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 reces- sive 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 regu- lator (CFTR).[4] There are more than 2000 mutations identified to date,[5] approxi- mately 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 estab- lishing the diagnosis in a minority of cases and because of this, healthcare pro- viders 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 popu- lation screening for maternal CF carrier status, prenatal ultrasound screening (that

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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

might reveal meconium ileus, meconium peritonitis, bowel ob- struction, or echogenic bowel), and newborn screening (NBS) has resulted in the routine diagnosis of asymptomatic or mini- mally 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 endorse- ments by the US Centers for Disease Control[7] and the CF Foundation,[8] the proportion of newly diagnosed patients iden- tified 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 Foun- dation in 2007 and published in *The Journal* in 2008,[9] indi- viduals 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 diag- nosed with CF by high levels of sweat chloride following a posi- tive 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 con- fusion for both families[15,16] and clinicians.[17] This group of infants, with varying levels of symptoms and a variety of *CFTR* mu- tations, has been the focus of discussions in the US and in Europe, with somewhat differing conclusions on both diag- nosis and management.[18,19] In addition, there has been a lack of international harmony regarding terminology, leading to con- fusion reflected in a recent article, entitled "Comparing the American and European diagnostic guidelines for cystic fi- brosis: 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 improve- ment in predicted survival requires careful attention to diag- nostic recommendations. Despite efforts to reach and sustain a consensus on diagnostic criteria, however, it has become in- creasingly clear during the past few years that CF Founda- tion 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 di- agnostic 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 chlo- ride 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 sce- nario has led to a new diagnostic term and management guide- lines, 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, sensitiv- ity 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 circum- stances, 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 pro- grams 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 al- gorithm 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 re- ferred for sweat testing if they display ultrahigh IRT levels (IRT/PAP-failsafe).[48] Pure biochemical IRT/PAP protocols nev- ertheless 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 com- bined 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 ex- perience, 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, per- sonal 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 neo- nates 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 trig- gered sweat testing, approximately 345 infants would undergo this diagnostic procedure. If ultrahigh IRT led instead to re- quired 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 spec- trum of risk for CF subsequent to a positive screen result. The likelihood of a "positive CF newborn screen" resulting in a di- agnosis 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 sup- porting genetic information, whereas others may have had ex- tensive 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 sup- ports 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 mis- labeling 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 in- valuable. Even though there may appear to be less need of a sweat test in the presence of meconium ileus because meco- nium ileus provides obvious evidence of a physiological defect, a sweat test revealing elevated chloride should still be the cri- terion to confirm the diagnosis. There have been many in- stances 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 work-up 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) reimburse- ment 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 diag- nosed 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. Re- quiring 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 per- formed as part of the diagnostic evaluation, even if a genotype was reported as part of the newborn screen (**Table I**, state- ment 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 muta- tions, a more in-depth genetic analysis may be useful. For example, if the c.350G>A mutation (legacy: R117H) is iden- tified, 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 posi- tive, 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 analy- sis 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 de- tected (**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 inter- pretations can be difficult. The mutation effects are not always clear-cut because of the presence of modifier genes, or envi- ronmental 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] Asymptom- atic 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 evalua- tion, 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 col- lected for analysis.

Although it is appropriate from the perspective of both phy- sician and patient to label newborns who screen positive with intermediate sweat chloride values and <2 CF-causing muta- tions 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* mu- tations 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 muta- tion 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 fi 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 geno- type (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 insuffi 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 un- derstanding 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 defin- ing the risk of CF for children with inconclusive genetic data. Gathering information about these biomarkers in early child- hood 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* vari- ants (and other genes) contribute to disease beyond our current understanding of CF.

Fecal Elastase (FE)

Demonstration of low FE levels <200 mg/g (in the absence of diarrhea) has been proposed as an indicator of pancreatic in- sufficiency and a diagnostic marker for CF. FE values fluctu- ate through the fi 12 months of life. In a study[76] of 61 infants diagnosed with CF through NBS, 48 infants (79%) had initial FE <200 mg/g; 13 of these 48 infants (27%) had a least 1 FE value >200 mg/g over the next several months before resolv- ing into levels <200 mg/g, and 4 of 48 infants (8%), on the other hand, displayed pancreatic-sufficient levels >200 mg/g by >9 months of age. In addition, 13 infants (21%) had initial FE >200 mg/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 insuffi who have "quantity not suffi " 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 mea- sured over the first 24 months of life in a cohort of 36 infants with CFSPID, all but one remained >200 mg/g, and none of

the babies later diagnosed with CF displayed low FE levels, re- maining pancreatic sufficient (Tanja Gonska, personal com- munication, October 2015).

