is not necessary; this is a change from previous CF Foundation diagnostic guidelines.[24,48]

Sweat Chloride Test Results ≥60 mmol/L

(6) <u>All populations:</u> *In individuals presenting with a positive newborn screen, clinical features consistent with CF, or a positive family history, a diagnosis of CF can be made if the sweat chloride value is ≥60 mmol/L.* Even though the sweat test is commonly used for diagnosis of individuals presenting with symptoms of CF, many newborns are reported as having CF based solely on a positive NBS result. However, NBS tests must always be considered as screening procedures and not diagnostic studies. The genetic analysis included as part of many NBS programs must not be relied upon for conclusive diagnosing and/or genotyping, as errors can arise from problems with Guthrie card labelling,[49,50] changes in the mutation panel used by the NBS program (eg, as described by Watson et al[51]), NBS laboratory errors including DNA misinterpretations, or detection of 2 *CFTR* mutations in *cis* (ie, on the same chromosome).[22,52,53] All of these problems have occurred and will occur again.

(7) <u>For newborns:</u> *Individuals who screen positive and meet sweat chloride criteria for CF diagnosis should undergo* CFTR genetic testing if the CFTR *genotype was not available through the screening process or is incomplete.* Genetic testing is an important part of the diagnostic work-up, and it is not uncommon for a positive NBS result to include the recognition of 2 CF-causing mutations. Even in the presence of a positive sweat test, the identification of 2 CF-causing mutations should be confirmed in a clinical genetics laboratory capable of performing in-depth genetic analysis when required to further define CF risk (eg, the length of polyT tracts with the c.350G>A [legacy: R117H] *CFTR* mutation).[54,55] Confirmation of genetic testing results with an FDA-approved companion diagnostic test also has additional value in therapy selection[56] and access.[57,58]

Sweat Chloride Test Results <30 mmol/L

(8) <u>For newborns:</u> *In individuals with a positive newborn screen, a sweat chloride <30 mmol/L indicates that CF is unlikely.* Sweat chloride testing may be repeated if indicated by family history, or if symptoms suggestive of CF occur.

(9) <u>All populations:</u> *Individuals with clinical features that may be consistent with CF who have a sweat chloride <30 mmol/L indicates that CF is less likely. It may, however, be considered if evolving clinical criteria and/or* CFTR *genotyping support CF and not an alternative diagnosis.* The level of sweat chloride below which CF is considered unlikely is 30 mmol/L for all age groups. This is a change from previous guidelines for individuals >6 months of age (the previous limit was 40 mmol/L) because patients have been definitively diagnosed with CF with chloride values in the 30-39 mmol/L range.

Details regarding the diagnosis of CF in the very rare individual with sweat chloride <30 mmol/L are published elsewhere.[22] Some *CFTR* mutations, such as c.3717 + 12191C>T (legacy: 3849 + 10 kb C- > T), are associated with low sweat chloride values; in these cases, an alternative diagnosis does not need to be ruled out.[9,59,60]

Sweat Chloride Test Results of 30-59 mmol/L

(10) <u>All populations:</u> *Individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range (30-59 mmol/L) on 2 separate occasions may have CF. They should be considered for extended* CFTR *gene analysis and/or CFTR functional analysis.* Individuals with sweat chloride concentrations in the intermediate range will need further study to establish or rule out a CF diagnosis.[12,61-63] Evidence may be provided by *CFTR* genotype[20] (an article in this Supplement provides a discussion of *CFTR* genetic testing and interpretation in detail[22]) or by further CFTR physiologic testing.[64-67] Other articles in this Supplement present a discussion of the demonstration of CFTR dysfunction including the use of nasal potential difference (NPD) or intestinal current measurement (ICM) on the screen-positive newborn[28] and information on the symptomatic patient.[9]

Next Steps for Intermediate Sweat Test Results

(11) <u>All populations:</u> *The latest classifications identified in the Clinical and Functional Translation of CFTR (CFTR2) project*[20] *should be used to aid with CF diagnosis: (1) CF-causing mutation: individuals with 2 copies on separate alleles will likely have CF (clinical sweat confirmation needed).* (A sweat chloride test result ≥30 mmol/L is confirmatory for patients with this genotype); (2) *mutation of varying clinical consequence: A mutation that in combination with a CF-causing mutation or another mutation of varying clinical consequence mutation may result in CF*; (3) *uncharacterized mutation/mutation of unknown clinical consequence: mutation that has not been evaluated by CFTR2 and may be disease causing or of variable clinical consequence or benign*; and (4) *non-CF-causing mutation: individuals with 1 or more are unlikely to have CF (as a result of that allele).*

The CFTR2 project provides a detailed characterization of *CFTR* mutations by collecting clinical and laboratory evidence of phenotypic consequence.[20] For each mutation, the CFTR2 website provides information and classification as listed above. The CFTR2 project is updated as mutation-specific functional analyses are completed. Also, because mutation categorization may change over time, it is important to confirm genotype interpretation on the most current version of the website. Mutations that are not analyzed as part of CFTR2 may still be interpretable if adequate research exists. For example, if a mutation is detected that is not annotated in CFTR2 and has been shown to be seen previously in patients with CF, has functional evidence that the nucleotide/protein change is deleterious; and does not occur commonly in databases of general (healthy) population, that mutation can be characterized as CF-causing.[22]

(12) <u>All populations:</u> *In individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, the identification of 2 CF-causing mutations (defined by CFTR2) is consistent with a diagnosis of CF. Sweat chloride testing is necessary, though, to confirm the diagnosis.* A sweat chloride test result ≥30 mmol/L is confirmatory in individuals with 2 CF-causing mutations on separate chromosomes. As stated above, there are situations in which repeated sweat chloride testing does not provide further clarity, such as in individuals with *CFTR* mutations known to be associated with normal or intermediate sweat chloride.[59,60] Another article in this Supplement provides further exploration of this topic.[9]

(13) <u>All populations:</u> *The absence of detection of 2 CF-causing* CFTR *mutations does not exclude a diagnosis of CF.* Because classification and identification of CF-causing *CFTR* mutations is ongoing, there are individuals with a CF diagnosis in whom 2 *CFTR* mutations have not been detected. Thus, even though the CFTR2 initiative has been a valuable step forward in improving the diagnostic characterization of patients with *CFTR* mutations, it does not

take the place of clinical observation and expertise. Other articles in this Supplement present more in-depth discussions on this topic. [9,22,29]

To explore further a CF diagnosis in individuals with a positive newborn screen, symptoms of CF, or a positive family history, intermediate sweat chloride values (30-59 mmol/L), and fewer than 2 CF-causing mutations, the committee recommends additional CFTR physiological testing that directly measures CFTR function, such as NPD and ICM.[68]

(14) <u>All populations:</u> *If further CF functional testing is needed (NPD and ICM), it should be performed in a validated reference center with trained staff certified by the CF Foundation Therapeutics Development Network or ECFS Clinical Trial Network.* When performed correctly, NPD can discriminate a wide range of CFTR function.[69,70] ICM also can be used to confirm a diagnosis of CF in the context of intermediate sweat chloride levels,[66-68,70-73] and may be useful when NPD testing is unsuccessful (eg, when attempting to conduct NPD testing in the uncooperative child) (I Sermet-Gaudelus, personal communication, October 2015). Few CF centers in the US are prepared to conduct these tests. However, the added value that the results have provided to situations of diagnostic uncertainty (especially in Europe where they are more widely used) suggests that there will be widespread uptake of the tests in the future. There are patients with intermediate sweat chloride test results and an undefined *CFTR* genotype for whom NPD or ICM testing could provide diagnostic clarity; these patients should be seen in centers certified for the test in their country. Another article in this Supplement presents further discussion of NPD and ICM testing.[28]

(15) <u>For newborns:</u> *In individuals with a positive newborn screen but variable or uncharacterized* CFTR *mutations (<2 CF-causing mutations), the diagnosis of CF can be made by demonstrating CFTR dysfunction (a sweat chloride ≥60 mmol/L or CF-typical NPD or ICM).* Identification of diagnostic levels for NPD and ICM measurements must be performed at the level of the reference center conducting the tests. Another article in this Supplement presents further discussion on this topic.[28]

For the Newborn with an Inconclusive Diagnosis

(16) <u>For newborns:</u> *The term CRMS is used in the US for healthcare delivery purposes and CFSPID is used in other countries, but these both describe an inconclusive diagnosis following NBS.* Newborn infants with a high level of immunoreactive trypsinogen (IRT) and inconclusive CFTR functional and genetic testing may be labeled either CRMS or CFSPID.[25,26,29] CFSPID describes the inconclusive nature of the condition in a manner that is easy for patients and families to understand and can be designated by *International Statistical Classification of Diseases and Related Health Problems, 10th Revision* (ICD-10) code P09. However, because of the US healthcare

system requirements,[74] CRMS (ICD-10 code E88.89) must be used in clinical settings in the US for continuing, follow-up care. These 2 terms are nearly identical, and the consensus committee recommends that the 2 terms be harmonized, for improved international communications and analysis of clinical outcomes. The term CRMS/CFSPID will be used throughout this Supplement and is recommended.[75]

(17) For newborns: *The term CRMS/CFSPID is reserved for individuals who screen positive without clinical features consistent with a diagnosis of CF.* The CRMS/CFSPID diagnosis should not be used in other clinical scenarios, including those involving individuals who have not received a positive NBS result, or individuals who have clinical symptoms attributable to CFTR dysfunction.[9]

(18) For newborns: *The definition of CRMS/CFSPID is an infant with a positive NBS test for CF and either: (1) sweat chloride value <30 mmol/L and 2 CFTR mutations, at least 1 of which has unclear phenotypic consequences; or (2) intermediate sweat chloride value (30-59 mmol/L) and 1 or 0 CF-causing mutations.* Individuals designated as CRMS/CFSPID initially appear asymptomatic and may never develop CF symptoms; however, they should be referred to a CF specialist at an accredited CF care center to ensure there are no hidden signs or symptoms of CF and to establish a plan for follow-up.[25,76]

Next Steps in the Newborn with CRMS/CFSPID Designation

(19) For newborns: *Children designated as CRMS/CFSPID should undergo at least 1 repeat sweat chloride test at CF centers with suitable expertise, such as an accredited CF center.* This test should be used to confirm the CRMS/CFSPID designation. Appropriate timing for the repeat sweat chloride test is discussed elsewhere in this Supplement.[29]

(20) For newborns: *Children designated as CRMS/CFSPID should have clinical evaluation performed by CF providers to identify the minority that may develop clinical symptoms.* This group of children must be monitored for development of symptoms, and surveillance evaluations conducted (respiratory tract cultures, imaging, and spirometry or lung-clearance index when age-appropriate). Measuring fecal elastase levels or following IRT or pancreatitis associated protein trends may be considered if clinically indicated to identify CF clinical manifestations (phenotypes) objectively.[11,25,76-78] CF cannot be diagnosed through the identification of elevated levels of IRT alone; elevated IRT can occur in the context of other tissue stress.[79,80] Another article in this Supplement presents information about appropriate timing for monitoring.[29]

(21) For newborns: *Children designated as CRMS/CFSPID can be considered for extended* CFTR *gene analysis (sequencing and or deletion duplication testing), as well as CFTR functional analysis (NPD/ICM) testing to further define*

their likelihood of developing CF. Other articles in this Supplement present information on the genetic tests that are useful in this scenario and useful functional analysis testing.[22,29]

(22) For newborns: *The decision to reclassify children designated as CRMS/CFSPID as CF is an integrated decision that should take into account functional assessment of CFTR (sweat chloride, and possibly NPD/ICM),* CFTR *genetic analysis, and clinical assessment by the CF clinicians caring for the patient.* The decision to change a designation from CRMS/CFSPID to CF is a difficult one and should be made by an experienced CF physician.[25,26,29]

(23) For newborns: *Genetic counseling should be offered to families of individuals followed for CRMS/CFSPID, including a discussion of the risk in future pregnancies.* The CF Foundation recommends that genetic counseling be offered to all families of newborns diagnosed with CF.[24] This is also important for families of newborns designated CRMS/CFSPID. Our understanding of the impact of various CFTR mutations is evolving and will continue to be clarified for many years. Genetic counseling is important for parents to understand the risk of a child having CF or being designated as CRMS/CFSPID in future pregnancies.[25,26]

(24) For newborns (research recommendations): *Infants with a designation of CRMS/CFSPID (by definition) do not have clinical features consistent with a diagnosis of CF and further research is needed to determine the prognosis and best practices for frequency and duration of follow-up.* There is inadequate evidence to recommend a standard period and frequency for follow-up of these individuals. Further research on this will require common definitions, and the merging of CRMS and CFSPID designations is, therefore, especially timely.

General Note for the Nonscreened Individual

(25) *For individuals presenting with CF symptoms, the same diagnostic criteria recommended for the screened population for sweat chloride testing,* CFTR *genetic analysis, and CFTR functional testing should be used to confirm a CF diagnosis.* Although NBS encompasses the majority of new diagnoses, diagnosis of CF in the nonscreened population, particularly those born before the initiation of NBS at all accredited CF centers, still occurs. (There will also be individuals that present with symptoms following a false negative CF NBS result[28,50] who should then be considered as in the nonscreened population.) In these individuals, the diagnostic algorithm (**Figure**) remains applicable. However, the assignment of a diagnosis of CF will be weighed against alternative diagnostic explanations of the presenting symptom or feature. Therefore, the pretest probability of CF will influence the interpretation of sweat chloride testing, CFTR genetic analysis, or CFTR physiologic testing. Definitive diagnostic criteria for nonscreened populations include the presence of CF symptoms or a family history and sweat

chloride ≥60 mmol/L OR presence of 2 CF-causing *CFTR* mutations and sweat chloride ≥30 mmol/L or physiologic testing demonstrating CFTR dysfunction.

The diagnosis of CF also can be appropriate if the above testing is not definitive, but CFTR dysfunction is the best explanation of the patient's symptoms. In keeping with the reasons for recommending genetic analysis of newborns diagnosed with CF (statement 7) or CRMS/CFSPID (statement 23), we suggest that all nonscreened individuals diagnosed with CF or a CFTR-related disorder also undergo genetic analysis, and they or their families be provided with genetic counseling to clarify the risk of disease in future pregnancies. Of course, as with all other diseases, it should be said that phenotype can vary in individuals with the same genotype.

For the Nonscreened Individual with an Inconclusive Diagnosis

There are scenarios in which a given patient may not meet the above diagnostic criteria to be diagnosed with CF but also cannot be "ruled-out" as not having CF. Although this situation is similar to infants with CRMS/CFSPID, those classifications are not appropriate for the nonscreened populations.

(26) *The diagnosis of CFTR-related disorder has been defined as a monosymptomatic clinical entity (congenital bilateral absence of the vas deferens/pancreatitis/bronchiectasis) associated with CFTR dysfunction that does not fulfill the diagnostic criteria for CF.* Individuals with a CFTR-related disorder[27] (generally mono-organ) should be assessed and followed by a CF physician to ensure that if any additional symptoms develop that are typical of CF, they are detected.[9]

(27) *Clinicians should avoid the use of terms such as classic/nonclassic CF, typical/atypical CF, and delayed CF, because* *these terms have no harmonized definition and could be confusing for families or caregivers.* In these and other situations, education on clinical entities and organ pathologies associated with CF and their relationship with CFTR-related disorder should be provided to patients, families, and primary care providers to aid in the early recognition of symptoms of CF. The CF Foundation reaffirms the view that it is essential to avoid confusion of parents and patients, and also caregivers, with imprecise terms like atypical or nonclassic because early diagnosis and more effective treatments can lead to relatively mild disease for many years even in c.1521_1523delCTT (legacy: F508del) homozygotes. However, it is understood that some European countries will continue to use such terminology as they pursue research on mild cases.

ICD-10 Codes for Individuals with CFTR Dysfunction

The ICD[81] system is a medical classification list created collaboratively by the World Health Organization to be "the international standard for defining and reporting diseases and health conditions. It allows the world to compare and share health information using a common language."[82] It is an alphanumeric system containing codes for diseases, signs and symptoms, abnormal findings, complaints, social circumstances, and external causes of injury or diseases. The ICD system is valuable, indeed essential, for many purposes including: (1) entry and continuation into the healthcare delivery mechanisms of some countries such as the US where the ICD codes are an integral and required component of billing; (2) coding death certificates internationally, thus, allowing assessment of mortality data; (3) epidemiologic research; and (4) medical economics research.

Table II. ICD-10 codes for use in individuals with CF and other CFTR dysfunctional diseases or disorders

Diseases/disorders	Primary ICD-10 code	Secondary ICD-10 code
CF, unspecified	E84.9	
CF, with meconium ileus	E84.11	
CF, with other intestinal manifestations (eg, DIOS)	E84.19	
CF, with pulmonary manifestations	E84.0	Use secondary code for details such as infectious organisms present (eg, B96.5 for *Pseudomonas aeruginosa*)
CF, with acute pneumothorax	E84.09	J93.83
CF, with pneumothorax not otherwise specified	E84.09	J93.9
CF, with hemoptysis	E84.09	R04.2
CRMS, metabolic disorder unspecified	E88.89	
CFSPID	P09 (abnormal findings on neonatal screening)* Or: E88.89 (if CRMS/CFSPID is adopted as the preferred terminology)	
CFTR-related disorder (Code the signs/symptoms as described but do not use E84.9)		
Pancreatitis, recurrent	K85.9	Z14.1 (CF carrier status)
CBAVD	Q55.4†	
Bronchiectasis, chronic acquired	J47.9	

DIOS, distal intestinal obstruction syndrome.
*Describes positive newborn screen result with an inclusive diagnosis but only applies to the newborn period and thus cannot be used in follow-up care.
†Preferred over N46.025 (azoospermia because of a systemic disease).

The most recent revision of the system, ICD-10, implemented in October 2015, provides more than 14 400 different codes and can be expanded to over 16 000 codes by using optional subclassifications. It is not possible to convert ICD *Ninth Revision* datasets to ICD-10. In the ICD-10 coding system, characters 1-3 indicate the category of disease; 4-6 indicate etiology, anatomic site, severity or other clinical detail of disease; and character 7 is a placeholder for extending the code to increase specificity. The designation "E" indicates endocrine, nutritional, and metabolic diseases, and "J" applies to diseases of the respiratory system.

Some CF specialists were engaged in the ICD-10 development process, but the degree of influence was limited, and coding for diseases or disorders caused by CFTR dysfunction is not ideal, including the absence of a code for CFTR-related disorder. The current ICD-10 code is undergoing revision to ICD *11th Revision* which is due to be completed in 2018. Participation is invited (http://www.who.int/classifications/icd/revision/en/), and we encourage involvement by CF caregivers.

A list of ICD-10 codes that should be used in the delivery of care for those disorders associated with *CFTR* mutations (that is, CF, CRMS/CFSPID, and CFTR-related disorder) is shown in Table II.

Summary of Revisions to the 2008 CF Foundation Guidelines

The basic strategy necessary for diagnosis of CF in the large majority of individuals remains unchanged from the process recommended in 2008.[24] However, some of the diagnostic tools presented in this document and the recommended application of those tools in more complex clinical scenarios do represent significant changes. A summary of the main changes to the 2008 diagnostic algorithm is presented in Table III.

Discussion

Although NBS is now widely implemented, the diagnosis of CF is not always clear. A sweat test is required for confirmation of CF; a sweat chloride level ≥60 mmol/L indicates a diagnosis of CF and a sweat chloride level <30 mmol/L indicates

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Table III. Summary of revisions to the 2008 CF Foundation guidelines for diagnosis of CF

Revisions to guidelines for screened populations

2015 Consensus	2008[24] Comparison
• Sweat testing: same recommendation in 2008, but is not being followed and is, therefore, re-emphasized here	• Sweat testing: should be done in everyone
• Sweat Cl⁻: < 30 mmol/L is normal threshold for all ages (exceptions occur)	• Sweat Cl⁻: < 40 mmol/L was normal threshold for ages ≥6 mo (exceptions occur)
• NPD/ICM: useful; testing should be conducted in a validated lab	• NPD: limited to contributory evidence; ICM: used only in research
• CFTR mutations: use CFTR2 mutation list, with guidelines given for mutations not included in CFTR2	• *CFTR* mutations: Used ACMG/ACOG panel of 23 mutations[51]
• Presumptive diagnosis of CF: can be made (NBS⁺ and 2 CF mutations or signs and symptoms of CF; or meconium ileus) and treatment started; diagnosis must be confirmed with a sweat test	• Not discussed
• Genetic analysis: recommended in addition to that done during NBS	• Genetic analysis: recommended if not part of NBS

Revisions to guidelines for CRMS/CFSPID

2015 Consensus	2008[24] Comparison
• CRMS = CFSPID: now a harmonized definition	• (Neither term in use)
• Repeat sweat testing recommended; NPD/ICM testing may be considered	• Repeat sweat testing: every 6-12 mo, but recommendation considered to be "evolving"
• Clinical assessment: by age 2 mo; duration and frequency of follow-up remains to be determined	• Clinical assessment: by age 2 mo; repeat every 6-12 mo

Revisions to guidelines for nonscreened population with inconclusive sweat chloride values

2015 Consensus	2008[24] Comparison
• Sweat Cl⁻: < 30 mmol/L is normal threshold for all ages (exceptions occur)	• Sweat Cl⁻: < 40 mmol/L was normal threshold for ages ≥ 6 mo (exceptions occur)
• Ancillary testing: NPD/ICM	• Ancillary testing: NPD only

Revisions to general definitions

2015 Consensus	2008[24] Comparison
• CFTR-related disorder: a symptomatic entity that does not meet diagnostic criteria for CF	• CFTR-related disorder: Individuals with 0-1 CF-causing mutations and clinical signs (possibly multiple-organ) suggestive of CF
• Avoid terms like "atypical" or "nonclassical" CF because there is no consensus definition of these terms	• Recommendation unchanged but greater emphasis now given to the importance of avoiding these imprecise, potentially confusing terms in the US.

ACMG/ACOG, American College of Medical Genetics/American Congress of Obstetricians and Gynecologists.

that CF is unlikely. In individuals who fall into the intermediate sweat chloride level, 30-59 mmol/L, genetic analysis is required. Further testing for CFTR function such as NPD and ICM may also be indicated but should be performed in a specialized center approved for such studies. Some infants with a positive NBS and sweat chloride levels from 30 to 59 mmol/L or even ≤29 mmol/L and inconclusive genetic testing may be designated as CRMS/CFSPID. Further research is needed to determine their prognosis, best practice, and frequency of follow-up. ■

Author Disclosures

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Appendix

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Cystic Fibrosis Diagnostic Challenges over 4 Decades: Historical Perspectives and Lessons Learned

Philip M. Farrell, MD, PhD[1], Terry B. White, PhD[2], Nico Derichs, MD[3], Carlo Castellani, MD[4], and Beryl J. Rosenstein, MD[5]

Objective Because cystic fibrosis (CF) can be difficult to diagnose, and because information about the genetic complexities and pathologic basis of the disease has grown so rapidly over the decades, several consensus conferences have been held by the US CF Foundation, and a variety of other efforts to improve diagnostic practices have been organized by the European CF Society. Despite these efforts, the application of diagnostic criteria has been variable and caused confusion.

Study design To improve diagnosis and achieve standardization in terms and definitions worldwide, the CF Foundation in 2015 convened a committee of 32 experts in the diagnosis of CF from 9 countries. As part of the process, all previous consensus-seeking exercises sponsored by the CF Foundation, along with the important efforts of the European CF Society, were comprehensively and critically reviewed. The goal was to better understand why consensus conferences and their publications have not led to the desired results.

Results Lessons learned from previous diagnosis consensus processes and products were identified. It was decided that participation in developing a consensus was generally not inclusive enough for global impact. It was also found that many efforts to address sweat test issues were valuable but did not always improve clinical practices as CF diagnostic testing evolved. It also became clear from this review that premature applications of potential diagnostic tests such as nasal potential difference and intestinal current measurement should be avoided until validation and standardization occur. Finally, we have learned that due to the significant and growing number of cases that are challenging to diagnose, an associated continuing medical education program is both desirable and necessary.

Conclusions It is necessary but not sufficient to organize and publish CF diagnosis consensus processes. Follow-up implementation efforts and monitoring practices seem essential. *(J Pediatr 2017;181S:S16-26).*

"Every physician's first duty is to diagnose—accurately and promptly. Diagnosis is the first step of treatment."[1] Although this principle of medical practice applies well to cystic fibrosis (CF), this relatively common genetic disease has presented diagnostic challenges ever since autopsy-based diagnosis was first reported by Andersen[2] in 1938, leading to the recognition and naming of the disease. In fact, there has been a surprising degree of difficulty encountered worldwide in establishing the diagnosis unequivocally. The advent of sweat electrolyte testing[3] provided considerable clarity over diagnosis based on demonstration of pancreatic insufficiency after duodenal intubation, but healthcare providers continued to be faced with uncertain cases and challenging diagnostic dilemmas. And, although the 5-decade-long quest to diagnose CF "accurately and promptly" became more feasible with the advent of newborn screening (NBS),[4] when evidence was published on how benefits of NBS[5] outweighed the manageable risks (as affirmed by both the Centers for Disease Control [CDC][6] and the US CF Foundation[7]), NBS expanded, and new diagnostic challenges ensued.

A diagnosis of CF has traditionally relied on recognition of characteristic clinical signs and symptoms, but the increased use of NBS and prenatal screening has resulted in diagnosis often before symptoms are recognized, with a consequent opportunity to foster normal growth and development. For example, in the US, approximately 64% of new CF diagnoses now follow a positive newborn screen.[8] According to consensus

CDC	Centers for Disease Control
CF	Cystic fibrosis
CFSPID	CF screen positive, inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
CRMS	CFTR-related metabolic syndrome
DNWG	Diagnostic Network Working Group
ECFS	European CF Society
ICD	*International Statistical Classification of Diseases and Related Health Problems*
ICD-9	*International Statistical Classification of Diseases and Related Health Problems, Ninth Revision*
ICD-10	*International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*
ICM	Intestinal current measurement
IRT	Immunoreactive trypsinogen
NBS	Newborn screening
NPD	Nasal potential difference
NSWG	Neonatal Screening Working Group

guidelines developed by the CF Foundation in 2007 and published in *The Journal* during 2008,[9] individuals identified by NBS can be diagnosed with CF by a sweat chloride value ≥60 mmol/L, by the identification of 2 CF-causing mutations, or by the presence of 1 CF-causing mutation plus a clinical finding in infants with an intermediate (30-59 mmol/L) sweat test result. Although the vast majority of screened infants can be unequivocally diagnosed with CF after a positive newborn screen and sweat test,[9,10] the decision is not clear-cut in a significant number of individuals,[11,12] leading to persistent challenges[13] and stress and confusion for both families[14,15] and clinicians.[16] This group, as well as symptomatic subjects identified without NBS who have varying levels of symptoms and a variety of CF transmembrane conductance regulator (*CFTR*) mutations, has been the focus of discussions in the US and in Europe, with differing conclusions on both diagnosis and management.[10,17-19] In addition, there has been a lack of international harmony regarding terminology, leading to confusion.[20]

Another recently recognized problem is that, despite the carefully developed CF Foundation consensus guidelines published in 1998[21] and 2008,[9] CF centers have not been applying them consistently. For instance, in the US, approximately 20% of new diagnoses in 2010-2012 were made after NBS but without a confirmatory sweat test.[11]

Because of these issues, the US CF Foundation decided to convene a CF diagnosis consensus conference in 2015, bringing together CF specialists from around the world in an effort to bring clarity to diagnostic algorithms, paying special attention to the diagnostic challenges presented subsequent to the widespread implementation of NBS in the US and other countries. During the planning phase of the 2015 CF Diagnosis Consensus Conference, it was decided that a detailed historical review would be performed to assess previous consensus-producing conferences with a goal of identifying reasons why published guidelines have been misunderstood and/or implemented inconsistently. The review focused on the processes and products of efforts organized by the US CF Foundation and generally published in *The Journal*.[9,21] In addition, the important contributions of the European CF Society (ECFS) were examined, especially the activities of their Diagnostic Network Working Group (DNWG) and the Neonatal Screening Working Group (NSWG). Thus, proceeding under the assumption that knowledge of history is a precondition for better outcomes, we reviewed and critiqued 3 previous CF Foundation diagnostic consensus conferences and current European practices and identified the lessons learned.

Initial Efforts of the CF Foundation to Establish Diagnostic Criteria for US CF Centers (1963-1976)

In 1963, a committee of 6 US experts was commissioned by the US CF Foundation to write a "Guide to Diagnosis and Management of Cystic Fibrosis" for use by a growing network of CF centers in the US.[22] The timing of this 1963 publication was related to the widespread introduction of routine sweat testing using the method of Gibson and Cooke[23] that was published in 1959. During the ensuing years, a number of issues developed about the safety and reliability of the sweat test. In addition, novel, intriguing research appeared in the literature on "CF factors"[24-26] and their applicability to diagnosis, as well as potential opportunities for screening. This led to a large multiworkshop conference[27] organized in 1974 by the National Institutes of Health and the US CF Foundation with an American committee of 16 broad "experts" and 69 others participating as "consultants." Thus, the impetus for the consensus conference in 1974 was a growing body of knowledge about CF combined with difficulties encountered in applying the sweat test. Even as the workshops proceeded, preliminary data were appearing in the literature on an entirely new laboratory method of facilitating the early diagnosis of CF through NBS using meconium albumin analyses.[28]

Lessons Learned
The landmark 1974 conference led to a number of recommendations (**Table I**) that still apply today, although some

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Table I. 1976 Consensus: conclusions and recommendations for CF consensus-development process*

1	"Information on the value of early diagnosis and early treatment is urgently needed."
2	All siblings should have diagnostic tests at CF centers.
3	Detailed long-term family studies are needed.
4	Studies need to be carried out on very young patients before the development of lung infections.
5	Communication and collaboration between investigators should be improved.
6	"The sweat test is an efficient diagnostic test when properly performed and when interpreted. . .in the light of the overall clinical picture."
7	"The major limitations. . . are due to technical limitations in the stimulation and collection of sweat."
8	"The quantitative pilocarpine iontophoresis test is recommended. . .corroborated by the clinical picture."
9	"A system for quality control of sweat testing for cystic fibrosis should be developed."
10	"To avoid burns. . . and electrical shocks. . ." several recommendations were made, including the importance of electrical current control, performing sweat tests in CF centers, etc.
11	"There are no tests that meet established criteria for general screening in utero or any time thereafter." (This conclusion changed soon thereafter in 1979 when IRT was reported as a valid NBS test applicable to dried blood specimens.[29])
12	"Neonates produce insufficient sweat to permit screening" by the sweat test before. . .about 4-6 wk." (This conclusion was subsequently disproven.[30])
13	"Research to develop a reliable and safe neonatal screening test and. . .antenatal test for CF is essential."
14	"A screening test for the CF heterozygote is needed so that carriers can benefit from family-planning counseling."

*Reprinted from Report of the Committee for a Study for Evaluation of Testing for Cystic Fibrosis[27] with permission from Elsevier Inc.

have been superseded by new knowledge, especially in our understanding of *CFTR* genetics. Clearly, the major conclusions and recommendations at that time centered on sweat test confirmation of the diagnosis after symptoms had appeared. However, limitations of sweat testing were acknowledged; as the well-known CF physician-researcher Dr Paul di Sant'Agnese stated, "the sweat test is only as good as the physician ordering it."[31] In addition, based on a study of outcomes following diagnostic delays,[32] some authorities such as Dr Harry Shwachman early on felt NBS was essential to achieving a diagnosis in time to improve outcomes. Other lessons learned from this conference included the conclusion that it was not appropriate to apply "CF factor" analyses or any other incompletely developed tests to make a CF diagnosis. Indeed, premature applications of potential diagnostic tests before validation and standardization should never be recommended.

Discovery of the *CFTR* Gene Leads to New Recommendations (1989-2000)

With the crucially important identification of the *CFTR* gene in 1989,[33-36] genetic analysis began to play an increasingly significant role in diagnosis. There was recognition of an expanded CFTR-associated phenotype (represented at that time mostly by congenital bilateral absence of the vas deferens), and development of new diagnostic techniques, such as nasal potential difference (NPD) measurement.[37] Attempts to categorize patients led to a number of imprecise or inadequately defined terms (such as classic/nonclassic CF, typical/atypical CF, mild CF, and delayed CF). Although genetic knowledge about CF and its epidemiology increased dramatically during the decade after the *CFTR* discovery, there was uncertainty in CF centers about: (1) how to apply genetic testing for diagnosis; (2) the disease-causing role of some mutations in the rapidly growing list of mutations being identified; and (3) whether or not prognosis could be predicted from genetic profiles. Faced with these new challenges, the US CF Foundation convened another consensus conference in 1996 that focused on assessing practical aspects of applying knowledge about *CFTR* genetics and that produced a helpful set of revised conclusions, recommendations, and criteria. This effort involved 10 experts, all from North America, who based their recommendations on updated genetic and NPD data. Published in *The Journal* in 1998,[21] the recommendations from this important consensus conference attempted to bring order to the diagnostic landscape. The consensus at that time was that the diagnosis of CF should be based on the presence of 1 or more characteristic clinical features (**Table II**), a history of CF in a sibling, or a positive NBS test result, plus laboratory evidence of an abnormality in the *CFTR* gene or protein.

The overall conclusion was that acceptable evidence of a CFTR abnormality included biological evidence of chloride channel dysfunction (such as abnormal sweat chloride concentration, defined as sweat chloride >60 mmol/L; or abnormal NPD) or identification of a CF disease-causing mutation[38] in each copy of the *CFTR* gene. It was emphasized that "The diagnosis of CF should be made only if there is an elevated sweat chloride concentration (>60 mmol/L) on 2 separate occasions in a patient with 1 or more clinical features consistent with the CF phenotype or a history of CF in a sibling." However, it was also stated that:

> The diagnostic criteria proposed here are not likely to cover every possible clinical scenario, and there will be clinical dilemmas. . .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 re-evaluation as appropriate.

Although NPD testing was not considered ready for routine use, it was concluded that "For patients in whom sweat chloride concentrations are normal or borderline and in whom 2 CF mutations are not identified, an abnormal NPD measurement recorded on 2 separate days can be used as evidence of CFTR dysfunction." Brief comments were also included on NBS, which by 1991 had become valid and feasible based on the immunoreactive trypsinogen (IRT) test combined with DNA analysis in some regions for *CFTR* mutations.[39]

Table II. 1998 Consensus: 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 (eg, 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)

CBAVD, congenital bilateral absence of the vas deferens.
*Reprinted from Rosenstein et al[21] with permission from Elsevier Inc.

Also at this time, the American College of Medical Genetics, in conjunction with the American College of Obstetricians and Gynecologists and the National Institutes of Health, developed a panel of mutations for use in population screening for CF carriers, based on allele frequencies of CF mutations in the US CF population.[36,38] Even though the American College of Medical Genetics guidelines were prepared for population CF carrier screening, and not for the identification of mutations in patients, genetic screening for carriers recognized the growing importance of *CFTR* genetic analysis in the CF diagnostic process.

Lessons Learned

This conference and the ensuing publication were timely, valuable contributions to medical practice in CF centers. However, it soon became recognized that many *CFTR* mutations thought to be CF-causing, were not associated with disease. Thus, the recommendations from this conference and the American College of Medical Genetics/American College of Obstetricians and Gynecologists[38,40] would need to be modified. Another problem, which was recognized more slowly, was that the lack of participation by European experts in the consensus process contributed to a division on some issues such as terminology. For instance, use of the term atypical has been, and remains, integral in European CF practice.

The Advent of NBS Changes the Diagnostic Strategy and Criteria (2001-2015)

In 1998, CF NBS was available in only 6 US states[41] and certain regional programs worldwide (such as Austria, Italy, the United Kingdom, and Australia), and a CF diagnosis was suggested by a positive NBS test result in fewer than 6% of new cases.[42] However, this was soon to change. Data were published from a randomized clinical trial in Wisconsin showing the benefits of early diagnosis through NBS,[43] and the CDC had become interested in the evidence and special opportunity to capitalize on the network of 114 CF centers for confirmatory diagnostic testing and follow-up care.[44] At that time, approximately 500 *CFTR* mutations had been identified,[45] with only 24 mutations classified as "CF-causing."[21] By 2007, CF NBS was available in 34 states in the US[46] and a growing number of European countries[10,47] and mandatory screening in every state was imminent because of CDC[6] and CF Foundation[7] recommendations. More than 1500 *CFTR* mutations had been identified, and there was greater awareness of the spectrum of mutations in specific population groups as well as an increased understanding of genotype–phenotype relationships. The dramatic, and in fact unprecedented, implementation of a NBS test that yielded genetic data led to more questions and challenges related to diagnostic criteria.

Consequently, the US CF Foundation convened another CF diagnosis consensus conference in 2007 and invited 18 participants, including 4 of the 10 from the 1996-1998 conference. Although predominately from the US, there were 2 Canadians, an Australian, and a European CF geneticist with NBS expertise. This group generated the US diagnostic guidelines in use before this publication (**Table III**) and recommended the process summarized in **Figure 1**.[9] Significant differences from the 1998 recommendations included a decrease in the level of sweat chloride recognized as suggestive of CF in infants (from 40 mmol/L in 1998[21] to 30 mmol/L in 2008[9]) and the requirement in 1998 for a sweat chloride test result >60 mmol/L on at least 2 occasions plus symptoms of the disease, or history in a sibling. In 2008, a second sweat test was not required for diagnosis if 2 CF-causing mutations were identified, demonstrating the importance that genetic analysis was having on diagnostic algorithms.

During the process of developing the 2008 guidelines, it was recognized that NBS introduced a new complexity and diagnostic dilemma, namely infants with abnormal screening tests because of elevated IRT levels but inconclusive sweat test and/or DNA results. Rather than address this complex situation in the diagnostic guidelines, it was decided that this issue of a "gray zone," affecting perhaps approximately 10% of infants with high IRT levels and a single *CFTR* mutation,[48] would be addressed by the CF Foundation through a separate US consensus

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Table III. 2008 US diagnostic guidelines*

Screened newborns: Infants with a positive newborn screen shall be diagnosed with CF if they have 1 of the following:
- sweat chloride ≥60 mmol/L
  - CFTR mutation assessment is recommended; in the absence of 2 CF-causing mutations, sweat chloride value should be verified by repeat sweat test
- sweat chloride = 30-59 mmol/L[†] if they also have at least 1 of the following:
  - 2 CF-causing mutations in trans
  - clinical findings suggestive of CF, such as fecal elastase <200 μg/g or respiratory tract cultures revealing CF-associated pathogens
- sweat chloride ≤29 mmol/L[‡] in the presence of 2 CF-causing mutations in trans

General population: Individuals shall be diagnosed with CF if they have 1 of the following:
- sweat chloride ≥60 mmol/L
- sweat chloride = 30-59 mmol/L[§] if they also have at least 1 of the following:
  - 2 CF-causing mutations (in trans, as stated in text[9])
  - significant clinical findings suggestive of CF, such as pancreatic insufficiency or respiratory tract cultures revealing CF-associated pathogen
- sweat chloride ≤39 mmol/L (after 6 mo of age) in the presence of 2 CF-causing mutations (in trans as stated in text[9])

*Reprinted from Farrell et al[9] with permission from Elsevier Inc.
†40-59 mmol/L in repeat sweat test after 6 mo of age.
‡≤39 mmol/L in repeat sweat test after 6 mo of age.
§40-59 mmol/L ≥6 mo of age.

CF Newborn Screening Result: **Age**
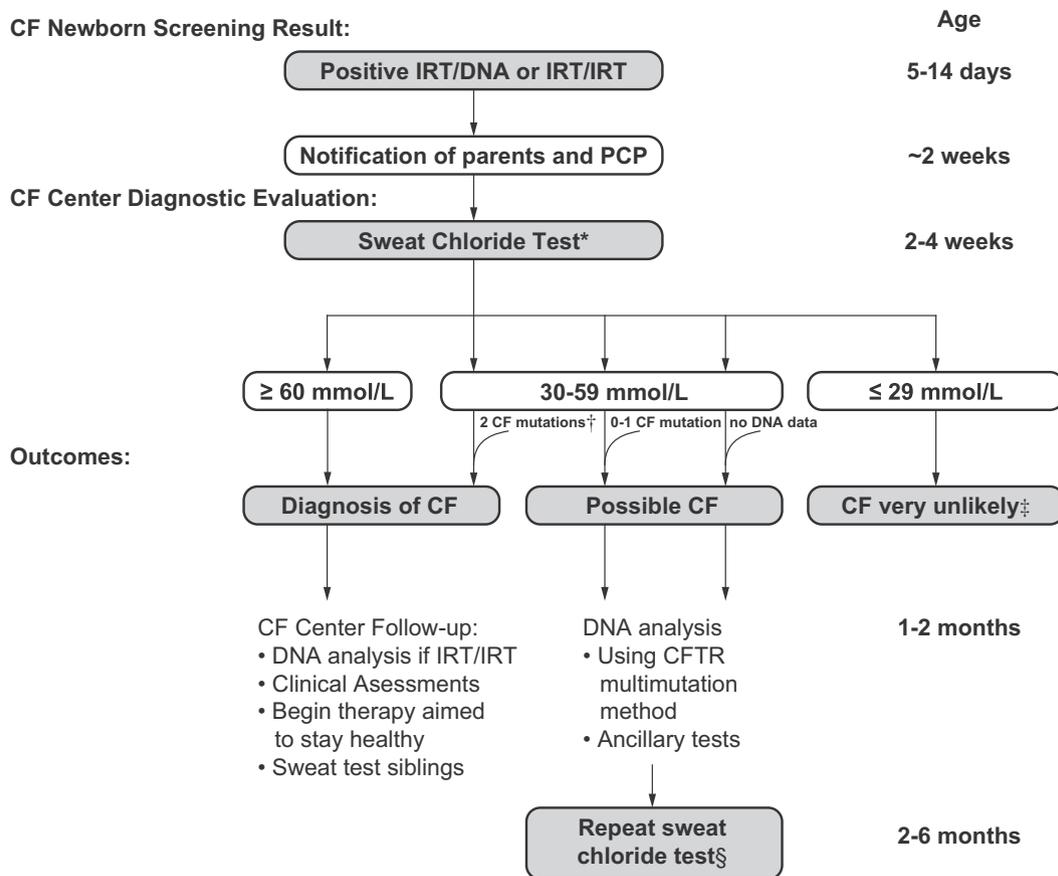
Figure 1. 2008 CF Foundation recommended diagnostic process for screened newborns. *If the baby is at least 2 kg and more than 36 weeks' gestation at birth, perform bilateral sweat sampling/analysis with either Gibson-Cooke or Macroduct method; repeat as soon as possible if sweat quantity is less than 75 mg or 15 μL, respectively. †CF mutation refers to a CFTR mutant allele known to cause CF disease. ‡The disease is very unlikely; however, if there are 2 CF mutations in trans, CF may be diagnosed. §After a repeat sweat test, further evaluation depends on the results as implied above. *PCP*, primary care physician. *Reprinted from Farrell et al[9] with permission from Elsevier Inc.

conference. An expert panel convened and, using a Delphi method,[49] recommended a new diagnostic term (CRMS for CFTR-related metabolic syndrome) and management guidelines, published subsequently in *The Journal*.[18] CRMS is the diagnosis used in the US to describe infants with elevated levels of IRT, but with sweat chloride levels below CF diagnostic levels, and fewer than 2 CF-causing mutations. Although this condition is not a metabolic disorder, the designation "metabolic syndrome" was established in part to have an *International Statistical Classification of Diseases and Related Health Problems* (ICD), *Ninth Revisions* (ICD-9) medical code (277.9) for US healthcare delivery system follow-up and billing purposes. In fact, this consensus-producing effort led to the general recognition that ICD coding was not included as part of the diagnosis considerations in previous CF Foundation-sponsored consensus conferences.

Despite the efforts to explain and apply CRMS as a diagnosis, this term has not been well accepted in Europe and some other countries because of concern about its appropriateness and a feeling that it was difficult for families to understand this

"syndrome label" as a descriptive term for a temporary diagnosis in an asymptomatic child requiring a further diagnostic step (which is yet to be decided). Thus, a different term, CFSPID, for CF screen positive, inconclusive diagnosis,[17] was developed in a Delphi process by the ECFS NSWG and introduced recently in Europe as an alternative to CRMS.

During the planning phase of the 2015 CF Diagnosis Consensus Conference, it was concluded that both CRMS and CFSPID must be considered diagnoses that should be accompanied by a clinical management plan. Indeed, the original consensus articles describing CRMS and CFSPID included action plans for follow-up care.[17,18] In addition, the CF Foundation organized a separate consensus conference that was focused on developing comprehensive care guidelines for infants diagnosed through NBS, extending to 2 years of age.[50]

Lessons Learned

The guidelines published in 2008 had significant impact as NBS programs proliferated in the US (extending nationwide by 2010) and also in other countries. However, the complexity

of the recommendations and the failure to incorporate the CRMS consensus statements into the general diagnostic guidelines resulted in 2 unlinked sets of recommendations and caused confusion. An additional diagnostic term, CFTR-related disorder, introduced by US authors in 2008,[51] contributed to the confusion, as it was used in a variety of ways by different authors before being precisely defined as a monosymptomatic entity by mainly European authors in 2011 after 2 consensus conferences.[52] Once again, the very limited representation of Europeans during the development of the 2008 CF Foundation guidelines led to less impact than anticipated. Another lesson learned was that the genetic data used for diagnostic purposes must be defined more precisely, as the explosion of information coming from the NBS programs demonstrated the wide array of phenotypic impacts of different *CFTR* mutations.[53,54] Finally, as these guidelines were disseminated and explained, it became clear that some confusion was attributable to the complexity and ambiguity of cases arising from NBS programs; in retrospect, it would have been wise to include case studies as examples of diagnostic challenges.

Consensus Recommendations and Practices of the European CF Society

The ECFS has established both a DNWG and a NSWG, which meet regularly and have published a variety of helpful articles on CF diagnosis. The DNWG was organized in 2004 and the NSWG in 2005. These groups have been responsible for recommending diagnostic criteria, methods, and practices for screened and nonscreened populations.[10,17,19,47,55-57] To reach consensus, they have used Delphi and other robust methods, including a variety of surveys and multidisciplinary conferences. Topics addressed by these ECFS groups include performance and follow-up recommendations of NBS, standardization of CFTR biomarkers such as NPD and intestinal current measurement (ICM) for difficult cases, as well as characterization of *CFTR* genotypes of unknown clinical relevance.

CF diagnostic algorithms were established by the ECFS in 2006 for the nonscreened population (**Figure 2**)[10] and in 2009 for screened infants (**Figure 3**).[19] Following the 2006 nonscreened algorithm can lead to 3 separate outcomes: "classic CF," "CF unlikely," or "CFTR dysfunction." A diagnosis of "classic CF" is given to patients with sweat chloride levels >60 mmol/L (as recommended in the 1998 CF Foundation consensus,[21] rather than ≥60 mmol/L as recommended in subsequent US guidelines[9]). "CF unlikely" is used to describe patients with normal sweat chloride values (≤29 mmol/L) or intermediate sweat chloride values (30-60 mmol/L for all ages), either 0 or 1 *CFTR* mutation and/or a normal NPD. Patients with intermediate sweat chloride concentrations require further study; NPD is recommended, and ICM may be considered to help clarify the diagnosis. Both measures of CFTR function appear to correlate well with CF genotype and phenotype[58] and potentially can be used to discriminate pancreatic-sufficient CF from the individual without CF in the setting of intermediate

sweat chloride values.[59,60] Both ICM and NPD require standardized techniques to be reliable, and the ECFS DNWG has designed standardized operating procedures for ICM[61] and NPD[62] that require validation in a multicenter setting.

In contrast to the potential outcome of "possible CF" described in the 2008 CF Foundation diagnostic guidelines, the ECFS algorithm recommends the use of NPD results to further classify individuals with intermediate sweat chloride values and less than 2 CF-causing *CFTR* mutations, potentially resulting in the third, gray zone, category of "CFTR dysfunction" or "nonclassic CF."[10] Subsequent validation of the European CF diagnostic algorithm revealed that the "CFTR dysfunction" group differs phenotypically from patients with intermediate sweat chloride values in whom CFTR function based on NPD testing was normal.[19] Despite recommendation of the terms "CFTR dysfunction" or "nonclassic CF" category in the 2006 ECFS algorithm, many European CF clinicians still use the term atypical CF. This highlights the need for better consensus in the currently ongoing update of the ECFS CF diagnostic guideline, which is being developed by Delphi consensus within the DNWG and NSWG, and which is expected to include recent European advances in using NPD and ICM as additional CFTR biomarkers for difficult CF diagnoses.

An additional contribution from the ECFS came after the results of 2 consensus conferences, which included worldwide experts, were published by Bombieri et al[52] describing a "CFTR-related disorder" as "a clinical entity associated with CFTR dysfunction that does not fulfil diagnostic criteria for CF." Examples of this would be certain monosymptomatic individuals with congenital bilateral absence of the vas deferens, diffuse bronchiectasis, or recurrent pancreatitis. Diagnostic algorithms for these groups[44] are shown in **Figure 4**. NPD and ICM values are not clear-cut in these individuals. The ECFS recommends that individuals who cannot be diagnosed with CF but are thought to be at risk of monosymptomatic disease because of CFTR dysfunction (suggested by symptoms or laboratory findings) should be categorized as having a "CFTR-related disorder."