Trypsinogen

Trypsinogen levels are already used to identify infants at high risk for CF and may be used to better advantage. Serum tryp- sinogen levels were serially examined over the first 36 months of life in 82 infants categorized as CFSPID and 80 infants di- agnosed with CF.[81] Overall, infants with CFSPID had signifi- cantly 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 re- mained 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 consen- sus 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 expe- rienced, 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 in- dicative of pathology (the test could not be completed in 3 chil- dren 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 identi- fi and intermediate sweat chloride levels demonstrated a con- nection 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 as- sociated 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 situa- tion, and analysis of CFTR function in the intestine (ICM) may be considered, aided by the fact that CFTR is highly ex- pressed in intestinal epithelia, offering high specifi y and sen- sitivity for the test.[83,84] Like NPD, ICM measurements must be

conducted in specific high quality reference centers with ex- perienced, 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 sen- sitive 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 vali- dated at present to recommend routine use and are not avail- able 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 applica- tion will undoubtedly occur.

Despite these advances, some infants still provide a diag- nostic 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 avail- able, 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 nonspecifi Chest computed tomography scans per- formed in infants diagnosed with CF following NBS through the Australian Respiratory Early Surveillance Team for CF in-

tensive surveillance program revealed 29.3% with bronchiec- tasis by 3 months of age,[93] although fi s differed in a United Kingdom cohort.[94] This finding is in keeping with earlier ob- servations 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 bac- teria 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 communica- tion, October 2015). At the time of the first culture, there was no significant difference between the bacterial species cul- tured from children with CF compared with those cultured from children with other lung diseases. However, when all sputum culture results from the fi 2 years were analyzed, cul- tures from children with CF were significantly more likely to contain *P aeruginosa*, *Enterobacter* species, *Escherichia coli*, *Kleb- siella 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 ret- rospective 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 Foun- dation Consensus Committee agreed upon the statements shown in **Table I** for diagnosing screened populations. Sweat tests, *CFTR* mutation analysis, and the ancillary tests de- scribed above may all prove valuable for establishing or con- fi ming 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 di- agnosis after NBS should be seen by the specialist CF team by 35 days of age and no later than day 58,[62] whereas the recom- mended 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 poten- tially fatal electrolyte depletion and hyponatremic dehydration[99] (**Appendix**, case study 3). Hence, it is important in some cir- cumstances to make a presumptive diagnosis of CF to enable

appropriate treatment and follow-up while pursuing diagnos- tic 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. Rec- ommendations 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 demo- graphic 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 informa- tion under consistent guidelines is critically important for all patients diagnosed with the disease, including those diag- nosed 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 impor- tant 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, geo- graphic 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* muta- tions 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 identi- fied 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 circum- stances 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 experi- enced 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 fi s 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 cat- egory 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 reclassi- fication of their genotype.

The guidelines described above should enhance the quality of data in the CFFPR. Other CF registries may wish to con- sider these recommendations. It would be ideal to have international harmony and consistency in the global CF registries. Perhaps the proper use of *International Statistical Classifica- tion of Diseases and Related Health Problems, 10th Revision* codes on an international scale, refl 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 • link to NBS video for families
http://www.nemours.org/service/medical/cvsticfibrosis.html	• 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 identifi 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 diffi To take advantage of burgeoning knowl- edge of the impact of various *CFTR* mutations and recent studies on cohorts with unclear CF diagnoses, the CF Foun- dation convened a committee of experts from around the world to update consensus guidelines on diagnosis of CF with an em- phasis on screened populations. The committee concluded that all diagnoses should be established by demonstrating CFTR dysfunction—by sweat chloride test where possible, or poten- tially by other methods such as NPD or ICM where neces- sary. 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 pro- viders and families, and ensure treatment is provided when needed, while avoiding medicalization of healthy infants. ■

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1.

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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 stuffi 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.

		Sweat Cl^-	Sweat Cl^-	Sweat Cl^-
Mutation 1	**Mutation 2**	**<30 mmol/L**	**=30-59 mmol/L**	**≥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?

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

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 screen- ing 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 pancre- atic elastase was low at <15 mg/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 ex- amination, 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 fi s 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.