The ECFS also has been responsible for 2 visionary "Garda conferences" that have contributed significantly to the application of CFTR genetic data in diagnostic processes and to the use of "best practices" in NBS.[55,63] In 2007, following the growing number of *CFTR* sequence variations detected by molecular assays and difficulties in understanding their actual significance, ECFS convened a consensus conference aimed at suggesting how to use and interpret mutation analysis in clinical practice.[63] An international group of CF clinicians and geneticists gave an account of the distribution of mutations and methods of analysis, addressed the role of genetic testing for establishing the diagnosis of CF and for outcome prediction, and provided recommendations on the bidirectional information flow between clinicians and molecular analysis laboratories. The resulting 2008 state-of-the-art publication[9] is now partially outdated in the sections on mutation distribution, which is better understood today, and on genetic analysis, which is going through a radical change thanks to next-generation sequencing technologies. Conversely, discussions
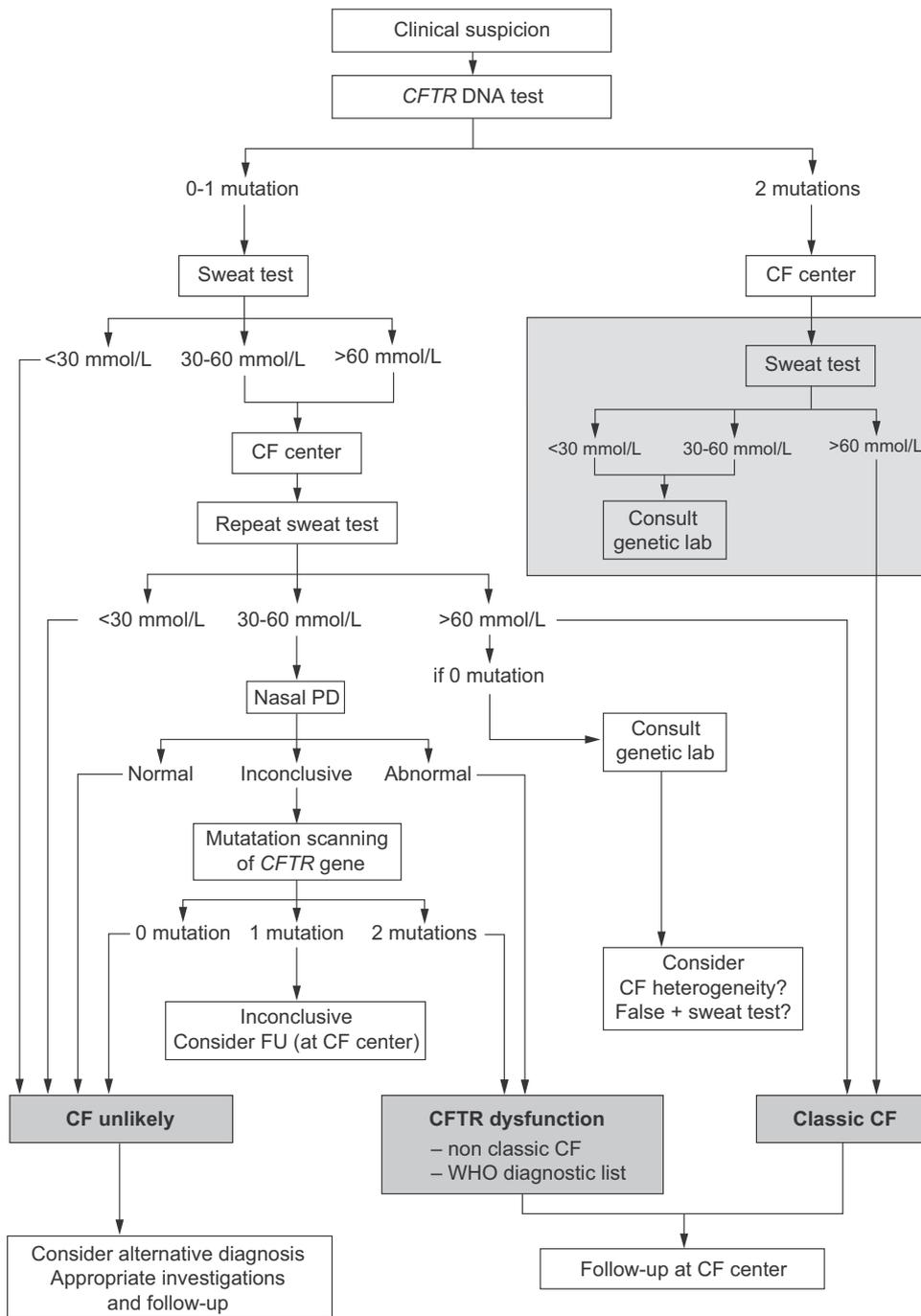
Figure 2. 2006 ECFS recommended diagnostic process for nonscreened population. *Reprinted from De Boeck et al[10] with permission from BMJ.

contained in other chapters retain their validity. The prominence of sweat chloride testing as a diagnostic tool, the limited role of mutation classes in predicting phenotype, and the shortcomings in reliably predicting the severity of pulmonary disease based only on *CFTR* genotype had been suggested before but reached a wider audience through the consensus publication.[63]

As an outcome of the 2007 Garda Conference, *CFTR* mutations were clustered into 4 groups according to their predicted diagnostic significance: (1) mutations that cause CF disease; (2) mutations that result in a CFTR-related disorder (with a partial overlap of groups 1 and 2); (3) mutations with no known clinical consequence; and (4) mutations of unproven or uncertain clinical relevance. In addition, suggestions were given on criteria to use to assess the probability that a given sequence variation might be CF-causing. These definitions were later adopted and adapted by the CFTR2 project.[53,64]

IRT at Birth
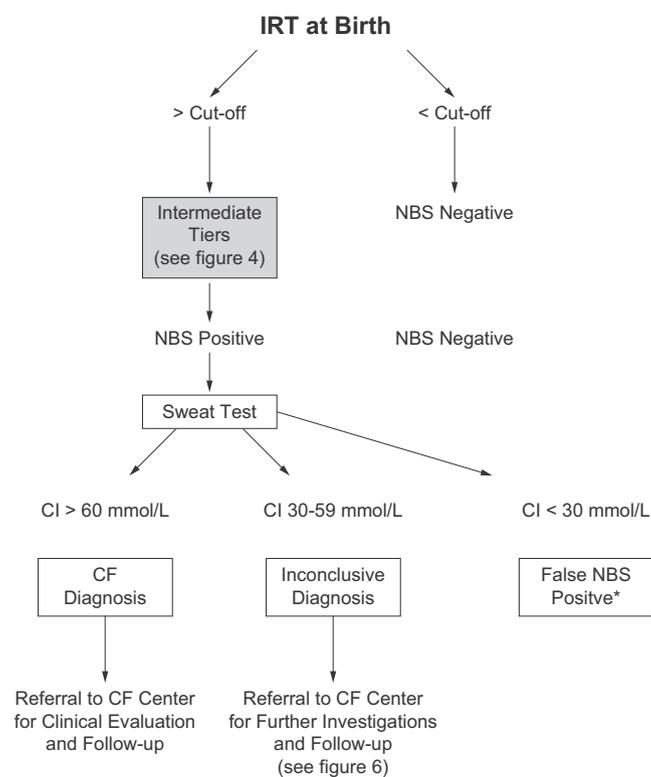
Figure 3. 2009 ECFS recommended diagnostic process for screened newborns. (Note that references to figures in this illustration refer to those in the original 2009 article.) *Reprinted from Castellani et al[55] with permission from Elsevier Inc.

One year later, a second ECFS Consensus Conference,[55] this time prompted by the proliferation of CF NBS programs and the heterogeneity of protocols and performances, outlined benefits, by-products and costs of NBS, examined advantages and disadvantages of different protocols, and suggested strategies to inform families. Problems and uncertainties connected with the identification of inconclusive cases elicited by NBS were also discussed and later further elaborated on by the ECFS NSWG. This in turn led to the use of the term CFSPID for asymptomatic infants with an inconclusive diagnosis after positive NBS.[17] There is ongoing ECFS activity on how, and for how long, to follow up infants with CFSPID.

Lessons Learned
Investment by the ECFS to organize a combination of consensus conferences and working groups on NBS and diagnosis has paid dividends worldwide. However, despite very impressive data on the potential value of new, very sensitive methods such as NPD and ICM, their dissemination has been limited. It is especially surprising that the US has lagged behind in the emerging field of evaluating CFTR functioning in situ with NPD and ICM methods for improving our diagnostic capability in certain cases such as infants with intermediate sweat chloride levels and inconclusive genetic data. Another lesson learned is that the Delphi consensus process, although

considered fairly robust, is quite time consuming. Finally, it appears that ECFS-generated products that we regard as "European" may not apply to some aspects of diagnosis and terminology (ie, atypical is not used throughout Europe even though it is favored in many countries).

ICD-Associated Healthcare Delivery Challenges and Recommendations for Using ICD, *10th Revision* Codes

Diagnostic consensus conferences, with the exception of the conference convened to define CRMS,[18] have lacked attention to the use of the ICD system. However, the ICD system was created by the World Health Organization to be "the international standard for defining and reporting diseases and health conditions. . .It allows the world to compare and share health information using a common language."[65] Thus, ensuring that ICD codes are understood and used appropriately in the many complex clinical scenarios encountered by CF caregivers internationally is vital to achieving clinical research objectives and optimizing access to and quality of care. Recognizing the need to encourage the common and correct use of ICD codes by CF caregivers, the issue was included during the development of recommendations from the 2015 US CF Diagnostic Consensus Conference.

The ICD system is updated on a regular basis. Revision of the ICD-9 codes for the ICD, *10th Revision* (ICD-10) system began in 1983, was completed in 1992, and implemented in the US on October 1, 2015. The delay was due to a variety of issues including the complexity of ICD-10, the arduous transformation processes involving both computer software upgrades and paperwork revisions, training needs, the rigorous regulatory requirements in the US, and the opposition of the American Medical Association. The next update of the code, to ICD, *11th Revision*, is due to be completed by 2018.

ICD-10 codes recommended for use in individuals with CF or CRMS/CFSPID can be found in the article in this Supplement by Farrell et al.[66] Although some CF specialists were engaged in the ICD-10 development process, the degree of influence was limited. Despite the creation of over 16 000 codes in the ICD-10 system, there is no code for a CFTR-related disorder, nor was there one in ICD-9. This unfortunate situation can be attributed to the timing of ICD-10 development vis-à-vis the definition of a CFTR-related disorder in 2011.[52]

Lessons Learned
International consensus is important to clarify our understanding of CF pathology and improve the care for individuals affected by CFTR dysfunction. The ICD coding system is an international language that must be used to enable clinical data to be reported accurately across member nations. A discussion of current ICD codes should be considered at future diagnosis consensus conferences, to enhance global understanding and ensure accurate diagnosis and research results.
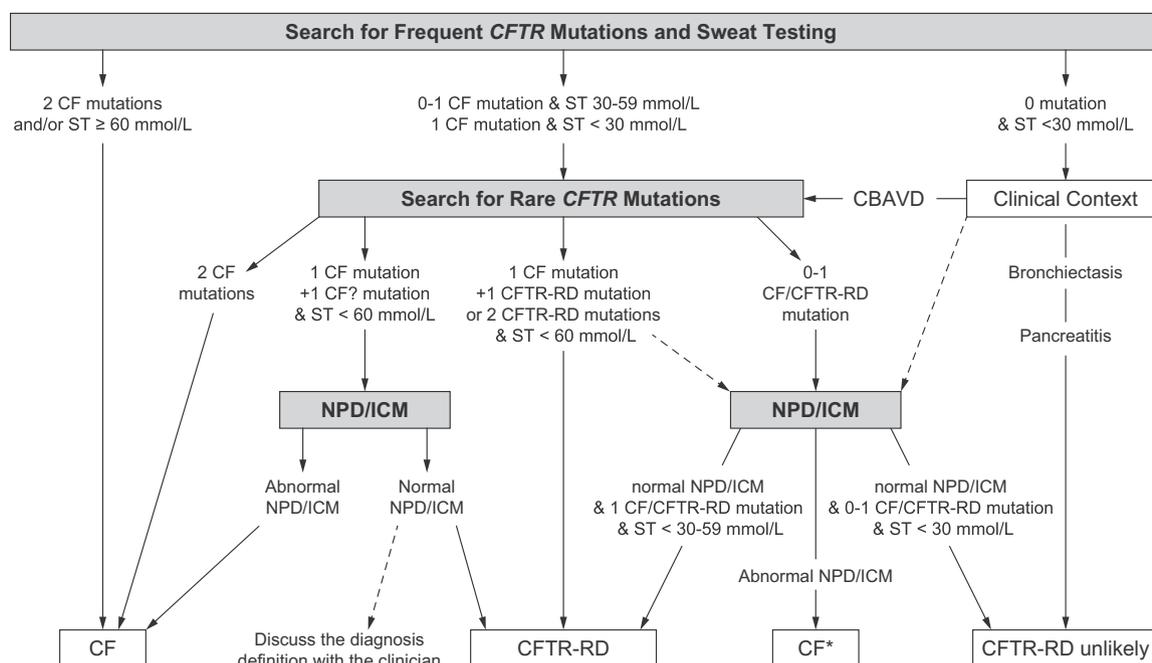
Figure 4. 2015 ECFS recommended process for diagnosis of CFTR-RD. Global diagnostic algorithm for CF and CFTR-RD. A global flow-chart of genetic and functional diagnostic testing in CF and CFTR-RD is presented. *CF? mutation*, mutation of unproven or uncertain clinical significance; *CF**, diagnosis of CF or consider this diagnosis; *CFTR-RD*, CFTR-related disorders; *ICM*, intestinal current measurement; *NPD*, nasal potential difference; *ST*, sweat test (repeated; false positive should be excluded/sought in a specialized center). *Reprinted from Bombieri al[52] with permission from Elsevier Inc.

Discussion

The US CF Foundation current diagnosis recommendations arose from 3 essentially concurrent consensus exercises published in 2008-2009, to be applied as an integrated "package."[9,18,50] The ECFS recommendations, emanating from their Diagnostic Network and Neonatal Screening Working Groups, have also contributed significantly to recommendations for diagnostic practices. Unfortunately, despite best intentions, the 2008 recommendations have been misunderstood by some CF specialist leaders and have not been applied consistently, leading to less-than-optimal outcomes for the CF population.

To improve the design, uptake, and proper use of new recommendations, a review was conducted of the past several decades of experience in creating CF diagnosis guidelines. A number of valuable lessons were learned, and 3 lessons in particular were used to inform the design of the 2015 CF diagnosis consensus process. First, although European recommendations have contributed to US decisions, criteria and terminology have varied, as reflected by the use of similar but not identical nomenclature for CRMS and CFSPID, and by the differential use of CFTR biomarkers such as NPD and ICM to clarify a difficult CF diagnosis. This problem was addressed by inviting more European participation than ever before, both during the conference itself, and afterward during open review periods while the report was being prepared. The

second lesson, that the recommendations were misunderstood and inconsistently applied is being addressed by the development of an implementation strategy. Finally, the lesson of confusion and errors resulting from incorrectly applied ICD codes was addressed by consultation with an ICD-coding expert and inclusion of the pertinent codes in the main diagnosis consensus document.[66] It is hoped that these efforts to understand and correct past oversights will improve development, uptake, and application of the 2015 diagnosis consensus guidelines. ∎

Author Disclosures

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Applying Cystic Fibrosis Transmembrane Conductance Regulator Genetics and CFTR2 Data to Facilitate Diagnoses

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Objective As a Mendelian disease, genetics plays an integral role in the diagnosis of cystic fibrosis (CF). The identification of 2 disease-causing mutations in the CF transmembrane conductance regulator (CFTR) in an individual with a phenotype provides evidence that the disease is CF. However, not all variations in CFTR always result in CF. Therefore, for *CFTR* genotype to provide the same level of evidence of CFTR dysfunction as shown by direct tests such as sweat chloride or nasal potential difference, the mutations identified must be known to always result in CF. The use of *CFTR* genetics in CF diagnosis, therefore, relies heavily on mutation interpretation.

Study design Progress that has been made on mutation interpretation and annotation was reviewed at the recent CF Foundation Diagnosis Consensus Conference. A modified Delphi method was used to identify consensus statements on the use of genetic analysis in CF diagnosis.

Results The largest recent advance in CF genetics has come through the Clinical and Functional Translation of CFTR (CFTR2) project. This undertaking seeks to characterize *CFTR* mutations from patients with CF around the world. The project also established guidelines for the clinical, functional, and population/penetrance criteria that can be used to interpret mutations not yet included in CFTR2's review.

Conclusions The use of *CFTR* genetics to aid in diagnosis of CF requires that the mutations identified have a known disease liability. The demonstration of 2 in *trans* mutations known to always result in CF is satisfactory evidence of CFTR dysfunction. However, if the identified mutations are known to be associated with variable outcomes, or have unknown consequence, that genotype may not result in a CF phenotype. In these cases, other tests of CFTR function may help. *(J Pediatr 2017;181S:S27-32).*

The diagnosis of cystic fibrosis (CF) in this and previous consensus statements relies on both a clinical presentation as well as evidence of CF transmembrane conductance regulator (CFTR) dysfunction.[1-3] As a genetic disease, CF is defined by mutation in the gene that codes for CFTR. Therefore, the presence of deleterious mutations in both copies of the *CFTR* gene does satisfy the diagnostic criteria for CF. It is important to keep in mind, especially in the evaluation of rare mutations, that the genetic evidence of CFTR dysfunction is indirect and must be inferred from what is known about the mutation. Even before the gene was identified, tests that measure the direct consequence of dysfunctional CFTR were shown to differentiate CF from non-CF.[4] This established the measurement of sweat chloride concentration as the "first-line" method to describe CFTR dysfunction, and it remains integral to CF diagnosis. However, there are circumstances such as newborn screening (NBS) in which sweat testing is impractical or impossible. As a result, *CFTR* genotyping has become an equally important part of CF diagnosis. The introduction of CFTR-modulating therapies that are specific to certain mutations also emphasizes the need for genotyping and mutation characterization.[5]

Initially, *CFTR* genotyping was done in individuals with clear phenotypic manifestations of CF who demonstrated CFTR dysfunction through sweat chloride measurement. Therefore, if a mutation was detected in the *CFTR* gene in a sample from an individual with CF, it was presumed to be CF-causing. This was accurate for most well-recognized mutations (such as c.1521_1523delCTT [legacy: F508del], c.1652G > A [legacy: G551D], or c.1624G > T [legacy: G542X]), but several important examples were recognized in which variants identified in *CFTR* were not independently causal of CF.[6,7] Also, the number of variants in *CFTR* expanded rapidly as genetic analysis was performed on more patients with CF and

CF	Cystic fibrosis
CFMD	CF Mutation Database
CFTR	CF transmembrane conductance regulator
CFTR2	Clinical and Functional Translation of CFTR
MVCC	Mutations of varying clinical consequence
NBS	Newborn screening
UNK	Mutation of unknown significance

in the general population. To address the need for the annotation of *CFTR* variants, the US CF Foundation assembled an international research group tasked with defining criteria for disease liability and annotating the mutations seen in patients with CF entered in registries. This research group, the clinical and functional translation of CFTR (CFTR2) team, has made an important contribution and has better informed genetic analysis as a part of CF diagnosis.

In this report, we outline the use of *CFTR* genetic analysis in the diagnosis of CF and other related conditions. The interpretation of *CFTR* genotyping will focus on the categories of mutations as described by the CFTR2 team. The CFTR2 analysis is a comprehensive annotation of mutations among patients with CF, but there are several important instances in which genetic analysis does not allow a definitive CF diagnosis, or in which information other than CFTR2 should be considered. For example, the use of genetic criteria in diagnosis is insufficient for mutations that are known to be associated with CF in some individuals but no phenotype in others, and for the many mutations not yet characterized by CFTR2. Therefore, the diagnostic challenge of difficult-to-interpret genotypes will remain.

Mutation Annotation

The initial repository for *CFTR* variants, termed the CF Mutation Database (CFMD),[8] began in 1990, shortly after the *CFTR* gene was identified. The CFMD content was assembled through voluntary contributions from research laboratories, genetic testing facilities, and clinicians. It is a comprehensive collection of variation in the *CFTR* gene, with graphic and text search features that incorporate both legacy-

mutation naming, as well as more recent naming rules that are consistent across the genetic community[9,10] (**Appendix**; available at www.jpeds.com). The database in some cases contains phenotypic information that was included with the submission. For example, it may report that a given mutation was noticed in an individual with infertility. Although this information might be helpful for rare mutations, these single cases do not always reflect the true disease liability of the mutations. The first reported case of a mutation may be the only one noted, even if subsequent occurrences of the mutations have differing phenotypes. Therefore, assessing the disease liability of *CFTR* variants based solely on whatever data have been submitted to CFMD is of limited usefulness toward CF diagnosis.

Although the CFMD constitutes an excellent existing repository of information on nucleotide variation in the *CFTR* gene, it was clear that a new approach was essential to comprehensively and consistently address the phenotypic and functional implications of *CFTR* variants. The CFTR2 project has assembled data from national registries of patients with CF, as well as large clinical databases from countries without a national registry, to collect, quantify, and describe the mutations reported in individuals with CF.[11] These registries and datasets are largely from Europe, North America, and Australia, but also contain representation from the Middle East, Asia, and South America. The mutations seen among this group of individuals with CF can be ranked by frequency to prioritize the analysis and annotation of mutations seen most commonly. Mutations are categorized as CF-causing if they meet clinical, functional, and population/penetrance criteria (**Table I**). Mutations are categorized as mutations of varying clinical consequence (MVCC) if they do not meet clinical and/or function criteria. Categorizing a mutation as non-CF-causing

Table I. Categories of mutations, as defined by CFTR2			
	Clinical criteria	**Functional analysis**	**Population/penetrance**
CF-causing	Mean sweat chloride ≥60 mmol/L in patients with the mutation present in *trans* with a known CF-causing mutation*	Mutation of the type expected to produce no protein (nonsense, frameshift, canonical splice)[†] OR Functional evidence of: • <10% wild-type CFTR mRNA present • <10% wild-type CFTR protein folding/processing • <10% wild-type CFTR-specific chloride conductance	Evidence suggests completely penetrant for CF: • Not seen in the nontransmitted allele in fathers of offspring with CF • Allele frequency in the population with CF higher than in the general population
MVCC	Variant may or may not meet CF-causing criteria Variant does not satisfy *both* clinical and functional criteria above (but may satisfy one or the other, or may satisfy neither)	Variant may or may not meet CF-causing criteria	Lack of CF phenotype in some individuals with mutation in *trans* with CF-causing mutation
Non-CF-causing	Clinical evidence not considered	Variant must not meet CF-causing criteria	Evidence that the variant is nonpenetrant: • Allele frequency in the population with CF lower than allele frequency in the general population • Observed as the nontransmitted allele in *trans* with a CF-causing mutation in the father of offspring with CF
Unknown	Analysis incomplete OR Unable to assign a disease liability characterization		

mRNA, messenger RNA.
*If too few patients with sweat chloride data, pancreatic function data are used.[20]
†Not all mutations that cause premature terminations will result in nonsense-mediated decay and no protein (ie, those with terminations in the last exon); in which case, laboratory-based functional analysis is required.

requires a different approach. Clinical criteria are not considered because it is likely that the patients' phenotypes result from an unidentified variant. Instead, characterization of non-CF-causing requires functional analysis and strong evidence from the general population that these variants are not fully penetrant for CF (that is, they do not result in CF when present in *trans* with a CF-causing mutation). A fourth category listed in Table I is unknown; the CFTR2 annotation project is ongoing, and analysis has not been completed for all *CFTR* variants, particularly those that occur at extremely low frequency.

Assigning mutations to these categories of disease liability has the benefit of simplifying genetic interpretation. It is consistent with current recommended practices for variant annotation by the American College of Medical Genetics, while keeping specificity toward the mutations' likelihood of causing CF (as opposed to any phenotype, which may not meet the definition of CF).[12] The following potential shortcomings of this classification hierarchy deserve consideration because they elucidate scenarios in which *CFTR* molecular genetic analysis may be less helpful in making a CF diagnosis or cases where the context of additional research may be needed.

The Penetrance of Variants Classified as Varying Consequence May Differ

For example, the variant c.3454G > C (legacy: D1152H) is well known to be associated with milder CF disease in some cases.[13-15] In comparison, the variant c.[350G > A;1210 − 12[7]] (legacy: R117H;7T) also is associated with milder disease but has been shown to have many more individuals that possess this variant with a CF-causing variant who do not have disease.[16]

There Is No Special Designation for Mutations Primarily Associated with CFTR-Related Disorders

The recognition of individuals with *CFTR* mutations and a CFTR-related phenotype who do not meet the diagnostic criteria for CF has led to the definition of CFTR-related disorders.[17] These individuals may have isolated male infertility, recurrent pancreatitis, or bronchiectasis. There are *CFTR* variants that more frequently (but not exclusively) result in these milder phenotypes.[18,19] In CFTR2, these variants have been classified as either MVCC or non-CF-causing. Prior reviews of the use of genetics in CF have assigned mutations to categories in a similar fashion as CFTR2 but include a category of mutations leading to CFTR-related disorders.[21,22] However, the authors of those reviews concede that many of the mutations assigned in the CFTR-related disorder category may also be included in another category. CFTR2 is focused more specifically on individuals with CF, and defining the disease liability relative to CF.

CFTR2 Is Not Comprehensive

Although the CFTR2 project is a comprehensive systematic annotation of disease liability, there is a great deal of published literature on mutations not yet included in CFTR2. This may occur for populations under-represented in CFTR2. Other research may be of considerable help, especially for mutations reported as "unknown" by CFTR2.

Penetrance

The genetic term "penetrance" refers to the proportion of people with a given genotype who will exhibit symptoms of a condition. For CF, this can be defined as the proportion of people with a specific mutation combination, (specifically, the individual mutation under analysis plus any known CF-causing mutation on the other allele) who will satisfy the diagnostic criteria for CF. Mutations or genotypes that always lead to CF are said to be 100% penetrant. The most common example of a mutation with 100% penetrance is F508del; this means that every person with the genotype F508del/F508del is expected to have CF. Other mutations, such as the more variable D1152H, are less than 100% penetrant for CF because not all people with this mutation, even in combination with a CF-causing mutation like F508del, will have CF. In this respect, a given mutation is expected to have different penetrance for CF- vs CFTR-related disorders.

Assessing penetrance requires an understanding of the frequency of individuals who have a given genotype but do not have CF (and are, therefore, not represented in CFTR2). There has been great progress in public repositories of genetic variation, which allows better estimates of the allele frequency of a given variant in general (presumably healthy and unaffected) populations. As part of the penetrance analysis for the CFTR2 assignment of disease liability, the allele frequency in individuals with CF is compared with the allele frequency in the general population as estimated using data from the 1000 genomes browser.[23,24] Mutations such as G551D are more common among individuals with CF. Therefore, the relative prevalence of patients with CF with this mutation can be accurately estimated by applying the Hardy-Weinberg principle to the allele frequency in European populations. For mutations such as c.2991G > C (legacy: L997F), the allele frequency in the general population is much higher than among individuals with CF. This suggests that many individuals carry this allele, in *trans* with a CF-causing allele, are not entered into registries of patients with CF, and are, therefore, not reflected in CFTR2. However, this comparison of the general to the population with CF does not include individuals who may have phenotype (such as infertility) because of L997F but are not entered into a registry of patients with CF.

Use of Genetics in CF Diagnosis

The US Cystic Fibrosis Foundation 2015 Diagnosis Consensus Committee voted to adopt the guidelines shown in Table II regarding the use of molecular genetic tests to aid in demonstrating CF diagnosis.[3]

Although it was not specifically designated in the consensus statements, it is the opinion of the authors that *CFTR* genotyping should be performed for all individuals diagnosed with CF, even if physiologic tests establish the diagnosis. The reason for this, beyond better understanding of the genetic epidemiology of this disease, is that currently available (and, presumably, future) therapies are able to modulate

Table II. 2015 CF Foundation diagnosis consensus conference recommendations for diagnosis of CF using CFTR2

Statement numbers	Consensus statements
11	The latest classifications identified in the CFTR2 project[25] should be used to aid with CF diagnosis: • CF-causing mutation: individuals with 2 copies on separate alleles will likely have CF (clinical sweat confirmation needed) • Mutation of varying clinical consequence (MVCC): a mutation that in combination with a CF-causing mutation or another MVCC mutation may result in CF • Uncharacterized mutation/mutation of UNK: mutation that has not been evaluated by CFTR2 and may be disease causing or of variable clinical consequence or benign • Non-CF-causing mutation: individuals with 1 or more are unlikely to have CF (as a result of that allele)
12	In individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, the identification of 2 CF-causing mutations (defined by CFTR2) is consistent with a diagnosis of CF. Sweat chloride testing is necessary, though, to confirm the diagnosis.
13	The absence of detection of 2 CF-causing *CFTR* mutations does not exclude a diagnosis of CF.

specific *CFTR* mutations. Genotyping errors are more likely to occur in NBS (state-run laboratories doing a large number of tests) than in genetic testing done for clinical purposes. Therefore, depending on the laboratories' quality metrics, repeat genotyping may be indicated to confirm the genotype identified through NBS.

In the event that an individual being considered for CF has a genotype that is not annotated by CFTR2, other literature should be consulted. If other research is lacking or equivocal, the clinician will need to rely more heavily on other tests to demonstrate CFTR dysfunction, such as sweat chloride testing or physiologic CFTR testing through nasal potential difference or intestinal current measures. Some specific genotype combinations and recommendations are described in **Table III**.

Genetic Tests for CFTR

As the use of genetic testing in medical practice has expanded, testing for more variation has become less expensive. Tradi-

tionally, testing for *CFTR* mutations was done by panel tests that detect a given number of mutations (usually 5-150 mutations). These panels can be customized to better detect local variants seen in the local populations. Sequencing is a comprehensive check of all nucleotides in the coding region of the gene and typically also includes noncoding regions where known mutations exist. Sequencing now is increasingly performed using "next-gen" technology. One application of this technology involves only reporting mutations with well-established disease liability as abnormal in the initial screening report, but with the ability to "go back" and reveal variants of uncertain disease liability if the clinician believes the clinical scenario warrants this.[27] Sequencing is more sensitive, but may not detect mutations in the noncoding region and also may not detect large deletions or duplications in the gene. Deletions and duplications can specifically be tested for by separate tests such as multiplex ligation-dependent probe amplification. Deletions and duplications may be detected by certain next-gen platforms, but this practice will vary by testing laboratory.[28]

Table III. Effects on diagnosis recommendations of different categories of *CFTR* mutations in the presence of a CF-causing mutation (in *trans*)

CFTR genotype		Recommendations for interpretation
Allele 1	**Allele 2**	
CF-causing mutation	Variant not characterized by CFTR2 (or categorized as "unknown")	This category includes mutations not annotated by CFTR2. Therefore, this genotype may or may not result in CF, depending on the disease liability of the uncharacterized variant. In some cases, there may be existing literature on the variant. If so, the same criteria used by CFTR2 can be used to define the pathologic potential for that variant. The literature on the variant should include clinical evidence (described in well-phenotyped patients with CF), functional evidence (either predicted to result in no protein, tested for RNA or protein levels, or tested for chloride conductance), and finally, population evidence. The population evidence can be investigated by looking for the variant in public databases such as 1000 genomes[23] or the Exome Aggregator Consortium.[26] A high allele frequency of the variant in these public databases would suggest that it is not fully penetrant.
	Mutation of varying clinical consequence	The likelihood that this genotype will result in CF will depend on the penetrance of the mutation with varying clinical consequence. In most cases, that is not well known. The clinical scenario becomes the key determinate of the diagnostic label. This can become challenging, especially if this genotype is detected in a newborn (eg, Extended Genetic Testing as Part of NBS in California). Because lung and pancreatic phenotypes progress over time, a clinical scenario that meets CF criteria may not occur until later in life.
	No variant identified	The key to interpreting a result in which only one variant identified is evaluating the extent of genetic testing. If only a panel containing common mutations was used, there may be an unidentified mutation. If there is suspicion from either clinical criteria or from CFTR physiologic testing, extended CFTR analysis should be performed. Sequencing and deletion duplication testing have a very high negative predictive value, but cannot completely exclude the CF.
	Non-CF-causing	The non-CF-causing mutations in CFTR2 were all identified in patients believed to have CF (enough to be entered in a registry). This occurred because the individual: (1) does not actually have CF, and the diagnosis is incorrect; (2) has mild organ system manifestations that do not typically meet diagnostic criteria for CF, but may be an example of a CFTR-related disorder; or (3) has CF, but 1 of the causative variants has not been identified (and the non-CF-causing variant is inappropriately assumed the culprit). This (2 mutations on the same chromosome) would be an example of a complex allele.

Complex alleles, in which multiple variants are present on the same chromosome, present an added diagnostic challenge. In some cases, disease liability is well recognized, but this is not often the case. For the mutation c.350G > A (legacy: R117H), it is well established that differing intron 9 (legacy intron 8) modifiers affect the severity and penetrance of this allele.[6,29] Another example of a complex allele is one containing the common variant L997F. Individuals who carry this and c.350G > T (legacy: R117L) on the same allele have a more severe phenotype than those carrying just L997F alone.[30] When multiple mutations are detected and there is suspicion of a complex allele, testing of the parents or siblings is needed to determine which variants are inherited together.

In general, genetic tests designed to identify the presence of one of a defined panel of *CFTR* mutations can be designed to be highly specific as they test for only *CFTR* variants that are known to cause CF. The sensitivities of these panels depend on how well the selected variants reflect the population being tested. Sequencing (and deletion/duplication testing) increases the sensitivity of the assay, but it is less specific because it may identify a *CFTR* variant that is uncharacterized or one that is known to be associated with varying clinical consequences. No genetic testing is completely infallible, and clinical suspicion should always take precedence.

Example: Extended Genetic Testing as Part of NBS in California

Because of the racial diversity in California, traditional NBS algorithms that rely on standardized panels of *CFTR* mutations were not sensitive enough to meet state NBS standards. In 2007, California initiated a CF NBS program that uses a 3-tiered approach: (1) a high immunoreactive trypsinogen level in a newborn's dried bloodspot; (2) which triggers use of a panel of 40 mutations selected from the mutations found in local CF clinics; (3) and then *CFTR* sequencing is performed for newborns with only 1 mutation identified from the California panel.[31] A consequence of this approach has been the detection of MVCC and (especially in the Hispanic community) previously unrecognized *CFTR* mutations that have not yet been classified.[32] Thus, the program has identified large numbers of infants with a positive newborn screen who do not fulfill current diagnostic criteria for CF, and who have mutations with as-yet-unknown likelihood of causing disease.

Of the 2 124 050 infants screened in the period from 2004 to 2011, 174 infants were diagnosed with CF on the basis of 2 panel mutations. Another 674 infants displayed 1 California panel mutation, with a second mutation found through *CFTR* sequencing. In 98 of these individuals, the genotype was determined to be non-CF-causing. In follow-up, these infants had no signs or symptoms related to CF, and none of them presented with high sweat chlorides or pancreatic insufficiency by 2 to 6 years of age.[33] In 60 infants, the mutation identified by sequencing was known to be CF-causing, and these individuals were diagnosed with CF. The remaining infants were grouped for further study according to the predicted effects of the second mutation: MVCC (n = 78), mutations of unknown significance (UNK; n = 244), and intron 9 5T variants (splicing efficiency mutation, n = 194).

In 2-4 years of follow-up, a small but significant percentage of children from the MVCC (4/78, 5%) and UNK (27/244, 11%) groups had been diagnosed with CF because of either having sweat chloride level ≥60 mmol/L or developing pancreatic insufficiency. These data highlight the challenge of uncharacterized mutations in screening: some unknown or uncharacterized mutations may cause CF, and others have evidence of being benign. The 5T variants are under analysis.

These outcomes highlight the potential benefit and challenges of using *CFTR* sequencing in NBS, which has expanded knowledge of *CFTR* variants in populations in which CF is uncommon but under-recognized. Sequencing will identify mutations that have no disease annotation or are associated with varying clinical consequences (MVCC). When used in screening, this will result in large numbers of patients with CRMS/CFSPID (Another article in the Supplement provides additional information on this topic.[34]) Because some children may develop different CF symptoms over the course of their lifetime, this presents the opportunity to detect disease before it occurs. However, the added sensitivity is countered by the distinct challenges resulting from the greater need for CF clinics to follow more children, and the potential consequences of over-labeling or "medicalizing" these asymptomatic children is not known. Because the prognosis for children in the MVCC and UNK groups described above is still not clear, further study is needed to better recognize features that will predict disease (or lack thereof) over the lifetime. Until this is done, sequencing is not a universal solution to establishing a CF diagnosis.

Discussion

The majority of individuals with CF can be diagnosed readily with sweat testing and with *CFTR* genetic analysis showing well-recognized CF-causing mutations. Genetic analysis can be difficult to interpret if mutations identified are rare and uncharacterized or are known to be associated with varying clinical consequences. The CFTR2 project has characterized mutations using clinical, functional, and population/penetrance criteria. Although in the final analysis the clinician's judgment must prevail, the genotypic information provided to the clinician is increasingly helpful, both in making the diagnosis and in choice of life-changing therapies that are becoming available for many individuals with CF. ∎

Author Disclosures

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Appendix

Mutation Terminology

Both the terms "mutation" and "variant" are commonly used to describe a genetic difference from what is typically seen in a population. These terms have different meanings in some contexts ("mutation" often describes a deleterious change, whereas "variant" does not) but are also frequently used interchangeably. To prevent confusion and misinterpretation, multiple groups now recommend the use of the terms "variant" or "sequence alteration" to describe a DNA change, therefore, avoiding the negative connotation of the term "mutation." However, most clinicians are more familiar and comfortable with "mutation"; thus, for the purposes of this report, this term will be used to describe any DNA change. Additional descriptors such as "CF-causing mutation" or "mutation of unknown significance" will be applied when known.

The *CFTR* gene was discovered before there was an attempt to standardize gene numbering and mutation nomenclature across all genes; thus, the original numbering and mutation naming system became outdated once the Human Genome Variation Society recommended standardizing these practices.[8] The CF community had already adopted a "legacy" numbering and nomenclature (eg, F508del, G551D, or 3849+10kbC->T). However, genetic testing laboratories generally report mutations using standardized Human Genome Variation Society terminology (F508del = c.1521_1523delCTT, G551D = p.Gly551Asp, or c.1652G>A, and 3849+10kbC->T = c.3717+12191C>T). The "c" refers to coding DNA nucleotide number, and the "p" refers to amino acid number. Although this can create confusion, resources such as CFTR2[20] and CFMD[8] can be consulted to translate mutations into different naming conventions.

Diagnosis of Cystic Fibrosis in Screened Populations

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Objective Cystic fibrosis (CF) can be difficult to diagnose, even when newborn screening (NBS) tests yield positive results. This challenge is exacerbated by the multitude of NBS protocols, misunderstandings about screening vs diagnostic tests, and the lack of guidelines for presumptive diagnoses. There is also confusion regarding the designation of age at diagnosis.

Study design To improve diagnosis and achieve standardization in definitions worldwide, the CF Foundation convened a committee of 32 experts with a mission to develop clear and actionable consensus guidelines on diagnosis of CF with an emphasis on screened populations, especially the newborn population. A comprehensive literature review was performed with emphasis on relevant articles published during the past decade.

Results After reviewing the common screening protocols and outcome scenarios, 14 of 27 consensus statements were drafted that apply to screened populations. These were approved by 80% or more of the participants.

Conclusions It is recommended that all diagnoses be established by demonstrating dysfunction of the CF transmembrane conductance regulator (CFTR) channel, initially with a sweat chloride test and, when needed, potentially with newer methods assessing membrane transport directly, such as intestinal current measurements. Even in babies with 2 CF-causing mutations detected via NBS, diagnosis must be confirmed by demonstrating CFTR dysfunction. The committee also recommends that the latest classifications identified in the Clinical and Functional Translation of CFTR project [http://www.cftr2.org/index.php] should be used to aid with CF diagnosis. Finally, to avoid delays in treatment, we provide guidelines for presumptive diagnoses and recommend how to determine the age of diagnosis. *(J Pediatr 2017;181S:S33-44).*

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disease in the US, occurring in approximately 1 in 4000 newborns.[1-3] Since 1989, it has become well known that CF is an ion channel disorder caused by mutations in the gene for the CF transmembrane conductance regulator (CFTR).[4] There are more than 2000 mutations identified to date,[5] approximately 10%-15% of which have so far been confirmed to be CF-causing alleles.[6] There has been a surprising degree of difficulty encountered worldwide in establishing the diagnosis in a minority of cases and because of this, healthcare providers continue to be faced with uncertain cases and challenging diagnostic dilemmas. Although the diagnosis of CF has traditionally relied on recognition of characteristic clinical signs and symptoms, the increased use of prenatal population screening for maternal CF carrier status, prenatal ultrasound screening (that

CF	Cystic fibrosis
CFFPR	CF Foundation Patient Registry
CFSPID	CF screen positive, inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
CRMS	CFTR-related metabolic syndrome
FE	Fecal elastase
ICM	Intestinal current measurement
IRT	Immunoreactive trypsinogen
NBS	Newborn screening
NPD	Nasal potential difference
PAP	Pancreatitis-associated protein
PFT	Pulmonary function test
VHIRT	Very high IRT

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might reveal meconium ileus, meconium peritonitis, bowel obstruction, or echogenic bowel), and newborn screening (NBS) has resulted in the routine diagnosis of asymptomatic or minimally symptomatic infants and a consequent opportunity to foster their normal growth and development. Since 2010 when nationwide CF NBS began in the US because of endorsements by the US Centers for Disease Control[7] and the CF Foundation,[8] the proportion of newly diagnosed patients identified through screening has progressively increased. In fact, in the US, approximately 64% of new CF diagnoses now follow positive NBS.

According to consensus guidelines developed by the CF Foundation in 2007 and published in *The Journal* in 2008,[9] individuals identified by NBS can be diagnosed with CF by a sweat chloride value ≥60 mmol/L, or a level of 30-59 mmol/L if they have 2 CF-causing mutations in the *CFTR* gene. Although the vast majority of screened infants can be unequivocally diagnosed with CF by high levels of sweat chloride following a positive newborn screen,[9,10] the decision is not clear-cut in a significant number of individuals.[11-13] Unclear diagnoses lead to treatment delays, persistent challenges,[14] and stress and confusion for both families[15,16] and clinicians.[17] This group of infants, with varying levels of symptoms and a variety of *CFTR* mutations, has been the focus of discussions in the US and in Europe, with somewhat differing conclusions on both diagnosis and management.[18,19] In addition, there has been a lack of international harmony regarding terminology, leading to confusion reflected in a recent article, entitled "Comparing the American and European diagnostic guidelines for cystic fibrosis: same disease, different language?"[20]

Although treatment advances over the past several decades have raised the median predicted survival age from the midteens in the 1970s to more than 40 years of age today in the US[21] and many countries in Europe,[22,23] and more than 50 years in Canada[24] and in addition new CFTR modulator therapies offer great promise,[25] achieving optimal outcomes for all ages depends on timely and accurate diagnosis.[26,27] Continued improvement in predicted survival requires careful attention to diagnostic recommendations. Despite efforts to reach and sustain a consensus on diagnostic criteria, however, it has become increasingly clear during the past few years that CF Foundation guidelines published in 2008 are not being used consistently and are considered obsolete by many clinicians.[14]

During the process of developing the 2008 guidelines, it was recognized that CF NBS introduced a new complexity and diagnostic dilemma, namely infants with abnormal screening tests because of elevated immunoreactive trypsinogen (IRT) levels but inconclusive sweat tests and/or DNA results. Some infants with a high IRT, for example, can display an initial sweat chloride level below the lowest accepted value for a potential CF diagnosis (30 mmol/L), even in the presence of 2 CF-causing mutations.[12,28] More common, however, are infants with high IRT levels and sweat chloride levels below CF diagnostic levels who have fewer than 2 CF-causing mutations.[12] This latter scenario has led to a new diagnostic term and management guidelines, published in *The Journal*,[19] in an article that created the term CFTR-related metabolic syndrome (CRMS).

In an effort to resolve the current diagnostic challenges following a positive CF NBS result, participants in the 2015 Diagnosis Consensus Conference included the following objectives in their mission: to develop revised guidelines for NBS-linked diagnosis, as well as for babies born after positive prenatal testing (ie, positive fetal diagnostic testing, including sweat test requirements and use of genetic data). Consensus recommendation statements that apply to the screened population, developed as a result of this conference[29] are presented in **Table I**.

The Many Potential Meanings of a Positive CF NBS Test

A positive CF newborn screen is a result that demands prompt follow-up to identify infants with CF. However, CF NBS programs vary considerably in design, and the type of NBS algorithm used to produce a positive screening result affects the positive predictive value, follow-up, and diagnostic processes.

All CF NBS programs begin with detection of a high IRT level in a dried blood specimen from the newborn. In the US, this is routinely followed either by a second IRT measurement (IRT/IRT) or by use of a variety of *CFTR* mutation panels (usually 23-40 mutations[30]) (IRT/DNA). IRT/IRT is used following approximately 10% of all US births, but its use is declining, because of lower sensitivity,[31] delayed completion,[32] and higher false-negative rate[33] compared with IRT/DNA NBS algorithms. A variation of the IRT/DNA method, called IRT/IRT/DNA, requires the demonstration of persistent hypertrypsinogenemia for 1-2 weeks before DNA is analyzed.[34] The time to diagnosis may be longer than in IRT/DNA programs, but a study suggests the IRT/IRT/DNA screen is more sensitive and detects fewer carriers.[34]

Once a positive CF NBS result has been found, sweat chloride testing must be performed to establish a CF diagnosis (**Table I**, statement 3). Some CF NBS programs in the US that use IRT/IRT have added sweat testing, combined selectively with DNA analysis, for follow-up to the biomarker screening. However, requiring sweat testing of all infants with positive IRT/IRT tests can be logistically problematic, such as when the infant does not live close to an accredited sweat test facility. Performing a sweat chloride test in infants receiving neonatal intensive care, who are more likely to have high IRT values because of nonspecific pancreatic stress,[35] can also be challenging, either because they are preterm or <2 kg in weight (**Table I**, statement 2), are on supplemental oxygen, or cannot leave the intensive care unit for the test. In these cases, *CFTR* mutation analysis can play a role in the initial evaluation even in CF NBS programs that measure biomarkers alone.

Most US CF NBS programs now include some form of DNA analysis in a second or third tier of screening.[36] The type of analysis performed depends on state laws and demographics of the population being screened,[37] but usually involves a panel of 23-40 of the most common CF-causing mutations. Some CF NBS programs subject the DNA to a more comprehensive genetic analysis.[38-40] Although a more detailed analysis can improve the detection of CF in nonwhite populations,[41] it can

Table I. 2015 CF Foundation diagnosis consensus conference recommendations related to diagnosis of CF in the screened population*

Statement numbers*	Consensus statements
2	Newborns with a positive CF newborn screen, to increase the likelihood of collecting an adequate sweat specimen, should have the test performed bilaterally and when the infant weighs >2 kg, and is at least 36 wk of corrected gestational age.
3	Newborns greater than 36 wk gestation and >2 kg body weight with a positive CF newborn screen, or positive prenatal genetic test, should have sweat chloride testing performed as soon as possible after 10 d of age, ideally by the end of the neonatal period (4 wk of age).
4	In infants with presumptive CF identified through NBS, CF treatment should not be delayed while efforts to establish a diagnosis of CF are initiated.
6	In individuals presenting with a positive newborn screen, clinical features consistent with CF, or a positive family history, a diagnosis of CF can be made if the sweat chloride value is ≥60 mmol/L.
7	Individuals who are screen-positive and meet sweat chloride criteria for CF diagnosis should undergo *CFTR* genetic testing if the *CFTR* genotype was not available through the screening process or is incomplete.
8	In individuals with a positive newborn screen, a sweat chloride <30 mmol/L indicates that CF is unlikely.
10	Individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range (30-59 mmol/L) on two separate occasions may have CF. They should be considered for extended *CFTR* gene analysis and/or CFTR functional analysis.
12	In individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, the identification of 2 CF-causing mutations (defined by CFTR2) is consistent with a diagnosis of CF. Sweat chloride testing is necessary, though, to confirm the diagnosis.
13	The absence of detection of 2 CF-causing *CFTR* mutations does not exclude a diagnosis of CF.
14	If further CF functional testing is needed (NPD and ICM), it should be performed in a validated reference center with trained staff certified by the CF Foundation TDN or ECFS Clinical Trial Network.
15	In individuals with a positive newborn screen but variable or uncharacterized *CFTR* mutations (<2 CF-causing mutations), the diagnosis of CF can be made by demonstrating CFTR dysfunction (a sweat chloride ≥60 mmol/L or CF-typical NPD or ICM).
18	The definition of CRMS/CFSPID is an infant with a positive NBS test for CF and either: • A sweat chloride <30 mmol/L and 2 *CFTR* mutations, at least 1 of which has unclear phenotypic consequences *OR* • An intermediate sweat chloride value (30-59 mmol/L) and 1 or 0 CF-causing mutations
19	Children designated as CRMS/CFSPID should undergo at least 1 repeat sweat chloride test at CF centers with suitable expertise, such as an accredited CF center.
20	Children designated as CRMS/CFSPID should have clinical evaluation performed by CF providers to identify the minority that may develop clinical symptoms.

CTN, clinical trial network; *ECFS*, European CF Society; *TDN*, therapeutics development network.
*Adapted from Farrell et al.[29]

also result in the detection of many more infants with unclear diagnostic results.[13]

An approach taken by some US CF NBS programs to improve sensitivity is the institution of a "safety net." (The term "failsafe" is also used,[36] although it must be cautioned that false-negative screening tests will still occur.) Safety net design differs between programs. In CF NBS programs using DNA analysis as a second tier of screening, if the DNA suggests CF, the infant is referred for diagnostic confirmation by sweat test. However, even if the second-tier DNA screen does not detect a panel mutation, infants with very high IRT (VHIRT) values may still be referred for diagnostic sweat chloride testing. These CF NBS programs include algorithms such as IRT/DNA/VHIRT,[42] IRT/IRT1↑/DNA,[43] or IRT/DNA/IRT.[44] In all cases, sensitivity and specificity of algorithms using VHIRT must be evaluated, as has been done in New York.[42]

The use of VHIRT as a safety net also has been used elsewhere, including France[45] and the United Kingdom.[46] In the United Kingdom, the national NBS program uses a safety net for infants with a VHIRT value but no mutations identified on a limited mutation panel. The United Kingdom approach is to undertake a repeat IRT measurement on a sample obtained on day 21 of life. If the IRT value remains elevated at this stage, the NBS is reported as positive, and the infant referred for diagnostic assessment. This safety net approach has been useful for identifying infants with CF from a diverse ethnic population but at the expense of reducing positive predictive value. The adoption of various safety net algorithms is being considered by at least 9 Euro-

pean nations (Olaf Sommerburg, personal communication, October 2015).

Because of diverse political and demographic circumstances, there are at least 32 separate CF NBS programs in Europe, using a host of different screening algorithms.[47-53] While still using IRT as the first tier of screening, some European programs have incorporated a second biomarker, pancreatitis-associated protein (PAP), into their CF NBS algorithms. Although issues have surfaced regarding PAP cut-off values,[47,52,54] there are significant advantages of adding PAP analysis as a second tier of screening, including decreased recognition of carriers.[55,56] However, using PAP analysis may also result in lower sensitivity. Methods to enhance sensitivity depend on the algorithm being used. While in France, Sarles et al[52] decreased the PAP cut-off values recently to reach sufficient sensitivity, programs in Germany incorporated a failsafe strategy in which not only infants with high IRT and high PAP are referred for sweat testing, but also infants with low PAP values are referred for sweat testing if they display ultrahigh IRT levels (IRT/PAP-failsafe).[48] Pure biochemical IRT/PAP protocols nevertheless show a poor positive predictive value. More than two-thirds of all European CF screening programs use DNA, in accordance with the European CF Society recommendations,[57] Programs can still benefit from the use of PAP when combined with genetic analysis as a third tier as demonstrated by Vernooij-van Langen et al[51] in 2012. Those protocols show not only sufficient sensitivity but also a positive predictive value comparable to that of IRT/DNA programs. Because of this experience, IRT/PAP/DNA protocols will be implemented as a

national screening program from 2016 and 2017 in Germany and in France, respectively. In a post hoc analysis, the effect of an ultrahigh-IRT safety net was demonstrated for the German IRT/PAP/DNA CF NBS program (Olaf Sommerburg, personal communication, October 2015). In this NBS program, a high IRT leads to PAP analysis as the second-tier screen, and elevated PAP leads to the third-tier screen, that is, analysis for 31 CF-causing mutations. Data obtained from 372 906 neonates screened from 2008 to 2015 in southwest Germany were used to compare the potential impact of an ultrahigh IRT safety net that could trigger either sweat testing or DNA analysis in the absence of an elevated PAP level. If ultrahigh IRT triggered sweat testing, approximately 345 infants would undergo this diagnostic procedure. If ultrahigh IRT led instead to required DNA analysis, only 79 infants would subsequently undergo sweat testing. In either case, 71 infants with CF would be detected.

The diversity of CF NBS algorithms leads inevitably to a spectrum of risk for CF subsequent to a positive screen result. The likelihood of a "positive CF newborn screen" resulting in a diagnosis of CF can vary hugely, from close to 100% (as may occur if 2 CF-causing mutations are identified) to around 1% (as may occur in infants with positive NBS results because of VHIRT[42]). Some infants present for follow-up without any supporting genetic information, whereas others may have had extensive genetic analysis performed. Regardless of the algorithm used, it must be emphasized that CF NBS is not a diagnostic test, and whether or not the baby has CF must be determined in follow-up care by diagnostic testing. As stated above, the essential component of this determination is the sweat test; the identification of a physiological abnormality not only supports the positive NBS result but may also help the family accept the diagnosis.[58] Clearly, the CF diagnosis is a serious one, and the sweat test provides an important safeguard to avoid mislabeling babies because of identity errors, or laboratory errors in IRT or DNA analysis.[59,60] Furthermore, the sweat test will be performed on siblings,[61] and comparison data can be invaluable. Even though there may appear to be less need of a sweat test in the presence of meconium ileus because meconium ileus provides obvious evidence of a physiological defect, a sweat test revealing elevated chloride should still be the criterion to confirm the diagnosis. There have been many instances where a neonatologist or surgeon did not properly inform the parents of an infant with meconium ileus about the high probability of CF, leading them to unrealistic expectations.

Thus, the next step in the follow-up of a positive NBS or prenatal test that suggests CF must be determination of the sweat chloride concentration. However, its interpretation and any additional tests needed to further explore the possibility of the CF diagnosis depend on the NBS algorithm used.

Confirming CF Diagnosis after Positive Newborn Screen without Detection of CF-Causing Mutations

Some positive results from a CF newborn screen do not include a DNA screen (for example, those using an IRT/IRT screen);

in this case, a sweat chloride test is directed, and the nature of the follow-up is determined by the chloride levels found (Table I, statements 6, 8, 10). If the sweat chloride level is ≥60 mmol/L, the infant has CF and CFTR genetic testing should be done (Table I, statement 7).

In most cases, if no CF-causing[6] mutations are found in a CF NBS program that includes DNA analysis, the infant would be considered screen-negative. A safety net (such as very high or ultrahigh IRT), however, could be triggered to direct a sweat chloride test, with follow-up again determined by the chloride levels found (Table I, statements 6, 8, 10).

Confirming CF Diagnosis after Positive Newborn IRT/DNA Screen with Detection of 2 CF-Causing Mutations

Even with the identification of 2 CF-causing mutations (Table I, statement 11), the next step in a diagnostic workup must be sweat chloride analysis (Table I, statement 12). Regardless of increased understanding of CFTR genetics, experts continue to emphasize the need for proof of CFTR dysfunction to complete the CF diagnosis.[14,62] Some CF NBS programs demonstrate very high adherence; in France, for example, sweat test results are reported for up to 95.4% of infants with 2 CF-causing mutations (excluding those with meconium ileus),[63] but the same is not true for all CF NBS programs.[58] A review of diagnostic practices in the European Union showed that only 13 of 26 CF NBS programs reported routinely including sweat testing for infants with 2 CF-causing mutations.[58] Four programs never conducted sweat testing in these infants, whereas it was sometimes conducted in 6 other programs. Analysis of data in the US CF Foundation Patient Registry (CFFPR) suggests a similar lack of adherence to this guideline: nearly 24% of US patients with 2 CF-causing mutations do not have associated sweat chloride results.[11] Although the policy of a CF NBS program may be to recommend sweat chloride confirmation, the responsibility for performing the test resides with the primary care provider and the CF clinician assuming care. If the decision is made by the diagnostician to assign a diagnosis of CF without sweat chloride testing, the CF NBS program is left without these data, as are the CF patient registries.

Improving adherence may require a better understanding of the potential challenges in sweat testing young infants and improved performance of sweat test procedures: (1) the sweat test itself remains challenging because of higher rates of insufficient sweat (quantity not sufficient) in neonates; (2) the diagnosis of CF already seems confirmed to some parents when they arrive with the baby at a CF center; (3) the presence of intermediate sweat chloride levels in babies with 2 CF-causing mutations can cause confusion in the family and primary care providers; (4) it may be difficult to order a sweat test if it has been postponed and a CF diagnosis has been presumptively made and recorded in a medical chart; (5) sweat test results do not impact follow-up modalities in these infants; (6) sweat test results, unlike genetic

analysis, do not provide utility for personalized medicine; (7) because of the high costs of analytic devices, sweat chloride testing often is not performed in countries that have limited resources or consider CF rare; and (8) reimbursement for sweat testing may be problematic; in many areas of the world, including some of the US, the sweat test is not part of NBS funding, and health insurance companies may not pay for the test, and certainly not for a repeat sweat test, if they believe it is unnecessary.

Despite these issues, it is clear that sweat tests can be performed successfully in most infants during the first month of life.[44,64] Rock and Farrell[64] reported that patients diagnosed in Wisconsin following positive CF NBS from 2004 to 2014 had sweat testing performed at a mean age of 21 days (SD of 16 days) and a median age of 16 days. There were 2 infants whose sweat testing was delayed, which skewed the data distribution (an infant who had meconium ileus with surgery and prolonged hospitalization, with sweat test at 55 days of age, and an infant born prematurely at 34 weeks' gestation, who was identified as a homozygous c.1521_1523delCTT [legacy: F508del] patient with birth weight of 2126 g and whose sweat test was delayed until 100 days of age to ensure a sufficient quantity of sweat). This study used the Gibson-Cooke method. Other data show similar, if not better results regarding adequate sweat quantity with the Macroduct collector (Wescor Inc, Logan, Utah).[65]

It is important to explore ways to improve adherence. Requiring sweat test results for entry into the European and US CF Foundation data registries might emphasize the need for the information for diagnosis. Thus, beginning in 2017, the CF Foundation will require that a sweat chloride value be entered for enrollment of a newly diagnosed patients in the CF registry.

In addition to the sweat test, *CFTR* gene analysis on DNA obtained directly from the infant should ideally be performed as part of the diagnostic evaluation, even if a genotype was reported as part of the newborn screen (**Table I**, statement 2). This recommendation is new but is appropriate in the expanding era of CFTR modulator therapy in which the genotype must be known unequivocally. Although this group of infants has 2 reported CF-causing mutations, some *CFTR* mutations (such as c.3717 + 12191 C→T [legacy: 3849 + 10kB C→T] and c.3454G>C [legacy: D1152H]) are known to result in an increased probability of a sweat chloride level well below 60 mmol/L, even into the "normal" range, in individuals with CF.[66-70] In the case of certain other mutations, a more in-depth genetic analysis may be useful. For example, if the c.350G>A mutation (legacy: R117H) is identified, exploration of the polyT status and possibly TG repeats is essential because of their effects on both function and penetrance.[71-73] Such in-depth genetic analysis is not always done as part of the diagnostic evaluation following CF NBS. However, because the polyT tract is highly significant in individuals with R117H, it should be added to the diagnostic evaluation to better identify, early on, infants with CF vs those who should be categorized as CRMS/CF screen positive, inconclusive diagnosis (CFSPID), with a risk of converting

to a CF diagnosis[74] (**Appendix**, case study 1; available at www.jpeds.com). There are also uncommon instances of 2 CF-causing mutations occurring in cis[60,75]; in this scenario, the sweat test would be normal and additional genetic analysis including parental testing could explain the result and prevent medicalization of this healthy infant.

Confirming CF Diagnosis after Positive Newborn Screen with Detection of 1 CF-Causing Mutation

Not all infants with CF will have 2 CF-causing mutations detected (**Table I**, statement 13). Because of the lack of clarity on the disease liability of various *CFTR* mutations, the sweat test is an especially crucial part of the diagnostic algorithm for this group of infants (**Table I**, statements 10, 15), but interpretations can be difficult. The mutation effects are not always clear-cut because of the presence of modifier genes, or environmental or epigenetic influences: the same mutations may be associated with CF in some patients, but with CRMS/CFSPID in others.[18]

In general, in this group of infants, a sweat chloride level ≥60 mmol/L is clearly indicative of CF (**Table I**, statement 15) and a sweat chloride level <30 mmol/L indicates CF is unlikely (**Table I**, statement 8). A sweat chloride level of 30-59 mmol/L, however, should lead to a second sweat test (**Table I**, statement 19). Often, this second sweat test will produce resolution of the intermediate screening test result, with a decrease to <30 mmol/L resulting in discharge from the program as healthy, or an increase to ≥60 mmol/L as diagnostic of CF. However, in some instances the sweat chloride levels remain intermediate and inconclusive. In this scenario (1 detected CF-causing mutation), extended genotyping, clinical evaluation by a CF specialist by 2 months of age, and another sweat chloride test repeated by 6 months of age are recommended to seek resolution.[18,19] Asymptomatic infants who continue to display intermediate sweat chloride levels (30-59 mmol/L) and whose genetic analysis does not provide clarity (<2 CF-causing mutations) should be categorized as CRMS/CFSPID and followed at a CF care center (**Table I**, statements 18 and 20). (More details on frequency of CRMS/CFSPID, appropriate diagnostic evaluation, and outcomes can be found in the report by Ren et al.[74]) Some CF NBS programs may use nasal potential difference (NPD) or other tests to clarify CFTR physiological dysfunction, particularly if insufficient sweat can be collected for analysis.

Although it is appropriate from the perspective of both physician and patient to label newborns who screen positive with intermediate sweat chloride values and <2 CF-causing mutations as CRMS/CFSPID, when patients display a clear history of CF-like lung disease plus intermediate sweat chloride and abnormal NPD/intestinal current measurement (ICM), they need CF management and must be diagnosed with CF.

Infants whose newborn screen has identified 2 *CFTR* mutations with ≤1 known to be CF-causing and who display

normal sweat chloride levels should also be categorized as CRMS/CFSPID and followed (Table I, statement 18).

Infants who are screen positive with 1 CF-causing mutation but who produce insufficient sweat for analysis should be retested as described in Farrell et al[29] (Appendix, case study 2).

Additional Tests

A small percentage of infants who are eventually diagnosed with CF do not meet definitive CF diagnostic criteria at the time of their first evaluation following a positive CF NBS result. Typically, in this group, sweat chloride levels do not provide the information needed to properly identify CF. Extended genotype (or any genotype) results may not be available initially, and even with the substantial progress of the past decade, will not be able to resolve all cases because of extremely rare alleles with insufficient clinical data or partially penetrant alleles with variable clinical consequences that cannot be predicted. Thus, we must turn to other possible biomarkers to provide an understanding of the level of CF risk for these families as well as appropriate care for the patients. Embracing a wide array of biomarkers may help clinicians face the challenge of defining the risk of CF for children with inconclusive genetic data. Gathering information about these biomarkers in early childhood would help evaluate the penetrance of CFTR variants and describe the full spectrum of disorders associated with CFTR mutations, extending our knowledge about how CFTR variants (and other genes) contribute to disease beyond our current understanding of CF.

Fecal Elastase (FE)
Demonstration of low FE levels <200 μg/g (in the absence of diarrhea) has been proposed as an indicator of pancreatic insufficiency and a diagnostic marker for CF. FE values fluctuate through the first 12 months of life. In a study[76] of 61 infants diagnosed with CF through NBS, 48 infants (79%) had initial FE <200 μg/g; 13 of these 48 infants (27%) had a least 1 FE value >200 μg/g over the next several months before resolving into levels <200 μg/g, and 4 of 48 infants (8%), on the other hand, displayed pancreatic-sufficient levels >200 μg/g by >9 months of age. In addition, 13 infants (21%) had initial FE >200 μg/g; 10 of these (77%) had pancreatic sufficiency at the end of the first year of life.

FE may be useful as an interim measure in those infants with pancreatic insufficiency who have "quantity not sufficient" sweat test results, permitting appropriate treatment until repeat sweat testing is successful. This strategy has been used in Switzerland.[77] However, despite the early enthusiasm for this biomarker,[78,79] FE is of limited value in diagnosing CF definitively, as many individuals with CF retain normal levels of FE.[76,80] It also has been disappointing when used to try to determine which infants, categorized as CFSPID from a newborn screen, will eventually be diagnosed with CF.[81] When FE levels were measured over the first 24 months of life in a cohort of 36 infants with CFSPID, all but one remained >200 μg/g, and none of

the babies later diagnosed with CF displayed low FE levels, remaining pancreatic sufficient (Tanja Gonska, personal communication, October 2015).

Trypsinogen
Trypsinogen levels are already used to identify infants at high risk for CF and may be used to better advantage. Serum trypsinogen levels were serially examined over the first 36 months of life in 82 infants categorized as CFSPID and 80 infants diagnosed with CF.[81] Overall, infants with CFSPID had significantly lower NBS IRT than did infants with CF. Furthermore, nine of the 82 (11%) infants with CFSPID were subsequently diagnosed with CF, and these patients had significantly higher serial serum trypsinogen levels than did those infants who remained in the group with CFSPID. Thus, serial trypsinogen levels may contribute useful information.

NPD and ICM
As in the 1996-1998 and 2007-2008 CF Foundation consensus development processes, the overwhelming importance of demonstrating CFTR dysfunction to confirm a CF diagnosis, combined with the limitations of sweat tests, creates great appeal for other CFTR functional assays. This is especially true for those assays providing added value from an in vivo strategy and a drug responsiveness component that increases sensitivity.

Measurements of CFTR (and the epithelial sodium channel) activity in nasal epithelium readily distinguish the healthy young infant from one with CF. In fact, NPD, when attempted in babies with intermediate sweat chloride levels by very experienced, skilled operators, can provide reliable results. In 1 study of 11 children (aged 3.5 ± 1.5 years) with nondiagnostic sweat chloride values, NPD testing was able to demonstrate normal CFTR function in 7 of the children; only 1 showed NPD indicative of pathology (the test could not be completed in 3 children because of poor cooperation) (Michael Wilschanski, personal communication, October 2015). Another study of NPD conducted in 23 young children (aged 3 months to 4 years) with high IRT values, 1 CF-causing mutation identified and intermediate sweat chloride levels demonstrated a connection between NPD results and clinical outcome.[82] Although NPD results were not interpretable in 2 of the children, 13 of the 21 remaining children (62%) had NPD scores in the CF range; 2 CFTR mutations were subsequently found in all 13 patients, and 9 of the 13 had developed chronic lung disease at follow-up. Of the 8 children (38%) with normal NPD scores, only 2 children had 2 CFTR mutations (both of which are associated with a wide spectrum of phenotypes), and none had developed a CF-like lung disease at follow-up. Repeated sweat test results were obtained in 5 of these 8 children; all had sweat chloride values <60 mmol/L. A CF diagnosis was ruled out in 6 of the 8 children.

However, NPD is not possible or reliable in every situation, and analysis of CFTR function in the intestine (ICM) may be considered, aided by the fact that CFTR is highly expressed in intestinal epithelia, offering high specificity and sensitivity for the test.[83,84] Like NPD, ICM measurements must be

conducted in specific high quality reference centers with experienced, very skilled personnel.[85,86] ICM can be used to confirm a diagnosis of CF in the context of intermediate sweat chloride levels.[87,88] Ion transport in the intestine is a very sensitive measure of CFTR function: only 10% of wild-type CFTR is necessary to prevent intestinal pathology in CF, and a very small gain in *CFTR* expression (from 1% to 5% of wild-type) results in large gains in chloride secretion (from 5% to 25% of wild-type levels).[83] Because of this sensitivity, ICM can be used to better characterize variants of unknown disease liability.

Combining results from ICM and NPD, when available, can provide an even clearer picture of the spectrum of CFTR function, from CF-causing to healthy levels (Isabelle Sermet-Gaudelus, personal communication, October 2015). If the ICM is normal, other pathologies, such as immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, should be considered.

NPD and ICM have the potential to be useful as surrogate outcomes because they are in vivo/ex vivo measurements and may play a useful supplementary role for the diagnosis of CF (**Table I**, statement 15). However, they are not sufficiently validated at present to recommend routine use and are not available on a wide scale because of the expertise and experience required to obtain reliable measurements (**Table I**, statement 14). Nevertheless, these are increasingly attractive, advanced research methods that will help us better understand the nature of the spectrum of CF disease and CRMS/CFSPID. And, with more routine use in European CF centers, greater application will undoubtedly occur.

Despite these advances, some infants still provide a diagnostic challenge. Thus, long-term follow-up evaluations of children with various levels of CFTR function are needed to determine the best approach for diagnosis and management of children without the signs and symptoms of established, unequivocal CF. The best approach may involve sensitive functional tests such as NPD and ICM tests in combination with genotype-phenotype studies (such as correlation with the CFTR2 database[6]) and data collected from CF registries.

Clinical/Respiratory Evaluations in Uncertain Cases

Clinical evaluations for respiratory pathology are of little value in cases of uncertain CF during infancy. Signs and symptoms are unlikely and nonspecific, although chronic cough should raise suspicion. An infant pulmonary function test (PFT) that demonstrates gas trapping or reduced forced expiratory flows could be of value, but the measurement is not standardized, the capability of performing an infant PFT is not widely available, and other obstructive lung problems (such as wheezing) can produce similar PFT results.[89] Lung clearance index using the SF_6 method for infants is sensitive, but again, not widely available (N_2 washout is not currently developed for infants).[90-92] Chest radiographs are worthwhile, but the changes are often subtle and nonspecific. Chest computed tomography scans performed in infants diagnosed with CF following NBS through the Australian Respiratory Early Surveillance Team for CF in-

tensive surveillance program revealed 29.3% with bronchiectasis by 3 months of age,[93] although findings differed in a United Kingdom cohort.[94] This finding is in keeping with earlier observations from autopsy-based studies.[95] However, a study of chest computed tomography in asymptomatic infants with CF suggested that this imaging is usually not warranted because the changes during infancy are typically very mild.[94] Thus, in uncertain cases, the radiation burden and risks of anesthesia are unlikely to be balanced by benefit.

Induced sputum or bronchoalveolar lavage performed in children with CF aged 3 to 7 years may reveal evidence of bacteria common in CF, including *Pseudomonas aeruginosa,* *Stenotrophomonas maltophilia,* and *Staphylococcus aureus.*[96] To understand the significance of culturing CF-typical bacteria in this population, respiratory samples collected over the first 2 years of life in children with either CF (n = 23) or non-CF chronic suppurative lung disease (n = 124) were compared by retrospective analysis (Hannah Blau, personal communication, October 2015). At the time of the first culture, there was no significant difference between the bacterial species cultured from children with CF compared with those cultured from children with other lung diseases. However, when all sputum culture results from the first 2 years were analyzed, cultures from children with CF were significantly more likely to contain *P aeruginosa*, *Enterobacter* species, *Escherichia coli*, *Klebsiella oxytoca*, and *Serratia* species than were control cultures. *P aeruginosa*-positive cultures were found in 32/124 (about 26%) of the children with non-CF lung disease compared with 17/23 (74%) children with CF, although chronic *P aeruginosa* infection was rare in either case. The conclusion from this retrospective analysis is that cultures from induced sputum may provide a useful disease marker in the infant with suspected CF.

Consensus Statements on Diagnosing CF in Screened Populations

Taking into account the available evidence, the 2015 CF Foundation Consensus Committee agreed upon the statements shown in **Table I** for diagnosing screened populations. Sweat tests, *CFTR* mutation analysis, and the ancillary tests described above may all prove valuable for establishing or confirming a CF diagnosis in children with positive prenatal testing or neonatal screening. The European CF Society Standards of Care state that the majority of infants with a confirmed diagnosis after NBS should be seen by the specialist CF team by 35 days of age and no later than day 58,[62] whereas the recommended US standard of care specifies diagnosis by 2-4 weeks without specifying care at a CF center.[9] Therefore, to ensure timeliness, follow-up evaluations needed to decide CF status should be completed within 2-4 weeks of age when infants are hopefully still in a preclinical stage. Even by 2 weeks, however, malnutrition may be present,[97,98] and there is a risk of potentially fatal electrolyte depletion and hyponatremic dehydration[99] (**Appendix**, case study 3). Hence, it is important in some circumstances to make a presumptive diagnosis of CF to enable

appropriate treatment and follow-up while pursuing diagnostic confirmation (Table I, statement 4).

Once a diagnosis is established, genetic counseling should be offered to families of all infants with CF or CRMS/CFSPID.[18,19,57,100] In other words, all families of CF NBS positive newborns should receive genetic counseling, whether the infant turns out to be a carrier or is truly affected. Recommendations have been published on methods of genetic counseling.[101,102]

Enrollment into the US CF Foundation Patient Registry

The US CF Foundation Patient Registry was created in 1966 to collect data on health outcomes, clinical care and demographic characteristics of people with CF who receive care at CF Foundation-accredited care centers.[103] Clear, reliable data must be entered into the CFFPR and other registries to permit quality analysis of CF NBS programs and clinical outcomes of all those with CF. Entering diagnostic information under consistent guidelines is critically important for all patients diagnosed with the disease, including those diagnosed immediately following a positive newborn screen, as well as those diagnosed at a later age, such as those with a false-negative newborn screen, or those who had a positive newborn screen and a negative initial sweat test. It is important to accurately record dates, test results, and treatment from the initial contact. Because CFFPR consent often is not obtained until the patient has been seen at the CF center for 1 or more visits, it is essential to ask families for consent as soon as possible to allow retrospective data (ie, before consent) to be entered in the registry, subject to institutional review board approval. Appropriate guidelines for data entry on consented patients diagnosed following prenatal screening or NBS are presented below.

Guidelines for Date of Confirmation of CF Diagnosis following Prenatal Testing

Prenatal testing showing 2 CF-causing *CFTR* mutations (in *trans*, as confirmed by parental testing) is generally adequate for a presumptive diagnosis of CF. In most cases, prenatal testing is done when both parents are known carriers either because of population screening with cascade testing or as a result of testing because of a positive family history. However, because of varying prenatal testing and reporting protocols, infants with prenatal diagnosis should have the date of diagnosis listed as the date of birth (ie, diagnosed at 1 day of age). In addition, infants with positive prenatal testing results should always have a sweat test performed for confirmation of the diagnosis.

Guidelines for Date of Presumptive Diagnosis in CF NBS

It is important to provide a date of presumptive diagnosis for the purpose of evaluating timeliness of diagnosis and treatment by CF centers in conjunction with NBS programs.

An infant can be presumed to have CF in any of the following clinical circumstances: (1) positive CF NBS test result reveals 2 CF-causing *CFTR* mutations; (2) CF NBS test is positive based on a specific state's algorithm, and there are clinical features consistent with CF, such as growth failure or malabsorption; or (3) infant has meconium ileus, with or without a positive CF NBS test.

In each of these cases, confirmation of diagnosis should proceed as quickly as possible, but treatment should not be delayed while awaiting diagnostic confirmation.

Guidelines for Date of Confirmation of CF Diagnosis Subsequent to NBS

A sweat test should be performed in all cases of presumptive diagnosis, as soon as possible. It may be delayed because of illness, prematurity, or, rarely, other circumstances (eg, geographic or weather-related) but should not otherwise be delayed. A CF diagnosis is considered confirmed only if: (1) sweat chloride is ≥60 mmol/L; (2) CF-causing *CFTR* mutations are identified and the sweat chloride value is ≥30 mmol/L; (3) 2 variable or uncharacterized *CFTR* mutations are identified and physiological testing such as NPD or ICM reveal CFTR dysfunction, and the sweat chloride value is ≥30 mmol/L; (4) 2 CF-causing *CFTR* mutations are identified from a blood specimen obtained directly from the affected infant during follow-up—a genotype report from the NBS program is not sufficient because of the risk of errors (parental testing may be required in some circumstances to verify that 2 mutations are in *trans*,[60] in cases where genotype and CFTR functional testing results are at variance [eg, normal sweat value and 2 *CFTR* mutations are present]); or (5) NPD or ICM values typical of CF are present (CF-typical values must be defined by an experienced center performing the test).

Guidelines for Date of Confirmation of CF Diagnosis Because of "Diagnostic Drift" or Because of Genetic Reclassification

A change to a CF diagnosis most often occurs when an infant initially categorized as CRMS/CFSPID develops clinical signs or symptoms of CF. In these cases, the CFFPR entry should note the date of clinical diagnosis of CF, as well as the date of the onset of clinical or laboratory findings that led to the change in diagnostic category (such as an increase in sweat chloride into the diagnostic range, or infection with *P aeruginosa*).

Rarely, children may move from the CRMS/CFSPID category to a CF diagnosis because of a new recognition of the pathologic consequences of their *CFTR* alleles. In these cases, the CFFPR entry should be updated to the date for reclassification of their genotype.

The guidelines described above should enhance the quality of data in the CFFPR. Other CF registries may wish to consider these recommendations. It would be ideal to have international harmony and consistency in the global CF registries. Perhaps the proper use of *International Statistical Classification of Diseases and Related Health Problems, 10th Revision* codes on an international scale, reflecting a clear application of

Table II. Educational resources for diagnosis of CF	
Sources	**Content**
www.cff.org	• clinical and research information for CF clinicians and primary caregivers • educational information for families regarding NBS, clinical care, and resources • CF community blog for people living with CF, their families, their friends, and their care team • links to NBS quality improvement videos
http://www.nemours .org/service/medical/ cysticfibrosis.html	• link to NBS video for families
https://youtu.be/ 2bQ40fKaGMU	• NBS video for primary care providers and other professional caregivers

diagnostic categories created by consensus, would contribute to harmony.

Educational Resources

The diagnosis of CF has become increasingly complex, as *CFTR* mutations resulting in a wide spectrum of dysfunction have been increasingly identified. To address this challenge and help educate CF centers and care providers in CF diagnosis, efforts will be put in place to facilitate implementation. Additional educational resources are also available (**Table II**).

Conclusions

Prompt diagnosis of CF is vital for optimizing outcomes. The widespread use of CF NBS has enabled the diagnosis of CF in most affected infants before obvious clinical signs, but diagnosis can be difficult. To take advantage of burgeoning knowledge of the impact of various *CFTR* mutations and recent studies on cohorts with unclear CF diagnoses, the CF Foundation convened a committee of experts from around the world to update consensus guidelines on diagnosis of CF with an emphasis on screened populations. The committee concluded that all diagnoses should be established by demonstrating CFTR dysfunction—by sweat chloride test where possible, or potentially by other methods such as NPD or ICM where necessary. Even in babies with 2 CF-causing mutations detected by NBS, diagnosis must be confirmed by demonstrating CFTR dysfunction. Guidelines for making a presumptive diagnosis were also developed. Following the recommendations for screened populations should provide clarity to CF care providers and families, and ensure treatment is provided when needed, while avoiding medicalization of healthy infants. ∎

Author Disclosures

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Appendix

Case Study 1

A 1-week-old female infant presents to the pediatrician for a first visit following an uneventful pregnancy and normal vaginal delivery. She has been steadily improving in the time that she breastfeeds and is currently now at birth weight after being several ounces down at her discharge on day 2. Parents are concerned that she sneezes and has some nasal stuffiness. The physical examination is within normal limits.

She was born in a state that uses IRT/DNA for CF NBS and was positive for 2 mutations: c.350G>A mutation (legacy: R117H) and c.1521_1523delCTT (legacy: F508del). The polyT intron analysis revealed 7T/7T.

What is the recommended next step by the pediatrician for the evaluation of this infant?

A. Referral to a CF center for evaluation and sweat chloride testing
B. Observe for 1 y and refer to CF center if clinical symptoms suggestive of CF develop
C. Send for detailed genetic analysis of CF gene
D. Send for sweat testing at accredited laboratory and observe for 1 y of life with referral to CF center if symptoms develop

A—correct
A sweat test is obtained at an accredited laboratory, and sweat chloride value is 33 mmol/L. The infant was seen at an accredited CF center where a negative history was obtained, and she had a normal physical examination.

What is the most likely diagnosis of this infant at this time?

A. CF
B. CRMS/CFSPID
C. Atypical CF
D. CFTR-related disorder

B—correct
The diagnosis of CRMS or CFSPID is given after a positive newborn screen and: a sweat test value less than 30 mmol/L and 2 CFTR mutations, at least 1 of which has unclear phenotypic consequences; or an intermediate sweat chloride value (30-59 mmol/L) and 1 or no CF-causing mutations. In this infant, the R117H with 7T is the mutation that is unclear in its clinical significance but in a few patients has been associated with the development of symptoms consistent with CF. The term CRMS/CFSPID is reserved for those screen-positive infants without clinical features consistent with CF. Following consensus guidelines from 2008 would have resulted in considering this infant not to have CF, and no follow-up would be recommended. The new guidelines are specific that this infant should receive an evaluation and education at an accredited CF center. This is a significant shift in guidance.
Children designated as CRMS/CFSPID should undergo at least 1 repeat sweat chloride test at a CF center with suitable expertise, such as an accredited CF center. This is often done when the infant is 6 months of age. Some centers repeat again at 2 years of age.
These infants and children may benefit from continued clinical evaluation by CF providers at the accredited CF centers along with regular care from their primary care providers to monitor for signs and symptoms of CF. A minority of these infants may develop changes in their physiologic testing along with clinical symptoms that ultimately lead to a diagnosis of CF.

Guide to initial diagnosis in cases where 2 mutations have been identified through NBS or no genes identified in IRT/IRT CF NBS algorithm:

Mutation 1	Mutation 2	Sweat Cl⁻ <30 mmol/L	Sweat Cl⁻ = 30-59 mmol/L	Sweat Cl⁻ ≥60 mmol/L
CF-causing	CF-causing	CF	CF	CF
CF-causing	MVCC	CRMS/CFSPID	CRMS/CFSPID	CF
CF-causing	Unknown significance (not in CFTR2)	CRMS/CFSPID	CRMS/CFSPID	CF
Not available	Not available	CF unlikely	CRMS/CFSPID	CF

Case Study 2

A 1-week-old male infant presents for his first visit to the primary care physician. He was born at 34 weeks' gestation and was screen-positive because of high IRT and 1 CF-causing mutation, F508del, on his CF newborn screen test. During this visit, he is noted to be feeding well and is a few ounces over his birthweight of 4 pounds (1.8 kg). His history is negative for respiratory symptoms, and there is no family history of cystic fibrosis.

His physician refers him to the local hospital for a sweat chloride test as per protocol. At 9 days of age, the test is completed, and results in quantity not sufficient for testing (QNS).

What is the next step for this infant?

A. Repeat testing when he is corrected 36 wk of age and is 2 kg in weight
B. Repeat testing at an accredited laboratory
C. Repeat testing when tolerating feedings and is well hydrated
D. All of the above

D—correct
The sweat test was performed when his weight reached 2 kg and was normal with a sweat chloride value of 12 mmol/L. The infant is considered to be a carrier for CF.

What should be done next for this infant?

A. Discuss the testing results with the parents and refer for genetic counseling
B. Repeat the sweat test at 6 mo of age
C. Send for expanded genetic mutation analysis
D. Refer all siblings for sweat testing to verify the negative result

A—correct
Newborns with a positive CF NBS test should be tested when at least 36 wk gestation and 2 kg body weight. Testing should be completed no later than 10-28 d of age at an accredited laboratory in a healthy full term newborn with a positive CF NBS test.
In this case, the carrier state was detected by CF NBS testing. The incidence of carrier testing has been found to be decreased in those states that use the sequence of IRT/IRT/DNA testing. Genetic counseling is recommended for the families of all infants that screen positive for CF including those with carrier status.
It is important to recognize that CF NBS testing, except perhaps for those areas that use IRT/expanded DNA testing, is not designed to detect the carrier state. A negative NBS test in the majority of cases does not exclude that the infant is a carrier of CF.

Case Study 3

A female infant was born at 38 weeks' gestation with a birth weight of 2.8 kg to African American parents. Prenatal screening for CFTR carrier status revealed that the mother carried a common disease-causing CFTR mutation—c.1652G>A

(legacy: G551D). The father was tested via a 23-mutation panel (American College of Obstetricians and Gynecologists) that detected common mutations and was found to have no mutations.

The infant had a positive CF NBS test with an elevated IRT and was positive for 1 mutation—G551D. She was followed by her primary care physician, and at 2 weeks of age, she was found to be breastfeeding well but the weight had decreased to 2.3 kg. A sweat test was ordered and had QNS for analysis. At 4 weeks of age, she was seen again and weighed 2.2 kg, and a repeat sweat test was again QNS. A third sweat test at 6 weeks of age was positive with results of 89 and 94 mmol/L.

She was then seen at a CF center and was started on pancreatic enzyme replacement therapy, albuterol, and chest physiotherapy. Further testing was completed. Fecal pancreatic elastase was low at <15 μg/g. She was found to have her mother's mutation as well as a rare second disease-causing mutation that her father had not been tested for during prenatal testing. Slow weight gain was then established during a subsequent visit to the CF center and her primary care physician.

At 3 months of age, the infant presented to an emergency department for poor feeding. She was noted to have a 3-day history of reduced oral intake and irritability without cough or rhinorrhea. Over the previous 24 hours, she had eaten very little by mouth and had only 2 wet diapers. On physical examination, she was afebrile but lethargic and had dry tacky mucous membranes. Her weight was at the first percentile for age. Her chest was clear, and her abdomen was soft and nontender.

The most likely etiology of the current findings in this infant with CF is:

A.	Chronic malnutrition
B.	Acute pulmonary exacerbation of CF
C.	Distal intestinal obstruction syndrome
D.	Electrolyte abnormalities

D—correct

This infant presents with acute changes in behavior and feeding suggestive of electrolyte abnormalities; she had not been prescribed supplemental salt, a recommendation of the CF Foundation clinical practice guidelines for infants with CF. A basic metabolic panel showed Na$^+$ 119 mEq/L (134-146); K$^+$ 1.9 mEq/L (4.2-6.4); Cl$^-$ <60 mEq/L (98-108); HCO$_3^-$ 49.6 mEq/L (23-30). An electrocardiogram revealed first degree atrioventricular block and a prolonged QTc interval. She was hospitalized in the intensive care unit for 1 wk and was hospitalized for a total of 4 wk.

This case illustrates risk factors for delayed diagnosis after a positive CF NBS. Because the infant was from a racial minority with a lower incidence of CF, and because her mother was a carrier of a *CFTR* gene mutation but her father was found to have a "negative" carrier test, her initial positive NBS test was thought to be reflective of a carrier status. Even though sweat testing was ordered within an appropriate time interval, the QNS values were thought to be due to inadequate weight gain, and she was not evaluated for the possibility that pancreatic insufficiency was the underlying cause of this poor postnatal weight gain.

Her diagnosis was delayed until after the first month of life and, in addition, salt was not prescribed. She subsequently developed a severe, life-threatening electrolyte imbalance and notably this occurred during the winter in a cold climate. In infants with a positive newborn screen and poor growth, it is appropriate to make a "presumptive diagnosis" of CF, perform appropriate diagnostic studies, and treat empirically with pancreatic enzyme replacement therapy and salt until the diagnosis can be either confirmed or ruled out.

Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and Cystic Fibrosis Screen Positive, Inconclusive Diagnosis

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Objective An unintended consequence of cystic fibrosis (CF) newborn screening (NBS) is the identification of infants with a positive NBS test but inconclusive diagnostic testing. These infants are classified as CF transmembrane conductance regulator-related metabolic syndrome (CRMS) in the US and CF screen positive, inconclusive diagnosis (CFSPID) in other countries. Diagnostic and management decisions of these infants are challenges for CF healthcare professionals and stressful situations for families. As CF NBS has become more widespread across the world, increased information about the epidemiology and outcomes of these infants is becoming available. These data were reviewed at the 2015 CF Foundation Diagnosis Consensus Conference, and a harmonized definition of CRMS and CFSPID was developed.

Study design At the consensus conference, participants reviewed published and unpublished studies of CRMS/CFSPID and used a modified Delphi methodology to develop a harmonized approach to the definition of CRMS/CFSPID.

Results Several studies of CRMS/CFSPID from populations around the world have been published in the past year. Although the studies vary in the number of infants studied, study design, and outcome measures, there have been some consistent findings. CRMS/CFSPID occurs relatively frequently, with CF:CRMS that ranges from 3 to 5 cases of CF for every 1 case of CRMS/CFSPID in regions where gene sequencing is not used. The incidence varies by NBS protocol used, and in some regions more cases of CRMS/CFSPID are detected than cases of CF. The majority of individuals with CRMS/CFSPID do not develop CF disease or progress to a diagnosis of CF. However, between 10% and 20% of asymptomatic infants can develop clinical features concerning for CF, such as a respiratory culture positive for *Pseudomonas aeruginosa*. Most studies have only reported short-term outcomes in the first 1-3 years of life; the long-term outcomes of CRMS/CFSPID remain unknown. The European CF Society definition of CFSPID and the CF Foundation definition of CRMS differ only slightly, and the consensus conference was able to create a unified definition of CRMS/CFSPID.

Conclusions CRMS/CFSPID is a relatively common outcome of CF NBS, and clinicians need to be prepared to counsel families whose NBS test falls into this classification. The vast majority of infants with CRMS/CFSPID will remain free from disease manifestations early in life. However, a small proportion may develop clinical features concerning for CF or demonstrate progression to a clinical phenotype compatible with a CF diagnosis, and their long-term outcomes are not known. A consistent international definition of CRMS/CFSPID will allow for better data collection for study of outcomes and result in improved patient care. *(J Pediatr 2017;181S:S45-51).*

During the development of the 2008 cystic fibrosis (CF) diagnosis consensus guidelines, it was recognized that the increased implementation of newborn screening (NBS) had led to a new and complex diagnostic dilemma of infants with abnormal NBS tests but inconclusive sweat tests and/or DNA test results.[1] Rather than address this complex situation in the diagnostic guidelines, a separate CF Foundation consensus conference was convened to address

CF	Cystic fibrosis
CFFPR	CF Foundation Patient Registry
CFSPID	CF screen positive, inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
CFTR-RD	CFTR-related disorder
CRMS	CFTR-related metabolic syndrome
ECFS	European CF Society
IRT	Immunoreactive trypsinogen
NBS	Newborn screening
PCP	Primary care provider

Table I. Consensus recommendations related to CRMS/CFSPID

Statement numbers*	Consensus statements
16	The term CRMS is used in the US for healthcare delivery purposes and CFSPID is used in other countries, but these both describe an inconclusive diagnosis following NBS.
17	The term CRMS/CFSPID is reserved for individuals who screen positive without clinical features consistent with a diagnosis of CF.
18	The definition of CRMS/CFSPID is an infant with a positive NBS test for CF and either: • A sweat chloride <30 mmol/L and 2 *CFTR* mutations, at least 1 of which has unclear phenotypic consequences OR • An intermediate sweat chloride value (30-59 mmol/L) and 1 or 0 CF-causing mutations
19	Children designated as CRMS/CFSPID should undergo at least one repeat sweat chloride test at CF centers with suitable expertise, such as an accredited CF center.
20	Children designated as CRMS/CFSPID should have clinical evaluation performed by CF providers to identify the minority that may develop clinical symptoms.
21	Children designated as CRMS/CFSPID can be considered for extended *CFTR* gene analysis (sequencing and/or deletion duplication testing), as well as CFTR functional analysis (NPD/ICM) testing to further define their likelihood of developing CF.
22	The decision to reclassify children designated as CRMS/CFSPID as CF is an integrated decision that should take into account functional assessment of CFTR (sweat chloride, and possibly NPD/ICM), *CFTR* genetic analysis, and clinical assessment by the CF clinicians caring for the patient.
23	Genetic counseling should be offered to families of individuals followed for CRMS/CFSPID, including a discussion of the risk in future pregnancies.
24	Research recommendation: Infants with a designation of CRMS/CFSPID (by definition) do not have clinical features consistent with a diagnosis of CF and further research is needed to determine the prognosis and best practices for frequency and duration of follow-up.

ICM, intestinal current measurement; *NPM*, nasal potential difference.
*Adapted from Farrell et al.[2]

this issue. An expert panel used the Delphi method and created a new diagnostic term, CF transmembrane conductance regulator (CFTR)-related metabolic syndrome (CRMS) and recommendations for its management.[3] CRMS is the term used in the US to describe infants with elevated immunoreactive trypsinogen (IRT) levels, but with insufficient sweat chloride or genetic data to support a diagnosis of CF. Although this condition is not a metabolic disorder, the designation metabolic syndrome was established in part to have an *International Statistical Classification of Diseases and Related Health Problems, Ninth Revision* medical code (277.9) for US healthcare delivery system follow-up and billing purposes. However, CRMS has not been accepted in Europe and some other countries because of concern about the appropriateness of the term and a feeling that it was difficult for families to understand. Thus, a similar term, CF screen positive, inconclusive diagnosis (CFSPID), was developed in a Delphi process[4] by the European CF Society (ECFS) Neonatal Screening Working Group and introduced recently in Europe as an alternative to CRMS.[5]

The planning committee for the 2015 Diagnosis Consensus Conference recognized that with the increasing use of CF NBS worldwide, CRMS and CFSPID have become important aspects of the CF diagnostic process. Therefore, the conference included a session to review recently published and unpublished data on populations with CRMS and CFSPID. An important goal of the conference was to develop a consensus to unify the definition of CRMS and CFSPID that could allow for collection of data from populations around the world and increase our understanding of the epidemiology and outcomes of CRMS/CFSPID. At the conclusion of the conference, consensus recommendations were crafted and agreed upon by electronic survey (**Table I**).

Harmonization of US and ECFS Terminology

In the US, the expert consensus panel specifically created a term that did not imply the infant has CF, whereas still acknowl-

edging that these infants required follow-up by CF specialists.[3] CRMS (*International Statistical Classification of Diseases and Related Health Problems, 10th Revision* code E88.89) was defined as an infant with hypertrypsinogenemia at birth who is asymptomatic, and who has either: (1) persistently intermediate sweat chloride levels (30-59 mmol/L if age <6 months or 40-59 mmol/L if age ≥6 months) and fewer than 2 CF-causing *CFTR* mutations; or (2) sweat chloride concentration <30 mmol/L and 2 *CFTR* mutations with 0 to 1 known to be CF-causing.

In Europe and some other countries, especially when international coding is not required for healthcare delivery, expert consensus differed slightly on how to define this group. In the initial ECFS consensus process,[4] it was recommended that these infants should not have a designation, but in the second exercise 5 years later,[5] it was clear that the majority of respondents believed a designation was needed. In the subsequent voting exercise (including CRMS as an option), there were 2 clear favorites: CF inconclusive diagnosis and CFSPID. An expert panel decided to amalgamate the 2 terms, and CF screen positive, inconclusive diagnosis (CFSPID) reached high levels of agreement in the subsequent round of the Delphi exercise, creating a category for infants who are asymptomatic, with hypertrypsinogenemia at birth,[5] and have either: (1) 0 or 1 *CFTR* mutations, plus intermediate sweat chloride (30-59 mmol/L); or (2) 2 *CFTR* mutations, at least 1 of which has unclear phenotypic consequences, plus a normal sweat chloride (<30 mmol/L).

The differences between the definitions of CRMS and CFSPID are minor and resolved by the improved characterization of *CFTR* mutations as disease-causing by the CFTR2 project. The CF Foundation recognizes that CFSPID is a term that may be helpful in describing this complex situation to parents and families. However, the term CRMS will continue to be required for entry of this group of individuals into the US healthcare system. Recognizing the 2 groups as 1 will

facilitate global collection of data from these individuals and improve our understanding of this population.

Infants will be designated as CRMS/CFSPID if they present a positive CF NBS test plus: (1) sweat chloride <30 mmol/L and 2 *CFTR* mutations with 0-1 CF-causing *CFTR* mutations; or (2) sweat chloride 30-59 mmol/L and <2 CF-causing *CFTR* mutations.

CFTR mutations that have been shown to be non-CF-causing should not be considered when applying the definition. Also, note that infants with CRMS/CFSPID who have sweat chloride levels of 30-59 mmol/L may have 0, 1, or 2 *CFTR* mutations, as long as they do not have 2 CF-causing mutations.

Risks and Benefits of Labels

There are risks to classifying infants with CRMS/CFSPID, including anxiety in parents and families, and increased health-care costs because of extensive and potentially expensive diagnostic evaluations. Currently, despite guidelines, there are different practices regarding the extent, detail, and frequency of these evaluations. A detailed work-up, including DNA sequencing and additional tests, such as nasal potential difference measurements and intestinal current measurements, may resolve the diagnosis of CF in some cases, but certainly many cases will continue to remain inconclusive, despite extensive testing.

Overall, however, creation of a category for these infants is considered beneficial because it allows these infants who cannot be clearly diagnosed with CF but may remain at risk of CF to be followed so that treatment, if needed, can be initiated quickly for optimal outcomes. Further, it allows for epidemiologic studies to be performed that will help define the disease risk and outcomes for this group of individuals.

To provide additional guidance, the consensus conference reviewed recent data from Europe, Canada, Australia, and the US on frequency of occurrence and outcomes of infants categorized as CRMS/CFSPID. These studies were used to generate recommendations for further testing in CRMS/CFSPID,

including genetic and ancillary CFTR functional studies that may help diagnose CF or identify infants likely to develop clinical features of CF (Table I).

Prevalence, Clinical Features, and Outcomes of CRMS/CFSPID

Nutritional indices in this population are generally good. An analysis of data on infants with CRMS reported during 2010-2012 to the US CF Foundation Patient Registry (CFFPR) showed that almost all infants with CRMS in the CFFPR had pancreatic-sufficiency, consistent with other recent studies of CRMS.[6] However, a higher-than-expected rate of respiratory cultures may be positive for bacteria typically associated with CF[6-9]; for example, in the CFFPR study, 11% of the infants in the group with CRMS had at least 1 positive *Pseudomonas aeruginosa* respiratory culture during the first year of life, a proportion higher than that reported in the population without CF, underlining the need for these infants to be followed.[6] Although it is important to ensure that these infants are monitored, the application of a CF diagnosis should be made with much thought, taking into account the implications for the infant and family with increased healthcare costs and raised anxiety. Follow-up requires a careful balance between surveillance and readiness to diagnose with CF and appropriately treat. Several recent studies (Table II) have provided information about CRMS/CFSPID prevalence and outcomes and longitudinal studies show that these infants do have a small risk of developing CF over time. The results of these studies, described briefly below, can help provide guidance on determining how this group should best be monitored.

CRMS/CFSPID Characteristics in US CF Foundation Patient Registry

The CFFPR collects data from all the accredited CF care centers in the US. In 2010, CRMS was added as a diagnostic category,

Table II. Summary of recent studies of CRMS/CFSPID

	Ren et al[6]	Levy et al[7]	Kharrazi et al[10]	Groves et al[11]	Ooi et al[8]
Study design	Prospective cohort	Prospective cross-sectional	Prospective cohort	Retrospective case control	Prospective case control
Location	US	Wisconsin	California	Australia	Multinational
Duration of follow-up (y)	1	20+	5	14*	3
CF	1540	300	373	225‡	3101
CRMS	309	57	553	29§	82
CF:CRMS	5:1	5.2:1	0.67:1	7.8:1	1.8:1
CRMS→CF	N/A	N/A	20	14¶	9†
CRMS→CF %	N/A	N/A	3.7%	48% (38%)	10.9%
Increased sweat chloride ≥60 mmol/L	N/A	Initial <30, n = 14 (27%) Initial 31-59, n = 38 (73%)	22 (3.2%)	4 (14%)	3 (33%)
P aeruginosa %	10.7%	39%	N/A	78.6%	14.6%
S maltophilia %	9.4%	N/A	N/A	N/A	4.9%
Pancreatic insufficiency	14 (4.5%)	0	3	4 (13.8%)	0
F508del/R117H	26.1%	36/57 (63%)**	N/A	4 (28.5%)	19.5%

N/A, not applicable.
*28% Lost to follow-up.
†Diagnosed as CF through reclassification of a second disease-causing mutation or increased sweat Cl⁻.
‡Number of infants with CF inferred based on reported annual rate of new diagnoses and timespan of study.
§CRMS definition was slightly different from CRMS/CFSPID.
¶Diagnosed through clinical signs and symptoms of respiratory disease or PI. Eight were diagnosed through respiratory symptoms.
**R117H(7T) as their second mutation with a class 1, 2, or 3 mutation as the first.

Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and Cystic Fibrosis Screen Positive, Inconclusive Diagnosis

S47

and Ren et al[6] reported the outcomes in infants with CRMS from 2010 to 2012. A total of 1540 infants met the diagnostic criteria for a CF diagnosis in that timeframe, and 309 met the criteria for CRMS (5:1). However, 40.8% of infants that fit a strict CRMS definition were actually classified as CF by the recording CF center, and 13 states entered a CF diagnosis inappropriately for at least 10% of their infants. Based on definitions established in the guidelines, 8 infants placed into the CRMS category should actually have been diagnosed with CF. It may be possible that clinical data not entered into the Registry affected the decision to classify an infant as CF. However, it is also possible that there is some confusion or disagreement with consensus guidelines. A more consistent approach to classification is important to reduce variations in CRMS/CFSPID incidence that can have a significant impact on registry data quality and epidemiology research efforts.

Growth and nutritional indices in the group with CRMS were normal, but some infants had positive respiratory cultures for CF associated pathogens, such as *P aeruginosa* (10.7%) and *Stenotrophomonas maltophilia* (9.4%). The genotype c.1521_1523delCTT (legacy: F508del)/c.[350G>A;1210-12[7]] (legacy: R117H;7T) was present in 26% of infants with CRMS, reflecting the variable phenotype associated with the R117H mutation. There were more Hispanic and non-White infants in the group with CRMS compared with the group with CF (19.1% vs 12.5% and 11.7% vs 6.5%, respectively). The relatively higher proportion of Hispanic infants in the group with CRMS, however, could be due to the large numbers of infants reported with CRMS from California, which has a large Hispanic population and uses an NBS algorithm with a greater probability of CRMS detection. The CFFPR higher proportion of African American infants in the group with CRMS could reflect the fact that African American infants tend to have higher IRT levels,[12] and CF itself is less common in the African American population, leading to a higher number of false-positive NBS tests.

CRMS/CFSPID in Wisconsin

Investigators from Wisconsin reported their experience with CF and CRMS in a cohort of 376 patients.[7] Most of these patients were diagnosed through NBS, but some were referred from neighboring states or came to attention through clinical features suggestive of CF. These patients were divided into 3 groups: CF, CRMS, and CFTR-related disorder (CFTR-RD).[13] CFTR-RD was defined as an infant or child with clinical features of CF in a single organ system and who had: (1) an intermediate sweat chloride concentration and/or 2 CFTR mutations of which no more than 1 was known to cause CF; or (2) a sweat chloride concentration below the upper limit of normal for age. There were 300 patients with CF (80% of the total), 57 infants with CRMS (15%), and 19 patients with CFTR-RD (5%). The median IRT level was significantly higher in the group with CF compared with the groups with CRMS and CFTR-RD (171 ng/mL vs 94 ng/mL in CRMS and 106 ng/mL in CFTR-RD; *P* < .001). Similarly, the mean sweat chloride concentration was also higher in patients with CF compared with these other groups (105 mmol/L vs 35 mmol/L for CRMS and 43 mmol/L for CFTR-RD; *P* < .001). Patients

with CF were significantly more likely to have pancreatic insufficiency (83% vs 0%; *P* < .001), failure to thrive (28% vs 2%; *P* < .001), and a respiratory culture positive for *P aeruginosa* (80% vs 39%; *P* < .001). Fourteen of the infants with CRMS had a sweat chloride concentration <30 mmol/L; mutation analysis revealed that 10 infants had F508del on 1 allele and 13 had R117H-7T/9T.

CRMS/CFSPID in California

The large, diverse California population provides additional insight into the population with CRMS.[10] California uses a 40-mutation genetic panel, specific to the state's population, in all infants with high IRT. Infants with 2-panel mutations are screen positive. Infants with only 1 California-panel mutation identified undergo gene scanning and sequencing, and only those with 1 or more additional *CFTR* mutations are considered to have a positive newborn screen. From 2007 to 2012, a total of 2 573 293 newborns were tested; 345 were diagnosed with CF (20 of whom were initially considered to have CRMS); 553 were categorized as CRMS (not including the 20 who were later given a CF diagnosis), and 1617 were designated healthy carriers. In addition, 28 infants were later diagnosed with CF following false-negative NBS results. Thus, in California, there was a rate of approximately 2 infants with CF for every 3 infants diagnosed with CRMS.

It is likely that the gene sequencing required by the California program is at least partially the cause of the identification of large numbers of infants categorized as CRMS, and it is useful to examine whether this results in an overall benefit to population health. Some of the infants are labelled as CRMS because of the presence of novel *CFTR* mutations whose disease-causing potential is unclear. Over the first 3 years of the California NBS program, 55 novel *CFTR* variants were identified in the 524 infants with 2 mutations detected.[14] Of the 424 infants with adequate follow-up, novel *CFTR* variants were detected about equally in the group of infants diagnosed with CF (14/110, 12.7%) during the 3-year study compared with those who stayed in the group with CRMS (28/279, 10%). Thus, in this population, the detection of novel *CFTR* variants resulted in the identification of 14 additional children with CF at the "cost" of adding another 28 children to the CRMS category who may not eventually become symptomatic. Further analysis of these infants has shown as expected that infants with a non-CF-causing *CFTR* mutation have benign outcomes, but that those with mutations of varying clinical consequences can potentially develop CF.[15,16] It is likely that factors other than CFTR play a role in this, but these are unclear at this point and only large long-term studies will provide clarity.

In the first 5 years of the California NBS program, progression from CRMS to a CF diagnosis because of clinical presentation has occurred in a relatively limited number of infants: In 20 infants with CRMS (5.8%), a diagnosis of CF was made due to 1 or more clinical symptoms, including failure to thrive/gastrointestinal involvement, respiratory involvement, or *P aeruginosa* infection (n = 7; 29%). Two of the 24 patients (8%) eventually had sweat chloride >60 mmol/L levels just above the diagnostic cut-off.[10]

CRMS/CFSPID in Australia

In New South Wales, Australia, infants are categorized as CFSPID if they have a high IRT on NBS, 1 copy of F508del, and an intermediate sweat chloride concentration of 30-59 mmol/L. A retrospective long-term follow-up of 29 infants in this group revealed approximately one-half (n = 14) were eventually diagnosed with CF and referred to as "delayed CF," although the course of CF was milder compared with NBS-positive CF cases.[11] From this study, the estimated risk of a future CF diagnosis is 48%, much higher than that found elsewhere in the literature (8%-15%). However, many of the infants were diagnosed as delayed CF based on nonspecific respiratory symptoms, such as cough.

At the 2015 CF Diagnosis Consensus Conference, investigators from Victoria, Australia, presented their experience involving 111 infants with positive NBS results (high IRT, 1 CFTR mutation) and sweat chloride concentration of 30-59 mmol/L. The clinical practice at that time (1994-2014) was to perform clinical assessment at 4-6 weeks of age, fecal elastase measurement, and extended CFTR mutation analysis. The infants were seen again at 6-12 months of age, and sweat testing was repeated. Infants with sweat chloride concentration <40 mmol/L, only 1 CFTR mutation found, and no clinical features of CF were considered carriers and referred for genetic counseling only. Infants with clinical features of CF or with a diagnostic sweat chloride concentration (≥60 mmol/L) or in whom a second CF-causing mutation was found were referred for CF care. Individuals who continued to display sweat chloride concentration of 30-59 mmol/L, or found to have a second CFTR mutation of unknown significance or indeterminate clinical features, were not considered to have CF and were referred for annual clinical assessment, with a repeated sweat test ordered when they reached 5 years of age. Over the 20-year study period, 15 children received a diagnosis of CF and 96 were determined to be healthy carriers. In the group of individuals with intermediate sweat chloride concentrations, the higher the value, the more likely the individual would eventually receive a CF diagnosis. The Victoria experience showed that the vast majority of these children never received a CF diagnosis, but a small minority did.

CRMS/CFSPID in Canada and Verona, Italy

A prospective, longitudinal study was performed in collaboration between Canada and the CF center in Verona, Italy. They identified 1-2 subjects with CFSPID for every 3 infants with CF. A study reported on the first 3 years' health outcomes of 82 individuals categorized as CFSPID compared with the health outcomes of 80 infants with CF diagnosed through NBS.[8] CFTR mutation rates did not differ between the CF and CFSPID groups; extensive gene analysis showed that 2 CFTR mutations were present in 96.3% of individuals in the group with CFSPID, compared with 92.5% in the group with CF. P aeruginosa and Staphylococcus aureus were isolated in 12% and 40%, respectively, of subjects with CFSPID. Although this was significantly less than in patients with CF (31% P aeruginosa and 71% S aureus), the frequency is higher compared with a healthy non-CF population.[17,18] In the CFSPID group, there

was a slight increase in sweat chloride concentration throughout the first 2-3 years of life. In 9 of the 82 children in the group with CFSPID (11%), CF was eventually diagnosed through abnormal sweat chloride results, reclassification of their CFTR mutations as CF-causing, or a combination. Infants that converted from the CFSPID category to a CF diagnosis had significantly higher serial sweat chloride levels ($P < .0001$) and serum trypsinogen ($P = .009$) levels than did individuals who remained in the CFSPID group. The authors demonstrated that sweat testing at age 2 years provided the clearest differentiation between infants who were likely to develop a positive sweat test and be reclassified as having CF compared with those who were unlikely to do so.

CRMS/CFSPID in France

The French CF NBS algorithm utilizes a 30-mutation genetic screen, specific to their population, in all high-IRT infants. Between 2002 and 2014, over 10 million babies were screened. A total of 1765 infants met the diagnostic criteria for a CF diagnosis in that timeframe, and 280 met the criteria for CFSPID based on the available US definition of CRMS (CF:CFSPID, 6.3:1.0). R117H was identified in 58% of infants labeled CFSPID, all with a polypyrimidine variant in intron 8 T7 in cis. The estimated risk of a future CF diagnosis has not been yet evaluated. A prospective nutritional, pulmonary, and diagnosis assessment of a national matched cohort CF/CFSPID is underway.

Recommendations for Follow-Up of Infants with CRMS/CFSPID

Following a review of data presented at the consensus conference, recommendations for follow-up of CRMS/CFSPID infants were developed by electronic survey of the consensus committee (Table I). The committee determined that when an infant is classified as CRMS/CFSPID, a clear explanation must be given to the parents and primary care provider (PCP).[3] Genetic counselling should be offered to the family. The children should undergo at least 1 repeat sweat chloride test at the CF center, and extended CFTR genetic analysis. In addition, second-tier CFTR functional analysis (nasal potential difference or intestinal current measurement) can be considered and performed by centers with expertise in these tests. Other clinical assessments, such as measurement of fecal elastase activity, can be performed as clinically appropriate. Parents and PCPs should understand that children classified as CRMS/CFSPID must be followed by a specialized CF care physician because some will develop manifestations of CF disease (Appendix; available at www.jpeds.com). However, there are currently not enough data to help stratification of the individuals at risk for developing CF disease. CF care centers should follow strict infection control guidelines for infants with CRMS/CFSPID to minimize exposure to individuals with CF and, thus, potential transmission of pathogens. Although guidelines for management of CRMS/CFSPID have been published, they are based on limited data.[3,4]

Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and
Cystic Fibrosis Screen Positive, Inconclusive Diagnosis

S49

It was apparent to the consensus committee that further research is needed to determine the prognosis and best practices for frequency and duration of follow-up of the infant with CRMS/CFSPID. To facilitate this, parents should be advised on the need to provide consent to include data on their infants in patient registries.

Discussion

In the past few years, several studies of CRMS and CFSPID have been reported from across the world. Although they vary in terms of patient characteristics, sample size, and study design, some common themes have emerged. CRMS/CFSPID is a relatively common outcome of CF NBS, and CF clinicians and PCPs need to be prepared to counsel families with these infants. The majority of these infants do not go on to develop clinical CF, but in some infants, the diagnosis of CF is eventually made through an increase in the sweat chloride concentration into the CF diagnostic range, or through reclassification of an infant's *CFTR* mutation as disease-causing based on increased knowledge of *CFTR* genetics. In addition, infants may be diagnosed subsequent to the development of clinical features that are concerning for CF. Because respiratory symptoms, such as cough and wheeze, are very common in infants and young children without CF, interpreting their significance in children with CRMS/CFSPID is challenging. To avoid potential transmission of CF-related respiratory pathogens, exposure of infants with CRMS/CFSPID to individuals with CF should be minimized during medical evaluation and follow-up.

Current studies suggest that most of the infants with CRMS/CFSPID do not develop disease symptoms in a 3-year follow-up period. However, there is a lack of longer term outcome data. Experiences from diagnosing adolescents and adults with CF or a CFTR-related disorder suggest that disease symptoms on these individuals can become apparent later in life. Studies of large cohorts of infants with CRMS/CFSPID are essential to better understand the relationship between CRMS/CFSPID, CF, and CFTR-RD and to provide more information to CF caregivers to counsel families of these infants. Families need to be well-informed of the increased risk for these infants as they grow. Some infants with CRMS/CFSPID may develop respiratory symptoms and acquisition of CF-typical respiratory pathogens. However, because of lack of data, we currently do not know how many of those will go on to develop CFTR-RD or CF.

A harmonized definition will facilitate research into the frequency and clinical outcomes of these infants, which should address a number of concerns:

- Will these patients remain free of significant symptoms, or will they demonstrate increasing pathology, "phase shifted" by a few decades?
- What factors are involved or promote this development and progression of pathology?
- Do the current recommendations for management, including testing and follow-up, provide the optimal balance between monitoring and detecting development of CF disease while not over medicalizing infants with CRMS/CFSPID?
- Should the CF care team adopt a more proactive approach for this infant or is the reactive approach advocated in current recommendations appropriate?
- What is the psychological impact of designating a potentially healthy individual with CRMS/CFSPID?
- What is the cost to the healthcare system of follow-up for infants with CRMS/CFSPID, and how should this be considered in the financing of CF NBS?
- When can we release an individual from a designation of CRMS/CFSPID? Even if they are asymptomatic as infants and children?
- What is the true risk of these infants developing single-organ disease as an adult consistent with a designation of CFTR-RD?

These important questions need answers if we are to monitor this group of children optimally. However, this consensus conference focused on diagnosis and definitions, not management. Thus, although the definitions of CRMS and CFSPID should be harmonized as described above, no recommendations on management were made at the consensus conference other than follow-up diagnostic testing. We appreciate that current management of CRMS/CFSPID may differ between the US and other countries. CF Foundation guidelines for the management of infants with CRMS were published in 2009, and it is likely that they will be revised in the near future to reflect the additional knowledge gained since that time. ∎

Author Disclosures

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Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and Cystic Fibrosis Screen Positive, Inconclusive Diagnosis

S51

Appendix

Case Study

The parents of an infant boy are notified that he screened positive for CF by their state department of health and presented to your pediatric office at 10 days of age for evaluation. Your state follows an IRT/DNA testing protocol and the screening results note that after an elevated IRT he was positive for 1 mutation: c.1521_1523delCTT (legacy: F508del).

The infant was born following a full-term uncomplicated pregnancy with a birth weight of 3.2 kg. The family history is negative for CF. His neonatal period was normal, and he passed meconium soon after birth.

Your examination reveals a negative history and a normal examination. His current weight is 6 ounces over his birth weight, and he is breastfeeding without concerns.

A sweat test is obtained at 2 weeks of age, and the laboratory notifies you that it is in the intermediate sweat chloride range at 32 mmol/L.

What is the most likely initial diagnosis of this infant?

A.	CF
B.	CRMS/CFSPID
C.	Negative NBS test
D.	CF carrier

B – correct

What should you do as the next step for care of this infant?

A.	Observe and repeat sweat test at age 2 years
B.	Observe for one year and refer to CF center if clinical symptoms suggestive of CF develop
C.	Referral to a CF center for evaluation and testing
D.	Send for chest radiograph and a throat swab for CF pathogens

C – correct

The definition of CRMS/CFSPID applies to this infant with a positive NBS, 1 CF causing mutation and an intermediate sweat test. The next step, referral to an accredited CF center, will initiate an evaluation by a CF provider, and very importantly, begin education regarding this complex NBS diagnosis for the parents and family of this patient. If the findings in the history and physical examination are normal, the center may elect to observe the infant and plan on a repeat sweat chloride test in several months. Genetic counseling would be recommended.

Often, further genetic testing with an expanded CF mutation panel is obtained at this visit or a subsequent visit and will help determine the presence of other CF-causing mutations not identifiable on the limited state screening panel. The center will usually follow the infant at regular intervals over the first several years, closely watching for signs and symptoms suggesting CF. Further testing will be completed as prompted by signs and symptoms or any changes on the subsequent sweat test that would indicate a potential change in physiologic status.

Diagnosis of Cystic Fibrosis in Nonscreened Populations

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Objective Although the majority of cases of cystic fibrosis (CF) are now diagnosed through newborn screening, there is still a need to standardize the diagnostic criteria for those diagnosed outside of the neonatal period. This is because newborn screening started relatively recently, it is not performed everywhere, and even for individuals who were screened, there is the possibility of a false negative. To limit irreversible organ pathology, a timely diagnosis of CF and institution of CF therapies can greatly benefit these patients.

Study design Experts on CF diagnosis were convened at the 2015 CF Foundation Diagnosis Consensus Conference. The participants reviewed and discussed published works and instructive cases of CF diagnosis in individuals presenting with signs, symptoms, or a family history of CF. Through a modified Delphi methodology, several consensus statements were agreed upon. These consensus statements were updates of prior CF diagnosis conferences and recommendations.

Results CF diagnosis in individuals outside of newborn screening relies on the clinical evidence and on evidence of CF transmembrane conductance regulator (CFTR) dysfunction. Clinical evidence can include typical organ pathologies seen in CF such as bronchiectasis or pancreatic insufficiency but often represent a broad range of severity including mild cases. CFTR dysfunction can be demonstrated using sweat chloride testing, *CFTR* molecular genetic analysis, or CFTR physiologic tests. On the basis of the large number of patients with bona fide CF currently followed in registries with sweat chloride levels between 30 and 40 mmol/L, the threshold considered "intermediate" was lowered from 40 mmol/L in the prior diagnostic guidelines to 30 mmol/L. The CF diagnosis was also discussed in the context of CFTR-related disorders in which CFTR dysfunction may be present, but the individual does not meet criteria for CF.

Conclusions CF diagnosis remains a rare but important condition that can be diagnosed when characteristic clinical features are seen in an individual with demonstrated CFTR dysfunction. *(J Pediatr 2017;181S:S52-7).*

Since the identification of cystic fibrosis (CF) as a pathologic entity in 1938,[1] diagnosis has been based on the appearance of signs and symptoms of the disease. For many decades, diagnosis occurred in infancy or early childhood, although by the 1960s, the disease was occasionally being identified in adults,[2-4] who were usually pancreatic-sufficient. The identification of the gene for the CF transmembrane conductance regulator (*CFTR*) in 1989[5-7] and subsequent discovery of mutations that can alter quantity and/or function of the protein to varying degrees,[8] as well as the recognition of modifier genes,[9] have led to demonstration of a wider spectrum of CF in individuals of all ages and ethnicities.[10] It is now clear that in individuals with residual function *CFTR* mutations, clinical manifestation of CF may develop later in life.[11] Furthermore, although the advent of widespread newborn screening (NBS) has dramatically changed the diagnosis for many infants born in the last decade or so, more than one-third of all US diagnoses in 2014 were not a result of NBS.[12] Criteria to establish a diagnosis of CF outside of NBS are needed because CF NBS is neither universal nor foolproof; false negatives can and do occur.[12,13] Thus, although physicians today may have less clinical suspicion as a result of CF NBS, a diagnosis of CF or related entities must remain a consideration in anyone who displays signs and symptoms of the disease, regardless of age, race, or whether they may have undergone NBS.

Diagnosis of the Nonscreened Individual

The process for diagnosis of CF in individuals that present with clinical symptoms rather than a positive newborn screen does not differ greatly from that

CF	Cystic fibrosis
CFTR	CF transmembrane conductance regulator
CFTR2	Clinical and functional translation of CFTR
CT	Computed tomography
FEV₁	Forced expiratory volume in the first second
ICM	Intestinal current measurement
NBS	Newborn screening
NPD	Nasal potential difference

Table I. 2015 CF Foundation diagnosis consensus conference recommendations related to diagnosis of CF in nonscreened populations*

Statement numbers*	Consensus statements	Comments
25	For individuals presenting with CF symptoms, the same diagnostic criteria recommended for the screened population for sweat chloride testing, *CFTR* genetic analysis, and CFTR functional testing should be used to confirm a CF diagnosis.	This represents a change in the cut-offs for sweat chloride testing. In previous statements, an "unlikely CF" sweat chloride was <40 mmol/L;[19] at present it is <30 mmol/L. See "Change in Sweat Chloride Range Definitions" section.
26	The diagnosis of CFTR-related disorder has been defined as a monosymptomatic clinical entity (CBAVD/pancreatitis/bronchiectasis) associated with CFTR dysfunction that does not fulfill the diagnostic criteria for CF.	This draws on the international effort to characterize this clinical scenario. See "Alternative Diagnoses" section.
27	Clinicians should avoid the use of terms like classic/nonclassic CF, typical/atypical CF, delayed CF because these terms have no harmonized definition and could be confusing for families or caregivers.	The European CF Society Diagnostic Network Working Group has maintained a nonclassic or atypical CF label.[20,21] See "Alternative Diagnoses" section for discussion.

CBAVD, congenital bilateral absence of the vas deferens.
*Adapted from Farrell et al.[14]

recommended by earlier diagnosis consensus criteria[13] (flow chart representing the diagnostic process recommended for all populations by the 2015 CF diagnosis consensus committee[14]). There is growing recognition that CF can present at any age, and in any race or ethnicity. In making the diagnosis, an appropriate clinical presentation needs to be linked with evidence of CFTR dysfunction. Since earlier consensus statements, several advances have evolved our experience with both the clinical presentation (as we have recognized a broader spectrum of CF and CFTR-related disorders) and with our understanding of the molecular and cellular pathophysiology of CFTR dysfunction (increased genetic annotation and improved physiologic testing of CFTR). This article will place those advances in the context of CF diagnosis in the era of expanded CF therapeutics.

As part of the US CF Foundation Diagnosis Consensus Conference, convened in Phoenix, Arizona, in October 2015, the criteria for CF diagnosis were reviewed. This review included recent advances in changes to diagnosis for screened individuals, as well as for nonscreened. A summary of the conference was organized according to consensus statements and voted on by participants in the conference, as well as opened to public comment. The summary review and other articles can be viewed as part of this Supplement.[14-18] The consensus statements pertaining specifically to nonscreened individuals are listed in **Table I**.

Steps to Establish CF Diagnosis

When the diagnosis of CF is being considered outside of the NBS context, the presenting signs and symptoms (**Table II**) play an important role in defining likelihood of CF. An individual with multiple typical-organ system manifestations of CF (bronchiectasis, sinus polyps, and pancreatic insufficiency) has a higher probability of having CFTR dysfunction as the explanation of their phenotype compared with someone with only atypical manifestations of CF (eg, isolated symptoms such as chronic cough or sputum production without bronchiectasis, recurrent pancreatitis) that may have alternative explanations. Therefore, diagnosis of CF can be heavily influenced by the pretest probability or how well the phenotype is consistent with CF as we understand it now.

Coincident with the consideration of presenting signs and symptoms for CF, the clinician must also compare these with

Table II. Clinical signs/symptoms that may signify CF

Presenting conditions	Common as first presentation of CF	Uncommon as first presentation of CF*
Family history	Sibling or parent with CF	Parent of a child diagnosed with CF
Sinus	Chronic sinusitis, nasal polyps	
Lower respiratory	Bronchiectasis, chronic or recurrent lower airway infection (especially *Pseudomonas* infection)[22]	ABPA, nontuberculous mycobacterial infection, asthma, chronic obstructive pulmonary disease
GI/lumen	Meconium ileus, distal intestinal obstruction syndrome	Abnormal motility, rectal prolapse
GI/hepatobiliary	Pancreatic insufficiency, recurrent pancreatitis	Elevated liver enzymes, ecchymosis, cirrhosis, prolonged neonatal jaundice, fat soluble vitamin deficiencies (may present as ecchymosis, anemia, edema, night-blindness, skin rash)
Reproductive	Male infertility because of obstructive azoospermia (CBAVD)	Female infertility
Other	Hyponatremic dehydration, failure to thrive	Pseudo-Bartter syndrome, aquagenic wrinkling of skin, digital clubbing

ABPA, allergic bronchopulmonary aspergillosis; *GI*, gastrointestinal.
*Many of the uncommon presentation clinical features are not uncommon in patients with CF (ABPA, nontuberculous mycobacterial infection, abnormal motility, clubbing, vitamin deficiencies), however, they are uncommon as isolated presenting complaint that ultimately is due to CF. Atypical mycobacterial infection has more commonly led to a diagnosis of CF in adults.[23,24]

alternative diagnoses. Comprehensive lists of alternative diagnoses are beyond the scope of this article and will differ according to the specific clinical presentation and the age of the patient. For example, an individual presenting with bronchiectasis will have a different differential diagnosis (immune deficiency, prior infection, ciliary dyskinesia, etc) than an individual with primarily pancreatitis (alcohol toxicity, gallstones, hypertriglyceridemia, etc). To evaluate other potential etiologies, the clinician may rely on tests that better characterize the presenting signs and symptoms, or that test for a specific alternative diagnosis. Those tests may include: (1) pulmonary function tests; (2) high-resolution chest computed tomography (CT); (3) respiratory tract cultures from sputum or bronchoalveolar lavage; (4) fecal fat quantification or elastase antibodies; (5) pancreatic imaging; (6) fat-soluble vitamin levels; (7) genital evaluation in males to evaluate for the bilateral absence of vas deferens; and (8) exclusionary testing for ciliary dyskinesia, immunodeficiencies, recurrent pancreatitis, celiac disease, and inflammatory bowel disease.

Informed with a pretest probability that compares the likelihood of CF vs an alternative explanation, the clinician will interpret genetic analysis and tests for CFTR function (sweat chloride testing, nasal potential difference [NPD], or intestinal current measurement [ICM]) to support or refute a CF diagnosis. Often, when a patient is referred for CF evaluation, some of these tests may have already been performed. A clinician would best interpret these tests in the context of a reasoned pretest probability. The tests of CFTR function may point in a clear single direction (eg, if the sweat chloride is >60 mmol/L and 2 CF-causing *CFTR* mutations are found, vs an alternative scenario of only 1 mutation found, and the sweat chloride and NPD are normal). Challenges arise if those CFTR tests do not provide a definitive answer. Examples of this could include an intermediate sweat chloride level (30-59 mmol/L), the detection of at least 1 *CFTR* mutation of varying or uncertain clinical consequence, or NPD/ICM that cannot be interpreted as positive or negative. In these circumstances, the clinician may refer to their pretest probability. It is important to note that diagnostic challenges should not delay potentially beneficial treatments.

Change in Sweat Chloride Range Definitions

A major change that resulted from the 2015 Diagnosis Consensus Conference was the adoption of a lower sweat chloride level as the upper limit of normal. The 2008 diagnosis consensus[19] recommended the use of ≥60 mmol/L chloride in the sweat as diagnostic of CF, with levels from 30 to 59 mmol/L as intermediate sweat chloride values in infants under 6 months of age, or 40-59 mmol/L in individuals over 6 months of age. After reviewing evidence accumulated in the interim (eg, Augarten et al,[25] Highsmith et al,[26] and Collaco et al[27]), the 2015 Diagnosis Consensus Conference Committee, however, now recommends the use of 30-

59 mmol/L as representing the intermediate sweat chloride level for all ages.

The basis for a sweat chloride level of <60 mmol/L in individuals who are diagnosed with CF because of a preponderance of clinical symptoms can be attributed in large part to the occurrence of *CFTR* mutations that do not result in a total loss of chloride channel activity. The Clinical and Functional Translation of CFTR (CFTR2) was established to determine the clinical and functional impact of various *CFTR* mutations.[8] Phenotype and genotype information are collected from patient registries, and disease-liability of each *CFTR* mutation is evaluated through a combination of in vivo (sweat chloride) and in vitro data (functional activity identified in cell-based systems). The *CFTR* mutations are sorted into 4 categories: (1) CF-causing (defined as resulting in CF when 2 copies are present in an individual); (2) a mutation of varying clinical consequence (defined as a mutation that, in combination with a CF-causing mutation or another mutation of varying clinical consequence may result in CF); (3) a mutation of unknown clinical consequence (defined as one that has not been evaluated by CFTR2); and (4) a mutation that is non-CF-causing (defined as not causing CF when present). Thus, consulting the CFTR2 database to determine the disease liability categories represented by an individual genotype may offer better insight into the questionable cases.

Table III provides an example of the clinical data accumulated in CFTR2, and shows the clinical characteristics of patients in CFTR2 with a diagnosis of CF, but who have a sweat chloride of <60 mmol/L. In general, these patients with intermediate sweat chloride concentrations have been diagnosed at an older age and have milder manifestations of disease. These data show some of the clinical manifestations likely in individuals with CF who are homozygous for c.1521_1523delCTT (legacy: F508del), the most common CF-causing mutation, but who have a nondiagnostic sweat chloride. It was on the basis of the 746 individuals with CF in CFTR2 with a sweat chloride between 30 and 40 mmol/L that the consensus committee supported a change of the lower limit of the "intermediate" range to 30 mmol/L from 40 mmol/L. A consequence of this is that individuals presenting with symptoms of CF and a sweat chloride level between 30 and 40 mmol/L who were previously considered unlikely

Table III. Association of lung function in patients with high, intermediate, or low sweat chloride values[8]

Sweat chloride	Patients* (n)	F508del† homozygous (n)	Mean FEV$_1$% predicted	Mean age (y)
41-59 mmol/L	3278	192	85.5	16.3
30-40 mmol/L	746	35	87	16.1
<30 mmol/L	52	40‡	88	16.1

*CFTR2 contains anonymized data on 88 664 patients with CF, gathered from 41 countries.
†Legacy name for the mutation c.1521_1523delCTT.
‡Although this may include some patients truly homozygous for F508del, the CFTR2 team believes this more likely represents errors either in genotyping or in sweat values.

to have CF, could be reconsidered as having a possible diagnosis of CF under the current guidelines. Of course, in subjects with a sweat chloride from 30 to 40 mmol/L, the physician must still rely on other criteria to establish or exclude a CF diagnosis. In general, this highlights that regardless of the test cut-offs used, CF diagnosis in nonscreened individuals needs to appropriately consider all diagnostic test results in the context of the clinical scenario.

Alternative or Unresolved Diagnoses

CFTR-Related Disorders
An important advance since the publication of the last US CF Foundation diagnostic consensus criteria is a better definition of CFTR-related disorders.[28] This is simply defined as a clinical entity associated with CFTR dysfunction, that does not fulfill diagnostic criteria for CF.[28] It has been particularly ascribed to clinical entities such as congenital bilateral absence of the vas deferens, recurrent pancreatitis, and disseminated bronchiectasis. This traditionally has been diagnosed in adults. An argument can be made that an individual with bronchiectasis as a manifestation of CFTR-related disorder should be labeled as CF. However, because there are many causes of bronchiectasis (including various immunodeficiencies, primary ciliary dyskinesia, and sequellae of pneumonia), the identification of a single CF-causing mutation, as exists in the parents of an individual with CF, is not uncommon. The clinician must be cautioned not to conclude too readily that a symptom is the result of CFTR dysfunction. Rather, it would be prudent simply to treat the individual following CF guidelines while the diagnosis is being resolved.

It also has been recognized that variance in the CFTR gene may play a contributing role in bronchiectasis and other organ pathologies. For example, among individuals with bronchiectasis, there is a higher than general-population incidence of CFTR mutations,[29-33] including mutations with only minor effects on CFTR function that are not classified as CF-causing, and that are seen commonly in the population. Similar genetic analysis shows CFTR mutations in patients with congenital bilateral absence of the vas deferens,[34-37] chronic sinusitis,[38] and pancreatitis.[39,40]

At this point, the distinction between CFTR-related disorders and conditions in which CFTR mutation or mutations are contributing to complex traits is blurred. Individuals that are carriers (only 1 mutated CFTR allele) may present with a manifestation of reduced CFTR function (for example, chronic sinusitis).[38] The clinical entity of CFTR-related disorder is an important recognition that CFTR variation may impact a much wider segment of the population, beyond those with the life-shortening Mendelian disease. However, in light of newly approved and future CFTR modulating therapies that can result in significant improvements in quality and length of life, it will be worth identifying the disease liability of an individual genotype, to determine whether the CFTR mutations may be the primary (or modifiable) cause of the phenotype. This will need to be studied with epidemiologic comparison of the genome of individuals with and without traits potentially related to CFTR dysfunction.[41]

Cases in Which a CF Diagnosis Cannot be Resolved
It would be useful to place CFTR-related disorders and complex traits in which CFTR may be playing a role, into an algorithm for CF diagnosis. In cases in which there is a clinical suspicion for CF, but sweat chloride testing, CFTR mutation analysis, and physiologic testing cannot rule in or rule out CF, the clinician is left with a decision. Ultimately in these cases, if the physician believes CF therapies or CF follow-up would benefit the patient, that should outweigh equivocal, nondiagnostic, test results.

The goal must be to achieve optimal treatment, especially of lung disease, rather than to engage in an ongoing debate about the best diagnostic label. The European CF Society Diagnostic Network Working Group has advocated for the use of the term "atypical" CF.[20,21] Although these cases may not follow the "typical" path of CF, patients who do not display 2 CF-causing mutations or do not have diagnostic sweat chloride test results can still have severe, life-limiting respiratory disease.[42,43] In fact, there are no CFTR mutations that can be exclusively characterized as "atypical" mutations (although some mutations may trigger more risk than others[44]). Thus, the diagnosis consensus committee has agreed (not universally) to recommend against the use of the terms atypical or borderline CF. Most, although not all, of the committee has concluded that if a CF clinician feels CF therapies and follow-up would benefit the individual and other potential diagnoses are ruled out, the CF diagnosis is appropriate. This certainly is influenced by the third-party payers in the US that may be more likely to cover expensive care if a CF diagnosis is used. Regardless, in individuals diagnosed with CF as an adult or with milder presentations, appropriate counseling should include that the life expectancy estimates derived from mostly patients with CF diagnosed early in life are less applicable in their situation.

Inconclusive Result after CF NBS
It is important to note that the uncertain diagnostic category that can result from positive newborn screens that do not meet criteria for CF (ie, CF-related metabolic disorder/CF screen positive, inconclusive diagnosis) does not apply to anyone presenting with CF-like symptoms.[14,18] However, individuals identified in NBS that do not have CF frequently have mutations that are seen more commonly in individuals with CFTR-related disorders.[45] Some of these individuals identified in NBS may go on to develop a CFTR-related disorder later in life. Future study of the likelihood of developing these conditions is needed, but this will require long-term follow-up. In the meantime, counseling parents of CFTR-related metabolic syndrome/CF screen positive, inconclusive diagnosis infants about this possibility is an important component of NBS programs. Clinicians considering CF in a nonscreened setting should ask questions about NBS history.

Special Considerations Regarding Adult Diagnosis

In the period from 1995 to 2005, there were 9766 new CF diagnoses, of which 811 (8.3%) were in adults, with a mean age of 34 years at the time of diagnosis.[42] Some examples of adult diagnoses can be found in the case studies shown in the Appendix (available at www.jpeds.com). Most adults (70.6%) present with commonly described respiratory symptoms, such as *Pseudomonas* lung infections and reduced lung function. Despite the delayed appearance of symptoms, the causes of death do not differ significantly from those experienced by patients diagnosed in childhood, with the vast majority of both caused by respiratory failure (87% following childhood diagnosis, 85% following adult diagnosis).[43] The importance of recognizing these later presentations of CF is that there is tremendous opportunity to intervene in progressive lung disease. Unlike asymptomatic babies who are diagnosed through NBS, these patients (or their parents) are actively seeking a diagnosis to facilitate treatment. A CF diagnosis often comes as a relief in these individuals.[46] Beyond this, establishing the diagnosis is critical to help these individuals access the specialized care, genetic counseling, and drugs they need. Without a CF diagnosis, there may be challenges getting insurance to pay for necessary therapies. The "diagnostic odyssey" is prolonged, and further unnecessary testing and uncertainty will ensue.

Discussion

Given the multitude of different organ systems that can be involved in CF, establishing a diagnosis in an individual presenting with symptoms does not lend itself well to protocols or algorithms, such as those used in newborn screening. The central tenant that an individual with a clinical scenario (symptoms, signs, and/or family history) and evidence of CFTR dysfunction (sweat test, *CFTR* genotype, CFTR physiologic testing with NPD or ICM) has CF is true regardless of the situation, but in nonscreened individuals, the diagnosis demands different clinician recognition and judgment. NBS has been a tremendous improvement in CF detection, but it will never eliminate the need to consider CF as a clinical diagnosis in individuals who were not screened or who were not identified. CF was recognized as a clinical entity before the genetic cause was determined. However, as the range of genetic variations identified in *CFTR* has expanded, so too must our understanding of the clinical entity. The clinician must now look beyond traditional presentations to consider milder cases of CF and situations in which CFTR dysfunction is playing a role in lung, gastrointestinal, or reproductive tract disorders. It is the goal of the CF community to refine these diagnostic criteria in a way that gets CF care and therapeutic advances to those that would benefit the most from them, but also to inform the clinician to help all those in whom the disease should be considered. ∎

Author Disclosures

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Appendix

Case Study 1

A 51-year-old male presents with a lifelong history of a productive cough and chronic rhinosinusitis. As a child, he was hospitalized on several occasions for dehydration and was unable to perform strenuous exertion in hot weather. As an adult, he suffered from recurrent pulmonary and sinus infections that failed to resolve without antibiotic treatment. He was unable to have children, and a semen analysis revealed azoospermia. He does not complain of constipation or diarrhea and tolerates a full diet. Prior evaluation by a pulmonologist demonstrated an obstructive pattern of airflow limitation, and he was diagnosed with asthma. On 2 occasions, a sputum culture was positive for a mucoid strain of *Pseudomonas aeruginosa*. There was no history of CF within his extended family.

A chest CT scan was significant for upper lobe bronchiectasis, worse on the right side. Pulmonary function tests showed a forced expiratory volume in the first second (FEV_1) of 57% predicted. Analysis of CFTR function demonstrated sweat chloride values of 80 and 82 mmol/L. Full CFTR sequencing confirmed the presence of c.350G>A (legacy: R117H) and c.1585-1G>A (legacy: 1717-1G>A) with an intron 8 poly (T) variant 5T and 7T. Extensive ancillary testing for other causes of bronchiectasis were nondiagnostic.

Which of the following statements is correct?

A.	He is unlikely to have CF because he is too old, lacks a family history, and does not have symptoms of pancreatic insufficiency
B.	His bronchiectasis is likely the result of recurrent childhood respiratory infections
C.	He meets diagnostic criteria for CF
D.	He meets the diagnostic criteria for CFTR-related metabolic syndrome and is unlikely to develop full CF

C – correct

This case is an example of a typical presentation for a CF diagnosis made in adulthood because of the presence of 1 or more residual-function *CFTR* mutations. With a background of obstructive azoospermia, by his sixth decade of life the patient has developed moderately severe bronchiectasis, chronic infection with typical CF pathogens, and extensive sinus disease. Failure to present with pancreatic insufficiency is common in adult-diagnosed patients, but he is at risk to lose pancreatic function over time. The diagnosis is confirmed by the presence of 2 disease-causing *CFTR* mutations and a sweat chloride <60 mmol/L. Although R117H is often mild when combined with intron 8 poly (T) variant 7T, it is much more severe when paired with the 5T, which is reflected by the relatively high sweat chloride values.

Even though postinfectious bronchiectasis remains common worldwide, it is rare in the US or Europe, and is essentially a diagnosis of exclusion. It would be incorrect to label this case CFTR-related metabolic syndrome, as he was not identified through NBS, and he fulfils all criteria for CF.

What is the next appropriate step for this patient?

A.	He should be referred to an accredited CF center
B.	He should receive comprehensive CF care including airway clearance, suppression of *P aeruginosa*, evaluation of sinus disease, and CF modulator treatment with ivacaftor should be initiated
C.	Genetic counseling should be offered to the patient, siblings, and extended family members as appropriate
D.	All of the above

D – correct

The patient should have access to CF center care, including all of the therapies listed above. In this case, he had an outstanding response to treatment, in particular ivacaftor. Genetic counseling is also an important consideration. Even if the patient does not have children, his siblings each have a 50% chance of being carriers.

Case Study 2

A 40-year-old woman presents with persistent productive cough. She reports that her cough has increased in intensity over the years. She has had a history of frequent upper respiratory infections, feels that these illnesses last much longer than in other family members, and often requires oral antibiotics for resolution of symptoms. She has symptoms of chronic sinusitis and rhinorrhea but has not pursued formal evaluation. She reports symptoms of intermittent diarrhea and constipation but without frank steatorrhea. She has never had pancreatitis and does not limit fat intake in her diet. She reports that her sister was given the diagnosis of CF and died in her 40s of progressive respiratory failure because of bronchiectasis. Her sister had chronic infection of her airways with *P aeruginosa* and had suffered a precipitous decline in her health following childbirth. The patient reports that her sister's physicians described her sweat chloride results as "borderline," and she had only 1 *CFTR* mutation identified on screening.

At presentation, her initial sputum culture demonstrated methicillin-sensitive *Staphylococcus aureus*, *P aeruginosa*, and *Mycobacterium avium* complex. A chest CT scan was significant for upper lobe predominant varicose, cylindrical bronchiectasis, with moderate mucoid impaction and air trapping. A sinus CT confirmed moderately severe pansinusitis. Her FEV_1 was 68% predicted. Analysis of CFTR function demonstrated sweat chloride values of 54 and 54 mmol/L. Full *CFTR* sequencing of the entire coding regions as well as common intronic mutation sites demonstrate only 1 copy of c.1521_1523delCTT (legacy: F508del), with an intron 8 poly(T) 7T 11TG and 9T 10TG. There were no large sequence duplications or deletions.

She was initiated on inhaled tobramycin for 1 month, and *P aeruginosa* was successfully eradicated. Subsequent cultures have continued to grow methicillin-sensitive *Staphylococcus aureus*. The *Mycobacterium avium* complex has not been recovered since the initial visit, but she has had many positive cultures for *Mycobacterium abscessus*. Airway clearance was initiated with a therapy vest in combination with dornase alfa and hypertonic saline. After 3 years, her FEV_1 has improved to 76%, with improved radiographic changes, decreased cough, and less frequent upper respiratory infections.

Which of the following statements is correct?

A.	She is only a CF carrier because full *CFTR* gene sequencing did not demonstrate 2 CF-causing mutations
B.	She does not meet diagnostic criteria for CF because her sweat chloride is <60 mmol/L
C.	The absence of detection of 2 CF-causing mutations does not exclude a diagnosis of CF
D.	Both A and B

C – correct

This case demonstrates the role of clinical judgment in establishing the CF diagnosis in certain individuals. The patient presents with a disease phenotype consistent with CF, combined with a strong family history in a first-degree relative. With currently available analysis, only a single *CFTR* mutation can be identified, but her sweat chloride test is grossly abnormal, although below the standard diagnostic cut-off. As our understanding of CFTR structure, function and regulation continues to grow, it is likely that at some point a second gene defect will be discovered in this individual, but in the meantime she should be given the diagnosis of CF and provided access to CF center care.

Which of the following statements is correct?

A. A sweat chloride of 54 mmol/L is typical of a carrier of a single copy of F508del
B. NPD testing is necessary to establish a CF diagnosis
C. She can be assigned the diagnosis of CF based on clinical presentation, family history and demonstrated CFTR dysfunction
D. She does not have CF and neither did her sister

C – correct

In general, CF carriers will not have an abnormal sweat chloride. NPD testing could confirm the degree of CFTR dysfunction assessed by the sweat chloride testing, but it should not delay the institution of CF therapies. The strong family history should not be dismissed unless a more plausible hereditary cause of bronchiectasis can be proven in this case. Her sweat chloride of 54 mmol/L is entirely consistent with her clinical presentation at this point in life.

Case Study 3

A 20-year-old woman presents with recurrent hemoptysis and a productive cough since age 11 years. She has had a history of many respiratory infections, and her episodes of bleeding have become more frequent and severe. She has chronic sinusitis and nasal polyps. She has symptoms consistent with typical steatorrhea and is underweight. She has never had pancreatitis. She denies a family history of CF.

At presentation, her initial sputum culture demonstrated *P aeruginosa* and methicillin-sensitive *Staphylococcus aureus*. A chest CT scan was significant for mild upper lobe bronchiectasis and scattered mucous plugging. Her FEV$_1$ was 70% predicted. Analysis of CFTR function demonstrated a mean sweat chloride value of 33 mmol/L. *CFTR* genotype revealed the presence of 1 copy c.1521_1523delCTT (legacy: F508del) and 1 copy c.3737C>T (legacy: T1246I).

She was initiated on inhaled tobramycin and standard airway clearance. Subsequent cultures have continued to grow methicillin-sensitive *Staphylococcus aureus*, and intermittently *P aeruginosa, P fluorescens/putida,* and *Serratia marcescens.* She was initiated on pancreatic enzyme replacement, with resolution of steatorrhea. After 2 years of CF center care, her FEV$_1$ has improved to 101%, with marked reduction in hemoptysis and decreased cough. She has gained 3 kg.

Which of the following statements are correct?

A. A sweat chloride of 33 mmol/L is too low to consider a diagnosis of CF
B. T1246I has not been established as "disease-causing" and therefore this patient is unlikely to have CF
C. The diagnosis of CF can be made on clinical judgment in patients with rare *CFTR* mutations of unknown significance
D. The benefit to an individual patient from standard CF therapies confirms the diagnosis of CF

C – correct

This case demonstrates a rare presentation of CF diagnosed early in adulthood. Her clinical features are typical for CF, including bronchiectasis, infection with usual CF pathogens, chronic sinusitis, and pancreatic insufficiency. Although her sweat chloride is relatively low, the diagnostic criteria developed at the 2015 CF Foundation Consensus Conference acknowledges the potential to develop CF with sweat chlorides ranging from 30 to 59 mmol/L. Even though 2 *CFTR* mutations were identified, the second is very rare and not well-characterized. However, there is no other plausible explanation for her combination of clinical findings combined with evidence of CFTR dysfunction. The diagnosis of CF best fits her clinical presentation at this time, and offers her access to therapies that are likely to extend her life. Her response to therapies is certainly fortunate, but many patients with bronchiectasis without CF may also respond to these treatments. Appropriate care takes priority over establishing a label, but it would be inaccurate to diagnose everyone who responds to CF therapies as having CF.

Which of the following statements is correct?

A. In patients with symptoms consistent with CF and low sweat chloride values or unusual CFTR mutations, it is important to evaluate for causes of their symptoms other than CF
B. It is unusual to see pancreatic insufficiency with a low sweat chloride value
C. If available, ancillary testing such as NPD could be helpful in this situation
D. All of the above

D – correct

In cases such as this, confidence in the CF diagnosis is increased if other typical causes of bronchiectasis have been ruled out. However, the presence of pancreatic insufficiency in early adulthood is not consistent with other diseases that result in bronchiectasis. In general, patients with sweat chloride values in this range develop bronchiectasis much later in life and are typically pancreatic sufficient. This case demonstrates that in the individual patient, a wide range of disease severity is possible. An NPD test may be of interest in this case but is likely to reflect the findings of the sweat chloride test.